The development of a cured, fermented sheepmeat sausage designed to minimise species and pastoral-diet flavours

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Table of Contents

List of Figures

List of Tables

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Statement of Originality

"I hereby declare that this submission is my own work and to the best of my knowledge, it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at any educational institution, except where due acknowledgement is made in the acknowledgement."

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Abstract

The purpose was product development to increase sheepmeat liking for consumers unhabituated to its characteristic flavour. The development has centered on a cured, fermented sausage, traditionally flavoured with garlic and rosemary, aiming to suppress the intrinsic species flavour (from branched chain fatty acids) and flavours (mainly skatole) from New Zealand's pastoral diets. However, appearance and texture are also important for liking. Therefore, the colour and textural properties of cured, fermented sheepmeat sausage were compared with those of beef and pork equivalents.

The problem of between-animal variation was overcome by comparing replicates made from meats bought on seven dates from different butchers. During fermentation over four days, samples were tested for growth of lactic acid bacteria, pH decrease, and colour and texture changes. There were no significant differences between the species.

Subsequently, the flavour of fermented beef sausage was assessed by consumers where the fat was pre-treated (or not as controls) with the ovine-characterising branched chain fatty acids (BCFAs), 4-methyloctanoic acid and 4-methylnonanoic acid, as well as with the pastoral flavour- characterising compound, skatole. Beef has no BCFAs and low concentrations of skatole, and was thus 'turned into sheepmeat' by addition of BCFAs and skatole.

The sausage mixture was in eight treatments that were combinations of cured vs. non-cured, flavoured vs. non-flavoured, added BCFAs/skatole vs. non-added. For sensory evaluation, 60 consumers tasted samples in a randomized design and completed a 9-point liking-score scale for each. Neither curing nor flavouring affected the liking of sausage treatments with or without added BCFAs/skatole. However, combined curing and flavouring significantly enhanced the liking of the added BCFAs/skatole treatment.

Thus, appearance and texture of fermented sheepmeat were the same as for other species; species and pastoral flavours in sheepmeat may be more acceptable to unhabituated consumers where fermented sausage is flavoured with at least traditional European flavours.

Chapter 1

Introduction

Brief history of food preservation

Moist foods are unstable in a number of ways for a number of reasons, both intrinsic and extrinsic to the food. These can result in changes in colour, texture, and flavour where flavour is a combination of odour and taste. Instability can also result in accumulation of a toxic microflora by way of colonisation of the human gut, or by direct toxicity of microbial products. The aim of food preservation is to delay adverse chemical, physical and microbial changes so as to extend the storage life of food. Throughout history and in the modern world, food preservation has been a major human activity.

The main preservation mechanisms are as follows: inactivation of enzyme and microbial activities by heating; irradiation; slowing down chemical, physical changes and microbial activity by cooling; lowering water activity; adding chemicals to prevent chemical, physical and microbial changes; using beneficial microorganisms to counter the development of spoilage and hazardous microorganisms; and application of packaging technology to create physical barriers to slow down any change. The most dominant of these methods are better known by the common names drying, smoking, chilling, freezing, canning, and curing and fermentation.

Drying

Drying is one of the oldest food preservation methods, which is still commonly used in the modern food industry. Drying can apply to a wide range of foods: meat, fruits, vegetables and crops, exemplified by beef jerky, dry banana chips, dry tomatoes, rolled oats etc. Although there are few early literature references to food drying as a means of preservation, Jopp (2010) reported a dried food sample that was dated 4000 years before present. Jopp also claimed that the Bible was the first book to record sun-drying used to preserve food. Drying is a simple, effective, and economic food preservation method, as it requires only heat from a source like sunlight to vaporise moisture in food. Dried food is in a storage-stable condition that is resistant to deterioration by bacteria and moulds. Food drying reduces water activity of food to prevent microbial growth (Rahman and Perera 2007). At a mechanistic level, low water activity equates to low molecular mobility that also minimises biochemical degradation due to enzymes and substrates endogenous to the food rather than the microorganisms.

Fresh foods normally have water activities between 1 and 0.95, thus all microorganisms can potentially grow. However, most microorganisms cannot grow where the water activity is lower than 0.91. Table 1 shows the minimum water activity required for the growth of pathogenic bacteria and moulds. It shows that even the salt tolerant *Staphylococcus aureus* cannot survive where the water activity is lower than 0.81. However, pathogenic moulds can survive in environment with lower water activity down to 0.76. Dried foods, which typically contain no more than 2.5% water have water activities range between 0.65 and 0.2. Only osmophilic yeasts may be grow where the water activity is between 0.65 and 0.6 (Rahman and Labuza 2007).

Table 1. Minimal water activity for the growth of pathogenic bacteria and moulds

Bacteria	Minimum water activity for growth
Bacillus cereus	0.93-0.95
Clostridium botulinum	0.93-0.97
C.perfringens	0.93-0.95
Salmonella spp.	0.92-0.95
Staphylococcus aureus	0.86-0.87
Vibrio parahaemolyticus	0.94
Moulds	
Alternaria alternate	0.9
A. flavus	0.78-0.8
A. parasiticus	0.82
A. orchraceus	0.77-0.83
Byssochlamys nivea	0.84
Penicillium cyclopium	0.81-0.85
P. viridicatu	0.83
P. orchraceus	0.76-0.81
P. cyclopium	0.82-0.87
P. martensii	0.79-0.83
P. islandicum	0.83
P. urticae	0.81-0.85
P. expansum	0.83-0.85
Stachybotrys atra	0.94
Trichothecium roseum	0.9

Source: Beuchat (1981)

Smoking

Smoking over a wood fire is also a food preservation technique with a long history. Smoking can involve exposure of the food to heat from a fire and thus at least the surface of the food would be dried to some extent, conferring some preservative effect by lowering water activity. However, smoking does not majorly reduce food moisture content; rather it generates compounds with an ability to prevent growth of microorganisms and lipid oxidation of foods (Cohen and Yang 1995). Wood smoking produces a family of phenolic compounds which effectively inhibit the growth of bacteria. (The composition of phenols from smoking depends on the nature of wood) (Rahman and Perera 2007). These chemicals in wood smoke also add flavour and colour to the food. In smoking's modern application, there are two categories of food smoking, exemplified by fish. These are cold-smoking and hot-smoking. As the name suggests cold-smoking applies smoke at relatively low temperatures, say 35°C, and in this situation, the fish remains raw. Hot-smoking also cooks the product. Salt is also usually applied to muscle foods prior to smoking (Rahman and Perera 2007) and this serves to lower water activity (Huang and Nip 2001) and inhibit some classes of microorganism. Overconsumption of smoked foods is a risk factor in stomach cancer because some the smoke components – polycyclic aromatic hydrocarbons for example – are carcinogens (McIlveen and Valley 1996). This problem can be overcome by using so-called liquid smoking. Liquid smoke is liquid extract of smoke substantially free of these hydrocarbons, but which contains the compounds responsible for antimicrobial activity (Rahman and Perera 2007), colour and flavour.

Chilling

Chilling is widely applied in modern food storage, both on an industrial scale and domestically. Chilling simply involves lowering the temperature as close to the freezing point of wet foods as possible (-1.5°C for raw meat), but due to costs of refrigeration and its control, this ideal is not often achieved, particularly in domestic situations. In its widest context, chilling can mean storage at any temperature below ambient. Chilling is a short-term preservation technique, which slows food deterioration due to biochemical changes endogenous to the food or changes due to exogenous factors, namely microorganisms. The growth rate of microflora on food is generally slowed by chilling (Herbert 1989; Russell 1990), and this is probably due to lower rates of reaction. The slower growth rate effect extends the time required for multiplying microorganisms to reach a hazardous concentration for human consumption. Most of the pathogenic bacteria cannot growth at 3°C, although *Listeria monocytogenes* and *Yersinia enterocolitica* are still able to grow (Russell and Gould 2003). However, food spoilage due to

yeasts and moulds occurs slowly even when the product starts to freeze and below that temperature (Herbert 1989). In modern food distribution systems, chilling is often applied with modified-atmosphere packaging (MAP), particularly for fresh fruit and vegetable preservation. Chilling, MAP salad is able to be stored at 4-7°C up to 7 days (Leistner 2007). Much of the New Zealand chilled meat export trade is conducted with MAP systems.

Freezing

Freezing of food involves storage below about -1.5°C and is accompanied by development of ice crystals and a simultaneous increase in the concentration of dissolved solutes in liquid water (Fennema 1975). The lower the temperature the more storage-stable the food becomes (Fennema 1996), but as with chilling of foods, low temperature storage is constrained by cost of refrigeration. Generally, at freezing temperatures the metabolism of microorganisms is inhibited strongly or prevented entirely. However, deterioration of foods by endogenous biochemical changes is slowed down, but not stopped. Moreover, the development of growing ice crystals, which occupy a volume about 9% more than water, leads to cell membrane damage, resulting in enzymatic and nonenzymatic damage leading to 'freezer burn' (Franks 1985). Where, ice crystals volatilise from the meat surface, leaving a dry parchment like appearance on the meat surface. Thus, the important problem of frozen preservation is ice crystal damage. When the food thaws, the cellular water leaks from the cell structure, and thus the food matrix is damaged. Thus freezing can adversely affect the quality of food.

Although it is believed that microbial activity is rare at low freezing temperatures, say -18°C, spores of *Clostridium* and *Bacillus* species, are unaffected by long-term frozen storage (Moharram and Rofael 1993; Peterson 1961; Sebranek 1996). The non-spore forming *Listeria monocytogenes* is even more resistant to frozen temperatures. The bacterium can survive in food after storage at -50°C (Gianfranceschi and Aureli 1996).

Canning

The long-shelf-life technology for preserving food was invented by Nicholas Appert in the 19th century in response to military needs (Barbier 1994), and was widely adopted particularly when the original glass jars were replaced by tin-coated steel cans. Canned food was and remains an important food preservation method for the military (National Canners Association Communications Services 1971). Canning can be applied to nearly all food types. Canned food is almost indefinitely self-stable, as it has almost nil enzyme activity, microbial activity, and very low oxygen content. Thus, food deterioration is unlikely to occur. Canning can also apply to a range of capacity from grams to kilograms. However, in Western societies domestic refrigeration

(chilling and freezing) became increasingly common from the 1960s and preservation by canning has subsequently become less popular in those societies (Hutton 2004). This is because canning necessarily causes organoleptic changes in many foods that chilling does not cause. Canning causes loss of desirable aroma and texture of foods, changes in food colours, and reduction of nutrients like some vitamins.

Canning is a commercial sterilization preservative method, employing metal and glass containers, and more recently retortable plastic pouches. It destroys microorganisms and spores to keep the foods safe at ambient temperature until consumption. Canning involves so-called retort temperatures to destroy microorganisms. For example, for non acid foods, the cans are heated to 121°C and held for at least 3 minutes to achieve sterility (Ramesh 2007).

Curing and fermentation

Historically, curing means preserving food through variations and combinations of drying, salting and smoking. Nowadays, the term curing has come to mean a preservation method using the combined effect of nitrate/nitrite and salt, to extend food storage-life and improve food flavour (Hutton 2004).

Fermentation is one of the oldest food preservation methods along with salting and curing, which is inherited from lands with warm climates. When cooling methods were unavailable, fermentation was a significant method to extend storage life. Worldwide, the main fermented foods are dairy products, meats, fish and vegetables (Hutton 2004). Curing and fermentation will now be discussed in more detail, with a particular emphasis on meat.

Meat curing and fermentation

Curing

The food preservation ability of salt has been well known for thousands of years. The origin of curing meat was credited as prevention of meat spoilage through the addition of common salt. The history of meat curing can be traced back to at least 2100 years before present, a document describing dry cured hams by Cato (Martin 2001). Analytically pure NaCl will not induce the characteristic red colour and flavour of cured meat. This was and remains due to impurities in salt: sodium nitrite and or/nitrite. Now cured meat is defined as the addition of purified salt plus nitrite/nitrate with the purpose of meat preservation, which is associated with specified colour and flavour (Martin 2001).

The quality of early cured meat was highly variable. It was not until the late 19th century, that curing formulations were developed in response to increased commercial production of cured meat. Variability was not wanted. Some rapid curing methods were also invented at this time, like brine injection. Modern curing involves lower salt and nitrite concentrations in the final product in response to health concerns (Pearson and Gillett 1996). Because these lower-concentration products are less storage-stable, modern products often require additional preservation boundaries such as vacuum packaging and refrigerated storage to assist in maintaining quality and safety before consumption.

Salt is the basic curing ingredient used for all types of curing products such as brine and dry curing. Salt binds water and increases the osmotic pressure, both effects manifest as a lowering of water activity. The change in osmotic pressure of microbial cells kills the non-salt tolerant bacteria and some moulds that cause food deterioration (Pearson and Gillett 1996). Also, salt is important for the flavour of meat products and the gelation of emulsion sausages (Shackelford 1989). But when salt is used alone, the meat products are dry, harsh and brown, due to the formation of metmyoglobin. For these reasons, salt is used with sugar/sweeteners and nitrate/nitrite.

Food-grade salt used for curing is highly purified, because impurities in salt can affect the appearance and flavour of products. Metals ions such as copper and iron can affect the colour development of cured meat. Moreover, these ions catalyse lipid oxidation, resulting in the development of rancid flavour (Andersen and Shibsted 1991).

The salt concentration in meat products is between 2 and 4% of the total product weight. Too much salt will result in very salty tasting products and too little salt adversely affects meat protein gelation and preservation. Recently, reduction of salt use in food has drawn a lot of attention. High salt food is a risk for hypertension. Sodium chloride use is associated with hypertension, and its substitute potassium chloride has been partially successful in the development of low salt foods. A 60% sodium chloride and 40% potassium chloride mixed salt was found to be the most favoured combination by consumers for reduced sodium foods (Pearson and Gillett 1996). The regulation stated that, product with sodium chloride 35% lower than equivalent products can claim the description ‘reduced sodium’ product. Although reduced sodium products are beneficial for hypertension, the taste of these products is deemed to be less salty (Pearson and Gillett 1996), and potassium imparts a bitter flavour. That is one reason why potassium chloride cannot fully replace sodium chloride.

Many types of sugars such as glucose, sucrose and corn syrup are used in cured meat products to overcome the harshness due to salt and to enhance product flavours. The amount of sugar added is self-limiting, depends on the taste required and the sweetness intensity of the particular sugars. Usually, the most economic way of addition is to use liquid sweeteners that can be pumped into brine (Martin 2001). Reducing sugars, of which sucrose is not one, also contribute to Maillard browning and caramelization, which may or may not be wanted in the final product.

The use of nitrite for food preservation was an unavoidable result of nitrite and nitrate occurring as impurities in salt. These result in the development of a pinkish-red meat colour (Binkerd and Kolari 1975). Because the concentration of these salt compounds was probably variable, product colour was also variable. At the time, manufacturers did not know the role of nitrite in colour development and preservation. Martin (2001) states that the first use of nitrate as a legal food additive substance was in 1908 by the Bureau of Animal Industry of the United States Department of Agriculture, and they permitted nitrate as a cured meat ingredient in later years (Kerr and others 1926). During the decade 1950 to 1960, the technology of curing was greatly improved as the chemistry of the process became better understood (Martin 2001). The active ingredients in meat curing are salt and nitrite. Nitrate can substitute for nitrite in the following way. The reductive environment and the microflora of meat reduce nitrate to nitrite. After this reduction, nitrite is further reduced to nitric oxide and water. Nitric oxide reacts with myoglobin to form mainly nitric oxide metmyoglobin, which imparts a brown colour at the initial stage of the cured meat process. Eventually, nitric oxide metmyoglobin is reduced to the pink-red nitric oxide myoglobin on heating, if applied, converts to the stable nitrosohemochrome which is also pink-red (Pearson and Gillett 1996).

The functions of nitrite in cured meats are lean meat colour stabilization, bacteria inhibition, flavour development and prevention of lipid oxidation. Originally, colour stabilization was the primary reason to add nitrite to cured meat products. However, it turns out that the most important outcome is to inhibit the growth of pathogenic bacteria and to improve product flavour.

A major role of nitrite is the inhibition of the growth of *Clostridium botulinum* (Sofos and others 1979), which is able to produce a neurotoxin that causes serious neuroparalytic disease (Tompkin and Christiansen 1976). The mechanism of nitrite inhibition involves at least the binding of iron ions by nitric oxide making it unavailable to *Clostridium* (Tompkin 1978; Reddy and others 1983). In addition, nitrite limits the metabolic exchanges on cell membranes.

Nitrite is believed to be involved in the characteristic cured meat flavour development but it is not acting directly as a flavour enhancer (McDougall and others 1975). The most recognised role of nitrite is the inhibition of fat oxidation that leads to rancid flavour. And certainly, nitrite improves the cured meat colour that improves acceptability of cured meat product appearance for consumers (Wasserman and Talley 1972). The effect of nitrite in preventing lipid oxidation is directly involved in the same reactions responsible for colour development of cured meat (Cross and Ziegler 1965; Watts 1954). The iron ions in myogolobin are fat oxidation catalysts, but when nitrite undergoes the reactions of colour formation, the iron is retained firmly bound in the haem of myoglobin and unavailable to oxidise fats to yield a rancid or 'warmed-over' flavour in cured meat products.

Toxicity of nitrosamines

In recent years, there has been concern over a health risk due to overconsumption of nitrate and ultimately its reduced form nitrite. The richest source of nitrate in the human diet is from vegetables (Richardson 1907). The human nitrite intake from cured meat is small compared with the intake from vegetables such as spinach, lettuce and beets, (Rubin 1977; White and Jr 1975) where bacteria in the mouth reduce nitrate to nitrite. The intake of nitrite from different sources: about only 10 to 21% of nitrite comes from cured meat, the rest coming from vegetables. As long as nitrate intake is below 300 mg per kilogram of body weight per day (Tannenbaum 1976) there is a very low risk of toxicity. Nitrite in excess affects the ability of the liver to store vitamin A and disturbs thyroid function (Emerick and others 1963). Further, nitrite can bind to haemoglobin in the blood (and to myoglobin in muscle) and limit the oxygen carrying capacity of blood (Martin 2001). Nitrite can also yield the carcinogenic nitrosamines. These are the result of reaction between a secondary amine and nitrite, shows in Figure 1.

Figure 1. Nitrosamine formation. The reaction of a secondary amine and nitrite to form a nitrosamine (Coultate 2002).

This reaction occurs in the gut and also occurs in cured meats, particularly where the temperature is raised as is the case with well fried bacon, but not in the case of cold-cured hams etc. (Martin 2001). Modern cured meat formulations often include ascorbic or erythorbic acid. These not only help to minimise fat oxidation, but also help to stabilise the cured colour and inhibit the nitrosation reaction in Figure 1. Although nitrite in cured meat has some health issues, there is no substitute to replace the unique properties of nitrite in the role of colour development and bacterial inhibition in cured meat products (Bailey and Swain 1973).

Fermentation

Application of fermentation has been known from ancient time in many cultures, the biological driver being food preservation to prevent starvation when fresh food was limited. Moist foods will not remain edible for long, owing to microbial colonisation. Moreover, deteriorated foods can be poisonous for humans. Fermentation is a low cost food preservation technique that can apply to a wide range of moist foods, including meat.

Dry fermented sausage is one of the oldest fermented foods. One of the most well known dry fermented sausages is salami, that took its name from the ancient Greek town of Salamis (Pederson 1979), and this fermented style remains widely accepted in Western countries (Smith 1987). In ancient Rome, leftover fresh meat was blended with salt then extruded into skin casing and hung inside a room to dry. People only knew that these dry sausages had a special flavour but did not understand the fermentation role of lactic acid bacteria. Around the same time, a fermented dry sausage named Lap Cheong was developed in China (Leistner 1986). None of these styles required cooling to minimise microbial colonisation, and this was presumably the reason for their widespread adoption, particularly as meat animals could not survive extreme winters without huge energy and resources supply. Fermentation captured and preserved the food value of an animal.

There are many fermentation pathways, each applicable to a given fermented food. In this thesis, discussion is limited to lactic fermentations. Lactic fermentation requires the action of a suitable bacterium acting on a carbohydrate source, that is either endogenous to the food or is added. In the case of milk, lactic acid bacteria form lactic acid by fermentation of lactose, a dimer of galactose and glucose. Thus, yoghurt is a lactic fermentation product from milk. Meat also contains some carbohydrate that can support the growth of lactic acid bacteria, but in modern food industry practice, artificially adding glucose as carbohydrate source guarantees a supply for the bacteria, and accelerates the growth rate of the bacteria and thus the fermentation to lactic acid.

In earlier times, the fermentation relied on the naturally occurring microflora on meat. The natural fermentation of sausage required experienced masters to control the fermentation quality. They ‘back slopped’ finished sausage to fresh sausage mixture to maintain a suitable fermentation flora (Bacus and Brown 1982). In modern practice, starter cultures with a known bacterial profile are applied (Zeuthen 2008) to guarantee fermentation. Starter cultures reduce the fermentation time, product variance and cost of fermented products. In addition, starter cultures improve the safety of fermented products, owing to their rapid dominance in the fermenting sausage.

The USA was the first country to use starter cultures isolated from natural fermentation products, and this traces back to the 1920s (Zeuthen 2008). The first academic report was published in 1940 by Jensen and Paddock. They patented the first use of mixed strains of *Leuconostoc spp*. The first starter culture in Europe developed by Niinivaara (1955) was named M53, which was a single strain of *Micrococcus*. In the USA, the first commercial starter culture was of *Pediococcus acidilactici* (Niven and others 1959). In the modern fermented food industry, mixed culture starters are used commonly to achieve different products requirements. At present, a wide range of fermented food exists in the market - vegetable food, animal products and dairy products. Table 2 summarise some common fermented foods and their principle microorganisms used as cultures.

Fermented foods still play an important role in the human diet, because fermented foods are shelf stable, have low energy consumption, are easily digested, have unique sensory properties and nutritional benefits. Actually, the market demand for fermented foods has increased recently, as consumers regard fermented foods as natural and beneficial for health (Granato and others 2010).

Table 2. Some common fermented foods

Food	Microorganism responsible
Alcoholic beverages	
Beer	Yeasts
Wine	Yeasts
Spirits	Yeasts
Sake	Moulds
Dairy products	
Yogurt	Lactic acid bacteria
Cheese	Lactic acid bacteria
Butter milk	Lactic acid bacteria
Fermented vegetables	
Pickle cucumber	Lactic acid bacteria
Sauerkraut	Lactic acid bacteria
Pickled olives	Lactic acid bacteria/(yeasts)
Fermented animal products	
Fermented sausage	Lactic acid bacteria
Fermented fish	Lactic acid bacteria
Bakery products	
Bread	Yeasts
Sourdough bread	Lactic acid bacteria + yeasts
Sauces	
Vinegar	Yeasts + acetic acid bacteria
Soy sauce	Moulds + yeasts + lactic acid bacteria

Source: Guizani and Mothershaw (2007)

Lactic acid bacteria (LAB) have been used to produce fermented foods throughout human history. These bacteria play a very important role in fermentation. LAB are Gram-positive, catalase-and oxidase-negative, and nonspore-forming bacteria, which favour anaerobic growth and use glucose as a carbon source to produce lactic acid as a metabolic end product (Guizani and Mothershaw 2007). Lactic acid and other organic acids also produced by LAB are antimicrobial substances in their own right, but also lower the pH. These combined effects inhibit or kill other competing microorganisms (Axelsson 1998). LAB can thus be used as a food preservative. According to the products of glucose fermentation, LAB can be divided into two groups: homofermentative and heterofermentative. Homofermentation produces lactic acid as the sole fermented product of glucose, whereas, heterofermentation produces lactic acid plus carbon dioxide and ethanol. Also, heterofermented LAB are important to produce aroma substances such as acetaldehyde (Guizani and Mothershaw 2007). Moreover, the carbon dioxide produced by heterofermentive LAB generates an anaerobic environment that suppresses the growth of aerobic bacteria (Steinkraus 1983).

Table 3. Common genera of LAB and their fermented food products

Genus	Food product
Lactococcus	Yoghurts, cheese
Leuconostoc	Kimchi/sauerkraut (fermented cabbage, pickles)
Lactobacillus	Yoghurts, salami
Streptococcus	Yoghurts, Parmesan cheese
Pediococci	Sausage
Enterococcus	Unhygienic fermented foods

Source: Bamforth (2005)

LAB not only produce organic acids (principally lactic acid) but also antimicrobial substances known as bacteriocins e.g. nisin. These substances are cationic amphipathic peptides that able to damage cell membranes of susceptible microorganisms, resulting in cell leakage and metabolic inhibition (Bamforth 2005). Table 3 shows the common LAB used in the food industry and examples of their application. There are also some potential health benefits induced by LAB, such as an enhanced B vitamin content, improvement of nutrient digestibility (e.g. lactose intolerance is avoided), and inhibition of tumour growth (Drouault and Corthier 2001).

Fermented meat

Fermented meat products are mainly fermented meat or fish sausages, using cultures of LAB either endogenous to the food or added. In general, fermented sausages are made from comminuted meat, salt, glucose and LAB. With the growth of LAB, the generation of lactic acid rapidly reduces the pH to between 4.0 and 4.5. This pH, and presumably the concomitant effect of lactate as undissociated lactic acid and bacteriocins, inhibits the growth of other microbial species, but not the LAB. Table 4 shows the limitation of pH for growth of some microorganisms. Other non-pathogens such as some staphylococci and yeasts can be added to fermentation mixtures to develop distinctive colours and flavours. More complexity can be added with curing, smoking and adding spices.

Table 4. The minimum of pH for the growth of some microorganisms

Microorganisms	Minimum pH
Salmonella typhi and *Clostridium botulinum*	4.5
Escherichia coli	4.4
Clostridia	4.0
LAB	3.0
Yeasts and moulds	1.5

Source: Hutton (2004)

According to the extent of drying, fermented sausages can be separated into three groups. The first group is dry fermented sausages, which require more than four weeks' fermentation and maturation; the water activity is less than 0.9. The best known examples are salamis, traditionally originating from countries like Italy, Hungary and Germany. The second popular group is the semi-dry sausage with water activity between 0.9 and 0.95, needing about three weeks' fermentation and maturation. This group includes most fermented sausages common in France, Spain, Holland and USA. The third group is less well-known than the first two, the undried sausage. Water activity is also between 0.9 and 0.95, with a fermentation and maturation time of less than two weeks, e.g. Spanish sobrassada (Lücke 2003).

The LAB genera *Lactobacillus*, *Pediococcus* and *Staphylococcus* are used commercially by the sausage industry. They are tolerant of low water activity due to the salt content and grow rapidly, leading to a reduction of pH. Moreover, the bacteriocins produced by LAB significantly inhibit food pathogens such as *Clostridium botulinum*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Nettles and Barefoot 1993). Normally, fermented sausages are nitrite cured. Non-pathogen *Staphylococcus* species are also included in the inoculums. They assist in reduction of nitrite-myoglobin complexes to stable forms (Hammes and Hertel 1998) as discussed in the section on Curing.

The characteristic flavour of fermented sausage is due to LAB fermenting the carbohydrate source to lactic acids and other organic acids that are together responsible for the typical sour flavour. Furthermore, after the pH is lowered by the growth of LAB, the tertiary and, secondary structure of meat proteins is adversely affected, commonly known as denaturation. Some proteins are hydrolysed to generate peptides and free amino acids (Nishimura and others 1988). In addition, fat oxidation progresses slowly throughout fermentation, and the addition of salt,

sugar, and spices contribute to flavour. Thus, these combinations define the flavour of fermented sausages (Toldrá and others 2001; Lücke 1985).

Preservation of sheepmeat for international markets

Worldwide, fermented sausages are almost all made from pork, beef and mixtures of pork and beef. The differences between these pork and beef sausages are derived from degree of drying, genera of LAB, fat to lean ratio, salt and sugar contents and use of spices based on cultures and geographical separation (Table 5).

Table 5. Typical fermented sausages in some countries

Country	Sausages	Distinctive feature
Denmark	Danish salami	High in fat and salt
France	Saucisse de Montbeliard	Smoked
Hungary	Kunsag	Black and white pepper, garlic and caraway
Italy	Mortadella	Visible fat cubes
Holland	Salami, cervelat	Finely chopped ingredients and high fat content
China	Lap Cheong	Only made in winter, soy sauce, alcoholic beverages
Thailand	Sai Ua	Curry paste

Source: Incze (2008) and Toldrá and others (2008)

Fermented sausages made from other animal meats are rare, but can be found in the Middle East. As a religious prohibition edict, pork is not allowed for consumption by Muslims, thus other animal meats are used for sausage making, such as sheepmeat. Also in northern European countries, Finland, Iceland and Norway that have very cold winters, there is an incentive to slaughter domestic/farm animals that might not survive. The meats of horse, reindeer and lamb are made into sausages (Toldrá et al. 2008). The plan in this thesis is to develop a fermented, cured sheepmeat sausage as a means of creating benefit.

New Zealand is one of the largest contributor to the global sheepmeat market; it occupies more than half of the international sheepment trade (McDermott and others 2008). The world market has a high demand for New Zealand lamb, because New Zealand Lamb (as the official brand) purportedly has an image of “clean, green, grass-fed, healthy, and doesn’t get any more free range than this” (Clemens and Babcock 2004). Europe is the largest importer of New

Zealand lamb. In 2006, 51% of total New Zealand lamb was shipped to Europe (Meat and Wool New Zealand Economic Service 2007). In the U.K., New Zealand lamb is one of the most well known brands of lamb among consumers (Meat and Poultry News 2003). Another consumer survey completed in U. K. claimed that consumers there like New Zealand lamb more than the lamb from other countries (Clemens and Babcock 2004).

Whereas lamb is popular, the two other categories of sheepmeat are not so popular. For historical reasons, the definition of sheepmeat is based on the British definitions of lamb, hogget and mutton according to animal maturity as determined by teeth development. Sheepmeat sourced from sheep less than 12 months is named lamb. 'Two tooth' animals, roughly between 12 and 24 months are termed 'hogget', whereas 'full mouth' animals are termed mutton. According to Young and Lim (2001), consumer perceptions of 'quality', 'flavour' and 'healthiness' on name and name alone all declined in the sequence: lamb > hogget > mutton. In markets, lamb commands the highest retail price, and hogget and mutton are much cheaper.

In seeking to add value to sheepmeat it would seem important to use the cheaper hogget and mutton, if the deficiencies in these two categories, real or perceived, can be overcome. Whereas mutton has the strongest distinguished sheepmeat flavour and toughest meat structure (Young and Braggins 1998), there is no significant difference in eating quality between lamb and hogget (Young and others 2006). In time, the distinction between lamb and hogget may be lost. This leaves mutton as the opportunity with which to add value.

The toughness of mutton can be overcome by extending cooking in casserole styles and by comminution. A fermented, cured sheepmeat sausage necessarily requires comminution, so that problem can be considered solved. Flavour is another matter. The characteristic sheepmeat odour/flavour is caused by branched chain fatty acids (BCFAs) (Wong and others 1975) and skatole (Young and others 1997; Young and others 2002). The main BCFAs involved are 4-methyloctanoic acid (4-MeO) and 4-methylnonanoic acid (4-MeN). Development of a fermented, cured sheepmeat sausage also provides the opportunity to suppress these flavours in mutton, potentially solving the flavour problem. An experimental design to test this opportunity raises a problem. If lamb or mutton were used, a 'control' lamb or mutton should be used that contained no BCFAs or skatole. No such sheepmeat has been identified. Therefore, the strategy used by Prescott and others (2001) was followed here. Prescott used grain-finished beef as the control and added BCFAs and skatole to simulate sheepmeat, on the basis that grain-finished beef contains no BCFAs and has very low concentrations of skatole. Grain-finished beef could not be

obtained, so conventional pasture-finished beef was used instead, realising that some skatole would be avoidably present in the control.

Salamis are cured, fermented sausages, which are subsequently partly dried and often smoked at a low, about 30°C, or higher temperatures, about 50 to 60°C. For the present study, the cured, fermented sausages were neither dried nor smoked, and thus represented an uncomplicated model – a fresh, cured, fermented sausage – for comparison of the textural and flavour properties.

The experimental plan

The purpose of the study is to add value to New Zealand mutton through comminution, curing and fermentation, and applying flavours to suppress sheepmeat flavour caused by BCFAs and skatole. For the initial physicochemical phase of the work (Chapters 3), the properties of sheepmeat sausage were compared with properties of equivalent sausages made from and beef and pork. The sheepmeat chosen was chilled lamb, rather than mutton, because the former was always available and, as discussed above, comminution eliminate any problem due to toughness. The sensory phase of the work (Chapters 4) created a synthetic mutton flavour by addition of BCFAs and skatole to beef (which is devoid of BCFAs), to explore the effect of curing and culinary flavour addition. Rosemary and garlic are commonly used flavours in lamb dishes and were chosen as the potential sheepmeat flavour suppressors.

The next chapter describes the materials and methods used in this work.

Chapter 2

Material and Methods

Chemicals

Salt, glucose, $Na_4P_2O_7.10H_2O$ (pyrophosphate) (Produits Pour Laboratoires, RHONE-POULENC, Decines, France, No. 28053) and sodium nitrite (Riedel de Haen AG, Seelze-Hannover, Germany, 7E 60421) were sourced from the AUT chemistry laboratory. A fermentation culture mixture (*Pediococcus pentosaceus* and *Staphylococcus carnosus*, BFL-F02 BactoFlavor®) was donated by Chr. Hansen Pty. Ltd. (Melbourne, Australia). Rosemary essential oil (FN11146) and garlic essential oil (FN11516) were produced by Lionel Hetchen (Essential Oils) Limited (UK) and were donated by Hawkins Watts Limited (Auckland, New Zealand). The branched chain fatty acids (BCFAs), 4-methyloctanoic acid (4-MeO) and 4-methylnonanoic acid (4-MeN) were donated by AgResearch Limited, Hamilton, as was 4-methylindole, also known as skatole. Man-Rogosa-Sharpe (MRS) medium, peptone water medium, Baird Parker medium (BP), MacConkey broth medium base, Eosin Methylene Blue (EMB) medium, trytone water medium, Brilliant Green Bile Broth (BGBB) medium were purchased from Fort Richard Difco Auckland, New Zealand.

Meat purchasing

There were three meat species (beef, lamb and pork) used in the first phase of the research. The muscle tissue used was from the forequarter for all species. On average, forequarter meat of these species contains a higher concentration of connective tissue (Wenham and others 1973) and for reasons explained in the Introduction – price among them – is better suited to adding value by way of comminution and fermentation. However, the forequarter comprises multiple muscles each with its own connective tissue profile. To narrow the selection further, shoulder cuts were asked for and supplied, realising that there would still be variability depending on the source animal and exact butchery cut. To minimise these sources of variation (animal, cut), seven purchasing trips made on seven different days (over a period of months) to seven different retail butchers. Orders were placed with the butchers three days in advance of purchasing and this ensured that sufficient carcass fat was included in each purchase.

At each purchase, the lean and fat from the three species were packed in separate plastic bags and transported to AUT Food Laboratory under chill conditions. As soon as the meat arrived, it was held at refrigeration temperature until processed on that day.

Modified syringe casing preparation

Making normal sausage using collagen casings requires a high skill level to evenly extrude the meat mixture into the collagen casing. Moreover, the scale of mass required is in the tens of kilograms, well above the masses involved in these laboratory experiments. The sausage casings were replaced by modified syringes, as first used by Khem (2009). The plastic syringes (BD Plastipak, 300865, Becton Dickinson, Ireland) have a nominal 50 mL capacity, but can hold 60 mL and 130 mm long with an internal diameter of 25 mm. To prepare the syringes, the piston is withdrawn and the tapered Luer-lock is excised by lathe. The barrel thus becomes an open-ended cylinder (Figure 2) into which the sausage mixture is to be extruded (and ultimately held in place by the reinserted piston – see below). Before extruding the sausage mixture into the syringe barrel, the barrel is lubricated with a thin layer of petroleum jelly. This acts as a release agent as the fermenting sausage is extruded periodically for the various tests.

The sausage mixture is extruded into the syringe barrel by a domestic Kenwood food processer using the nozzle attachment which is the output of the worm drive (Figure 3). Care is taken to avoid air bubbles. After the barrel is filled, the piston is replaced and forced back to the 60 mL mark. The extruded excess sausage mixture is cut off with a clean spatula. After wiping the barrel clean, the open-end is first sealed with one layer of Parafilm®, then overlaid with a 50-mm square sheet of aluminium foil. A rubber band is used to hold the aluminium foil firmly onto the syringe barrel (Figure 4). These seals are designed to maintain an anaerobic environment.

Figure 2. A modified syringe barrel. The syringe barrel on the left was cut from a normal syringe shown on the right.

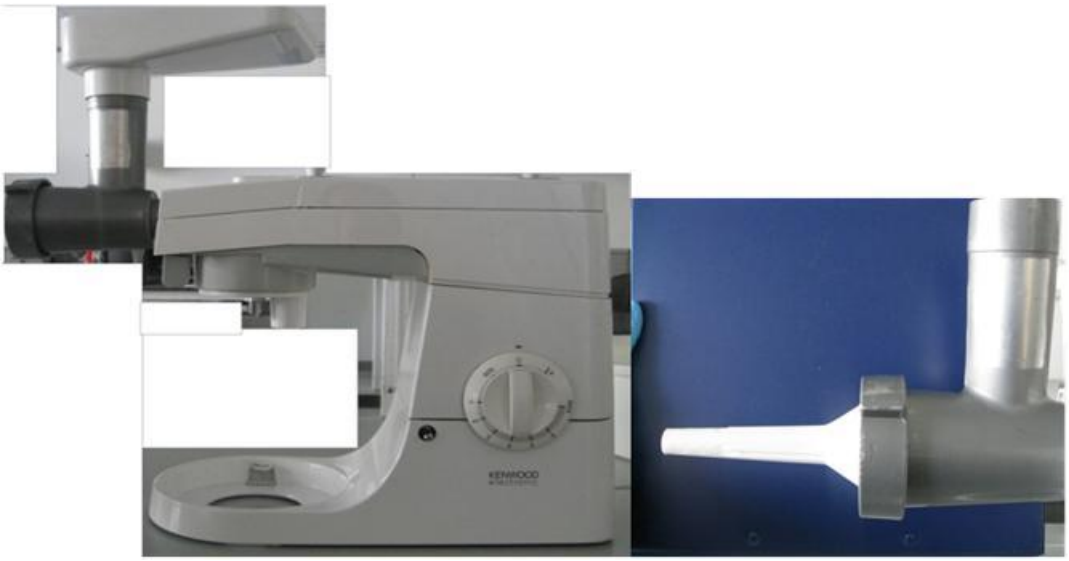


Figure 3. Mincing equipment. The domestic Kenwood food processer was used to prepare minced raw sausage (left) and the extruder nozzle (right) was used to fill the syringe barrels.

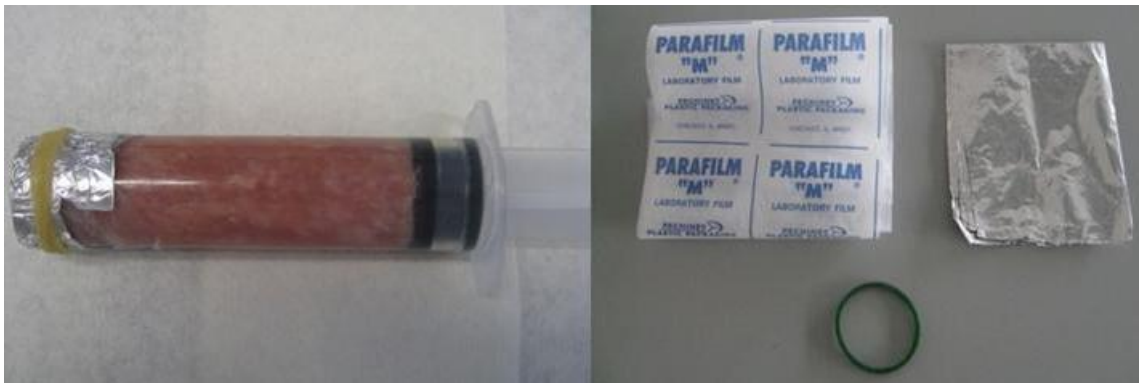


Figure 4. A sample of sausage. A syringe barrel filled with sausage mixture and sealed with Parafilm®, aluminium foil and a rubber band.

Sausage meat preparation

Disposable gloves were worn at all time when handling meat and its products. Some fatty tissues remained attached to lean and vice versa, and a limited attempt was made to fully separate the two components, based on colour. However, separation was not perfect but was approximately constant between species. After trimming, lean and fat were cut into cubes about 15 mm on edge. Lean and fat cubes were distributed in suitable quantities among plastic vacuum barrier bags (D. M. Dunningham, Auckland, New Zealand), and labelled as beef lean, beef fat etc. After evacuation, the packs were kept in a -80°C freezer. It was unnecessary to store at such low temperature. However, this freezer was the only facility available, and maintenance of quality overtime could be guaranteed.

A domestic Kenwood food processer (KM300) with a mincer attachment was used for lean and fat mincing, and for extruding the sausage mixture into the modified syringes described earlier. Knives, cutting boards, mincing parts, spatulas, large mixing bowls and other utensils in contact with meat were washed hygienically and dried with paper towels. The mincing barrel, a 4-mm cutting plate, and the cutting accessories were held dry in a refrigerator at 4°C to keep them cool. At the point of using the lean and fat cubes, the barrier bags were submerged in warm water to the point of thawing. After thawing, the cubes were minced through the 4-mm cutting plate, lean before fat with no intermediate cleaning. Utensils were washed and dried between species.

The ratio of lean and fat was 2.13 to 1 and together comprised 94% of the final formulation (Table 6). After the lean and fat had been combined in sufficient quantities for the experiment in hand, the other sausage ingredient quantities were calculated. All ingredients were placed into a Kenwood mixing bowl, and mixed at maximum speed for 5 minutes. The sausage mixture was then passed through the mincer again fitted with plate and cutter, followed by extrusion into the opened-ended syringe barrels.

Table 6. Basic formula of sausage meat mixture

Ingredient	Proportion of sausage mixture by weight (%)
Lean	64
Fat	30
Salt	4
Glucose	2
$Na_4P_2O_7.10H_2O$	0.2
Sodium nitrite	0.01
Fermentation culture	0.01

For the physicochemical work, five syringe sausages were made for each meat species at each of the seven days of meat purchase. Three syringes were used for daily physicochemical analyses and two were used for texture analysis at the end of fermentation. After the syringe barrels were sealed (see earlier), they were placed horizontally on trays and incubated at 30°C in the dark for 96 hours. The remaining sausage mixture after filling the syringes was used to generate the Day 0 data. Every 24 hours, a 20 mm length of sausage was extruded from each of the three of syringe barrels for each species. These cylinders were further dissected to yield sufficient quantities for each physicochemical test. The 20-mm syringe-extruded cylinders represented fermentation Days 1, 2, 3, and 4. After syringe extrusion, the syringes were resealed and returned to the incubator. Texture analysis required 30-mm samples that were extruded conveniently from the two syringes dedicated to texture.

Physicochemical analyses

Colour measurement

The colour was measured by a reflectance spectrophotometer (Model 45/0 Hunterlab ColourFlex, Reston, VA, US). L*, a* and b* were the parameters used to describe reflectance, redness/greenness and yellowness/blueness, respectively. The discs were placed in the centre of a glass crystallising dish (Schott, Duran, Germany) which was used to hold the samples for colour measurements. The empty dish was placed on the glass platen and covered with the cylindrical hollow black shroud. The colour was measured five times to yield blank colour data. A 5-mm high x 25-mm diameter sausage layer was cut from the 20-mm extruded sample from each of the three syringes. The 5-mm discs were then placed centrally in the dish, covered and three readings were recorded per disc. The blank L*, a* and b* data were averaged, as were the three equivalent readings from each disc. The blank mean was subtracted from the sample means

from each syringe. Finally, the three corrected means of the three syringes per species were used to calculate the final mean and standard deviation for each species at each time point.

Texture profile analysis

Arising from the work of group of General Foods (U.S.A.), Bourne (1978) finally modified and described texture profile analysis in an academic published paper. The texture profile analysis was measured with a TAXT Plus Texture Analyser (Stable Microsystems, U.K.). This measurement was performed only at Days 0 and 4. For each meat species, three identical samples were prepared, 30-mm long with syringe-governed diameter of 25 mm. A cylindrical aluminium flat surface probe with 50-mm diameter was attached to the analyser mobile shaft. For each measurement, a sample was placed on a flat sheet of glass placed on the instrument's base. The test was done at ambient temperature. It comprised two compression cycles under the following conditions: probe speed 5 mm s^{-1}; threshold 0.294N, surface sensing force 0.971 N; 50% strain (Khem 2009) (the disc was compressed to 2.5 mm); the interval between the two compressions was 1 second (Riebroy and others 2007). The data were transmitted to a computer with Exponent 32 software installed. For each sample, hardness, adhesiveness, springiness and cohesiveness were measured and calculated from the force-time curves by the software.

pH determination

According to the method described by Benjakul and others (1997), a 5-g portion from each working sample was dispersed into 50 mL of deionised water, and the pH measured by a pH meter (Meterlab, U.K.) fitted with a conventional glass electrode.

Detection of the growth of fermentation culture

MRS agar was used to determine the growth of *Pediococcus pentosaceus* which is a lactic acid bacterium (LAB) commonly used in fast fermentation meat products (Raccach 1981). This test was done in the first two of the seven trials only, with the purpose of deciding if the chosen LAB was able to grow and fully ferment under the formulation and incubation conditions.

A 5-g portion was collected from each working syringe sample and transferred aseptically into a sterile plastic pouch. For each sample, 45 mL of 0.1% peptone water was added and the mixture was dispersed by a laboratory blender (Seward Stomacher® 100) at normal speed for 1 min (AOAC 2000). From this 1 in 10 dilution (10^{-1}), further sterile decimal dilutions were made up to 10^{-7}. Starting at the 10^{-3} dilution, 1 mL of suspension was transferred to a Petri dish and mixed with about 15 mL of molten MRS agar at around 50°C. After the agar was set, all MRS

agar plates were incubated in a CO2 atmosphere at 30°C for 48 hours. LAB counts were expressed as colony forming units per gram (cfu g^{-1}).

Sensory evaluation

Sheepmeat and spice flavours

As explained in the Introduction the meat chosen for this work was beef rather than sheepmeat. To reiterate, the characterising feature of sheepmeat is the presence of BCFAs that are absent from beef (Prescott et al. 2001) and, in the case of pasture-raised animals, the presence of skatole. In a previous study of hedonic responses of Japanese and New Zealanders to BCFAs and skatole as meat components, the base material was grain-finished beef lean and fat. BCFAs and skatole were added to these in defined concentrations to mimic intense sheepmeat flavours. Grain-finished beef lean and fat could not be sourced realistically at the AUT, so standard pasture-finished beef was used instead, accepting some background skatole. Moreover, the physicochemical data generated in the syringe sausage work showed that beef and lamb had closely similar properties (pH, texture and colour).

The aim of sensory evaluation was to show if fermentation, curing and spicing, individually and a combination, were able to reduce the unwelcome sheepmeat flavour and thus enhance the acceptability of sheepmeat consumption.

Based on concentrations used by Prescott et al (2001) the finial concentrations of 4-MeO, 4-MeN and skatole in the sausage mixture were 5, 0.35 mg kg^{-1}, and 80 µg kg^{-1}, respectively (Table 7). The prepared solutions were stored in airtight glass containers and suitable volumes were added to the fat before mincing etc. to achieve the final concentrations required (Table 7). Addition was achieved with a glass 10 µL syringe, whereby small aliquots were injected into multiple fat pieces with the intention of achieving a homogeneous distribution after mincing and mixing.

Table 7. Preparation of BCFAs and skatole and their concentrations in ethanol and the final sausage

	Mass (mg)	Volume of ethanol (mL)	Concentration (mg L^{-1})	Final concentration in sausage (mg kg^{-1})
4-MeO	709	7.26	97,660	5.00
4-MeN	68	10.53	6,460	0.35
Skatole	14.90	10.18	1,460	0.08

Garlic and rosemary are spices commonly used in Western-style lamb dishes, and were chosen as the flavour enhancers. To minimise disturbance of the sausage basic formula ratio, essences were used so that they had no effect on sausage weight. Garlic and rosemary essential oils are natural flavourings obtained by hexane extraction and distillation of garlic bulbs (*Allium sativum L.*) and rosemary herb (*Rosemarinus officinalis L.*), and standardised for volatile oil content and flavour.

Based on the recommendation of Tyrrell (2007a; 2007b), the essences were added to salt and not directly to the sausage mixture. The masses added were 0.02% of salt in the formulation (Table 6) and this translated to a concentration of 0.004% in the final sausage.

Preparation of sausage samples

For each panellist, a 10-g sample slice fresh, cured, fermented sausage form each treatment was required. Sixty panellists were hoped for. In order to prepare enough sausage for 60 panellists and to allow for waste, enough samples of the eight treatments for 80 panellists were prepared. Thus, a minimum 800 g of sausage mixture was made for each treatment. As the sausage mixture was to be cut into 10-g cubes, the sausage mixtures were fermented in a 1-kg airtight flat cuboid plastic box suitable for domestic food storage. For each treatment, one backup box was prepared, thus, two boxes of sausage mixture were made for each treatment. So in total, 16 boxes were prepared and labelled as described later. Each sensory evaluation sample was evacuated by a vacuum packer (Vacuum Packaging Systems, TYPE VMS 123) in a vacuum barrier bag, before incubation at 30°C for 96 hours, exactly as was done with the syringes (Figure 5). After incubation, the boxes were held unopened at refrigeration temperatures until needed for microbiological assay and sensory evaluation.

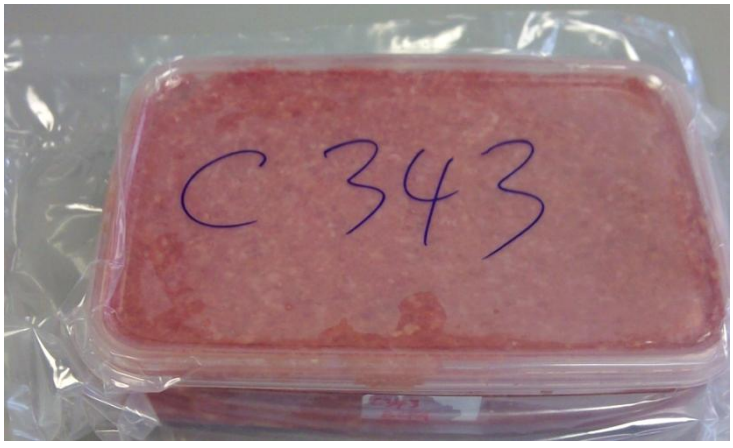


Figure 5. A sample of sensory evaluation sausage mixture. A 1-kg airtight flat cuboid plastic box was filled with sausage mixture and vacuum packed in a barrier pouch.

The beef lean and fat were purchased from a local butcher shop. After the required quantity of lean and fat were calculated, randomised pieces were divided the lean and fat into two lots. The addition rates for BCFAs and skatole were calculated (Table 7) and added to one lot by way of fat. The other lot was injected with equal volumes of ethanol. (The calculations for production of the samples were facilitated by the use of a comprehensive spreadsheet, attached as an electronic appendix in the library version of this thesis. A master sheet of mass of sausage ingredients for about 1kg sausage mixture is shown in Appendix 1).

Both the BCFAs/skatole and non-BCFAs/skatole lots were divided into four equal sub lots. Lots from the BCFAs/skatole lot were labelled as 470, 511, 343 and 130 as described later. The other four lots were labelled 370, 133, 879 and 078. Then for each numbered treatment, the rest of the ingredients were added according to Table 6 and Table 8. All eight treatments were carefully mixed by hand for at least 10 minutes. Each mixture was then passed through the Kenwood mixer with 4-mm mincing plate before filling the boxes. Thus, each treatment was minced twice overall.

Hygiene screening

Prior to the sensory analysis process, the 16 boxes (eight treatments) were screened to ensure the food was safe to eat. Tests were conducted for *Staphylococcus aureus*, total coliform and faecal coliforms. For each of the 16 boxes, a 25-g sample was collected aseptically from under and on the surface of the sausage and transferred to a 400 mL sterile plastic pouch. Then 225 mL of 0.1% peptone water was added and the mixture dispersed in a stomacher (Seward Stomacher® 400) at normal speed for 90 seconds. Decimal dilutions were prepared from this, 10^{-1} to 10^{-4}.

For *Staphylococcus aureus* detection, 0.1 mL of each dilution was applied to BP agar plates by a standard spread plate technique. Plates were incubated aerobically at 37°C for 48 hours. The presence of any black, shiny and convex colony would be considered a positive result.

For total and faecal coliforms analysis, 1 mL of each dilution was added into triplicate MacConkey broth tubes. These were incubated at 35°C for 48 hours. A broth changing from purple to yellow with gas formation would be considered as positive for coliforms. From any positive MacConkey broth, one loopful of suspension was transferred subsequently into a Brilliant Green Bile Broth (BGBB), which was incubated at 44.5°C for 48 hours. The presence of turbidity with gas would indicate faecal coliforms. Concomitantly, 1 mL of positive MacConkey broth suspension was transferred into a tryptone water broth and incubated at 44.5°C for 48 hours. Finally, a few drops of Kovac's reagent were added to the tryptone water. A

red ring on the surface would also indicate faecal coliforms. Thus, growth in BGBB and production of production of indole from tryptophan in the tryptone water was interpreted as positive faecal coliforms.

The positive results of MacConkey broths gave the most probable number (MPN) of total coliforms. The positive results of BGBB and trytone water confirmed the presence of faecal coliforms. When traced back to which MacConkey broth dilutions contained faecal coliform, it was possible to calculate the MPN of faecal coliforms.

Details of sensory evaluation

Eight randomised three-digit codes were needed to represent eight different treated samples. These eights codes were chosen from the Yellow Pages® phone numbers. The Yellow Pages® directory was opened to any page and the last three digits of the phone numbers in one column were chosen. Table 8. shows the codes, samples with treatments and tasting Bays.

Table 8. The eight sausage treatments and their blinding codes. Shaded blocks indicate an active state.

Sensory bay	Fermented	Cured	Spiced	BCFAs/skatole	Blinding code
A	■			■	470
	■				370
B	■		■	■	511
	■		■		133
C	■			■	343
	■				897
D	■	■	■	■	130
	■	■	■		078

Heavy shading means a factor is applied in that treatment

The expected number of panellists for the sensory evaluation was 60. A vertical 9-point liking scale was used for scoring the liking of the eight sausage meat treatments. According to a fully randomised design, (see Chapter 3) each of the panellists was required to taste all of the eight treatment samples and complete the sensory evaluation form. In the form (Appendix 2), they were asked to choose from: like extremely, like a lot, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike a lot and dislike extremely. In addition, gender and age category data were collected. The categories were: male and female for

gender; 18-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64 and 65+ for age groups. After the tasting analysis was completed, the nine liking description phases were converted into numbers 1 to 9 as dislike extremely to like extremely, respectively. The results were compared by taking the means and standard deviations of the eight sausage samples.

To eliminate order effects in tasting, a fully balanced randomised trial was used. According to the design described by Wakeling and MacFie (1995), each of the eight sausage samples were equally presented in every tasting order. The tasting orders of each bay were equally in the first, second, third and fourth positions. Further, the order of tasting the two samples in each bay was alternated. For example, in Bay A, the two samples were 470 and 370. If a panellist tasted 470 first; the next panellist in this bay would taste 370 first. Therefore, the tasting order was repeated after each eight panellists. For this sensory test, eight cycles were prepared, a possible 64 panellists.

If the prepared sausage treatments reached the hygiene requirements of the Microbiological Reference Criteria for Food (Ministry of Health October 1995), the food could be used for sensory evaluation. All the eight treatments were cut into a 25 x 40 x 5 mm slices. Each two samples presented within one bay were placed in the correct tasting order on a single 180 x 125 x 12 mm flat white foam tray, just above their three-digit codes. Filled trays were kept at 4°C prior to tasting. During sample tasting, panellists were required to use tap water and apple slices to clean any leftover flavour in their mouths before tasting the next sample and between bays. The samples exhibited various intensities of red, because treatments in A and B were uncured and in C and D (brown) were cured (red) (Table 8). While the comparisons within A, B, C and D were between cured samples or between uncured samples, the aim of randomising bay order (A, B, C then D for panellist 1; D, C, B then A for panellist 2 etc.) was to permit between-bay comparisons as well as the more obvious within-bay comparisons. The difference in colour might have biased judgement of different bays. In order to blind the colour affect, the light used in each bay was red with other light sources in the tasting room dulled (Figure 6).

The immediate reward for panellist participation was a chocolate bar for each panellist and a chance to win a $50 cash prize by ballot. Only the panellist winning the prize would be notified.

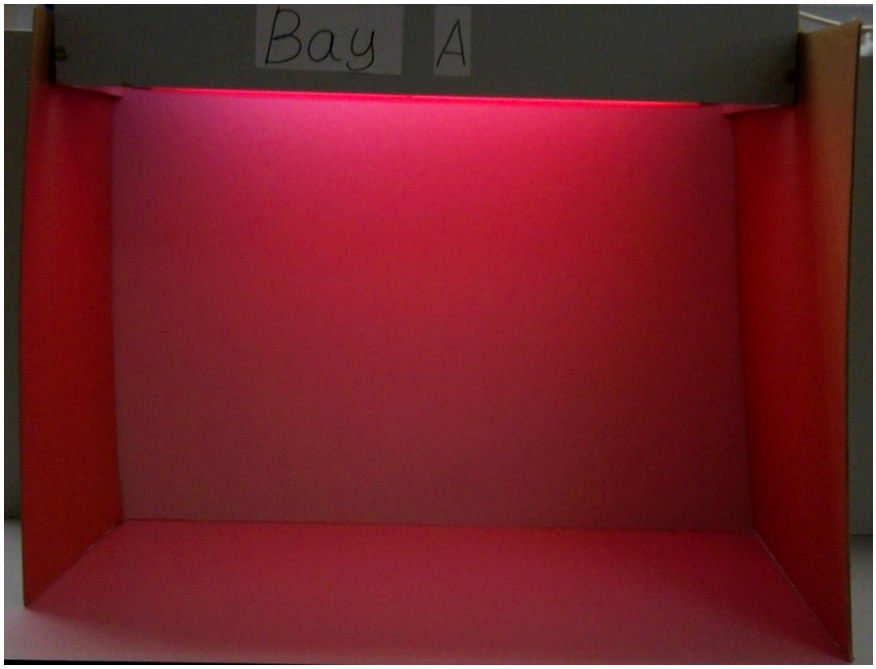


Figure 6. A red-light bay. This is one of four red-light bays used in this sensory trial.

Data analysis

The data collected during this research were first marshalled and analysed by basic routines in Microsoft Excel. More detailed analysis of variance was performed with Minitab 15 (Minitab Inc., State College, Pennsylvania). In the case of sensory evaluation, factors explored were the effects of curing, spicing and sheepmeat flavours addition (Table 8).

Chapter 3

Physicochemical Results and Discussion

The detection of growth of LAB

The sausage mixtures included a fermentation culture mixture comprising *Pediococcus pentosaceus* and *Staphylococcus carnosus*. Only the *P. pentosaceus* is active in lactic acid formation from the added glucose. The other bacterium *S. carnosus* is a non-pathogen, and it is also commonly used as a fermented meat product starter for helping the development of flavours, reducing nitrate to nitrite, and minimising rancidity flavour due to fat oxidation (Hammes and Hertel 1998). In reducing nitrate to nitrite *S. carnosus* also contributes to cured meat colour formation. LAB are weak in nitrite and nitrate reductase activities (Lücke 1985).

The detection of the growth of the LAB added (*P. pentosaceus*) and any endogenous LAB was done in the first two trials to confirm they were multiplying in the incubated sausage mixture. The increase in LAB numbers during fermentation is reported in Figure 7. The initial number of LAB in all the three species (lamb, pork and beef) was between 7 and 8 log cfu g^{-1} ($P < 0.000$). Clearly, the LAB number increased markedly in Day 0 and Day 2, and then the rate of increase slowed down. By Day 4, the number exceeded 9-log cfu g^{-1} ($P < 0.001$ compared with Day 0). Thus, the numbers of LAB were about 100 times higher after four days of fermentation. Both the initial and final counts of LAB in all three species were similar.

Samelis and others (1998) reported a traditional Greek salami with no added starter had an initial LAB number of about 5-log cfu g^{-1}. The finial LAB count was about 8.5-log cfu g^{-1}, it was more than 1000 times higher. In contrast, research by Johansson and others (1994) reported that the initial number of LAB of fermented pork sausage with starter was about 6-log cfu g^{-1}. Also, they reported that the growth rate of LAB in the first 48 hours was the fastest (0.75-log cfu g^{-1} day^{-1}) growth to ultimately reach 8-log cfu g^{-1}. The overall increase was about 100 times, consistent with the results obtained here. Thus, the *P. pentosaceus* was able to grow well in all three species of meat.

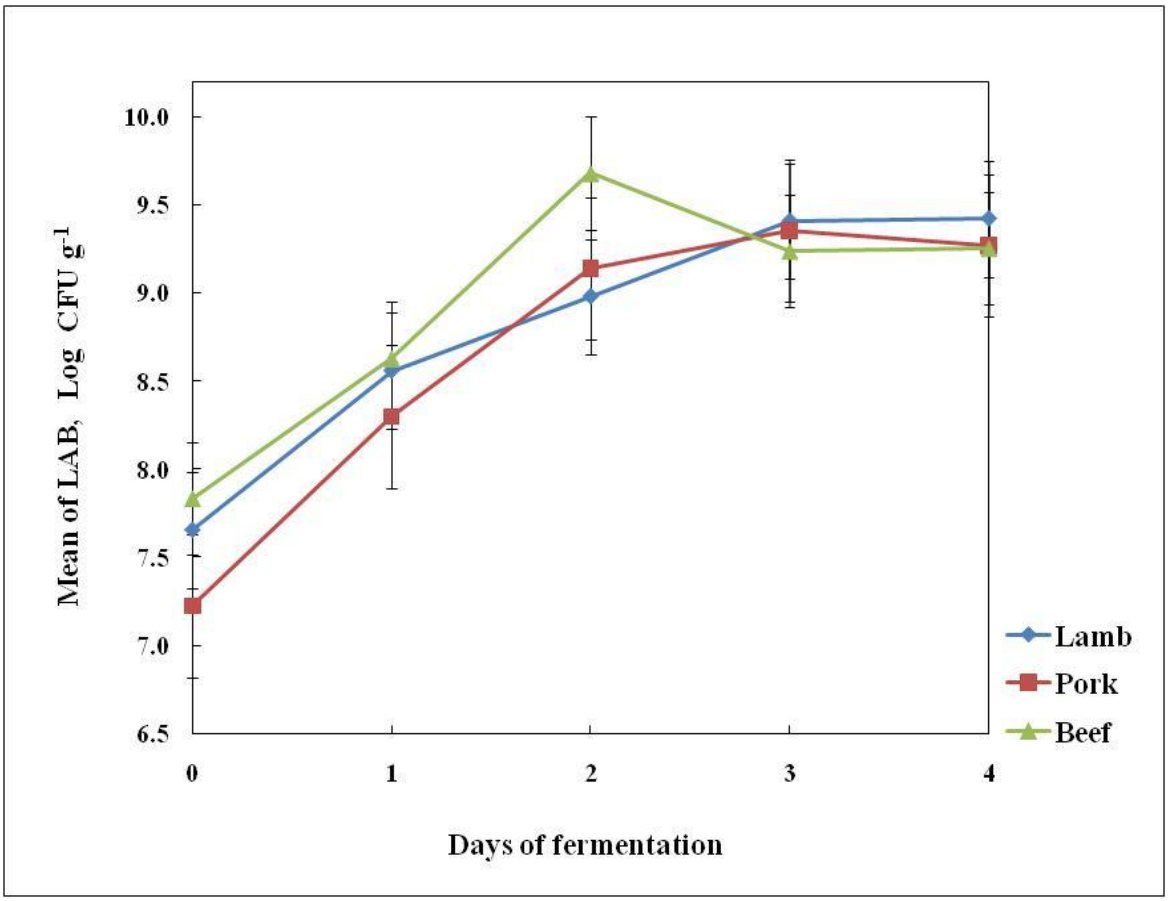


Figure 7. The LAB growth curve. The growth of LAB in sausages mixture made from the three species. Data points are means and vertical bars are standard deviations for two replicates.

Changes in pH

The initial mean pH values of the sausage mixtures of the three species of meat were between 5.5 and 6 (Figure 8). The pH of the lamb sausage was numerically higher than of pork and beef, but the differences were not significant ($P > 0.05$). After Day 0, pH values of the three species started to fall, with the greatest fall between Day 1 and Day 2. In contrast, the growth of LAB was rather similar between Day 0 and 1 and between Day 1 and Day 2 (Figure 7). This does not necessarily mean that the LAB were more active in producing lactic acid between Day 1 and Day 2 (Figure 8), because the sausage mixtures are buffered due to their protein and phosphate contents. By Day 2, the three species had fallen to around to 4.5, and these pH values were roughly constant through to Day 4. The difference in changes of pH values between the species were not significant ($P > 0.05$), but the large differences between fermentation days were significant ($P < 0.05$).

The pH decrease confirmed the growth of LAB, which indicated the added carbohydrate glucose had been anaerobically metabolised to organic acids, especially lactic acids, by LAB (Riebroy and others 2005). The decrease in pH was important to assure the fermented sausages of the three species were safe to eat. Almost no food pathogenic and spoilage bacteria can grow at pH values below pH 4.6 (Riebroy et al. 2005).

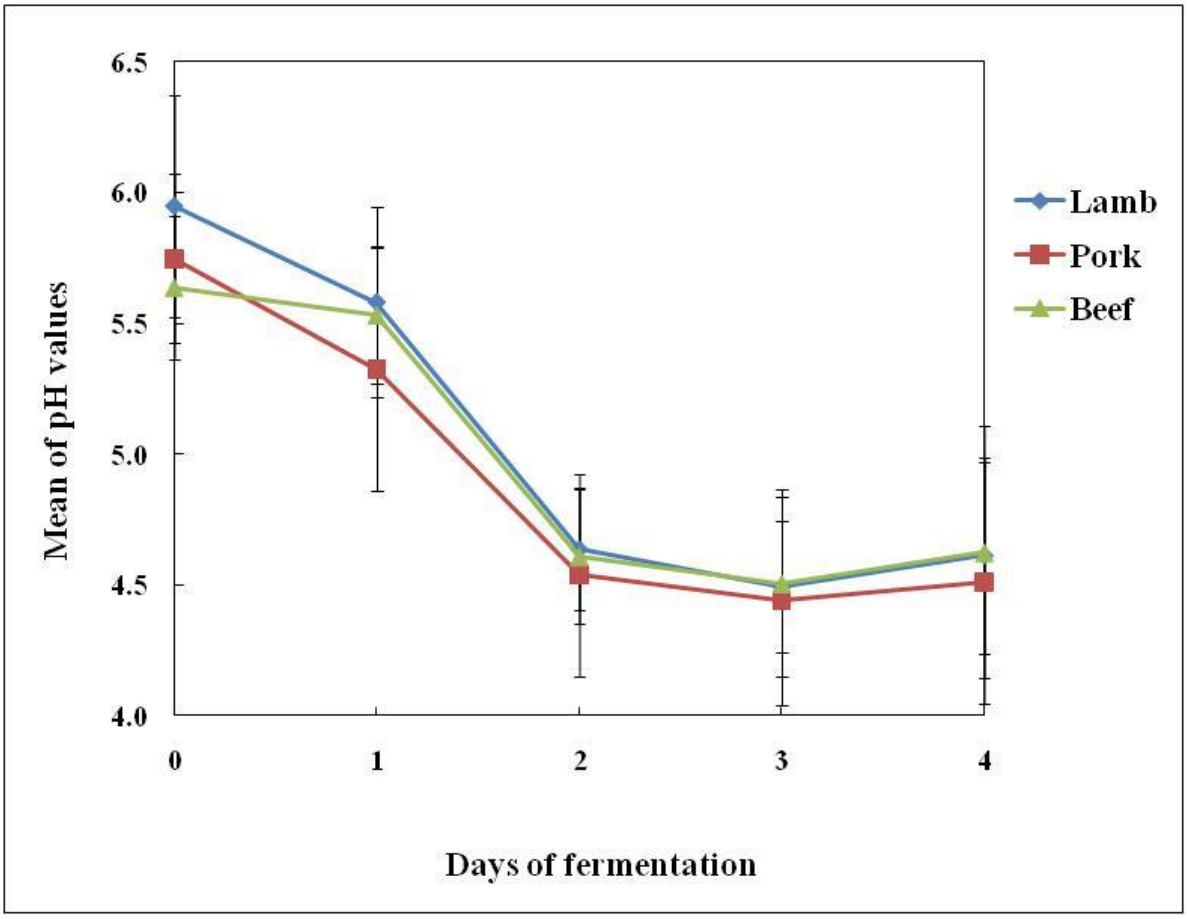


Figure 8. The pH changing curve. pH values during fermentation decreased of sausage mixture made from the three species. Data points are means and vertical bars are standard deviations for seven replicates.

The changes in colour during fermentation

The reflectance (L*) of the sausages from the three species was generally unchanged between Day 0 and Day 4 ($P > 0.05$) (Figure 10). Standard deviations were particularly large on Day 0.

The Hunter a* values of all three species were positive at all times, meaning that the meats were more red than green, as was obvious by inspection. In fact, at Day 0, the mixtures were

obviously brown due to the formation of metmyoglobin. This was due to the (sodium) nitrite added to the sausage mixture as one of the ingredients. Nitrite is a strong reducing agent, and it prevents fat oxidation. Under the fermentation conditions, which were dark and nearly complete absence of air, nitrite formed nitric oxide (NO). Myoglobin (+ Fe^{2+}) (red) will be oxidised by NO to (+ Fe^{3+}) (brown colour), which happens at the early stage of fermentation.

Figure 9 shows the possible pathways of cured meat colour generation. With the growth of LAB, pH reduction occurs, and generates nitrous acid (HNO_2) according to the following equation:

$HNO_2 \leftrightarrow H^+ + NO_2^-$

In the acid conditions (LAB generate lactic acid), the equilibrium shifts to the left, so HNO_2 is produced from NO_2^- (Ranken 2000). This in turn generates some NO (Figure 9 left side). Under the conditions of fermentation, metmyglobin may be reduced back to myoglobin: metmyglobin reductase may be active in reducing metmyglobin to myoglobin, utilising NADPH from glycolysis. NO-myoglobin is ultimately formed, which is a stable typical pinkish cured colour (Pearson and Gillett 1996). NO-myoglobin is the pink colour that dominates Hunter a* values in this fermentation system.

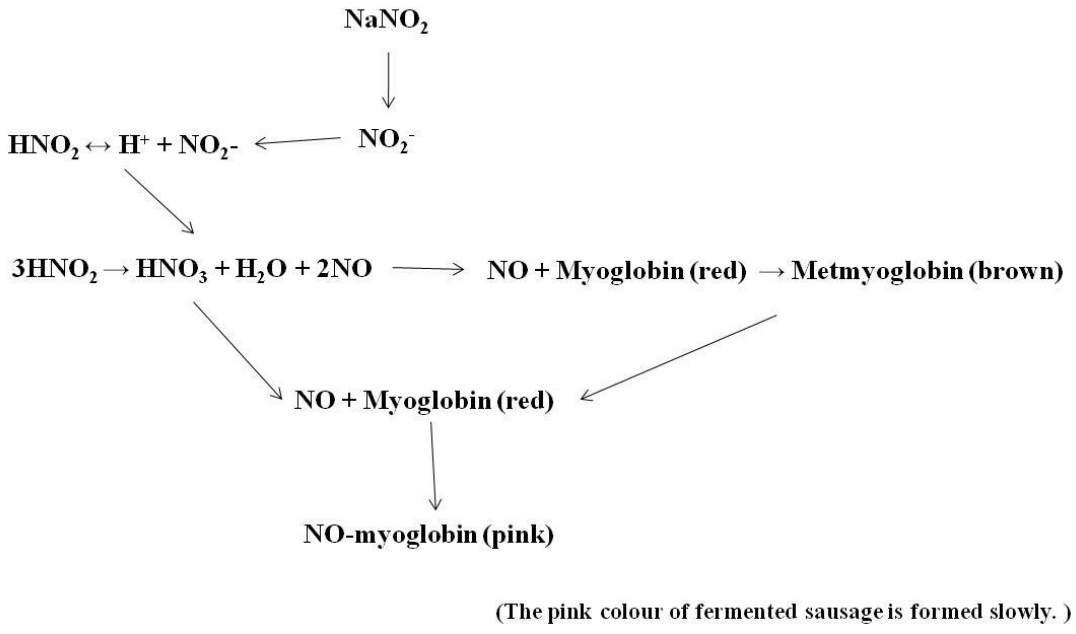


Figure 9. Cured meat colour formation. The changes of nitrite added to the three species sausage samples during curing and fermentation. Adapted from Ranken (2000).

As a result, Hunter a* values steadily increased from Day 0, with an overall statistical significance of $P < 0.05$ (Figure 11). There was no statistical difference between the species but there were hints that the kinetics were different for pork compared to lamb and beef.

Browning of meat is accompanied by an increase in b* values (in the positive range) relative to a* (Young and West 2001), such that the hue angle (arc tan b*/a*) – the colour as is perceived as hue – increases. As the contents of the syringes increased in redness with time (Figure 11), yellowness tended to decrease (Figure 12), but the change in yellowness was not as marked. Note that Figure 11 and Figure 12 are scaled identically. But reflecting the yellow component of a brown pigment (Ranken 2000; Young and others 1999), the yellowness of all three species fell between Day 0 and Day 2, but then plateaued in the case of lamb and pork. However, the yellowness of the beef treatment increased slightly to Day 4 (Figure 12) such that the difference between beef on one hand and pork and lamb on the other was statistically different ($P < 0.01$).

The results and discussion above for colour have focused on the red lean component of the sausage mixture, and ignored the effect of fat. Beef fat from animals raised on pasture is prone to accumulation of yellow carotenoids (Gimeno and others 2000) and the beef fat used in this work was observed to be generally yellower than the pork and lamb fat. However, this was probably not the reason for the higher b* value of beef sausage on Day 4. A marked yellowness arising from beef fat would be observed as a higher b* value on Day 0. This was not observed (Figure 12).

Overall, the colour properties of fermented, cured sausage made from the three species were much the same. It is a surprise to find that the colour properties were not related to species. Work by Gimeno, Ansorena, Astiasaran, & Bello (2000) showed that L*, a*, b* values for five brands of Spanish dry fermented pork sausages were closely similar, and this was attributed to the closely similar lean content of the final sausage mixture. The lean content of the three species used here was identical and this appears to be the main factor that governs colour. The colour of the lean meats used in sausage preparation was not monitored, but it is common experience in New Zealand that pork on retail display is typically a lighter red than that of beef and probably lamb. This did not translate to lower Hunter a* values (Figure 11). However Young and West (2001) reported three research studies that showed that domestic cattle, sheep and pig muscles respectively contained 2 to 5, 3 to 7 and 3 to 6 mg of myoglobin per g of muscle. These potential differences in myoglobin content may be the source of wide variation in redness experienced in the Young and West study. In the present study, given that meat was purchased on seven

occasions over seven weeks. Thus, the redness variation of lean meat was minimized, and the difference of redness between the three species was effectively controlled in this research.

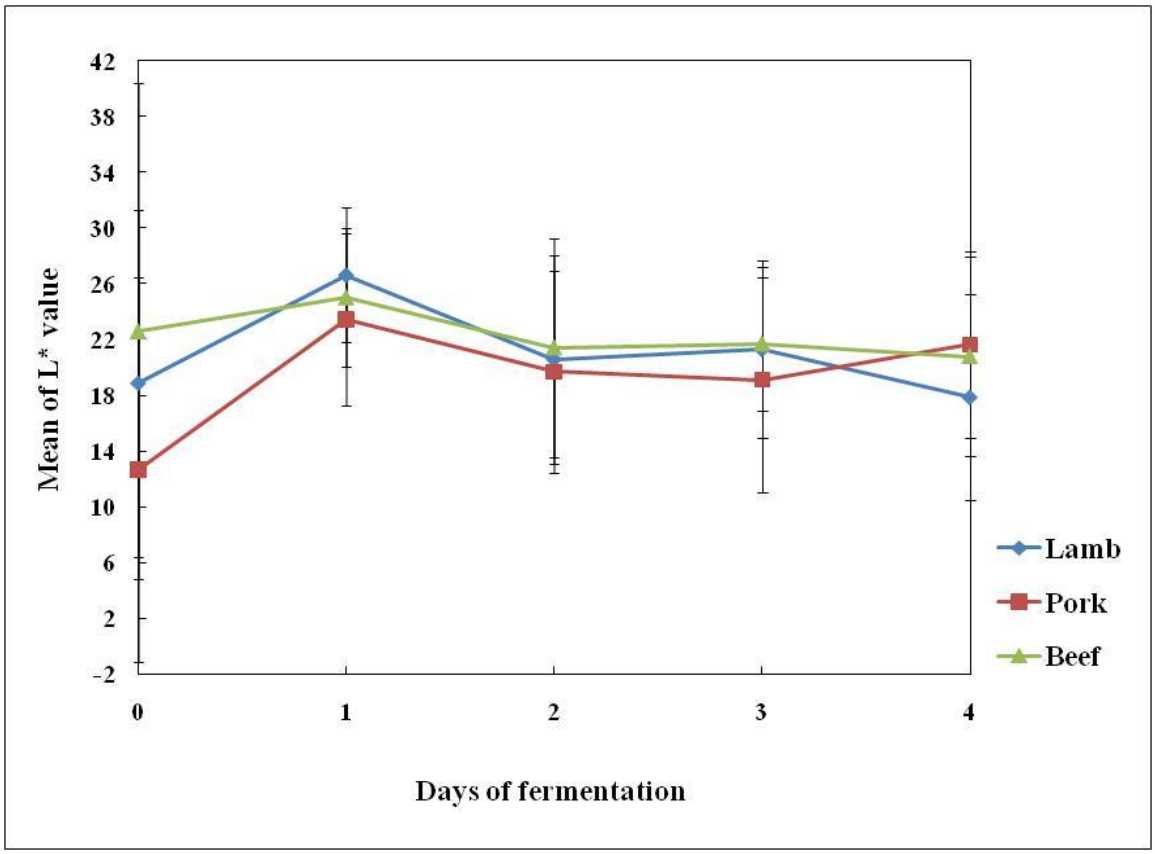


Figure 10. The changes in reflectance. The reflectance changes of sausage mixture made from the three species during fermentation. Data points are means and vertical bars are standard deviations for seven replicates

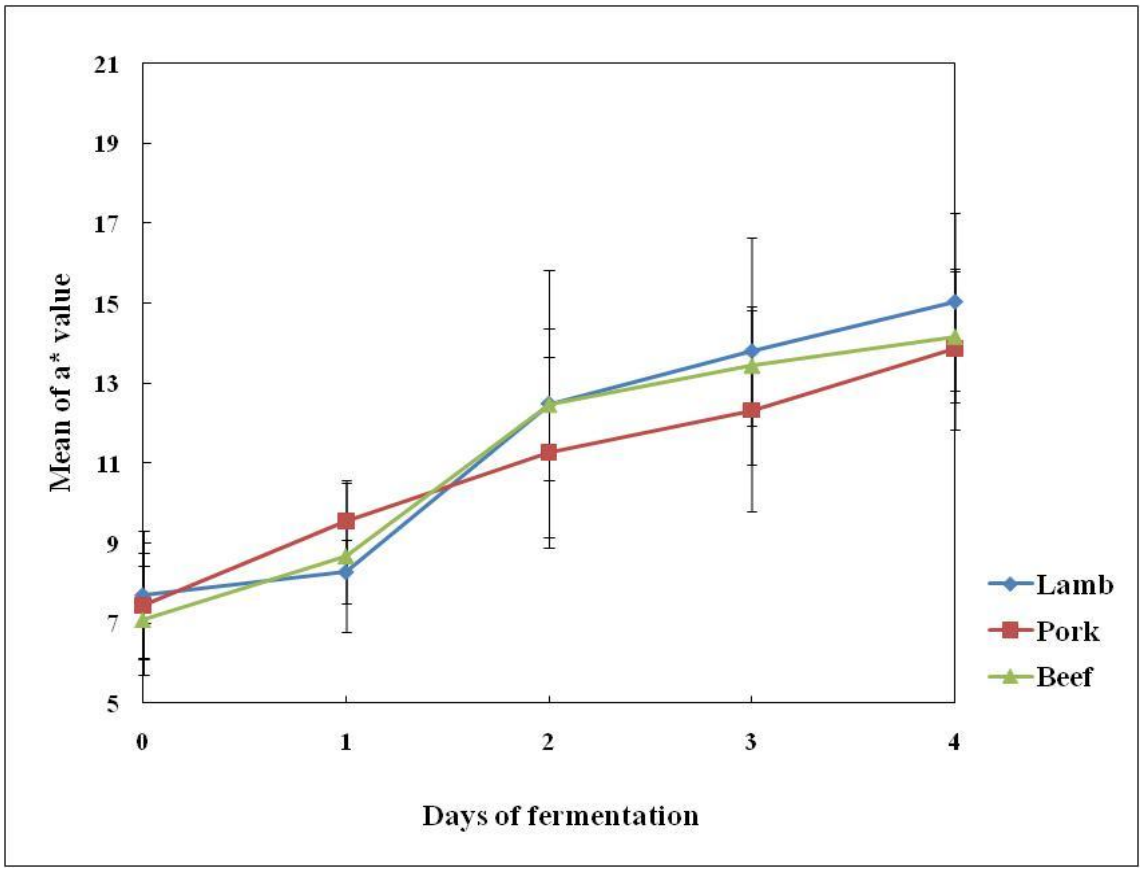


Figure 11. The development of red colour. Hunter a* values indicate the red colour development during fermentation of the three species. Data points are means and vertical bars are standard deviations for seven replicates.

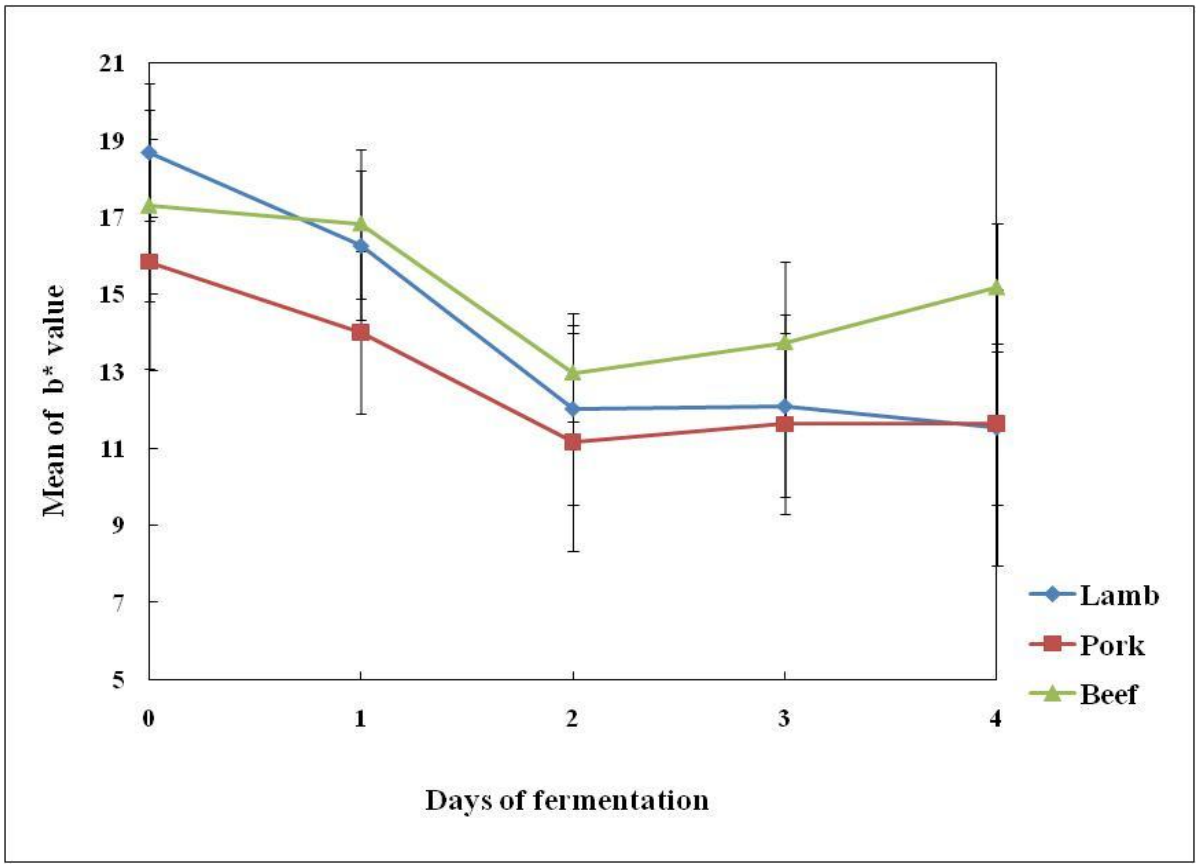


Figure 12. The changes in yellow colour. Hunter b* values indicate the development of yellowness during fermentation of the three species. Data points are means and vertical bars are standard deviations for seven replicates.

Texture

The instrumental texture profile analyses (TPA) of sausages made from the three species were measured to determine hardness, springiness, adhesiveness and cohesiveness (Bourne 1978). Figure 13 shows a typical force-time curve of an extruded syringe sausage sample. The hardness is the maximum force required to compress the sample on the first compression. Thus, in Figure 13, the first major peak shows the hardness of sample, force in gram (1g equates to 0.00981 Newton). Springiness relates to the reshaping ability of sample after the compressive force has been removed. The springiness is not directly shown in Figure 13; Distance 2 divided by Distance 1 (D2/D1) calculates it. The dimensionless value always lies between 0 and 1 where 1 is perfectly elastic. Cohesiveness, which also lies between 0 and 1, is the ratio of the work (energy) required for compression in the two compressions. In Figure 13, cohesiveness is that area under the second curve divided by the area under the first (A2/A1). Adhesiveness is a

measure of the stickiness of sample to surfaces. Adhesiveness clearly relies on the nature of contacted surfaces.

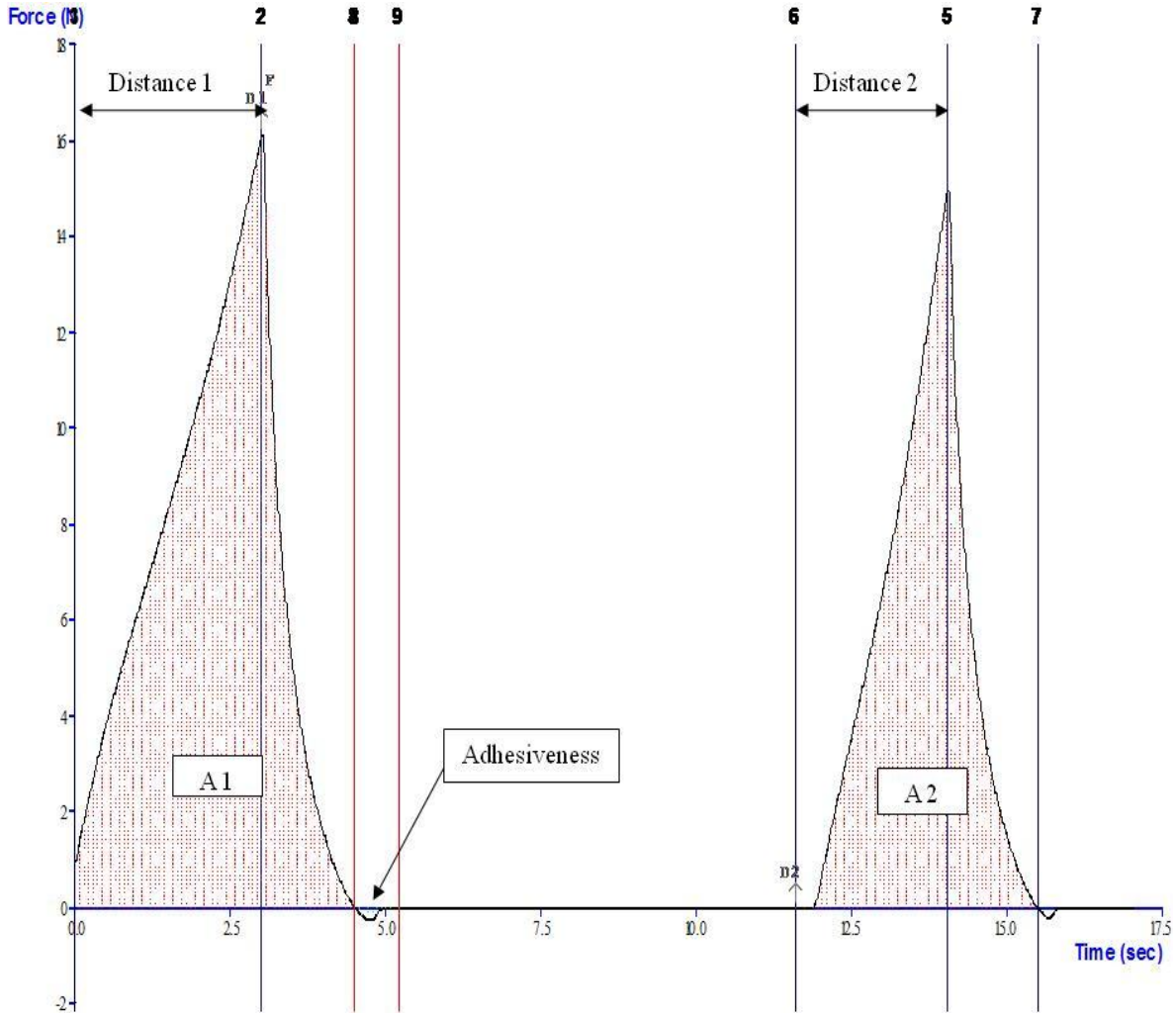


Figure 13. An example of a force-time curve. One 30-mm extruded syringe sausage sample was compressed twice during one compression analysis (two peaks); this curve was drawn by the TAXT Plus Texture Analyser by computer.

The hardness, springiness, cohesiveness and adhesiveness results are shown in Figures 14, 15, 16, and 17, comparing Day 0 and Day 4. The hardness values at Day 4 for the three species were about four times the Day 0 values (Figure 14). The difference between days was highly significant ($P < 0.001$) in spite of large variances within species as indicated by the standard deviations. There was no difference between species ($P > 0.05$). The increase in hardness is caused by denaturation and gelation of meat proteins like myosin heavy chain in response to low pH in a salt-rich environment (Toldrá et al. 2001).

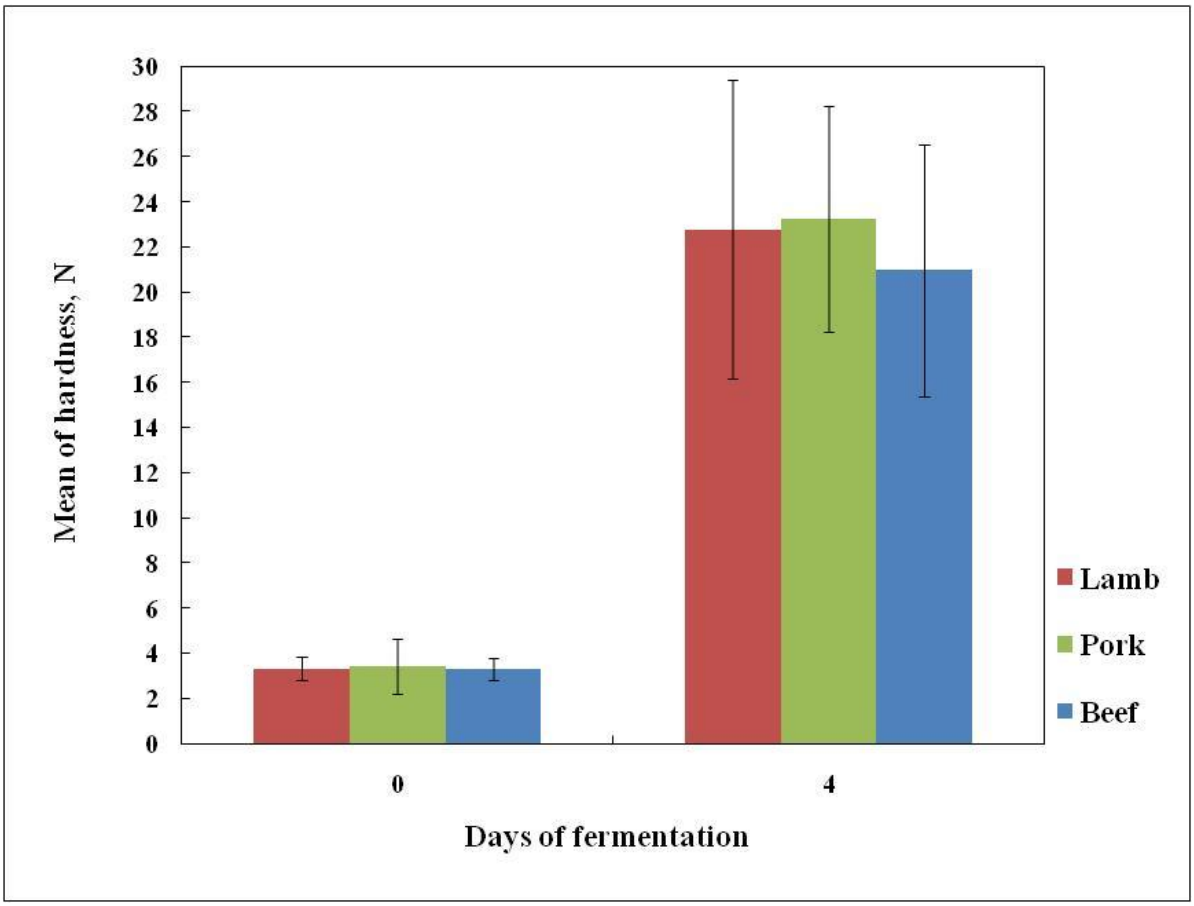


Figure 14. The results of hardness. Changes in hardness of sausage mixture extruded from syringes at Day 0 and Day 4 for the three species. Data values are means and vertical bars are standard deviations for seven replicates.

The springiness of the three species is shown in Figure 15. Clearly, the springiness measured on Day 4 was similar to Day 0 values for the three species. Numerically, the results on Day 4 were slightly lower than on Day 0, but the difference was not significant ($P > 0.05$). Overall, the unfermented mixtures and the fermented sausages were all similarly springy, returning to 90% of the original height after the first compression.

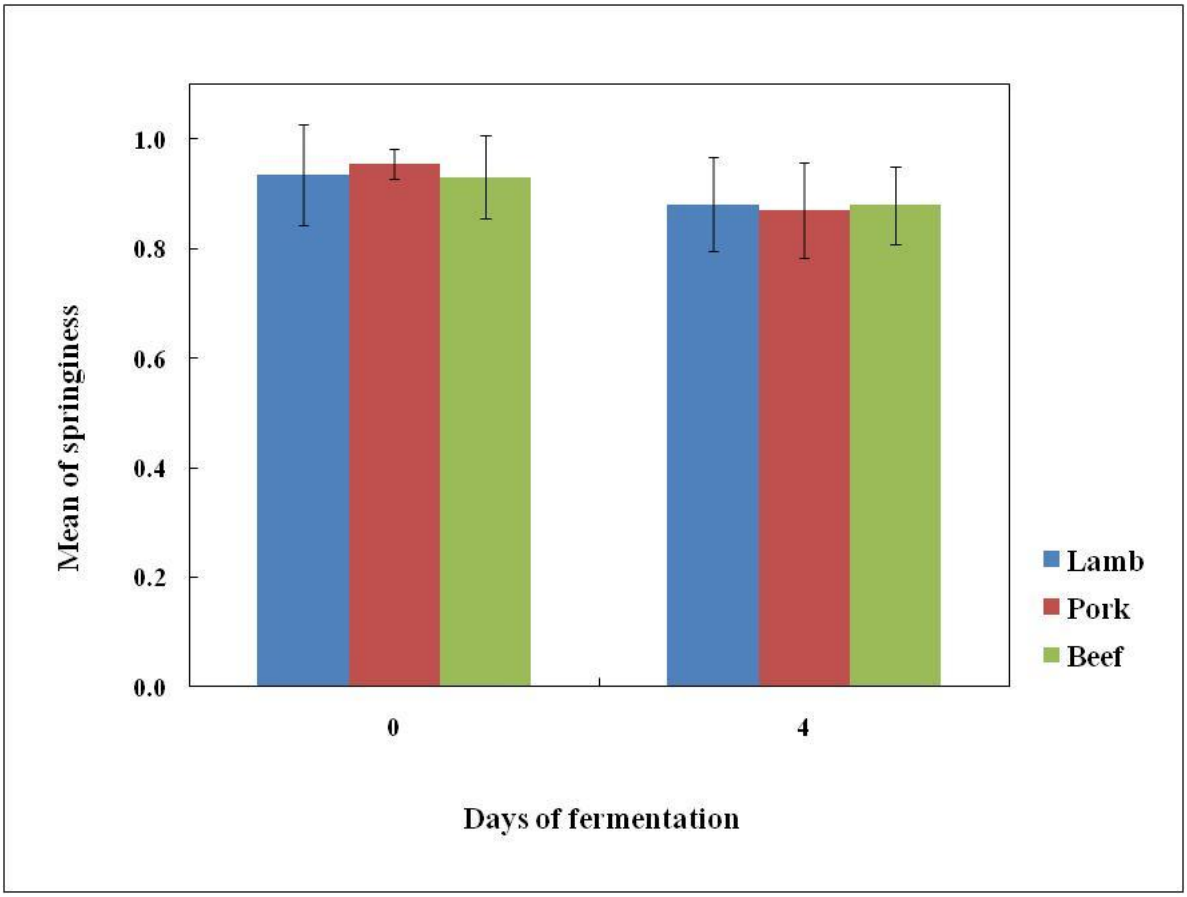


Figure 15. The results of springiness. Changes in springiness of sausage mixture extruded from syringes at Day 0 and Day 4 for the three species. Data values are means and vertical bars are standard deviations for seven replicates.

Figure 16 shows the cohesiveness at Day 0 and Day 4 of the three species. For all species, the cohesiveness at Day 4 was higher than on Day 0 ($P < 0.001$). This was especially true for pork sausage, where the cohesiveness on Day 4 was about twice the value on Day 0. However, the differences between species on any one day were not significant. The cohesiveness increased in parallel with the hardness. The harder sausages on Day 4 were less prone to rupture, presumably in response to gelation that helps to retain shape.

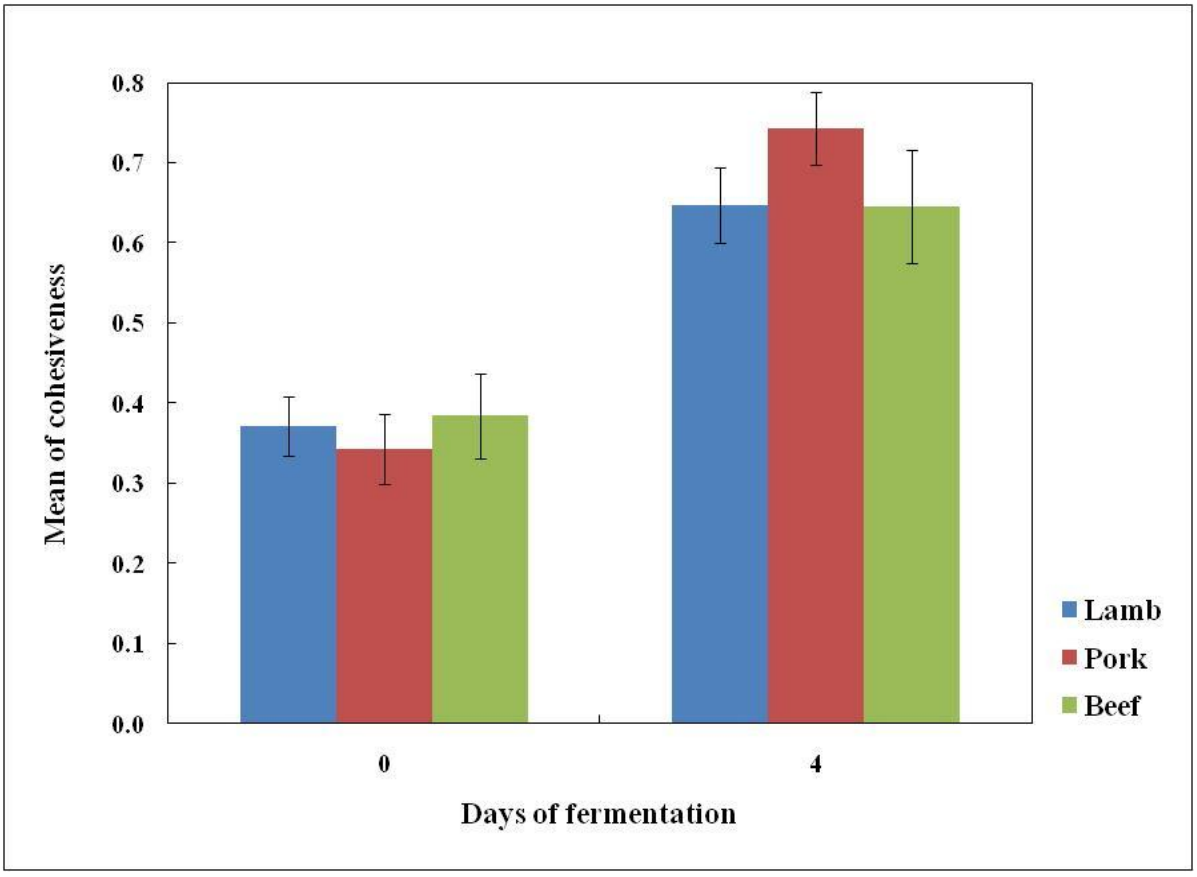


Figure 16. The results of cohesiveness. Changes in cohesiveness of sausage mixture extruded from syringes at Day 0 and Day 4 for the three species. Data values are means and vertical bars are standard deviations for seven replicates.

As discussed above, adhesiveness is the measure of stickiness. The nature of the anodised aluminium probe, the glass base surface and the effect of gravity were such that the compressed sausage discs adhered to the glass rather than the probe. The adhesiveness results are shown in Figure 17. High negative values indicate high adhesiveness. These sausages samples were much less adhesive on Day 4 than on Day 0 ($P < 0.01$). However, the results between species within the same analysis day were not significantly different, and the standard deviations between species for the two days were large, particularly on Day 0. Looking at the pork results in isolation shows a three-fold decrease in adhesiveness and a two-fold decrease for lamb and beef.

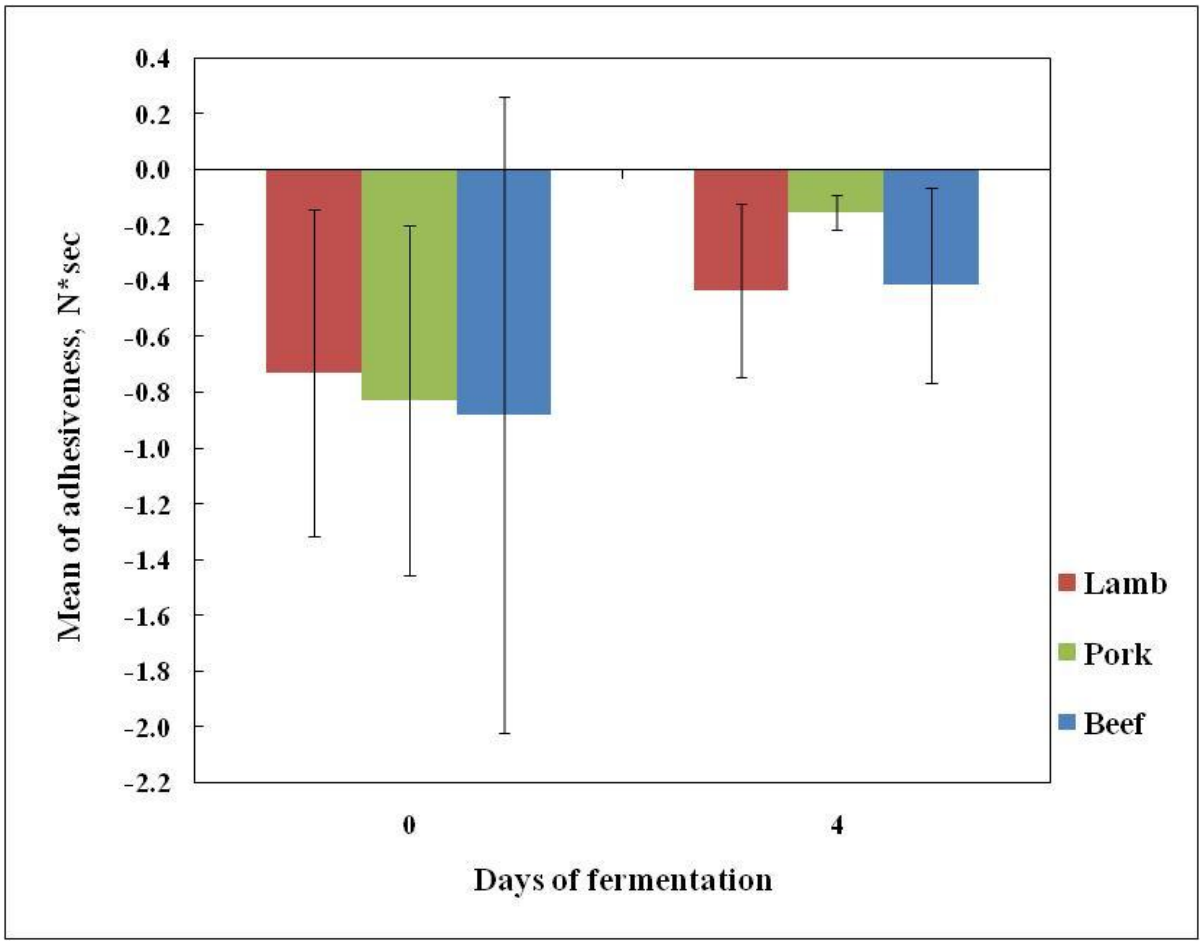


Figure 17. The results adhesiveness. of Changes in adhesiveness of sausage mixture extruded from syringes at Day 0 and Day 4 for the three species. Data blocks are means and vertical bars are standard deviations for seven replicates.

Although the differences between the three species were not statistically significant for all the texture profile parameters, pork sausage appeared to be slightly different on average from lamb and beef equivalents. If more replicates were analysed, it is likely that a significant difference would emerge between pork compared with lamb and beef. The origins of these putative differences between species would derive from the gelation property differences of porcine and ruminant forequarter muscle proteins. There are no known prior research reports comparing low pH- and high salt-generated gelation properties of porcine and ruminant forequarter muscle proteins, although it is well known that fast-twitch and slow-twitch myosins from within one species (beef) have markedly different heat-mediated gelation properties (Young and others 1992). Moreover, the chemical and subsequently the rheological properties of fat from porcine and ruminant forequarter muscles are on average different (Figure 18) (Enser

and others 1996). Pork fat typically has a higher proportion of unsaturated fatty acids, resulting in a softer fat at a given temperature compared with ruminant fat (Wood and others 2004).

Figure 19. shows the different melting points of some C18 fatty acids. The saturated fatty acid stearic (18:0) has the highest melting point at about 70°C, whereas the unsaturated fatty acid linoleic (18:1) melts at low ambient temperatures. Moreover, linoleic acid (18:2) and α-linolenic acid (18:3) are even melted below 0°C (Wood et al. 2004). The higher the concentration of saturated fatty acid in fat composition, the higher the temperature required to melt the fat. Figure 20 indicates that the proportion of stearic acid and melting point of fat have a positive relationship (Enser and Wood 1993).

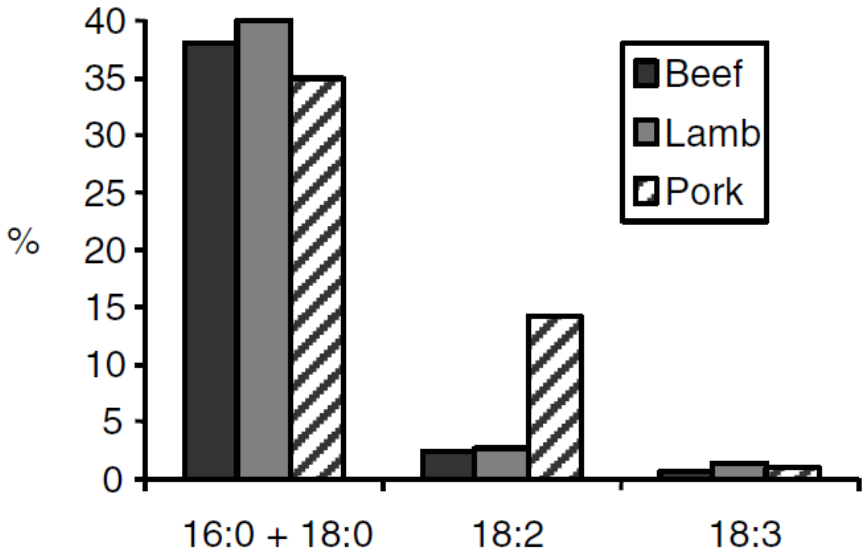


Figure 18. The fatty acids compositions of beef, lamb and pork. The blocks indicate composition of selected fatty acids in beef, lamb and pork. Lamb and beef have higher saturated fatty acid content 16:0 (palmitic) and 18:0 (stearic)) than pork. Pork contains much more polyunsaturated fatty acid, 18:2 (linoleic acid), than lamb and beef (Enser et al. 1996).

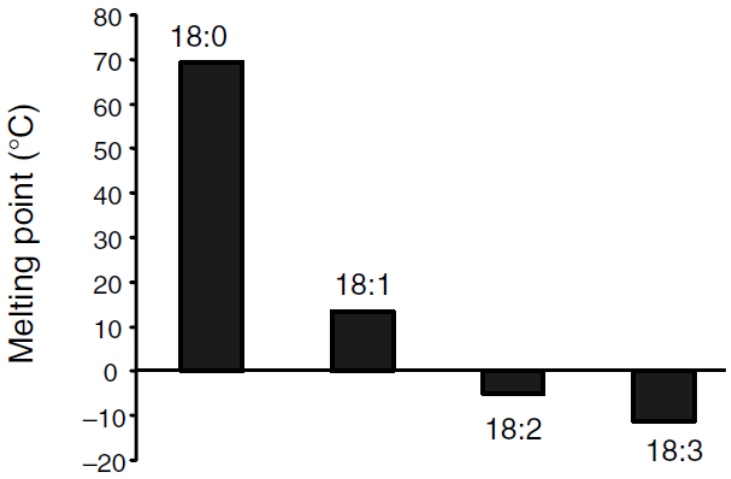


Figure 19. The melting points of some fatty acids. The blocks showed the difference in melting points of some C18 fatty acids (Enser et al. 1996).

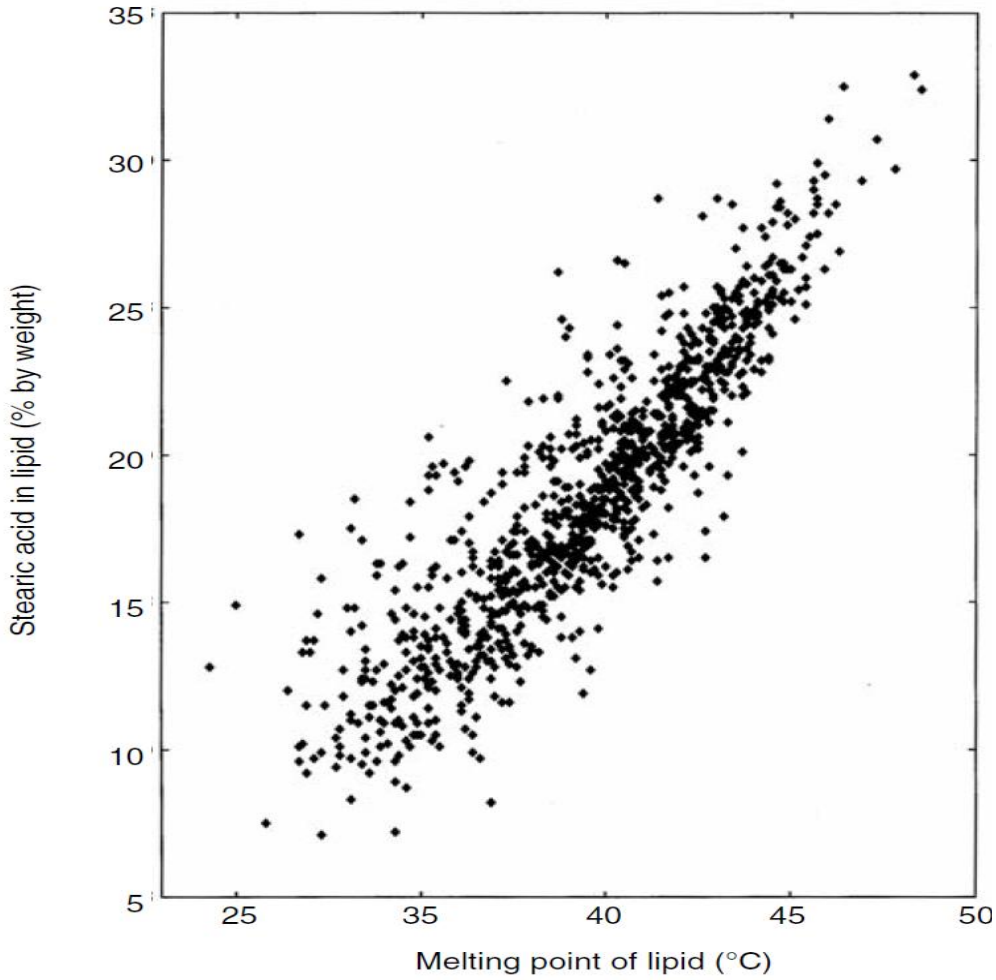


Figure 20. The relationship between melting point of lipid and stearic acid. The graph above showed a positive relationship between the proportion of stearic acid and the melting point of fat (lipid) (Enser and Wood 1993).

In summary, the results of the growth of LAB, and changes in pH, Hunter colour values (L*, a* and b*), and texture (hardness, springiness, cohesiveness and adhesiveness), were very similar between the three species. This indicates that it should be possible to develop a fermented, cured lamb sausage with closely similar physicochemical properties to existing pork equivalents. A fermented, cured lamb sausage would be unlikely to be sold in the form developed for experimental purposes (Khem 2009). Rather, fermented sausages are often dried (and smoked) to some extent to modify texture and flavour. However, it seems likely that a fermented, cured lamb sausage would behave in the same way as a pork equivalent when dried and smoked.

Thus, the preparation of a fermented, cured lamb sausage presents no textural or colour problems, but the sensory acceptability remains unknown.

Experiments to examine this potential problem are described in the next chapter.

Chapter 4

Sensory Evaluation Results and Discussion

Introduction

The last chapter (Chapter 3) reported the physiochemical results of the three species (lamb, pork and beef). All the sausage samples were fermented well. The pH values dropped to about 4.5 for all samples. The colour was found to be not significantly different between the three species, but redness developed with increasing time of fermentation for all three species. The Texture Profile Analysis showed that after fermentation, the three species sausage samples became harder, chewier and less sticky, however, no change in springiness was observed.

Thus, the physicochemical results reported in Chapter 3 strongly suggests that the properties of lamb, pork and beef fermented/cured sausages were similar, such that lamb sausages could be made equivalent to the existing wide range of pork and beef sausages available internationally. However, as discussed in Chapter 1, sheepmeat (and goat meat) fat has a fatty acid profile that includes low proportions of branched chain fatty acids (BCFAs) that when present as free fatty acids – in cooked meat and meat products generally – give a characteristic species flavour that is disliked by many unhabituated consumers. Moreover, New Zealand ruminant meat production systems are based on pasture, such that the tryptophan-derived compound skatole can accumulate in body fat. Skatole has a gamey, even faecal note that, as with BCFAs, is disliked by unhabituated consumers. The average New Zealander is well adjusted to these flavours, but this is not the case for many consumers in existing and potential export markets.

Chapter 1 also described an experiment where Prescott and others (2001) analysed the influence of BCFAs and skatole on sheepmeat by comparing the liking score of two groups of consumer panellists, Japanese and New Zealanders, for sheepmeat supplemented with different levels of BCFAs and skatole. The aim was to simulate enhanced sheep meat and pastoral flavours in a highly controlled way. For both populations, the liking score decreased with the increasing concentration of skatole. The Japanese were unhabituated to sheepmeat consumption, so the decrease in liking score began at a low concentration of added skatole. By comparison, New Zealanders were only affected by a high skatole concentration. In order to explore the effect of fermentation, curing and spicing on consumer liking of fermented lamb sausage, the same approach of using spiked beef to simulate strong sheep meat and pastoral flavours was adopted here. However, there was a difference in experimental method. In the work of Prescott and

others (2001), the beef was sourced from a barley diet based feedlot, a beef source known to be very low in skatole. This meat could not be sourced for the present work, and the meat used would have contained a background concentration of skatole. Its concentration was unknown, but because the meat was from beef cattle slaughtered in early April 2010, the concentration was expected to be minimal compared with, say, meat obtained from cattle slaughtered in between July and September. This knowledge is based on unpublished dairy industry data which show that skatole concentration in cream (another bovine fat) peaks at that time of the year owing to pasture conditions in late winter/early spring (O.A. Young, personal communication).

Thus, the fat fraction of the sausage mixtures were spiked – or not– with controlled quantities of 4-methyloctanoic acid (4-MeO) and 4-methylnonanoic acid (4-MeN) to simulate sheep meat flavour, and a controlled quantity of skatole to simulate enhanced pastoral flavour. Two other variables were "cure/no cure" and "spice/no spice" as fully described in Chapter 2. Thus, there were eight treatments presented blind to 60 panellists in a fully randomised design. Panellists assessed liking on a 1 to 9 scale. The aim of sensory evaluation was to show whether the individual effects of curing and spicing and their combinations were able to enhance acceptability of fermented sheep meat products.

The eight treatments were prepared in a kitchen with no formal hygiene control, so before the eight treatments could be presented to the panellists, the hygiene of the products had to be verified.

Hygiene screening for samples used in sensory evaluation

The 16 containers of fresh cured, fermented sausage meat mixture (8 different treatments samples and one backup for each treatment) of sensory evaluation samples were designed for human consumption. Therefore, the safety state of materials was the first criterion for the sensory evaluation. As described in Materials and Methods, sausage samples were tested for *Staphylococcus aureus*, total coliforms and faecal coliforms. BP agar plates were used for the *S. aureus* analysis. No black, shiny, and convex colonies were observed on any of the BP agar plates. Thus, all the boxes of sausage intended for the sensory trial were probably free of *S. aureus*.

MPN tests were used for the detection of total coliform and faecal coliforms. Each of the positive MacConkey broths (yellow with gas) was subcultured to BGBB and tryptone water broths.

All the inoculated BGBB and tryptone water broths were observed negative after incubation. Therefore, there probably were no faecal coliforms present. The MPN results of the total coliform tests are reported in Table 9. According to the Microbiological Reference Criteria For Food (Ministry of Health October 1995), the low number of total coliforms in all treatments indicate the treatments had been processed under sanitary conditions.

Table 9. The screening test results for total coliforms in MPN

Treatment					
All combinations of factors					
Ferment	Cure	Spice	BCFA /skatole	Number of MacConkey broth positive results[1] (duplicates)[3]	Results calculated from the MPN table[2], per g
■			■	3, 0, 0	23
				(2, 0, 0)	9.1
				2, 0, 0	9.1
				(3, 0, 0)	23
		■	■	0, 0, 0	<3
				(0, 0, 0)	<3
		■		0, 0, 0	<3
				(0, 0, 0)	23
	■		■	3, 0, 0	23
				(3, 0, 0)	23
				2, 0, 0	9.1
				(2, 0, 0)	9.1
		■	■	0, 0, 0	<3
				(0, 0, 0)	<3
		■		1, 0, 0	3.6
				(1, 0, 0)	3.6

Heavy shading means a factor is applied in that treatment.
[1] Values represent positive results from 10^{-2}, 10^{-3}, 10^{-4} dilutions
[2] See MPN Table in Appendix 4
[3] Backup/duplicate sample of each treatment

Hedonic sensory trial

Sixty panellists participated in the sensory evaluation trial. All tasted all of the eight treatment samples and completed the sensory evaluation form, they were all asked for these, gender, age group and liking score. The mean of liking score and standard deviations of each of the eight treatment samples are presented in Table 10. Data were also analysed for variance due

to main effects (cure, spice, BCFA/skatole) and their interactions. Heavily shaded areas mean that factor was applied to the treatment, and lightly shaded areas mean that factor was applied to only half the treatments. According to Table 10, cure had no significant effect ($P = 0.817$) on the liking score of sausage treatments whether BCFA/skatole was added or not. However, spice significantly improved the liking score for all the treatments, with or without BCFA/skatole ($P < 0.001$). The presence of BCFA/skatole brought down the liking score ($P = 0.003$). The differences of liking score between the eight treatments can perhaps be more clearly seen in Table 11. The star marks indicate the means of liking score for the eight treatments. The 95% confidence interval is based on pooled standard deviation; here confidence intervals indicate 95% of the population mean of liking scores for each treatment lie within these intervals. Within each bracket, the dotted line indicates that the distance between sample mean of liking score ($\bar{x}$) and the population mean (μ) of liking score is less than $1.96\frac{\sigma}{\sqrt{n}}$ (σ, pooled standard deviation, here is 1.778; n, number of sample, here is 60). Thus, the 95% confidence limits for the population mean of liking score, $\mu = \bar{x} \pm 1.96\frac{1.778}{\sqrt{60}}$.

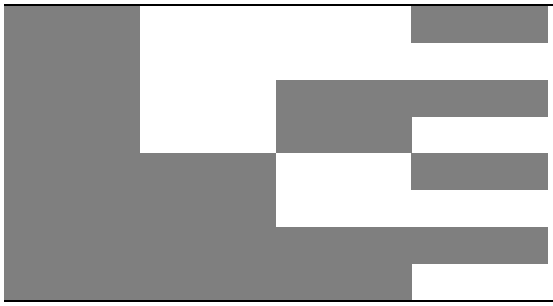
In Table 11, the neutral (none) effect of cure is obvious as is the positive effect of spice. The latter is particularly true when BCFA/skatole is added in the two top treatments in Table 11. Liking is markedly reduced when BCFA/skatole is included. Another way of looking at this is to inspect the interactions (Figure 21, Table 10). Table 10 reports there was very clearly no interaction between spice and BCFAs/skatole ($P = 0.457$), illustrating the overwhelming advantage of using spice where BCFA/skatole was present. Thus, the lines on the BCFA/skatole x spice graph (Figure 21) were essentially parallel. In contrast, the lines on the BCFA/skatole x cure graph converged when cure was applied. This hints that curing can slightly reduce disliking due to BCFA/skatole. The interaction of spice and cure suggests that curing reduces the negative effect of non-spiced fermented sausage (Figure 21). It must be emphasised however that these effects were not statistically significant and remain as only hints of effects. The role of curing will be further discussed in the final chapter, where its commercial value will be examined.

Summary of the sensory results

The BCFA/skatole reduced the liking scores of treatments. Although some treatments with BCFA/skatole were not statistically demonstrated to be different from treatments without, Table 11 shows that BCFA/skatole treatments were scaled lower. On the other hand, treatments containing spices were liked the most. Spices (garlic and rosemary) significantly improved the acceptability of meat modified with sheepmeat flavour. Thus, spices were able to suppress

sheepmeat flavour. However, cure did not enhance the acceptability of treatments where either BCFA/skatole or spice were involved. The role of cure here was to generate the typical redness of cured meat that improved the appearance of samples. Nevertheless, cure was impotent to prevent fat oxidation, which leads to rancidity of sausage. Cure was also the active component that controls the growth of pathogens and moulds. Commercial aspects of these results will be discussed in the next and final chapter.

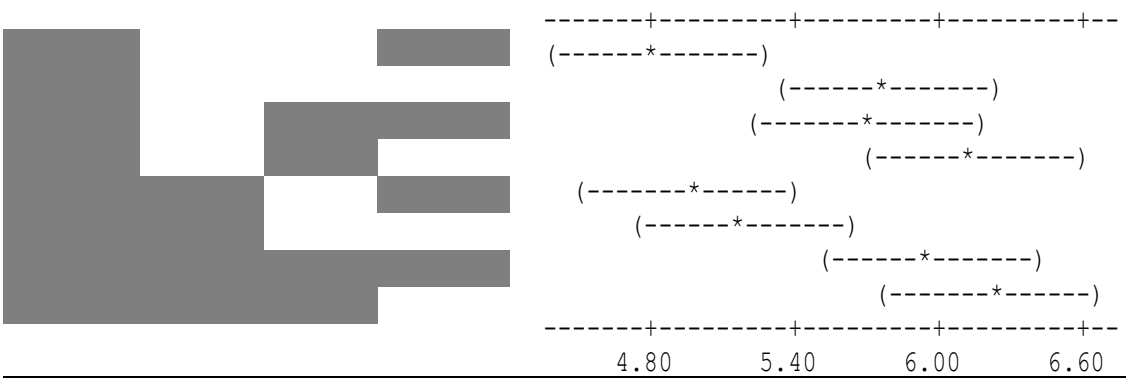
Table 10. The liking scores of eight treatments

Treatments				
All combinations of factors				
Ferment	Cure	Spice	BCFAs/ skatole	Mean liking score ± SD
				4.82 ± 1.89^{a} 5.78 ± 1.60^{b} 5.70 ± 1.87^{abc} 6.15 ± 1.87^{bcd} 4.95 ± 1.89^{abce} 5.18 ± 1.72^{abcef} 5.95 ± 1.66^{bcdf} 6.22 ± 1.70^{bcd}
Analysis of individual factor				Mean liking score ± SD
Effect of cure as a factor				
				5.58 ± 1.81^{a} 5.61 ± 1.87^{a}
Effect of spice as a factor				
				6.00 ± 1.78^{a} 5.18 ± 1.81^{b}
Effect of BCFAs/skatole as a factor				
				5.35 ± 1.88^{a} 5.83 ± 1.76^{b}
Analysis of variance				*P*
Main effects				
Overall effect of treatments				< 0.000
Effect of cure				0.817
Effect of spice				< 0.000
Effect of BCFAs/skatole				0.003
Interactions				
Cure x spice				0.232
Cure x BCFAs/skatole				0.169
Spice x BCFAs/skatole				0.457

Heavy shading means a factor is applied in that treatment; light shading means only half have that factor applied.

[a, b] Means in a treatment comparison with different superscripts are significantly different at $P < 0.05$

Table 11. The differences of liking scores between eight treatments

Treatment				
All combinations of factors				
Ferment	Cure	Spice	BCFA /skatole	Individual 95% confident intervals for liking score mean based on the pooled standard deviation[a]
				

Heavy shading means a factor is applied in that treatment
[a], pooled standard deviation = 1.778

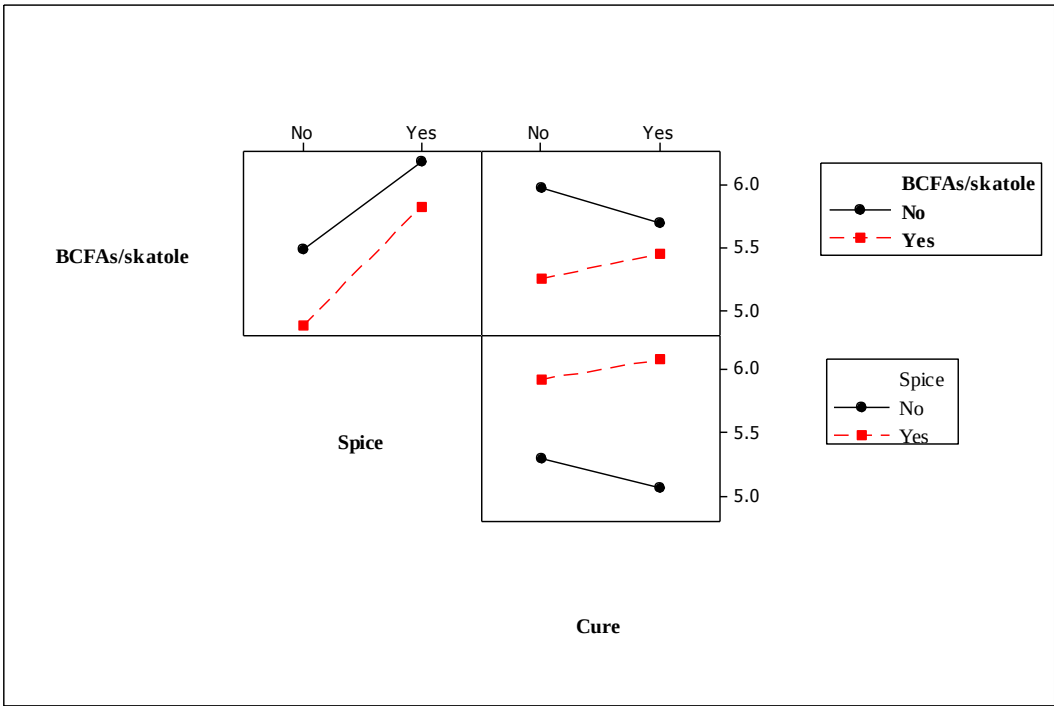


Figure 21. Interaction plots of liking score means for cure, spice and BCFAs/skatole.

Chapter 5

Conclusion

Fresh fermented and cured lamb sausages were very similar to pork and beef equivalents, according to the physicochemical (growth of LAB, pH and colour) and texture (hardness, springiness, cohesiveness and adhesiveness) results. Thus, it is processing possible to produce a fermented and cured lamb sausage with close physicochemical and texture properties to those found in existing pork equivalents, such as salamis. However, the fermented and cured sausages prepared, based on the methods, which have been described in Chapter 2 (Materials and Methods), were wet sausage. Because these lamb sausages were fermented in appropriately sealed modified syringe barrels with no maturation, no liquid was able to evaporate through the plastic barrels. Thus, these lamb sausages were high in moisture and water activity. Nevertheless, these fermented cured lamb sausages were developed for experimental purposes only. Further experiments will be required to choose an appropriate casing and to design a maturation period. This has not been included in this thesis research. Despite the high moisture content, these fermented and cured lamb sausages have the potential to behave similarly to salami style after drying.

The characteristic sheepmeat flavour, contributed mainly by BCFA/skatole, affected the liking scores of all treatments. Thus, when BCFA/skatole was present, consumers tended to scale the sausage samples lower. However, spices (garlic and rosemary) were shown to suppress this characteristic sheepmeat flavour at statistically significant levels. The description of spiced sausage was “like slightly” (see Chapter 4, Table 10 and the Appendix 2). Therefore, the unpleasant characteristic sheepmeat flavour is likely to be solved by spices.

Although curing was not an effective flavour enhancer for these fermented lamb sausages, its role was to generate typical pinkish-red cured meat colour, which improved the appearance of the sausages. Curing (nitrite) plays a very important role in prevention of fat oxidation, which leads to rancidity of sausage, and in preventing the growth of pathogens and moulds. Moreover, the curing agent nitrite is a highly efficient and economic substance. A small portion of nitrite applied to the sausage mixture can produce sausages with a stable red colour, low level of fat oxidation and low risk of pathogens. These benefits secure nitrite as an irreplaceable food additive.

In this research, LAB performed well as a starter in the fermentation process. According to the results presented in Chapter 3, LAB initially reduced the pH of sausages rapidly and the pH continued to decrease throughout the fermentation. The application of LAB is using beneficial microorganisms to counter the development of spoilage and hazardous microorganisms. Thus, both low pH level and the possibility of bacteriocins produced by the LAB are efficient food preservations. Also, fermentation provides a unique flavour for processed meat products, one of the important reasons why fermented foods have been popular for centuries. In addition, fermented foods have potential health benefits, which have drawn a lot attention from consumers in recent years. Fermentation and curing together are the primary preservative techniques in this development of lamb sausage (see Chapter 1, Fermentation). Even though the fermented and cured lamb sausages produced in this study were high in moisture and water activity, no food pathogens or spoilage organisms were detected.

Although New Zealand sheepmeat has an image of "Clean, green, grass-fed, healthy, and doesn't get any more free range than this" (see Chapter 1, Preservation of Sheepmeat for International Markets), the sheepmeat produced in New Zealand is exported to the rest of the world in the forms of chilled and frozen packages only. There is a lack of processed sheepmeat or added value products transported overseas. It may be that a few processed sheepmeat products have been shipped outside New Zealand, but they are rare. Chilling and freezing are common preservation methods, but they consume a lot of energy and if the energy source fails, the foods are at risk. Deterioration and spoilages due to microorganism growth take place in a short time. Fermented, cured and dried sheepmeat sausage is likely to solve these problems. Fermentation and curing can ensure the safety of sheepmeat products. After the sausage is dried during maturation, it becomes shelf-stable at ambient temperature and no cool temperature storage is necessary. Fermentation and maturation are time consuming procedures that may need up to two weeks for the manufacturing process. However, fermented, cured and dried sheepmeat sausage has a reasonably long, low energy, low risk, and economic shelf-life in the market. Finally, even though maturation takes time, it adds value to the meat. Consumers are familiar with the concept that 'Better food costs more'.

As has been explained in Chapter 1 in Preservation of Sheepmeat for International Markets, sheepmeat is the name given to lamb, hogget and mutton. Whereas lamb is the best in quality and flavour, consumers select sheepmeat on name and preference declines in the sequence: lamb > hogget > mutton. So lamb is already sold at a high price in chilled or frozen form. Only hogget and mutton are low priced sheepmeats and it is desirable to raise their value. Therefore, hogget

and mutton are more likely to be used for production of fermented, cured and dried sheepmeat sausage. Under this circumstance, a new problem on package labelling will be introduced. As this sausage will be made from hogget or mutton or their combination, and the name of the sausage must describe the nature of this product (Food Standards Australia and New Zealand 1987), a possible name will be "Fermented, cured and dried hogget/mutton sausage". But consumers are not attracted to the names hogget and mutton; they would probably not purchase such a product. It will be possible to use the name 'sheepmeat' to replace 'hogget/mutton', or adding small quantity of lamb that is no less than 5% weight of total sausage weight to claim the name 'Lamb sausage' (Food Standards Australia and New Zealand 1987). However, the true amounts of ingredients used must be stated in the ingredient list, thus, the percentages of hogget, mutton and lamb must be printed alongside the ingredient names. The analysis of a suitable name to express the true nature of the fermented, cured and dried sheepmeat sausage was not part of this thesis research. This challenge is for future researchers and marketing professionals to find the best name (hogget and mutton could be named "ovine") to help this product achieve its maximum benefits.

Appendix 1

The following table is a master sheet showing all sausage ingredients required for sensory evaluation samples. For each treatment, about 1kg sample was prepared and filled in an airtight plastic box. The first part of the table (shaded in light grey) was the basic formula of sausage mixture (Table 6). The ingredients shaded with light pink colour were added to sausage mixture according to Table 8.

The completed calculations were facilitated by the use of a comprehensive Excel spreadsheet, attached as an electronic appendix in the library version of this thesis. The electronic spreadsheet is active so when if the mass of lean is changed, the rest of ingredients are modified relevantly.

	Mass (g)	
Lean	1277	
Fat	599	
Salt	79.8	
Pyrophosphate	4.0	
Glucose	39.9	
Culture	0.20	
Total mixture weight	2000	
Volume of 4-MeO in EtOH added	0.11	mL
Mass of 4-MeO added	0.011	g
Final concentration of 4-MeO (ppm)	5	ppm
Volume of 4-MeN in EtOH added	0.11	mL
Mass of 4-MeN added	0.001	g
Final concentration of 4-MeN (ppm)	0.35	ppm
Volume of skatole in EtOH added	0.11	mL
Mass of skatole added	0.159	g
Final concentration of skatole (ppb)	79.56	ppb
Sodium nitrite	0.20	g
Garlic	0.40	g
Rosemary	0.40	g

Appendix 2

A sample of Sensory evaluation form

Sensory Evaluation of Sausage Samples

You are panellist number ☐

Gender: Male Female (please circle one)

Age group: (please tick the box nearby)

18-24	☐	45-49	☐
25-29	☐	50-54	☐
30-34	☐	55-59	☐
35-39	☐	60-64	☐
40-44	☐	65+	☐

How much do you like the flavour of these sausage samples?

Taste the samples from left to right and tick the box that best describes your liking or disliking.

Follow the sequence of Bays shown in the four following pages

Please drink water and eat apples slices to clean the flavour left in your mouth in between samples.

Now go to the Bay shown on the next page…..

Bay A

Taste the samples from left to right and tick the box

	470	370
Like extremely	☐	☐
Like a lot	☐	☐
Like moderately	☐	☐
Like slightly	☐	☐
Neither like nor dislike	☐	☐
Dislike slightly	☐	☐
Dislike moderately	☐	☐
Dislike a lot	☐	☐
Dislike extremely	☐	☐

Remember to clean your palate with apple and water

Bay B

Taste the samples from left to right and tick the box

	511	133
Like extremely	☐	☐
Like a lot	☐	☐
Like moderately	☐	☐
Like slightly	☐	☐
Neither like nor dislike	☐	☐
Dislike slightly	☐	☐
Dislike moderately	☐	☐
Dislike a lot	☐	☐
Dislike extremely	☐	☐

Remember to clean your palate with apple and water

Bay C

Taste the samples from left to right and tick the box

	343	897
Like extremely	☐	☐
Like a lot	☐	☐
Like moderately	☐	☐
Like slightly	☐	☐
Neither like nor dislike	☐	☐
Dislike slightly	☐	☐
Dislike moderately	☐	☐
Dislike a lot	☐	☐
Dislike extremely	☐	☐

Remember to clean your palate with apple and water

Bay D

Taste the samples from left to right and tick the box

	130	078
Like extremely	☐	☐
Like a lot	☐	☐
Like moderately	☐	☐
Like slightly	☐	☐
Neither like nor dislike	☐	☐
Dislike slightly	☐	☐
Dislike moderately	☐	☐
Dislike a lot	☐	☐
Dislike extremely	☐	☐

Remember to clean your palate with apple and water

Appendix 3

Microbiological Reference Criteria for Food of New Zealand (Ministry of Health October 1995)

The following terms as used by the International Commission on Microbiological Specifications for Foods (ICMSF) are used in these reference criteria.

n = The number of sample units which must be examined from a lot of food to satisfy the requirements of a particular sampling plan.

c = The maximum allowable number of defective sample units. When more than this number is found, the lot is rejected by the sampling plan.

m = Represents an acceptable level and values above it are marginally acceptable or unacceptable in the terms of the sampling plan.

M = A microbiological criterion which separates marginally acceptable quality from defective quality. Values above M are unacceptable in the terms of the sampling plan and detection of one or more samples exceeding this level would be cause for rejection of the lot.

Manufactured, cured or fermented meat - ready-to-eat

Coagulase producing staphylococcus (/g)	n = 5	c = 2	m = 10^2	M = 10^3
Faecal coliform (/g)	n = 5	c = 2	m = 20	M = 2 x 10^2

Appendix 4

The MPN table (Brooks and McIntyre 2005)

THREE-TUBE MOST PROBABLE NUMBER TABLE showing most probable number of bacteria in the ***centre*** dilution

No. of Tubes Positive in			MPN Index	No. of Tubes Positive In			MPN Index
SERIE S	**SERIES**	**SERIE**		**SERIES**	**SERIES**	**SERIE**	
0	0	0	< 0.03	2	0	0	0.091
0	0	1	.03	2	0	1	0.14
0	0	2	.06	2	0	2	0.20
0	0	3	.09	2	0	3	0.26
0	1	0	0.03	2	1	0	0.15
0	1	1	0.061	2	1	1	0.20
0	1	2	0.092	2	1	2	0.27
0	1	3	0.12	2	1	3	0.34
0	2	0	0.062	2	2	0	0.21
0	2	1	0.093	2	2	1	0.28
0	2	2	0.12	2	2	2	0.35
0	2	3	0.16	2	2	3	0.42
0	3	0	0.094	2	3	0	0.29
0	3	1	0.13	2	3	1	0.36
0	3	2	0.16	2	3	2	0.44
0	3	3	0.19	2	3	3	0.53
1	0	0	0.036	3	0	0	0.23
1	0	1	0.072	3	0	1	0.39
1	0	2	0.11	3	0	2	0.64
1	0	3	0.15	3	0	3	0.95
1	1	0	0.073	3	1	0	0.43
1	1	1	0.11	3	1	1	0.75
1	1	2	0.15	3	1	2	1.20
1	1	3	0.19	3	1	3	1.60
1	2	0	0.11	3	2	0	0.93
1	2	1	0.15	3	2	1	1.50
1	2	2	0.20	3	2	2	2.10
1	2	3	0.24	3	2	3	2.90
1	3	0	0.16	3	3	0	2.40
1	3	1	0.20	3	3	1	4.60

1	3	2	0.24	3	3	2	11.00
1	2	3	0.29	3	3	3	>24.00

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