Effect of Different Carbohydrates on the Properties of Cured, Fermented Sheepmeat Sausage

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Abstract

The purpose of this thesis was product development to define the basis of a novel sheepmeat sausage, fermented and cured. The development has centred on the role of the carbohydrate sources, traditionally using glucose but in the present study, the carbohydrate sources, potato, sweet potato (kumara) and yam were used. Kumara showed the great capacity to generate fermentable sugars after microwave heating, and given the pivotal role of kumara in pre-European New Zealand Maori culture that remains important contemporary in New Zealand cuisines, this tuber was chosen for sausage development. The rationale for this was that the novel sausage should display features of geographical exclusivity as a point of difference.

The basic formulation comprised lean and fat components of sheepmeat, heated kumara, salt, sodium pyrophosphate, sodium nitrite, and lactic acid bacteria in descending order of addition abundance. The mixtures were extruded into 50 mL plastic syringes, where the needle end of the barrel had been excised by a lathe. The lubricated barrel was overfilled to 60 mL, capped with a layer of film and aluminium foil and fermented at 31°C. After four days fermentation, samples were monitored in physical and chemical parameters.

Three New Zealand kumara varieties, red (skinned), orange (flesh) and gold (flesh) kumara were used to develop model fermented sheepmeat sausage. Fermentation occurred with each variety, but did not significantly affect colour parameters and textual properties. Due to cost, red kumara were used for the next stage experiments to identify properties of fermented sheepmeat sausages affected by cooking time and the concentration of red kumara. According the physical and chemical results (pH, colour and texture), cooking time and kumara variety did not have a significant effect on the propreties. However, the concentration of kumara had an influence on hardness.

The results point to commercial opportunities and further research with kumara. To improve the product quality and to show geographical exclusivity, further research could be done by associated preservation methods, casings, and spices and herbs which are commonly used in New Zealand, such as garlic, rosemary and pepper or New Zealand specific herbs, such as horopito. In addition, sensory studies should also be performed before the products could be tested in the market.

Statement of Originality

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institutions of higher learning, except where due acknowledgement is made in the acknowledgement".

Signed _____

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Chapter 1

Introduction

1.1. The New Zealand sheepmeat industry and sheepmeat preservation history

New Zealand has a sophisticated sheepmeat industry. The New Zealand climate is favourable for pasture growth and it plays a pivotal role in sheep production because pasture provides virtually the whole diet of sheep (Hathaway, 1997). Meat from pasture-fed sheep is marketed as 'naturally-raised' and 'healthy', although the objective differences from feed-lot raised animals are typically limited to fat content and fat flavour.

Although New Zealand is not the largest producer of sheepmeat, it is the world's largest exporter of sheepmeat (Irish Food Board, 2010), variously sold as lamb, hogget and mutton. Sheepmeat export makes a large contribution to New Zealand's economy. Total meat exports comprised approximately 13% of the national total exports by value in 2009, and was worth NZ$5.14 billion (New Zealand Trade & Enterprise, n.d.). Bovine (beef) and ovine (sheep) meats are the main export products. Almost 90% of ovine (sheep) meat is exported and the main export destinations are the European Union (EU), North Asia, North America and the Middle Eastern (Morris, 2009; Spedding, 2010). Specifically, about 180,000 tonnes of sheepmeat were exported to the EU in 2010, accounting for more than 85% of overall imported sheepmeat into that union (Agrifax, 2010). New Zealand sheepmeat imports accounted for about 16% of USA sheepmeat consumption in 2010 (Spedding, 2010). Although China's sheepmeat flock is the largest in the world (FAOSTAT, 2011), almost 50% of total imported sheepmeat was supplied by New Zealand to China in 2009 (RedFern Associates, 2010), although China's sheepmeat flock is the largest in the world (FAOSTAT, 2011).

Export of sheepmeat requires its preservation because of the time between slaughter and consumption in foreign markets can be many weeks, even months. New Zealand is remote from Northern Hemisphere markets and export by sea freight requires weeks in transit. New Zealand started to establish sheep flocks for local consumption from 1840 onwards, but as production increased ways had to be found to preserve the meat, with a

longer term view to export (McLintock, 1966). Following Australian experiences, the canning technique was applied from about 1869 (McLintock, 1966). Sheepmeat was first transported to Britain in cans in 1870, but with mixed success (McLintock, 1966). Refrigeration applied in meat industry stemmed from the 1870s in America and then spread into Europe and Southern Hemisphere, where in New Zealand the very first frozen export shipment to Britain in 1882 was a technological and commercial success (Nicol & Saunders, 1966). The export trade in frozen mutton and lamb developed steadily and by 1889 the number of carcasses exported exceeded 1 million. In these early years, mutton carcasses made up the bulk of the shipments, but by 1900 the lamb trade was firmly established (McLintock, 1966).

In the last century the frozen trade expanded enormously (Maurovi, 2007), but from the 1970s was gradually replaced by chilled meat exports, that depended on very precise temperature control and packaging that excluded air or otherwise controlled the atmosphere around meat (Bell, 2001). Chilling and freezing techniques remain the cornerstone of sheepmeat preservation in New Zealand and its export trade, but there are many other methods of meat preservation available. One of these is fermentation, and that is the subject of this thesis. However, before describing the aims of the present study, a review of the reasons to preserve meat and how meat preservation is done is presented.

1.2. The characteristics of meat deterioration and the need to preserve

Once the conversion of muscle to meat occurs, the storage life is limited to a few days or weeks depending on the ambient temperatures. Without meat preservation, meat will spoil, mainly due to microbial activity but also due to oxidation of meat components on exposure to air. Although a few microbial species are beneficial for meat in the context of human consumption – as described more in the later sections – the majority of microbial species contribute the unattractive changes in meat (Table 1).

Table 1. Superficially recognisable symptoms of microbial spoilage of meat

Oxygen status	Type of microorganism	Symptoms of spoilage
Present	Bacteria	Slime on meat surface Gas production Off-odours and taints Discoloration by destruction of meat pigments or growth of colonies of coloured organisms Fat decomposition
Absent	Bacteria	Putrefaction accompanied by foul odours Gas production Souring
Present	Yeasts	Yeast smile Off-odours and tastes Discoloration Fat decomposition
Present	Moulds	Surface stickiness and 'whiskers' Odours and taints Discoloration Fat decomposition

Source: Haines (1937)

From a huge literature, it is clear that these destructive changes to meat result from the growth in bacteria, moulds and yeasts in different atmospheric environments, and are additional and interactive with changes caused by enzymes endogenous to meat. Of the types of microbes, bacteria are mainly responsible for meat deterioration. This is due to rapid growth of bacteria by replication and variable capacity to grow in the presence or absence of oxygen in air. Bacteria can secrete collagenase to hydrolyse the connective tissue between the fibre bundles, leading to partial solubilisation of meat. Subsequently, gas production can occur. Deamination and decarboxylation of amino acids and proteins are also caused by bacteria, giving rise to compounds such as carbon dioxide, hydrogen, ammonia, hydrogen sulphide and indole. Hydrogen sulphide and indole are the cause of

foul smells. In addition, discoloration of meat can be attributed to hydrogen sulphide oxidising myoglobin to porphyrins that creates a green colour on the surface of meat.

Moulds and yeasts are favoured by using low storage temperatures. Among the genera of moulds resulted in meat deterioration are *Thamnidium*, *Mucor*, and *Rhizopus* which produce 'whiskers' (Van Laack, 1994), *Cladosporium, Sporotrichium* and *Penicillium*, which generate black, white and green patches, respectively (Lawrie, 1998; Van Laack, 1994). Yeasts can cause the development of brown spots due to their action on haem. Also yeasts are related with souring that is mainly caused by lactobacilli and enterococci.

Hence, inhibiting or deterring the activity of the spoilage microbes by creating unfavourable conditions is primary principle of meat preservation. Apart from microbes, some other factors are responsible for the meat deterioration. The preservation also inhibits activity of endogenous enzymes, chemical reaction that may spoil meat, and invasion and spoilage by insects and rodents (Sivasankar, 2004).

1.3. A brief history of meat preservation

There are five ancient methods of meat preservation: drying, smoking, salting, chilling and fermentation. Drying in air to lower water activity is probably the earliest method of preserving meat, followed by smoking (Sleight & Hull, 1982). In the Neolithic period, smoking was used as a means of preserving meat and fish (Sleight & Hull, 1982). Smoking can also be seen as a form of drying, because the high temperatures accelerated the drying process. Fires also kept insects away from meat and fish and created new flavours, although the latter would undoubtedly not be the motive for smoking. Much later the ancient Egyptians learned to use salting combined with and sun drying to preserve food (Pearson & Gillett, 1996). This technique is also known as curing, which greatly extends the storage life and improved the flavour of food. The ancient Romans were the first to first adopt the chilling as a method of food preservation by using ice and snow to store food (Pearson & Gillett, 1996). The ancient Chinese made the first fermented sausages about 5000 before present (Varnam & Sutherland, 1995).

Modern methods of food preservation were triggered by the development of canning in 1809 (Pearson & Gillett, 1996). Subsequently, advanced scientific technology has contributed to the improvement and/ or innovation of meat preservation. Newer methods of meat preservation are freeze drying, irradiation, and chemical or microbiological additives. These newer meat preservation techniques will be described in the next section.

1.4. Techniques of preserving meat

Cassens (1994) classified preservation of meat into three categories: physical, chemical and microbiological, as shown in Table 2. In contrast, Lawrie (2006) classified preservation of meat into three categories: temperature control, moisture control and direct microbial inhibition. These can be identified in different places in Table 2. According to Gould (2000), preservation methods comprise three techniques: techniques that slow or prevent the growth of microorganisms, techniques that inactivate microorganisms and techniques that restrict access of microorganisms to products. Again these can be identified in Table 2, which will serve as the basis of this review.

Table 2. Major preservation methods of meat

Physical	Chemical	Microbiological
Heating	Curing	Competition
Canning	Smoking	Fermentation
Chilling	Antioxidants	Antimicrobials that occur naturally
Freezing	Sulphite	Bacteriocins
Control of water activity	Sorbates	Genetic engineering
Microwave application	Lactate	
Irradiation	Acidulants	
Ultrasound, high pressure and high-voltage pulses	Carcass rinses	
Packing		

Source: Cassens (1994)

Commonly used means of meat preservation as described in Table 2 are discussed in the next section.

1.4.1. Physical methods of meat preservation

The physical methods of preservation are achieved by changing physical parameters such as control the temperature (cooling or heating), control of moisture content, and oxygen.

1.4.1.1. Control of temperature

To create an environment that inhibits microbial growth by controlling temperature is an effective and simple method to preserve meat. Table 3 shows microorganisms associated with meat deterioration and their optimal growth temperature. Obviously, either cold or heat treatment can create an unfavourable environment to the growth or survival of spoilage microorganisms. Therefore, heating and cooling are applied in commercial meat industry to preserve meat and extend storage life of products.

Table 3. Microorganisms associated with meat deterioration

	Growth interval	Microorganism
Psychrophiles	-5 to 35ºC	*Pseudomonas* sp. *Achromobacter*
Mesophiles	15 to 45ºC	*Eschericia coli* *Bacillus subtilis*
Facultative thermophiles	24 to 54ºC	*Steptococcus thermophilus* *Clostridium perfringens*
Thermophiles	45 to 75ºC	*Clostridium thermosaccharolyticum* *Bacillus stearothermophilus*

Source: Bem & Hechelmann (1995)

Heating is an ancient and remains a commonly used preservation method (Hubbert, Hagstad, Spangler, Hinton, & Hughes, 1996). In terms of temperature increase, four commercial heating processes are usually employed to food products: blanching, cooking, pasteurisation and sterilisation (Legarreta, 2004). Of all heating processes, canning is a

form of sterilisation, which probably is the most efficient means of preserving meat (Legarreta, 2010). Three steps are involved in sterilisation by canning: heating to above 100°C, maintaining that temperature for a minimum time, and cooling. The thermal processing inhibits activity of enzymes and destroys the microbes and their spores. This can be done sealed tin cans, aluminium cans, or glass or plastic containers (Cassens, 1994). Canning ensures meat can be stored almost indefinitely at any ambient temperature, which is a unique advantage compared with other meat preservation methods (Legarreta, 2004).

In contrast to heating, the main aims of refrigeration are to slow or inhibit growth of microbes and enzymatic activity by reducing temperature to below the optimum temperature of spoilage microbes (Cassens, 1994). The refrigeration can be classified into two categories, chilling/cooling and freezing in terms of storage temperature. After slaughter, carcass are normally chilled in the air in a chamber held at between 0 and 5 ºC with rapidly moving dry air (Cano-Muñoz, 1991), which promotes surface drying and thus reduces microbial growth. At the temperatures well below the freezing point of meat (-1.5°C), meat deterioration due to microbes is severely retarded and deterioration due to oxidative rancidity and endogenous enzymatic processes is similarly slowed (Zhou, Xu, & Liu, 2010). Freezing supports a much longer preservation period than chilling. Compared with any other long-term preservation method, freezing has a better effect on maintaining the flavour, texture and nutritive properties of products (Hogan, Kelly, & Sun, 2005). However, ice crystal damage that is manifest as excess drip on thawing remains an intrinsic problem with freezing (Hogan et al., 2005).

1.4.1.2. Control of moisture content

Dehydration, or drying, is also a common preservation technique that works by lowering the moisture content of meat. This in turn lowers water activity that inhibits the growth of microbes. It also causes a decrease in molecular mobility (Fennema, 1996), thereby slowing enzyme activity such as hydrolytic activity on fat and protein in meat. Dehydration includes hot air-drying ambient air-drying, and freeze-drying as the cold extreme. The last is particularly effective, although costly, because molecular mobility is low at low temperatures. The moisture content of hot air-dried meat falls to around 5%

while freeze-dried to 1% (Hubbert et al., 1996). The optimum water activity for successful bacterial growth ranges from about 1.0 to 0.75, while yeasts and moulds can grow (slowly) at water activity of 0.62 (Scott, 1957). Thus, to comprehensively prevent microbial growth depends on stringent control of final moisture content. Dehydration is commonly combined with other preservation methods such as curing, smoking, fermentation, heating and cooling.

1.4.1.3. New physical techniques

Irradiation

Irradiation, also known as ionising irradiation, is the most effective postharvest intervention methods for inactivation pathogens in meat that is not treated by other methods (Lee & Ahn, 2009). The ionising irradiation includes three forms of high-energy radiation: gamma-rays, X-rays and electron beams (Osterholm & Norgan, 2004). Satin (2002) pointed out that microbial toxins, viruses and spores would still survive in meat and meat product at irradiation less than 10 KGy. Thus, irradiation must be combined with other preservation methods as a control method to ensure meat safety and extend the storage life. As a part of overall meat manufacturing program, irradiation could reduce the risk of cross-contamination and survival of pathogens that resulted from improper cooking, cooling during storage and transportation (Molins, Motarjemi, & Käferstein, 2001).

Ultrasound

Ultrasound technology has a range of applications in the meat industry, such as low temperature pasteurization, inactivation of enzymatic activity, meat tenderization and meat brining (Zogul, Zogul, & Boga, 2011). This approach shows more rapid and effective inhibition of microbes than heating treatment (Mason, Paniwnyk, & Chemat, 2003) and can improve textural prosperities of meat such as tenderness and cohesiveness (Chemat, Zill, & Khan, 2011). However, this technique has a high energy cost and unlike irradiation cannot be a stand-alone process (Zogul et al., 2011).

1.4.2. Chemical techniques

1.4.2.1. Curing

Curing is an ancient and enduring method of meat preservation and a wide range of curing ingredients are applied in curing. Curing works by lowering water activity (Honikel, 2010). Of the curing ingredients, the most used curing agent is common salt, sodium chloride. Salt added to meat inhibits the growth of many microbes. Salt used in curing in earlier times was impure, and contained many other cations and anions. Most notably when the salt contained nitrate, the cured meat became a stable red colour. Flavour was also improved. Honikel (2007) reported that several researchers in the 19th century developed the concept that nitrate was responsible for the colour change but only after it was reduced by microorganisms associated with the raw meat, which becomes anoxic after slaughter due to the consumption of residual oxygen by the still-active mitochondria in muscle cells. Hoagland (1910, 1914) proposed the scheme in Figure 1 that has endured to the present day (Honikel, 2010).

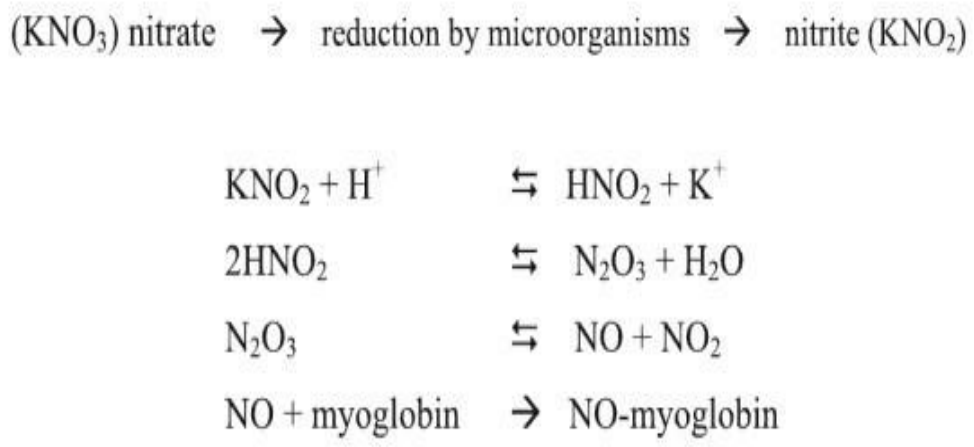

Figure 1. The action of nitrate in cured meat products (Honikel, 2010)

Without doubt, nitrite-cured meats suppress fat oxidative deterioration more than non nitrite-cured meats. There are four different mechanisms to propose the fat retardation as summarised by Pegg and Shahidi (2004): (1) formation of a stable complex between nitrite

and heme pigments; (2) stabilisation of unsaturated fat acids within tissue membranes; (3) interaction of nitrite as a metal chelator to bond trace metals in meat and liberated non-heme iron from denatured heme pigments; and (4) formation of nitroso and nitrosyl compounds in meat.

When purer forms of salt became available to the food industry, if colour changes were wanted, nitrate – or more commonly nitrite – was added to the salt, and in controlled amounts governed by food regulations. Salt can also be used with sugars, phosphates, hydrolysed vegetable proteins, lactates and potassium sorbate and spices, each fulfilling a technological function that could extend to flavour.

1.4.2.2. Smoking

Smoking and curing are closely interrelated in preserving meat and are often practiced together (Pearson & Gillett, 1996). Certain chemicals from wood smoke, such as phenols and acids, play a significant role in preservation due to their bactericidal and bacteriostatic properties. These components also act as antioxidants that minimise oxidative changes particularly to fats, and contribute directly and indirect to flavour and colour (Pearson & Gillett, 1996). Besides, smoking decreases the moisture content to between 10 and 40%, depending on the exact smoking procedure (Belitz, Grosch, & Schieberle, 2009). This moisture loss lowers water activity, retarding microbe proliferation and so extends storage life of meat and meat products.

1.4.3. Microbiological techniques

Of the microbial methods of preservation listed by Cassens (1994) (Table 2), one is particularly relevant to this project, fermentation.

1.4.3.1. History of fermentation

The word ‘fermentation’ originally derives from the Latin fevere, which means boiling (Sanchez, 2008). The ancients learned to make fermented sausages dating from over 2000 years ago in Mediterranean countries and China (Varnam & Sutherland, 1995). Around 900 years ago, people started to use salt in combination with fermentation (Varnam &

Sutherland, 1995). The meat fermentation technology was subsequently introduced into northern and western Europe, and then spread to the Americas.

Compared with other preservation methods, the fermentation process is simpler, more economical and requires lower energy input (Sanchez, 2008). Five outcomes of the fermentation process were concluded by Steinkraus (1994) to be: (1) food preservation; (2) improvement on the physical and organolectic properties of food (appearance, flavour, odour and textural properties); (3) reduction of toxins; (4) enhancement on nutrients such as proteins, essential fatty acids and vitamins; and (5) decrease in energy demands and cooking time.

Table 2 also lists bacteriocins as a microbial method of preservation. Bacteriocins are proteinaceous antimicrobial compounds generated by lactic acid bacteria used in fermentation but are not lethal to the producer microbes (Cahill, Uption, & McLoughlin, 2001). Thus, lactic acid bacteria not only inhibit the growth of various microbes by lowering pH, but also generate bacteriocins that inhibit potentially competing bacteria in the meat product (Hugas, 1998). Various isolated bacteriocins such as sakacin, pediocin, curvatcin or mesenterocin have been applied in meat industry with some success (Drosinos, Skandamis, & Mataragas, 2009), but that technology is beyond the scope of this project.

1.4.3.2. Meat fermentation processes

Meat fermentation processes in use today are fundamentally little different from the processes used in ancient times. The manufacture of fermented sausages only contains a few steps (Figure 2): (1) selecting, weighing and mixing the ingredients; (2) stuffing the mixture into casings; (3) fermentation under controlled conditions (i.e. temperature, humidity); and (4) post-fermentation maturation. Comminuted lean meat and its fat are mixed with salt, a source of fermentable carbohydrate, spices and other flavours, and extruded into casings. These are held at a controlled temperature, typically 30°C. Air is largely excluded from the interior of the casing so that aerobic metabolism is inhibited. Rather than generation of carbon dioxide water, the carbohydrate is converted to lactic acid. In the presence of salt and the absence of air, the lactic acid bacteria proliferate at the

expense of other microbes and lower the pH as lactic acid accumulates over a period of days. Fermented sausages are usually partially dried or/and smoked during or after fermentation.

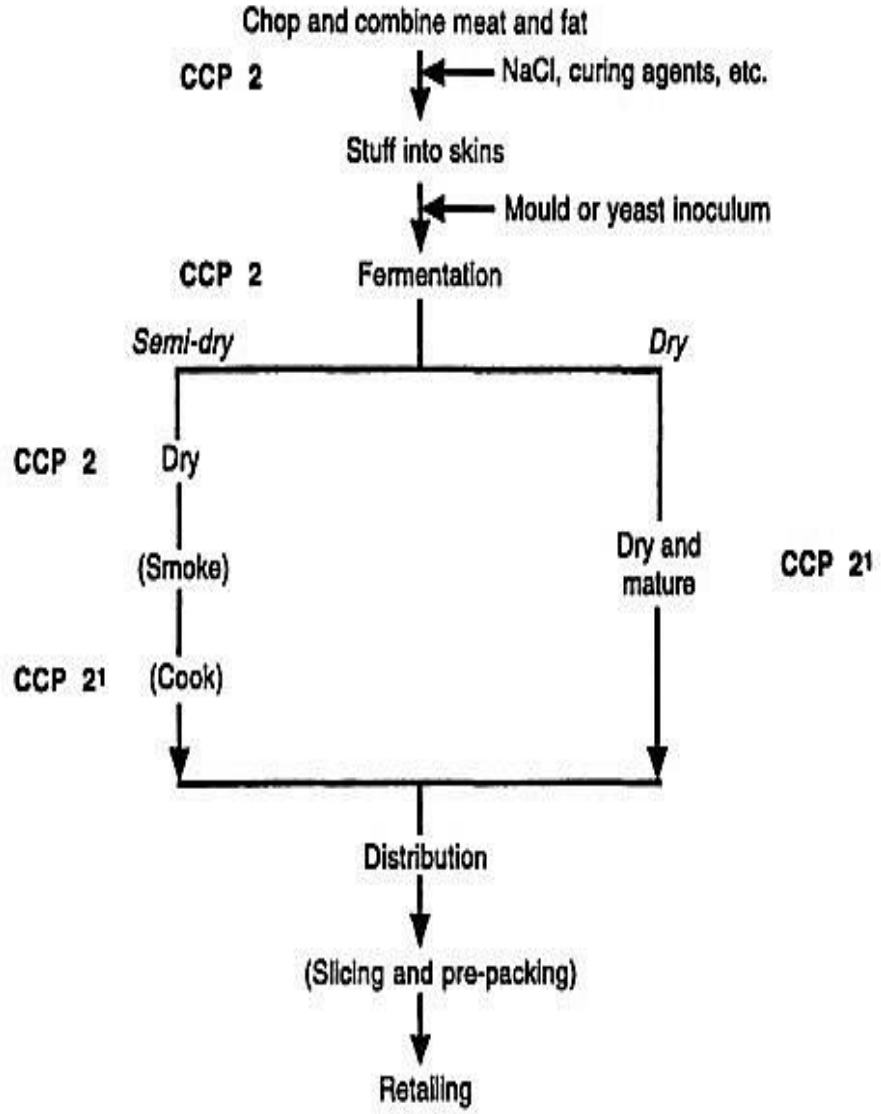

Figure 2. Basic sausage fermentation process (Varnam & Sutherland, 1995). CCP refer to critical control points that must be controlled to ensure product safety.

1.4.3.3. Classification of fermented sausages

There are many criteria that can be used to classify fermented sausages, such as timing of manufacture, final water content, final water activity, fineness of chop, diameter of sausage, application of smoke, use of moulds in maturation etc. Fermented sausages are commonly divided into three categories: spreadable; sliceable with short-term processing; and sliceable with long-term processing in terms of the first three criteria (Lücke, 2003; Varnam & Sutherland, 1995) (Table **4**).

Table 4. Classification of fermented sausages

Type	A*w*	Fermentation time (weeks)	Surface mould growth	Smoked	Example	Origin
Dry	< 0.9	> 4	Yes	No Yes	Salami	Italy Hungary
			No	Either	Dauerwurst	Germany
Semi-Dried	0.9 to 0.95	< 4 1.5 to 3	Yes No	No Usually	Various Most fermented sausages	France, Spain Holland, USA, Scandinavia
Undried	0.9 to 0.95	< 2	No	Either	Sobrasada	Spain

Source: Lücke (2003)

1.4.3.4. Ingredients and additives

Only five ingredients are essential: meat, salt, carbohydrate, curing agent and starter culture. Although fermented sausages can be made without addition of starter culture, it is applied in most large-scale manufacturing to ensure a more consistent product (Hutkins, 2006).

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Lean and fat meat

Meat plays a pivotal role in the characteristics of final products such as sensory, nutritional, safety and health aspects (Ruiz, 2007). Specially, meat provides proteins that contribute to maintain and replace the tissues in body and fats that lead to flavour development. Meat provides the bulk of the final product matrix. The ratio of lean to fat meat is 2 to 1 in most manufactured of fermented sausages and the content of lean meat is usually 50 to 70% (Demeyer, 2004). Of all types of meat, pork is the most widely except where prohibited by religious edicts, followed by beef. In New Zealand, deer and chicken meat are also used, but there are no sheepmeat-dedicated products.

Salt

Salt partially solubilises the myofibrillar proteins of the muscle in such a way as to improved gelation when the pH is lowered by lactic fermentation and/or heating as well as increasing the osmotic pressure such that spoilage by bacteria is suppressed and a typical salty flavour is imparted (Bamforth, 2005). A salt concentration of 2 to 3% of is typical, but can range as high as 8%.

Carbohydrate

Carbohydrates are used as the obligatory carbon and energy source in microbial fermentation for meat products, resulting in lactic acid accumulation that reduces pH. The type and concentration of carbohydrates added strongly affects the rate and extent of pH reduction (Toldrá, 2002). Simple sugars such as glucose and sucrose are favoured by fermentable bacteria because these carbohydrate can be readily transported through the bacterial cell wall (Spedding, 2010). According to Toldrá (2002), readily fermented sugars like sucrose and glucose (dextrose) result in a very fast fall in pH, while carbohydrates like lactose and dextrin (starch fragments) result in a slower pH fall (**Error! eference source not found.**). Apart from fermentation, excess carbohydrate interacts with salty flavour to mask the taste of salt (Bamforth, 2005). However, carbohydrates will act as Maillard reactants during any heating stages in sausage production to impact on colour and flavour (Bamforth, 2005).

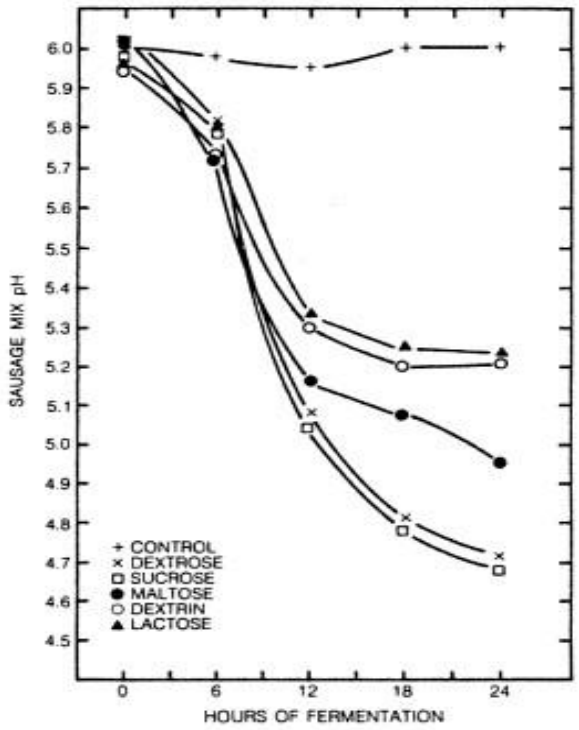


Figure 3. One percent of a suitable fermentable sugar is sufficient to lower the pH to 4.7 in just one day of fermentation (Acton, Dick, & Norris, 1977).

Nitrate, nitrite and phosphate

As described in the section 1.4.1.2, nitrate, or more accurately nitrite, promotes the typical colour of preserved meats through the formation of nitric oxide compounds by reaction with the haem of myoglobin. Moreover, through minimisation of fat oxidation off-flavours due to aldehydes and ketones are avoided, meaning that the flavour is stable. Nitrite also inhibits the growth of some potential pathogen in meat, notably *Clostridia* (Christiansen, Johnston, Kautter, Howard, & Aunan, 1973). The mechanism of colour change in cured fermented sausages resulted from nitrite will be described in details in Chapter 4 to explain the changes on colour parameters during the fermentation of sheepmeat sausages made with kumara.

The utilisation of phosphates is widely used in the cured meat industry. According to McCormick (1983), there are three basic functions of phosphates. First, phosphates provide some degree of buffering capacity, which stabilise the final pH. Second,

phosphates sequester and bind metal ions and thus protect meat from oxidative rancidity and microbes that need those metals. Third, phosphates serve as polyanions to enhance the ionic strength. Apart from these functions, phosphates increase the water-binding capacity of the protein, which leads to a stabilisation of the myofibrils.

Starter cultures

Table 5. Microbes as starter cultures for fermented sausages

Type of microbe	Microbial species	Useful metabolic activity	Benefits
Lactic acid bacteria	*Lactobacillus lantarum* *Lactobacillus sake* *Lactobacillus pentosus* *Lactobacillus curvatus* *Pediococcus acidilactici*	Formation of lactic acid	Inhibition of pathogenic and spoilage bacteria Acceleration of colour formation and drying
Catalase-positive cocci	*Staphylococcus carnosus* *Staphylococcus xylosus* *Micrococcus varians*	Nitrate reduction and oxygen consumption Peroxide destruction Lipolysis Nitrate reduction	Colour formation and stabilization Delay of rancidity Aroma formation Removal of excess nitrate
Yeasts	*Debaryomyces hansenii*	Oxygen consumption Lipolysis	Delay of rancidity Aroma formation
Moulds	*Penicillium nalgiovense*	Oxygen consumption Peroxide destruction Lactate oxidation Proteolysis Lipolysis	Colour stability Delay of rancidity Aroma formation Aroma formation Aroma formation

Source: Lucke and others (1990)

The significant evolution of the fermented meat was led by Louis Pasteur's 19th century discovery that microorganisms were fundamentally important to fermentation

processes (Sanchez, 2008). This outstanding finding demonstrated that chemical reactions in the fermentation process were triggered through the activity of enzymes produced by microorganisms. The addition of starter cultures into meats has four advantages: (1) improving safety (inactivation of pathogens), (2) enhancing stability to extend storage life by inhibiting undesirable changes brought about by spoilage microbes and/or abiotic reactions, (3) providing diversity (modification of the raw material to obtain new sensory properties), and (4) providing health benefits (through positive effects on the intestinal flora) (Lücke, 2000). Table 5 shows different microbes as starter cultures for fermented sausages and their benefits. Thus, for these reasons the use of a culture would be essential for a consistent commercial product.

In 1940s, study on the use of starter cultures began (Cocconcelli & Fontana, 2010). The first generation of meat starter cultures was composed of *Lactobacillus plantarum* and members of the genus *Pediococcus*. The second generation comprised *L. sakei* and coagulase-negative staphylococci. Nowadays, the second generation is widely employed in industrial fermented sausage manufacturing.

1.5. Potential carbohydrate sources

As mentioned in the section 1.4.3.4, carbohydrate plays a key role in fermentation. Khem (2009) used ground steamed rice as a carbohydrate source for three fermented fish sausages made from hoki (*Macruronus novaezealandiae*), kahawai (*Arripis trutta*), and trevally (*Pseudocaranx dentex*), and relied on wild bacteria on fish, hands and equipment etc. Fermentation did not occur and the sausage mixtures rapidly putrified. The cause of this failure was not identified. One proposal was that the endogenous bacteria associated with New Zealand's fish value chain to retail were incapable of hydrolysing gelatinised rice starch, unlike the endogenous bacteria associated with the domestic production of fermented fish sausage in Khem's native Cambodia. The problem was overcome by using glucose as the carbohydrate source. Equally the problem might have been overcome by using a starter culture.

Carbohydrate-rich root crops like potato, cassava, sweet potato (kumara), yam and

taro, might also be a source of fermentable carbohydrate, but like rice, only after heating to gelatinise the starches. For example, the very name 'sweet potato' implies generation of fermentable sugars after heating. Carbohydrates in raw kumara root make up 80 to 90% of the dry matter. Heating of the tuber, which is necessarily wet heating due to the tuber's moisture content, will accelerate hydrolysis of kumara starch. Of all fermentable sugars, the concentration of maltose increases most significantly as shown in Table 6. The concentration of maltose increased to between 4.02 and 14.12% of the fresh weight from a very low of initial concentration. In another study, Shen & Sterling (1981) reported that in Garnet, another sweet potato variety, reducing sugars increased from 0.4 to 7.3% during baking.

Table 6. Composition of sugars in raw and cooked sweet potato tubers

Cultivar	Sugar concentration (%, w/w)							
	Glucose		Fructose		Sucrose		Maltose	
	Raw	Cooked	Raw	Baked	Raw	Baked	Raw	Baked
Centennial	0.24	0.27	0.30	0.43	4.10	5.17	0	9.33
Jasper	0.44	0.42	0.43	0.41	3.63	5.14	0	7.75
Travis	1.50	2.73	1.15	1.99	2.87	3.26	0	4.02
Jewel	1.22	1.29	1.01	1.20	2.78	3.98	0	7.55
White Star	0.40	0.39	0.39	0.40	2.25	3.35	0	14.12
Rojo Blanco	0.95	1.22	0.65	0.97	1.30	1.59	0	10.77
Tongan	0.45	0.37	0.33	0.26	2.03	2.43	0.64	7.09

Source: Picha (1985).

Moreover, cooking will result in a progressive inactivation of amylase inhibitors in kumara tubers (Rekha & Padmaja, 2002). As a result, α-amylase secreted by fermentative or other bacteria acting on the starch would not be inhibited (Kreuzer & Massey, 2008), so generating fermentable sugars.

Ruiz (2007) observed that 0.5 to 0.7% of carbohydrate content in a fermentable sausage mixture can yield pH values lower than 5.0, while 0.3% cannot support

fermentation successfully. If sweet potato comprised 15% of a sausage mixture, the final concentration of reducing sugars derived from whole sweet potato baked would be about 1.1%. This would theoretically support fermentation. The microwave-cooked kumara could clearly be a potential substitute for glucose as a carbohydrate source in fermented sausages.

1.6. Objectives of the study

This research aims to develop a fermented, cured sheepmeat sausage that can be fermented from added starch that has been partially hydrolysed. The initial carbohydrate sources were potato, yam and kumara. The latter used with a view to identify the product in New Zealand.

The research is divided into two phases. First, experiments were conducted to determine the effect of microwave cooking on the concentration of fermentable sugar formation. Having defined a suitable hydrolysed source, the sausages were prepared. The effects of tuber variety, cooking time and concentration of carbohydrates were compared. The physical and chemical properties of the sausages were monitored. These properties include pH, colour and texture profile. On the basis of results, marketing of the potential novel sheepmeat sausage made with carbohydrate source is discussed. The intended outcome was a high value sausage formulation with some geographical exclusivity, which could serve as commercial primer.

Chapter 2

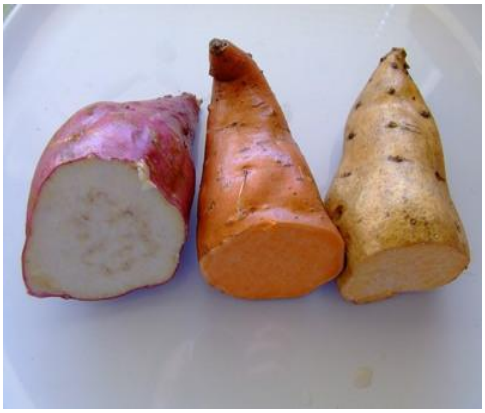
Materials and Methods

2.1. Ingredients and chemicals

Boneless lamb forequarter meat from pasture-raised lambs was purchased from retail butchery. The butchers separated the fat from the lean, although this separation was imperfect, and subsequently minced each fraction through a 4-mm mincing plate. The carbohydrate sources were kumara, potato and yam (Figure 4), which were variously bought from a supermarket and vegetable shops in Auckland as required. Three kumara varieties were used: red (red skin, white flesh; orange (orange skin, orange flesh); gold (gold skin, light gold flesh). The single potato variety was white (pale fawn skin, white flesh) and yams were unspecified varieties.

(a)

(b)



(c)

Figure 4. Carbohydrate sources: (a) White potato; (b). Yam; (c). Red kumara (left), orange kumara (middle), gold kumara (right)

Salt (NaCl) was purchased from supermarket. Glucose, sodium pyrophosphate, and sodium nitrite were analytical grades from the AUT University store. The culture used was fast fermenting culture BFL-F02 (FactoFlavor, No. 2908742, Germany). Flavourings used for the sensory trial were standardised concentrates of rosemary and garlic essential oils (FN11146 and FN11516, respectively, Lionel Hitchen Ltd., UK) which were donated by Hawkins Watts Ltd., Auckland.

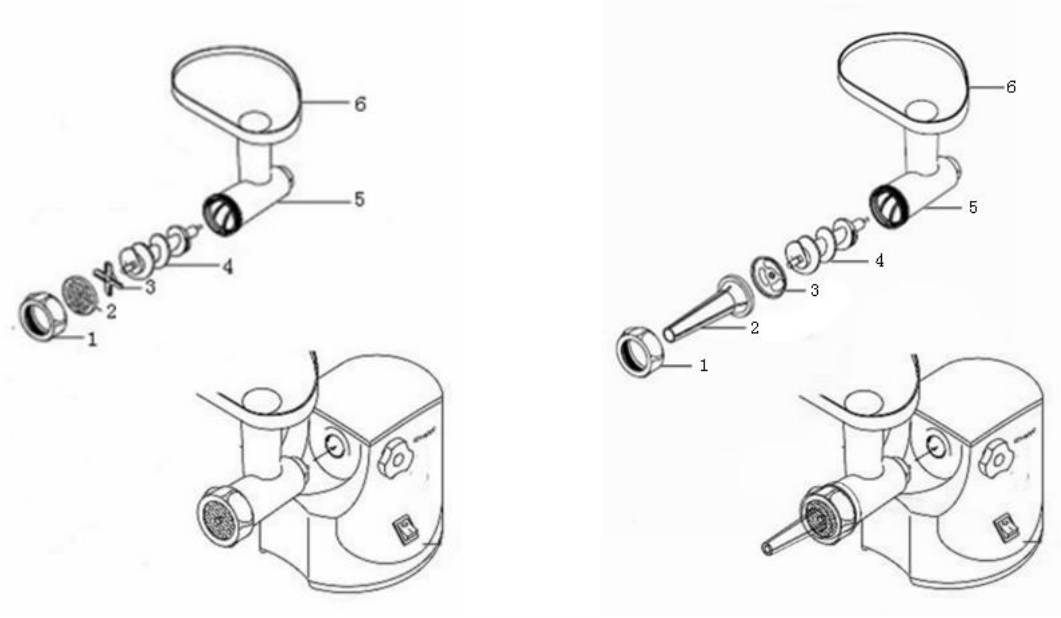
2.2. Treatment of carbohydrate sources to generate fermentable carbohydrate

To support fermentation, the starch in these tubers must be hydrolysed to some extent to generate fermentable sugars such as glucose, fructose and maltose. Thus, prior to making the fermented sheepmeat sausages, a range of different cooking times was applied to kumara, potato, and yam. Hydrolysis of starch was monitored by a diabetic's meter in that glucose generation was considered a proxy indicator of oligosaccharides that might also be fermentable.

The tubers were peeled and cut into pieces measuring approximately 2 x 2 x 3 cm with a mass of 12.5 ± 0.1 g. Three replicates were prepared for each treatment. The pieces were each placed in an individual 250-mL flask and 30 mL of deionised water was added. The replicate flasks were covered with a small watch glass to minimise evaporation during heating, and placed radially on a microwave oven carousel (Samsung Timesaver 800W, Korea). The cooking times (0, 2, 4, 6, 8, 10 min) were applied on a high power setting (800W). After heating, the flasks were cooled to room temperature by immersion in cold water. Each piece and its cooking fluid were blended in situ with an Ultra-Turrax dispersing element (IKA® T25 Basic) for 30 seconds at 24,000 rpm. After quantitatively transferring the sample into a 100-mL volumetric flask, the slurries was diluted to volume with deionised water and mixed well. These slurries were filtered through Whatman 540 paper to yield a clear supernatant for glucose determination. This was achieved with an ACCU-CHEK Advantage Diabetes Meter and test strips (ACCU-CHEK Advantage II, Germany). The extracts were diluted as required if the meter returned a 'HI' value meaning that it was the high side of the dynamic range limit.

2.3. Sausage preparation equipment and casing

The mincing equipment was a domestic Kenwood food processor (Model, Kenwood, U.K.). All cutting and extruding parts were clean, dry and chilled prior to any working. The Kenwood meat mincing attachment comprises a mincer body, scroll, cutter, mincer screen, and a ring nut to correctly adjust the contact between cutter and tray (Figure 5a). While, the extrusion arrangement consisted of a ring nut, mincer body, scroll, nozzle, base plate and tray (Figure 5b). The mixture is extruded into the casing barrels by the nozzle in the front of the cutter and plate.

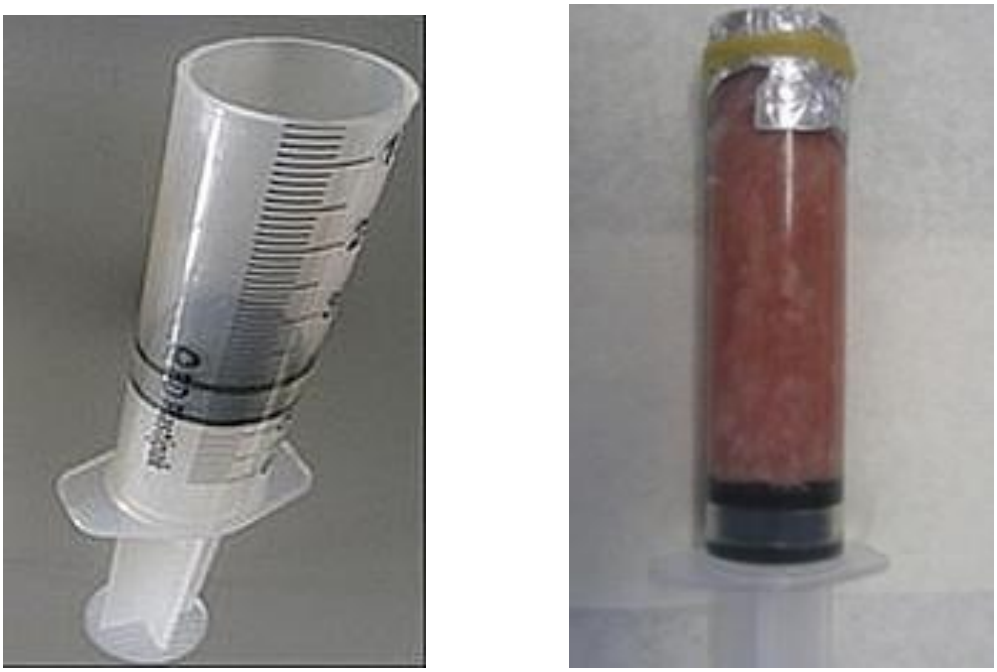


(a) (b)

Figure 5. Kenwood mincing equipment
(a). Mincing arrangement: 1.ring nut, 2.mincer screen, 3.cutter, 4.scroll, 5.mincer body, 6.tray
(b). Extrusion arrangement: 1.ring nut, 2.nozzle, 3.basic plate, 4.scroll, 5.mincer body, 6.tray

Cellulose or traditional collagen casings are commonly used in commercial production of fermented sausages, but were unsuitable for the small scale of production in the present study. Moreover, these casings do not create sausages of exactly even diameter, and allow a limited ingress of air onto the sausage surface, creating heterogeneity

in the sausage that would complicate textural analysis of sausage slices. To avoid all these problems with fermented fish sausage, Khem (2009) developed a novel syringe casing (Figure 6a) that was subsequently used by Lu (2010) with pork, beef and sheepmeat. This novel barrel is a simple but effective casing device for research on this small scale.



(a) (b)

Figure 6. (a). The novel syringe casing developed by Khem (2009); (b). The novel syringe casing of experimental fermented sheepmeat sausages

The novel casing was modified from the normal plastic syringes (BD Plastipak, BD, Ireland) whose capacity and internal diameter were 50 mL and 25 mm, respectively. The front end of the syringe is cut off on a lathe. A thin layer of petroleum jelly is laid on the interior of the modified barrel so as to easily extrude fermented sausage when and as required. After extruding ingredients of mixture, the barrels were isolated from air with a layer of Parafilm© covered with two layers of aluminium foil, with the entire assembly held by a rubber band.

2.4. The basic sausage formulation

Table 7. The basic ingredient concentration of fermented lamb sausages shows the basic sausage formulation for the most common content of carbohydrate source, shown here for kumara (Table 7). In this common formulation, the carbohydrate source content was 15%, but this varied from experiment to experiment, as will be specified in Chapter 3. Also glucose was included in some experiments and spices were added in the sensory trial work.

Table 7. The basic ingredient concentration of fermented lamb sausages

Ingredient	Quantity (g 100 g^{-1})
Lamb meat lean	54
Lamb meat fat	27
Carbohydrate source	15
Salt	4
Sodium pyrophosphate	0.2
Sodium nitrite	0.01
Culture	0.01

However, the content of salt, sodium pyrophosphate, sodium nitrite, and culture bacteria in lamb meat sausage was unvarying at 4, 0.2, 0.01, and 0.01%, respectively. The content of lean and fat was reciprocally changed with the content of the carbohydrate source. However, the ratio of lean to fat was consistently 2 to 1.

2.5. Basic method of fermented sheepmeat sausage manufacture

As is described in Chapter 3, heated kumara generated the highest concentration glucose in the time range chosen, suggesting that it would support fermentation the best. For this and other reasons, kumara was the tuber of choice for further work. The model system described in the section 2.2 was useful to determine glucose concentration but was unsuited to produce useful quantities of heated kumara. The routine method of generating

fermentable kumara was as follows. As is reported in Chapter 3, three unpeeled kumara were heated in the microwave oven for 8 min. After cooling, they were coarsely peeled the desired mass and accurately weighted. They were then added to the other ingredients in a mixing bowl. After the ingredients were dispersed hygienically by gloved hands, the mixture was passed through the Kenwood food processer to be minced one more time so as to further disperse the ingredients. The minced filling was stuffed into the novel syringe barrels by the extrusion arrangement (Figure 6Figure 5b), which were sealed as described above (Figure 6b). The sausages were then placed in an incubator held at 30 ± 1°C for 96 h.

2.6. Chemical analysis

2.6.1. pH

The pH values of samples were taken by dipping a combination electrode of portable pH meter (Meterlab, U.K.) into slurry prepared from a 5 g of sausage in 50 mL of deionised water. Dispersal to a slurry was achieved by disruption with a wooden stick and shaking over 2 min at room temperature.

2.7. Physical analysis

2.7.1. Colour analysis

For colour measurements, three colour coordinates were obtained: lightness (L*), redness/greenness (a*), and yellowness/blueness (b*) by using a reflectance spectrophotometer, a Model 45/0 Hunterlab ColourFlex (Hunterlab, Reston, Virginia). Measurements were done in a dish measuring (Schott, Duran, Germany). First, replicate measurements were made for the empty dish. To do this, the dish was placed approximately in the centre of measuring area of the spectrophotometer and covered with black shroud. The dish was then moved slightly and another replicate measurement made to a total of five replicates. Mean L*, a* and b* were calculated. These were the blank values. Sausage samples from three replicate fermenting syringes were then cut into discs 10 mm high x 25 mm diameter. The discs were centrally placed in the dish and triplicate measurements made. These data were averaged to obtain the mean for a given day from one

fermentation replicate which was then corrected for the colour of the empty dish by subtraction. In this way, three replicate values were derived that were the raw data for statistical analysis. Hue angle and chroma (colour intensity) were also calculated from these raw data.

2.7.2. Texture profile analysis

Textural properties were determined according to the Textural Profile Analysis (TPA) test summarised by Bourne (1978) by permission of A.S. Szczesniak. A TA-XT Plus (Stable Microsystems, U.K.) was used for this work. The sausage, extruded as required from a syringe, was accurately sliced into 30-mm-high cylinders whose 25-mm diameter was determined by the internal diameter of the syringe. The cylinders were individually placed, flat surface down, on a flat sheet of glass on top of the texture analyser's platen. These cylinders were compressed with a 50-mm diameter plunger whose flat surface was made of anodised aluminium. They were compressed twice at room temperature to half of their original height. In all tests, the pre-test speed was 1 mm sec^{-1}, the plunger test speed (first compression, first withdrawal, second compression) was 5 mm sec^{-1}, plunger post-test speed (second withdrawal) was 10 mm sec^{-1}, strain was 50%, and surface sensing force was 0.049 N. The program for the TPA test was the Texture Exponent 32 software (Stable Micro System, U.K.) that also processed the raw data generate derived data that were downloaded to an Excel file.

In Figure 7, the hardness value (N) is the maximum force of the first compression cycle. The cohesiveness ability of sample relates to how well a sample withstands the second deformation compared with its first deformation. It is the dimensionless ratio of the positive force areas under the second to first compression portions excluding areas during withdrawal (Area 2/Area 1). Thus, Areas 1 and 2 represent the energy required to compress the cylinder on the first and second compression cycles, respectively. The first negative force area (Area 3) on the first deformation is defined as adhesiveness (mN s), which is a measure of the stickiness of the cylinder to the glass plate and the anodised surface of the plunger.

Springiness (elasticity) is a measure of the extent to which a cylinder in this case reverts to its original height when the compressive force is removed. The most typical measurement is determined by the distance (Length 2) that a sample recovers to its original height (Bourne, 2002), but this applies only where the original sample dimensions are identical. A safer measure, however, is the dimensionless ratio Length 2 to Length 1, to compensate for any variation in cylinder height. Because the sausages involved in this experiment are solid at 96 hours, chewiness at 96 hours is calculated as the force (N) required masticating a product to a steady state before swallowing:
springiness × hardness × cohesiveness.

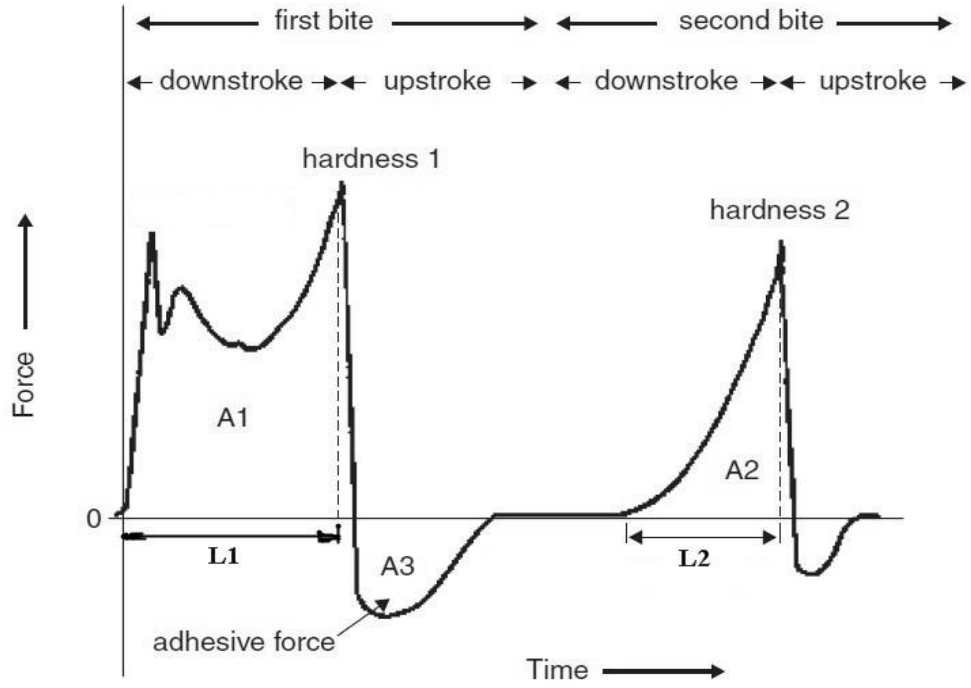


Figure 7. A typical force-time curve of a double compression test (Pons & Fiszman, 1996). The Y-axis is expressed in g force but all data are ultimately expressed in SI units

2.8. Statistical analysis

Data were statistically analysed for means, variance, correlation and principal components using Excelstat, a program embedded in Microsoft Excel and Minitab 15

(Minitab Inc., State College, Pennsylvania).

Chapter 3

Results and Discussion

3.1. Detection of glucose content in carbohydrate sources during microwave cooking

As discussed in Chapter 2, starch or similar carbohydrate in uncooked tubers is unlikely to be fermented by bacilli, although it is obvious that moulds can metabolise ungelatinised starch in uncooked tubers. As for free glucose, the content of glucose in raw tubers is likely to be too low to support fermentation. For example, glucose accounts for 0.5 to 1.5% in potato tubers (Lisińska & Leszczyński, 1989). If potato tubers were only 15% of a sausage mixture, the final amount would be a low 0.075 to 0.225% based on that value. To support fermentation, the amount of sugar added to dry or semi-dry sausages are typically in the range of 0.5 to 2.0% (Spedding, 2010). According to Marianski & Marianski (2009), the amount of fermentable sugars such as glucose should be a minimum of 0.75%. If a very low amount of fermentable sugar was added, undesirable microorganisms would grow to spoil meats.

Heating gelatinises starch in tubers, and also generates metabolisable sugars and denatures certain antinutritional factors such as trypsin inhibitor acitivity in sweet potato (Palaniswami & Anil, 2008). Hence, prior to fermentation, it is important to at least gelatinise starch in tubers by heating. At the same time, the smaller saccharide moieties will be generated, the smallest being glucose and the next smallest being maltose. However, only glucose was measurable by the assay method chosen because the diabetic meter is specific for only glucose.

3.2. Calibration of a blood glucose meter for glucose in tuber extracts

Known volumes (50mL) of glucose solution were mixed with the unknown concentrations of glucose in tuber extracts and the combined concentration was determined with the by diabetic meter. After calculation, the correction factor was found by dividing the meter reading by the true glucose concentration. Figure 8 shows the correction factor at each of the times. Because the values were close to unity at all times the raw data were

accepted as accurate.

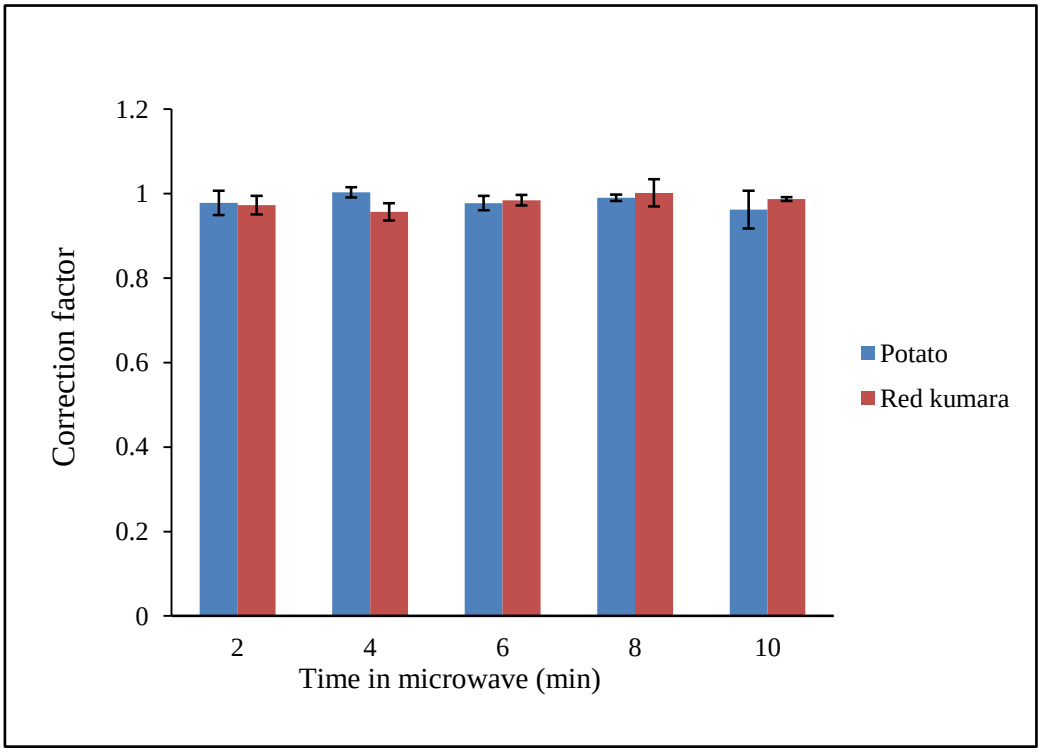


Figure 8. The correction factors in red kumara and potato tubers for each microwave cooking treatments. Values are means of triplicate mixed solution of known glucose solution and tuber extracts and error bars are standard deviations

3.3. Changes of glucose formation in cooked tubers

Figure 9Figure 9 shows the changes of glucose generation in potato and red kumara pieces over 10 min of microwave cooking at 800 W. Without microwave heating (0 min), the glucose in kumara and potato was – when extracted and made to the standard volume – at or below the lowest possible concentration ('LO') that the meter could return a value for. This translated to 2.2 mg g^{-1}, and was less than the value 5.16 mg g^{-1} reported above. The graph shows 2.2 mg g^{-1} for both red kumara and potato. The concentration at 2 min was much higher, especially for kumara. The concentration in kumara was in fact so high that kumara extracts that to be diluted to a final volume of 250 mL to avoid the meter returning a

'HI' value outside the dynamic range of the meter. Potato extracts were suitably concentrated in a final volume of 100 mL. After 2 min, the yield of glucose in red kumara reached a peak (39.4 mg g^{-1}) at 4 min and then gradually dropped. The concentration of glucose in potato appeared to plateau at 10 mg g^{-1} at about 2 min.

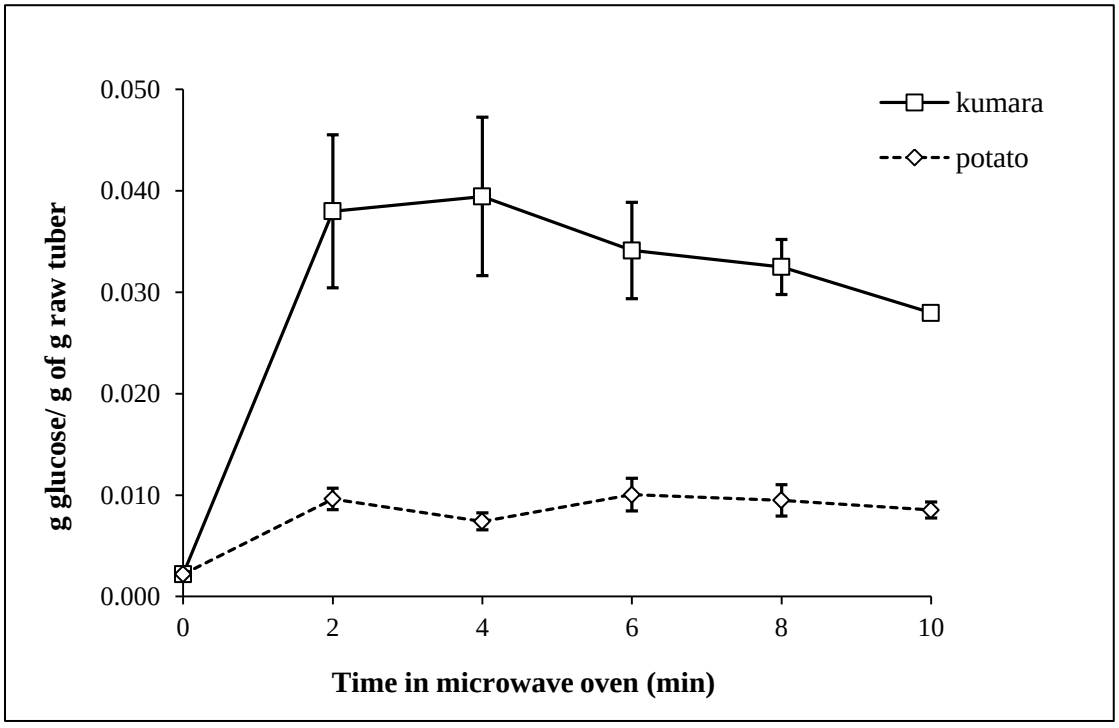


Figure 9. Glucose concentration in red kumara and potato pieces during microwave cooking. Values are means of triplicate tuber pieces and error bars are standard deviations

It was expected that the glucose concentration would continue to rise as the heat from microwave cooking should continue to slowly hydrolyse the starch, and should certainly no decline in concentration. The reason for this is probably as follows. It was observed that the surface colour and texture of tuber pieces were affected by microwave cooking, becoming darker and tougher with time. Tuber pieces were soft and easily blended by the Ultra-Turrax dispersing element at 4 and 6 min, but were too tough to be easily dispersed at 8 and 10 min. Thus the concentrations at 8 and 10 min may be an underestimate. Although water was included in each cooking flask, there was evidently not enough available moisture

in the atmosphere surrounding each tuber piece to maintain high moisture content in the tuber pieces. In short they progressively dried. This was reflected in the colour of the tuber pieces, which became yellower with time. The colour change strongly suggests that glucose was being lost to the Maillard reaction, which is well known to be favoured by low water activities. Glucose's carbonyl group can react with amino groups in proteins particularly at high temperatures. Also, caramelisation reactions, which are effectively dehydration reactions of – in this case – glucose, may also cause loss of glucose. Caramelisation reactions also lead to generation of yellow/brown colours.

The concentration in yam for all cooking time treatments was so low that yam extracts was diluted to a final volume of only 50 mL, the lowest manageable volume, in attempt to obtain a value. The meter still returned a 'LO' value. Yam was therefore eliminated as a potential carbohydrate sources for making fermented sheepmeat sausage.

Salt-assisted and acid-assisted methods have been used in conjunction with microwave heating. Yu and others (1996) reported that 4.0 mL of 10% starch suspended in dilute hydrochloric acid (0.5 M) was completely fractured into smaller units such as dextrans and glucose within 5 min under microwave irradiation of 2.45-GHz (20 to 40% full power). Li and others (2001) subsequently found that inorganic salts accelerated acid hydrolysis of starch under microwave conditions. The 3-mL starch suspension comprised 200 mg starch, 2 mL of 0.05% HCl and 1 mL of salts solution. For most inorganic salts, 3 min was the optimum microwave time to break down starch into glucose and other reducing sugars. However, the amount of glucose was reduced after 4 min of microwave and no glucose was detected at 10 min. This phenomenon was similar with what happened when potato and kumara generated glucose under microwave irradiation in the current experiments. The loss of glucose may be attributed to the instability of glucose at the higher temperature (Li et al., 2001). The glucose was oxidised rapidly. The loss of glucose may be attributed to the caramelisation and not the Maillard reaction because amine concentrations are negligible in starch.

Based on glucose generation, as determined by the diabetes meter, red kumara was the optimum carbohydrate for further work, yielding a soft glucose-rich product when heated

with microwave at 4 min (Figure 9).

3.4. Preparation of kumara for sausage production

The changes method was well suited to monitor changes in glucose concentration as a representative fermentable sugar. It was however poorly suited to generate quantities needed for sausage products. It remained to determine a suitable cooking times for large pieces of kumara, and this was based on the texture and flesh colour shown to be best is the changes experiments, a soft texture and yellow colour. Therefore, three similar kumara about (200 g each) was placed on the microwave oven to be heated for 4, 6, 8, 10, 12 min each time. After heating, kumara was cut off. The flesh was compared in terms of texture and colour. Consequently, flesh of unpeeled kumara under 8-min microwave heating was the similarity with red kumara tubes after 4-min microwave heating.

If kumara comprises 15% of a sausage mixture, the final concentration of glucose derived from whole kumara cooked for 8 minutes would be about 0.6% based on Figure 9. At the same time there would an unknown concentration of other fermentable sugars like maltose, which could not be measured with the diabetes meter. Therefore 0.6% would likely be a lower limit for fermentable sugar. However, it remained to be demonstrated that these concentrations were sufficient to support fermentation (assuming for the moment that the culture was incapable of hydrolysing gelatinised starch).

To show this or otherwise, three syringes of fermented sausages were made according to the basic sausage formulation. After four-day incubation, pH of all samples was reduced to a low value of 4. According to Leistner and Roedel (1975), the safety pH of meat products was low than 5.0. That confirmed 15% of kumara could supply enough fermentable sugars (glucose and maltose, plus any resulting from starch hydrolysis by the culture) to support fermentation, and the low pH suggested that lower concentrations of kumara would also be successful. However, this possibility was not tested at that time. It was of more immediate interest to show which kumara variety might be most useful for product development, red, orange and gold kumara (Figure 4). The focus was colour.

3.5. Physical and chemical properties of fermented sheepmeat sausage due to kumara variety

For the comparison of kumara variety, triplicates of whole kumara around 200 g were microwave cooked for 8 min as described in the previous section and the kumara concentration was 15%.

3.5.1. Determination of pH

As discussed in Chapter 1, carbohydrates are the fermentation substrate for LABs. The LAB count typically increases from 10^3 to 10^5 cfu g$^-$1 to a final 10^7 to 10^9 cfu g^{-1} during fermentation where starter cultures are not added (Toldrá, 2002).

In Khem's study (2009) with the application of LAB culture in fermented fish sausages; there were increases in LAB count from 10^2 to 10^3 cfu g^{-1} to a 10^7 to 10^9 cfu g^{-1} in all three fish species (trevally, kahawai and hoki) during fermentation. In Lu's study (2010), with sheepmeat fermented sausage inoculated with a starter culture, the final LAB count reached 10^9 cfu g^{-1} from initial count of 10^7 to 10^8 cfu g^{-1}.

As LABs proliferate, reducing sugars are fermented to organic acids like lactic acid which results in a marked pH fall but which is buffered by the meat proteins. It is not essential to monitor the growth of LABs, but judged by pH fall there was clearly enough fermentable sugar available for the bacteria to grow, whether available from cooking and/or derived by hydrolysis of gelatinised starch by the bacteria. Hence, to confirm that fermentation was successful, pH was monitored with fermentation time.

As shown in the Figure 10, pH of the three kumara variety treatments decreased to 4 during the first two days of fermentation. This indicated that after 8 min cooking, 15% of each of the three kumara varieties supported the fermentation successfully. Additionally, a rapid decrease in pH to below 5.3 inhibits the growth of pathogenic bacteria such as *Salmonella* and *Staphylococcus* (Lücke, 2000), such that fermented sausage is inherently safe.

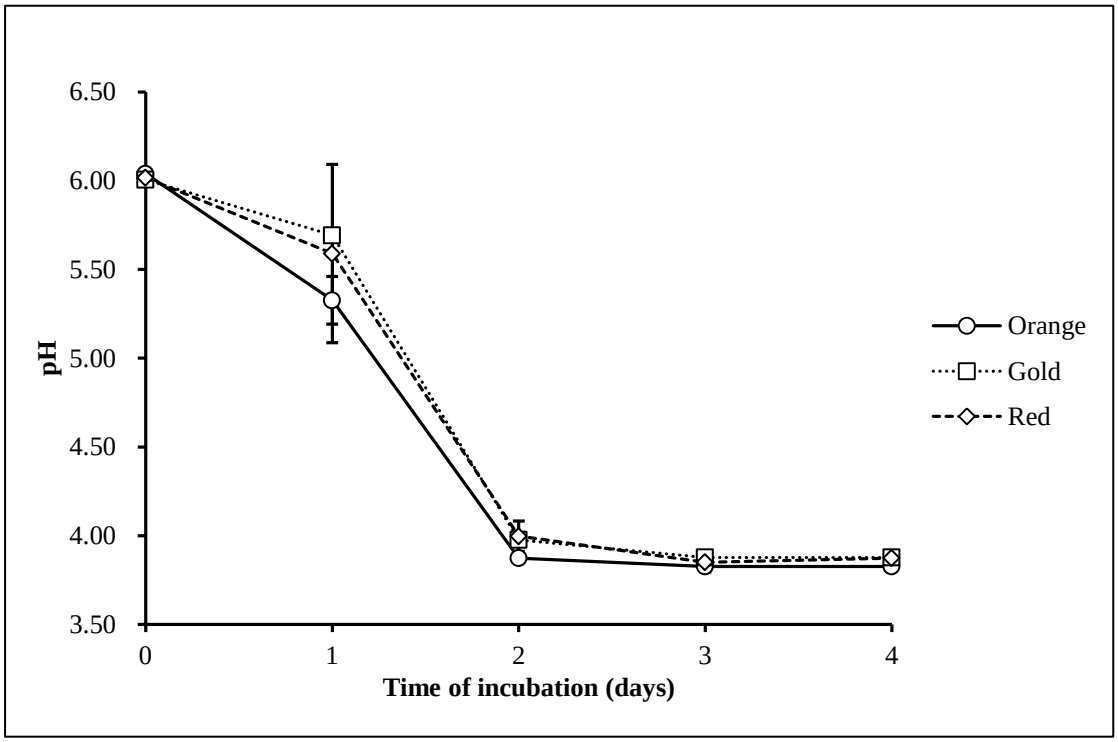


Figure 10. The pH development of fermented sheepmeat sausage from three varieties of kumara during fermentation. Values are means of triplicate tuber pieces and error bars are standard deviations

3.5.2. Colour

The colour of fermented sausage is affected by several factors such as concentration of curing agents, species(s) of fermentation bacteria, ratio of lean to fat meat, and fermentation period (Marianski & Marianski, 2009). Colour of adjunct ingredients might also be important, and this factor was explored here.

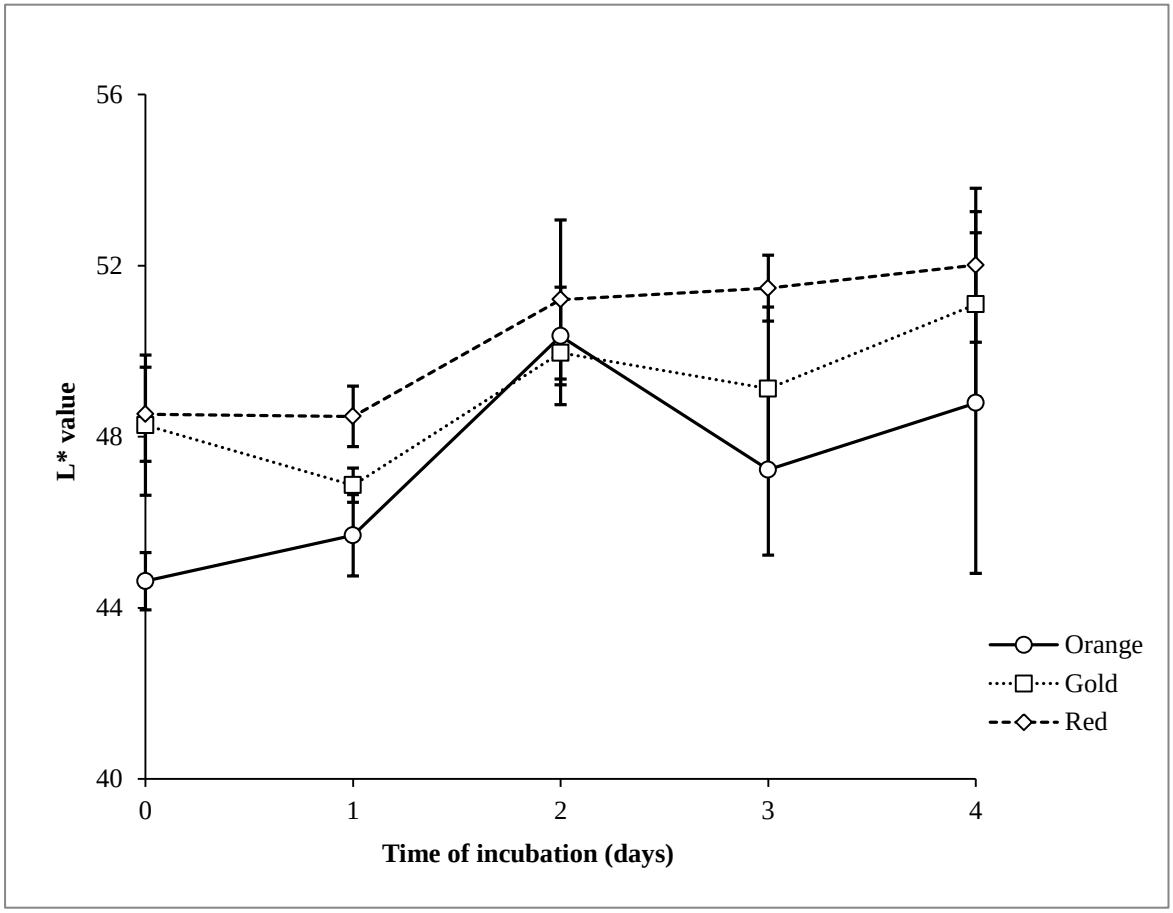


Figure 11. Lightness development (L*) of the fermented sheepmeat sausages during fermentation. Values are means of triplicate fermentations and error bars are standard deviations

Figure 11 shows the changes of L* values of fermented sausage during fermentation. Generally, all three kumara varieties showed a small numerical increase in L* value (gold and orange kumara: $p < 0.001$, red kumara: $p > 0.05$) during four day-fermentation. The L* values of the white-fleshed red kumara steadily increased, while the other varieties showed more fluctuation. At Day 4 – the only important day for a marketable product – red kumara had the highest numerical lightness (L*), followed by gold kumara and orange kumara the lowest value. However, the differences were not statistically different ($p > 0.05$) among the varieties, but could still be commercially important. Compared with the orange and gold kumara, red kumara flesh was lighter after microwave heating, which probably explains the highest numerical L* value among the three kumara sausage treatments.

The changes of redness (a*) of fermented sheepmeat sausages are shown in Figure 12. At all times, orange kumara had the highest numerical a* value, followed by gold kumara and red kumara lowest. Red kumara refers to the colour of the kumara. Importantly red kumara are distinctly white-fleshed and this will account for the lowest a* at Day 0. The a* values of all kumara rose sharply within the first day of fermentation ($p < 0.001$), then were subsequently rather static. At the Day 4 there were no significant differences among the three treatments ($p > 0.05$).

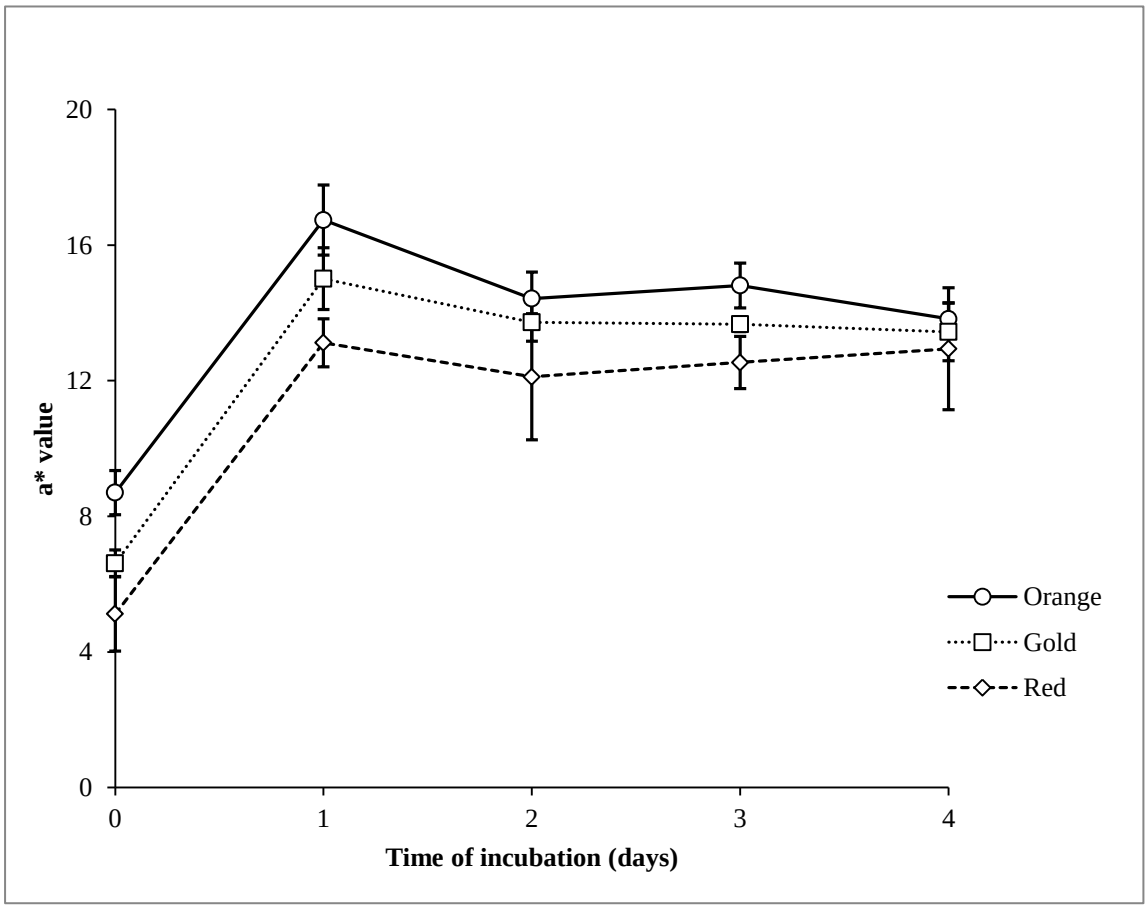


Figure 12. Redness development (a*) of the fermented sheepmeat sausages during fermentation. Values are means of triplicate fermentations and error bars are standard deviations

The a* value is mainly related to different chemical states of myoglobin. There are four major forms of interest here: myoglobin (purple red), oxymyoglobin (bright red), metmyoglobin (brown) and nitrosyl myoglobin (NOMb) (red). At the beginning of

fermentation, the myoglobin is initially oxygenated and thus is red, arising from the inclusion of air during sausage preparation in mincers and mixers. The oxymyoglobin subsequently reacts with added nitrite to create the brown metmyoglobin and nitrate (Wood, 1998). At low pH the metmyoglobin then reacts with nitrous acid and/or nitric oxide and endogenous and/or exogenous reductants (e.g. ascorbate) to yield red NOMb, the NO group occupying the sixth ligand position of haem (Wood, 1998). The increase in a* value with time was almost certainly caused by the accumulation of bright red NOMb with pH fall. In a study on Spanish-type dry-cured sausage, Pérez-Alvarez and others (1999) found an increase in redness during fermentation and a slight drop on redness during ripening.

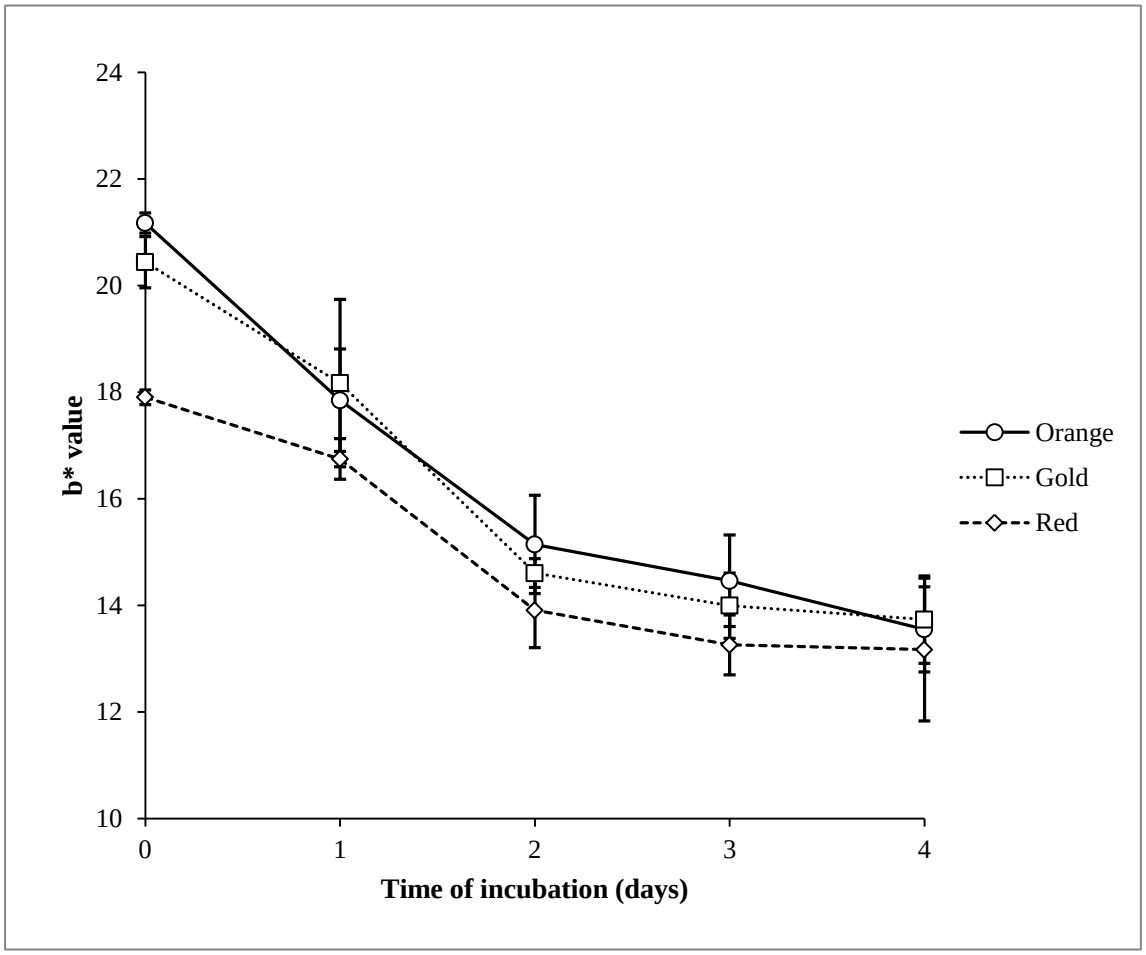


Figure 13. Yellowness development (b*) of the fermented sheepmeat sausages during fermentation. Values are means of triplicate fermentations and error bars are standard deviations

Figure 13Figure **13** shows the changes of yellowness (b*) of sheepmeat sausage during fermentation. At Day 0, the white-fleshed red kumara was also the least yellow as well as being the least red (Figure 12). There was a sharp drop in b* values for all three kumara varieties fermented sheepmeat sausages during the first two days. At Day 3 and 4, all three varieties showed a subsequent slight decrease in b* values. However, there was no statistically significant difference in b* value among the varieties at Day 4 ($p > 0.05$), contrasting sharply with the result at Day 0. A decrease in b* values indicates a decrease in the intensity of yellowness, which in meat appears as browning due to the metmyoglobin discussed above. Pérez-Alvarez and others (1999) showed a similar fall in yellowness during fermentation in a Spanish-type dry-cured sausage.

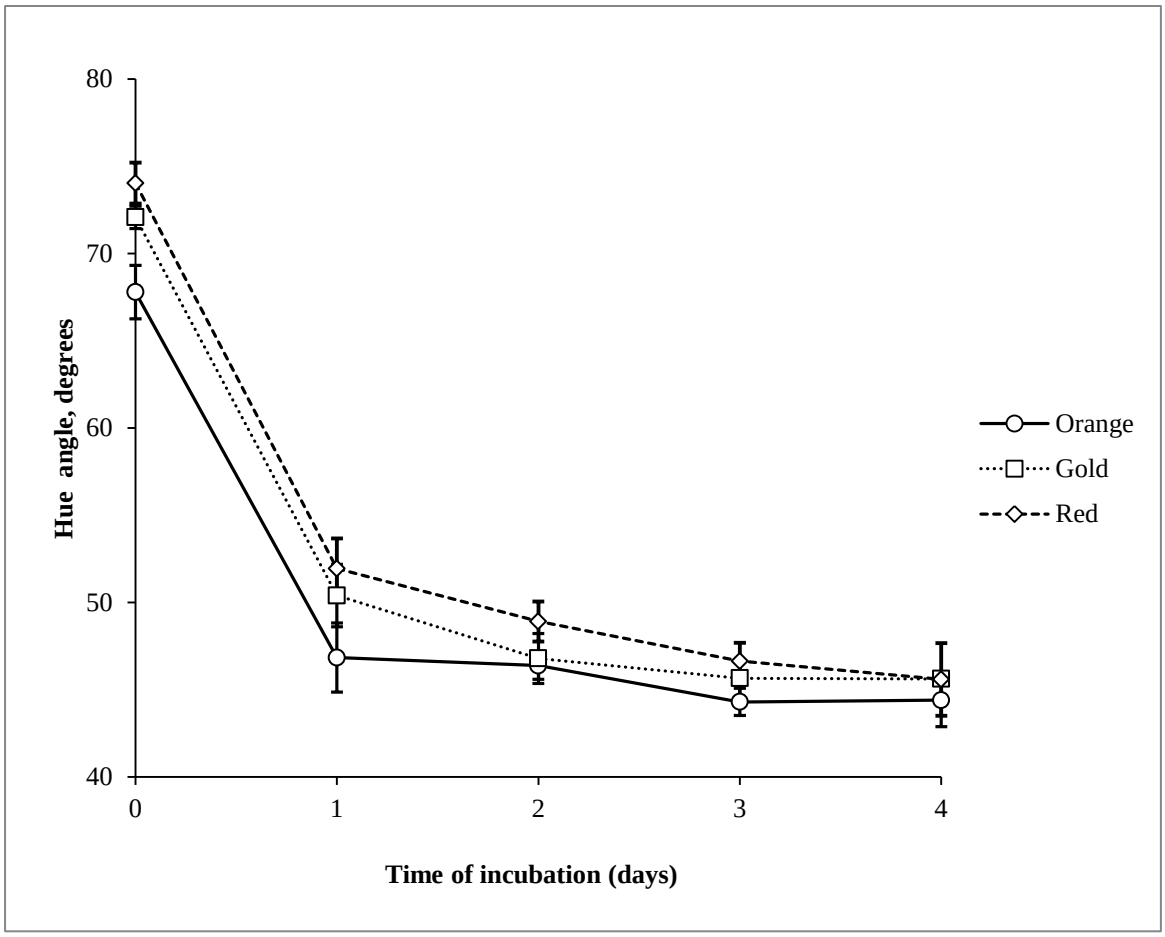


Figure 14. Hue angle development of the fermented sheepmeat sausages during fermentation. Values are means of triplicate fermentations and error bars are standard deviations

The changes of hue angle with fermentation time is shown in Figure 14Figure **14**. At Day 0, the orange-fleshed orange-skinned kumara had the lowest hue angle, indicating that red pigments dominated over yellow pigments. By contrast, what pigments there were in red-skinned, white-fleshed kumara were such that yellow pigments dominated over red pigments. There was a large decrease in hue angle for all kumara varieties ($p < 0.001$). However, at Day 4 the differences were not significant ($p > 0.05$).

In the present laboratory study, the source of kumara was not important factor of colour changes at Day 4. In a commercial application, it is unlikely that Day 4 sausage would be marketed in the form achieved at this time. Thus, a commercial fermented sheepmeat sausage might be prepared in a moisture-permeable casing and the sausage

would likely be stored to achieve a partially dried salami-style with complex flavour development. Colour will change under these circumstances, but it seems very unlikely that kumara type would affect colour on storage, given that at Day 4, all colour parameters were the same (Figure **11**Figure 12 and Figure 13, Figure 13 and Figure 14). Thus, the type of kumara to be used is not important to fermented sheepmeat sausage in terms of colour.

3.5.3. Textural analysis

The textural properties of fermented sheepmeat sausage as affected by fermentation and kumara variety are shown in Table 8. The way these data were extracted from the force-distance outputs of the texture analyser were described in chapter 2.

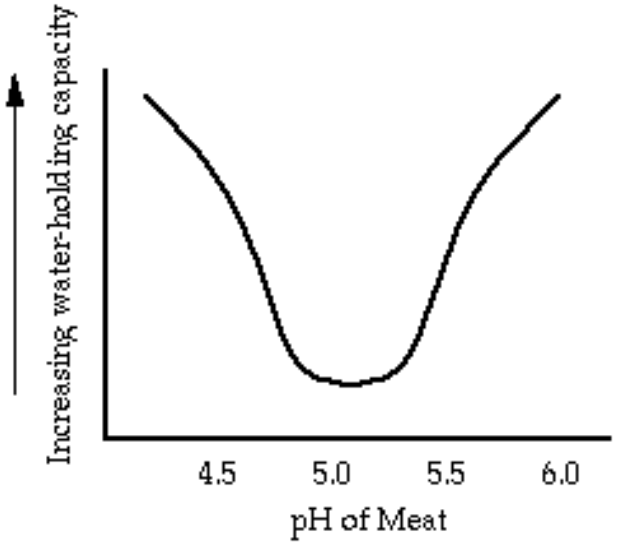


Figure 15. Effect of pH on water holding capacity of meat (Elton, John, David, & Edward, 2001)

Generally, all three kumara varieties showed a significant rise in hardness due to fermentation ($p < 0.01$ at least), where values were typically doubled. The difference between varieties in hardness at Day 4 was not significant ($p > 0.05$). The increase in hardness could be brought about by the lowering of pH during fermentation. The dominant muscle protein myosin is partially solubilised by the added salt and the

water-holding capacity increases as meat pH progressively decreases (Figure 15) (Elton et al., 2001). These two changes result in an acid-induced gelation of denatured proteins and thus a major change in textural properties (Gimeno, Astiasarán, & Bello, 1998).

Adhesiveness is a measure of the degree of stickiness, in this case to the glass platen on the analyser's base or the anodised aluminium crushing probe, whichever was the least sticky. At Day 0, the three varieties had a similar stickiness ($p > 0.05$), that reduced at Day 4 ($p < 0.05$ at least), but again with no significant differences between varieties on that day ($p > 0.05$).

Springiness is the relative height recovery after the first compression, where a springiness of 1.00 represents a perfectly elastic body. Springiness did not change with time except in the case of red kumara, where it increased ($p < 0.01$), but the difference appeared unimportant.

Cohesiveness is used to measure the degree to which particles hold together after chewing (Carpenter, & Cheney, 1998) and is the ratio of the area of the second compression to the first. The higher the value the more cohesive it is. The cohesiveness of the sausages was typically halved on fermentation ($p < 0.01$ at least), but with no significant difference due to varieties on Day 4.

Chewiness is defined as the product of hardness, springiness and cohesiveness (Bourne, 2002), and is commercially relevant only on Day 4. There was a significant difference between the varieties on Day 4, with the orange variety being the chewiest. However, the numerical differences between the varieties were minor.

It is difficult to interpret the above results in respect of commercial fermented sausages because these undergo some degree of drying. The only comparable study was that by Lu (2010) where fermented sheepmeat sausages were produced by the same syringe system as used here, and the textural test conditions were identical. Moreover, the lean to fat ratio was also the same. At Day 4, the hardness, adhesiveness, springiness and cohesiveness were ranged from 15 to 27 N, -0.1 to -0.8 N s, 0.82 to 0.93 and 0.55 to 0.7, respectively. Only hardness was very different from the results obtained here, between

44.3 and 74.7 N. There could be several reasons for this. First, the current formulation contained 15% added cooked kumara, whereas the Lu formulation contained none, relying only on 2% glucose to support fermentation. Second, the pH values at Day 4 were very different, 4.6 for Lu (2010) and 4.0 in the present study. Either of both these factors could be the cause of a difference.

Riebroy and others (2004, 2005) showed that high acceptability of the undried Thai-style fermented fish sausage was positively related to hardness. If that relationship also applies to the current undried product, then on the face of it, a low pH sausage containing 15% kumara would be texturally more attractive than the product made by Lu (2010).

On the basis of results, red kumara was marginally the best carbohydrate source to augment the hardest and chewiest cured, fermented sheepmeat sausage. Moreover, in terms of the market price, red kumara is usually NZ$1 to 2 kg^{-1}, cheaper than the other two varieties in the supermarket. Thus, considered within price, the lowest price would be the first choice among the same quality and characteristics of ingredients for the further industrial manufacture processing. Thus, the red-skinned white-fleshed kumara was chosen for much of the subsequent work.

Table 8. Changes in texture of fermented sheepmeat sausage before and after fermentation as affected by kumara varieties. Data shown are means ± standard deviations

Kumara variety	Fermentation	Hardness (N)	Adhesiveness (N s)	Springiness	Cohesiveness	Chewiness (N)
Orange	Before	12.03 ± 1.12	-1.35 ± 0.15	0.83 ± 0.03	0.78 ± 0.08	
	After	64.94 ± 8.83	-0.13 ± 0.08	0.83 ± 0.01	0.53 ± 0.03	28.40 ± 2.10
Effect of fermentation		***	***	NS	**	
Gold	Before	25.65 ± 2.54	-1.73 ± 0.88	0.71 ± 0.04	1.10 ± 0.16	
	After	57.22 ± 5.75	-0.15 ± 0.16	0.78 ± 0.01	0.56 ± 0.07	24.52 ± 4.66
Effect of fermentation		***	*	NS	**	
Red	Before	23.99 ± 1.11	-1.02 ± 0.04	0.71 ± 0.01	1.12 ± 0.07	
	After	59.87 ± 13.49	-0.08 ± 0.05	0.82 ± 0.03	0.50 ± 0.06	25.09 ± 1.46
Effect of fermentation		**	***	**	***	
Effect of kumara variety after fermentation		NS	NS	**	NS	**

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, not significant

3.6. Physical and chemical properties of cured, fermented sheepmeat sausages made with red kumara due to microwave heating time

In the section 3.4, an 8-min microwave heating was regarded as the optimum cooking time for whole kumara tubers. However, that was based on visible observation and touchable detection not texture and colour parameters detected by physical machines. Therefore, in this part experiment, the microwave heating time was considered as a further factor which could influence on pH, colour and textural parameters of fermented sausages.

For the microwave heating comparison, three time treatments were chosen: 4, 8 and 12 min at 800 W. For each time, three unpeeled red kumara were microwave cooked. Then the flesh of kumara was scooped and transferred to a beaker. The cooked interiors of the kumara were used for sausage preparation, where the concentration of kumara was 15%.

3.6.1. Determination of pH

Table 9. Changes in pH of fermented sheepmeat sausage as affected by microwave heating time of red kumara

Heating time (min)	Fermentation	
	Before (Day 0)	After (Day 4)
4	5.93 ± 0.01	3.95 ± 0.02
8	5.94 ± 0.00	3.94 ± 0.03
12	5.94 ± 0.01	4.00 ± 0.05
Effect of heating time	NS	NS

NS, not significant

The pH values are presented in Table 9, comparing Day 0 and Day 4. With all three treatments, pH values dropped significantly to around 4.0 after the 4-day incubation ($p < 0.001$), confirming there was an adequate accessible carbohydrate source from each

heating time (Table 9). There was no statistical difference in pH values between the three heating time treatments either at Day 0 or Day 4 ($p > 0.05$).

3.6.2. Colour

As shown in Table 10, heating time did not affect colour parameters ($p > 0.05$). Thus, any time between 4 and 12 min could be useful for fermented sausage production.

Table 10. Colour parameters of fermented sheepmeat sausage as affected by microwave heating time of red kumara

Heating time (min)	Colour parameters			
	L*	a*	b*	Hue angle h^o
4	47.55 ± 0.98	14.15 ± 0.15	12.89 ± 0.04	42.34 ± 0.38
8	47.66 ± 1.51	14.74 ± 0.35	13.04 ± 0.13	41.50 ± 0.86
12	48.15 ± 0.69	14.14 ± 0.30	13.04 ± 0.13	42.68 ± 0.47
Effect of Heating time	NS	NS	NS	NS

NS, not significant

3.6.3. Textural analysis

All heating time treatments showed a significant increase in hardness and adhesiveness (all $p < 0.01$), but marginal or no change in springiness and cohesiveness after fermentation (Table 11). After fermentation, the textural attributes were unaffected by the microwave heating time ($p > 0.05$ for all). This suggested that the dominant structural component of the fermented sausage was the myofibrillar gel, and that retrogradation of kumara starch, if any, was a negligible effect.

These textural data for 15% kumara in Table 11 can be directly compared with the textural data for red kumara in Table 8. The hardness values in particular were very different from the values in the kumara variety treatment. This difference will be discussed in chapter 4.

To summarise the results of this section, the colour and textual properties were not affected by the microwave heating time. However, 8 min was chosen as the standard for these reasons: the kumara flesh remained tough to the touch at 4 min and that time might be too short to destroy all the antinutrient factors ; at 12 min the flesh was drier than wanted and was not easy to disperse with the equipment used.

The next major variable to be examined was the effect of kumara concentration on sausage properties.

Table 11. Changes in texture of fermented sheepmeat sausage as affected by kumara cooking time. Data shown are means ± standard deviations

Heating time (min)	Fermentation	Hardness (N)	Adhesiveness (N s)	Springiness	Cohesiveness	Chewiness (N)
4	Before	13.03 ± 1.28	-2.29 ± 0.23	0.91 ± 0.02	0.66 ± 0.10	
	After	115.68 ± 10.79	-0.12 ± 0.00	0.90 ± 0.01	0.52 ± 0.02	54.41 ± 5.19
Effect of fermentation		***	***	NS	NS	
8	Before	14.69 ± 2.90	-1.87 ± 0.57	0.83 ± 0.04	0.91 ± 0.19	
	After	117.09 ± 22.79	-0.15 ± 0.05	0.86 ± 0.04	0.56 ± 0.09	54.16 ± 25.05
Effect of fermentation		**	**	NS	*	
12	Before	13.18 ± 0.77	-2.27 ± 0.77	0.86 ± 0.12	0.86 ± 0.37	
	After	123.46 ± 16.94	-0.16 ± 0.02	0.89 ± 0.01	0.63 ± 0.05	68.48 ± 6.11
Effect of fermentation		***	**	NS	NS	
Effect of heating time after fermentation		NS	NS	NS	NS	NS

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, not significant

3.7. Physical and chemical properties of fermented sheepmeat sausage due to concentration of red kumara

Unpeeled whole kumara around 200 g were cooked in a microwave for 8 minutes, and blended with the other ingredients at concentrations of 0, 7.5, 15 and 20%, in all cases replacing the fat and lean. To ferment the 0%-kumara treatment, glucose was added to this treatment – and the other three concentration – at the rate of 3 g 100 g^{-1}. Thus the ingredient group comprising fat plus lean, kumara and glucose made up a constant 96% of the mixture and the group salt, pyrophosphate, nitrite and culture contributed the remaining 4%.

The pH value and texture parameters were measured before and after fermentation, while colour parameters were determined only after fermentation.

3.7.1. Determination of pH

Table 12. Changes in pH of fermented sheepmeat sausage as affected by concentration of red kumara

Concentration of kumara (%)	Fermentation	
	Before (Day 0)	After (Day 4)
0	5.87 ± 0.02	4.18 ± 0.02
7.5	5.90 ± 0.02	4.10 ± 0.02
15	5.95 ± 0.01	4.12 ± 0.01
20	6.00 ± 0.02	3.94 ± 0.03
Effect of kumara concentration	NS	***

***, $p < 0.001$; NS, not significant

Table 12 shows pH values of sheepmeat sausage before and after fermentation. The initial pH ranged from 5.87 to 6.00 but was statistically unaffected by kumara concentration. There was a large decline in pH to Day 4, as expected for a successful fermentation. Although kumara concentration similarly had no significant effect on fermented pH, here

was some indication that increasing concentration of kumara resulted in a lower pH ($p < 0.05$). As reported by Puolanne and Kivikari (2000) meat has high buffering capacity due to proteins, carnosine and orthophosphate, but starch has little or no capacity because it has no acidic or alkaline titratable groups. Because the concentration of fermentable sugar was not limiting (all treatments contained at least 2% glucose) the cause of this effect may be due to the reduced buffering capacity of the sausage as the concentration of lean meat decreased with concentration of kumara increasing.

3.7.2. Colour

The concentration of kumara had a significant effect on the colour at Day 4. The L* value increased with increasing concentration of the white-fleshed kumara, but In terms of redness, a* value decreased with increasing the concentration; however, 15%-kumara treatment showed a slight increase compared with 7.5% of kumara. In contrast with a* value, b* and hue angle were increased insignificantly with increasing the level concentration of kumara.

Table 13. Colour parameters of fermented sheepmeat sausage as affected by concentration of white-fleshed red kumara

Concentration of kumara (%)	Colour parameters			
	L*	a*	b*	Hue angle h^o
0	43.86 ± 0.16	15.14 ± 0.13	11.61 ± 0.19	37.48 ± 0.62
7.5	45.51 ± 0.86	14.61 ± 0.32	11.85 ± 0.16	39.06 ± 0.94
15	47.33 ± 0.19	14.81 ± 0.52	13.11 ± 0.54	41.52 ± 0.81
20	48.03 ± 1.08	14.27 ± 0.23	13.09 ± 0.23	42.54 ± 0.71
Effect of kumara concn.	NS	NS	NS	NS

NS, not significant

Sheshetaxy and Faid (2010) used germinated lentil to replace beef to make dry fermented sausage. After fermentation and air-drying, the L* and a* values decreased with increasing concentration of germinated lentil (from 0 to 10 to 15%), while the b*

value were showed an inverse trend. The colour of the germinated lentils was not stated. In the present experiment, the increase in L* (from Day 0 to Day 4) can be attributed to the reflectance due to the white flesh of kumara.

3.7.3. Textural analysis

As shown in Table 14, all kumara concentration treatments showed a significant ($p < 0.001$) increase in hardness after fermentation and a significant decrease in adhesiveness (negative values tend to zero) ($p < 0.001$). However, springiness and cohesiveness were hardly affected by fermentation. After fermentation (Day 4), only hardness and cohesiveness were significantly affected by kumara concentration ($p < 0.05$ for both). The pattern was clear for hardness. It decreased with kumara concentration again suggesting that only the gel structure of meat was positively contributing to hardness, possibly due to a lack of retrogradation in kumara starch. When kumara is moist cooked, by microwave or conventional infrared radiation, the starch forms a gel, as with any moist-cooked starch.

Retrogradation of kumara starch gel would probably cause an increase on hardness of a sausage containing starch. Starch comes in two molecular forms: branched and linear which relate with amylose and amylopectin, respectively (Murphy, 2000). These two forms play different roles in starch retrograditon. Amylose contributes to the short-term rheological and structural changes, whereas amylopectin the long-term changes (Gudmundsson, 1994). If proportion of amylopectin is higher and then is easy to see why increasing kumara causes a decrease in hardness. In kumara, the ratio of amylose to amylopectin is typically18 to 82 (Goswami, Anandjiwala, & Hall, 2004). Moreover, it was found that maltose, glucose, sucrose and fructose can impede retrogradation (Biliaderis, 2009), presumably by interfering with the realignment of the amylose. As was shown earlier in this chapter, cooking kumara results in an increase in glucose concentration. The meter used to measure glucose concentration was specific for only glucose, and neglects fructose and small polysaccharides like maltose. Thus, decrease in hardness due to an increase in kumara concentration appears to be simply due to the dilution of the meat gel by the continuingly soft kumara.

Table 14. Changes in texture of fermented sheepmeat sausage as affected by kumara concentration. Data shown are means ± standard deviations

Kumara concn. (%)	Fermentation	Hardness (N)	Adhesiveness (N s)	Springiness	Cohesiveness	Chewiness (N)
0	Before	13.70 ± 0.90	-2.48 ± 0.19	0.88 ± 0.01	0.72 ± 0.05	
	After	168.53 ± 15.54	-0.29 ± 0.17	0.91 ± 0.03	0.76 ± 0.16	117.6 ± 34.9
Effect of fermentation		***	***	NS	NS	
7.5	Before	14.09 ± 0.61	-2.18 ± 0.42	0.86 ± 0.06	0.70 ± 0.12	
	After	158.32 ± 5.48	-0.10 ± 0.01	0.90 ± 0.01	0.68 ± 0.05	97.3 ± 7.0
Effect of fermentation		***	***	NS	NS	
15	Before	12.03 ± 2.54	-3.15 ± 0.28	0.97 ± 0.08	0.52 ± 0.11	
	After	122.57 ± 22.08	-0.17 ± 0.01	0.88 ± 0.01	0.83 ± 0.04	89.6 ± 19.7
Effect of fermentation		***	***	NS	NS	
20	Before	15.19 ± 2.39	-1.97 ± 0.25	0.84 ± 0.05	0.77 ± 0.06	
	After	105.22 ± 27.70	-0.24 ± 0.14	0.87 ± 0.02	0.57 ± 0.08	53.9 ± 20.9
Effect of fermentation		***	***	NS	*	
Effect of kumara concn. after fermentation		*	NS	NS	*	NS

*, $p < 0.05$; ***, $p < 0.001$; NS, not significant

This argument however, overlooks the possible role of fat and pH. The fat content of sausage is inversely related to hardness, because fat will not form a gel. For example, in the case of cooked frankfurters, hardness dropped with increasing the content of fat (Matulis, McKeith, Sutherland, & Brewer, 1995). However in those studies the ratio of fat to lean varied, whereas in the present study the ratio was a constant. Another complexity was pH. In a study to compare chorizo-Spanish dry-cured pork sausage with three levels of added glucose (0.1, 0.5, and 1.0%), González-Fernández and others (2006) found that the hardness tended to increase with increasing the content of glucose. Throughout fermentation and associated drying over 21 days, the respective pH values for these sausages decreased in the order 0.1%, 0.5% and 1% glucose, and while hardness increased in the same order. The González-Fernández's data for pH have been plotted in Figure 16Figure 16, but the equivalent detailed hardness data were not presented. However, the overall hardness data, for increasing glucose concentration were 129, 156 and 164 N, respectively. This decrease on pH was presumably responsible for the increasing hardness.

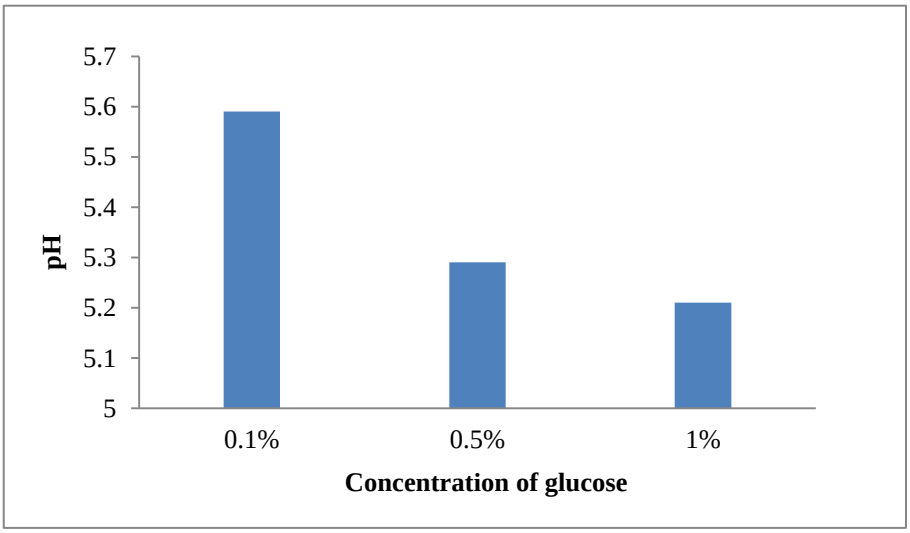


Figure 16. Influence of concentration of glucose on pH of *chorizo*

However, the gross differences in hardness between the present results (Table 14) and those of Lu (2010) using the same casing system were most easily explained by the

big difference in pH, around 4.5 for Lu (2010) and 4.1 as shown in Table 12. According to a pH model of texture, 0% of kumara should have the lowest hardness after fermentation and 20% kumara the highest. This was clearly not the case, so dilution of the meat gel by soft kumara is clearly the dominant effect in the textural changes in Table 14.

Chapter 4

Overall Discussion

4.1. Cooked kumara as a carbohydrate source

In this study, cooked kumara was used as an ingredient of the fermented sheepmeat sausage instead of glucose as a carbohydrate source. There were three reasons for this. The first is that while sweet potato is grown commercially throughout the world. It is commonly used in New Zealand as a roasted vegetable (kumara) with roast lamb. Thus kumara and lamb are culturally linked, and has been adapted to a fermented product. The second reason is that lamb (or any meat) is the most expensive ingredient in a meat product, and added carbohydrate obviously lowers the cost. Third, cooked kumara has been shown to generate high concentrations of fermentable sugars (Figure 9), eliminating the need to add glucose as an ingredient. Glucose as an ingredient would represent a negligible cost, but the advantage of not using glucose is that it needs not appear in the ingredient list; the importance of this is discussed late in the section 4.4.

However, the cooked kumara yielded a concentration of fermentable carbohydrate that was found to be high because it led to a low final pH.

4.2. Excessively low pH in fermented sheepmeat sausage made with kumara

After 4 days fermentation, the ultimate pH of fermented sheepmeat sausage made with kumara ranged between 3.9 and 4.2, such that the lowest pH was associated with the highest kumara concentration. This range is low when compared with typical pH values of fermented meat products and would be responsible for an over-acidic flavour. There are three likely reasons for this very low pH. The first reason could be the addition of starter culture. The utilisation of start culture results in a rapid and uniform acidification (Josephsen & Jespersen, 2004). According to Jay and others (2005), the final pH of fermented sausages was in the range of 4.0 to 4.5 when starter cultures were use, while the fermentations without starter cultures had pH values between 4.6 and 5.0.

The second reason is that kumara itself, rather than the available sugar is important in generating an excessively low pH. When 15% kumara was used to explore colour, texture and pH, the pH was around 3.9. Table 6 in Chapter 1 showed that the fermentable sugar concentration in cooked kumara is typically 14%. When present in the fermentable mixture at 15%, the upper limit tested here, the final concentration would be around 2.1%, high enough to drive pH to 3.9. In the experiments showing the effect of kumara concentration on sausage properties, four kumara concentrations were tested: 0, 7.5, 15 and 20%. Because 0% kumara treatment did not contain any fermentable sugars, it was necessary to add glucose for the fermentation. Thus, 2% of glucose was added in all four treatments in the kumara concentration experiment. With 0% kumara, 2% of glucose was enough to drive the final pH to 4.2, which is higher than the 3.9 achieved with kumara. Thus, kumara appears to stimulate the fermentation. There may be many chemical factors in kumara that stimulate fermentation but one reason stands out. There is a high concentration of manganese in kumara, typically 0.27 mg 100 g^{-1} (U.S Department of Agriculture, 2007). This trace element has been shown to enhance the acid production by lactic acid bacteria with fermentation (Zaika & Kissinger, 1984).

An excessively low pH may also lead to other problems. The very low pH might be the reason these fermented sheepmeat sausages made with kumara were harder than is typical for fermented meat products. Very hard fermented meat products may be considered as undesirable by older consumers, whose teeth frequently suffer with age.

4.3. Further development of fermented sheepmeat sausage made with kumara

4.3.1. Nitrite and pyrophosphate

Sodium nitrite and sodium pyrophosphate were included in all fermentations described in this thesis. There several reasons to maintain these ingredients. Nitrite and pyrophosphate acts as antioxidants, and it is the lack of fat oxidation that contributes to the characteristic fermented sausage flavour, as described in Chapter 1. Nitrites create a characteristic cured red colour the consumers expect in a fermented

sausage. Pyrophosphate is an analogue of adenosine triphosphate and as such has a solubilising effect on myosin and actin, so that sausage formulations are easier to mechanically handle prior to fermentation. Finally, along with salt, nitrite has a bacteriostatic effect, inducing inhibition of undesirable microbes.

4.3.2. Modifying strong sheepmeat flavour

As described in chapter 1 meat from sheep and goats has a characteristic odour and flavour which produced by branched chain fatty acids. Further, an additional flavour is introduced to the meat because the animals were raised on pasture. This chemical is 3-methylindole, also known as skatole, a decarboxylation product of dietary tryptophan (Young, Berdagué , Viallon, Rousset-Akrim, & Theriez, 1997). Sheepmeat has a strong odour and flavour that to a great extent results in low consumption of sheepmeat (McMillin, Huang, Ho, & Smith, 1999) in markets where there is a wide choice of meats. Thus, in order to expand sheepmeat consumer base, it is necessary to modify the intensity of sheepmeat odour.

There is good evidence that older sheep – mutton as opposed to lamb – have stronger flavoured than younger sheep (Young & Braggins, 1998; Young et al., 2006), and since mutton would be the meat of choice in the fermented sheepmeat/kumara sausage, reduction of flavour in that meat is the topic of interest.

The literature is varied on this and many ways of reduction are reported in the patent literature, as summarised by Young and Braggins (1998) and McMillin and others (1999). Methods described include repetitive washing dilute alkali to remove fat by flotation, and soaking in a 0.1% solution of either malate, fumarate or succinate, or addition of 0.05 to 5% of asparagine, glutamine, alanine or glycine. Each of these treatments increases cost, and in the case of repetitive washing induces of the onset of oxidative rancidity (Young, pers. comm.). This is credible because comminution to expose particles of fat involves exposure to air and oxygen is also soluble in water. Another treatment was described by Young and Cummings (2008) and Young and others (2009). Addition of 2% of the pentose sugar xylose results in a marked

reduction in sheepmeat flavour, but to achieve this effect the meat has to be cooked, and the same would apply to the amino acid treatments. In the case of xylose, the Maillard reaction (Maillard & Gautier, 1912) generates flavours that suppress or otherwise modify sheepmeat flavour.

Herbs and spices represent the other useful way to suppress or modify sheepmeat flavour. Also, herbs and species are usually potent antioxidants. Coggins (2001) noted that there are no definitions that clearly distinguish between the meaning of spices and herbs, so the word spice is used here can be taken to mean herb or spice primarily used to enhance or change flavour. Spices can be divided into four categories in terms of function, as shown in Table 15. Spices can also contribute to inhibition of the growth of undesirable bacteria, to retardation of oxidative rancidity, and to colour (Marianski & Marianski, 2009). In addition, spices can accelerate fermentation through provision of micronutrients, notably manganese (Zaika & Kissinger, 1984).

Table 15. Categories of spices

Categories	Representative spices
Hot and/or pungent	Cayenne pepper, ginger, horseradish, onion, garlic
Aromatic	Bay, cinnamon, clove, nutmeg, pimento (allspice), mace
Herbaceous	Anise, basil, caraway, cumin, dill, laurel leaf, rosemary, sage, marjoram, thyme, tarragon
Colour	Paprika, saffron, turmeric

Source: Coggins (2001)

Spicing is an inexact science and it is possible that any combination of spices may suppress sheepmeat flavour where this is required. In Lu (2010) for example, sheepmeat was successfully flavoured with traditional herbal spices garlic and rosemary, where many of the consumers were culturally unfamiliar to sheepmeat. Zhao and

others (2011) reported previous work that found that a mixture of 0.5% ginger, 0.5% cumin, 1.0% scallion (an *Allium*) and 0.4% dried tangerine peel (Chinese spice) could effectively suppress the strong smell of roast sheepmeat. Hampikyan and Ugur (2007) reported that a mix of garlic powder, coriander seeds, cumin, pepper and nutmeg were used to made sucuks, a Turkish fermented sausage made with lamb. It is not clear whether this latter mix suppressed, enhanced, or was neutral in respect of sheepmeat flavour, although the first seems most likely.

Coggins (2001) also pointed out that the use of specific spices is often indicative of certain geographical or ethnic origin. From author's experience in her home country China, anise, cinnamon, clove, ginger, tangerine peel, garlic, fennel, hawthorn and tree leave are frequently added to mask sheepmeat flavour. In New Zealand, garlic, rosemary, thyme and oregano are common spices used with sheepmeat.

Of all the spices available, one spice that should be used is garlic. Smith and Young (1993) analysed recipes for lamb dishes throughout the world's major cuisines and found that garlic was very commonly added to sheepmeat dishes, much more than for beef. Whether this suppresses sheepmeat flavour or otherwise alters its perception is not known, but its universality suggests it should be a mandatory spice ingredient here. Without doubt, black pepper is the most popular herbal spice applied to fermented sausages at 0.2 to 0.3% (Marianski & Marianski, 2009) and should be considered in the kumara/sheepmeat formulation. Rosemary has the highest antioxidant activity among herbal spices, containing several antioxidants: carnosic acid, carnosol, rosemarinic acid and rosmanol (Shylaja & Peter, 2004). This spice is frequently linked with lamb and they are used in formulation for cooking such as stews, fries and soup (Clarke, 1994). Thyme and oregano are also traditionally used with sheepmeat in Western culinary traditions (Smith & Young, 1993).

Horopito (*Pseudowintera colorata*) is a spice unique to New Zealand, and is the leaves of a shrub (Figure 17Figure **17**a). Horopito contains a biologically active chemical component, the sesquiterpene dialdehyde called polygodial (Figure 17b), which has a hot and spicy taste, and leaves a burning sensation in the mouth

(Berry-Kilgour, 2002). In Japanese cuisine, polygodial is a common component in peppery spices. In New Zealand, horopito is used as a condiment on cooking, but is rather limited to the gourmet market. Apart from that, horopito had medicinal applications for the native Maori population of New Zealand. Extracts of horopito were used in the medical treatment of fungal skin ailments, wounds, ulcers, cuts, stomach ache and toothache. Whatever its mode of action for each of these ailments, polygodial has potent fungicidal activity that extends to yeasts (Lee, Lee, Lunde, & Kubo, 1999; McCallion, Cole, Walker, Blunt, & Munro, 1982).

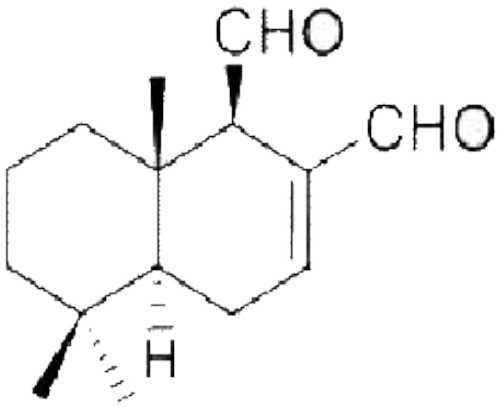
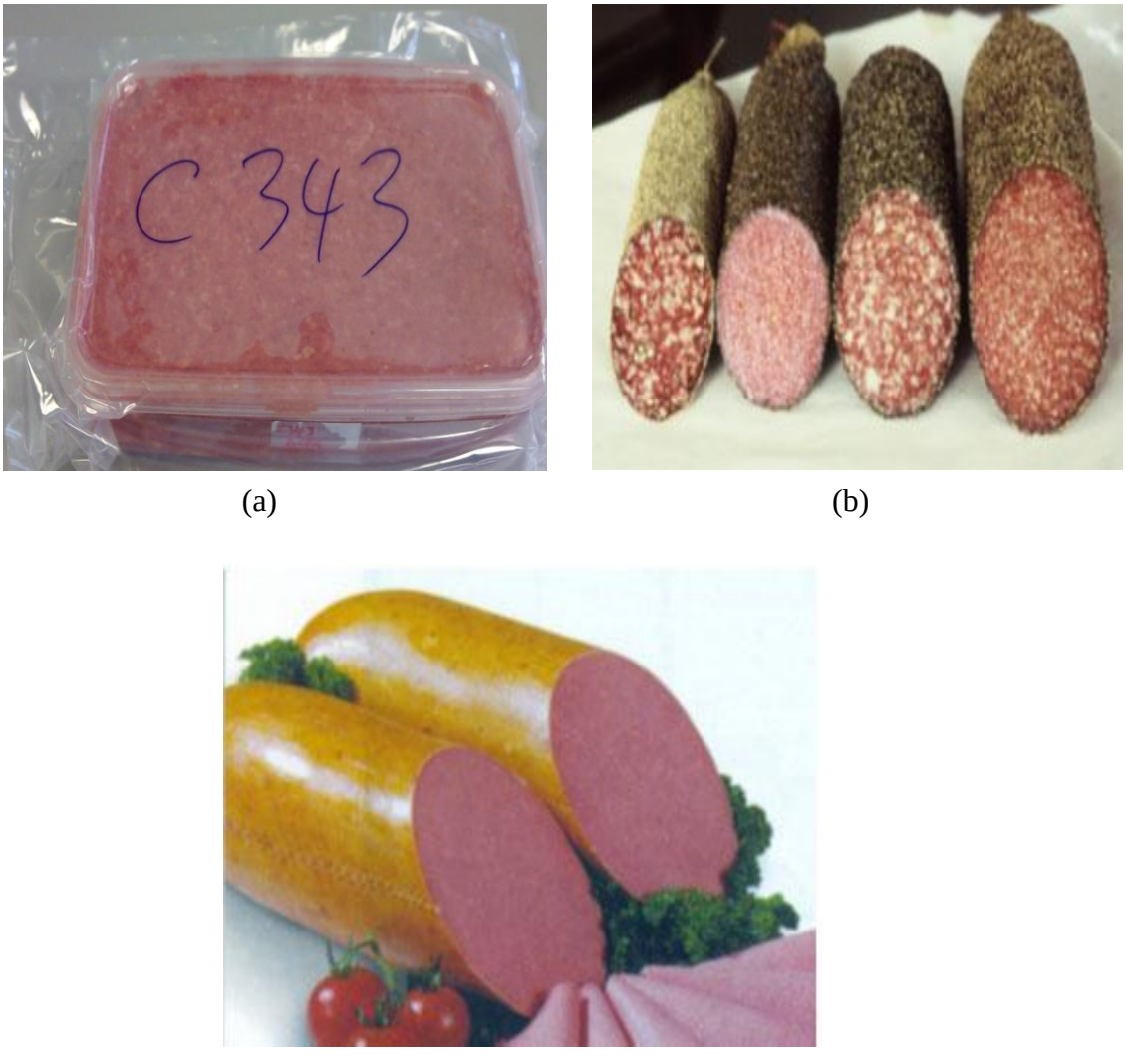


Figure 17. (a). Horopito leaves, a unique Zealand native spice；(b). The molecular structure of polygodial

To sum up, in further development of a commercial cured and fermented sheepmeat/kumara sausage, garlic, black pepper, rosemary, thyme and oregano could be variously used to mask or improve flavour. Whether horopito also has these properties or not is not known, but horopito could be used as well as or in place of pepper to generate a hot spicy note.

4.3.3. Choice of casings and associated preservation methods for creating novel sheepmeat/kumara sausages

In this study, the water impermeable plastic syringe was used as a model casing that enabled extrusion as required. However, it is unsuited to any commercial application. In choosing a casing there are three broad possibilities that will generate undried, semi-dry and dry sausages (Figure 18Figure **18**).



(a) (b)

(c)

Figure 18. (a). A sample of undried fermented sausage (Lu, 2010); (b). Semi-dry sausage; (c). Dry sausage

For an undried fermented sheepmeat/kumara sausage, the fermentation time would be approximately four days as in the present study. The mixture would be extruded into synthetic casings such as polyethylene, polypropylene and or other water impermeable polymer casings. These synthetic casing could prevent water loss and also be more convenient and cheaper methods of packing compared with other casings. It is noted that drying and conventional smoking would not be possible. However, liquid smoke could be used and has the advantages that it contains a lower content of harmful compounds such as polycyclic aromatic hydrocarbons (PHAs) than traditional smoke (Gomaa, Gray, Rabie, Lopez-Bote, & Booren, 1993; Simko & Brunckova, 1993). Polycyclic aromatic hydrocarbons are known to prevent occurrence of cancer from exposure to PAHs atmosphere and intake of smoked foods (Wenzl, Simon, Anklam, & Kleiner, 2006).

With semi-dry and dry sausages, the non-edible collagen, cellulose and fibrous casings would be used. These casings can support associated preservation methods such as drying and conventional (non liquid) smoking. Drying and smoking would aid fermentation as means of preservation. Smoking contributes to inhibition of growth of spoilage microbes on the surface and a delay of oxidation (Lücke, 2003). Fermented sausages can be smoked at either lower or higher temperatures.

The semi-dry and dry fermented sausages take days and weeks to ripen, respectively (Toldrá, 2002). Drying decreases water activity, which prevents spoilage beyond that obtained by pH reduction from fermentation. Moreover, complex enzymatic and non-enzymatic reactions occur to generate fat oxidation products and proteolytic fragments that all contribute to flavour (Toldrá, 2002). Harmless moulds and yeasts (species of *Penicillium, Candida, Debaromyces*) can also be used to form a powdery microbial coat (Li et al., 2001). These microbes penetrate the surface of the casing and further contribute to flavour development and also inhibit the survival and growth of spoilage bacteria. Importantly however, if horopito were used as a spice, these moulds and yeasts such as *Candida* might not grow because of polygodial's potent fungicidal activity.

4.4. Marketing

In response to advertising campaigns seeking points of difference in otherwise generic food products, many customers prefer foods containing perceived ‘natural’, rather than ‘artificial’ additives and ingredients. From a logical chemical perspective these distinctions are arbitrary or even meaningless, but in order to create a market it is probably sensible to adopt the ‘spirit of the time’ (zeitgeist). Apart from ‘natural’, people are increasingly requiring food products made with fewer ingredients. The claims on food label such as ‘simple’, ‘simply’, ‘no additives’ are favoured by consumers. In the present study, replacing fermentable sugar with kumara would not only match the simplicity trend but satisfy customer willingness of ‘natural’ ingredients as well. Pyrophosphate and nitrite would have to be declared on an ingredients label and there is nothing that can be done about those two ingredients. Spicing represents an opportunity. Garlic is a macro ingredient, and will be listed as a senior ingredient. Spicing with junior ingredients represents an opportunity. Rather than listing the vague term ‘spices’, which is allowable under New Zealand food law (FSANZ, 2011), there is an excellent opportunity to list the spices in detail, such as horopito.

Thus it would be comparatively easy to create a distinctive New Zealand-specific sausage, but a major problem remains. Stemming from the colonial past, sheepmeat is classified into three meat types in New Zealand and Australia: lamb, hogget and mutton. Lamb is the meat of a sheep up to approximately 12 months of age, hogget is from sheep between approximately 12 and 24 months, and mutton is from sheep older than that. Lamb is the most expensive, followed by hogget, followed by mutton, and mutton would be the choice of meat in a commercial sausage on the basis of cost. When eaten as primal cuts there is no doubt that mutton is a stronger flavoured meat probably because of the increase in branched chain fatty acids in the fatty tissue (Morris, 2009). However, the flavour distinction between lamb and hogget is essentially nonexistent (Young, Hopkins, & Pethick, 2005). Nevertheless, there is a distinction in the minds of consumers, certainly in New Zealand, as discussed below.

At Fieldays 2000 in Hamilton, New Zealand, a survey was conducted to research

consumer perception of seven meat types from 405 respondents (Lim, 2001). The consumers did not see or taste the meats. They simply scored their perceptions on a 1 to 5 scale where 1 was lowly rated and 5 was highly rated. Of the three questions posed for consumers, only one – tastiness – has an arguably real meaning, but the other two – quality and healthiness – are in common usages in spite of their lack of defined meaning. The ballot as presented to consumers was randomised, but the results are expressed in Figure 19 to clearly show the preference differences between lamb, hogget and mutton. Consumer preference in all questions decreased markedly with an increase in the age of sheep.

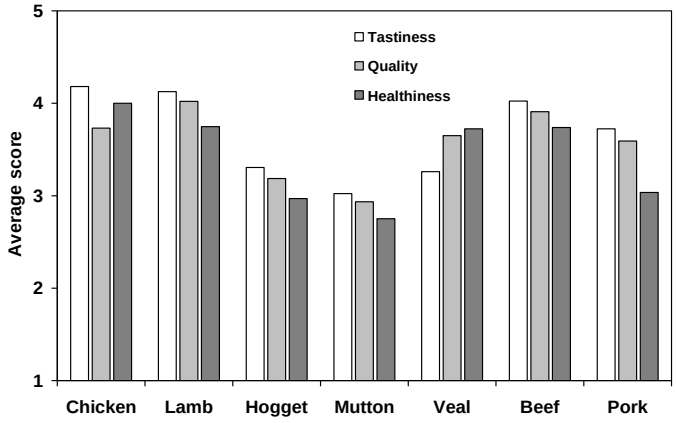


Figure 19. Consumer perception and preference of seven meat types. 1 was the least like and 5 was the highest like. Data are means for 400 consumers. Standard deviation bars have been deleted for clarity, but many of the differences were highly significant (Lim, 2001)

If mutton were used as the cheaper base material in a cured, fermented, spiced, kumara/sheepment sausage, it is highly unlikely that the stronger flavour of mutton would be evident. That is evident from the results of Lu (2010). However, the name mutton would have to be used in the ingredient list and 'lamb' could not be used as. The data in Figure 19 show that the name 'Mutton and kumara' (salami) would be a

marketing problem. Short of a law change there is nothing that can be done about this problem. Although added kumara would lower the cost, the expensive ingredient lamb would still mainly account for the price of fermented sausage. The final price would be higher than most of fermented sausage products in the market. For this, the market for this product would be aimed at specialty markets such as Nosh and Farro Fresh rather than Pak n Save and Countdown.

4.5. Future work

Each of the developments discussed above in this section would demand further research to understand physical, chemical and microbiological properties of different product options. After the product options were narrowed, hedonic sensory trials would be conducted to test the liking of colour, flavour, texture and overall liking.

Chapter 5

Conclusion

The first experiments showed that of the carbohydrate sources (potato, kumara and yam), kumara presented a best opportunity to serve as a carbohydrate source of fermented sheepmeat sausages. In the second phase, fermented sheepmeat sausage has been made with three New Zealand kumara varieties, red, orange and gold kumara. For each variety, pH had a sharply fallen below 4.5 after one day fermentation, which indicated the fermentation was successful. By Day 4 the pH values had stabilised at around pH. At Day 4, kumara variety did not significantly affect colour parameters and textual properties. On the basis of price alone, red kumara was used for the third phase experiments to identify properties of fermented sheepmeat sausages as affected by cooking time and the concentration of kumara. According the physical and chemical results (pH, colour and texture), cooking time and kumara variety did not have a significant effect on the fermented sheepmeat sausages. However, the concentration of kumara had an influence on hardness. The work was limited to sporadic purchases of kumara, and the effects – if any – of factors like season, geographical source and storage prior to use cannot be known from the present research.

The fermented sausages made in the present study were ‘wet’ due to the plastic casings used. Thus, they would have a high water activity, which contrasts with typical fermented beef and pork sausages that are typically semidried in their water-permeable casings. Further experiments should be conducted to test a variety of casing and maturation conditions. A ‘geographically distinct’ (although not truly unique) fermented sheepmeat sausage can clearly be made, but two problems remain. The first is that consumers unhabitutated to sheepmeat flavour are common throughout the world and many of these consumers live in societies able to pay the high prices anticipated for geographically distinct. Work with spicing, by other researchers, has shown that it is possible to overcome perceived intrinsic flavour defects. Thus sensory trials should be carried to find customer preference of the kumara-fermented sheepmeat sausages for

spicing. In this respect there is an opportunity to further add to geographical distinctiveness by using a unique New Zealand spice, horopito.

The second problem is the names given to sheep of different ages. To be cost effective the kumara-fermented sheepmeat sausages would be made from mutton, animals older than two years, and in Australasia, at least, that name would have to be used on ingredients lists. Emphatically the name 'lamb' could not be used. Research has shown that the name 'mutton' would be a marketing disaster.

Looking beyond this problem – which could be solved by a change in commercial legislation relating to sheepmeat products as opposed to primal cuts – kumara-fermented sheepmeat sausages could be a starting point to add value to one of New Zealand's major commodity exports.

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