PRODUCTION OF SALAMI FROM MEAT OF AQUATIC AND TERRESTRIAL MAMMALS

by

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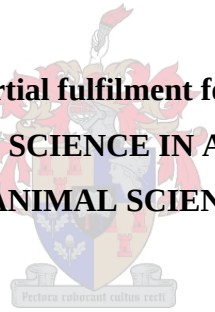

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

Signature:_____ Date:_____

SUMMARY

The aim of this study was to develop a product using alternative red meat species, aquatic and terrestrial mammals, which would be acceptable to the consumer and suitable from a food safety aspect. Many of these alternative species are harvested seasonally. A product which is shelf stable needs to be developed to provide a supply of this meat all year round. The species used in this investigation were the Cape fur seal (*Arctocephalus pusillus pusillus*), the Grey seal (*Halichoerus grypus*) from the northern hemisphere, the Minke whale (*Balaenoptera acutorostrata*), horse, beef, mutton, blesbok (*Damaliscus dorcas phillipsi*) and springbok (*Antidorcas marsupialis*).

Muscle (*m. pectoralis*) of Cape fur seal pups has a higher percentage fat (4.2g/100g) than the bulls (2.4g/100g), but similar levels of protein (23.2g/100g). Bull blubber samples have a higher percentage protein (26.6g/100g) than the pups (14.6g/100g), but a lower fat percentage (67.1g/100 g) compared with the pups (77.2g/100g). In the Cape fur seal bull meat, saturated fatty acids (SFA) contribute 33mg/100g, monounsaturated fatty acids (MUFA) 29mg/100 g and polyunsaturated fatty acids (PUFA) 38mg/100g of the total fatty acid content. In pups, the three fractions are 39, 30 and 31 mg/100g for SFA, MUFA and PUFA, respectively.

*Salami,prepared using exclusively seal meat, or seal meat with beef and pork, was produced in a pilot study, using two commercially available starter cultures. The pH values of all three batches started off at ca. 5.6, and dropped to 4.*3. Water activity readings started off at 0.96 and dropped to 0.91 after 21 days. Salami produced from the meat of the Grey (Havert) seal and Minke whale, using three starter cultures, had recorded pH values (in both species), which started off between 5.68 and 5.92, and dropped to between 4.5 and 4.8 over the 21 days. Water activity showed an initial value of 0.96, which dropped to 0.90 after 21 days. The final force (N/cm^2) that was needed to compress the salami samples was double that of the initial force required for the same species and starter culture combination. The raw seal meat contained 349.6 (mg/100 g sample) SFA, 271.6 (mg/100g sample) MUFA and 175.8 (mg/100g sample) PUFA, whilst the raw whale meat contained 312.3, 251.9 and 179.6 (mg/100g sample) SFA, MUFA and PUFA respectively.

Fifteen batches of salami were made from horse, beef, mutton, blesbok and springbok, respectively, and starter cultures of *Lactobacillus curvatus* DF 38 (batch I), active bacteriocin

producing *Lactobacillus plantarum* 423 (batch II) and then a mutant variation of *Lactobacillus plantarum* 423m, which did not produce the bacteriocin (batch III). Batch I had a higher final pH value (4.66), after 23 days, whereas the values for batches II and III were similar (4.42 and 4.46 respectively). On day 23 the water activity value was 0.90 for all starter cultures. Horse salami, in batch I, was the leanest in terms of fat content (34.34g/100g salami), with mutton salami having the highest fat content (37.52g/100g salami). Blesbok salami had the highest fat content in batch II (42.77g/100g meat), with beef the leanest (35.71g/100g meat). Salami made from horse and springbok proved to be the most desirable in terms of chemical composition, especially fatty acid profiles, with regard to P: S and n-6: n-3 ratios.

Similar growth patterns in colony forming units (cfu) were recorded for *L. plantarum* 423, *L. plantarum* 423m and *L. curvatus* DF38 in MRS broth (Merck) at 30^{o}C, although batch I reached asymptotic growth earlier. The percentage of *L. plantarum* 423 compared with the total population of microflora in mutton salami remained almost the same (80-95% variety) during the entire fermentation and maturation process. In horse salami, *L. plantarum* 423 was present at relatively low cell numbers (55-50% on day 1 and before smoking), but increased to 70% after smoking and stabilized to 70-80% for the remaining fermentation period. In beef salami, cell numbers in batch II decreased slightly during the first five days (from 95 to 70%), followed by an increase to 90%. In springbok salami, cell numbers in batch II remained fairly stable at 80-90%. In blesbok salami, batch II slowly decreased during the first three days, from 88% to 70%, then increased to 92% after 12 days and stabilized for the rest of the fermentation period. Similar results were recorded for batch I.

Analytical sensory evaluation concluded that the salami prepared using starter culture I resulted in end products with lower sensory qualities. Salami prepared using blesbok and mutton also resulted in end products with lower sensory qualities and was perceived as significantly lower in salami flavour ($P \leq 0.05$) and higher in venison-like and mutton-like flavour respectively. The blesbok samples were rated significantly higher ($P \leq 0.05$) in sour meat aroma, sour meat flavour and venison-like flavour than the rest of the samples. The blesbok salami was rated significantly lowest for colour compared with the rest of the samples. The tastes of the springbok and horse salami were significantly ($P \leq 0.05$) more acceptable than those of the beef and blesbok salami.

OPSOMMING

Die doel van die studie was om, deur gebruik te maak van alternatiewe rooivleisspesies afkomstig van die see en land, 'n produk te ontwikkel wat beide dieetveilig en vir die verbruiker aanvaarbaar is. Aangesien van hierdie spesies seisonaal geoes word, moes die produk ook stabiel wees om voorraad dwarsdeur die jaar te voorsien. Die spesies wat tydens die studie ondersoek is, het die Kaapse pelsrob (*Arctocephalus pusillus pusillus*), die Grysrob (*Halichoerus grypus*) van die noordelike halfrond, die Minke-walvis (*Balaenoptera acutorostrata*), perd, bees, skaap, blesbok (*Damaliscus dorcas phillipsi*) en springbok (*Antodircas marsupialis*) ingesluit.

Spiere (*m. pectoralis*) van Kaapse pelsrobkalfies het 'n betekenisvolle ($P \leq 0.05$) hoër persentasie vet (4.2g/100g) bevat as dié van pelsrobbulle (2.4g/100g), maar dieselfde hoeveelheid proteïen (23.2g/100g). Spekmonsters van pelsrobbulle het egter 'n betekenisvolle ($P \leq 0.05$) hoër proteïeninhoud (26.6g/100g) gehad as dié van die kalfies (14.6g/100g), maar 'n betekenisvolle laer vetinhoud (67.1g/100g vs 77.2g/100g). In die bulsvleis van die Kaapse pelsrob het die totale vetsuurinhoud bestaan uit 33mg/100g versadigde vetsure (VVS), 29mg/100g mono-onversadigde vetsure (MOVS), en 38mg/100g poli-onversadigde vetsure (POVS). In die geval van kalfies was hierdie waardes onderskeidelik 39, 30 en 31mg/100g.

In 'n loodsondersoek is tradisionele salami voorberei deur gebruik te maak van robvleis alleenlik of robvleis gekombineer met bees- of varkvleis en twee kommersiële aanvangskulture. pH-waardes van al drie produkgroepe het vanaf 'n aanvangswaarde van 5.6 afgeneem tot 4.3. Wateraktiwiteitswaardes was aanvanklik 0.96 en het afgeneem tot 0.91 na 21 dae. In die geval van salami wat van Grysrob en die Minke-walvis gemaak is, het die pH-waardes vanaf die aanvanklike 5.68 en 5.92 na 21 dae afgeneem tot 4.5 en 4.8 respektiewelik. Die finale krag (N) wat nodig was om die salamimonsters saam te pers was dubbel die aanvanklike waardes vir dieselfde kombinasie van spesie en aanvangskultuur. Rou robvleismonsters het onderskeidelik 349.6mg/100g VVS, 271.6mg/100g MOVS en 175.8mg/100g POVS bevat teenoor 312.3, 239.9 en 179.6mg/100g in die geval van rou walvisvleis.

In 'n daaropvolgende studie is drie groepe van vyf verskillende tradisionele salamis gebruik wat bestaan het uit die vleis van vyf verskillende spesies, naamlik perd, bees, skaap, blesbok en springbok, terwyl drie verskillende aanvangskulture gebruik is, naamlik *Lactobacillus curvatus* DF38 (groep I), aktiewe bakteriosienproduserende *Lactobacillus plantarum* 423 (groep II) asook 'n muteerde variasie van *Lactobacillus plantarum* 423 wat nie bakteriosien produseer nie (groep

III). Na 23 dae het groep I 'n hoër finale pH-waarde gehad (4.66) teenoor groepe II en III wat bykans dieselfde was (pH 4.42 en 4.46 onderskeidelik). Op dag 23 na vervaardiging was die wateraktiwiteitswaarde 0.90 vir al die aanvangskulture. Perdesalami van groep I het die laagste vetinhoud gehad (34.34g/100g) teenoor skaapsalami wat die hoogste was (37.52g/100g). In groep II het blesboksalami die hoogste vetinhoud vertoon (42.77g/100g) terwyl beessalami die laagste was (35.71g/100g). Springbok- en perdesalami het die gewenste chemiese samestelling gehad, veral ten opsigte van vetsuurprofiele, met verwysing na P:S en n-6:n-3 verhoudings.

Dieselfde groeipatrone ten opsigte van kolonie-vormende eenhede (kve) is waargeneem vir *L. plantarum* 423, *L. plantarum* 423m en *L. curvatus* DF38 in MRS-kragsop by 30^{o}C alhoewel groep I vroeër eksponensiële groei bereik het. In skaapsalami het die persentasie van *L. plantarum* 423 ten opsigte van die totale mikro-flora populasie bykans dieselfde (varierend tussen 80% en 95%) gebly tydens die volle fermentasie- en maturasieproses. In die geval van perdesalami het *L. plantarum* 423 aanvanklik lae selgetalle getoon (55% op dag een en voor beroking). Dit het egter tot 70% toegeneem na beroking en op 70-80% gestabiliseer vir die res van die fermentasieperiode. In beessalami van groep II het die persentasie kultuurselle effens afgeneem tydens die eerste vyf dae (van 90% tot 74%) waarna dit tot 90% toegeneem het. By die springboksalami van groep II het die selpersentasie redelik stabiel gebly – tussen 80-90%. In die geval van blesboksalami van groep II het die selpersentasie tydens die eerste drie dae stadig van 88% tot 70% afgeneem, waarna dit tot 92% op dag 12 toegeneem en op hierdie vlak gestabiliseer het vir die res van die fermentasieperiode. Soortgelyke resultate is vir groep I aangeteken.

Volgens die analitiese sensoriese evaluasie is vasgestel dat die salami wat met aanvangskultuur I voorberei is, die swakste sensoriese kwaliteit vertoon het. Dieselfde waarneming is gedoen ten opsigte van salami wat van blesbok- en skaapvleis berei is. In laasgenoemde twee gevalle was die waarnemings ook dat die produkte 'n betekenisvolle ($P \leq 0.05$) swakker salamigeur gehad het en 'n sterker skaap- en wildsvleisgeur as die res van die monsters. Die blesbokmonsters is ook die laagste geëvalueer ten opsigte van kleur in vergelyking met die res van die monsters ($P \leq 0.05$). Die smaak van springbok- en perdesalami was meer aanvaarbaar ($P \leq 0.05$) teenoor dié van bees- en blesboksalami.

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LIST OF CONTENTS

NOTES

The language and style used in this thesis are in accordance with the requirements of the scientific journal, *Meat Science*. This thesis comprises a compilation of manuscripts, where each chapter is an individual entity and some repetition between the chapters has, therefore, been unavoidable.

Papers emanating from results of this study have been presented at the following symposia and congresses:

1. K.S.C. Koep, L.C. Hoffman, E. Slinde & L. Dicks (2004). Chemical properties of the meat and blubber of the Cape fur seal (*Arctocephalus pusillus pusillus*). Annual joint GSSA – SASAS, June 2004, Goudini Spa, Western Cape, South Africa.

2. K.S.C. Koep, L.C. Hoffman, E. Slinde & L. Dicks (2004). Chemical properties of the meat and blubber of the Cape fur seal (*Arctocephalus pusillus pusillus*). International Congress of Meat Science and Technology (ICoMST), August 2004, Helsinki, Finland.

Chapter 1

Introduction

1.1. Introduction

The South African and international meat market is presently experiencing a substantial increase in the demand for game and other exotic meat types as healthier alternatives to traditional red meat species. Over time, economic and social changes in civilisations have led to the transformation and modification of nutritional demands in many societies. Nowadays consumers favour meat that is authentic, tasty, rich in protein and low in lipid and cholesterol content. Therefore, the purchase of alternative sources of red meat, as opposed to products from the traditional species of red-meat-producing animals, is becoming more acceptable.

Growing consumer interest in non-traditional meat products underlies the purpose of this investigation into the processing potential of species such as seal, horse and certain species of game animals (blesbok and springbok). Five of the seven species (Cape fur seal, Grey seal, Minke whale, blesbok and springbok) used in this study are not farmed intensively, and are therefore considered as wild game (either aquatic or terrestrial). Such animals have had no direct daily contact with humans and therefore contain minimal chemical substances in their bodies usually administered by man. Given these differences, comparisons in terms of suitability for processing can be drawn between these wild species and conventional red meat species such as beef and mutton. Therefore, this study focuses on a comparative analysis of the following species: Cape fur seal, the Grey seal, the Minke whale, beef, mutton, horse, blesbok and springbok.

The Cape fur seal (*Arctocephalus pusillus pusillus*) is harvested commercially in Namibia, on the south western coast of Africa. At present, there is a large demand for the hides of the pups and bulls, but the rest of the carcass is processed to carcass meal, traditionally used to supplement the diets of ruminants. Due to the occurrence of BSE (*Bovine spongiform encephalopathy*), there has been a decrease in the use of animal by-products in any animal feed. An alternative use for the meat is thus desirable.

As the meat has been shown to be healthy, it is lean meat that contains high concentrations of the macro-minerals calcium, phosphorous, iron and selenium (Robinson, 1996), the possibility of processing the meat into a food source presents itself. This would make seal harvesting a more

acceptable practice, as it would entail optimal utilisation of the entire carcass of the animal and would provide a supply of nutritious meat to the consumer. Due to the fact that seal harvesting is a seasonal occurrence, shelf-stable products need to be developed that will ensure that the ongoing demand for this meat type is met by a regular supply.

As regards wild game species, Eloff (2002) reported that the annual gross profit of the South African game industry amounted to R843 million in the year 2000. Game meat sales contributed only R20 million (2.4%) to this amount, with non-commercial hunting delivering the highest gross profit, followed by live-game sales and trophy hunting. Since the contribution of game meat production (jerky hunters and game meat sales) constitutes 67.5% of the total income of the game industry of South Africa (Potgieter, 2001), research is required to investigate the possibility of utilising wild game species in processed products, such as salami.

Salami has a long shelf life and is a fairly safe product. Salami may be defined as a mixture of meat and fat particles, combined with a mixture of NaCl, curing agents and spices that have been stuffed into either artificial or natural casings. Starter cultures are often used to aid in the fermentation and maturation of the sausage. An appropriate selection of meat ingredients is very important for the production of sausages of uniform quality. Fermented sausages traditionally contain 50-70% lean meat. Any meat species may be used, and the raw meats generally used for the production of salami are usually the higher valued meat cuts that are free from collagen. Pork is favoured in countries such as Europe, the USA and China. Elsewhere in the world, mutton and beef are the preferred species. Chicken and other poultry meat is also used in the production of fermented sausages.

After drying of the mixture, the final product may contain up to 50% fat, which makes fat an important ingredient of fermented sausage (Campbell-Platt, 1987). This may lead to problems such as rancidity, with a direct effect on the shelf life of the product. This plays a key role when considering the use of seal meat, as the diet of aquatic mammals consists mainly of fish and their tissue contains more polyunsaturated fatty acids than that of their terrestrial counterparts. Although these fatty acids are very popular amongst health conscious consumers, they are a challenge in the food industry when it comes to preventing the rancidity of products.

The most common species of starter cultures used in the fermentation of beef and pork sausages are strains of *Lactobacillus, Pediococcus* and non-pathogenic S*taphylococcus* and *Micrococcus* spp. (Lücke, 1986). The major role of lactic acid bacteria in the sausage is to produce organic acids, primarily lactic acid, from carbohydrates via the Embden-Meyerhof pathway (Campbell-

Platt, 1987). This results in a decreased pH and contributes to the retardation of the development of undesirable micro-organisms.

As the consumer nowadays demands access to ready-to-eat foods and fast foods, processed foods are becoming evermore popular and the use of alternative red meat species in these processed products is of particular interest to the food industry. Due to the fact that some similar meat products often use the lower valued cuts of a carcass, optimal and cost-effective carcass utilisation can therefore be realised. This is especially important when considering non-traditional meat species, such as the seal.

1.2. Objectives

No literature could be found on the nutritional value of the meat of the Cape fur seal, and therefore a baseline investigation into its chemical composition (Trial 1, Fig. 1) was undertaken. The viability of using seal meat for processing into meat products, such as salami, could open up the possibility of a more optimal utilisation of the carcass (Trial 2, Fig. 1).

Similarly, the Grey seal (*Halichoerus grypus*) and the Minke whale (*Balaenoptera acutorostrata*) are found in the oceans of the northern hemisphere and are harvested seasonally, as the Cape fur seal is in the southern hemisphere. The chemical properties of the meat of these two aquatic mammals needed to be determined prior to assessing the suitability of using these animals in the production of salami (Trial 3, Fig. 2).

Lastly, the suitability of using non-conventional terrestrial mammals, such as blesbok and springbok, and more conventional species such as beef, mutton and horse, in the production of salami was investigated (Trial 4, Fig. 3). The reactions of specific starter cultures in the different meat types were analysed concurrently, and researched more specifically under controlled laboratory conditions, to identify the individual reactions relevant to the processing conditions under which traditional salamis are produced (Trial 5, Fig. 3). An aim of this study was, therefore, to be able to pin-point starter cultures (which would optimise the production of salami) utilising different non-traditional meat species. The conditions under which the research was done were strictly monitored and controlled to eliminate any external factors, which could influence the study.

1.3. Methodology

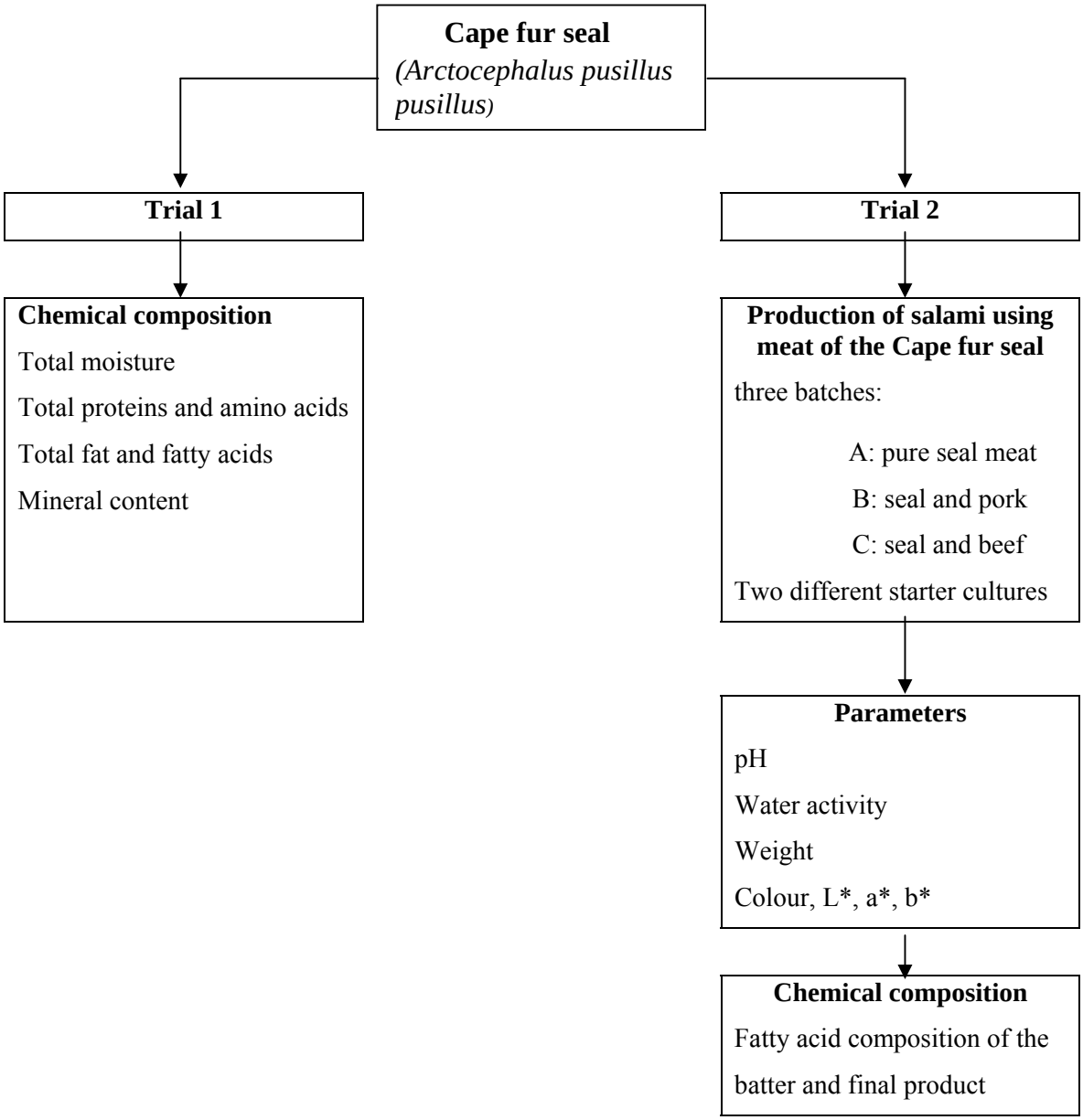

Figure 1. Investigation of the meat of the Cape fur seal and its potential for the production of traditional salami.

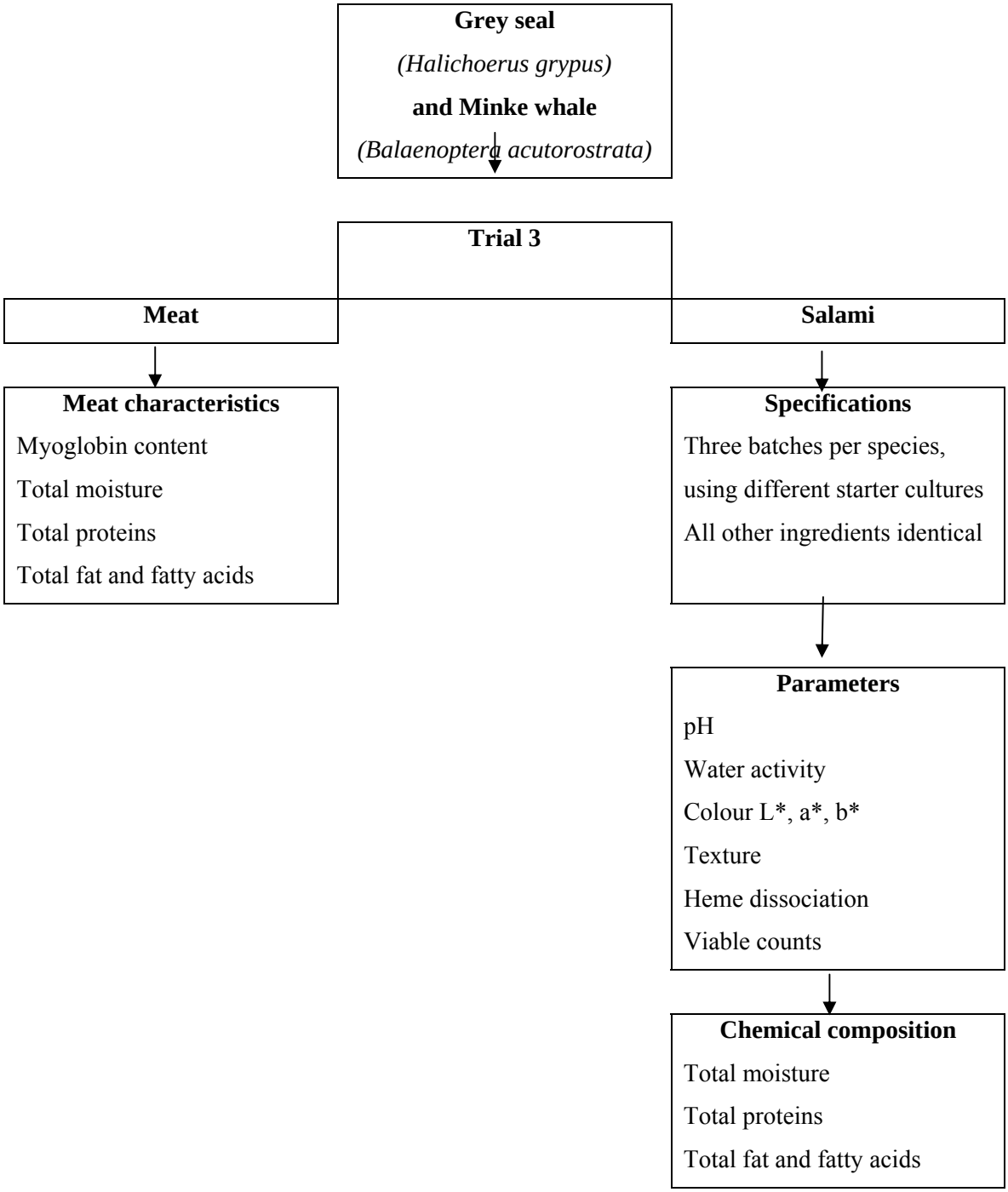

Figure 2. Investigation of the meat of the Grey seal and Minke whale and their potential for the production of traditional salami.

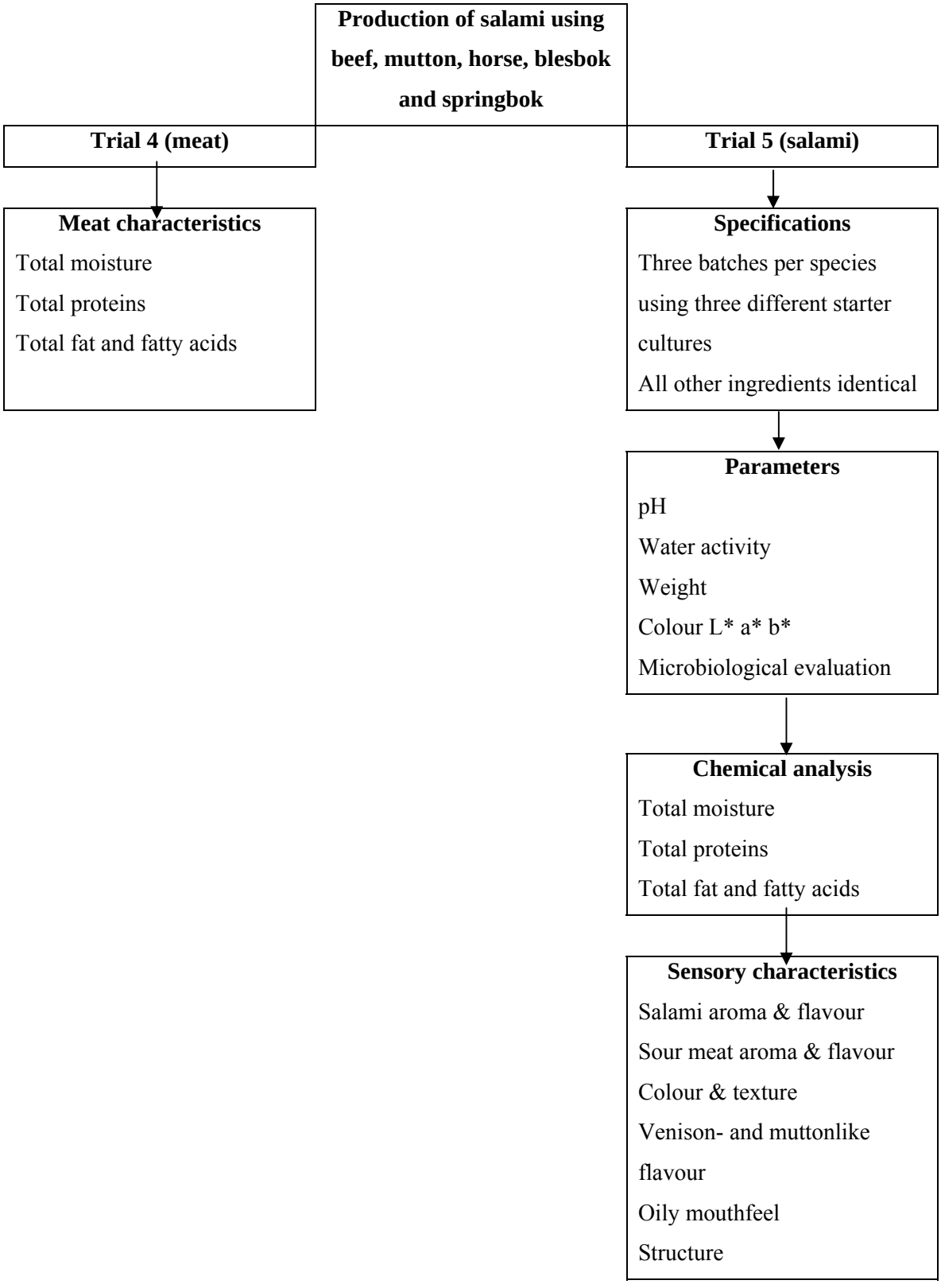

Figure 3. Investigation of the meat of horse, beef, mutton, blesbok and springbok and the potential for the production of salami.

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Chapter 2

Literature review

1. INTRODUCTION

Judging by archaeological findings and cave paintings across the globe, as well as orally transmitted accounts of religious ceremonies and legends from numerous cultures, meat seems to have formed part of mankind's staple diet since pre-historic times. Humans have consumed a very wide range of animals in one form or another, drawing on both wild and domestic groups of mammals, poultry and fish to fulfill their essential requirements for protein, calories and fats. Over time, mans' consumption of this natural resource has evolved considerably; and agricultural activity has impacted on the production, management and storage of the food sources, resulting in a proliferation of meat products. In recent times, as the processing of animal flesh has become more sophisticated, varied and complex, it is becoming more difficult to identify specific species in meat products. Cuts are either displayed as fresh or frozen, processed to varying degrees (sous-vide, marinated, dried, smoked, salted, etc.), and prepared ready-to-eat products are becoming increasingly available.

Over time, economic and social changes across civilisations have led to nutritional demands being transformed and modified substantially. As a result it is becoming more acceptable to purchase alternative sources of red meat as opposed to products from traditional red meat animals. Tastes and preferences have changed and nowadays consumers favour meat that is genuine, tasty, rich in protein and low in lipid and cholesterol content. Various alternatives are being explored to meet this new set of consumer demands.

This presents a challenge to meat producers and scientists, as meat production potential, in general, depends on the growth and development of an animal, whereas carcass quality is mainly influenced by the distribution of muscle, fat and bone in the body. Muscle, and to a lesser extent fat, are the major edible tissues of the carcass; bone being a non-edible tissue, with its proportions in the carcass affecting the proportions of muscle and fat (Mahgoub & Lu, 1998).

Increasing consumer interest in non-traditional meat has shifted the interest to investigating the production potential and processing ability of species such as seal, horse and certain game animals, e.g. blesbok and springbok. Some of these animals are not farmed intensively, and have

had no direct contact with humans. This implies that their bodies do not contain chemical substances that could have been administered by human beings. Comparisons need to be drawn between the meat of these species and that of conventional red meat species, such as beef and mutton, with regard to quality, palatability and desirability in terms of changing consumer tastes. Consumers nowadays also demand access to ready-to-eat, fast foods, resulting in processed foods becoming increasingly popular, and thus the use of alternative red meat species in these processed products is of additional and particular interest to the meat industry. As processed meat products usually contain the lower valued cuts of a carcass, optimal carcass utilisation can be realised by making use of these sections of meat. This fact also addresses cost-related concerns of meat producers.

In response to these changing expectations and desires from the side of the end-user, cured, fermented and dried products from different species (Paleari *et al*., 2000) have recently appeared on the market and are being sold, alongside traditional red meat products. Venison products are being sold worldwide. However, the supply of this meat source is dependent to some extent on the seasonal hunting and harvesting times in different parts of the globe.

Commercial harvesting of game within South Africa is an annual occurrence, and alternative products need to be researched in utilising this meat. The harvesting of seals in Namibia is set to continue, and an alternative use of the carcass is called for. At present, not all the meat of these animals is being utilised. The abundance of seals in their territorial Norwegian waters is leading to the legalisation of commercial harvesting of these animals and the controlled harvesting of the Minke whale has been resumed (Björndal & Conrad, 1998). Most of these mammalian species are harvested seasonally, and therefore the meat is only available for limited periods of time. Products need to be developed that are shelf stable, to provide a year-round supply of the meat. Salami, a fermented sausage, is such a product.

Traditional salami has a pH of less than 5.0 and a water activity of less than 0.9, which results in it being shelf stable at ambient temperatures.

2. POTENTIAL PRODUCTION OF SALAMI FROM ALTERNATIVE RED MEAT

Comminuted raw meats, which are fermented into various sausages, require the addition of salt, nitrate/nitrite, and specific lactic-acid bacteria as starter cultures. If fermented correctly, pathogens will be destroyed or their numbers decreased to improve the quality of the product and increase its shelf life. In addition, desirable sensory properties develop, such as the cured pink colour, distinct flavour and firm texture, characteristic of this type of product (Holzapfel, 1998).

2.1. Background to salami

Salami is defined as a mixture of meat and fat particles containing salt (NaCl), curing agents, and spices stuffed into either artificial or natural casings. Starter cultures are used to aid in the fermentation and maturation of the sausage. Fermentation processes were developed to enhance the production of dried meats at a time when drying was the only means of preservation. It is thought that fermentation was first used by the Chinese approximately 2 000 years ago, with salt and nitrate being introduced around the 13th century. The name salami is derived from the Latin word – *sale*, meaning salt (Campbell-Platt, 1995).

A large variety of salami has been developed worldwide. A common basis for classifying fermented sausages is the length of processing, final water content and water activity (a_w) level. Based on these criteria, salami is divided into the following categories: spreadable; sliceable, short processed; and sliceable, long processed (Table 1).

Table 1

Classification of fermented sausages adapted from Campbell-Platt (1987)

	Spreadable	Sliceable, short processed	Sliceable, long processed
Process lggength	3-5 days	1– 4weeks	12-14 weeks
Final water content	34-42%	30–40%	20-30%
Final a_w level	0.95 – 0.96	0.92 – 0.94	0.82 – 0.86
Examples	Teewurst, Mettwurst (Germany)	Summer sausage (US), Thuringer (Germany)	Salami, Saucisson (France), Chorizo (Spain)

2.2. Ingredients

An appropriate selection of meat ingredients is very important for the production of sausages of uniform quality. Salami traditionally contains 50-70% lean meat, and any meat species can be used for the production of sausages. Pork is favoured in countries such as Europe, the USA and China. Elsewhere in the world, mutton and beef are the preferred meat species. Chicken and

other poultry meat is also used in the production of fermented sausages. Meat used in the production of salami should be suitable in terms of water-holding capacity, pH value and colour. When pork is used, the initial pH value should be between 5.6 and 6.0, which is lower than that of other red meat species.

After drying, the final product may contain up to 50% fat, which makes fat an important ingredient of fermented sausages. This may lead to problems such as rancidity, which has a direct effect on the shelf life of the product. As cited by Morrissey *et al*. (1998), the relative oxidation rates of fatty acids containing 1, 2, 3, 4, 5 or 6 double bonds are 0.025, 1, 2, 4, 6 and 8, respectively (Horwitz, 1986). Therefore, both palmitoleic and stearic acid would be more susceptible to oxidation than would fatty acids containing no double bonds. The changes in quality are realised by adverse changes in flavour, colour, texture as well as nutritive value, and the possible production of toxic compounds (Gray, Goma & Buckley, 1996; Morrissey, *et al.*, 1998). However, oxidation is vital in developing desirable flavours of dry cured ham or fermented sausages (Chizzolini, Novelli & Zanardi, 1998; Ladikos & Lougovois, 1990). During sausage processing a few factors influence the rate of lipid oxidation. These are the actual composition of raw meats, the grinding action and the addition of exogenous components such as salt, nitrite, spices and antioxidants (Kanner, 1994).

Many substances can be added to act as anti-oxidants. These include ascorbic acid/ascorbate, which also acts as a curing agent and stabilises the colour, and tocopherol, used due to its different properties compared to ascorbic acid.

Eight vitamins of vitamin E occur in nature, of which four are tocopherols. The other four are tocotrienols (Ball, 1996). These compounds consist of substituted ring systems linked to a phytyl side chain. The four main forms are designated alpha, beta, delta and gamma, depending on the number and position of the methyl groups on the chromanol ring. The acetate ester of α-tocopherol, instead of the free alcohol, is used in food products due to its greater stability (Ball, 1996).

The alkyl radical (R) is overactive in an aerobic environment for any competing species to successfully reduce R to RH before oxygen is added to establish the peroxy radical (ROO). In this form, ROO is quite a stable free radical, which reacts slowly with target polyunsaturated fatty acids (PUFA). This is the accepted point of action for free-radical scavenging antioxidants such as phenolic tocopherol. Tocopherol is able to reduce ROO to ROOH so easily that tocopherol is competitive with biologically sensitive targets such as the unsaturated lipids, RH,

even at levels 10 000 times lower (Branen *et al*., 2002). Tocopherols are soluble in lipids and in aqueous solutions. Therefore, tocopherol is an important tool in controlling rancidity in products with a high fat (PUFA) content.

As the diet of aquatic mammals consists mainly of fish, their tissues contain more PUFA than the tissues of their terrestrial counterparts. Although these fatty acids are very popular in health conscious consumers, they are a challenge in the food industry when it comes to preventing rancidity of products and thereby increasing the shelf life of products.

Salt (NaCl) is generally added to the batter at a concentration of 2.5 – 3.0%, salt being the single most critical non-meat ingredient. Salt is added to extract the soluble proteins from the meat particles, thereby forming an adhesive film which aids in the cohesion with the other ingredients of the sausage. Nitrite is also added regularly and is an important factor in the formation of the colour of many fermented meat products and the retarding of lipid oxidation. The maximum level of nitrite allowed in sausages is between 120 and 150ppm (Campbell-Platt, 1987). Both NaCl and nitrite play vital roles in creating a favourable environment for the development of lactic acid bacteria in the early stages of fermentation and retard the growth of undesirable micro-organisms.

Commonly used spices in the production of salami globally are cumin seed, fennel seed, dried garlic, mustard, and pepper. In some countries, producers may also use other ingredients such as: red wine, berry extracts, and artificial flavourants to influence flavour and mask unpleasant flavours (Arboles & Julia, 1992).

Commercially produced starter cultures from various bacterial strains are used in the production of fermented sausages, and are usually added to the batter shortly after the salt, in a liquid form, to ensure equal distribution of the bacteria.

2.3. Use of starter cultures

The first commercially used meat starter culture in the United States, in 1957, comprised a single strain of *Pediococcus acidilactici* (Niven *et al*., 1959; Everson *et al.,* 1970). The first starter culture in Europe was also made up of a single strain of *Kocuria*, named M53 (Niinivaara *et al*., 1964). Thereafter, a variety of combinations of starter cultures were developed to produce a broad spectrum of metabolic properties.

At present almost all commercial production of fermented sausages is carried out with the use of selected starter cultures or inoculants. Starter cultures are available as either a frozen concentrates or lyophilised dry powder. Commercially used starter cultures are usually cocktails of two or more different micro-organisms and sometimes even different strains of the same species. The most commonly used cultures are strains of homofermentative *Lactobacillus* spp., *Pediococcus* spp., *Lactococcus* spp., and *Micrococcus* spp. (used to reduce nitrate to nitrite). Specific examples are *Lactobacillus plantarum, Pediococcus acidilactici* and *Lactococcus lactis* subspp. *lactis* (Xiong & Mikel, 2001).

i) Lactic acid bacteria

The two *Lactobacillus* spp. most commonly used are *Lactobacillus sakei* and *Lactobacillus curvatus*. These are psychotrophic, with optimal growth at approximately 30^{o}C, which makes them favourable in European conditions. *L. plantarum* and *Pediococcus* spp. are mesophilic, with optimal growth temperatures between 30 and 35^{o}C, which renders them more favourable in countries with a warmer climate.

There are certain criteria that lactic acid bacteria starter cultures should meet when being included in food products (see Table 2). Various species have been, and are, used as inoculants in sausage fermentations, of which *L. plantarum, L. curvatus, Pediococcus damnosus* and *P. acidilactici* are the most popular (Campbell-Platt, 1987).

The fermentation of carbohydrates leads to the following desirable results: (a) production of organic acids, which lead to the reduced pH value; (b) production of favourable organoleptic compounds; (c) coagulation of meat proteins, thereby decreasing the water holding capacity and thus, facilitating the drying process, which affects the texture and firmness of the end product, and (d) red colour formation due to the reaction of nitrogen monoxide with the heme group in myoglobin (pH 5.4 to 5.5).

The synthesis of bacteriocins acting against undesirable micro-organisms is a vital property, which is desired when selecting starter cultures for the fermentation of meat products. Bacteriocinogenic strains of *L. sakei, L. curvatus, L. plantarum,* and *Pediococcus* spp. are known, but are not extensively used in commercial practices.

Table 2

Criteria characteristic of lactic acid bacteria starter cultures used in sausage fermentations (Campbell-Platt, 1987)

1	Compete effectively with indigenous lactic acid bacteria.
2	Produce adequate quantities of lactic acid.
3	Grow in the presence of up to 6% NaCl.
4	Tolerate $NaNO_2$ at concentrations of up to 100mg/kg.
5	Grow between 15 ^{o}C and 40^{o}C, with an optimum between 30 ^{o}C and 37^{o}C.
6	Homo-fermentative.
7	Non-proteolytic.
8	Do not produce large quantities of H_2O_2.
9	Catalase-positive.
10	Reduce nitrate.
11	Enhance flavour of the finished sausage.
12	Do not produce biogenic amines.
13	Do not produce slime.
14	Antagonistic to pathogenic and other undesirable micro-organisms.
15	Tolerant of, or synergistic with, other starter components.

ii) Micrococcaceae

Selected strains of *Kocuria* and *Staphylococcus* spp are used as starter cultures. Due to the metabolic activity of *Staphylococcu*s under anaerobic conditions, it is more competitive than *Kocuria*. Colour formation and stabilisation, as well as aroma development, are the primary effects these bacteria have on fermenting sausages. This is due to their catalase and nitrate and nitrite reductase activities, a process that has been researched by Neubauer & Goetz (1996).

However, *micrococcacea* are not proteolytically active and play a more active role in lipid metabolism and the generation of volatile aromatic components (Johansson *et al.,* 1993; Stahnke, 1995).

2.4. Preparation of fermented sausage batter

There are two main factors that need to be taken into account in the preparation of the batter of these sausages: 1) the need for the sausage to lose water easily during drying, and 2) the high fat content of the batter.

Lean meat is usually cut to an average diameter of 3mm at temperatures between –4°C and slightly above 0°C, to prevent water from binding. The fat is cut while still frozen, at a temperature of *ca.* -8°C. This temperature is required to prevent smearing and the coating of the meat particles with a layer of fat, which would lead to a decreased water loss from the particles. Salts, spices and other additives are then added, with special attention being given to the equal distribution of each into the batter.

The batter is then stuffed into casings, which can be of varying diameters and made from different materials such as artificial cellulose or natural intestine. The casing must allow evaporation of water, penetration of smoke and shrinkage during drying. It is advisable to keep the temperature of the batter during stuffing below 1°C, to avoid smearing of the fat (Price & Schweigert, 1987).

2.5. Fermentation of sausage

During fermentation, active growth and metabolism of lactic acid bacteria is encouraged, and this leads to a decrease in pH. The major role of lactic acid bacteria in the sausage is to produce organic acids, primarily lactic acid, from carbohydrates, via the Embden-Meyerhof pathway (Campbell-Platt, 1987). This results in a decrease in pH and contributes to the retardation of the development of undesirable micro-organisms. The decreased pH also leads to a reduced water-holding capacity of proteins and ensures correct desiccation of the product. To achieve this, *ca*. 5% of a carbohydrate source, e.g. dextrose, is added to the mixture to serve as an energy source (Campbell-Platt, 1987).

When fermentation proceeds correctly, growth of lactic acid bacteria is rapid, and levels of 10^6–10^8 cfu (colony forming units)/g are reached after 2–5 days of fermentation. The decrease in pH leads to the destruction of *Pseudomonas* and other acid-sensitive Gram-negative bacteria within 2–3 days, although more acid tolerant genera, including *Salmonella*, can persist for longer periods. The amount of lactic acid bacteria found in the sausage declines after reaching the peak. The course of fermentation due to inoculations of starter cultures of lactic acid bacteria is essentially the same as that of natural fermentation, the one advantage being that lactic acid bacteria may dominate earlier in the process (Campbell-Platt, 1987).

2.6. Drying and maturation of sausage

The ripening and drying time of fermenting sausage depends on the type of starter cultures used, as well as the type of product. The period allowed for the product to ripen also depends on the diameter of the sausage, and can take anything from 20 days to 3 months. There are three

distinct groups of fermented sausages (Flores, 1997): (a) rapid (<7 days), (b) regular (approximately 3 weeks), and (c) slow (up to 3 to 4 months).

Rapid ripening systems, which use controlled drying chambers and improved starter cultures, are increasingly replacing traditional fermentation technology. Although the reduced fermentation time is of advantage to the producer, rapidly ripened sausages tend to have an intense acid flavour (Sanz *et al.,* 1998), which is not acceptable to all consumers (Arboles & Julia, 1992).

The physio-chemical and sensory qualities of fermented sausage rely heavily on the extent of drying of the product. An important factor when drying sausage is that the rate of moisture loss from the surface should be equal to the rate at which moisture moves from the sausage interior. Rapid drying is only possible when the pH value is low and the correspondingly low solubility of proteins enables moisture loss. The drying of a sausage also depends on the diameter of the sausage. Drying is executed at low temperatures, normally below 20^{o}C (Flores, 1997).

Smoke is applied to many types of semi-dry sausages during the drying stage. The results of smoke application to meat products can be summed up as: improved flavour and colour enhancement, antimicrobial, and anti-oxidising actions (Price & Schweigert, 1987). The main reason for smoking meat is to preserve it, but smoking also provides flavour and colour. Smoke is also applied to inhibit mould growth by drying the surface and by the deposition of antimicrobial phenols, carbonyls and low molecular weight organic acids. Phenolic compounds decrease the extent of fat oxidation. Smoking also affects the organoleptic qualities of the sausage. The pyrolysis of cellulose and hemicellulose in the casing of the fermenting sausage produces carbonyls (Price & Schweigert, 1987). These are important in the development of colour of the meat when smoked, as the carbonyls are absorbed into the surface of the sausage. The reaction between carbonyls and amino groups is similar to the Maillard reaction, and is enhanced by the increase in temperature and dryness of the product (Gilbert & Knowles, 1975).

Two types of smoking are considered in the production of fermented sausages, i.e. hot and/or cold smoke application. During the hot smoke application, smoke is applied during the thermal processing of the product. Cold smoking is usually used for products that have undergone thermal processing or require only low levels of thermal processing. Salami, which falls under the category of a dry fermented sausage, undergoes low levels of thermal processing (Campbell-Platt, 1978).

2.7. Chemical and physical changes during production of fermented sausages

Lactic acid production and the consequent drop in pH influence the organoleptic properties of fermented sausages. Lactic acid results in a 'tangy', acidic characteristic. Lactic acid has the potential of enhancing saltiness and the low pH values may change flavour by restricting proteolytic and lipolytic enzyme activity (Lücke, 1986).

As the consistency of the sausage relies upon the pH and the water activity (a_w) level, these factors need to be closely monitored to ensure optimum consistency. A pH value below 5.4 and a_w level of less than 0.9 are required. The a_w is a measure of the partial vapour pressure of the foodstuffs, compared to that of pure water at its surface. Water molecules are loosely orientated in pure liquid water and can easily rearrange. When solutes such as NaCl are introduced to aid in the reduction of water activity, they orientate the water molecules around them and make them less available for use by micro-organisms. Reduction of the a_w level during drying is a function of water loss and increase in solute concentration (Campbell-Platt & Cook, 1995). A decrease in a_w will inhibit the growth of undesirable micro-organisms, and therefore rapid drying of the product is desirable. Lu & Townsend (1973), for example, removed as much water as possible from meat prior to fermentation by freeze-drying it. As water is required for fermentation, this strategy did not produce suitable sausage. Other strategies to reduce water activity levels are by reducing the water binding capacity of the batter by adding Pale, Soft, Exudative (PSE) meat (Townsend *et al.,* 1980). Chin *et al*. (1995) reduced the drying time period by up to 30%, regardless of environmental temperatures (17, 19 and 22^{o}C), by drying pepperoni sausages under vacuum.

The a_w level of fresh sausage batter is high, dependant on solute concentration and fat content. This condition is favourable for the development of *Micrococcus* or *Staphylococcus* in the first stages of fermentation, but is enough to inhibit other micro-organisms. A decrease in the a_w level during drying is caused by loss of water and an increased solute content (Campbell-Platt, 1978).

Decreased solubility of proteins is due to pH decrease, leading to an increased gel forming ability, which is enhanced by the addition of NaCl. Myofibrillar proteins show the largest decline in solubility at low pH values and can be considered more important with regards to consistency than sarcoplasmic proteins (Gilbert & Knowles, 1975).

The flavour of fermented sausages is most likely due to the free fatty acids and carbonyl compounds. Formation of these is aided by lipases. In many sausages intense lipolysis occurs during ripening. As lipolysis continues, a decrease in triacylglycerol fatty acids corresponds to

an increase in diacylglycerols, free fatty acids and, to a lesser extent, monoacylglycerols. Higher temperatures increase lipolytic activity, although apparent lipolytic activity drops. This is brought about by temperature dependant feedback inhibition of lipases by free fatty acids (Lücke, 1986).

Unsaturated fatty acids are exclusively involved in oxidative changes in the fat of fermented sausages. Oxidation leads to the formation of lipid peroxides and carbonyl compounds, which are linked to an increased peroxide value. An increased peroxide value is highest when *Lactobacillus* is present (Horwitz, 1986).

An increased rate of auto oxidation of myoglobin to metmyoglobin is brought about by the low pH value, which destabilises myoglobin. The heme group of the pigment dissociates at the low pH of fermented sausages and the observed colour is primarily due to the formation of nitrosomyoglobin or nitroso-heme (Slinde & Nordal, 1978).

According to Verplaetse (1994) there are internal and external factors influencing flavour development. Internal factors are said to be chemical or microbiological, the external being the physical, such as the climatic conditions throughout the process.

Apart from the effects of spices and salts, carbohydrate, lipid and protein catabolism results in the flavour associated with fermentative sausages due to the action of microbial and endogenous meat enzymes (Toldra *et al*., 1998). Other reactions, such as auto oxidation, also form flavour components without direct enzymatic participation (Toldra *et al*., 1998).

The HACCP (Hazard Analysis Critical Control Point) approach is used to locate certain points throughout the process, which are critical for optimum food safety. These points are based on the hygiene risks, which form part of any production chain. One of the main factors in the control and optimisation of fermented sausage production is pH. Bello & Sanchez-Fuertes (1996) came up with a mathematical model (see equation below) to describe the acidification occurring during the ripening of dry fermented sausage. This model could be applied to large scale production units, where it could be used on random samples to suggest how the process is continuing.
The following equation is applied:

$$Y = Y_o - k^*_1 e^{\{-e[-k^*2(x-k3)]\}} + k^*_4 e^{\{-e[-k^*5(x-k6)]\}}$$

where: Yo initial value of pH at the beginning of the ripening

x time of curing process (days)

k_1 reduction of pH from an initial level to minimum value reached

k_2 rate of reduction with relation to pH

k_3 time at which the decline of pH is maximum

k_4 increase of pH from the minimum value to the level at which the sausage experiences last day of ripening

k_5 rate of relative increment to pH

k_6 time in which the increment of velocity of pH is maximum

Dry fermented sausage is a very popular product on the international market and differences exist in each country as to which type of fermented sausage is preferred. However, the main production factors,, i.e. pH, water activity and sensory qualities, remain the same (Arboles & Julia, 1992).

Technological advances are constantly being made to ensure a product that is safe and acceptable to the consumer. The development of new techniques, such as the measurement of pH, water activity, colour and microbiological assessments of any meat product, lead to ongoing improvements in this industry. At the same time, however, the many meat-product recipes and methods that originated many years ago have to be taken into consideration when producing a traditional product. Considerably more scientific research is required with regard to the suitability of particular meat types for certain processing systems, and how these can be improved, to secure a continuous supply of consistently high quality meat products.

2.8. Composition and nutritive value of salami

Campbell-Platt (1987) suggested that salami should have the composition and nutritive values as listed in Table 3. These values are, however, dependant on the type and quantity of ingredients used in the processing of the products.

Table 3 shows that the moisture, protein and fat are the three most abundant ingredients in traditional salami. All these ingredients, but in particular the latter two, are determined by the type of meat and lard used. Pork fat is preferred as a fat source. Meat types may vary in quality (cut) and species. Alternative animal species are being used more often in the new millennium. Some species that have been identified in this investigation as having potential value are seal, whale, blesbok and springbok. These species are discussed in detail in the following section, as well as the more commonly used beef, horse and mutton.

Table 3

Suggested composition and nutritive value (per 100g) of traditional salami (Campbell-Platt, 1987)

Component	Mass
Moisture	24-35g
Protein	23-27g
Fat	53-57g
Carbohydrate	2-3g
Fiber	0
NaCl	5-7g
Energy	660-700kcal or 2.6-2.9MJ
Na	2500mg
K	210mg
Ca	70mg
Mg	18mg
P	220mg
Fe	1mg
Cu	0.3mg
Zn	2mg
S	170mg
Thiamin	0.3mg
Riboflavin	0.3mg
Niacin	6mg
Vitamin E	0.4mg
Vitamin C	0
Vitamin B_6	0.2mg
Pantothenic acid	1mg
Vitamin B_{12}	1μg
Biotin	4μg
Free folic acid	3μg
Total folic acid	4μg

3. ALTERNATIVE RED MEAT SPECIES

3.1. Aquatic mammals

3.1.1) Seals and sea lions

During the previous century many control programmes, aimed at reducing or limiting the numbers of seals or cetaceans, were implemented around the world. These activities took various forms, from bounties and culls, lasting several decades (e.g. a bounty on harbour seals *Phoca vitulina* in eastern and western Canada), to once-off hunts sponsored by governments (an open season on New Zealand fur seals *Arctocephalus forsteri* in 1946). Culls were implemented through existing commercial hunts. Authorities would subsidise a hunt to encourage it to take more animals than it otherwise would have, or a marine mammal harvest was managed with the aim of reducing, rather than merely maintaining, the marine mammal population. However, increasingly, these practices came under criticism from animal rights groups during the second half of the twentieth century (Vogel & Koch, 1992).

Seal products have traditionally comprised food delicacies and fur products made mainly from pelts. Oils have also been produced and used largely in commercial/industrial applications. However, this utilisation is changing, and presently a stronger emphasis is being placed on seal oils for human consumption and various uses are being found for seal meat. In the fashion industry a broader range of fur, leather and suede products is being manufactured from the pelts. This is a strong indication of the growing interest in the full utilisation of the animal.

In the late 1970's, it became apparent that many commercial fish stocks were being seriously endangered and suggestions were made to reduce populations perceived as competing with fisheries, to improve fisheries' yields. This occurred despite the influence of strong internationally based anti-harvesting groups. However, conservation and animal welfare organisations objected to such culls on ecological and ethical grounds, and there is still an ongoing difference of opinion among scientists about their potential effect (Butterworth et al., 1995).

As a result of these controversies, "sustainable utilisation" is presently defined as harvesting only the natural annual growth of a population, without depleting it to such a low level that the growth is greatly reduced (Butterworth *et al*., 1995). "Culling" is the term used when the aim is to reduce the population size. In both processes, animals of both sexes and all ages may be killed, but females may be targeted especially to achieve the fastest reduction in numbers.

According to Shahidi & Synowiecki (1993), the quality of seal meat is comparable to or better than that of other sources of animal protein, with its intramuscular lipids. The latter are present in the blubber and are an excellent source of omega-3 fatty acids. A myoglobin content of up to 10% provides the opportunity to utilise seal meat as a colour contributor in meat products utilising meats of lesser colour quality or potential (Synowiecki *et al*., 1992).

The level of iron in the meat is also especially high (Shahidi & Synowiecki, 1993), which makes it an even more desirable commodity, especially in countries where meat constitutes a large part of the population's diet. The main contributors to this absorbable dietary iron are haemoglobin and myoglobin. Heme iron is the form in which the human body most easily absorbs iron, as the availability of nonheme iron is affected by promoting and inhibiting components in the diet (Conrad *et al*., 1967). As seal meat is very rich in myoglobin and haemoglobin (Synowiecki *et al*., 1992), due to the need of the seal's body to carry more oxygen in the muscle than terrestrial mammals, it can be deduced that seal meat is a rich source of heme iron. One of the main hemoproteins in muscle is myoglobin, which serves as an oxygen reservoir in live animals. It is not only a source of iron, but also functions as a catalyst in the auto-oxidation of lipids (Shahidi & Hong, 1991).

Seal oil is very high in omega-3 fatty acids, which make it a valuable health commodity. In countries where the refining and purification of seal oil is not undertaken, it is used in industrial applications. Seal meat is very high in protein and is used in a variety of ways – including the manufacture of food products for human consumption (Shahidi & Synowiecki, 1993).

One of the major concerns regarding the utilisation of marine mammal tissue is the increasing threat of persistent organic pollutants (POPs) in many of the world's seal populations, such as in the Californian sea lions (*Zalophus californianus*), Wadden Sea harbour seals (*Phoca vitulina*), Baltic ringed seals (*Phoca hispida*) and Grey seals (*Halichoerus grypus*) (DeLong *et al.,* 1973; Reijnders, 1986).

POPs comprise a group of synthetic organic compounds, produced for industrial and agricultural purposes, or are by-products of other industrial processes. Many of these compounds are found in the high-lipid organs of animals, as they are fat-soluble (lipophilic) and continual; they therefore accumulate efficiently in the thick blubber layers of marine mammals (Tanabe *et al*.,

1988). Large amounts of POPs are also transferred to young via the very rich milk of their mothers (Addison & Brodie, 1987).

Polychlorinated biophenyls (PCBs) and 1,1,1-trichloro-2,2,-bis[p-chlorophenyl] ethanes (DDTs), especially, are regarded as environmental pollutants due to their chemical stability and persistence in the environment. The levels of these undesirable compounds are especially high in marine mammals, as these are situated at the apex of the aquatic food web and the concentrations of these compounds increase along the food chain (Nyman *et al.,* 2002). This accumulation of POPs along the food chain is highly dependent on the ability of the various organisms to metabolise them. Marine mammals are thought to be less efficient in metabolising POPs than other aquatic organisms (Tanabe *et al*., 1988). In humans, these chemicals have been known to cause genetic mutations and therefore defects and abnormalities in appearance and behaviour.

Pathological changes and various abnormalities, such as an increased disposition to infectious disease, have been associated with abnormally high POP levels in wild marine mammal populations (Bergman *et al*., 1992; Mortensen *et al*., 1992; Olssen *et al*., 1994; Jepson *et al*., 1999). Literature studies on harbour seals have revealed that, although there is no evidence that contaminants directly cause mortality in any marine mammal population, there are negative effects on reproduction, the immune system, hormonal and vitamin A status (Reijnders, 1986; Brouwer *et al*., 1989).

In the aquatic environment, trace elements such as mercury and cadmium, derived from natural or anthropogenic sources, are known to bioaccumulate along the food chain (Dietz *et al*., 1996). High levels of these metals have been observed in marine mammals, while levels of other metals are usually low (AMAP, 1998).

Many metals occur in large quantities in biota, and many of these are essential for the organism. Others have no known biological function, and some of these may be harmful to the exposed organism. In studies on metals in marine mammals, mercury, cadmium and lead have been a major focus due to their known danger and toxicity to humans and other mammals (AMAP, 1998; O'Shea, 1999). However, their concentrations have been below levels that are toxicologically relevant or no associated effects have been observed (AMAP, 1998).

However, since the ban of PCBs and DDTs in countries around the Baltic Sea, their levels, especially those of DDTs, have decreased in this ecosystem (Bignert *et al*., 1998) and the

concentration of these chemicals in marine animals has decreased accordingly, contributing towards its quality and desirability as a potential source of protein.

i) The Cape fur seal (*Arctocephalus pusillus pusillus*)

Arctocephalus pusillus pusillus, the South African fur seal, is found along the coasts of Southern Africa and Namibia only. It is the sole resident seal species in Southern Africa. Breeding colonies are distributed from Black Rocks (33°50'S, 26°16E) in Algoa Bay, on the southeast coast near Port Elizabeth in South Africa, all the way to Cape Cross (21°46'S, 13°58E) in Namibia. The species range does, however, extend further north to Cape Frio (18°26'S, 12 °00E), near the northern border of Namibia with Angola.

The Cape fur seal is a marine mammal that is equally at home on land or sea. Males of this species are an average of 2.3m in length and weigh from 200 to 350kg. Their coat is grey or black in colour and lighter on the underside (Schliemann, 1990). Female Cape fur seals are smaller, weighing an average of 120kg and measuring an average of 1.8m long. Their coats are brown with lighter shading on the underside (Schliemann, 1990).

Fur seal harvesting is one of the oldest 'fishing' practices in South Africa. The Dutch were the first commercial sealers and killed approximately 45 000 Cape fur seals in 1610 alone (Shaughnessy & Best, 1981). The European settlement at the Cape in 1652 resulted in most of the seals in and around Table Bay being destroyed (Shaughnessy & Best, 1982). British and American sealers moved in around the late 18th and early 19th centuries and exploited west coast seal herds (Best & Shaughnessy, 1979; Shaughnessy & Best, 1982). Up to and including the turn of the 20th century, *Arctocephalus pusillus pusillus* were almost extinct, as about 23 colonies had been exterminated and the total number of animals on the colonial islands was reduced to approximately 20 000 (Shaughnessy & Best, 1982).

In the early 1980s, four mainland colonies, out of a total of 53 colonies around Southern Africa's vast coastline, remained and consequently produced 75% of all pups presently born in Southern Africa. This was due to extensive culling of the animals, which then moved and re-established themselves elsewhere. Whilst the mass movement of hundreds of thousands of seals was taking place, 54% of the known breeding colonies became extinct. At this time, seal pup fur was a most profitable commodity and the well-known Cape Fur seal officially became the South African fur seal. This has meant that 20 out of an original 37 offshore island colonies have become entirely extinct over the past 60 years. Currently, 97% of all Southern African Fur seals are found on the

west coast and a mere 3% on the east coast of South Africa. Cape fur seals in South Africa are protected by Appendix II of CITES.

The population is thought to be 1.5–2 million animals, of which 67% are found along the coast of Namibia (Butterworth & Wickens, 1990). Of the 25 breeding colonies, six are on the mainland and the others on small islands. Of the ten haul-out sites, only four are on islands (Oosthuizen, 1991). According to Butterworth *et al.* (1988), the population increases at a rate of 4% per annum.

Harvesting is an economically driven activity, by which specific age classes are selected for use in the manufacture of products. In the case of seals, adult cows have no economic value, leaving only pups and bulls to be killed, the pups for their pelts and the bulls primarily for their genitals, which fetch high prices in the Far East as aphrodisiacs, as well as for their leather and their oil.

'Common-sense' arguments that culling seals will benefit the fisheries are based on mythological views of predators, which have been unsubstantiated by most scientific evidence. Research conducted in other fisheries has indicated that the complexity of marine food webs, as well as the diversity of seal diets, could mean that increased seal numbers may lead to positive effects on commercial fish stocks.

In the South Atlantic, South African fisheries biologists and the fishing industry were concerned that Cape fur seals were depleting the hake *Merluccius* spp. stock. Initially, there was a call for the culling of seals. The policy was reversed, however, when it was realised that the seals were involved in a complex relationship which could well be increasing hake production (Butterworth *et al.* 1988; Anonymous, 1991; Punt, 1994).

For the past five years, the annual Namibian quota for the culling of the Cape fur seal has been 30 000 pups and 3 500 bulls. The harvesting season extends from September to the end of November, when the pups are at the point of being weaned from their mothers, who are pregnant with the next pups at that time. These quotas are scientifically reviewed every five years, and are based on annual seal population counts. Many more seals are hunted, illegally, by fishermen, and these approximate numbers are also taken into consideration in the annual quota. As the harvesting is destined to continue, research is required in order to make it a more acceptable practice (W. Burger PO Box 955, Luderitz, Namibia, personal communication).

ii) The Grey (Havert) seal (*Halichoerus grypus*)

The Grey seal is classified as being a part of the Mammalia class, of the order pinnipedia and belongs to the family *phocidae*. It is a large seal, whose diet consists primarily of fish and which lives in the coastal seas of the temperate North Atlantic. It is believed that approximately fifty percent of the global Grey seal population is found in British waters, and the western North Sea (De Jong *et al*., 1997).

Adult males in the eastern population can measure up to 2.5m in length, and have a weight of up to 310kg. The females measure up to 2.1m and weigh up to 180kg. Males are known to reach sexual maturity between 4 and 6 years, and females between 3 and 5 years.

This seal species was, in historical times, indigenous to the south-eastern North Sea, but population numbers gradually decreased from the early middle ages, due to increased human predation (Reijnders *et al*., 1995). It occurs on both sides of the Atlantic Ocean, and is divided into three populations. The western Atlantic population occurs in the Canadian Maritime Provinces, the eastern Atlantic population is found around the coasts of the UK and Ireland, and on the coasts of the Faroe Islands, Iceland, Norway, and northwestern Russia as far as the White Sea. The third population is located in the Baltic Sea and is quite distinct from the Atlantic population (Seal Conservation Society, 2000).

Estimated numbers of these populations are: 150 000 in the western Atlantic population, 130 000-140 000 in the eastern Atlantic population, and approximately 75 000 in the Baltic Sea. This produces a total estimate of 290 000-300 000 Grey seals. This seal species is listed as an Appendix III species under the Bern convention.

The Grey seal is closely related to the Harbour seal (*Phoca v. vitulina),* with one major difference being that the pups of the Harbour seal are born and reared on temporarily flooded ground, whereas those of the Grey seal are more like the pups of the ice-breeding arctic phocids that bear immature, white-coated pups, which require a permanently dry area to mature (McLaren, 1975). Grey seals breed most frequently on sandy or rocky shores, but can also be found on various kinds of sea ice (Riedman, 1990).

One factor which is extremely relevant to the survival and ecology of these seals in the south-eastern North Sea, specifically, is the shortage of adequate breeding habitat, an area which is undisturbed, permanently dry and in close proximity to open water. At present, pups are born and reared in areas that are prone to being flooded (t'Hart *et al*., 1988; Vogel & Koch, 1992).

The Grey seal is not known to travel far distances; most of its travel is directed towards haul-out sites and following food (McConnell *et al.,* 1999).

This species is also regarded as being responsible for depleting fish stocks in its habitat. Although large-scale commercial harvesting of the seal has not occurred in recent years, there are frequent calls for the culling of Grey seals, particularly in Canada, the UK and Ireland, based primarily on commercial fishing interests. Another argument in favour of the culling of these animals is the fact that the Grey seal is a known host to the codworm parasite, causing large-scale codworm infestation in cod and flatfish stocks (McLaren, 1975).

Apart from this, Grey seals are frequently shot by fishermen to prevent them from doing damage to nets, traps and catch, and this form of shooting is legal in most countries within the Grey seal's domain. Marine debris also causes major problems to the seals due to entanglement. Furthermore, seals are prone to sporadic disease outbreaks, for example, many hundreds of seals died due to the epizootic of Phocine Distemper Virus in 1988, resulting in a 12% reduction in Grey seal pup production (Seal Conservation Society, 2000).

3.1.2) Minke whale (*Balaenoptera acutorostrata*)

According to Rice (1998), there are two species of Minke whale: the Northern Minke whale *Balaenoptera acutorostrata*, and the Antarctic Minke whale *Balaenoptera bonaerensis*. At least two subspecies of the Northern Minke whale occur: the North Atlantic form *B. acutorostrata acutorostrata* and the North Pacific form *B. acutorostrata scammoni.* The common name is derived from the Norwegian 'Minkehval'.

The Minke whale is the smallest member of the rorquals, with a body length of between 8 and 10m and a weight of 8-13.5t. It is stocky, but slender, with a small, narrow triangular head and pointed, paddle-like flippers. The dorsal fin is relatively tall, and sickle-shaped, and is set about two thirds of the way along the back. The body colour is dark slate-grey, with paler grey to white on the undersides and throat, on which there are between 50-70 grooves. Each flipper usually bears a bright white band, which is noticeably absent in the subspecies *acutorostrata bonaerensis*. There are 460-720 baleen plates per animal, the longest of which is 30cm in length (Stewart & Leatherwood, 1985).

The Minke whale is found along the polar ice-edge to the tropics, and is frequently encountered quite close to the coast. It is a non-migratory species, known to follow its food source, both inshore and offshore. This aquatic mammal inhabits polar, tropical and temperate waters,

moving according to the seasons to and from the North Atlantic in search of food, at times segregated into sexes (Stewart & Leatherwood, 1985).

Minke whales seem to feed very little in warm waters. In both the northern and the southern hemisphere, the preferred diet is euphausiids, although those in the former will also take shoaling fish and free-swimming molluscs. In the North Pacific, the whale also feeds on sand lance, sand eel, krill, salmon, capelin, mackerel, cod, herring and a number of other fish species. In the Antarctic, their main diet consists of krill (Stewart & Leatherwood, 1985).

The gestation period of the Minke whale is approximately 10 months. Calves are about 2.4-2.7m at birth. Sexual maturity in females takes place when they reach a length of 7.3m and in males when their body length is 6.7-7.0m, with the exact age at sexual maturity being unknown. Females can give birth every year and they will suckle their young for 4-5months.

This species of whale is seen as one of the more abundant ones, although exact stock sizes are disputed. The following estimates are available (Bjorndal & Conrad, 1998):

- North Pacific: 17 000-28 000. This stock is considered a *Protection Stock* by the International Whaling Commission (IWC), due to the high uncertainties in these estimates.
- Northeast Atlantic: 70 000-186 000. The IWC estimated this population at 90 000-135 000 in 1996. The central Atlantic stock in 1995 was estimated at 60 000, whilst a 1990 survey only estimated this same population at 28 000. The difference was attributed to a population increase, as well as improved census methods.
- Southern Hemisphere: 200 000-416 700.

Minke whales have been hunted in the North Atlantic, since the Middle Ages, when they were captured using nets. Modern whaling for Minke whales began in the 1920s, and between this time and 1973, the Norwegian fleet harvested approximately 94 000 animals. In the 1980s, a quota was set at 2 500 whales in the North Atlantic (Klinowska, 1991). Since the moratorium on commercial whaling was introduced in the 1985-1986 season, they have been harvested in reduced numbers under scientific permission. In June 1993, the commercial hunt for Minke whale by the Norwegians recommenced. The following table (Table 4) indicates actual numbers culled over the last 60 years:

Table 4

Annual harvest of Minke whales from the Northeast Atlantic stock, 1938-1997 (High North Alliance, 1998)

Year	Harvest	Year	Harvest	Year	Harvest
1938	1345	1958	4338	1978	1383
1939	915	1959	3062	1979	1786
1940	539	1960	3233	1980	1807
1941	2109	1961	3092	1981	1770
1942	2133	1962	2975	1982	1782
1943	1612	1963	3059	1983	1688
1944	1348	1964	2463	1984	630
1945	1782	1965	2114	1985	634
1946	1833	1966	1902	1986	298
1947	2556	1967	1758	1987	279
1948	3487	1968	1986	1988	29
1949	3840	1969	2014	1989	17
1950	1990	1970	1890	1990	5
1951	2751	1971	1799	1991	0
1952	3324	1972	2175	1992	92
1953	2433	1973	1558	1993	217
1954	3499	1974	1410	1994	273
1955	4309	1975	1426	1995	217
1956	3654	1976	1884	1996	388
1957	3624	1977	1698	1997	503

The hunt in 1993 involved the Minke whale stock in the Northeast Atlantic, which migrates along the coast into the Barents Sea. The average weight of these animals ranged from 1263.5kg to 1772.5kg. However, these whales were not specifically chosen for their size (Bjorndal & Conrad, 1998). The resumption of the harvest resulted from long discussions with the IWC that had met in Iceland (1991), in Scotland (1992) and in Japan (1993), resulting in the votes being in favour of the continuation of the moratorium on commercial whaling (Bjorndal & Conrad, 1998) on each occasion. The issue of whaling has become a managerial rather than an environmental one for the Norwegians.

As a result, interested countries such as Norway, Iceland and Japan have all undertaken programmes with regards to the science of whaling. This is done in accordance with the IWC schedule, in terms of which age, sex and the pregnancy status of the whale females which need to be determined. This type of information is vital to estimate mortality rates, age of sexual maturity, and the calving intervals of the females. The species under most scrutiny in these discussions was the Minke whale. Due to its high population numbers, it is regarded as a prime candidate for commercial harvesting (Bjorndal & Conrad, 1998).

In 1996, the IWC appointed an international group of scientists who reached a decision on the estimate, based on a total stock in 1995, of 112 000 Minke whales in the Northeast Atlantic, with confidence limits of 90 000 to 135 000 (High North Alliance, 1998). This study provided key information to the Norwegian government with regard to the decision to resume whaling and it rested on two assumptions:

1) that the Minke whale stock was abundant, and
2) that whaling could be undertaken on the basis of sustainable harvesting, which would eliminate the risk of extinction of the species.

The export of any whale products has been prohibited by Norway, as the Minke whale is a member of CITES Appendix II, and therefore all the meat has to be sold on the domestic market. This restriction has considerably reduced revenues, due to the higher price that meat, and especially the blubber, would have brought in Japan. There has been evidence to suggest that increases in the supply of whale meat would lead to a decrease in price, unless effective marketing strategies are employed. However, this situation would change dramatically, if the export market were to be reinstated.

3.2. Terrestrial mammals

3.2.1) Blesbok & springbok

Game farming in South Africa is becoming extremely popular, largely due to the increased popularity of ecotourism, and the high costs of feeding commercial livestock species. Many farmers have decided to move towards the farming of game species such as springbok, *Antidorcas marsupialis*, the blesbok, *Damaliscus dorcas phillipsi*, and the impala, *Aepyceros melampus.* There are various reasons for the fact that the South African meat market and export industry are experiencing an increasing demand for game meat, including: the different tastes of game meat (Pauw, 1993), the decline per capita supply of other high quality red meat (Onyango, Izumimoto & Kutima, 1998), and the low fat content of game meat (Schönfeldt, 1993).

According to Berry (1986), various uses are made of the wildlife present on farms through a number of activities, such as trophy hunting, non-trophy recreational hunting, live animal sales and venison (game meat) production. It is estimated that trophy hunting provides the greatest net return, followed by live animal sales, non-trophy recreational hunting and venison production. Since the contribution of game meat production (jerky hunters and game meat sales) constitutes 67.5% of the total income of the game industry of South Africa (Potgieter, 2001), research is required to investigate the possibility of utilising these species in processed products as well. Due to the increase of game farming and ecotourism, farmers invest in many

game species, but due to the absence of natural predators, population numbers have increased to such an extent that culling often becomes a necessity, as grazing and space are limiting factors on many farms.

The fat content of many red meat types is associated with health risks, projecting a negative image of meat to the consumer. There has been a global decrease in the per capita consumption of red meat, compared to that of white meat and other non-meat proteins (Schönfeldt, 1993). As the meat of wild animals, game, has a lower fat content (2-3g per 100g meat) compared to traditional red meat species, it may provide a suitable alternative for health conscious consumers (Schönfeldt, 1993). Seasonal harvesting of surplus animals of a specific species could provide a constant source of the meat on a larger and economically more profitable scale (Hoffman & Bigalke, 1999).

The springbok is a game animal of considerable importance with regard to its harvesting potential and, therefore, its impact on the meat industry (Rautenbach, 1980). It is presently the game animal that is the most extensively cropped in South Africa and, therefore, most research relating to South African game meat has been undertaken on this species. Their habitat preference is dry woodland and forest, montane communities, moist lowland, swamps, lakes and river edges (Talbot *et al.,* 1965). Springbok are not often found in dense bushveld like impala, making their cropping easier than that of other ungulates.

Despite the ease of obtaining game, it is important to consider that producing meat from wild ungulates or domestic livestock requires that the same factors, such as yield, chemical composition and meat quality, are taken into account, especially with regard to the demands related to potential export markets (Hoffman, 2000).

In 1996, for example, the sale of red meat in the U.K decreased by 11%. This might have been triggered by an increased general consumer awareness of the quality attributes of the red meat being purchased, and an increased specific awareness with regards to consuming infected or contaminated meat (Viljoen, 1999). *Bovine spongiform encephalopathy* (BSE), a chronic, degenerative disease that attacks the central nervous system of cattle, causing death in cattle and in humans, was a main contributor to this awareness. BSE is a disease transmitted to humans via the consumption of red meat. Another incident in Europe that led to a decrease in red meat consumption resulted from meat tainted with dioxin ending up in the food chain and on the consumer's table (Windhorst, 2001).

In the light of the above, and because South African wildlife holds no threat of possible BSE infection, it is also free of possible contaminates caused by pollution, and, can be classified as organic meat, the commercial potential of South African game products is very high.

According to van Zyl and Ferreira (2003), the ratio of protein in the carcass of blesbok and springbok antelope is higher than that of commercially-farmed species such as beef and mutton. It was also found that, compared to the meat of traditional red meat species, these game species have a better dressing percentage and more desirable lean meat production.

A dressing percentage of 56% can be attained by springbok of 12 weeks of age, of which approximately 83% of the carcass is lean, 13% bone and 4% fat. The growth curve of springbok begins to decrease at 28 weeks, but compared to domestic stock, it shows a continuous increase in carcass lean up to maturity, which peaks at 84% and 82% of the mature body weight in males and females respectively (Von la Chevallerie & Van Zyl, 1971). Maximum growth rate is attained before the animal is one year old, at which stage the mean mature body masses of 31.5kg and 27.1kg for males and females, respectively, are achieved.

The influence of the fat content on meat quality is of critical importance to the consumer. Springbok meat is low in saturated and high in poly-unsaturated fatty acids, and has low concentrations of two saturated fatty acids, namely myristic (C14:0) and palmitic (C16:0), which contain attributes contributing to increased serum cholesterol levels (Viljoen, 1999).

According to Smit (2004), blesbok meat has a mean fat content of 1.42g/100g, a mean protein content of 22.39g/100g and a mean collagen content of 1.67g/100g. This indicates that the meat should be considered as a healthy alternative source of protein (Smit, 2004).

3.2.2) Horse

Horsemeat was a main part of the human diet in many European countries during pre-Christian times, except in Islamic or Jewish countries. Horsemeat is considered unclean under Mosaic Law, as it does not conform to the requirements for an animal to be cloven-hoofed and cud-chewing at the same time. In pre-Christian times, the consumption of horsemeat in northern Europe was an integral feature of Teutonic religious ceremonies, especially those worshiping the god Odin (Martuzzi *et al*., 2001).

In 732 A.D., Pope Gregory III made great attempts to put an end to the pagan practices of horsemeat consumption, and some nations, such as the people of Iceland, were reluctant to

welcome Christianity due to this prohibition. In a few countries, the repercussions of this edict by the Catholic Church have remained even now, and the prejudice towards horsemeat has developed from taboos to avoidance or abhorrence. From the year 1928 onward, legislation in Italy prohibited the sale of horsemeat together with other meat sources from the same store. But since 1999, law 526 (12th paragraph), abolished this legislation, permitting the conventional sale of horsemeat (Martuzzi *et al*., 2001).

Despite all such attempts from religious leaders to curb the practice, horsemeat has been a part of the diets of many European countries for centuries. Countries such as Italy, France, Belgium and the Netherlands, have always regarded the consumption of horsemeat as part of their culture. Horsemeat is one of the two food-animal species under mandatory USDA (United States Department of Agriculture Food Safety and Inspection) inspection in the USA, the other species being goat (http://www.igha.org/USDA.html).

One of the distinguishing features of horses as red-meat-producing animals is that they are not specifically and systematically bred for the purpose of producing meat for human consumption in many countries of the world, but are slaughtered after they have served their purpose, be it racing or breeding. This results in variations in the quality of the meat, as their diets differ according to their age, breed, and temperament, and the work which they were required to perform.

According to Martuzzi *et al*. (2001), the breeding of horses solely for the purpose of meat production is not considered convenient by Italian breeders, and, therefore, Italy imports many horses from countries such as Poland for slaughtering purposes. Horses in Italy are normally slaughtered as foals of approximately six months of age, with a weight of 250-300kg. Compared with a beef carcass of the same weight and degree of fatness, a horse carcass has a higher percentage of subcutaneous and body cavity fat and a lower percentage of intermuscular and intramuscular fat. An Italian survey showed that the meat derived from sport horses seemed to be regarded more favourably by the consumer. This was due to the more intense colour, subtler muscle fibres and a lower fat content. The organoleptic characteristics of the meat from older horses were no different to those animals 30 months old (Martuzzi *et al*., 2001).

Commercial retail cuts from a horse carcass are comparable to those of beef. The meat is leaner, with a sweet flavour and aroma. Good quality horsemeat is very tender, but highly variable, due to the high variation in this species. The most popular cuts of horsemeat are taken from the hindquarters, the tenderloin, sirloin, fillet steak, rump steak and rib. Less tender cuts are ground and used in the production of sausages or pet food (http://www.igha.org/USDA.html).

In countries with a large racing industry, e.g. South Africa, there are many surplus horses, which are ideally suited for slaughter, due to their physiological age, and, therefore, possess optimal meat quality characteristics. Many of these horses are now abandoned and end up in shantytowns in front of carts and are not optimally cared for. If there was a suitable, economically beneficial alternative for breeders, such as specialised horse abattoirs, then South Africa could become a net exporter of horsemeat to countries where there is a high demand for it. This would be economically beneficial to the country, as well as the racing industry.

In the USA, as in many other western countries, the general public has an aversion to the consumption of horsemeat. However, presently, the horsemeat industry is competing with the beef and pork industries, in terms of the quantities of fresh meat exported. In 1994, 109 353 pounds of horsemeat was exported from the USA. In Sweden, more horsemeat is sold than lamb and mutton combined (http://www.igha.org/USDA.html). According to Badiani *et al* (1997), the chemical composition of horsemeat, including cholesterol, as listed in Table 5, supports Bodwell and Anderson's contention (1986), that 100g raw horsemeat would provide 40% of the recommended daily protein requirements for adults of both genders on a varied diet.

Table 5

Approximate composition, cholesterol content, and energy value of raw horsemeat (per 100g) (Badiani *et al.,* 1997, USDA, 1997)

Component	Average amount
Moisture, g	70.9
Protein, g	19.8
Lipids, g	6.63
Ash, g	0.98
Cholesterol, mg	61
Energy value, kcal	140
Calories	175
Fe, mg	5
Na, mg	55

Badiani *et al* (1997) showed that the PUFA/MUFA/SFA ratio for horsemeat was 0.54/1.34/1, compared to that of beef (0.12/1.1/1), lamb (0.07/0.9/1), and veal (0.2/1.3/1) (AA.VV, 1987).

3.2.3) Beef and mutton

Beef, mutton and pork, are regarded as the traditional meat species consumed all over the world and, despite religious beliefs in certain places that prohibit their use as food, are eaten to a certain extent in every country. South Africa is viewed as one of the countries consuming large amounts of red meat *per capita* per year (Table 6).

Beef production systems vary; they include those based on free-range conditions, as well as semi-intensive and intensive production methods. Consumer demand has dictated that the meat be lean, with a minimum of fat cover, tender, nutritious, palatable and, not least importantly, be relatively cheap (Barge, 2004).

According to Barge (2004), beef is still marketed internationally as an undifferentiated product. The protein content of beef is approximately 21%. Much of the fat in beef is found intramuscularly, which is commonly known as marbling, a fat deposition that makes beef highly acceptable to the consumer.

The United States Department of Agriculture (USDA) (http://www.igha.org/USDA.html) has released the following nutritional facts about beef. The meat is low in sodium, with high amounts of iron, zinc as well as manganese. It contains adequate amounts of protein, vitamin A, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, phosphorous, copper and selenium. It is one of the red meat species that has a high cholesterol concentration of approximately 275mg in a 100g portion of raw meat.

Table 6

Per capita annual consumption (kg) of red meat and pork in South Africa (Hallowell, 2001)

Year	Beef	Pork	Mutton
1970	24.5	3.5	9.6
1980	21.5	2.9	11.2
1991	18.1	3.4	14.4
2000	13.5	3.0	19.4

Sheep were probably amongst the first animals to be domesticated by man. They can be found living under a wide range of environments throughout the world and, as with goats, their system of husbandry has changed very little over the centuries in most countries, which, in the main, can

be classed as an extensive grazing system. The latter is the most natural for two of the three main species of meat animals, these being cattle, sheep and pigs, with cattle and sheep benefiting from extensive grazing systems.

Presently, the principal sheep-producing countries in the world are (in millions): China 127.2; Australia 126.3; Iran 51.5; New Zealand 48.8; India 45.5; Turkey 33.8; Pakistan 29.8; South Africa 29; UK 28.8; Sudan 23.4; Ethiopia 21.7; Spain 21.3 (FAO Production Yearbook, 1996).

The desirable features required in both lamb and mutton carcasses of any breed are short stocky plump legs, a thick full loin, and broad full back, thick fleshy ribs with a wide breast and shoulder, a good depth of chest cavity, a short plump neck, and overall lean content (Bouton *et al*., 1978).

The quality of lamb meat is dependent on key characteristics of the animals from which the products are derived, such as chronological age (Bouton, Harris, Ratcliff, & Roberts, 1978; Ono *et al.,* 1984), slaughter weight (Jeremiah, Tong, & Gibson, 1998) as well as sex (Butler-Hogg, Francombe, & Dransfield, 1984; Dransfield *et al*., 1990).

According to Hoffman *et al*. (2002), since 2000 there has been a rapidly decreasing per capita consumption of mutton in South Africa. This provides a challenge to the producers, processors and retailers, as they have to meet changing consumer demands and follow global trends, such as leanness, organic meat production and traceability of animals.

Salami usually contains beef and pork as main meat ingredients. However, mutton is used in the production of sausages, especially in Scandinavian countries.

4. MOTIVATION FOR THIS STUDY

It is apparent that there is an ever-increasing demand for the provision of a variety of high quality meat and meat products. Therefore, research is needed to determine whether species of animals other than the traditionally used mutton, beef and pork might serve as alternatives in red meat production systems in order to meet this demand. If the meat of non-traditional animals could be utilised optimally, there would be increased public acceptance of controlled culling systems. Many of these are naturally living animals, which have had little or no experience with man, and they are therefore untreated in terms of antibiotics and/or hormones and their flesh is uncontaminated. As consumers are becoming more aware of chemical residues in meat, as well as the manner in which the animal they eat lived prior to dying, these alternative species could become highly sought after in terms of quality and safety.

Producers are constantly striving for products with an increased shelf life and stability, securing economic advantages due to the continuous supply, of the amount and type required by the consumer. Therefore, this study was undertaken to obtain more information on the possibility of using alternative terrestrial mammalian species, as well as to investigate the feasible utilisation of aquatic mammalian species in the production of a stable and safe product such as traditional salami. Factors such as pH, water activity, microbiology and sensory evaluation were examined to establish facts about these meat types and their adaptability to this type of processing. Salami is a dry fermented sausage, which has pH values as well as water activity too low for harmful bacteria to develop.

Most of the species used in these investigations are harvested according to season, which usually implies a non-continuous supply of meat and meat products from these animals. However, if products which would be acceptable from a food safety perspective could be developed using these species, the harvesting of these animals would serve additional purposes other than for only the trophy or skin. This would make harvesting more acceptable ethically, and providing economic benefits to producers and the countries involved in such production.

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Chapter 3

CHEMICAL COMPOSITION OF THE MEAT AND BLUBBER OF THE CAPE FUR SEAL (*Arctocephalus pusillus pusillus*)

3.1. Abstract

The aim of this study was to investigate the chemical composition of the meat and blubber of the Cape fur seal (*Arctocephalus pusillus pusillus*), to establish whether it would be fit for human consumption. Meat and blubber samples were taken from the *Pectoralis* muscle of freshly culled carcasses of ten Cape fur seal pups and ten Cape fur seal bulls, and fat, protein, moisture and fatty acid content were determined. The muscle of the pups contained a higher percentage of fat (4.2g/100g) than that of the bulls (2.4g/100g), but a very similar percentage of protein (23.2g/100g). The blubber samples of the bulls contained a higher percentage of protein (26.6g/100g) than that of the pups (14.6g/100g), but a lower fat percentage (67.1g/100g) than that of the pups (77.2g/100g). In the Cape fur seal bull muscle, saturated fatty acids (SFA) contributed 33% and monounsaturated fatty acids (MUFA) 29% to the total fat content. Polyunsaturated fatty acids (PUFA) contributed 38%. In the muscle of the pups, the different fractions were calculated to be 39, 30, and 31% for SFA, MUFA and PUFA, respectively. In the seal muscle and blubber the n-6:n-3 ratio was much higher in the tissue of the bulls (2.10 and 2.69, respectively) than that of the pups (0.59 and 0.84, respectively). The polyunsaturated: saturated fatty acid ratio (P:S ratio) of the muscle and blubber of bulls and pups was >0.70, with the muscle of the pups having a value of 0.80, and that of the bulls 1.15. The blubber of pups and bulls had a P:S ratio of 1.44 and 1.19 respectively. The toxin content for the Cape fur seal, in terms of organochlorine components, was considerably lower than that of the Canadian seal. Both have PCB#153 as the highest PCB congener. No organochlorine component of the Cape fur seal was found in quantities higher than 13.7ng/g oil, whereas the maximum amount for the same PCB was 87.2ng/g in Canadian seal oil. The results of this trial revealed that the meat of the Cape fur seal could be considered fit for human consumption.

Keywords*:* Cape fur seal, sustainable harvesting, protein, fat, organochlorines

3.2. Introduction

The Cape fur seal (*Arctocephalus pusillus pusillus*) is harvested commercially in Namibia on the south-western coast of Africa. There is a large demand for the hides of the pups and bulls. The remainder of the carcass is processed to carcass meal. Due to the occurrence of BSE (*Bovine*

spongiform encephalopathy), there has been a decrease in the use of animal by-products in any animal feed. Therefore, finding an alternative use for the meat is desirable.

The meat is healthy and lean containing high amounts of the macro-minerals calcium, phosphorous, iron and selenium (Robinson, 1996), which would make it suitable for further processing into products for the human food chain. This would make harvesting a more acceptable occurrence, as it would mean that more of the carcass of the animal would be utilised as a source of nutritious meat to the consumer.

Little research has been conducted on the meat qualities of marine mammal meat and none whatsoever could be sourced on the meat of the Cape fur seal. Some literature is available on other pinnipeds, their physiology, geography, environment and life cycles, some comparable to the Cape fur seal. As the Cape fur seal is a sub-species of the sea lion, there are characteristics which distinguish it from other marine mammals.

The Cape fur seal is harvested commercially each year in Namibia, and this sustainable harvesting is destined to continue. The current culling numbers are 30 000 pups and 3 000 bulls per year. The harvesting is conducted in a scientific manner and is strictly monitored. As mentioned, the meat is no longer utilised; it is presently discarded. Information is therefore needed on the dress out percentages and chemical characteristics of the meat and blubber, as these would influence the potential processing methods used in any commercial production line. The age and gender of the animals used for these purposes could also play a role, and this too, requires research.

Namibia exports most of its pup skins to the Far East, where they are used in the clothing industry. Fish oil capsules are successfully sold in the pharmaceutical industry and are a well-established supplement in the diet of many people around the world. The omega-3 fatty acids in fish oil have a prophylactic action on thrombosis, and the hardening of arteries. The consumption of whale and seal oils benefits the immune system and improves the viscosity and coagulative properties of blood (NAMMCO, 1998). However, very little information exists on the fatty acid composition of the Cape fur seal.

One of the major concerns regarding the utilisation of marine mammal tissue is the increasing threat of persistent organic pollutants (POPs) contaminating the tissue in many of the world's seal populations (DeLong *et al.*, 1973; Reijnders, 1986), such as in the Californian sea lions

(*Zalophus californianus*), Wadden Sea harbour seals (*Phoca vitulina*), Baltic ringed seals (*Phoca hispida*) and Grey seals (*Halichoerus grypus*).

POPs consist of a group of synthetic organic compounds produced for industrial and agricultural purposes, or are by-products of other industrial processes. Many of these compounds are found in the high-lipid organs of animals as they are fat-soluble (lipophilic) and continual, thereby accumulating efficiently in the thick blubber layers of marine mammals (Tanabe *et al*., 1988), making them especially vulnerable to POP contamination. Large amounts of POPs are also transferred to the young seals via the milk of their mothers (Addison & Brodie, 1987).

In an article by Vetter *et al*. (1999), PCB levels accounted for almost 20-75% of the chlorinated hydrocarbon levels found in the blubber of Cape fur seals. PCB# 153 was found to be the most abundant PCB congener, accounting for approximately 40% of the PCB congeners analysed. Monitoring the organochlorine levels of marine mammals from Africa has not been extensively researched (Henry & Best, 1983; Cockcroft *et al*., 1989; Borrell *et al*., 1997). Aucamp *et al*. (1972) confirmed that DDT and other organochlorines have been used in South Africa since the 1950s. Reports indicate that DDT has been used in Namibia (<10 tons) and lindane in South Africa (10-100 tons) (Voldner & Li, 1995).

The aim of this study was to determine the chemical composition of the meat and blubber of the Cape fur seal. The potential exists to use a larger percentage of the animal than just the hides. Before this can be realised, baseline information on the chemical composition of its meat and blubber is required.

3.3. Materials and methods

Ten Cape fur seal pups, approximately eight months old, of both sexes, and ten Cape fur seal bulls, between two and four years of age, were used. As selective harvesting is not allowed, the pups were not differentiated by gender. All pups were of similar age, all at point of weaning. The bulls were estimated to be between 2 and 4 years old, none being sexually mature.

The animals were harvested using standard procedures; the pups were hit on the head once to result in loss of consciousness, and the bulls were shot with a .22-calibre rifle with a silencer. Thereafter, all animals were stuck in the heart to bleed within 30 seconds of being stunned. They were then transported to a skinning shed where they were eviscerated and the hide removed. At this point, samples of muscle were collected from the *Pectoralis* muscle, and samples of blubber from the ventral side of the carcass. Samples were stored in separate vacuum bags and identified

according to the specific animals, vacuum packed and frozen at -5^{o}C (once the meat samples had all reached room temperature).

3.3.1. Proximate analysis

The moisture and protein contents (g/100g meat) of all the samples were determined according to the Association of Official Analytical Chemist's Standard Techniques (AOAC, 1997). The moisture content was determined by drying at 105°C for 24 hours. To establish the protein content, dried and defatted samples were used; samples were ground with a pestle in a mortar until a fine powder was obtained. 0.100mg of the powder was taken per animal and inserted into a foil wrap designed for the Leco protein analyser (Leco Fp-528). The protein was determined as Nitrogen, which was multiplied by 6.25 to determine the protein concentration in the sample. For fat determination, samples were homogenised in a blender and a chloroform:methanol (2:1) extraction used (Lee *et al*., 1996).

3.3.2. Amino acid determination

The amino acid composition was determined, using a modification of the method of Bidlingmeyer, Cohen & Tarvin (1984), on a defatted, dried meat sample, using a Waters high performance liquid chromatography (HPLC) system.

The meat sample was defatted by solvent extraction, according to the method of Lee *et al*. (1996). The sample was hydrolysed with 6 N HCl in a vacuum-sealed tube for 24 hours at 110°C. Thereafter, the sample was centrifuged (15 000 rpm for 5 minutes) and dried under vacuum for 90 minutes to two hours. The pH was adjusted by adding 20µl solution of 2:2:1 ethanol:water:triethylamine and the sample was dried for a further 90 minutes to two hours.

The resulting sample was derivatised by adding 20µl of 7:1:1:1 ethanol:water:triethylamine: phenylisothiocyanate derivatising solution which was allowed to react at room temperature (26°C) for 10 minutes prior to drying under vacuum (minimum of 3 hours). The sample was resuspended in 200µl of Picotag sample diluent (Waters, Millford, MA, USA) and an 8µl sub-sample was then injected for separation by HPLC under gradient conditions using two buffers. Buffer A was a sodium acetate buffer (pH 6.4) containing 5000ppm ethylenediaminetetraacetic acid (EDTA), 1:2000 triethylamine and 6% acetonitrile. Buffer B comprised 60% acetonitrile with 5000ppm EDTA. Other conditions were:1525 HPLC with a binary gradient delivery, 717 auto-sampler and Injector, 1500 column heater, 2487 dual wavelength UV detector). The data were analysed using Breeze software (Waters, Millford, MA, USA).

3.3.3. Mineral determination

The samples used for mineral analysis were ashed, before being dissolved in 3 N HCl and diluted to appropriate concentrations required for mineral analysis by the AOAC method No. 968.08 (AOAC, 1997). Three macroelements (Na, K, and Mg) and two trace elements (Fe and Zn) were determined, using a Varian, spectra AA 250, plus atomic absorption spectrophotometer equipped with hollow cathode lamps specific to each element and an air-acetylene flame. The instrument settings, as well as all other experimental conditions, were in accordance with the manufacturer's specifications. The wavelengths (nm) used for the detection of each mineral were: K, 766.4; Na, 588.9 or 330.2; Mg, 285.2; Fe, 248.3; Zn, 213.9.

3.3.4. Fatty acid determination

The fatty acid content was determined using the method described by Tichelaar *et al*. (1998). After thawing the meat, the lipids in a 2g sample were extracted with chloroform/methanol (CM 2:1; v/v). All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. A polytron mixer (Kinematica, type PT 10-35, Switzerland) was used to homogenise the sample for 30 seconds within the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard to quantify the individual fatty acids.

A sub-sample of the extracted lipids was transmethylated for two hours at 70°C, using methanol/sulphuric acid (19:1; v/v). After cooling, the resulting fatty acid methyl esters (FAME) were extracted with water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen.

The FAME were purified by TLC (silica gel 60 plates) and analysed by GLC (Varian Model 3300 equipped with flame ionisation detection), using a 60 m BPX70 capillary column of 0.25mm internal diameter (SGE, Australia). The hydrogen gas flow rate was 25ml/min; and the hydrogen carrier gas rate 2-4ml/min. Temperature programming was linear at 3°C/min, with an initial temperature of 150°C, a final temperature of 220°C, an injector temperature of 240°C and a detector temperature of 250°C. The FAME in the total lipids were identified by comparison of the retention times with those of a standard FAME mixture (Nu-Chek-Prep Inc., Elysian, Minnesota).

3.3.5. Toxin evaluation

Seal oil was collected from the processing plant in Namibia, and taken for analysis to the Institute of Marine Research in Bergen, Norway. This oil had been extracted from the blubber, with heat. Additional blubber samples had been collected from ten *Phoca vitulina* seals

(harvested in Canada for another study), in order to investigate the toxins present in these animals at the Institute of Marine Research in Bergen, Norway (unpublished data).

Chlorinated dioxins and furans, as well as non-*ortho*- and mono-*ortho*- substituted polychlorinated biphenyls (NO- and MO-PCB, respectively) were analysed, using procedures described previously (MacDonald *et al*., 1997; Addison, Ikonomou & Stobo, 1999). The procedures involved a succession of column chromatographic extraction and clean-up steps, followed by high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) analysis. Results are presented as ng/g oil.

3.3.6. Statistical analysis

The two-sample T-test was used on SAS (statistical analysis systems, 1990) to compare the chemical and fatty acid values of the bulls and pups.

3.4. Results and discussion

It was noted that the carcasses of seal pups dress out to very low percentages (49%), compared with the carcasses of other species such as cattle, sheep and pigs. The dress out percentage in this case included the head, and the flippers. All viscera and the hide were removed. This is because at present, the whole carcass is either used for the production of carcass meal or discarded and no particular cuts are required. If the head and flippers were removed, as is done with these other species, this dress out percentage would decrease even further.

On dissecting the relevant animals, it was noticeable that the meat of the pups was a lot lighter in colour than that of the bulls. However, the meat of the pups was darker than that of traditional domestic animals. It was also noted that the blubber, present as a subcutaneous fat layer in both age groups, came in the form of a thick, snow-white layer. There was a predominant fat depot around the tail. Most of the excess dietary energy is stored subcutaneously as a form of insulation. The pups used for this investigation were all at the point of being weaned and none of them would have had to dive deep or swim far to find food. Therefore, their bodies had not yet been required to maintain high levels of oxygen in their muscles, and this might explain the lighter colour of the meat. Their staple diet had to date consisted of milk, and no other food sources, e.g. fish, would have been consumed to affect the colour or flavour of blubber or meat. The myoglobin content of up to 13.4mg/g (Synowiecki *et al*., 1992) provides the opportunity to utilise seal meat as a colour contributor in meat products utilising meats of lesser colour quality or potential.

3.4.1. Proximate analysis

Table 1

Mean (±standard deviation) and P values indicating differences between the chemical composition (g/100g sample) of the meat (predominantly the *m.pectoralis)* and blubber of ten Cape fur seal pups and ten Cape fur seal bulls

	Meat			Blubber		
	Moisture	Protein	Fat	Moisture	Protein	Fat
Pups	73.0±1.6	23.3±1.6	4.2±1.8	8.0±2.0	14.6±3.0	77.2±1.9
Bulls	74.3±1.6	23.6±1.5	2.4±0.9	6.0±1.6	26.6±4.4	67.1±4.8
P- value	0.0023	0.4825	0.0000	0.0001	0.0000	0.0000

Chemical analysis of tissues of the Cape fur seal (Table 1) revealed a high protein concentration in the blubber, especially that of the bulls (26.6g/100g) when compared to that of the pups (14.6g/100g), but a lower fat content, of 67.1g/100g and 77.2g/100g respectively. The meat (consisting mostly of the *m. pectoralis*) of the pups contained a higher level of fat (4.2g/100g) than that of the bulls (2.4g/100g), but had a very similar protein content (23.2g/100g).

As the seal is a marine mammal, it mainly stores fat subcutaneously. The protein content in the blubber is not consistent with that of superficial muscle, but rather of connective tissue. On drying, the blubber became rubbery rather than brittle. As connective tissue increases with age of the animal, the protein content in the blubber of the bulls would be higher than that of the pups. As seen from the P values in Table 1, all components of the tissues differed ($P<0.05$), except the protein concentration in the muscle.

3.4.2. Amino acid determination

Table 2

Mean values (g/100g meat sample) of the amino acid profile of the *m. pectoralis* of ten Cape fur seal bulls

Essential amino acids	Mean ± std. dev	Non-essential amino acids	Mean ± std. dev
Histidine	0.7 ± 0.1	Alanine	2.1 ± 0.2
Isoleucine	0.6 ± 0.1	Arginine	1.0 ± 0.1
Leucine	1.8 ± 0.2	Aspartic acid	2.0 ± 0.2
Lysine	1.5 ± 0.2	Glutamic acid	3.0 ± 0.3
Methionine	0.6 ± 0.1	Glycine	2.0 ± 0.3
Cystine	0.2 ± 0.0	Proline	1.1 ± 0.1
Phenylalanine	0.7 ± 0.0	Serine	1.3 ± 0.1
Threonine	1.2 ± 0.1		
Tyrosine	0.5 ± 0.1		
Valine	1.0 ± 0.1		

There are eight amino acids (isoleucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) essential for maintaining the nitrogen balance in young adults (Rose, Wixom, Lockhart & Lambert, 1955). The proportion of essential amino acids determines the nutritional quality of a food protein. In light of recently adjusted estimates of the amino acid requirements for human adults proposed by Pellett & Young (1990), seal meat would be a valuable source of essential amino acids, especially leucine and histidine, but seems to contain little cystine.

Table 2 shows that the four major amino acids noted were glutamic acid, alanine, aspartic acid and glycine, which contributed 3.0, 2.1, 2.0 and 2.0g/100g meat respectively. Leucine (1.8g/100g meat) was the major essential amino acid in seal meat, followed by lysine (1.5g/100g meat). The table shows data for bulls only, as no samples were analysed for the pups.

3.4.3. Mineral determination

Meat is known to play an important role in a balanced human diet, as it contains higher concentrations of minerals, especially Fe and Zn, compared to plants (Lin *et al*., 1989; Simonsen, Hamm & Rogowski, 1988; Williams, 1987). According to literature published by the American Meat Institute Foundation (1960), K appears to be the most important mineral, followed by P and Fe. As seen in Table 3, Mg was found to be the most predominant mineral present (634mg/100g meat) in the seal meat analysed. This value was approximately five times higher than K (174mg/100g meat). The concentration of sodium (49.9mg/100g meat) was also higher compared to that of iron (4.1mg/100g meat) and zinc (2.9mg/100g meat).

Table 3

The mean mineral composition (mg/100g meat) of the *m. pectoralis* of ten Cape fur seal bulls

Mineral	Mean ± std. dev
Iron (Fe)	4.1 ± 0.2
Magnesium (Mg)	634.0 ± 6.0
Potassium (K)	174.0 ± 2.4
Sodium (Na)	49.9 ± 0.5
Zinc (Zn)	2.9 ± 0.2

The best sources of zinc in the human diet are meat, poultry and seafood. Approximately 20-40% of zinc is absorbed from a meat source, which makes it the main contributor to dietary zinc intake in developed countries (Higgs, 2000). According to Lawrie (1998), the zinc contents of pork, beef and mutton are 2.4, 4.3 and 2.1mg/100g respectively.

Red meat is considered one of the best sources of iron in the human diet, with 50-60% being in the heme form (Higgs, 2000). Seal meat can, therefore, be considered a rich meat in terms of heme iron content. Heme-iron is absorbed by a more efficient mechanism than non-heme iron, which is common in plants. Absorption of iron from meat sources is 15-25%, as to 1-7% from plant sources (Higgs, 2000).

The iron content in seal meat, as seen in Table 3, is much higher than that of any traditional red meat species, being 4.1mg/100g, compared with that of pork, beef and mutton, with values of 1.4, 2.3 and 1.0mg/100g respectively (Lawrie, 1998).

The standard deviations for the different mineral contents are quite large (Table 3), which might be caused by the fact that body mineral composition is affected by age, feeding regimen, breeding season and geographical differences. In the animals used for this trial, possible slight age differences within the group of bulls sampled, as well as differences in their body conditions, might have contributed to these variations. These factors are also known to influence the mineral and chemical composition of muscles.

3.4.4. Fatty acid determination

As stated by Shahidi, Synowiecki, Amarowicz & Wanasundara (1994), the deposition of lipids in the seal, is found subcutaneously, in the form of blubber. This consists mostly of neutral lipids (98.9%), and small quantities of polar lipids. According to Shahidi & Synowiecki (1993), seal meat is comparable to, or better than, other sources of animal protein in terms of its intramuscular lipids. Furthermore, those lipids present in the blubber are excellent sources of omega-3 fatty acids (Shahidi *et al*., 1994a). This was substantiated by the results in this trial (see Tables 4 and 5).

Table 4 shows the mean values in mg/100g of the fatty acids in the *m. pectoralis* and blubber of Cape fur seal bulls, and pups. In muscle, palmitic acid was the most dominant fatty acid, followed by eicosapentanoic (EPA) and then palmiteleidic and oleic acids, in similar concentrations. One major difference between the blubber of the bulls and pups was that the pups had a much higher concentration of docosohexaenoic (DHA) acid.

Table 4

Mean values ± SD of the fatty acid composition (mg/100g muscle or blubber) of the *m. pectoralis* and blubber of ten Cape fur seal bulls and pups

Fatty acid		Bulls		Pups	
		muscle	blubber	muscle	blubber
SFA	16:0	194.4± 0.6	1326.7±5.6	626.9±1.8	2213.7±5.9
	18:0	119.9± 0.2	377.9±1.4	214.1±0.5	405.8±0.9
	20:0	34.2± 0.2	58.1±0.4	25.1±0.1	37.1±0.2
	22:0	nd	93.2±1.8	5.6±0.1	57.6±1.0
	24:0	40.5±0.6	52.1±0.3	12.7±0.1	51.2±0.1
MUFA	16:1 n-7	39.7±0.2	819.5±4.0	164.7±0.7	943.5±2.4
	18:1 n-9	213.0±0.8	813.8±8.7	395.8±4.0	2204.0±14.6
	20.1 n-9	34.2±0.2	239.1±2.0	58.6±0.4	299.8±1.5
	22:1 n-9	29.8±0.1	246.7±2.0	44.8±0.4	148.6±1.0
	24:1 n-9	27.7±0.3	38.6±0.2	19.8±0.1	33.0±0.2
PUFA	18:2 n-6	70.1±0.2	296.1±2.6	232.6±0.8	176.7±0.3
	18:3 n-3	9.2±0.1	60.8±0.1	17.1±0.1	71.2±0.2
	18:3 n-6	18.7±0.2	23.2±0.2	13.0±0.06	17.5±0.1
	20:2 n-6	16.3±0.1	35.2±0.1	14.1±0.1	26.4±0.1
	20:3 n-3	51.5±0.3	96.4±0.7	109.9±0.4	200.9±0.7
	20:4 n-6	22.4±0.3	67.8±0.7	25.3±0.4	8.9±0.1
	20:5	68.5±0.4	898.8±4.5	171.3±0.7	811.3±1.4
	22:2	nd	28.0±0.1	9.8±0.08	14.60±0.1
	22:5	69.3±1.0	573.5±4.3	71.6±1.1	446.6±4.9
	22:6	120.0±0.7	187.0±9.2	45.7±2.1	2208.0±4.7
Total	ΣSFA	389.0	1908.0	884.4	2765.4
	ΣMUFA	344.4	2157.7	683.7	3628.9
	ΣPUFA	446.0	2266.8	710.4	3982.1
	ΣTUFA	790.4	4424.5	1394.1	7611.0
	DFA	910.3	4802.4	1608.2	8016.8
	P:S	1.15	1.19	0.80	1.44
	n-6	127.5	422.3	75	229.5
	n-3	60.7	157.2	127	272.1
	n-6: n-3	2.10	2.69	0.59	0.84

SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; TUFA = Total Unsaturated Fatty Acids; DFA = Desirable Fatty Acids (C18:0 + TUFA); P: S = PUFA: SFA; nd = not detected

Table 5 is a representation of the same data from Table 4, but in percentage format. Table 5 also incorporates the P values of the muscle and blubber when compared between the two age groups. There are highly significant differences in the muscle fatty acid values between bulls and pups. The only fatty acids not significantly different in the muscles are behenic (22:0), oleic (18:1 n-9), erucic (22:1 n-9), linoleic (18:2 n-6), α-linolenic (18:3 n-3), γ-linolenic (18:3 n-6), (20:2 n-6), and DPA (22:5) fatty acids.

Table 5

Percentage composition of the fatty acid composition (% of total fatty acids identified) of the *m. pectoralis* and blubber of ten Cape fur seal bulls and ten pups

Fatty acid		Bulls		Pups		P value	P value
		muscle	blubber	muscle	blubber	muscle	blubber
SFA	16:0	16.9	17.3	24.0	22.0	0.00	0.11
	18:0	10.8	5.7	8.4	4.1	0.00	0.81
	20:0	2.8	1.1	1.0	0.4	0.05	0.29
	22:0	0.4	1.0	0.2	0.5	0.42	0.04
	24:0	2.9	0.9	0.5	0.5	0.03	0.22
MUFA	16:1 n-7	3.3	9.5	6.1	9.4	0.07	0.63
	18:1 n-9	18.1	8.8	13.7	18.8	0.80	0.35
	20.1 n-9	3.0	2.7	2.0	2.6	0.00	0.19
	22:1 n-9	2.5	3.3	1.7	1.6	0.10	0.22
	24:1 n-9	2.1	0.6	0.8	0.3	0.06	0.09
PUFA	18:2 n-6	6.3	4.7	8.9	1.8	0.95	0.44
	18:3 n-3	0.7	1.1	0.7	0.7	0.41	0.06
	18:3 n-6	1.6	1.1	0.5	0.4	0.18	0.42
	20:2 n-6	1.3	0.6	0.5	0.3	0.61	0.90
	20:3 n-3	4.5	1.4	4.5	2.0	0.05	0.01
	20:4 n-6	1.9	1.5	1.0	0.1	0.00	0.13
	20:5	5.4	10.4	6.3	8.4	0.00	0.16
	22:2	1.4	0.5	0.4	0.2	0.00	0.24
	22:5	4.6	6.4	2.2	3.9	0.12	0.26
	22:6	9.5	22.2	16.7	22.4	0.00	0.32

Significant differences ($P<0.05$) were found in the meat fatty acid composition when comparing pups and bulls (Table 5). Significant differences ($P<0.05$) were found in the fatty acids of both the muscle and blubber, although the differences were higher in the muscle. The fatty acids found to differ, were mainly polyunsaturated and mono-unsaturated acids. As the diet between adults (fish) and young (milk) differs, this could be the main contributor to this result.

In the blubber fatty acid values, there were fewer significant differences ($P>0.05$). The only fatty acids significantly different ($P<0.05$) in the blubber of the pups and bulls, were behenic (22:0), 20:3 n-3 and γ-linolenic (18:3 n-6) acid. The bulls had the higher concentration of all three, compared with the pups.

In the bull *m. pectoralis,* the SFA contributed 33%, the MUFA 29%, and the PUFA 38%, to the total fatty acid composition. However, in the pups, the PUFA was lower (31%) and the SFA (39%) higher than the MUFA (30%), which was similar to that of the bulls. This is explained by the diet of the young seals, which were all on the point of being weaned. One major difference between the blubber of the bulls and pups was that the latter had a higher concentration of docosohexaenoic (DHA) fatty acid, which has been found to be vital for optimal development of

the central nervous system and visual performance in humans (Kim & Edsall, 1999). Once again, this could be related to their diet of mother's milk. As pups need to gain weight and blubber to protect them against the cold water, the milk can be assumed to be extremely rich in lipids.

The fatty acid composition of red meat has little or no influence on the market value of a carcass at present, as carcasses of domestic livestock species are valued according to a specific grade or classification, taking into consideration age of the animal and degree of fatness of the carcass. However, there is an increasing demand for meat products with a certain fatty acid profile. The actual qualities (chemical and physical) of the fatty acids affect the sensory and storage qualities of meat (Banskalieva *et al*., 2000), and it is known that the flavour of meat is also influenced by its fatty acid composition (Melton, 1990). Unsaturated fatty acids have an increased expectation for oxidation and thus rancidity, which influences shelf life.

PUFA, such as the omega-3 fatty acids, have recently become increasingly popular in human diets, due to their apparent health benefits. The ratio of PUFA to SFA (P:S ratio) was higher in the blubber of the pups than the bulls (1.44 and 1.15). However, in the *m. pectoralis*, the bulls had a higher value than the pups (1.15 compared to 0.80). From a human dietary perspective, the aim is to bring the P:S ratio of meat closer to >0.70, and the n-6:n-3 ratio to <5.0 (Raes *et al*., 2004; Sanudo *et al*., 2000). According to Enser *et al*. (1998), the meat from ruminant animals usually has a lower P:S ratio. As seen in Table 4, the P:S ratio of both the muscle and blubber of the bulls and pups was >0.70. The n-6:n-3 ratio was higher in the tissue of the bulls (2.10 and 2.69 respectively), than that of the pups (0.59 and 0.84 respectively).

Human plasma cholesterol levels are correlated to the fatty acid composition of their diet (Flynn *et al*., 1985). In general, MUFA and PUFA do not result in increased cholesterol levels, but high levels of long-chain SFAs do (Grundy & Denke, 1990). It has been reported that palmitic acid (C16:0) increases cholesterol levels, but that stearic acid (C18:0) does not (Rowe *et al*., 1999). The importance of these particular fatty acids is becoming ever more evident in their utilisation for value-added application in the food and pharmaceutical domains. The n-3 fatty acids decrease serum triacylglycerol (TAG) and cholesterol levels (Kim & Edsall, 1999). They have been used in the treatment and prevention of arthritis, rheumatism, hypertension, inflammatory and immune disorders (Senanayake & Shahidi, 2001). Guidelines for consumers suggest the reduction in intake of n-6 PUFA to n-3 PUFA and the intake of short- and medium- chain SFA (Gibney, 1993).

3.4.5. Toxin evaluation

Seal oil samples (*ca.* 50ml) were taken from 200-litre barrels and sent away for analysis of the organochlorines in Bergen, Norway. The values obtained for the Canadian seal oil were results from an independent study, obtained from the laboratory in Bergen. The organochlorine content of the Namibian cape fur seal and the Canadian seal are depicted in Table 6.

Table 6

Organochlorine components of seal oil from the Namibian Cape fur seal and Canadian seal (ng/g oil) (Institute of Marine Research, Bergen, Norway)

Component	Cape fur seal	Canadian seal*
PCB#28	0.8	13.4
PCB#52	1.1	14.6
PCB#101	2.5	17.5
PCB#105	1.2	2.0
PCB#118	4.9	23.5
PCB#138	8.3	53.2
PCB#153	13.7	87.2
PCB#156	0.7	2.6
PCB#180	4.2	18.6
ppDDD	13.1	17.3
ppDDT	16.2	23.8
Dieldrin	1.0	1.7
HCB	0.6	61.3
a-HCH	0.1	79.1
b-HCH	8.6	16.5
g-HCH	1.0	5.5
Transnonachlor	18.5	165.5

*unpublished data

According to Nyman *et al*. (2002), the liver: blubber ratio in seals is equal to one, which implies that the PCB and DDT burden is at equilibrium in the body. PCB#28 is the most prevalent PCB in humans, and is known to have a neurotoxic effect. PCB#52 is found in the environment and is metabolised rapidly in the mammalian body (Promochem, 1999). As can be seen in Table 6, the Cape fur seal has very low levels of toxins in its oil compared with the oil from the Canadian seal. This is consistent with results reported by Vetter *et al*. (1999). No organochlorine component of the Cape fur seal was found in larger quantities than 13.7ng/g oil, whereas the maximum amount for the same PCB was 87.2ng/g in the Canadian seal. The lowest concentration of an organochlorine component in the oil of the Cape fur seal was 0.8ng/g (PCB#28), whereas the lowest value for the Canadian seals was 2.0ng/g (PCB#105). According to Vetter *et al*. (1999), the reason for such low organochlorine levels in the Namibian Cape fur seal might be due to the fact that the Benguela current dries out the coastline, resulting in a belt of non-arable land, 100-150km wide. This implies that there is no direct organochlorine input

except for rivers**,** which is also unlikely, since the regional input would remain limited mainly to the Orange River in South Africa.

The amount of toxins found in the blubber of the Cape fur seal from Namibia (175µg/kg) used in this study, were lower than the mean values reported in the blubber of harbour seals from Iceland (1546µg/kg), ringed seals from Spitsbergen/Arctic (1226µg/kg), and only slightly higher than those reported for Weddell seals (*Leptonychotes weddellii*) from the Antarctic (105µg/kg) (Luckas *et al*., 1990).

3.5. Conclusion

Seal meat can be regarded as an exotic red meat species. No information on the chemical composition of the meat of the Cape fur seal could be sourced. The results of this investigation indicate that the seal is an animal whose meat and blubber characteristics make it suitable for use in the human food chain. It can be regarded as an aquatic venison species, which has had no artificial additives in its feed or administered otherwise, and can, thus, be viewed as organic meat. As people are becoming increasingly aware of the origins of any meat they eat, the fact that the seal has had no contaminating human contact whatsoever counts heavily in its favour.

This research has shown that, potentially, the seal can be regarded as an important contributor toward the mineral, amino acid and polyunsaturated fatty acid requirements of humans. The research findings show that seal meat is lean, with very high protein content, with most of the fat being stored subcutaneously as blubber. It can therefore be viewed as a healthy, low fat alternative to other traditional red meat species such as beef, mutton and pork.

With its low toxin content the oil could be processed into capsules and sold commercially for human consumption, providing another potential alternative use for this animal. As also seen in this investigation, seal blubber has optimum ratios of n-6:n-3, P:S and high levels of desirable fatty acids. This indicates that the oil produced from this blubber would be a healthy alternative for the consumer.

Countries with concessions to harvest seals, such as Namibia, could benefit from the full utilisation of the animal. More research is needed with regard to the processing of the meat, as well as the blubber, into products suitable for human consumption, because harvesting is currently carried out in line with the main interests of the hide and animal feed industries and no regard is presently paid to the cleanliness and hygiene of these operations. However, if

harvesting were to become more orientated towards producing meat for the human food industry, there would be a need for a comprehensive design of a processing plant specifically geared towards seal utilisation. Apart from this, commercialisation of seal meat harvesting would have substantial ramifications in terms of job opportunities and increased economic returns and foreign exchange revenues for the countries involved.

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Chapter 4

PRODUCTION OF SALAMI FROM MEAT OF THE CAPE FUR SEAL

4.1. Abstract

This is a pilot study investigating the utilisation of Cape fur seal *(Arctocephalus pusillus pusilus)* meat in the production of salami. Seal meat, or seal meat combined with beef or pork, with two different commercial starter cultures were used. Changes in pH, water activity and colour were monitored over 21 days in a controlled temperature environment. The pH of all three meat batches decreased from *ca.* 5.6 to *ca.* 4.5 during fermentation. Water activity levels decreased from 0.96 to 0.91 during the same period. No drastic changes were recorded in the colour of the fermented meat. Production of salami from seal meat is possible. However, more research is required on the use of seal blubber instead of pork fat due to the fatty acid composition of marine mammal tissue compared with that of other mammal species.

Keywords*:* salami, Cape fur seal, fermentation

4.2. Introduction

As harvesting of seals is a seasonal occurrence, meat from this species needs to be processed into a product that will have a long shelf life. Salami is a meat product which complies with this criterion. Salami can be defined as a blend of meat and fat particles, NaCl, curing agents, and spices stuffed into either artificial or natural casings. Starter cultures, such as lactic acid bacteria, are often used to aid in the fermentation and maturation of the salami.

Fermentation processes were developed to enhance the production of dried meats when drying was the only means of preservation. The major role of lactic acid bacteria in the sausage is to produce organic acids, primarily lactic acid, from carbohydrates (Campbell-Platt, 1987). This leads to a decrease in pH, which contributes to the retardation of the development of undesirable micro-organisms. Decreased pH is also correlated with a reduced water-holding capacity of proteins and, thus, results in essential desiccation of the product.

For the making of sausage, pork is favoured in countries such as Europe, the US and China. Elsewhere in the world, mutton and beef are the preferred choices. Chicken and other poultry

meat is also used in the production of fermented sausages. The meat used in the production of salami should be suitable in terms of water-holding capacity, pH value and colour.

After drying of the salami, it may now contain up to 50% fat, which makes fat an important ingredient of fermented sausage. This can lead to problems such as rancidity, which has a direct effect on the shelf life of the product. There are many substances that can be added to act as anti-oxidants, one of which is ascorbic acid, which may also act as a curing agent and in stabilising the colour.

Common salt (NaCl) is generally added to the batter at a concentration of 2.5-3.0%, making it the single most critical non-meat ingredient. Salt extracts soluble proteins from meat particles, thus forming an adhesive film to aid in cohesion of the different ingredients in the sausage. Nitrite is important in the determination of colour of many fermented meat products, and in retarding lipid oxidation. The maximum level of nitrite allowed in sausages is between 120 and 150ppm (Campbell-Platt, 1987). Both NaCl and nitrite play vital roles in creating a favourable environment for the development of lactic acid bacteria in the early stages of fermentation and to retard the growth of undesirable micro-organisms.

Commonly used spices are cumin seed, fennel seed, dried garlic, mustard, and pepper. In some countries, producers also use other ingredients to influence taste as well as mask unpleasant flavours: red wine, berry extracts, as well as artificial flavourants .

Fermented sausages are generally produced as dry or semi-dry products, although some are intermediate (Jay, 1978). There is a continuous spectrum of reduced moisture contents between different products, with drier products having a water activity of approximately 0.8, which increases the shelf life of the product, this being a great advantage in the hotter regions of the world, such as South Africa (Campbell-Platt, 1987).

The most common starter culture species used in the fermentation of beef and pork sausages are strains of *Lactobacillus, Pediococcus*, non-pathogenic S*taphylococcus* and *Micrococcus* (Lücke, 1986). The organic acids produced by these bacteria prevent the development of pathogenic micro-organisms (Schillinger & Lücke, 1987), aid the cohesion of meat particles (Townsend *et al*., 1980) and contribute to the colour change of fermented sausages.

According to Shahidi & Synowiecki (1993), seal meat is comparable with or better than that of other sources of animal protein, in terms of its intramuscular lipid composition (fatty acids). The

fatty acids also present in the blubber are excellent sources of omega-3 fatty acids (Shahidi *et al*., 1994). A myoglobin content of up to 13.4mg/g provides the opportunity to utilise seal meat as a colour contributor in meat products using meats of lesser colour quality or potential (Synowiecki *et al*., 1992).

As the harvesting of seals is seasonal, a product such as salami needs to be developed with a shelf life that would allow a year-round supply of this commodity. The aim of this study was to determine whether seal meat could lend itself to the production of traditional salami. This had to be researched with regard to the ability of a particular starter culture reacting favourably with the meat. Chemical reactions within the meat, including change in pH and water activity (a_w), and colour and moisture loss, also had to be monitored.

4.3. Materials and methods

The seal meat used in this trial was obtained from a cull commissioned by the Department of Nature Conservation of the Western Cape.

The seals were shot with a .22 calibre rifle by a professional hunter, and stuck to bleed within 30 seconds of being shot. Evisceration occurred on site. The carcasses were subsequently taken to a commercial meat processing plant, where the production of the salami took place. This process took place under the same conditions applying to the production of commercial salami.

4.3.1. Starter cultures and growth conditions

The starter culture LS 25 (Freddy Hirsch, P.O. Box 2554, Cape Town, 8000, South Africa), used in two of the three batters (namely the salami produced from pure seal meat and the one made with seal and beef), is a cocktail consisting of *Lactobacillus* spp. L110 and *Staphylococcus.*

The other starter culture, called 'OPTIstart PLUS' from Raps in Germany (Freddy Hirsch, P.O. Box 2554, Cape Town, 8000, South Africa), was used in the seal and pork salami. It consists of a mixture of *Lactobacillus* spp. L110*, Micrococcus Varians* M86 and *Debaryomyces hansenii*.

The starter cultures came in powder form and were suspended in water, according to the sizes of the relevant batches, before being added to the batter during preparation.

4.3.2. Meat preparation and fermentation

In this trial, the frozen seal meat was chopped to a particle size of 3-4mm in diameter in a commercial, two-speed 25-litre bowl cutter. Curing salt was added to the mixture before frozen

pork back fat was added. After chopping to a particle size of 5-8mm diameter, a mixture of spices was added. The three batches were prepared in succession, so as to minimise the variation in the production environmental conditions.

The three batches differed (see Table 1); batch A contained mostly seal meat, using starter culture LS 25, batch B contained some pork, using 'OPTIstart' starter culture, and batch C contained some beef, using the same standard starter culture, LS 25, as batch A.

The batter was stuffed into collagen casings (60mm diameter), using a commercial sausage stopper. The different batters were treated in the same way and sausages were stuffed at 15cm intervals. *Ca*. 21 sausages per batter were produced.

The salami was fermented for 24 hours at 20-22^{o}C and 97-99 % RH, and cold smoked for two days at 18-24^{o}C and 45-60 % RH. It was then ripened for a further 20 days at 16-18 ^{o}C and 40-60% RH. Samples were taken from the batter (day 1), and subsequently at three-day intervals during the fermentation period, and the relevant parameters measured (see 4.3.3). The sausages were also weighed every three days, to observe moisture loss.

4.3.3. Recorded parameters

In this investigation, pH values were taken, in triplicate, using an insertion probe (Crison model 507, Crison instruments, South Africa). The instrument was calibrated using standard calibration solutions of pH 4 and 7. The probe was cleaned with distilled water between each reading, to ensure that no fat particles from the sausage batter were left in the nozzle to affect further readings.

Water activity values were measured, using a Novasina ms1-aw (Labotec, P.O. Box 790, Howard Place, 7450, South Africa) handheld instrument. This instrument was calibrated according to the manufacturer's instructions. Approximately 5g of sample was placed into a 2.5cm-diameter plastic dish, which was then inserted into an enclosed space.

Colour measurements (L*, a*, b* values) were recorded, using a CIELab Colorimeter (Gardner, Columbia, USA). The salami was cut into sections, 2.5cm thick, and colour readings were taken from the sliced surfaces. Readings were collected from three different areas on each slice to obtain an approximate value for each factor. In the L*a*b* colour space, colour difference can be expressed as a single numerical value, $\Delta E^*_{ab.}$ The ΔE^*_{ab} value was calculated according to the following equation:

$$\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2}$$

The chroma and hue angles were also calculated as psychometric correlates of perceived chroma and hue, using the a* and b* colour values (Hunter and Harold, 1987; Setser, 1984):

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$$

$$\text{Hue angle} = \tan^{-1}(b^*/a^*)$$

The readings were taken in triplicate from the first section, the centre and the end of the sausage respectively. The orientation was determined by the position of the sausage during hanging on the rack in the chamber. In terms of this, the first section was attached to the loop from which the sausage hangs. The readings were taken from these different positions, in order to note if there were any changes resulting from the orientation. As no differences were found, the values were pooled for further comparisons.

Weights were recorded up to a three decimal point accuracy, and the same scale was used throughout the trial. The same pH probe, and water activity instrument, were used to ensure consistency of results, and to minimise variation due to possible instrumental error.

Table 1

Quantities (g) of various constituents used to make the seal salami

Constituents	Batches		
	A	B	C
Seal meat	6000	3000	3000
Pork fat	3000	1600	1600
Fresh beef mince	500	500	800
Commercial starter culture	18		18
OPTI start starter culture		15.6	
Salt	200		200
Spice (pepper, paprika) and salt		312	
Spices (pepper, paprika)	128		128
Pork meat		3000	
Beef meat			3000
Mustard seed		50	
Red wine			250
Total	9846	8477.6	7996

4.4. Results and discussion

As this was an exploratory investigation, the three salami batches had no common main effect and no statistical analysis was therefore applied to the results to test for differences between

batches. However, standard regression equations using SAS (1990) were used to try and quantify changes with time.

4.4.1. Water activity and pH correlation

From a food safety aspect, food is regarded as safe and shelf-stable at either a water activity (a_w) of <0.9 or pH values of <5.0. Both a_w and pH values should be below the stated values to prevent bacteria from developing any further. With regard to collected data, pH is the most important and readily obtained variable used to monitor fermentation progress in salami (Acton *et al.,* 1977; De Ketelaere *et al*., 1974). Production of lactic acid results in a decrease of meat pH to a final pH of between 4.8 and 4.9, and stimulating the aggregation of muscle proteins, which forms the typical texture of salami sausage due to this coagulation (Campbell-Platt, 1987). The content of lactate in meat used for the processing of salami varies in meat with different meat characteristics: pale, soft and exudative meat is acidic, whereas dark, firm and dry meat is more alkaline. These are important variables to take into account when selecting ingredients for the production of fermented sausages (Nordal & Slinde, 1980).

Inoculant bacteria, such as Micrococci and *Staphylococcus carnosus* are vital in the initial stages of fermentation, as they are active in reducing nitrate to nitrite and also stabilise the colour of the product. The presence of lactic acid bacteria helps in decreasing the pH of the meat and thus making conditions unfavourable for other Gram-negative bacteria (Campbell-Platt, 1987).

The consistency of fermented sausage is also determined by the pH value of the product, and the a_w level, the consistency being ideal at a pH value <5.4 and a_w level <0.9. Therefore, the pH value is seen to be the major determinant of consistency in high-acid, semi-dry sausages, and the a_w level in low-acid, dry sausages (Campbell-Platt & Cook, 1995).

The water activity is a measure of the partial vapour pressure of the foodstuffs compared to that of pure water at its surface. Water molecules are loosely orientated in pure liquid water and can easily rearrange. When solutes are introduced, these orient the water molecules around them and make them much less available for use by micro-organisms (Gracey *et a*l., 1999). Reduction of the a_w level during drying is largely a function of water loss and consequent increase in solute concentration (Campbell-Platt & Cook, 1995).

The rate of the pH drop in all three batches, prepared using different starter cultures, was similar. At day 10, there was a slight increase in the a_w levels in all batches, but they all evened out again by day 13. In all batches, the initial pH was approximately 5.6, with an ultimate pH value of 4.4

(see Figs. 1, 2, 3). Linear regression lines were found to give the best goodness of fit, and the intercept and gradient of these equations are depicted in Table 2.

The sudden increase in a_w shown in Figs. 1-3 might have been due to a malfunctioning of the maturation chambers at the plant, such as a power failure or a system fault. A change in the a_w level would not rectify itself immediately, and could take three days. This would correspond with the data collected. However, records from the processing plant did not show any technical problems during that particular time.Alternatively, the water activity meter may have malfunctioned, although it was the same instrument, used in exactly the same manner.

Another possibility is that at this pH level, muscle proteins are near their iso-electric point and could be releasing more water from their structure. This would be indicated by an immediate increase in a_w after a decline, due to the released water around the cells, which is recorded by the water activity meter when the measurement is taken. This water will remain there, as the cells are not able to reabsorb the water which they have released due to the pH value at that point. This would result in an increased level of a_w, which would then gradually decrease again, due to the standard drying procedures involved in the production of fermented sausages. Another possibility is that the salt added on the day of production of the sausages absorbs water, which is released as it dissolves, thus resulting in a surge in the water activity. This phenomenon could not be substantiated, as no a_w information could be sourced from the available scientific literature.

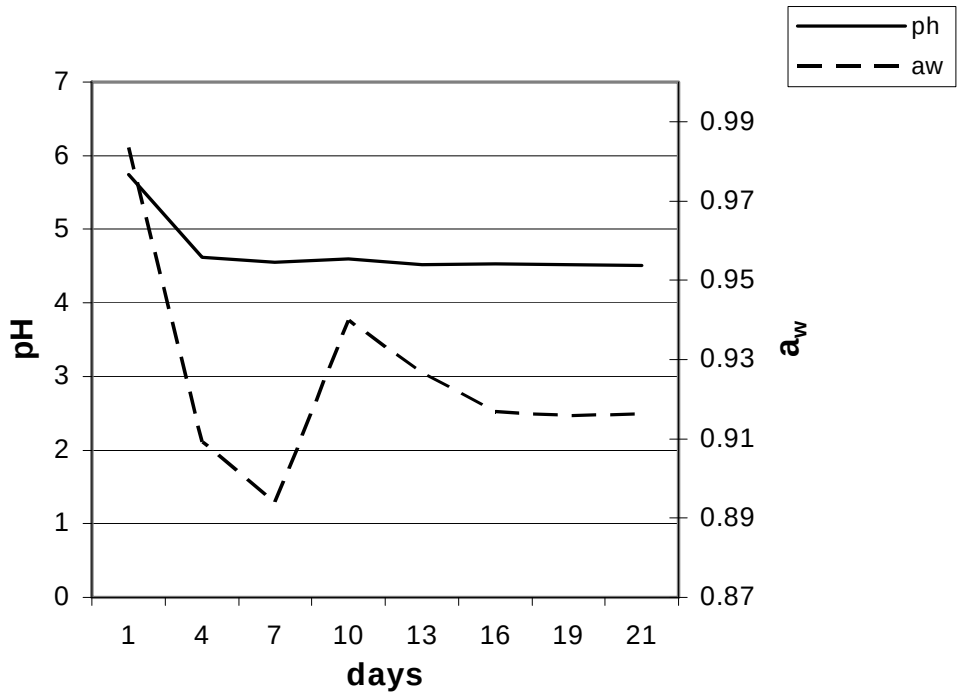

Figure 1. Water activity and pH values in salami made from seal meat (batch A) over 21 days

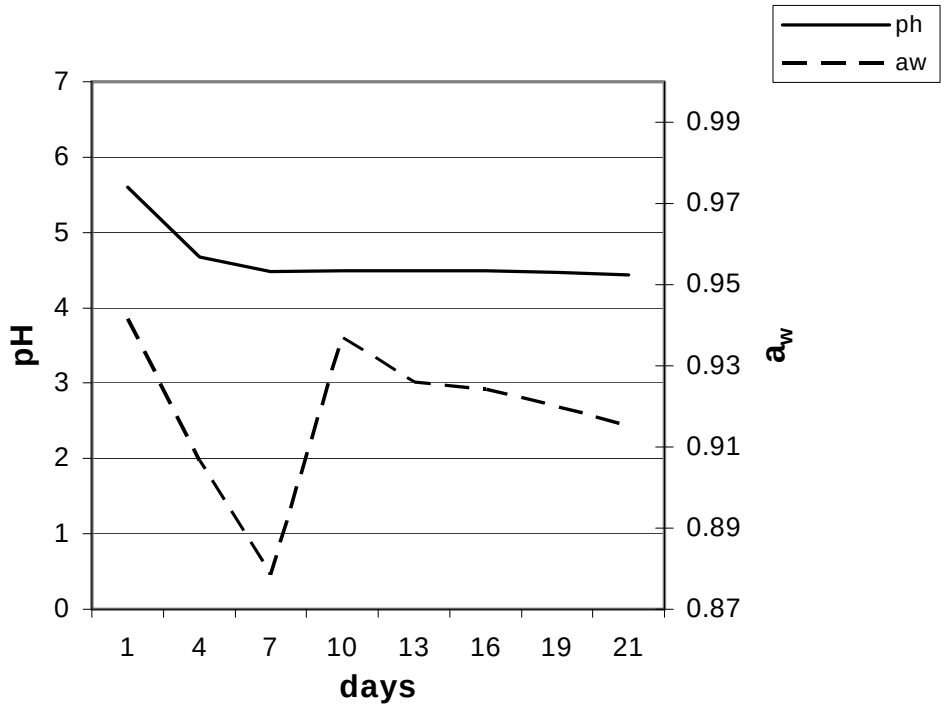

Figure 2. Water activity and pH values in salami made from seal and pork meat (batch B) over 21 days

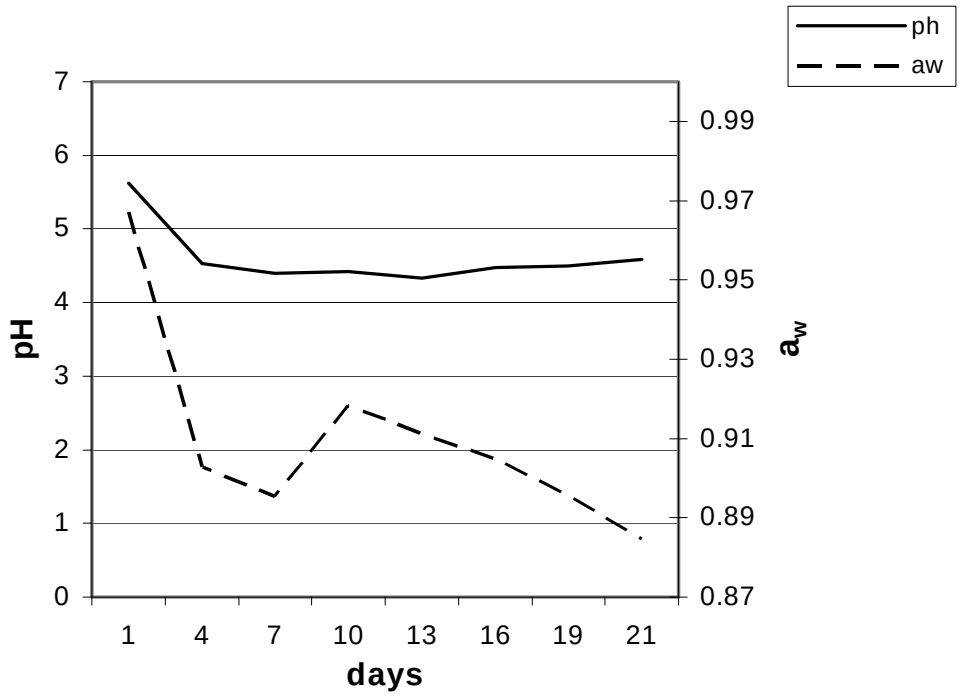

Figure 3. Water activity and pH values in salami made from seal and beef (batch C) over 21 days

Table 2

Regression line data for the pH values of all three seal salami batches over 21 days

Batch	Intercept (a)	Gradient (b)	R^2 value
A	5.1975	-0.1107	0.4095
B	5.1286	-0.1084	0.4566
C	4.9983	-0.0866	0.2567

A= pure seal meat
B= seal meat with pork
C= seal meat with beef

4.4.2. Colour variation between the three different batches of salami

From the selection of ingredients, it can be assumed that batch A will appear the darkest, due to the high level of seal meat in it. Batch C should also be darker than batch B, as beef has a darker colour than pork. The CIELab colour space was used to determine colour change in the sausages. Measurements were taken on the batter prior to stuffing and then at regular intervals. As times between the preparation of the batter to the time of stuffing differed between batches, there could be discrepancies in the colour measurements due to the action of nitrite on the product and the time it had to have an effect. Therefore, it was decided to omit the first colour value of the batter. In this colour space, L* (Fig. 4) indicates lightness and a* (Fig. 5) and b* (Fig. 6) are the chromaticity coordinates, a* being sensitive to red and green, whereas b* is sensitive to yellow and blue chroma/hues. As the colour parameters indicate linear changes, linear regression equations were fitted to the data (Table 3).

Formation of heme-containing pigments in fermented sausages follows the same pathways as in other nitrite-containing meat products. The low pH value has a strong influence, initially destabilising myoglobin and increasing the rate of auto-oxidation to metmyoglobin. The heme group dissociates at the pH value of many fermented sausages and colour is primarily attributed to nitrosomyoglobin (Slinde & Nordal, 1978). Previous studies on dissociation curves for myoglobin and haemoglobin in citric acid buffer showed that the heme group dissociates from the proteins at lower pH values comparable with those normally found in dry sausages during fermentation (Slinde & Nordal, 1978). There was a decrease in absorbance values, due to the dissociation of the heme from myoglobin or haemoglobin.

Hutchings (1994) suggested that a decrease in the L* value corresponds to a decrease in whiteness, or lightness. According to Slinde & Nordal (1978), the dissociation of the heme group would result in a decreased lightness, i.e. darkening, and thus an L* value decline. It can be seen that batch B showed a slight decline at day ten. This would correlate with a pH value <5, which

would indicate that dissociation of heme had occurred, and which would be more visible in the lighter salami, such as the one with pork, which is batch B.

Fig. 4 shows that all three batches showed a decrease in lightness over time. Batches A and C had very similar gradients, -0.2674 and –0.278 respectively (Table 3), indicating that they had a similar degree of darkening. As pork is a lighter meat to begin with, its rate of darkening was less, as less pigment is available to oxidise to a darker state. Thus, this batch (B) had a gradient of –0.2286, less than that of the other two batches. The L* value of batch A was much lower than that of B and C, as was the intercept. This would correlate positively with the higher content of seal meat in this batch, which contained very high concentrations of hemoprotein (average 60mg/g^{-1}) (Synowiecki *et al.*, 1992).

According to Slinde (1987), the black colour found in salami is due to a hydrophobic component, and as seal meat is such a rich source of pigment, haemoglobin would be such a component. The dissociation of heme from haemoglobin and myoglobin would result in the L* value changing. A pH decline would result in the dissociation of these components. The red core found in many salami, at pH <5, would indicate the reductiveness of this part of the sausage, and thus the heme group would be less likely to dissociate from the haemoglobin or myoglobin, and not form the darker colour. The L* value also represents the reflection and absorbance as the surface changes due to the loss of water, which leads to decreased reflectance and absorbance on the surface of the sample.

The a* value is usually the value which is the most accurate when relating values to actual visual experience by the observer (Precise Colour Communication, 1998). A difference of 1-1.5 is taken to be able to be recognised as a difference visually. Fig. 5 shows that the a* value of all three batches decreased over time, with batch C showing the fastest decline, with a gradient of -0.5315, and batch B showing very little change, with a gradient close to 0 (-0.0631). Batch A, the batch containing the highest concentration of seal meat, showed a slight decrease over time, with a gradient of –0.1046 per time unit. According to Hutchings (1994), an increase in the a* value is correlated to an increase in the red colour of what it is being measured. The negative gradient in these batches would therefore indicate that the red colour was changing to a darker, browner colour, due to formation of metmyoglobin.

Fig. 6 illustrates the behaviour of the b* values of all three batches. The trend of all three batches appeared similar over the first four days. Thereafter, batch B increased slightly, while the other two batches decreased. This indicates that the yellow fraction of batch B was more prominent.

Batch B's b* value continued to increase throughout maturation, whereas the other two batches had decreasing b*-values, correlating with an increase in the blue fraction. Batch C showed nearly no change at all, with a gradient of 0.0042, indicating very little change in the yellow fraction over time. However, due to its high intercept, 7.9896, it contained a greater concentration of yellow than the other two batches, A and B, with intercept values of 4.8927 and 7.1129 respectively. Batch A had the largest rate of change over time, with an upward trend of 0.1359.

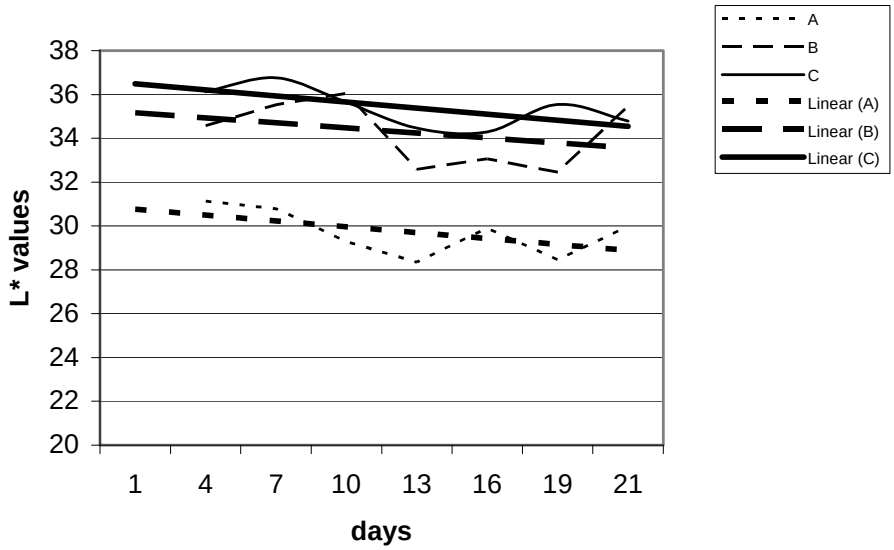

Figure 4. L*-values of batches (A, B, C) over time, as well as linear regression lines of the data

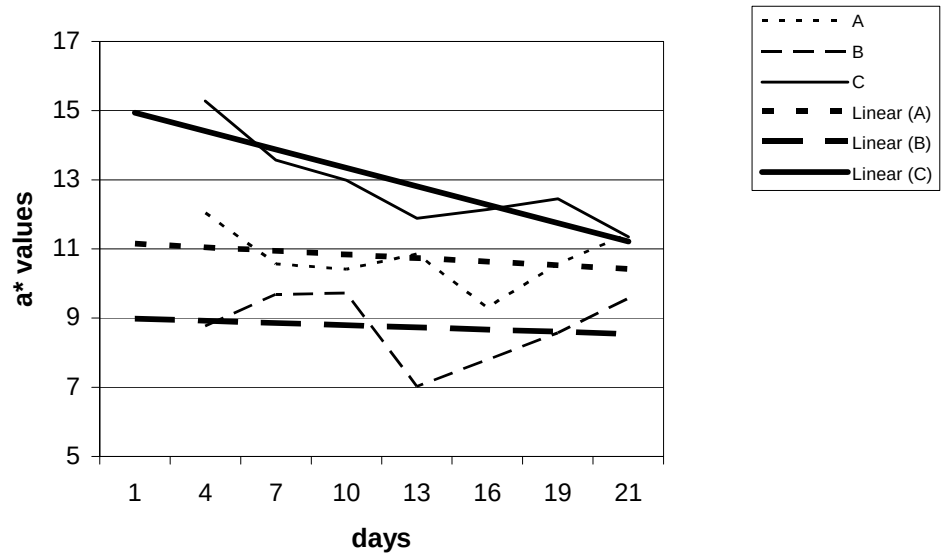

Figure 5. a*-values of batches (A, B, C) over time, as well as linear regression lines of the data

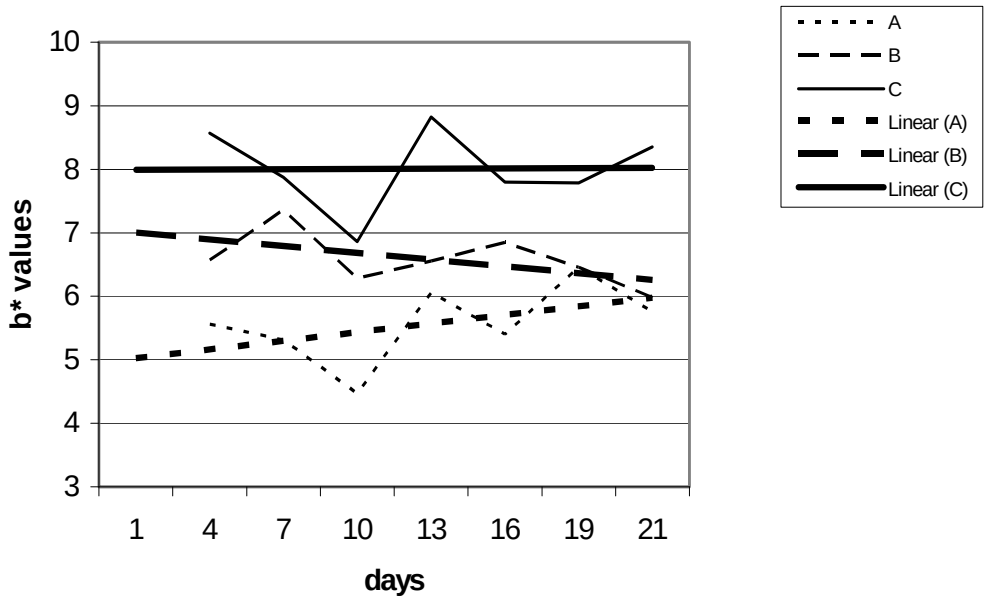

Figure 6. b*-values of batches (A, B, C) over time, as well as linear regression lines of the data

In the L*a*b* colour space, colour difference can be expressed as a single numerical value, $\Delta E^*_{ab.}$ This indicates the size of the colour difference, but not how the colours differ (Fig. 7) (Precise Colour Communication, 1998). High ΔE^*_{ab} values are indicative of greater colour difference. It is evident that all three batches were similar in behaviour regarding colour difference across the three-week time interval. As ΔE^*_{ab} values are a combination of a* and b* values, it can be expected that batch C will show the largest gradient, and batch B the smallest. All three batches showed a decreasing trend over time, with the largest decrease being seen in batch C (-0.4358), and the least in batch B (-0.2551), whilst that of batch A (-0.2679) was intermediate.

Hue (Fig. 8) is the term used to describe the attribute of a visual perception, according to a specific area, or proportions of two of the primary colours for the classifications of red, yellow, green and blue (Precise Colour Communication, 1999; Hutchings, 1994). An increase in hue indicates a slight shift towards the yellow spectrum, which would be interpreted as an increase in the brown colour in terms of visual appearance. Accordingly, batch C would be the batch showing a shift towards the yellow spectrum, which would agree with Fig. 6. The other two batches showed a decrease, with a gradient of -6.2363 and -3.3063 for batches A and B respectively, and with both batches having a similar intercept (98.05 and 94.577 respectively).

An increase in chroma is indicative of a trend away from greyish colour, and increased saturation, being visually interpreted as a more vivid red (Hoffman & Mellett, 2003), or it can be described as the colourfulness of an object relative to its brightness (Hutchings, 1994). Fig. 9 depicts the change of chroma over time. Batch B shows a smooth increase (gradient 4.0911) over 21 days. The line moves smoothly along the L-plane, which correlates to the L*value in Fig. 4. This would correlate to the R^2 value of batch B (Table 3), of 0.9977, which indicates a nearly perfectly linear rate of change over time. However, batch B had a much larger rate of change than batch C, which has a gradient of only 1.9379. Batch A shows little variation over time, but remains fairly even, with a gradient of –0.0302. Thus, it can be said that batches B and C became less grey, and redder in colour. This would be due to the action of nitrite on the colour of the sausage, which would be clearer in the lighter meat types, pork and beef. As pork is the lightest meat, it showed the most rapid increase in chroma over time.

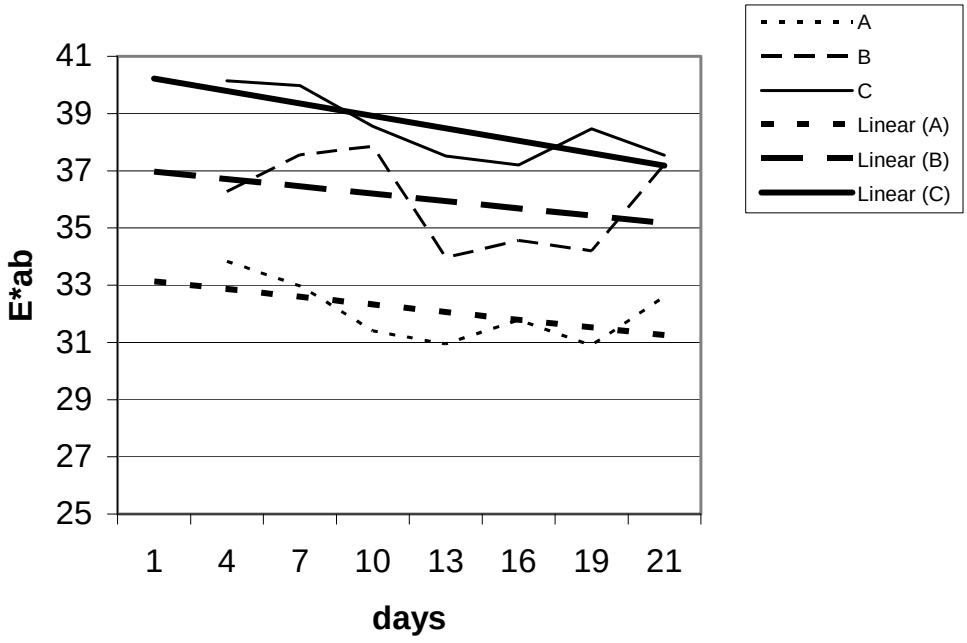

Figure 7. ΔE^*_{ab} value of batches A, B, C over time, as well as linear regression lines of the data

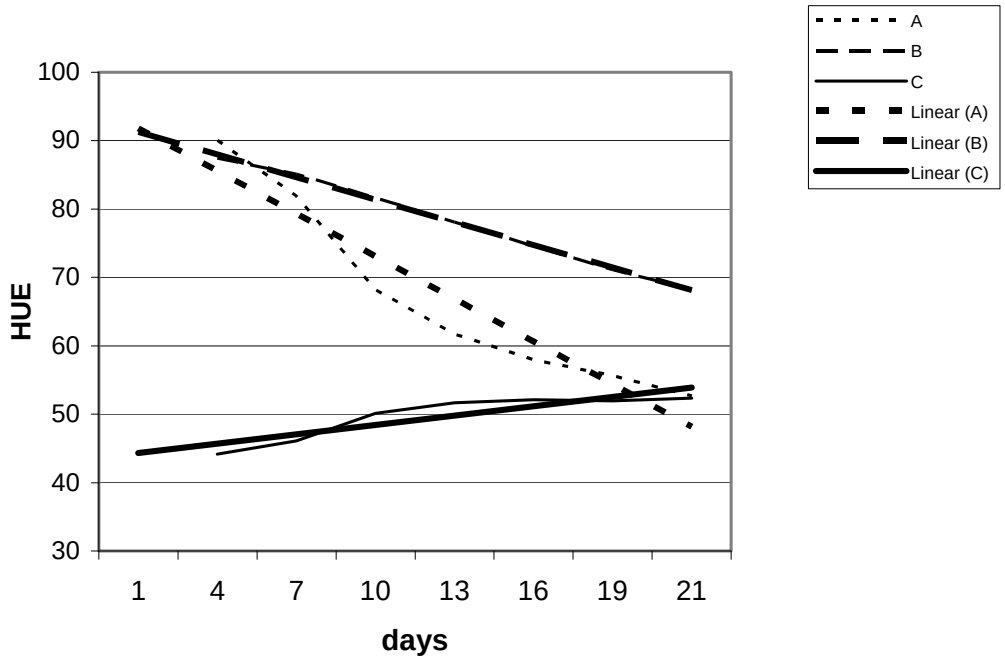

Figure 8. Hue values of batches A.B,C over time, as well as linear regression lines of the data

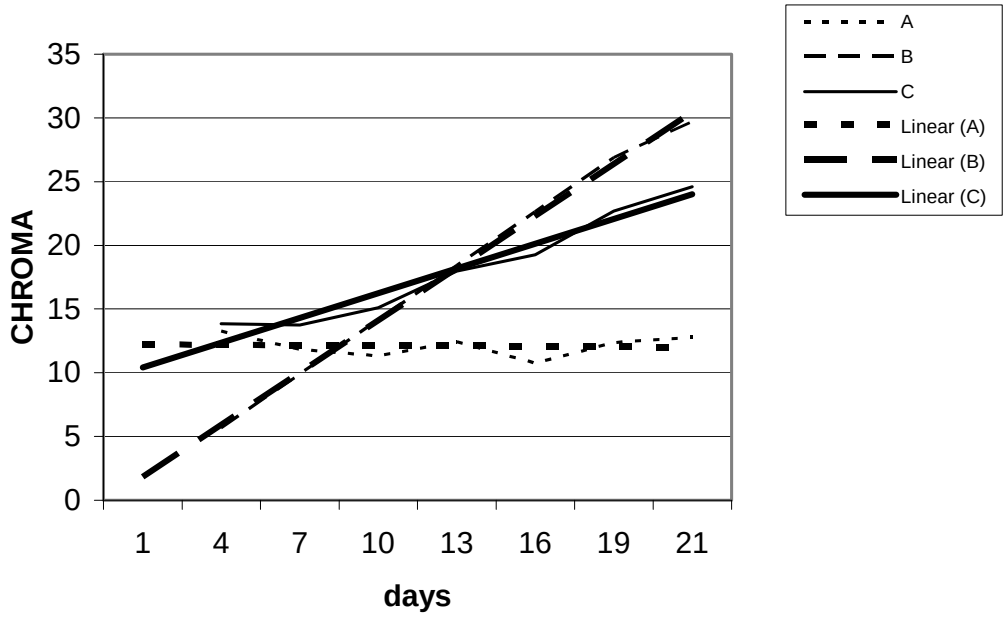

Figure 9. Chroma values of batches A, B, C over time, as well as linear regression lines of the data

Table 3

Regression line data for the L*, a*, b*, $\Delta E^*_{ab,}$ hue and chroma values of all three salami batches containing some degree of seal meat, over a period of 21 days

Parameter	Batch	Intercept (a)	Gradient (b)	R^2 value
L* values	A	31.033	-0.2674	0.2906
	B	35.387	-0.2286	0.1063
	C	36.763	-0.2780	0.4377
a* values	A	11.263	-0.1046	0.0690
	B	9.047	-0.0631	0.0173
	C	15.468	-0.5315	0.7691
b* values	A	4.893	0.1359	0.2183
	B	7.113	-0.1070	0.2807
	C	7.990	0.0042	0.0002
ΔE^*_{ab} values	A	33.401	-0.2679	0.2721
	B	37.219	-0.2551	0.1084
	C	40.466	-0.4358	0.6272
Hue	A	98.050	-6.2363	0.9115
	B	94.577	-3.3063	0.9977
	C	42.972	1.3646	0.7960
Chroma	A	12.264	-0.0302	0.0056
	B	-2.273	4.0911	0.9977
	C	8.489	1.9379	0.9520

4.4.3. Weight loss

Fig. 10 shows every third consecutive day's weight loss over a period of 20 days. All three batches showed similar weight losses over time, although batch A had a slightly lower weight loss during the first four days. This similarity in weight loss could be ascribed to the environment in the chamber (being set at fixed % RH) dominating any potential variation within the different salamis and thus over-riding their potential effect.

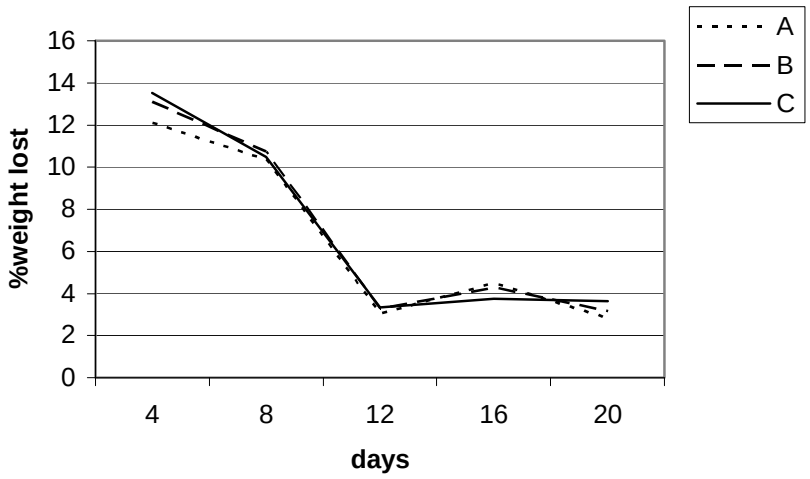

Figure 10. Percentage weight loss over time of all three salami batches A, B and C over 21 days

4.5. Conclusion

The results presented in this investigation show that the production of fermented, processed products is possible using the meat of the Cape fur seal. As this was a pilot study, some variables were omitted, such as microbiological examination of the batter and the finished product. As the seal is a wild animal, found under extensive conditions, its meat may have contained and attracted micro-organisms other than those found in the meat of intensively produced, traditional red meat producing species. It is vital to incorporate starter cultures into products produced from these species, as these could eliminate any resident bacteria. Further research is required into the microbiological characteristics of seal meat.

Significantly, these findings regarding the quality and palatability of seal meat products open up the possibility of using seal meat on a larger, commercial scale, leading to the more effective utilisation of this species, as seals are momentarily only hunted for their skins and genitalia. This opportunity for potential revenue generation, in turn, might make the harvesting of these animals a more acceptable practice globally, especially if it were known that the whole animal was being utilised fully and that proper use was being made of the meat.

Certainly, such an undertaking is not without challenges:

a) Seal flesh is dark in colour, which would require investigation into consumer acceptance of a product containing such dark meat. Consumers need to be completely satisfied with the sensory characteristics of a product before any other quality variables enjoy priority (Chambers & Bowers, 1993).

b) Presently, the supply of meat is seasonally dependent, as concessions to harvest seals are regulated strictly. Although this means in effect that securing a regular supply of meat throughout the year would be less likely, this in turn might well increase the desirability and value of the available meat.

It is possible that these potential drawbacks might also be offset by the fact that seal meat is regarded as healthy and wholesome, due to its lean meat content and high concentration of polyunsaturated fatty acids (see Chapter 3). Using seal meat in salami form would also ensure a continuous supply of this meat to the consumer, as it is shelf stable. In a world populated by people who are increasingly aware of the quality, purity and naturalness of their diets and the meat they consume, these features could be used as strategic factors in any campaign to market seal meat products commercially.

4.6. Acknowledgements

I would hereby like to thank the following persons and/or companies for their assistance in this trial: The company Roelcor and Roers, who supplied the materials other than the seal meat, as well as staff and their expertise in the production and continued fermentation of the salami; Mr. Herman Jonker, professional hunter contracted by Nature Conservation in the Western Cape, who realised the importance of this project and assisted in obtaining the seals, whose meat was used for this investigation; and Liezel and Greg, fellow students, for dissecting a seal, which was deemed not suitable for human consumption due to advanced decomposition.

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Chapter 5

PRODUCTION OF SALAMI FROM GREY SEAL (*Halichoerus grypus*) AND MINKE WHALE (*Balaenoptera acutorostrata*) MEAT

5.1. Abstract

Salami was produced from the meat of the Grey (Havert) seal and Minke whale. Three different starter cultures (*Lactobacillus curvatus* HJ5, *Lactobacillus plantarum* L-74 and Gilde MF 1115, which consists of *Lactobacillus sakei*) were used in separate meat fermentations. Changes in pH, water activity, colour, cell numbers, weight loss and texture were recorded over 21 days. The initial pH of the salami was between 5.68 and 5.92 and decreased to between 4.5 and 4.8 after 21 days. Water activity decreased from 0.96 to an average of 0.90 during the fermentation period. The texture of the salami doubled over this period, as recorded by an increase in force (N) required to compress the meat. The raw seal meat contained 349.6mg saturated fatty acids (SFA), 271.6mg monounsaturated fatty acids (MUFA) and 175.8mg polyunsaturated fatty acids (PUFA) per 100g meat, whilst the raw whale meat contained 312.3mg SFA, 251.9mg MUFA and 179.6mg PUFA in the same sample size. The polyunsaturated:saturated fatty acid (P:S ratio) recorded for seal and whale raw meat was 0.503 and 0.575, respectively, whilst the n-6:n-3 ratio was 1.458 and 3.010, respectively. The seal salami had P:S ratios of 0.580, 0.649 and 0.618, and the whale salami P:S ratios of 0.679, 0.659 and 0.697 for starter cultures A, B and C, respectively. The n-6: n-3 ratios of both salami types were much higher than the ideal of <0.5. The results indicate that meat from both the Grey seal and the Minke whale is suitable for the production of salami. However, more research is required to investigate the use of seal and whale blubber instead of pork fat to develop an unique seal- or whale-only product.

Keywords: salami, Grey seal, Minke whale, fermentation, fatty acid, chemical composition

5.2. Introduction

The harvesting of seals and whales is a seasonal exercise and products need to be developed that will provide a year-round supply of meat. Salami is a meat product that has a long shelf life. According to Campbell-Platt (1987), products with a decreased water activity (a_w) level show decreased rates of mould development, bacteria and yeast growth. Products with a_w levels of <0.9 and with pH values of <5.0 are generally assumed to be safe from a health point of view in terms

of microbiological and bacterial contamination (Campbell-Platt, 1995). Salami can be defined as a blend of meat and fat particles, NaCl, curing agents, and spices stuffed into either artificial or natural casings. Salami traditionally contains 50-70% lean meat. The meat used in the production of salami should be suitable in terms of water-holding capacity, pH value and colour.

After drying of the mixture, the final product may contain up to 50% fat, which makes fat an important ingredient of fermented sausage. This can lead to problems such as rancidity, which has a direct effect on the shelf life of the product. Substances that can be added to act as anti-oxidants, one of which is ascorbic acid (Campbell-Platt, 1995), may also act as a curing agent and in stabilising the colour. Tocopherol can also be used, due to its different properties compared to ascorbic acid. Tocopherol is hydrophobic and is soluble in lipids, which makes it suitable for use in products with a high fat content.

The most common starter culture species used in the fermentation of beef and pork sausages are strains of *Lactobacillus, Pediococcus*, non-pathogenic S*taphylococcus* and *Micrococcus* (Lücke, 1986). Organic acids produced by these bacteria prevent the development of pathogenic micro-organisms (Schillinger & Lücke, 1987), aid the cohesion of meat particles (Townsend *et al*., 1980) and contribute to the colour change of fermented sausages.

The major role of lactic acid bacteria in the sausage is to produce organic acids, primarily lactic acid, from carbohydrates (Campbell-Platt, 1987). This results in a decrease in pH and contributes to the retardation of the development of undesirable micro-organisms. The decreased pH also results in a reduced water-holding capacity of proteins and, thus, results in correct desiccation of the product.

As the diet of aquatic mammals consists mainly of fish, their tissues contain more polyunsaturated fatty acids than those of their terrestrial counterparts. Although these fatty acids are very popular amongst health-conscious consumers, they are a challenge in the food industry when it comes to preventing rancidity of products and, therefore, increasing the shelf life of products.

The Grey (Havert) seal (*Halichoerus grypus*) is classified as being a part of the Mammalia class, of the order pinnipedia and belonging to the family of the *phocidae*. It is a large seal that lives in the coastal seas of the temperate North Atlantic. It is believed that approximately fifty percent of the world population is found in British waters (De Jong *et al*., 1997).

According to Shahidi & Synowiecki (1993), in terms of nutritional quality, seal meat is comparable or better than that of other sources of animal protein suitable for human consumption. Its intramuscular lipids and those present in the blubber, are excellent sources of omega-3 fatty acids (Shahidi *et al*., 1994). A myoglobin content of up to 10% provides the opportunity to utilise seal meat as a colour contributor in meat products utilising meats of lesser colour quality or potential (Synowiecki *et al*., 1992).

The Minke whale (*Balaenoptera acutorostrata*) is found from along the North polar ice-edge to the tropics, and is frequently encountered quite close to the coast. Since the middle ages, Minke whales have been hunted in the North Atlantic, when they were captured using nets. Modern whaling for Minke whales began in the 1920's, and between this time and 1973, the Norwegian fleet harvested approximately 94 000 whales. In the 1980's, a quota was set at 2 500 whales in the North Atlantic (Klinowska, 1991). Since the moratorium on commercial whaling was introduced in the 1985-1986 season, these whales have been harvested in reduced numbers with scientific permission. In 1996, the commercial hunt for Minke whale by the Norwegians recommenced.

Little research has been done on the meat quality parameters and processing attributes of aquatic mammal species. The aim of this study was, therefore, to determine if seal and whale meat would be suitable for the production of salami. Factors monitored included the ability of starter cultures to react favourably with the meat, pH, a_w, microbiology, colour development and moisture loss.

5.3. Materials and methods

5.3.1. Raw materials

The seal and whale meat required for this trial were obtained through commercial sources in Tromsø, northern Norway. The meat was frozen and packed before being sent to Oslo. The pork fat was obtained from a local slaughtering plant near Oslo.

5.3.2. Starter cultures and growth conditions

The starter cultures used were *Lactobacillus curvatus HJ5* (batch A), *Lactobacillus plantarum L-74* (batch B) and *Gilde MF 1115* (batch C), which consists of *Lactobacillus sakei.* The bacteria were grown in MRS Broth (Merck), where they were incubated for 24 hours at 30°C. The cells were harvested at an optical density (OD_{600nm}) of 1.6-1.9 and resuspended in 10ml sterile distilled water. An aliquot of 0.3ml cell suspension (10^5 to 10^6 cells/g salami mixture) was used as an inoculum.

5.3.3. Meat preparation and fermentation

Frozen lean meat (7kg) of either seal or whale, was added to a 20-litre KILIA bowl cutter, and chopped to a particle size of approximately 3-4mm at low speed. Thereafter, tocopherol (0.4g), dextrose (120g), 15g spices (pepper, paprika and garlic), 20g herbs (rosemarine, thyme and sage) and ascorbic acid (2g) were added and mixed at low speed for approximately 30 seconds. The starter cultures, salt (150g) and NO_2^- (nitrite) were added to the mixture prior to the fat (3kg), which was then added and chopped at low speed for approximately two minutes to result in fat particles of approximately 2-4mm in diameter.

The batter was then stuffed into 7mm-diameter cellulose casings, using an automated FATOSA sausage stopper able to hold 15 litres. The temperature of the batter immediately prior to stuffing was between -0.8 and 0^{o}C. The sausages were made in lengths of 20.0-25.5cm, with weights between 610g and 911g. All sausages were marked with regard to species and starter culture. Three sausages of each batch were marked and placed on the rack, in order to ascertain the average weight loss during the process. These sausages were weighed (in grams) at the start (day 0) and then regularly on days 6, 9, 13 and 21 of the trial. The sausages were left at room temperature for the first 18 hours after preparation, to allow the surfaces to dry.

On the morning of day two, a sample of each batch was taken to test for pH and a_w. The remaining sausages were placed into a controlled chamber (22^{o}C and 95% RH) to allow the starter cultures to develop. Samples were analysed each day for the first seven days, with regards to pH and a_w.

On day five, the sausages were cold smoked (18^{o}C at 60% RH) with oak chips. Thereafter, the sausages were allowed to mature. During the first week of maturation, the temperature remained at 18^{o}C, with the RH at 88%. During the second week, the temperature remained constant, but the RH decreased to 80%. During the last week of maturation, the RH decreased to 75%.

5.3.4. Recorded parameters

Readings were collected on specific days throughout maturation. The acidification of the blend, due to lactic acid bacterial growth, was recorded by measuring the pH using a Beckman 31 insertion probe.

The a_w readings were recorded with an AquaLab CX-2 water activity analysing apparatus. Colour measurements were taken from slices of the salami from days 7, 13 and 21, using the

Chroma magic program, with a Minolta CR 300 OG hand-piece attached to a computer. Readings were collected as L*, a* and b* values.

Cell numbers were monitored from the first day, by using plating procedures on MRS Biolab agar growth media. Viable cell numbers were recorded immediately following incubation at 25^{o}C, and at three-day intervals thereafter.

Texture analysis was recorded on days 6, 9, 12 and 21 using a TX-XT2 texture analyser. Slices of each salami, approximately 1.5cm thick, were used.

5.3.5. Chemical analysis

The moisture and protein content (g/100g meat sample) of the raw materials, batter and final salami were determined for all the samples according to the Association of Official Analytical Chemist's Standard Techniques (AOAC, 1997). The moisture content was determined by drying samples at 105^{o}C for 24 hours. To determine the protein content, dried and defatted samples were grounded with a pestle in a mortar until a fine powder was obtained. 0.100mg of powder per animal was inserted into a foil wrap designed for the Leco protein analyser (Leco Fp-528, Leco Corporation). The protein was determined as Nitrogen, which was multiplied by 6.25 to calculate the protein concentration in the sample. Total fat content was determined by the method of Lee *et al*. (1996) using chloroform: methanol (2:1) as solvent.

5.3.6. Myoglobin content and dissociation of heme from myoglobin

Myoglobin extraction was conducted on thawed meat samples. This was done to ascertain the percentage myoglobin present in the muscle of these animals. According to the method described by Krzywicki (1982), thawed meat was cut into small (3mm cube) pieces, of which 5g was weighed out in duplicate per sample, and placed into 50ml polypropylene centrifuge tubes. 25ml ice cold sodium phosphate buffer (pH 6.8, 0.04M) was added to the tube containing the sample and homogenised for 40-45 seconds at low speed, ensuring that the meat was mixed thoroughly in the solution. The sample was stored at 4^{o}C for one hour. Thereafter, the sample was centrifuged at 9000 rpm for 45 minutes at 4^{o}C, and the supernatant filtered through Whatman no. 1 filter paper. Individual absorbances were read at 700 (A_{700}) and 525 (A_{525}) nanometer, using phosphate buffer as the blank. The following equation was used to calculate the % myoglobin present in the sample:

$$\text{Myoglobin (mg/ml)} = (A_{525}-A_{700}) \times 2.303 \times \text{dilution factor}$$

In the case of the whale meat, the absorbance reading was acceptable (<1) without any further dilution. The seal solution, however, was opaque, and a dilution had to be made until an acceptable reading was found. This dilution was 20% seal filtrate and 80% phosphate buffer as diluent.

The dissociation of heme from myoglobin and haemoglobin was also monitored throughout the processing of the salami on days 1, 5, 13 and 20. Extraction of the hydrophilic colour components was undertaken as by Slinde & Nordal (1978); 10g substrate was homogenised with 40ml 50mM sodium phosphate buffer (pH 6.9) and the solution cleared by centrifugation at 15 000 rpm (4^{o}C) for 10 minutes. Thereafter, the supernatant beneath the lipid layer was extracted once, using a pipette. The pigment concentration was determined by using a spectrophotometer to scan the Soret region (360-430nm).

5.3.7. Fatty acid determination

The fatty acid content of the samples was determined using the method described by Tichelaar *et al*. (1998). After thawing the raw meat, batter and salami samples, the lipids in a 2g sample were extracted with chloroform/methanol (2:1; v/v). All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. A polytron mixer (Kinematica, type PT 10-35, Switzerland) was used to homogenise the sample within the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard to quantify the individual fatty acids.

A sub-sample of the extracted lipids was transmethylated for two hours at 70°C, using methanol/sulphuric acid (19:1; v/v). After cooling, the resulting fatty acid methyl esters (FAME) were extracted with water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen.

The FAME were purified by TLC (silica gel 60 plates) and analysed by GLC (Varian Model 3300 equipped with flame ionisation detection), using a 60 m BPX70 capillary column of 0.25mm internal diameter (SGE, Australia). The hydrogen gas flow rate was 25ml/min; and the hydrogen carrier gas rate 2-4ml/min. Temperature programming was linear at 3°C/min, with an initial temperature of 150°C, a final temperature of 220°C, an injector temperature of 240°C and a detector temperature of 250°C. The FAME in the total lipids was identified by comparison of the retention times to those of a standard FAME mixture (Nu-Chek-Prep Inc., Elysian, Minnesota).

5.3.8. Statistical analysis

The non-linear procedure (Proc NLIN) of SAS (1990) was used to fit exponential decay regression models to the rate of salami pH decline over time. The model fitted was of the form:

$y = a + be^{(ct)}$,

where y is the dependant variable (pH) and t the time (day), a the intercept and b the gradient.

The two-sample T-test was used on SAS (Statistical Analysis Systems, 1990), to compare the chemical and fatty acid values of the seal and whale salami, using the three different starter cultures. Various regression lines were fitted to the relevant data to determine which type would suit the specific data set best. Linear regression lines had the best goodness of fit, and were, therefore, used for most data sets. Gradient, intercept and R^2 values were then calculated for these regression equations.

5.4. Results and discussion

5.4.1. pH, water activity, colour and microbiological measurements

pH is the most important as well as most readily-obtained variable used to monitor fermentation progress in salami (Acton *et al.,* 1977; De Ketelaere *et al*., 1974). The production of lactic acid results in a decrease of meat pH to a final pH of between 4.8-4.9, as well as stimulating the aggregation of protein, which forms the typical texture of sausage due to this coagulation (Campbell-Platt, 1987). The presence of lactic acid bacteria help in decreasing the pH of the meat and thus making conditions unfavourable for other Gram-negative bacteria (Campbell-Platt, 1987).

The consistency of fermented sausage is largely determined by the pH value of the product, as well as the a_w level, the consistency being ideal at a pH value <5.4 and an a_w level <0.9. The pH value is the major determinant of consistency in high-acid, semi-dry sausages, and the a_w level in low-acid, dry sausages (Campbell-Platt & Cook, 1995).

The pH and a_w levels were always recorded on the same day, in order to correlate the values more accurately. In both the seal (Fig. 1) and the whale salami (Fig. 2), starter culture A (*Lactobacillus curvatus* HJ5) had the highest final (21 days) pHs of 4.72 and 4.70 respectively. Starter culture B (*Lactobacillus plantarum* L-74) had the lowest ultimate pH values in the seal and whale salami (4.58 and 4.52 respectively). Starter culture C (Gilde MF 1115) showed a more rapid pH decline in the seal salami than in that of the whale.

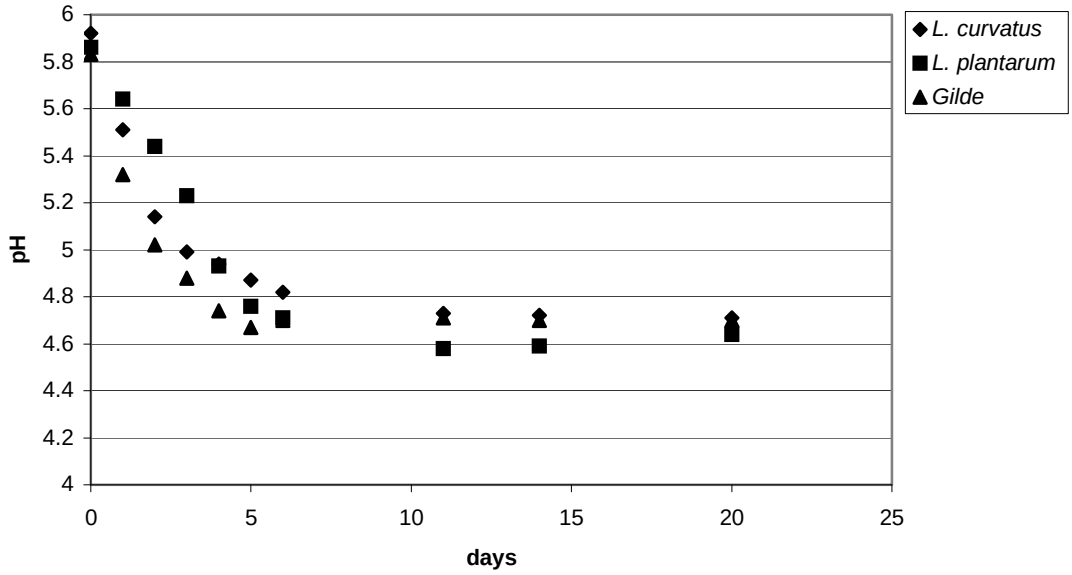

Figure 1. pH values of seal salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) over 21 days

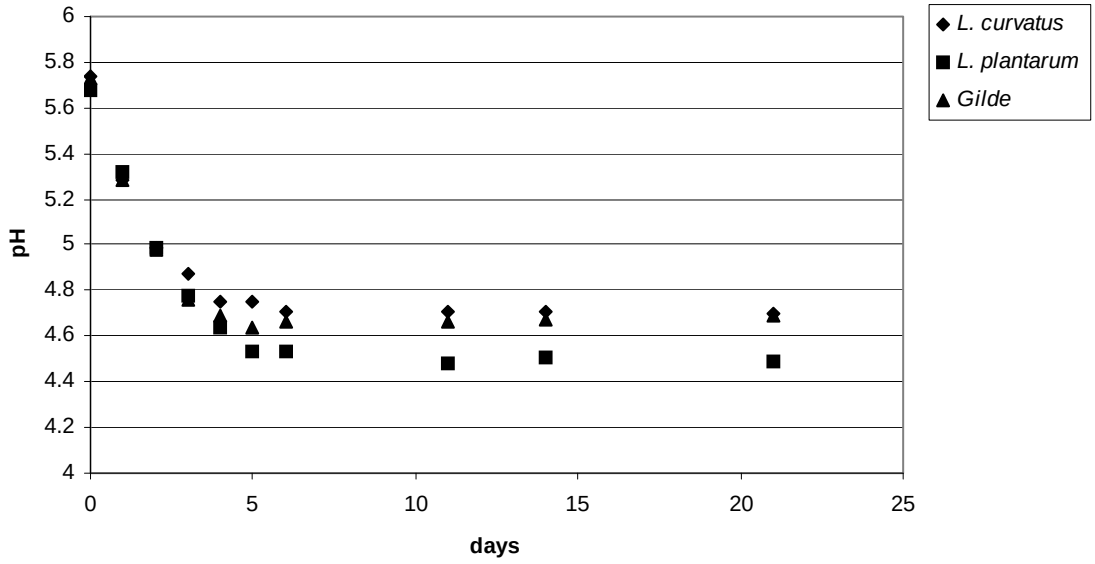

Figure 2. pH values of whale salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) over 21 days

Research done on Felino salami by Dellaglio *et al*. (1996) showed that the maximum pH of their salami was 6.36, with the minimum value being 5.40. These final pH values of their sausages were too high; they should ideally be less than 4.5, which the seal and whale salami were.

The conversion criteria were met for all the exponential decay models fitted for the pH decline of the salami with time. The probability values (Pr > F) for the equations were all <0.0001, and the

various equations for the two mammal species and three starter cultures are given in Table 1. As expected, the initial pH readings were all very similar, whilst the calculated initial pH values did not differ greatly from the actual values measured.

For both species, starter culture B had a slower rate of pH decrease (Table 1) although this effect was more pronounced in the seal salami (-0.290 vs -0.477 units per day). This also resulted in the whale salami with starter culture B having the lowest calculated ultimate pH (4.478), which was similar to the actual pH measured.

Table 1

The calculated constants for the exponential equations fitted to the pH decline of the seal and whale salami made from three different starter cultures (A: *L. curvatus*, B: *L. plantarum* and C: Gilde) over 21 days

Species	Starter Culture	Measured pH (ave)		$y = a + be^{ct}$		
		Day 0	Day 21	a	b	c
Seal				4.662 ± 0.021	1.824 ± 0.077	-0.438 ± 0.029
	A	5.91	4.72	4.743 ± 0.024	1.832 ± 0.108	-0.539 ± 0.045
	B	5.85	4.61	4.541 ± 0.031	1.860 ± 0.074	-0.290 ± 0.022
	C	5.84	4.69	4.677 ± 0.010	2.221 ± 0.071	-0.631 ± 0.025
Whale				4.611 ± 0.127	1.982 ± 0.086	-0.577 ± 0.032
	A	5.69	4.70	4.703 ± 0.004	1.979 ± 0.042	-0.645 ± 0.017
	B	5.69	4.51	4.478 ± 0.010	2.001 ± 0.047	-0.477 ± 0.016
	C	5.71	4.69	4.645 ± 0.011	2.048 ± 0.082	-0.636 ± 0.031

The a_w patterns of the three starter cultures in the seal salami were very similar (Figs. 3 and 4), although starter culture B generally showed a lower a_w than the other two. In the whale salami (Fig. 4) starter culture B was slightly lower at the beginning, and starter culture A had the highest values over the first two days. Thereafter, the a_w of all starter cultures followed a similar pattern. Starter B showed a very rapid decline in a_w after day 15. This corresponds with the day after the RH was decreased from 80 to 75% in the chamber. However, the other two starter cultures did not show such a rapid decline.

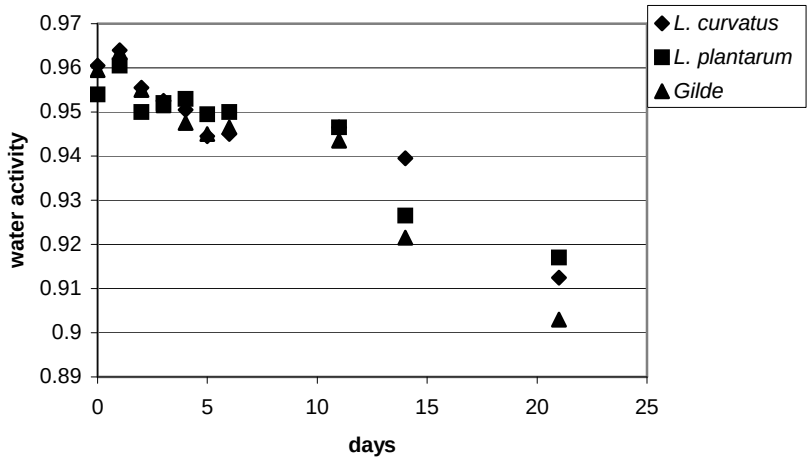

Figure 3. Mean a_w values of seal salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) over 21 days

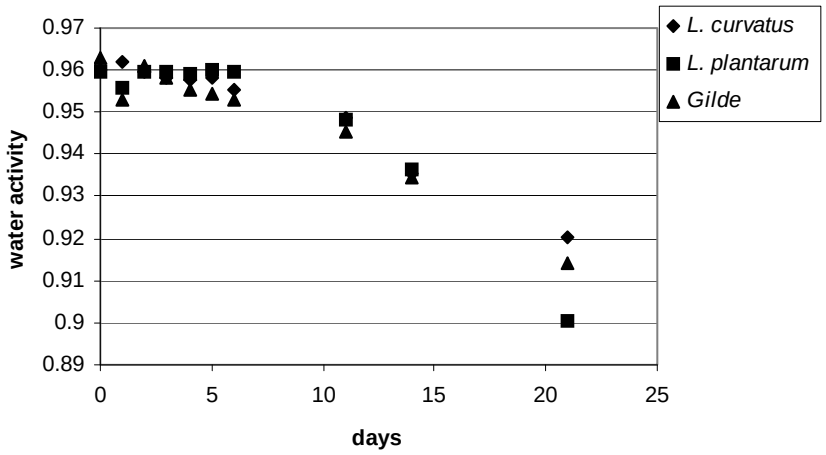

Figure 4. Mean a_w values of whale salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) over 21 days

For the change in a_w with time, linear regression equations were calculated, as they gave the best goodness of fit according to the R^2 values. It can be seen that the rate of change, gradient (b), over time was identical in both species (Table 2) using starter culture A (-0.020). For all the salamis (species and starter culture), the intercept values, i.e. initial a_w levels, were similar, as they reflect the values on day 0, and the different starter cultures would not have had sufficient time to show an effect at this point. The R^2 values for the a_w and the pH values were all close to 1, indicating a good representation of the fit of the linear regression to the data.

Table 2

Linear regression line data, intercept (a), gradient (b) and goodness of fit (R^2) for salami water activity data for both mammal species and all three starter cultures (A: *L. curvatus*, B: *L. plantarum* and C: Gilde)

Species	Measurement	Starter culture	a	b	R^2
Seal	a_w	A	0.9605	-0.0020	0.8717
		B	0.9586	-0.0019	0.8880
		C	0.9614	-0.0026	0.9351
Whale	a_w	A	0.9646	-0.0020	0.9487
		B	0.9672	-0.0026	0.8421
		C	0.9636	-0.0021	0.9314

Formation of heme-containing pigments in fermented sausages follows the same pathways as in other nitrite-containing meat products. The low pH-value has a strong influence, initially destabilising myoglobin and increasing the rate of auto-oxidation to metmyoglobin (Slinde & Nordal, 1978). The heme group is dissociated at the pH value of many fermented sausages and colour is primarily attributed to nitrosomyoglobin (Slinde & Nordal, 1978). Previous studies of the dissociation curves for myoglobin and haemoglobin in citric acid buffer show that the heme group dissociates from the proteins at lower pH values comparable to those normally found in dry sausages during fermentation.

According to Slinde (1987), the black colour found in salami is due to a hydrophobic component, and, as seal meat is a rich source of pigment, myoglobin would be regarded as such a component. The dissociation of heme from haemoglobin and myoglobin would result in the L* value changing. A pH decline would cause the dissociation of these components. The red core frequently found in many salami (at a pH <5) would indicate the reductiveness of this part of the sausage, and thus the heme group would be less likely to dissociate from the haemoglobin or myoglobin, and thereby causing the darker colour. It was observed that the whale salami had such a red core, indicating that it has fewer reductive properties than the seal.

Hutchings (1994) suggests that an increase in the L* value corresponds to an increase in lightness. A low L* value would, therefore, indicate a darker substrate. It is also suggested that a decrease in the L* value could be correlated to a pH value of less than 5, which would be indicative of the dissociation of heme from myoglobin or haemoglobin. Whale meat, being a lighter meat than seal meat, would have a more pronounced effect than the darker meat of the

seal, which contains a higher concentration of haemoprotein levels (average 60mg/g^{-1}) (Synowiecki *et al*., 1992).

Figs. 5 and 6 show the L* values of the seal and whale salami respectively. Whale meat has a lighter, redder colour than seal meat, and as the L* value correlates to lightness, the lighter whale meat had higher L* values ranging between 33.94 and 37.02, whereas the darker seal meat had lower L* values, ranging between 26.24 and 32.88. It can be expected that colour changes in the whale salami would differ from that of the seal, and be more visible to the human eye, due to these high L* values.

As seen in Fig. 5, starter cultures B (*L. plantarum*) and C (Gilde) showed a decrease in the L* value over time. In starter culture A (*L. curvatus*) the L* value fluctuated over time. Furthermore, in the second week of maturation, both starter culture A and C showed a slight increase, whereas culture B showed a decrease in the seal salami. In the third week it was observed that the L* value of starter culture A increased, whereas both starter cultures B and C showed a decrease.

In Fig. 6, for the whale salami, all three starter cultures showed an increase in their L*values in the second week of maturation. During the third week, both A and B showed a very definite decrease, but C showed a continued increase in L* value, until day 21.

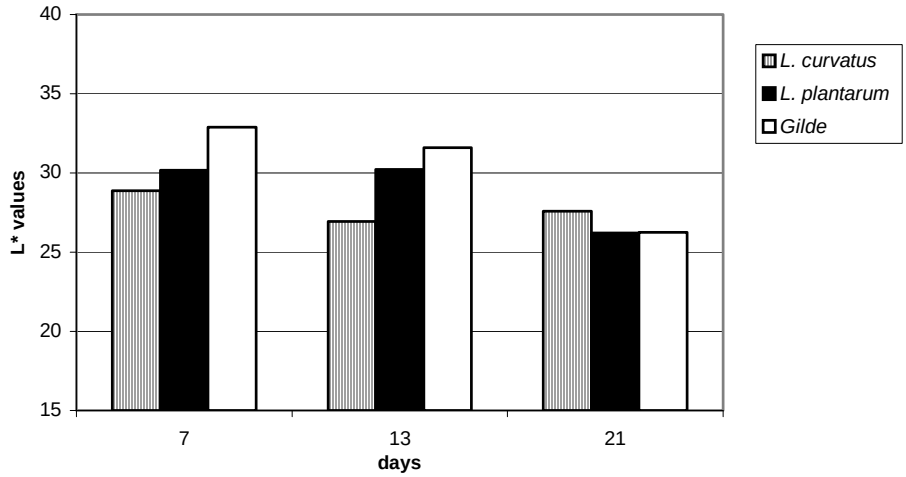

Figure 5. L* values of salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) made of seal meat, over a period of 21 days

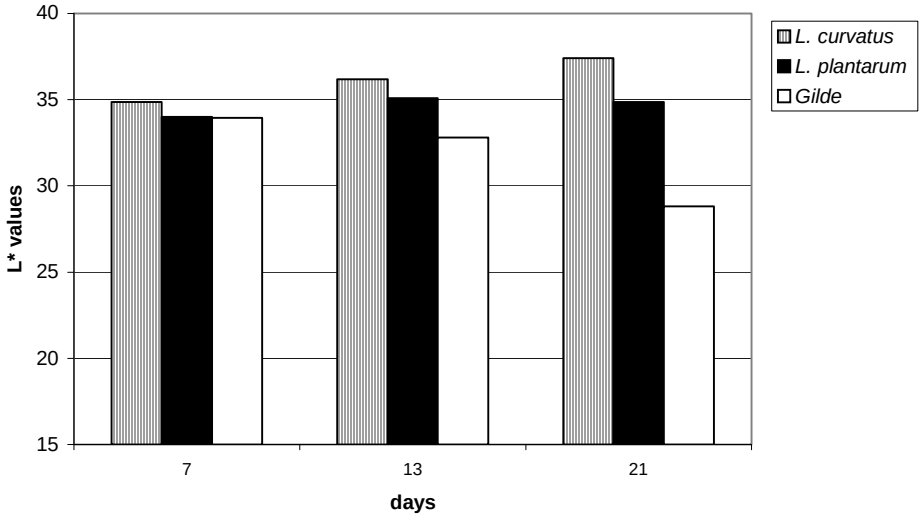

Figure 6. L* values of salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) made of whale meat, over a period of 21 days

The a* value is usually the objective value which most easily depicts the actual (subjective) visual experience of the human eye. A difference of 1-1.5 units is commonly recognised as a visual difference. Figs. 7 and 8 show clearly that the a* value is higher and more variable in the whale meat than in the seal. The values for the seal salami were very low, between 2.3 and 4.3, whereas the values for the whale were higher, between 2.5 and 5.7. This is probably due to the initial lighter colour of the whale meat, with greater potential to show colour changes visible to the human eye.

Starter culture A (*L. curvatus*) showed a slight decrease in a* value over time in the seal and whale salami. Starter culture B (*L. plantarum*) also showed a decrease over time in both species, although the decrease was somewhat greater than for starter culture A. Starter culture C (Gilde) showed an increase in both seal and whale salami over the first two weeks and then decreased in the seal, but continued to increase in the whale salami.

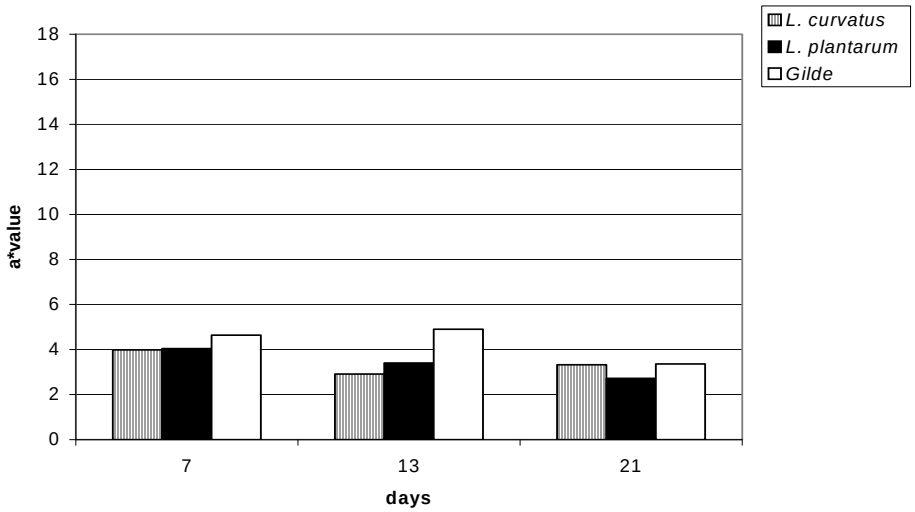

Figure 7. a* values of salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) made of seal meat, over a period of 21 days

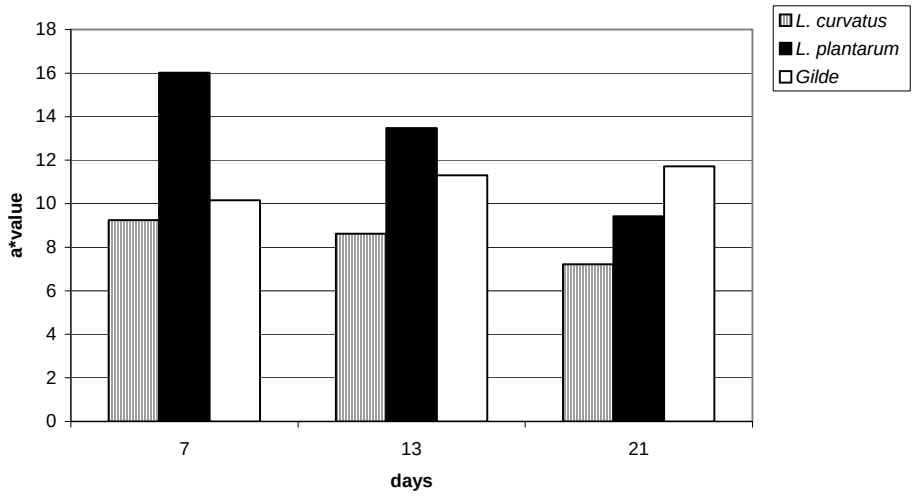

Figure 8. a* values of salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) made of whale meat, over a period of 21 days

Figs. 9 and 10 present the b* values for the salamis; the values for seal were lower than for whale. *L. curvatus* (starter culture A) showed a decrease in the first two weeks for both species, then plateaued in whale (Fig. 10) and increased slightly in the seal salami (Fig. 9). *L. plantarum* (starter culture B) decreased throughout the trial period of 21 days in both species. The Gilde starter culture (C) decreased steadily over time in the seal salami, but increased in the first two weeks in the whale salami, before decreasing substantially in the last week. The whale had higher b* values than the seal (Figs. 9 and 10), which indicated more yellow in the whale meat than the seal, or, due to the lighter colour of the whale meat, that it was easier to detect the

yellowness. Once again, starter culture B showed the same pattern in both species, with a gradual decrease over time. Starter culture A showed the same trend as for the a* values, and starter culture C had a different pattern in both species.

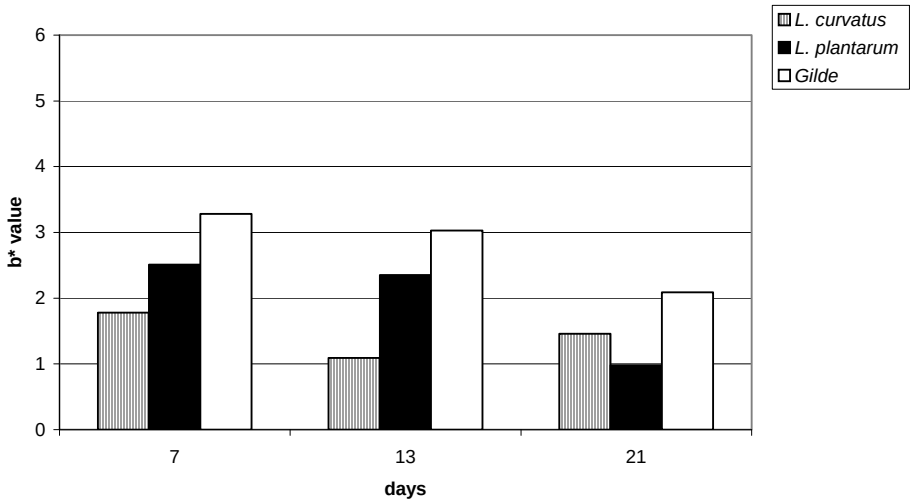

Figure 9. b* values of salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) made of seal meat, over a period of 21 days

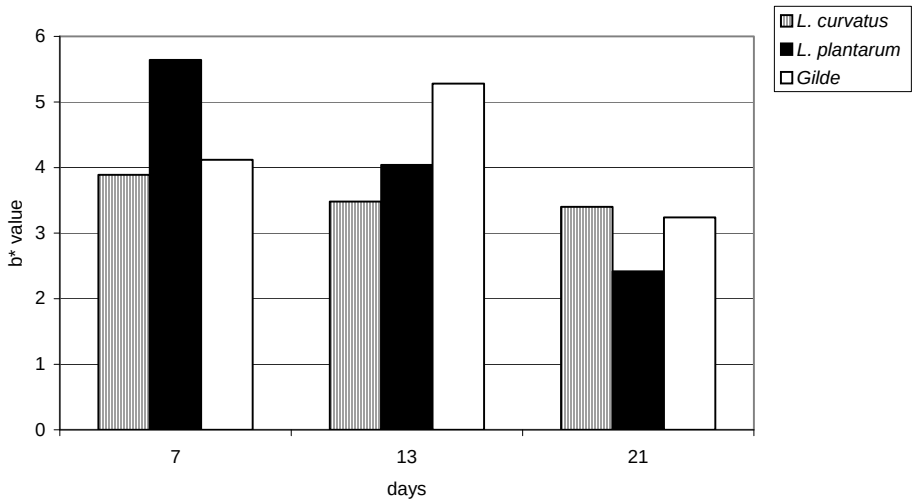

Figure 10. b* values of salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) made of whale meat, over a period of 21 days

The myoglobin extraction resulted in whale muscle having a myoglobin content of 1.5%, and seal having a much higher content of 14.2%. This result for the seal is consistent with a report by Synowiecki *et al*. (1992), who found the myoglobin concentration of mechanically separated seal meat to be 13.2%.

Fig. 11 shows the change in absorption of myoglobin (408nm) in both seal and whale salami using starter culture A over 21 days, and how it decreased, as the heme dissociates over time from the myoglobin molecule. During the first five days there was a rapid decline in the absorption. This correlates with a rapid decline in pH (Figs. 1 and 3), due to the rapid increase in lactic acid bacteria numbers over the same period (Figs. 12 and 13). This decline, as stated by Slinde & Nordal (1978), is due to dissociation of the heme and nitrosoheme from the myoglobin molecule, caused by the pH decrease as discussed. As seal meat has a higher myoglobin content than whale, it would show a lower absorbance than the whale, even when processed into salami.

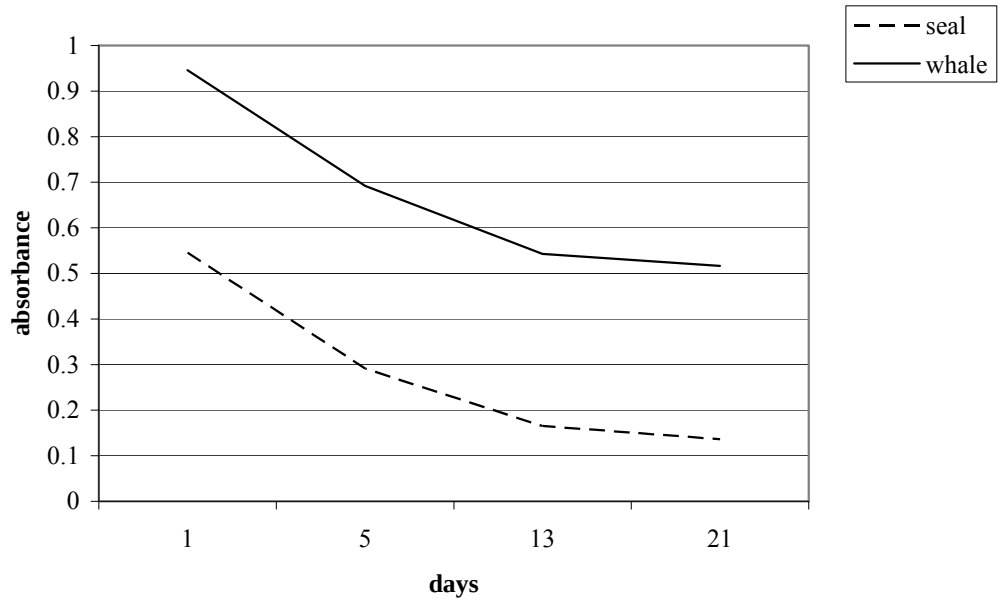

Figure 11. Absorbance (408nm) of myoglobin of seal and whale salami using starter culture A, over 21 days

The development of lactic acid bacteria (on MRS-agar plates) in the salami made of the two species, using the same three starter cultures, was observed throughout the production period. In Figs. 12 and 13, the viable counts of lactic acid bacteria over 21 days are shown. There was a rapid increase in bacterial activity over the first five days. This correlates positively with a decrease in pH over the same time period (Figs. 1 and 2). After the peak at five days, there was a decrease in bacterial numbers, which gradually evened out, and then remained at the that level. In both species it was apparent that starter culture B had the highest number of colony forming units, and starter culture C, the lowest. This remained consistent throughout in both species. This initial increase in lactic acid bacteria, followed by a decrease, has been noted by Dicks *et al*.

(2004). This change is ascribed to the initial multiplication of bacteria, and as the carbohydrate source is utilised, the numbers start to decrease.

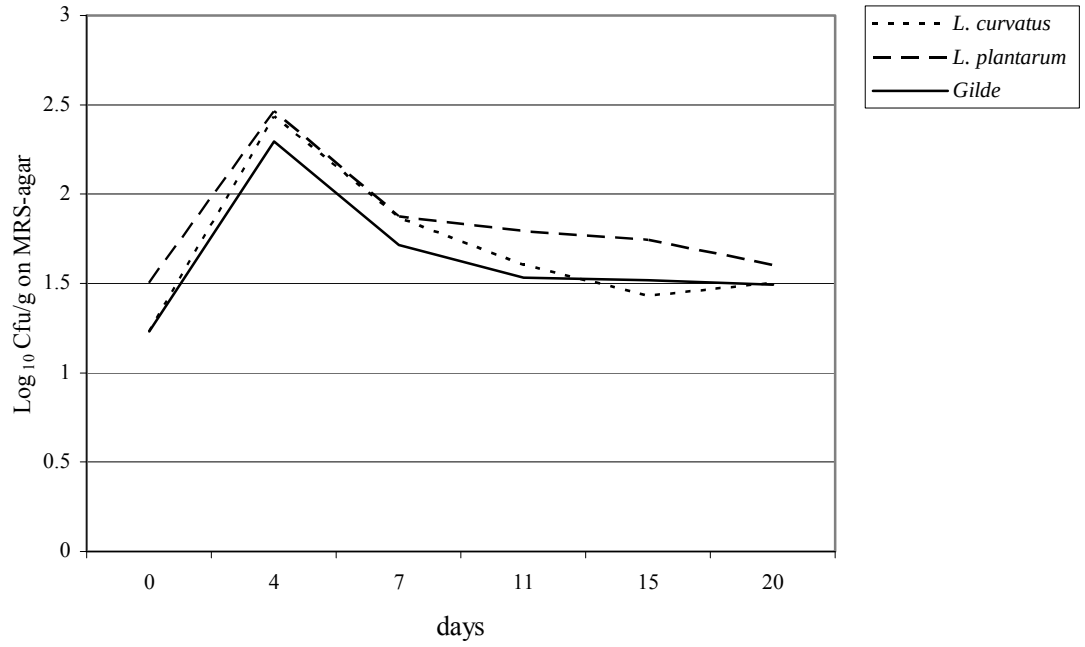

Figure 12. Viable counts of lactic acid bacteria in seal salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) over 21 days

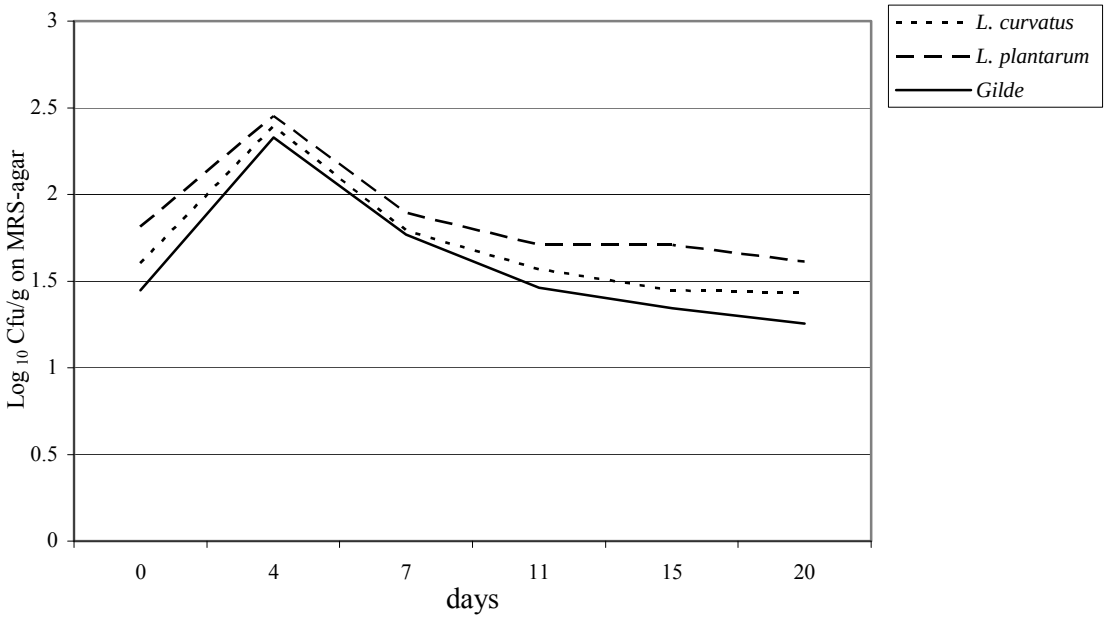

Figure 13. Viable counts of lactic acid bacteria in whale salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) over 21 days

5.4.2. Chemical analysis

Chemical analysis of the raw materials, the salami batter, and the final product, showed that seal meat is similar in fat to, and slightly higher in protein than, whale meat. This corresponds to the values of the relevant salami batters and sausages (Table 3), as exactly the same additives were added to both to produce sausages. As the sausages ferment, and dry out, moisture is lost. The moisture lost over the 21 days was higher in the whale salami (20.6% vs 17.0%), which also had the higher protein content (Table 3) than the seal salami in the final product. When calculated on a dry matter (DM) basis, whale salami contained 94.23% protein, and seal salami 95.49%.

Table 3

Chemical analysis values of the raw materials used during the making of the salami and the final salami, using starter culture A

Species	Sample type	Moisture %	Protein %	Fat %	Ash %
Seal	meat	71.2	27.5	0.3	1.0
	batter (day 1)	54.5	19.4	24.6	1.0
	salami (day 21)	54.3	23.5	30.3	1.0
Whale	meat	74.0	24.5	0.6	1.0
	batter (day 1)	57.5	18.0	23.5	1.1
	salami (day 21)	54.2	26.2	27.3	1.0

5.4.3. Weight loss and texture

Weights of allocated sausages were recorded on specific days and cumulative weight losses calculated. As seen in Figs. 14 and 15, the seal salami showed a lower cumulative weight loss than the whale: the highest weight loss was 26% for the seal salami, and 31% for the whale salami. However, the seal and whale salami were very similar in their moisture content (Table 3) on day 21, although there was a larger discrepancy in the batter. This is ascribed to the forced drying conditions, which lead to more homogenous products after the fermentation period. The tempo of weight loss of the different starter cultures within a species run nearly parallel to each other (Figs. 14 and 15), and therefore one can assume that weight loss was not influenced by starter culture. All sausages lost more moisture in the last week. This could be due to the change in RH of the maturation chamber, resulting in increased desiccation of the salami.

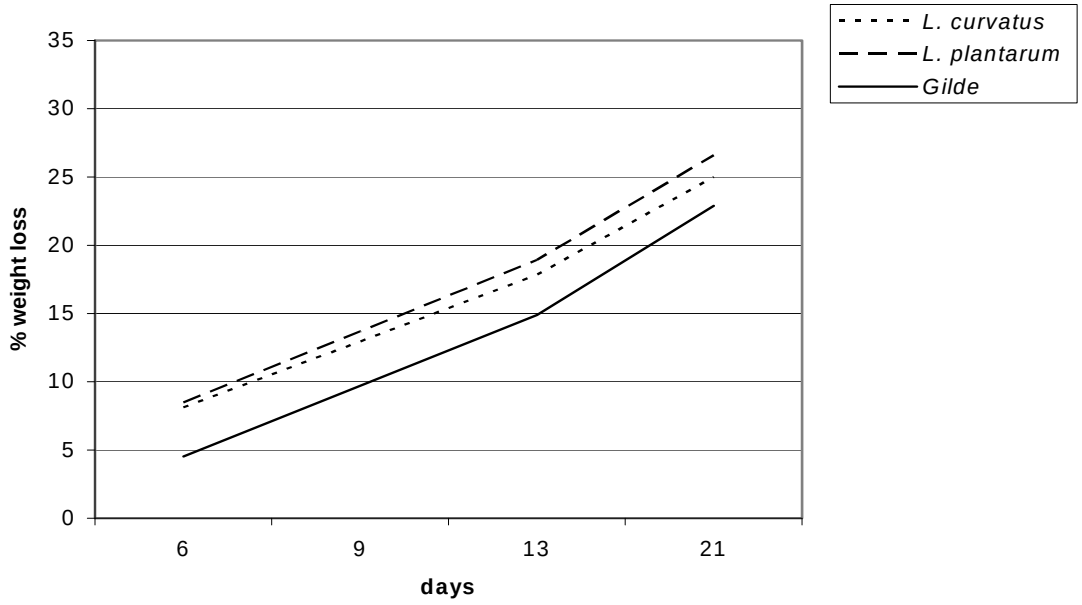

Figure 14. Cumulative weight loss over 21 days of seal salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde).

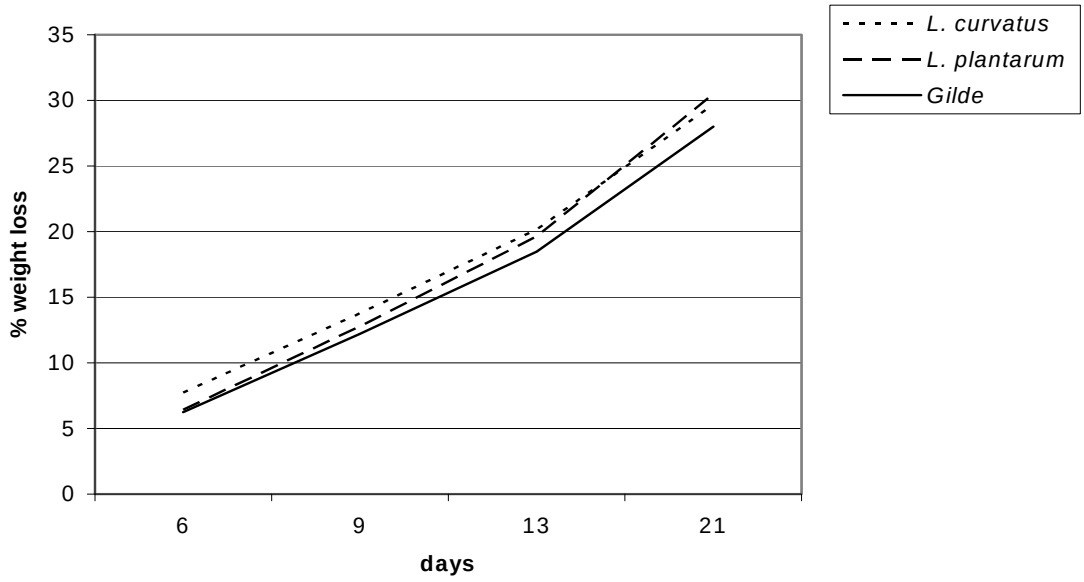

Figure 15. Weight loss over 21 days of whale salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde).

The linear regression lines fitted to the weight loss data are depicted in Table 4.

Table 4

Weight loss data of the salami batches A (*L. curvatus*), B (*L. plantarum*) and C (*Gilde*) of both species, expressed as a linear regression with intercept (a), gradient (b) and goodness of fit (R^2 value)

Species	Starter culture	a	b	R^2 value
Seal	*L. plantarum*	893.10	-50.70	0.99
	L. curvatus	795.14	-47.90	0.99
	Gilde	885.23	-46.69	0.99
Whale	*L. plantarum*	768.96	-51.47	0.99
	L. curvatus	847.10	-57.96	0.99
	Gilde	934.66	-59.33	0.99

Figs. 16 and 17 portray the force required to compress slices of salami, approximately 1cm thick, over 30 seconds. Texture of the sausages was analysed by the amount of force required to compress equal slices of the different sausages. As with weight loss, this parameter also corresponds to a_w. The higher the force required to compress a sample, the lower the moisture content of the sample. The force needed was nearly identical for the seal and the whale salamis. All starter cultures showed an increase in force required over the same period of time, with starter culture A (*L. curvatus*) requiring an initial force of 60N and a force of >100N after 21 days. This is due to a decreased water activity and accompanied loss of moisture (Figs. 2, 4, 14 and 15).

In the seal salami (Fig. 16) on day 6, prepared with starter culture B (*L. plantarum*), required less force than starter culture C (Gilde). On day 13 this changed, and the salami with starter culture B required more force than for starter culture C. This continued up to the last measurement on day 21. In the whale salami (Fig. 17), starter culture B (*L. plantarum*) consistently required the most force, starter culture C (Gilde) required slightly less, and starter culture A (*L. curvatus*) the least, to compress similar pieces of salami. This can be linked to the a_w values, where starter culture B sausages showed a lower a_w than the others, whilst starter culture A sausages showed higher a_w levels.

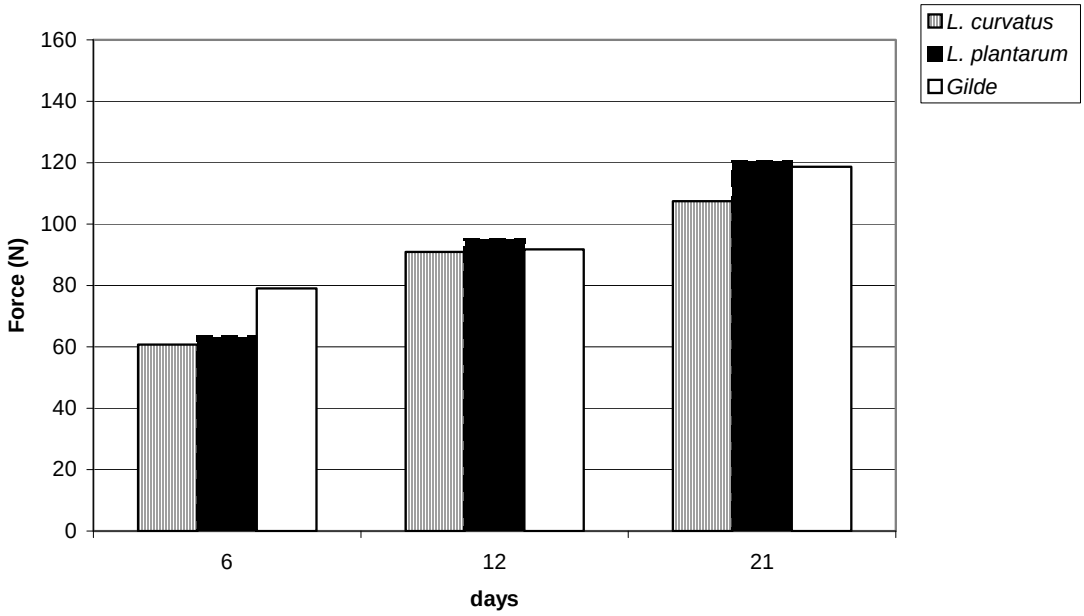

Figure 16. Force (N) used to compress seal salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) over 21 days

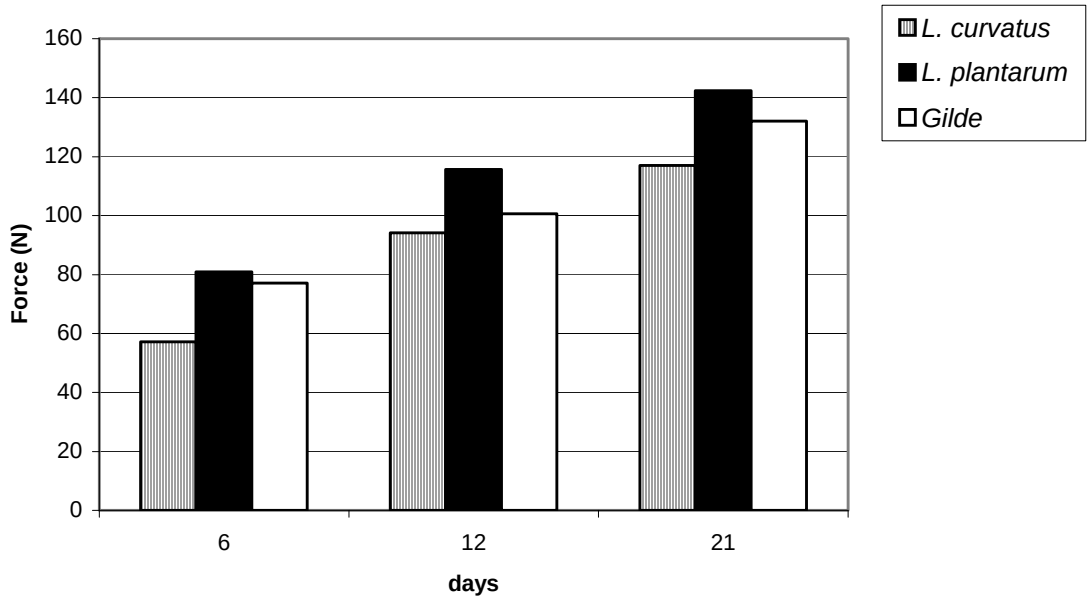

Figure 17. Force (N) used to compress whale salami batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde) over 21 days

5.4.4. Fatty acid determination

Polyunsaturated fatty acids (PUFA), such as the omega-3 fatty acids, have recently become increasingly popular, due to their apparent health benefits. Plasma cholesterol levels are correlated to the fatty acid composition of the diet (Flynn *et al*., 1985). In general,

monounsaturated fatty acids (MUFA) and PUFA do not result in increased cholesterol levels, but high levels of long-chain saturated fatty acids (SFA) do (Grundy & Denke, 1990). It has been reported that palmitic acid (C16:0) increases cholesterol levels, but that stearic acid (C18:0) does not (Rowe *et al*., 1999).

The importance of these particular fatty acids is becoming evermore evident in their utilisation for value-added application in the food, and pharmaceutical, domains. The n-3 fatty acids have been found to decrease serum triacylglycerol and cholesterol levels (Kim & Edsall, 1999). They have been used in the treatment and prevention of arthritis, rheumatism, hypertension, inflammatory and immune disorders (Senanayake & Shahidi, 2001).

Guidelines for consumers suggest the reduction in intake of n-6 PUFA to n-3 PUFA, and the intake of short- and medium-chain SFA (Gibney, 1993). As the fatty acid composition of human food sources is becoming increasingly important in terms of human health, the aim is to bring the P:S ratio of meat closer to the recommended value of >0.70, and the n-6: n-3 ratio of <5.0 (Raes *et al*., 2003).

In Table 5, the whale and seal meat used in the production of the salami have similar fatty acid compositions (mg/100g). The SFA, MUFA and PUFA values (mg/100g) are 349.2, 271.6 and 175.8 for raw seal meat respectively, and 312.3, 251.9 and 179.6 for raw whale meat respectively. When comparing seal and whale meat, they have similar desirable fatty acid (DFA) values (516.84 and 500.91mg/100g respectively).

The P:S ratio is also similar for both the seal and the whale, being 0.50 and 0.58 respectively. This would place both species close to the recommended value of >0.70. Similarly, the n-6:n-3 ratio of 1.46 and 3.01 respectively falls within the recommended limits (<5.0) (Raes *et al*., 2003). Pork fat has a SFA content of 4116.9 mg/100g, which is much higher than that of seal or whale meat, and will therefore result in a higher SFA content of the final salami. The P:S ratio of the pork fat used was 0.6, with an n-6: n-3 ratio of 8.3, which was also much higher than that of seal or whale meat. These ratios make both seal and whale meat a desirable alternative red meat in terms of fatty acid composition.

Table 5

Fatty acid composition of major raw materials (mg/100g sample) used in the production of the salami

Fatty acid		Seal meat	Whale meat	Pork fat
16:0	Palmitic	115.6	115.6	2599.7
18:0	Stearic	69.5	69.5	1276.8
20:0	Arachidic	13.6	22.7	27.6
22:0	Behenic	28.3	4.9	1.9
24:0	Lignoceric	122.2	99.7	210.9
16:1 n-7	Palmitelaidic	40.7	19.2	282.2
18:1 n-9	Oleic	165.0	186.7	5228.3
20.1 n-9	Gondoic	29.6	21.7	106.2
22:1 n-9	Erucic	24.0	8.2	26.3
24:1 n-9	Nervonic	12.2	16.1	6.4
18:2 n-6	Linoleic	46.9	47.4	2072.8
18:3 n-3	a-Linolenic	6.5	6.3	209.2
18:3 n-6	y-Linolenic	7.0	2.8	7.9
20:2 n-6		2.3	7.0	83.5
20:3 n-3	Mead	33.4	20.6	54.1
20:4 n-6	Arachidonic	22.3	23.8	27.7
20:5	Eicosapentaenoic	44.9	45.9	12.3
22:2		5.1	4.0	4.6
22:5		27.3	21.8	15.0
22:6	Docosahexaenoic	39.6	35.5	31.3
ΣSFA		349.2	312.3	4116.9
ΣMUFA		271.6	251.9	5649.4
ΣPUFA		235.4	179.6	2486.9
ΣTUFA		507.0	431.5	8136.3
DFA		576.5	501.0	9413.2
P: S		0.5	0.6	0.6
n-6		78.4	80.9	2191.8
n-3		40.0	26.9	263.3
n-6: n-3		2.0	3.0	8.3

SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; TUFA = Total Unsaturated Fatty Acids; DFA = Desirable Fatty Acids (C18:0 + TUFA)

Few fatty acids show differences between species, these being behenic acid (C22:0), erucic acid (C22:1 n-9) and arachidonic acid (C20:4 n-6). This could be due to the different diets of seal and whale. Seals consume primarily fish, and whale mainly krill and small crustaceans (Klinowska, 1991).

There is an increasing demand for meat products with a certain fatty acid profile. The actual qualities of these products (chemical and physical), affect sensory and storage qualities of meat (Banskalieva *et al*., 2000), and the flavour of meat is influenced by its fatty acid composition (Melton, 1990). Unsaturated fatty acids have an increased tendency towards oxidation and rancidity, which influences shelf life. The data was transformed from actual quantities to percentage of total fatty acid identified for purposes of comparison (Table 6).

Table 6

Percentage fatty acid composition of the major raw materials used in the production of the salami

Fatty acid		Seal meat	Whale meat	Pork fat
16:0	Palmitic	11.04	11.63	20.79
18:0	Stearic	3.89	1.93	2.26
20:0	Arachidic	23.89	25.16	2.00
22:0	Behenic	6.64	6.99	10.21
24:0	Lignoceric	15.77	18.78	41.82
16:1 n-7	Palmitelaidic	4.48	4.77	16.58
18:1 n-9	Oleic	0.66	0.28	0.06
20.1 n-9	Gondoic	0.63	0.64	1.67
22:1 n-9	Erucic	1.30	2.28	0.22
24:1 n-9	Nervonic	2.82	2.18	0.85
18:2 n-6	Linoleic	0.22	0.70	0.67
18:3 n-3	α-Linolenic	3.20	2.07	0.43
18:3 n-6	γ-Linolenic	0.21	2.40	0.22
20:2 n-6		4.29	4.62	0.10
20:3 n-3	Mead	2.71	0.49	0.02
20:4 n-6	Arachidonic	2.30	0.82	0.21
20:5	Eicosapentaenoic	0.49	0.41	0.04
22:2		11.68	10.03	1.69
22:5		1.17	1.62	0.05
22:6	Docosahexaenoic	2.61	2.19	0.12

Tables 7 and 8 show the fatty acid composition (mg/100g sample) of both the seal and whale salami on day 0, and day 21, whilst the percentages of total fatty acids in the sample are depicted in Tables 9 and 10 respectively. It is possible that lactobacilli play a role in fat hydrolysis during dry fermentation of sausages, due to their ability to cleave short-chain fatty acid triglycerides and diglycerides. Although the concentration of these glycerides is quite low in the original fat of the sausages, they could increase due to the action of bacterial and meat lipases during the ripening phase (Sanz *et al*., 1988). Therefore, it is difficult to monitor fatty acid changes during maturation and fermentation of a product. For the seal salami, the only significant difference over the 21-day period was in palmitic acid (16:0), which was only slightly significant, with a P value

of P=0.06. For the whale salami, the fatty acids showing significant differences were behenic (22:0) and gondoic (20:1n-9) fatty acids.

Table 7

Fatty acid composition (mg/100g sample) of the seal batter and the final seal salami (day 21) for batches A (*L. curvatus*), B (*L. plantarum*) and C (Gilde)

Fatty acid	Batter (day 1)			Salami (day 21)		
	A	B	C	A	B	C
16:0	2008.8	1287.5	1611.5	1970.3	1612.7	1658.1
18:0	1178.0	652.0	805.3	1079.0	786.1	810.1
20:0	78.7	14.5	21.3	23.9	21.4	24.6
22:0	9.2	11.0	3.9	64.4	6.6	14.9
24:0	29.0	38.7	132.0	38.4	83.1	253.7
16:1 n-7	14.0	201.4	5.8	196.7	234.6	187.4
18:1 n-9	3426.3	2292.3	2822.1	3644.7	3114.8	2866.8
20.1 n-9	93.2	71.2	108.7	97.1	84.3	87.0
22:1 n-9	48.7	11.9	13.9	35.4	20.9	54.6
24:1 n-9	10.2	7.3	7.4	5.9	18.9	27.6
18:2 n-6	1251.7	864.4	1196.7	1454.6	1264.0	1218.5
18:3 n-3	122.8	93.2	123.7	134.8	137.7	127.5
18:3 n-6	9.8	4.2	4.7	5.0	4.3	7.2
20:2 n-6	60.4	36.4	54.6	54.8	53.3	59.0
20:3 n-3	26.8	43.2	33.3	61.7	61.7	75.7
20:4 n-6	44.7	10.9	42.5	7.1	17.0	19.5
20:5	10.6	32.8	29.1	26.8	26.3	58.6
22:2	12.5	2.3	21.1	14.0	4.4	9.7
22:5	26.4	13.6	6.8	9.6	18.9	77.2
22:6	61.6	41.6	14.2	73.5	41.1	52.5
ΣSFA	3303.8	2003.6	2574.0	3175.9	2509.8	2761.4
ΣMUFA	3592.4	2584.1	2957.9	3979.7	3473.4	3223.4
ΣPUFA	1627.3	1142.5	1506.7	1841.2	1628.8	1705.3
ΣTUFA	5219.7	3726.6	4464.6	5820.9	5102.1	4928.7
DFA	6397.7	4378.6	5269.9	6899.9	5888.2	5738.8
P: S	0.5	0.6	0.6	0.6	0.6	0.6
n-6	1366.7	915.8	1298.4	1521.5	1338.6	1304.2
n-3	149.5	136.4	157.0	196.5	199.4	203.2
n-6: n-3	9.1	6.7	8.3	7.7	6.7	6.4

SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; TUFA = Total Unsaturated Fatty Acids; DFA = Desirable Fatty Acids (C18:0 + TUFA)

Table 8

Fatty acid composition (mg/100g meat) of the whale batter and the final whale salami (day 21) made from three starter cultures (A: *L. curvatus*, B: *L. plantarum* and C: Gilde)

Fatty acid	Starter culture					
	Batter (day 1)			Salami (day 21)		
	A	B	C	A	B	C
16:0	1410.4	1566.9	1865.1	1653.9	1530.7	1942.4
18:0	731.2	888.8	1045.0	773.9	783.9	783.9
20:0	12.8	21.4	20.3	14.9	13.2	19.6
22:0	2.7	3.7	55.5	4.1	3.2	19.5
24:0	30.4	31.1	36.7	43.4	61.4	46.4
16:1 n-7	141.8	152.5	166.2	195.5	168.7	208.7
18:1 n-9	2416.7	3058.4	3668.4	3095.8	3108.8	3700.9
20.1 n-9	64.1	75.9	92.2	81.8	69.4	90.2
22:1 n-9	18.7	30.3	30.3	22.3	15.5	26.7
24:1 n-9	9.3	6.4	10.8	7.6	13.9	9.8
18:2 n-6	909.1	1088.7	1429.9	1337.9	1173.0	1555.3
18:3 n-3	87.6	110.5	132.5	138.7	118.4	163.3
18:3 n-6	3.8	6.6	5.2	6.1	6.2	7.2
20:2 n-6	32.2	41.7	64.8	56.8	51.4	67.0
20:3 n-3	37.2	43.8	52.3	51.8	76.8	58.4
20:4 n-6	11.9	16.9	19.3	20.1	2.8	22.5
20:5	37.1	78.2	34.0	34.0	43.1	35.0
22:2	5.5	4.5	5.8	3.4	8.6	6.5
22:5	9.3	45.0	5.4	5.3	8.5	6.2
22:6	37.2	47.2	20.6	37.0	88.8	38.9
ΣSFA	2187.4	2511.8	3022.6	2490.2	2392.5	2811.8
ΣMUFA	2650.7	3323.5	3967.8	3403.0	3376.3	4036.2
ΣPUFA	1171.0	1483.0	1769.8	1691.2	1577.6	1960.3
ΣTUFA	3821.6	4806.5	5737.6	5094.3	4954.0	5996.5
DFA	4552.8	5695.3	6782.6	5868.2	5737.9	6780.4
P: S	0.5	0.6	0.6	0.7	0.7	0.7
n-6	957.0	1153.9	1519.2	1421.0	1233.3	1652.0
n-3	124.8	154.2	184.7	190.5	195.2	221.7
n-6: n-3	7.7	7.5	8.2	7.5	6.3	7.5

SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; TUFA = Total Unsaturated Fatty Acids; DFA = Desirable Fatty Acids (C18:0 + TUFA)

In the seal salami, the P:S ratio increased over the 21 days, whilst the n-6:n-3 ratio decreased. As a P:S value of >0.70 is regarded as desirable, the seal salami with P:S ratios of 0.58, 0.65 and 0.62 for starter cultures A, B and C respectively, could be viewed as an excellent alternative to more traditional species of salami on the market. The whale salami showed the same trend in

P:S ratios, and n-6:n-3 ratios, showing final P:S ratios of 0.68, 0.66 and 0.70 for starter cultures A, B and C respectively. The n-6:n-3 ratios of both species of salami were higher than the ideal of <0.5, due to the high amount of pork fat in the mixture, with a much higher n-6: n-3 ratio than the raw meat of both species (Table 5).

Tables 9 and 10 show the percentage values of the fatty acid content of the batter and final salami of both the seal and the whale salami respectively. Student's T-test (SAS, 1990) was done on these percentage values, to compare the batter fatty acid composition with that of the final salami. There were few significant differences (P<0.05) between the fatty acid values of the batter and the final salami of either the seal or whale. In Table 9, only palmitic acid (16:0) was significantly different after 21 days (P=0.06). In Table 10, there were two fatty acids that differed significantly after 21 days, these being behenic (22:0) and gondoic (20:1 n-9) fatty acids.

Table 9

Percentage fatty acid composition of the seal batter and the final seal salami (day 21)
(T-test executed irrespective of starter culture, to compare results for day 1 and day 21)

Fatty acid	Starter culture						T-test (P-value)
	Batter (day 1)			Salami (day 21)			
	A	B	C	A	B	C	
16:0	23.06	21.68	22.09	21.48	20.62	21.02	0.06
18:0	0.16	3.39	0.08	2.14	3.00	2.38	0.31
20:0	2.87	4.21	3.43	2.73	3.20	3.17	0.32
22:0	13.52	10.98	11.04	11.76	10.05	10.27	0.31
24:0	39.33	38.60	38.69	39.73	39.83	36.35	0.85
16:1 n-7	14.37	14.55	16.41	15.86	16.16	15.45	0.36
18:1 n-9	0.11	0.07	0.06	0.05	0.06	0.09	0.47
20:1 n-9	1.41	1.57	1.70	1.47	1.76	1.62	0.65
22:1 n-9	0.90	0.24	0.29	0.26	0.27	0.31	0.40
24:1 n-9	1.07	1.20	1.49	1.06	1.08	1.10	0.24
18:2 n-6	0.69	0.61	0.75	0.60	0.68	0.75	0.88
18:3 n-3	0.31	0.73	0.46	0.67	0.79	0.96	0.11
18:3 n-6	0.51	0.18	0.58	0.08	0.22	0.25	0.14
20:2 n-6	0.12	0.55	0.40	0.29	0.34	0.74	0.63
20:3 n-3	0.11	0.18	0.05	0.70	0.08	0.19	0.34
20:4 n-6	0.56	0.20	0.19	0.39	0.27	0.69	0.50
20:5	0.14	0.04	0.29	0.15	0.06	0.12	0.58
22:2	0.33	0.65	1.81	0.42	1.06	3.22	0.54
22:5	0.12	0.12	0.10	0.06	0.24	0.35	0.28
22:6	0.30	0.23	0.09	0.10	0.24	0.98	0.45

Table 10

Percentage fatty acid composition of the whale batter and the final whale salami (day 21)

(T-test executed irrespective of starter culture, to compare results for day 1 and day 21)

Fatty acid	Starter culture						T-test (P-value)
	Batter (day 1)			Salami (day 21)			
	A	B	C	A	B	C	
16:0	22.67	20.83	20.75	21.21	20.39	21.09	0.49
18:0	2.28	2.03	1.85	2.51	2.25	2.27	0.13
20:0	4.02	3.32	2.78	3.21	3.33	2.71	0.51
22:0	11.75	11.82	11.63	9.92	10.44	10.57	0.00
24:0	38.84	40.66	40.81	39.70	41.41	40.19	0.71
16:1 n-7	14.61	14.48	15.91	17.16	15.62	16.89	0.08
18:1 n-9	0.06	0.09	0.06	0.08	0.08	0.08	0.32
20:1 n-9	1.41	1.47	1.47	1.78	1.58	1.77	0.02
22:1 n-9	0.21	0.28	0.23	0.19	0.18	0.21	0.16
24:1 n-9	1.03	1.01	1.03	1.05	0.92	0.98	0.37
18:2 n-6	0.52	0.55	0.72	0.73	0.68	0.73	0.14
18:3 n-3	0.60	0.58	0.58	0.66	1.02	0.63	0.21
18:3 n-6	0.19	0.22	0.21	0.26	0.04	0.24	0.70
20:2 n-6	0.60	1.04	0.38	0.44	0.57	0.38	0.36
20:3 n-3	0.04	0.05	0.62	0.05	0.04	0.21	0.53
20:4 n-6	0.30	0.40	0.34	0.29	0.21	0.29	0.10
20:5	0.09	0.06	0.06	0.04	0.12	0.07	0.82
22:2	0.49	0.41	0.41	0.56	0.82	0.50	0.13
22:5	0.15	0.09	0.12	0.10	0.18	0.11	0.76
22:6	0.15	0.60	0.06	0.07	0.11	0.07	0.33

5.5. Conclusion

From this research it may be deduced that the production of salami is possible by utilising the meat of these aquatic mammal species, namely the Grey seal and the Minke whale. Both seal and whale meat are rich in desirable fatty acids. This, together with their other chemical properties, such as protein and fat content, make them healthier than traditional red meat species. However, as these animals are protected species, concessions for harvesting them will determine how much meat will be readily available for processing.

Although there were obvious and expected differences between the two species in terms of chemical and physical properties, vital parameter values were found to be favourable in both species, using all three starter cultures. The largest differences between starter cultures A (*L. plantarum*), B (*L. curvatus*) and C (Gilde) were between starter culture C and the others. This

indicates that starter culture C influenced the colour and other physical parameters and it can therefore be used to manipulate colour in such products if required.

The favourable results of this investigation regarding the potential and the quality of seal and whale meat products, opens up the possibility of using these species on a larger, commercial scale, resulting in more effective utilisation of both, especially seals, which are presently only hunted for their skins and genitalia. The possibility of manufacturing a product such as salami, using the meat of these species, could mean a continuous supply of a highly nutritious and healthy product to the consumer. The fact that the carcasses of these animals can be fully and effectively utilised might make the harvesting of these animals a more acceptable practice globally, especially in countries that have concessions, as does Namibia.

Certainly, such an undertaking is not without challenges: seal flesh is dark in colour, which would require investigation into consumer acceptance of a product containing such dark meat. Customers need to be completely satisfied with the sensory characteristics of a product before any other quality variables enjoy priority (Chambers & Bowers, 1993). Presently, the supply of meat is also seasonal as concessions to harvesting these animals are regulated strictly. Although this means, in effect, that securing a regular supply of meat throughout the year would be less likely, this, in turn, might well increase the desirability and value of the available meat.

However, it is possible that any potential drawbacks might be overcome by the fact that seal and whale meat are regarded as healthy and wholesome, due to their lean meat content and high concentration of PUFA. Certainly, the desirability by the customer of products made from these species would have to be determined before large-scale production can commence, with consumer perception and acceptance being the key to the success of any product.

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Chapter 6

Production of salami using meat from beef, mutton, horse, blesbok and springbok
a) Chemical composition and physical measurements

6a.1. Abstract

Salamis were was produced by using meat from horse, beef, mutton, blesbok and springbok. Three different starter cultures were used: *Lactobacillus curvatus* DF 38 (batch I); *Lactobacillus plantarum* 423 (batch II), which produces plantarin 423, a bacteriocin; and a bacteriocin-negative mutant of *Lactobacillus plantarum,* strain 423m, (batch III). Changes in cell numbers, pH, a_w, colour (L* a* b* values), colony forming units (cfu), fatty acid profiles, and chemical analysis were recorded over a period of 23 days. A difference in pH and a_w values was seen between starter cultures; batch I had a higher final pH value (4.66), whereas batches II and III were similar (4.42 and 4.46 respectively). On day 23, the a_w value was 0.90 for all fermented salami. The L* and a* values did not differ greatly for the three starter cultures, nor between species. The b* values, however, did show a distinct difference on day 6. Salami fermented with starter cultures II and III showed a dramatic decline in b* value, whereas a more gradual decline was recorded in batch I. Horsemeat was the leanest with only 1.38g fat/100g, whereas blesbok contained 4.12g fat/100g. The highest desirable fatty acid (DFA) content was recorded in the salami made from horsemeat. The polyunsaturated:saturated fatty acid (P:S) ratios were highly variable on day 23, compared with the batter (day 0), with horse salami having the highest ratio (0.08) and blesbok salami the lowest (0.05). The n-6:n-3 fatty acid ratios also changed, with mutton having the highest ratio, and horse the lowest after 23 days.

Keywords*:* fermented sausage, game species, horsemeat, starter cultures

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6a.2. Introduction

The harvesting of game animals, such as blesbok and springbok, is a seasonal occurrence and products such as salami need to be developed to extend the shelf life of these, and thereby supplying a continuous supply of a specific product.

Suitable meat ingredients are vital to produce blends of meat and fat particles, resulting in sausages that are consistent in quality. Usually, salami contains between 50-70% lean meat, often a combination of different species.

The most commonly used starter culture species in the fermentation of beef and pork sausages are strains of *Lactobacillus, Pediococcus*, non-pathogenic S*taphylococcus* and *Micrococcus* (Lücke, 1986). The major role of lactic acid bacteria in the sausage is to produce organic acids, primarily lactic acid, from carbohydrates (Campbell-Platt, 1987). This leads to a decrease in pH and contributes to the retardation of the development of undesirable micro-organisms (Schillinger & Lücke, 1987). The decreased pH is also correlated with a reduced water-holding capacity of the proteins, resulting in correct desiccation of the product, and also aids in the cohesion of meat particles (Townsend *et al*., 1980) as well as contributes to the colour change of the fermented sausages.

Economic and social changes in civilisations have led to nutritional demands being transformed and modified. It is becoming more acceptable to purchase alternative sources of red meat, as opposed to meat of only the traditional species of red meat producing animals. Further, modern consumers like to be informed of the nutritional make-up of the food they consume (Horbańczuk *et al*., 1998).

Increasing consumer interest in non-traditional meat products provides a reason for the investigation into the production possibility of species such as seal, horse and certain species of game animals (blesbok & springbok). Three of these species are not farmed intensively, and therefore can be considered as wild game animals. This implies that they have had no direct contact with humans, or contain any chemical substances in their bodies, which could have been administered by human beings. Comparisons need to be drawn between these species and conventional red meat species, such as beef and mutton, with regards to the production possibilities of utilising these meat species in commercial plants producing traditional salami. Since the contribution of game meat production (jerky hunters and game meat sales) constitutes 67.5% of the total income of the game industry of South Africa (Bothma, 2002), research is required into the possibility of utilising these species in processed products, too. As many alternative red meat species are harvested seasonally, salami made from these species would provide a continuous supply of meat products of these species.

As the consumer nowadays prefers to have ready-to-eat, fast foods, processed foods are becoming evermore popular, and the use of alternative red meat species in these processed products is of particular interest to the industries. The objective of this investigation was, therefore, to assess the potential of the five different meat species in the production of traditional salami, and the suitability of three different starter cultures in the production thereof.

6a.3. Materials and methods

6a.3.1. Raw materials

Meat from horse, beef, mutton, blesbok (*Damaliscus dorcas phillipsi*) and springbok (*Antidorcas marsupialis*), was sourced from commercial suppliers a week prior to production of the salami. The batch sizes were approximately 7.5kg each, comprising lean meat (5.0kg), and pork fat (2.5kg).

The spices (128g), salts (200g), the anti-oxidant and ascorbic acid (2.5g) were obtained from a local supplier (Freddy Hirsch, P.O. Box 2554, Cape Town, 8000, South Africa). Fifteen batches were produced from the five species and three starter cultures within one day so as to ensure that environmental effects were similar.

6a.3.2. Starter cultures and growth conditions

Lactobacillus curvatus DF38 (batch I), *Lactobacillus plantarum* 423 (batch II) (Van Reenen *et al*., 1998), and a mutant cured from plasmid p423 and unable to produce plantaricin 423 (strain 423m) (batch III) (Dicks *et al*., 2003), were used as starter cultures. Curing of strain 423 from its plasmid was undertaken by incubation in the presence of 0.125-8µg/ml novobiocin, according to the method described by Ruiz-Barba *et al.* (1991). Strains 423, 423m and DF38 were cultured in MRS broth (Biolab, Biolab Diagnostics, Midrand, South Africa). Cultures were stored at -80°C in MRS or BHI broth, supplemented with 15% (v/v) sterile glycerol.

Starter cultures were prepared by inoculating 2 l MRS broth with 5% (v/v) of a 12 hour-old culture. Incubation was for 24 hours at 30^{o}C, without shaking. The cells were harvested (8000 x *g*, 20 minutes, 4^{o}C), washed twice with sterile physiological salt (0.85% m/v NaCl) and resuspended in 200 ml of the same solution. Cell suspensions were stored at 4°C until used.

6a.3.3. Meat preparation and fermentation

Five kilograms of horse, beef, mutton, blesbok and springbok were mixed with 2.5kg pork back fat, and minced to a particle size of 3mm. The meat and fat were kept at -4°C to -8°C and minced while still frozen.

Half of the quantity of lean meat was added to a “Scharfen” 3-litre bowl cutter, and cut for 15 revolutions (120 seconds) with the salt and curing salt having been added, until the meat had a consistency of approximately 2mm. Thereafter, the speed was increased for another 60 seconds. The remaining half of the lean meat was then added and cut for 15 revolutions (120 seconds).

The fat, and spices, were added next and cut until the fat particles were no larger than 8mm. At low speed, the inoculum (10^5-10^6cells/g) was added into the mixture as a fluid, using a pipette. Ascorbic acid was also added separately to the batter. The entire mixture was then mixed for a further 30 seconds at slow speed, to ensure maximal mixing of ingredients.

The batches were prepared in the order of all species per one starter culture, to ensure that no contamination of starter cultures occurred. After each batch, all the apparatus were cleaned and after each starter culture, the apparatus were sterilised.

The batch was then stuffed into 6mm cellulose casings, using an automated stopper. Sausages of approximately 10cm were produced. One batch resulted in an average of 18 sausages. To ensure consistency of the product, one person was responsible for preparation of the batter, and another for stuffing the salami.

All salami was clearly marked, indicating species and starter culture. Samples were taken from the initial lean meat of each species, the pork fat and from each salami batch for fatty acid analysis, proximate chemical analysis and microbiological assessment. The pH and water activity readings were taken from each batter.

It was decided that colour measurements would not be taken on the first day, as the time between preparations of the batters and stuffing varied between batches, which resulted in the varying of the level of nitrite-induced colour change.

The sausages were sweated for two days, at 95% RH and 21^{o}C, in a chamber with controlled airflow and climate. Thereafter, they were smoked using cold smoke (18°C and 60% RH), from commercial oak shavings, for 2 hours. Salami samples were taken prior to smoking, as well as immediately after smoking. These samples were then taken to the laboratory for pH, water activity, colour and microbial growth determinations.

After smoking, the salami was once again exposed to high humidity (95% RH and 21^{o}C), to activate bacterial fermentation. A week thereafter, the sausages were placed into a maturation chamber at 70% RH and 10^{o}C. Before going into maturation, samples were taken for data analysis. Over the next 14 days, samples were collected every three days for analysis. Thereafter, the salami was deemed suitable for human consumption (23 days after the initial mixing of the batters); it was vacuum packed and stored at 4 ^{o}C until used for further analysis.

6a.3.4. Recorded parameters

In this investigation, pH values were recorded, in triplicate, using an insertion probe (Crison model 507, Crison instruments, South Africa). The instrument was calibrated using standard calibration solutions of pH 4 and pH 7. The probe was cleaned with distilled water between each reading, to ensure that no fat particles from the sausage batter were left in the nozzle to affect further readings.

Water activity (a_w) values were measured using a Novasina ms1-aw handheld instrument (Labotec, P.O. Box 790, Howard Place, 7450, South Africa). This instrument was calibrated according to the manufacturer's instructions. For the readings, approximately 5g of sample was placed into a 2.5-cm plastic dish, which is inserted into an enclosed space.

Colour measurements were recorded, using a CIELab Colorimeter (Gardner, Columbia, USA). (L*, a*, b* values). This instrument was calibrated using the standard white tile, according to the manufacturer's instructions. The salami was cut into 2.5cm-thick slices, and the colour taken from the sliced surface. Readings were collected as L*, a* and b* values from three different areas on the slice, to obtain an approximate value for each measurement.

The moisture, protein and ash content (g/100g meat) were determined according to the Association of Official Analytical Chemist's Standard Techniques (AOAC, 1997). Moisture content was determined by drying samples at 105^{o}C for 24 hours. Thereafter, the remaining sample was placed into an oven at 400 ^{o}C for six hours, to cremate it for ash determination. To determine the protein content, dried and defatted samples were used. They were ground by means of a pestle in a mortar, until a fine powder was obtained. Per animal, 0.100mg was weighed off and inserted into a foil wrap designed for the Leco protein analyser (Leco Fp-528, Leco Corporation). Protein was determined as nitrogen, multiplied by 6.25, to determine the protein concentration in the sample. For fat (g/100g meat) determination, samples were homogenised in a blender using the chloroform: methanol (2:1) extraction procedure of Lee *et al*. (1996).

The fatty acid content was determined using the method described by Tichelaar *et al*. (1998). After thawing the meat, the lipids in a 2g sample were extracted with chloroform/methanol (2:1; v/v). All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. A polytron mixer (Kinematica, type PT 10-35, Switzerland) was used to homogenise

the sample within the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard to quantify the individual fatty acids.

A sub-sample of the extracted lipids was transmethylated for two hours at 70°C, using methanol/sulphuric acid (19:1; v/v) as transmethylating agent. After cooling, the resulting fatty acid methyl esters (FAME) were extracted with water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen.

The FAME were purified by TLC (silica gel 60 plates) and analysed by GLC (Varian Model 3300 equipped with flame ionisation detection), using a 60 m BPX70 capillary column of 0.25mm internal diameter (SGE, Australia). Gas flow rates for hydrogen were 25ml/min; and hydrogen carrier gas 2-4ml/min. Temperature programming was linear at 3°C/min, with an initial temperature of 150°C, a final temperature of 220°C, an injector temperature of 240°C and a detector temperature of 250°C. The FAME in the total lipids was identified by comparing the retention times with those of a standard FAME mixture (Nu-Chek-Prep Inc., Elysian, Minnesota).

6a.3.5. Statistical Analysis

Linear and non-linear regressions were fitted to all data and the gradient (b), intercept (a) and goodness of fit (R^2 value) calculated (Statistical Analysis Systems, 1990). It was found that the goodness of fit was highest for the linear regression lines. Linear regressions were therefore used, and are discussed further.

6a.4. Results and discussion

6a.4.1. pH and water activity

Maturation of the sausages over approximately 23 days lowered the a_w and pH of the product significantly. The texture changed from a soft batter to a hard, sliceable salami, and salami-specific flavour compounds were formed during this period (Dainty & Blom, 1995). With regard to collected data, pH is the most important and most readily obtained variable used to monitor fermentation progress in salami (Acton *et al.,* 1977; De Ketelaere *et al*., 1974).

Figs. 1 and 2 show the pH and a_w for starter culture II only. Rates of change were similar for all three starter cultures. As seen in Fig. 1, the pH values and their decline over 23 days were similar (starter culture I) for all five species, except for the horse salami, whose pH decline had a less steep gradient than that of the other species. Similar patterns were observed (Table 1) using starter cultures II and III. As in starter culture I, horse salami had higher pH values than salami

made from the other species. The final (23 day) pH values were 4.45 and 4.55 for starter cultures II and III respectively, whilst the pH values for the other species ranged between 4.18 and 4.22.

Table 1 shows the linear regressions for pH and a_w. Most of the R^2 values indicated a good fit for the linear regressions to the data. The rate of pH change for mutton was the greatest (all three starter cultures), and that of the horse salami the smallest. The rate of change in a_w was similar between both the starter cultures and the species used in producing the salami. However, the mutton salami made with *L. curvatus* DF38 (batch I) showed a very low rate of desiccation.

The average final pH (day 23) value of *L. curvatus* (salami I) was 4.66. Using *L. plantarum* as inoculant (salami II) resulted in an end pH of 4.42. The mutant strain (salami III) had a final pH of 4.45. These values corresponded favourably with the fact that starter cultures II and III were the same, as the pHs of their sausages were similar, where starter culture I resulted in a higher end pH. With all three starter cultures, the sausages containing horsemeat showed the highest pH throughout the trial period. In Table 1 the intercept values were highest for the horse salami, and lowest for the blesbok salami.

As expected, the a_w values of starter cultures II and III were similar (Tables 1 and 2), as the mutation of the bacteriocin should not have an effect on this value. *L. curvatus* produced a slightly higher ultimate a_w than *L. plantarum*. In Table 1, the gradient values were all very similar, although those for starter culture I were slightly higher than those for starter cultures II and III.

A sudden increase in a_w was seen in all the salamis between days three and seven, irrespective of species or starter culture. The reason for this is not clear, but might be attributed to a malfunctioning of the maturation chambers at the plant, such as a power failure or a system fault. A change in the a_w level would not rectify itself immediately, and could take up to three days. This would correspond with the data collected. However, records from the processing plant did not show any technical problems during that particular time. Alternatively, the water activity meter may have malfunctioned, although it was the same instrument, used in exactly the same manner, for these and the subsequent readings.

Table 1

The intercept (a), gradient (b) and goodness of fit (R^2 values) of the linear regression equations of different types of salamis, for the pH and water activity (a_w), taken over 23 days

Starter culture & species	pH			a_w		
	a	b	R^2	a	B	R^2
L. curvatus DF 38						
Horse	5.6959	-0.0330	0.8627	0.9688	-0.0024	0.7213
Beef	5.5477	-0.0528	0.8336	0.9671	-0.0022	0.7000
Mutton	5.5950	-0.0563	0.7365	0.9781	-0.0022	0.8111
Blesbok	5.4728	-0.0481	0.8200	0.9648	-0.0025	0.9054
Springbok	5.6285	-0.0499	0.9250	0.9610	-0.0020	0.7023
L. plantarum 423						
Horse	5.6371	-0.0517	0.9217	0.9547	-0.0022	0.9342
Beef	5.4269	-0.0552	0.8930	0.9647	-0.0023	0.7789
Mutton	5.4862	-0.0600	0.8320	0.9573	-0.0019	0.6587
Blesbok	5.4076	-0.0549	0.8114	0.9603	-0.0019	0.6974
Springbok	5.5644	-0.0591	0.8917	0.9518	-0.0019	0.5894
L. plantarum 423m						
Horse	5.6304	-0.0471	0.9081	0.9539	-0.0019	0.6410
Beef	5.4751	-0.0586	0.8272	0.9463	-0.0019	0.7043
Mutton	5.4604	-0.0570	0.8218	0.9365	-0.0011	0.4613
Blesbok	5.4412	-0.0556	0.8341	0.9418	-0.0018	0.8517
Springbok	5.5642	-0.0604	0.7593	0.9535	-0.0023	0.8894

Another possibility is that at this pH, muscle proteins are near their iso-electric point and could be releasing more water from their structure. This would be characterised by an immediate increase in a_w after a decline, due to the released water around the cells, which is recorded by the water activity meter when the measurement is taken. This water will remain there, as the cells are not able to reabsorb the water, which they have released due to the pH at that point. This increased a_w would then gradually decrease again, due to the standard drying procedures involved in the production of fermented sausages. This theory could not be substantiated, as no a_w information could be sourced from the available scientific literature.

Fig. 2 shows the a_w levels of the batch prepared using starter culture I over the same time period. There was an initial decline, followed by an incline on day 3, which then gradually declined again. Table 2 shows the pH and a_w values measured for the different species, and starter cultures

on days 0 and 23. As the starter cultures should not yet have had an effect on pH or a_w values on day 0, any differences could be attributed to species. The horse salami had the highest initial a_w (0.98). With the exception of horse I, the salamis all had low pH values (<4.65) on day 23. Due to forced drying conditions, all the salamis had similar a_w levels after 23 days (either 0.91 or 0.90). The beef salami showed a delay in final decline between day 17 and 21, but did end up with a similar value at day 23.

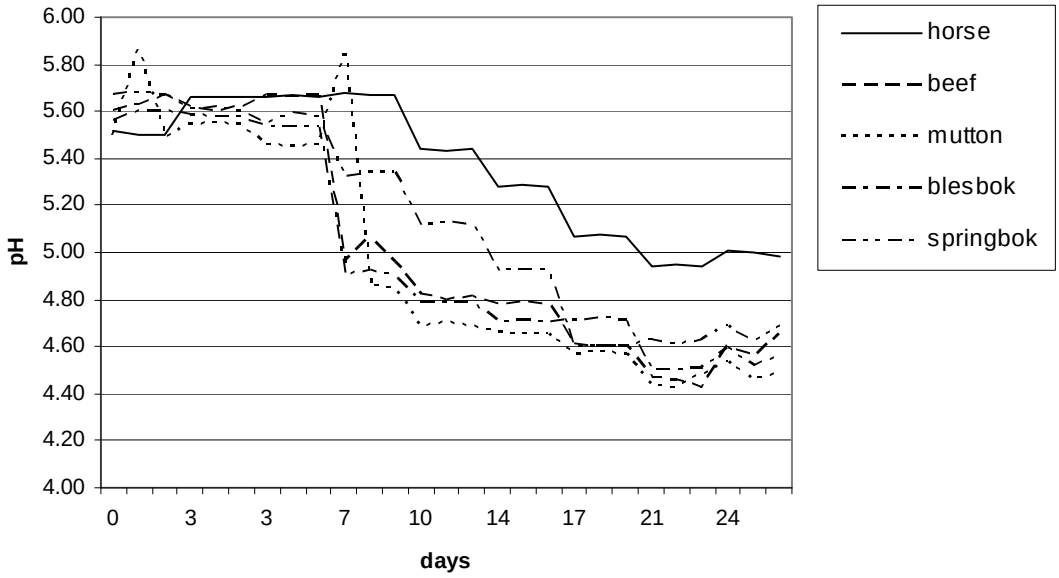

Figure 1. Salami pH values taken from five species over 23 days, using starter culture I

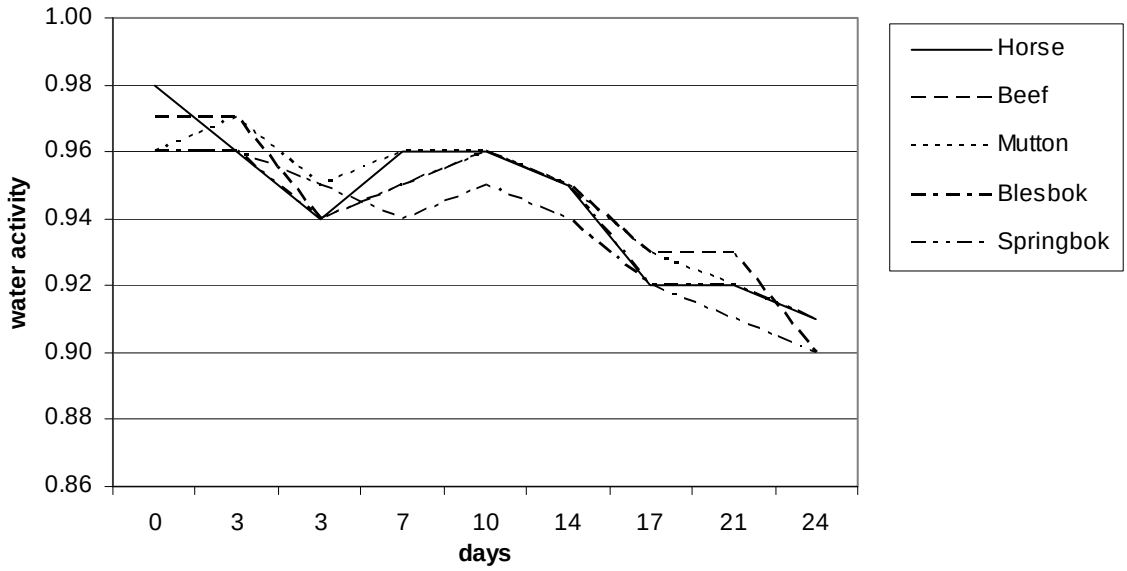

Figure 2**.** Salami a_w values of all species over 23 days, using starter culture I

Table 2

Average pH and water activity measurements taken on day 0 and day 23 for the five species of salami, prepared using three different starter cultures

Starter culture & species	pH		a_w	
	Day 0	Day 23	Day 0	Day 23
L. curvatus DF 38				
Horse	5.50	5.00	0.98	0.91
Beef	5.63	4.56	0.97	0.90
Mutton	5.86	4.46	0.96	0.91
Blesbok	5.60	4.52	0.96	0.90
Springbok	5.68	4.62	0.96	0.91
L. plantarum 423				
Horse	5.56	4.55	0.96	0.90
Beef	5.47	4.35	0.97	0.90
Mutton	5.56	4.36	0.96	0.91
Blesbok	5.39	4.38	0.97	0.91
Springbok	5.50	4.39	0.97	0.90
L. plantarum 423m				
Horse	5.57	4.65	0.97	0.90
Beef	5.48	4.36	0.96	0.90
Mutton	5.39	4.32	0.95	0.91
Blesbok	5.40	4.35	0.94	0.90
Springbok	5.51	4.41	0.96	0.90

6a.4.2. Colour evaluation

Regression lines were fitted to the colour data; the linear regression line had the highest R^2 value, but was still too low to be acceptable as an appropriate representation of the data (Table 3).

Meat colour is known to change during the ageing, curing and ripening period in salami (Ruiz de Huidobro *et al.*, 2003). The colour of meat is a vital point of judgment for the consumer (Clydesdale, 1991) and is dependent on the concentration and oxidation state of myoglobin, and on the meat structure. A higher L* value indicates increased lightness. Horse had the lowest L* values throughout and beef and mutton the highest. This is due to the innate characteristic of the meat, where horsemeat is a darker meat than beef and mutton. The game meat showed intermediate lightness, with the blesbok being lighter than the springbok. The change in L* values (over 23 days) of all five species salami using starter culture I (Fig. 3), indicated definite differences in lightness between species. Horse was the darkest meat with the lowest L* values, whilst beef was the lightest. Springbok salami showed the largest fluctuations over time, with beef being most stable.

Table 3

Regression line parameters, intercept (a), gradient (b) and goodness of fit (R^2 value) for colour values of the five species of salami, prepared using three starter cultures

Parameter	Batch	Intercept (a)	Gradient (b)	R^2 value
L. curvatus DF38				
L*value	Horse	39.123	0.0689	0.0239
	Beef	50.470	0.0106	0.0019
	Mutton	48.378	0.0950	0.1089
	Blesbok	47.242	0.1140	0.1705
	Springbok	43.823	0.0350	0.0033
a* value	Horse	14.798	-0.0475	0.0410
	Beef	12.789	0.0388	0.6060
	Mutton	13.984	0.0149	0.0067
	Blesbok	13.478	-0.0230	0.2228
	Springbok	12.952	0.1046	0.2056
b* value	Horse	10.919	-0.0942	0.3506
	Beef	12.889	-0.1805	0.4771
	Mutton	12.072	-0.1118	0.3299
	Blesbok	12.091	-0.1845	0.4276
	Springbok	11.538	-0.0994	0.3608
L. plantarum 423				
L* value	Horse	41.683	-0.0480	0.0060
	Beef	49.912	0.0881	0.1974
	Mutton	48.080	0.1053	0.0555
	Blesbok	44.875	0.2407	0.2446
	Springbok	44.180	0.1970	0.3252
a* value	Horse	14.787	-0.0569	0.2466
	Beef	13.15	0.0714	0.3652
	Mutton	13.988	-0.0135	0.0068
	Blesbok	15.732	-0.0878	0.1773
	Springbok	14.558	-0.0471	0.2316
b* value	Horse	10.696	-0.1202	0.4226
	Beef	13.419	-0.2437	0.4760
	Mutton	12.339	-0.1999	0.4747
	Blesbok	12.744	-0.2117	0.6057
	Springbok	11.865	-0.1739	0.6432
L. plantarum 423m				
L* value	Horse	44.789	-0.0928	0.2110
	Beef	50.331	0.0817	0.0274
	Mutton	50.498	-0.0251	0.0069
	Blesbok	49.659	-0.0068	0.0008
	Springbok	47.267	-0.0778	0.0663
a* value	Horse	13.610	0.0652	0.2010
	Beef	14.111	0.0333	0.3220
	Mutton	13.597	0.0373	0.1597
	Blesbok	12.576	0.1033	0.2173
	Springbok	12.424	0.0347	0.0528
b* value	Horse	10.225	-0.0544	0.1583
	Beef	12.458	-0.1688	0.3470
	Mutton	12.680	-0.1639	0.5368
	Blesbok	12.589	-0.1528	0.3782
	Springbok	11.333	-0.1327	0.8255

Table 3 shows that for starter culture II, all species, except beef, showed a negative trend in rate of change over time, whereas for starter culture III, all had a positive gradient.

Fig. 4 shows the a* values of the same sausages, indicating that the horse salami had the greatest fluctuations in red-green colour tones, probably due to the dark nature of the meat type. The a* values were lowest for the beef salami, except in starter culture III. As this intercept is a reflection of the batter on day 0, an average could be taken of all a* values of the different species, as starter culture would not yet have resulted in a change in colour. When averages were calculated, beef and springbok were the least red meat, with average a* values of 13.35 and 13.31 respectively. Horse was the most red, with an average a* value of 14.40. According to Strange, Benedict, Gugger, Metzger & Swift (1974), meat quality is positively related to the a* value.

Fig. 5 shows the b* values of the five species, prepared using starter culture I. All species displayed a definite negative trend over the same time period. The R^2 values for the b* values higher than for the L* and a* values, but still not high enough to consider a linear or non-linear regression as suitable fit to the relevant data. The goodness of fit of the linear regressions could be low due to the high variability in colour measurements, caused by the high variability within a specific sample measured. This variability is probably caused by fat and muscle pieces that make up the intricate colour mosaic of salami. Horse salami had the lowest b* value throughout, whereas beef had the highest, similar to Fig. 3. Some salami, for instance the mutton, had smaller fat particles and, thus, a smoother surface than the other species. No literature could be sourced which describes the change in L*a*b* colour during the fermentation of salami.

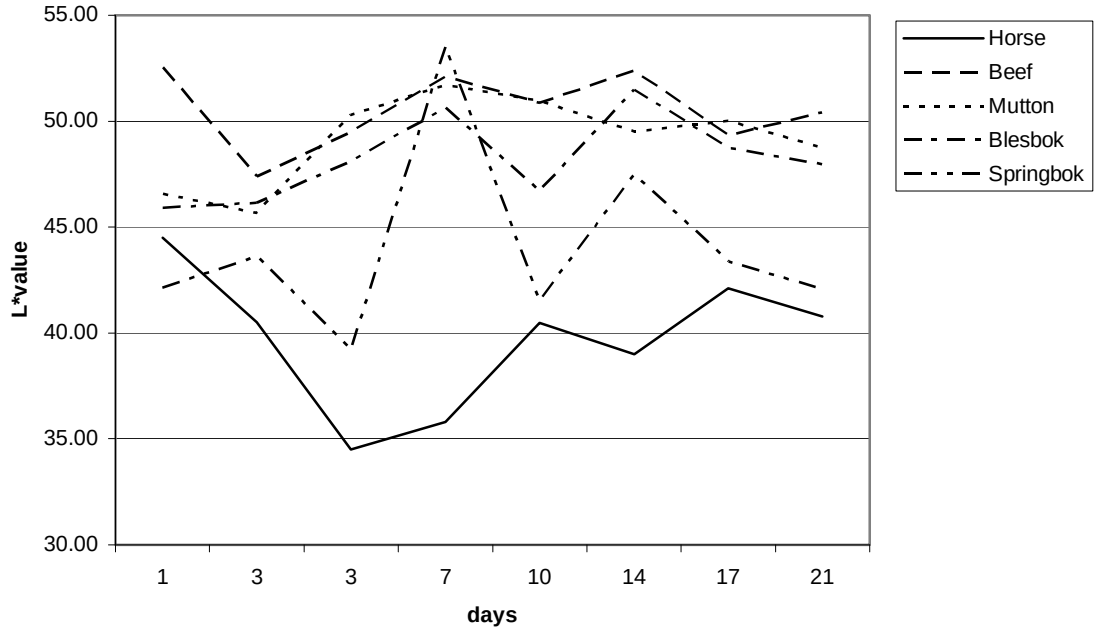

Figure 3. L* values of the five species used for the production of salami, using *L. curvatus* DF38, over 23 days

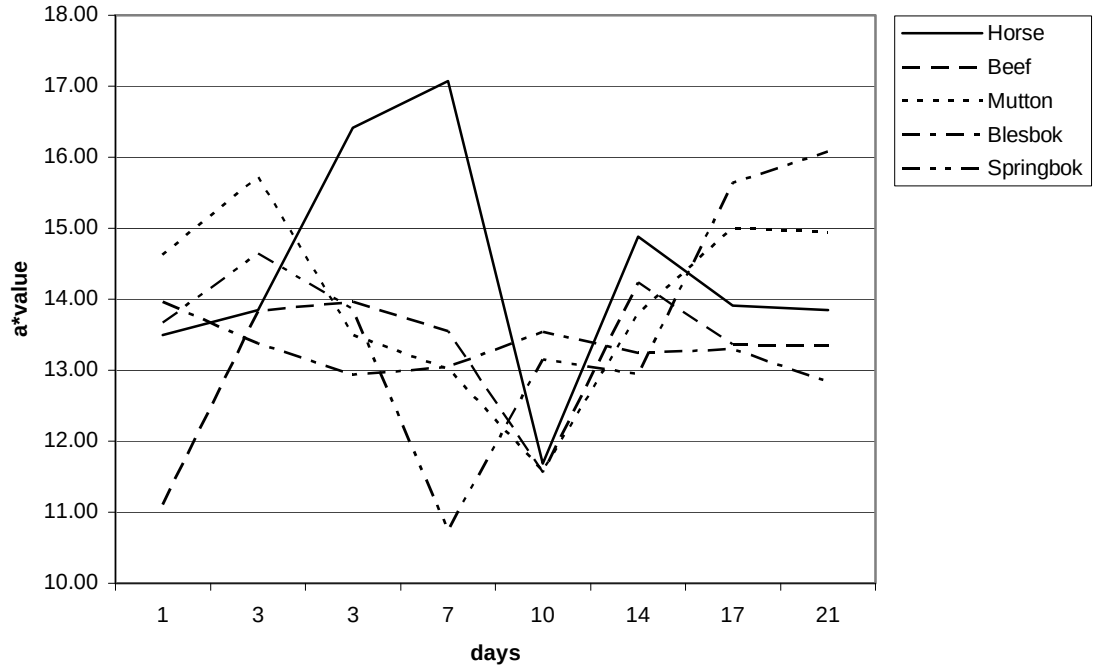

Figure 4. a* values of the five species used for the production of salami, using *L. curvatus* DF38, over 23 days

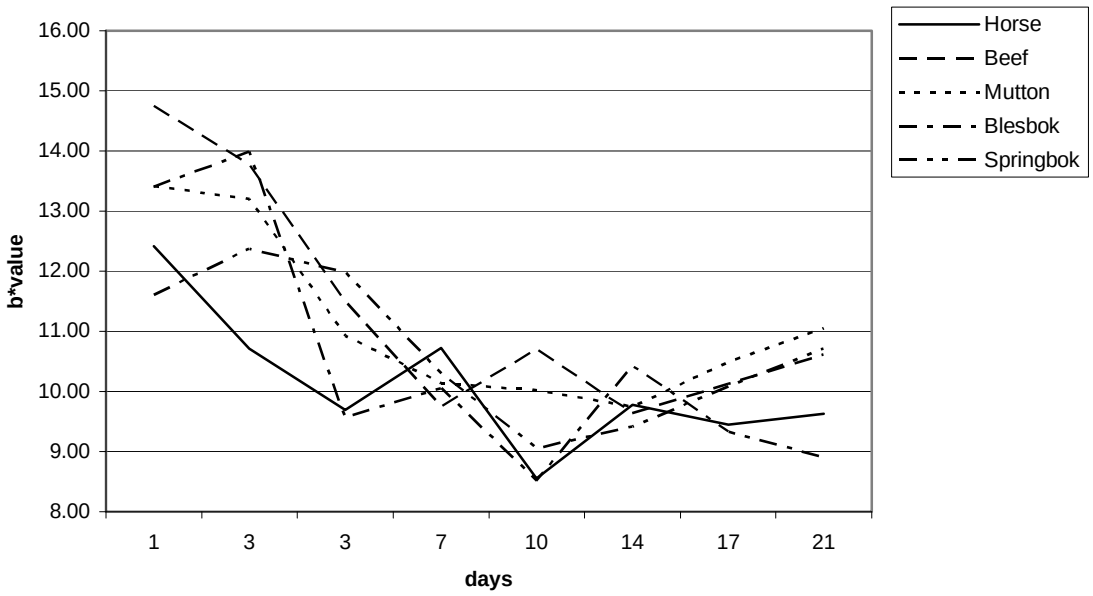

Figure 5. b* values of the five species used for the production of salami, using *L. curvatus* DF38, over 23 days

6a.4.3. Chemical analysis

In the proximate chemical analysis of the major raw materials used, horsemeat was the leanest, with only 1.38g fat/100g meat, whereas blesbok, with 4.12g fat/100g meat, had the most fat. Blesbok meat had the highest protein content, 22.15g/100g, whilst horse meat had the second highest protein concentration, 21.40g/100g. As neither of these animals is bred intensively for meat production, they would not be expected to have a high fat content. Game meat is known to be a lean meat, and the blesbok meat used in this study could have been from animals shot during the rainy season when their condition would have been at its optimum and the animals at their fattest, which would explain the chemical composition. When compared with the species normally used for salami production in South Africa, mainly beef, these other meat species would compare favourably and could be expected to be suitable for the production of salami. As fat is added in the preparation of the batter, the fat content of the actual raw (lean) meat used is not of that much relevance. Rather it is the physical attributes, such as water holding capacity and pH, which would determine the ultimate suitability of the meat of a certain species.

Proximate analysis of the raw materials used in the production of the salami was undertaken to evaluate the qualities of the individual species. Chemical composition in terms of moisture, protein, fat and ash of the five different meat types, and the pork fat used in the production of the sausages are depicted in Table 4.

Table 4

Means for the proximate chemical composition of the raw materials (g/100g) used in the production of the salami

Species	Moisture	Protein	Fat	Ash
Horse	75.10	21.40	1.38	1.46
Beef	75.32	20.84	2.67	1.27
Mutton	74.78	20.25	2.87	1.26
Blesbok	71.56	22.15	4.22	2.04
Springbok	74.75	19.94	3.14	1.42
Pork fat	14.50	6.79	78.01	0.64

Table 5 indicates the mean, and standard deviation of the species salami as pertaining to proximate chemical composition. This table also indicates the difference in chemical parameters within the salami between day 0 (batter) and day 23 (salami). Due to the drying conditions of the processing chambers, the final moisture values were more similar between species than they were on day 1. The protein and fat content increased relative to the moisture lost over this period.

During the chemical analysis of the batter and the finished product, it was noticed that the batch using starter culture I lost an average of 39.46% moisture over 23 days, batch II lost an average of 40.28%, and batch III 40.66%. The moisture content of the final salami varied from 29.2% (blesbok) to 34.6% (springbok), which corresponded with results reported by Meynier *et al*. (1999), whose salami's moisture content range from 26.0 to 45.5%. These researchers attribute the large variation in moisture content to differences in ripening between species (beef and mutton). The protein and fat contents of their sausages ranged from 22.7 to 30.8% and from 26.4 to 36.0% respectively. In this investigation, the protein content varied between 20.9 (springbok) and 24.2% (horse) and the fat content from 35.6% (horse) to 40.4% (blesbok).

Due to the high fat content of the blesbok meat, its moisture content was lower than that of the other species. As the pork fat used was subcutaneous fat, it contained some skin and connective tissue, which would result in relatively high protein and low fat content measured in the initial raw ingredient.

Table 5

Mean (g/100g) ± standard deviation of the proximate analysis results of the species on day 0 (batter) and 23 (salami)

Species	Batter (day 0)				Salami (day 23)			
	Moisture	Ash	Fat	Protein	Moisture	Ash	Fat	Protein
Horse	52.4	4.3	26.7	14.5	31.9	4.7	35.6	24.2
	±0.9	±1.6	±4.3	±3.2	±1.5	±0.1	±1.1	±0.5
Beef	53.4	3.2	28.7	13.1	30.1	4.9	36.6	23.5
	±1.0	±0.1	±4.0	±3.0	±1.9	±0.1	±0.8	±2.3
Mutton	55.0	3.6	25.1	14.2	33.6	4.9	36.4	22.3
	±1.0	±0.1	±0.1	±0.2	±1.2	±0.1	±1.0	±0.5
Blesbok	51.9	3.9	27.3	14.4	29.2	5.4	40.4	21.2
	±0.1	±0.5	±0.2	±0.8	±2.1	±0.7	±2.7	±1.1
Springbok	53.3	3.4	26.9	15.4	34.6	3.9	36.3	20.9
	±0.7	±0.1	±0.7	±3.0	±3.2	±0.8	±0.1	±1.9

6a.4.4. Fatty acid determination

The fatty acid composition of meat has little or no influence on the market value of a carcass at present, as carcasses of traditional red-meat-producing species are valued according to a specific grade. There is an increasing demand for meat products with a certain fatty acid profile, as the actual fatty acid qualities (chemical and physical) affect the sensory and storage qualities of meat (Banskalieva *et al*., 2000). It is also known that the flavour of meat is influenced by its fatty acid composition (Melton, 1990). Unsaturated fatty acids have an increased susceptibility for oxidation and thus rancidity, which influence shelf life.

It is possible that lactobacilli play a role in fat hydrolysis during dry fermentation of sausages, due to their ability to cleave short-chain fatty acid triglycerides and diglycerides. Although the concentration of these glycerides is quite low in the original fat of the sausages, they could increase due to the action of bacterial and meat lipases during the ripening phase of the sausages (Sanz *et al*., 1988). It is therefore difficult to monitor fatty acid changes during maturation and fermentation of a product.

Table 6

The fatty acid content of the raw materials (mg/100g meat) used in the production of the various species of salami

Fatty acid		Pork fat	Horse	Beef	Mutton	Blesbok	Springbok
SFA	16:0	2878.8	336	276.5	391	364.2	414.6
	18:0	351.8	183.3	201.6	288	274.7	327.5
	20:0	1480.5	4.1	7.2	3.4	8.6	3.4
	22:0	4.4	21.1	47.2	5.0	6.4	67.0
	24:0	18.8	2.5	25.5	86.2	36.7	2.3
MUFA	16:1 n-7	6.6	28.4	47.2	25.3	22.2	35.6
	18:1 n-9	101.3	304.3	364.2	583.5	572.6	536.3
	20.1 n-9	24.8	7.2	14.1	11.4	10.4	3.4
	22:1 n-9	83.7	4.1	7.2	14.8	11.3	3.7
	24:1 n-9	60.7	2.1	3.0	13.1	13.5	9.0
PUFA	18:2 n-6	64.1	344.1	175.3	136.2	132. 8	265.1
	18:3 n-3	20.2	72.9	21.8	33.9	35.3	31.8
	18:3 n-6	14.7	2.7	3.0	1.7	3.3	1.8
	20:2 n-6	18.8	16.4	20.0	12.1	14.1	12.1
	20:3 n-3	7.5	63.5	7.6	44.3	89.1	88.5
	20:4 n-6	2.1	8.7	8.0	1.7	2.1	9.3
	20:5	18	15.3	30.1	18.1	21	30.3
	22:2	4.9	2.3	2.4	17.5	3.0	8.3
	22:5	1.0	24.7	5.3	1.2	9.7	3.5
	22:6	20.6	33.5	9.0	7.0	6.3	2.9
Total	ΣSFA	4734.3	547.0	558.0	773.6	690.6	814.8
	ΣMUFA	277.1	346.1	435.7	648.1	630.0	588.0
	ΣPUFA	171.9	584.1	282.5	273.7	183.9	453.6
	ΣTUFA	449.0	930.2	718.2	921.8	813.9	1041.6
	DFA	800.8	1113.5	919.8	1209.8	1088.6	1369.1
	P: S	0.04	1.1	0.5	0.4	0.3	0.6
	n-6	99.7	371.9	206.3	151.7	152.3	288.3
	n-3	27.7	136.4	29.4	78.2	124.4	120.3
	n-6: n-3	3.6	2.7	7.0	1.9	1.2	2.4

SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; TUFA = Total Unsaturated Fatty Acids; DFA = Desirable Fatty Acids (C18:0 + TUFA)

Table 6 shows the fatty acid compositions (mg/100g meat) of the major raw materials used. Springbok showed the highest SFA content (814.8mg/100g meat) of all five species, and mutton the highest overall MUFA content (648.1mg/100g meat), with horse showing the highest concentration of PUFA (584.1mg/100g meat). Horsemeat also contained the lowest amount of SFA (547.0mg/100g meat) and lowest amount of MUFA (346.1mg/100g meat). Fatty acid analyses were conducted on the raw materials (meat samples and pork fat) and results are shown in Table 6, according to species and degree of saturation. Table 7 shows the percentage fatty acid values of the raw materials used. Palmitic acid (C16:0) was the dominant fatty acid present in all five species. Horsemeat had the highest PUFA:SFA (P:S) ratio (1.1) of all the raw materials

used, whereas pork fat had the lowest (0.04). Beef was seen to have the highest n-6:n-3 ratio of the meat used, with blesbok having the lowest value. In terms of DFA content, springbok meat had the highest concentration, and pork fat the lowest.

Table 7

Percentage values of the raw materials used in the production of traditional salami

Fatty acid		Pork fat	Horse	Beef	Mutton	Blesbok	Springbok
SFA	16:0	55.5	22.7	21.7	23.1	24.2	22.3
	18:0	6.8	12.4	15.8	17.0	18.3	17.6
	20:0	28.6	0.3	0.6	0.2	0.6	0.2
	22:0	0.1	1.4	3.7	0.3	0.4	3.6
	24:0	0.4	0.2	2.0	5.1	2.4	0.1
MUFA	16:1 n-7	0.1	1.9	3.7	1.5	1.5	1.9
	18:1 n-9	2.0	20.6	28.5	34.4	38.1	28.9
	20.1 n-9	0.5	0.5	1.1	0.7	0.7	0.2
	22:1 n-9	1.6	0.3	0.6	0.9	0.8	0.2
	24:1 n-9	1.2	0.1	0.2	0.8	0.9	0.5
PUFA	18:2 n-6	1.2	23.3	13.7	8.0	8.7	14.3
	18:3 n-3	0.4	4.9	1.7	2.0	2.3	1.7
	18:3 n-6	0.3	0.2	0.2	0.1	0.2	0.1
	20:2 n-6	0.4	1.1	1.6	0.7	0.9	0.7
	20:3 n-3	0.1	4.3	0.6	2.6	5.9	4.8
	20:4 n-6	0.0	0.6	0.6	0.1	0.1	0.5
	20:5	0.3	1.0	2.4	1.1	1.4	1.6
	22:2	0.1	0.2	0.2	1.0	0.2	0.4
	22:5	0.0	1.7	0.4	0.1	0.6	0.2
	22:6	0.4	2.3	0.7	0.4	0.4	0.2

Tables 8 and 9 represent the fatty acid composition (mg/100g sample) of the batter (day 0) and finished product (day 23). As the same amount of pork lard was added to each batter, all species of meats would be affected in the same way due to the dilution effect of the fat. The high SFA content of pork fat (Table 6) increased the SFA content of each batter, influencing the DFA and P:S ratios. The batter containing the horsemeat had the highest SFA content, and mutton the lowest (Table 8). The blesbok batter had the most DFA content and mutton the least. In terms of P:S ratios, all batters were between 0.4 and 0.5. The n-6:n-3 ratio for horse salami was the highest, whilst mutton had the lowest ratio. None of the samples of batter or salami conformed to the ideal P:S ratio of >0.70. This is possibly due to the high amount of pork fat in the mixture, giving it undesirable properties (Table 8). All species in the final salami had n-6: n-3 values of <5.0, indicating that these products are beneficial with regards to food healthy standards. The lowest n-6:n-3 ratio, i.e. the best one, was noted in the horse salami prepared using starter culture II (0.65). This would be considered the healthiest salami in terms of beneficial fatty acids.

As the fatty acid composition of human food sources is becoming increasingly important in terms of human health, the aim is to bring the P:S ratio of meat closer to the recommended value >0.70, and the n-6:n-3 ratio of <5.0 (Raes *et al*., 2004). The ideal P:S ratio is >0.70, but as very few species conform to this ideal, the recommended value for P:S ratio has thus been adjusted to 0.45 (Wood & Enser, 1997). According to Enser *et al*. (1998), the meat from ruminant animals usually has a low P:S ratio, whereas game meat is known to have a higher and more positive one, due to a high PUFA content (Girolami et *al*., 2003). In Table 6, the P:S ratio of the ruminants was lower than that of horse (1.1). The n-6:n-3 ratio of the horsemeat, mutton, blesbok and springbok were <5.0, which make them highly suitable for human consumption. The beef, similarly, had an n-6:n-3 ratio of >5.0.

The fatty acid content of the salami after 23 days is shown in Table 9. A comparison between Tables 8 and 9 indicates how the fatty acids changed due to oxidation in the fermenting sausage. Springbok salami contained the highest concentration of SFA after 23 days, whilst the sausage containing horsemeat had the second highest. The highest DFA concentration was found in the salami made from horsemeat. The P:S ratios were more variable after 23 days than they were on day 0, with horse salami having the highest ratio (0.08) and blesbok salami the lowest (0.05). The n-6:n-3 ratios also changed, with mutton having the highest n-6:n-3 ratio, and horse the lowest. This was in direct contrast with day 0, where the opposite was seen (Table 8).

According to Knapp & Melly (1986), PUFA have bactericidal effects and their toxicity increases with increasing unsaturation. The susceptibility and severity of lipid oxidation depends on the degree and amount of unsaturated fatty acids present (Kanner, 1994; Gray *et al*., 1996). Talon *et al*. (2000) substantiated this, by suggesting that the rate of oxidation decreased in the order linolenic, linoleic and oleic fatty acids. In sausage, free fatty acids increase during maturation. Johansson, Molley, Geenen & Demeyer (1996) suggest that linolenic and linoleic acids represent approximately 1 and 12 % respectively of the total free fatty acids, and, therefore, account for a significant percentage that will oxidise during sausage processing. In Tables 10 and 11, the percentage values of linolenic and linoleic fatty acids on day 0 in the batter and day 23 in the end product, indicate that linoleic acid (18:2 n-6) and both α- and γ linolenic acids (18:3 n-6 and n-3) showed an increase over time. Both had similar values to begin with, which were closer to 1% than 12%. This might be due to a variation in ingredients used in the making of the sausages in this investigation, compared with those used by Johansson, Molley, Geenen & Demeyer (1996). Tables 10 and 11 show the same fatty acids as in Tables 8 and 9, but are expressed as a percentage of the total fatty acids identified.

Table 8

The fatty acid content (mg/100g meat) of the batter used in the production of the different species salami

Fatty acid	H I	B I	M I	BB I	SB I	H II	B II	M II	BB II	SB I	H III	B III	M III	BB III	SB III
16:0	1754	1769	1583	1747	1862	1710	1820	1780	2108	1666	1897	1891	1804	2021	1772
18:0	180	186	161	169	207	200	182	180	217	202	208	201	191	200	197
20:0	951	968	804	1022	957	865	968	939	1109	825	938	1017	908	1156	926
22:0	3488	2969	2702	3318	3593	3394	3001	2521	3560	3506	3599	2877	2741	3211	3441
24:0	11	10	6	4	8	11	10	4	4	11	12	3	3	6	11
16:1 n-7	2	7	6	6	6	4	4	6	6	6	5	2	7	5	4
18:1 n-9	53	63	8	126	59	59	61	3	124	64	56	64	3	95	125
20.1 n-9	21	21	17	16	23	23	23	11	17	21	24	17	18	24	24
22:1 n-9	158	72	8	68	132	136	62	10	63	133	148	57	9	65	133
24:1 n-9	130	17	9	45	69	123	29	11	71	75	134	27	12	51	72
18:2 n-6	97	93	35	32	83	92	125	30	109	75	86	58	21	92	94
18:3 n-3	27	17	74	88	19	25	14	73	21	18	22	13	87	19	16
18:3 n-6	15	23	42	51	49	13	13	42	57	43	20	21	60	52	42
20:2 n-6	39	27	20	48	33	47	25	35	32	41	25	28	45	36	22
20:3 n-3	11	12	10	14	11	13	20	12	19	19	10	6	30	12	14
20:4 n-6	3	5	4	4	1	3	4	3	11	2	4	1	6	7	5
20:5	44	21	3	40	37	40	19	2	40	33	61	22	22	29	51
22:2	7	5	5	7	9	5	4	4	5	6	9	4	4	4	6
22:5	21	20	28	33	20	23	21	33	45	21	38	14	2	26	21
22:6	19	14	26	24	9	23	12	29	30	13	13	8	27	17	17
ΣSFA	6384	5902	5256	6260	6632	6180	5981	5424	6998	6210	6659	5989	5647	6594	6997
ΣMUFA	364	180	48	261	289	345	179	41	281	299	367	167	49	240	358
ΣPUFA	283	237	247	341	271	284	257	263	369	271	288	175	304	294	288
ΣTUFA	647	417	295	602	560	629	436	304	650	570	655	342	353	534	646
DFA	827	603	456	771	767	829	618	484	867	772	863	543	544	734	843
P: S	0.04	0.04	0.05	0.05	0.04	0.05	0.04	0.05	0.05	0.04	0.04	0.03	0.05	0.04	0.04
n-6	154	148	101	135	152	155	167	110	209	161	135	108	132	187	163
n-3	38	29	84	102	30	38	34	85	40	37	32	19	117	31	30
n-6: n-3	4.05	5.10	1.20	1.32	5.08	4.08	4.91	1.29	5.23	4.35	4.22	5.68	1.13	6.03	5.43

SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; TUFA = Total Unsaturated Fatty Acids; DFA = Desirable Fatty Acids (C18:0 + TUFA)

H = horse, B = beef, M = mutton, BB = blesbok, SB = springbok, I, II and III = starter cultures I, II and III

Table 9

The fatty acid composition of the salami (mg/100g meat) 23 days after processing using the five different species

Fatty acid	H I	B I	M I	BB I	SB I	H II	B II	M II	BB II	SB I	H III	B III	M III	BB III	SBIII
16:0	2283	2416	2237	2016	2564	2565	2822	2317	1982	2582	2038	2966	1862	2625	2840
18:0	227	246	247	221	215	146	278	227	230	255	208	321	219	205	288
20:0	925	1240	1197	1029	1447	910	1565	1202	994	1457	996	1520	1082	900	1590
22:0	4345	25	3905	3176	4258	4078	25	3968	26	4651	3848	35	3588	3097	4798
24:0	17	12	11	11	6	23	15	6	12	7	15	17	17	18	8
16:1 n-7	13	11	11	11	7	16	10	7	12	9	13	9	6	20	8
18:1 n-9	4	4	5	4	148	5	4	6	5	155	4	6	5	6	142
20.1 n-9	29	1	21	22	28	27	3	24	28	36	28	3	19	27	35
22:1 n-9	247	15	13	154	92	248	27	11	161	94	258	14	2	132	107
24:1 n-9	187	135	121	194	122	197	196	114	190	115	180	177	83	178	96
18:2 n-6	141	8	190	65	131	93	14	106	66	170	126	15	325	40	165
18:3 n-3	194	114	91	137	83	238	127	92	144	86	102	80	91	146	83
18:3 n-6	10	63	58	75	58	18	72	59	82	80	19	74	68	90	67
20:2 n-6	32	31	22	32	49	37	27	35	32	42	30	27	28	59	55
20:3 n-3	2	11	23	32	35	4	16	19	26	36	6	24	19	42	35
20:4 n-6	5	10	8	7	7	10	8	12	5	3	5	3	6	3	9
20:5	3	3	28	13	28	3	7	22	18	22	5	7	26	10	41
22:2	14	5	6	16	5	14	5	3	12	9	12	3	3	22	3
22:5	28	30	4	23	2	27	34	1	22	30	23	28	1	13	27
22:6	3	28	34	7	29	4	24	28	5	36	4	30	28	4	27
ΣSFA	7797	3939	7597	6453	8490	7722	4705	7720	3244	8952	7105	4859	6768	6845	9524
ΣMUFA	480	166	171	385	397	493	240	162	396	409	483	209	115	363	388
ΣPUFA	432	303	464	407	427	448	334	377	412	514	332	291	595	429	512
ΣTUFA	912	469	635	792	824	941	574	539	808	923	815	500	710	792	900
DFA	1139	715	882	1013	1039	1087	852	766	1038	1178	1023	821	929	997	1188
P: S	0.06	0.08	0.06	0.06	0.05	0.06	0.07	0.05	0.13	0.06	0.05	0.06	0.09	0.06	0.05
n-6	188	112	278	179	152	158	121	212	185	295	180	119	427	192	296
n-3	196	125	114	169	118	242	143	111	170	122	108	104	110	188	118
n-6: n-3	0.96	0.90	2.44	1.06	1.29	0.65	0.85	1.91	1.09	2.42	1.67	1.14	3.88	1.02	2.51

SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; TUFA = Total Unsaturated Fatty Acids;
DFA = Desirable Fatty Acids (C18:0 + TUFA);
H = horse, B = beef, M = mutton, BB = blesbok, SB = springbok, I, II and III = starter cultures I, II and III

Table 10

Percentage values of the batter (mg/100g meat) used in the production of the salami

Fatty acid	H I	B I	M I	BB I	SB I	H II	B II	M II	BB II	SB I	H III	B III	M III	BB III	SBIII
16:0	21.5	28.0	28.5	25.5	23.2	21.5	28.4	31.1	27.6	21.0	22.1	29.9	30.1	28.4	21.9
18:0	2.2	2.9	2.9	2.5	2.6	2.5	2.8	3.1	2.8	2.6	2.4	3.2	3.2	2.8	2.4
20:0	11.6	15.3	14.5	14.9	11.9	10.9	15.1	16.4	14.5	10.4	10.9	16.1	15.1	16.2	11.4
22:0	42.7	47.0	48.7	48.4	44.8	42.6	46.8	44.0	46.5	44.3	41.9	45.4	45.7	45.0	42.5
24:0	14.1	0.2	0.1	0.1	10.5	14.6	0.2	0.1	0.1	14.5	15.0	0.0	0.1	0.1	13.8
16:1 n-7	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.0
18:1 n-9	0.6	1.0	0.1	1.8	0.7	0.7	1.0	0.1	1.6	0.8	0.7	1.0	0.1	1.3	1.5
20.1 n-9	0.3	0.3	0.3	0.2	0.3	0.3	0.4	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3
22:1 n-9	1.9	1.1	0.1	1.0	1.6	1.7	1.0	0.2	0.8	1.7	1.7	0.9	0.2	0.9	1.6
24:1 n-9	1.6	0.3	0.2	0.7	0.9	1.5	0.5	0.2	0.9	0.9	1.6	0.4	0.2	0.7	0.9
18:2 n-6	5.5	5.3	2.2	1.8	4.5	5.4	6.9	1.7	5.2	4.5	4.5	3.1	1.2	4.6	5.3
18:3 n-3	0.3	0.3	1.3	1.3	0.2	0.3	0.2	1.3	0.3	0.2	0.3	0.2	1.5	0.3	0.2
18:3 n-6	0.2	0.4	0.8	0.7	0.6	0.2	0.2	0.7	0.7	0.5	0.2	0.3	1.0	0.7	0.5
20:2 n-6	0.5	0.4	0.4	0.7	0.4	0.6	0.4	0.6	0.4	0.5	0.3	0.4	0.8	0.5	0.3
20:3 n-3	0.1	0.2	0.2	0.2	0.1	0.2	0.3	0.2	0.2	0.2	0.1	0.1	0.5	0.2	0.2
20:4 n-6	0.0	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.1
20:5	0.5	0.3	0.1	0.6	0.5	0.5	0.3	0.0	0.5	0.4	0.7	0.3	0.4	0.4	0.6
22:2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
22:5	0.3	0.3	0.5	0.5	0.2	0.3	0.3	0.6	0.6	0.3	0.4	0.2	0.0	0.4	0.3
22:6	0.2	0.2	0.5	0.3	0.1	0.3	0.2	0.5	0.4	0.2	0.2	0.1	0.5	0.2	0.2

Table 11

The fatty acid profile of the salami 23 days after initial processing

Fatty acid	H I	B I	M I	BB I	SB I	H II	B II	M II	BB II	SB I	H III	B III	M III	BB III	SBIII
16:0	26.2	54.8	27.2	27.8	27.5	29.6	53.5	28.1	48.9	26.1	25.7	55.3	24.9	34.4	27.2
18:0	2.6	5.6	3.0	3.1	2.3	1.7	5.3	2.7	5.7	2.6	2.6	6.0	2.9	2.7	2.8
20:0	10.6	28.1	14.5	14.2	15.5	10.5	29.6	14.6	24.5	14.8	12.6	28.4	14.5	11.8	15.3
22:0	49.9	0.6	47.4	43.8	45.7	47.1	0.5	48.0	0.6	47.1	48.6	0.7	48.0	40.6	46.0
24:0	0.2	0.3	0.1	0.2	0.1	0.3	0.3	0.1	0.3	0.1	0.2	0.3	0.2	0.2	0.1
16:1 n-7	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.3	0.1	0.2	0.2	0.1	0.3	0.1
18:1 n-9	0.0	0.1	0.1	0.1	1.6	0.1	0.1	0.1	0.1	1.6	0.1	0.1	0.1	0.1	1.4
20.1 n-9	0.3	0.0	0.3	0.3	0.3	0.3	0.1	0.3	0.7	0.4	0.4	0.1	0.3	0.4	0.3
22:1 n-9	2.8	0.3	0.2	2.1	1.0	2.9	0.5	0.1	4.0	1.0	3.3	0.3	0.0	1.7	1.0
24:1 n-9	2.1	3.1	1.5	2.7	1.3	2.3	3.7	1.4	4.7	1.2	2.3	3.3	1.1	2.3	0.9
18:2 n-6	6.2	0.3	8.5	3.2	5.1	3.6	0.5	4.6	3.3	6.6	6.2	0.5	17.5	1.5	5.8
18:3 n-3	2.2	2.6	1.1	1.9	0.9	2.7	2.4	1.1	3.6	0.9	1.3	1.5	1.2	1.9	0.8
18:3 n-6	0.1	1.4	0.7	1.0	0.6	0.2	1.4	0.7	2.0	0.8	0.2	1.4	0.9	1.2	0.6
20:2 n-6	0.4	0.7	0.3	0.4	0.5	0.4	0.5	0.4	0.8	0.4	0.4	0.5	0.4	0.8	0.5
20:3 n-3	0.0	0.2	0.3	0.4	0.4	0.0	0.3	0.2	0.6	0.4	0.1	0.4	0.3	0.5	0.3
20:4 n-6	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.1	0.0	0.1
20:5	0.0	0.1	0.3	0.2	0.3	0.0	0.1	0.3	0.4	0.2	0.1	0.1	0.3	0.1	0.4
22:2	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.0	0.3	0.1	0.2	0.1	0.0	0.3	0.0
22:5	0.3	0.7	0.0	0.3	0.0	0.3	0.6	0.0	0.5	0.3	0.3	0.5	0.0	0.2	0.3
22:6	0.0	0.6	0.4	0.1	0.3	0.0	0.5	0.3	0.1	0.4	0.1	0.6	0.4	0.1	0.3

6a.5. Conclusion

The five species used in this trial, and the three different starter cultures, all responded favourably to the conditions to which they were subjected. Differences were observed between species and between starter cultures, this being indicative of the unique properties of the various parameters. Greater differences were recorded between starter cultures I and II, and I and III. This is not surprising, as starter cultures II and III were both *L. plantarum*, whilst starter culture I was *L. curvatus*. According to Dicks *et al*. (2003), *L. plantarum* is a good starter culture, functioning especially well in meat with high initial pH values. This investigation substantiates this observation.

Of the species used, horsemeat responded most favourably to the expectations in the production of traditional salami. Blesbok showed the least favourable characteristics, from the chemical analysis, and fatty acid content point of view.

The species researched, especially the horse and springbok, would lend themselves well to the production of a processed meat product such as salami. In this way, especially the springbok, which is harvested seasonally, can provide a year-round supply of meat to the consumer. The species researched are healthy species alternatives to other more conventional red meat species products.

6a.6. Acknowledgements

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Chapter 6

Production of salami using meat from beef, mutton, horse, blesbok and springbok
b) Microbiological investigation

6b.1. Abstract

Salami was produced using meat from horse, beef, mutton, blesbok and springbok. Three different starter cultures were used: *Lactobacillus curvatus* DF 38 (batch I), *Lactobacillus plantarum* 423 (batch II), which produces plantarin 423, a bacteriocin, and a bacteriocin-negative mutant of *Lactobacillus plantarum,* strain 423m, (batch III). Similar growth patterns were recorded for *L. plantarum* 423, *L. plantarum* 423m and *L. curvatus* DF38 in MRS broth (Merck) at 30^{o}C, although batch I reached exponential growth earlier. Production of plantaricin 423 and curvacin DF38 (400 and 200 AU/ml, respectively) was recorded after 3 hours. The activity of plantaricin 423 increased from 400 to 3200AU/ml as the pH decreased from 6.3 to 4.2 during logarithmic growth. The percentage of *L. plantarum* 423, compared with the total population of microflora in mutton salami, remained constant (80-95% variation) during the entire fermentation and maturation process. In horse, *L. plantarum* 423 was present at relatively low cell numbers (55-50% on day 1 and before smoking), but increased to 70% after smoking, and stabilised at 70-80% for the remaining fermentation period. In beef, batch II decreased slightly during the first 5 days (from 95 to 70%), followed by an increase to 90%. In springbok salami, batch II remained fairly stable at 80-90%. In blesbok salami, batch II slowly decreased during the first three days from 88% to 70%, then increased to 92% after 12 days and stabilised for the rest of the fermentation period. Similar results were recorded for batch I. The cell numbers of batch I remained stable in all five salami types (between 75 and 95%). A slow decrease in cell numbers was recorded in mutton, beef and springbok salami at 48 days (68, 70 and 80%, respectively), followed by stabilisation. A difference in one log cycle was recorded between the latter salami and a control sample (no bacteriocin present). Results recorded for horse salami did not show a reduction in viable cell numbers of *L. innocua* F when compared to the control sample.

Key words: *L. plantarum* and *L. curvatus*, salami

6b.2. Introduction

Lactic acid bacteria are found in a variety of natural and fermented food products (De Vuyst & Vandamme, 1994). Many of these strains produce desirable organoleptic compounds and, in the case of meat, facilitate cohesion of the meat particles and contribute to colour change (Zeuthen, 1995). The organic acids and other secondary metabolites do not only enhance the aroma of the fermented meat, but also extend its shelf life (De Vuyst & Vandamme, 1994). Some lactic acid bacteria produce bacteriocins, i.e. small peptides with antibacterial properties (Tagg *et al*., 1976; Klaenhammer, 1988; De Vuyst & Vandamme, 1994). A few reports on bacteriocin-like peptides with anti-mould and anti-yeast properties have also been published (Girafa, 1995; Atanassova *et al*., 2003; Fazeli *et al*., 2004; Mäyrä-Mäkinen & Suomalainen, 1995).

The fermentation of beef, pork, turkey and ostrich meat by strains of *Lactobacillus*, *Pediococcus*, and non-pathogenic *Staphylococcus* and *Micrococcus* spp. are well documented (Lücke, 1996; Böhme *et al*., 1996; Dicks *et al*., 2004). In a previous study (Böhme *et al*., 1996), ostrich meat salami was produced with a mixed starter culture of *Lactobacillus curvatus*, *Lactobacillus sakei* and *Micrococcus* spp., with single cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* (Dicks *et al*., 2004). In these two studies, the control of *Listeria monocytogenes* and *Listeria inocua* was clearly demonstrated.

Listeria monocytogenes is a major concern in fermented meat (Aymerich *et al*., 1998; Johnson *et al*., 1990); it is commonly found in cold-smoked products, usually in low numbers. Salting and cold smoking, used to prevent the growth of spoilage and pathogenic organisms, have proved ineffective in the control of *Listeria monocytogenes* (Guyer & Jemmi, 1991). Some strains survive at pH values as low as 4.5 (Ahmad & Marth, 1990), grow between 1^{o}C and 45^{o}C (Seeliger & Jones, 1986), in the presence of salts as high as 12%, (McClure et *al*., 1989), and at a water activity between 0.93 and 0.96 (Guyer & Jemmi, 1991). Infection with *Listeria monocytogenes* may lead to listeriosis, which is fatal to 20-30% of all patients diagnosed with the disease (Rocourt, 1996). In this investigation, *Listeria innocua F* was used. According to Vaz-Velho *et al*. (in press) this strain can be used to replace *Listeria monocytogenes* (due to laboratory constraints), if the latter pathogen cannot be used.

Several bacteriocin-producing strains of *Lactobacillus* (Hugas *et al*., 1995; Böhme *et al*., 1996; Dicks *et al.,* 2004), *Pediococcus* (Bhunia *et al*., 1988; Harris *et al*., 1989), *Leuconostoc* (Coffey *et al*., 1998), and *Carnobacterium* (Vaz-Velho *et al*., 2005; Duffes *et al*., 1999) have been used as starter cultures in fermented meat.

In the present study, *L. plantarum* 423, isolated from sorghum beer (Van Reenen *et al*., 1998), and *L. curvatus* DF38, isolated from Italian salami, were used as starter cultures in the fermentation of beef, horse, mutton and game (blesbok and springbok) meat. Strain 423 produces plantaricin 423, which is active against several *Lactobacillus* spp. and a few food spoilage organisms, including *Listeria monocytogenes* (Van Reenen *et al*., 1998). Strain DF38 produces the bacteriocin curvacin DF38, which has also proved to be effective in the preservation of ostrich meat salami (Dicks *et al*., 2004).

Increasing consumer interest in non-traditional meat products motivates the investigation into the processing potential of species such as horse, mutton, beef and certain species of game animals (blesbok & springbok). Two of these species are not farmed intensively, and therefore can be considered as wild game animals. Comparisons need to be drawn between these species and the conventional red meat species such as beef and mutton with regards to the potential of utilising these meat species in the commercial meat producing industry. Since the contribution of game meat production (jerky hunters and game meat sales) constitutes 67.5% of the total income of the game industry of South Africa (Bothma, 2002), research is required to investigate the possibility of utilising these species in processed products. As many alternative red meat species are harvested seasonally, traditional salami made from these species would ensure a continuous supply of a form of this meat from these species.

The aim of this investigation was therefore to determine the performance and growth of *L. plantarum* 423 and *L. curvatus* DF38 in the different meat types and evaluate plantaricin 423 and curvacin DF38 as preservatives in these salami.

6b.3. Materials and methods

The sourcing of the ingredients and processing of the salamis prepared for this investigation are discussed in detail in Chapter 6a.

6b.3.1. Starter cultures

Lactobacillus plantarum 423 (batch II) (Van Reenen *et al*., 1998), a mutant cured from plasmid p423 and unable to produce plantaricin 423 (strain 423m) (batch III), and *Lactobacillus curvatus* DF38 (batch I) (Dicks *et al*., 2004) were used as starter cultures. Curing of strain 423 from its plasmid was undertaken by incubation in the presence of 0.125-8μg/ml novobiocin, according to the method described by Ruiz-Barba *et al.* (1991). *Listeria innocua* strain F was used as indicator organism. Strains 423, 423m and DF38 were cultured in MRS broth (Biolab, Biolab Diagnostics,

Midrand, South Africa). *Listeria* innocua was cultured in BHI broth (Biolab). Cultures were stored at -80°C in MRS or BHI broth, supplemented with 15% (v/v) sterile glycerol.

Starter cultures were prepared by inoculating 2L MRS broth with 5% (v/v) of a 12 hour-old culture. Incubation was for 24 hours at 30°C, without shaking. The cells were harvested (8000 x *g*, 20min, 4°C), washed twice with sterile physiological salt (0.85% m/v NaCl) and resuspended in 200 ml of the same solution. Cell suspensions were stored at 4°C until used.

6b.3.2. Growth of starter cultures and bacteriocin production

In vitro production of plantaricin 423 and curvacin DF38 was studied by growing *L. plantarum* 423, *L. plantarum* 423m and *L. curvatus* DF38, respectively, in 250 ml MRS broth at 30°C. At specific time intervals, samples were taken to determine the optical density (OD_{600}) and pH of the culture. At the same time, 10ml of the culture was centrifuged (8000 x *g*, 20min, 4°C,) and the activity of the bacteriocins in the cell-free supernatants expressed in arbitrary units (AU)/ml, as described by Todorov *et al*. (1999). *Listeria innocua* F was used as target organism.

6b.3.3. Monitoring of changes in bacterial cell numbers during fermentation

At selected time intervals (every three days), a salami of each animal species and starter culture was removed from the maturation chamber and a section of meat aseptically sampled to represent the outer and inner sections of the salami. A sample of 10g was suspended in 90ml sterile physiological salt and blended for two minutes. The suspension was serially diluted and plated onto MRS agar (Biolab), supplemented with Delvocid (Gist-Brocades B.V., Delft, The Netherlands) to prevent yeast growth. The plates were incubated for 48 hours at 30°C and the number of viable cells determined. All cell counts were conducted in triplicate.

6b.3.4. Determining the percentage bacteriocin-producing strains

Plates with colonies between 50 and 300 were overlaid with 10ml BHI agar (Biolab), inoculated with $1x10^6$cfu *Listeria innocua* F per ml. The plates were incubated at 37°C for 24 hours. Colonies surrounded with inhibition zones were counted separately and the bacteriocin-producing cells calculated as a percentage of the total number of cells per gram meat.

6b.3.5. Growth of Listeria innocua F

A sample of salami (4.5g) was suspended in sterile physiological salt (4.5 ml) inoculated with 1.0ml *Listeria innocua* F ($1x10^6$ cfu/ml) and blended for two minutes. The suspension was incubated for 24 hours at 20°C. The samples were serially diluted, plated onto LEB and the

number of viable cells of *Listeria innocua* determined from colony counts after 48 hours of incubation at 37^{o}C.

6b.3.6. The effect of smoke on starter cultures

Strains 423, 423m and DF38 were cultured in 20 ml MRS broth (Biolab) for 24 hours at 30^{o}C. The cells were harvested (8000 x *g*, 20min, 4^{o}C) and washed twice with sterile physiological water. The cells were re-suspended in sterile physiological salt to approximately 10^{6} cfu/ml. The cell suspensions were subjected to smoke produced by commercial oak wood chips for one hour at 20^{o}C. The number of viable cells was determined before and after smoking. Cell suspensions treated with filter-sterilised air served as controls.

6b.4. Results and discussion

6b.4.1. Growth production

Lactic acid bacteria's major role in the production of salami is related to carbohydrate source metabolism, which results in the acidification of the meat batter. This reaction leads to hygienic stability, due to the reduced pH value, and results in the characteristic taste of the final product. Meat proteins coagulate at low pH and water holding capacity is decreased, which aids the drying process, affecting texture and firmness of the end product, as well as leading to the typical red colour of salami by favouring the reaction of nitrogen monoxide with myoglobin (pH 5.4 to 5.5).

Similar growth patterns were recorded for *L. plantarum* 423, *L. plantarum* 423m and *L. curvatus* DF38 in MRS broth (Merck) at 30^{o}C (Fig. 1). All three strains started going into stationary growth after approximately 12 hours. Production of plantaricin 423 and curvacin DF38 (400 and 200 AU/ml, respectively) was recorded after three hours (Fig. 1). After 12 hours, the bacteriocin activity of the latter two strains had increased to 3 200 and 1 600 AU/ml respectively (Fig. 1).

The culture pH of all three strains decreased from 6.3 to 3.6 during the first 12 hours of fermentation (Fig. 1). Similar results for bacterial growth, bacteriocin production and changes in pH have been reported for the bacteriocin-producers *Enterococcus faecium* RZS C5 (Leroy & De Vuyst, 2002), *L. plantarum* ST13BR (Todorov & Dicks, 2004), and *L. pentosus* ST151BR (Todorov & Dicks, 2004).

The activity of plantaricin 423 increased from 400 to 3200 AU/ml, as the pH decreased from 6.3 to 4.2 (Fig. 1). This is most probably due to a less stringent binding of the bacteriocin to the cell

wall at low pH (Verellen *et al*., 1998). Although the activity of curvacin DF38 also increased with the decrease in pH with time, this activity was lower than that of plantaricin 423.

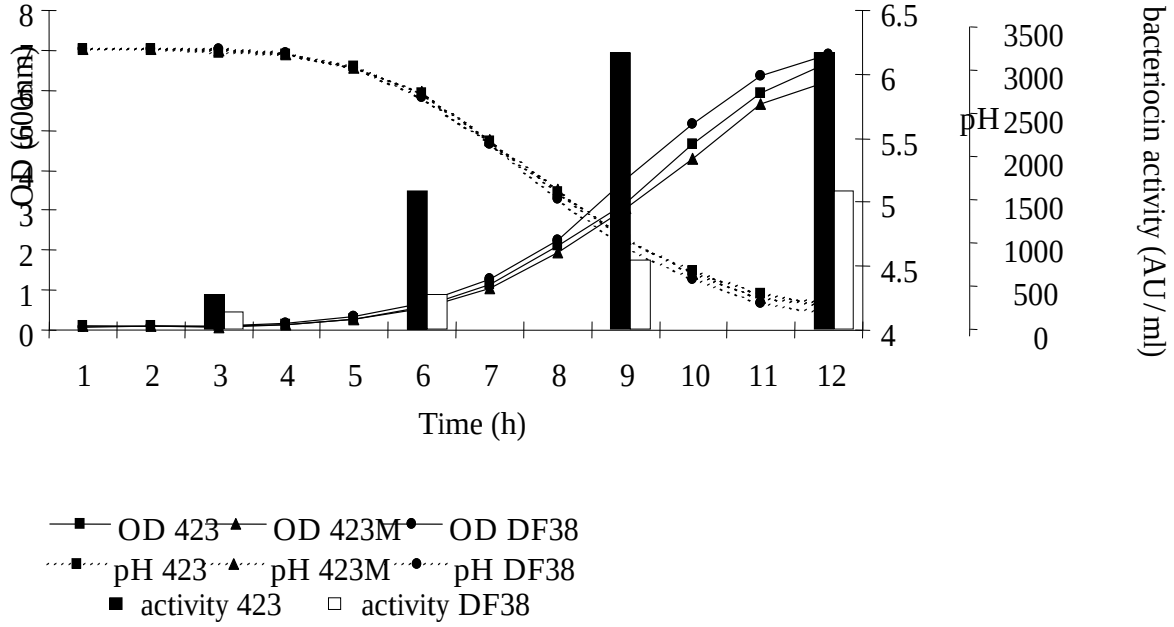

Figure 1. The growth of *L. plantarum 423, L. plantarum 423m* and *L. curvatus DF 38* under non-regulated pH, as well as the production of bacteriocin 423 and curvacin DF 38

6b.4.2. Microbiology

The total microbial count of *L. plantarum* 423 bacteria present in the mutton salami remained stable throughout the fermentation process whereas that of the horse salami, which had much lower values to begin with, increased greatly during smoking, whereafter it stabilised. Beef salami showed a decrease in starter culture cfu over the first five days, but then increased and stabilised. Springbok and blesbok showed little variation and stabilised rapidly. Similar results were seen using *L. curvatus* DF38.

A similar effect was observed by Skytta *et al*. (1991) in ground meat using a supernatant containing bacteriocin from a *Pediococcus damnosus* culture. The bacteriocin inhibited the growth of *Listera monocytogenes* for up to two weeks. A bacteriocin produced by a meat strain of *Lactobacillus sakei* inhibited the growth of *Listeria monocytogenes* in a pork meat product (De Martinis & Franco, 1997). Fiorentini *et al*. (2001) monitored the influence of a bacteriocin produced by *Lactobacillus plantarum* BN on the shelf life of refrigerated bovine meat. The latter

bacteriocin was also found to be effective against several pathogens in culture media (Skytta *et al*., 1991; De Martinis & Franco, 1997; Fiorentini *et al*., 2001).

The number of viable cells recorded for the three starter cultures are shown in Figs. 2a, b and c. Batch II (Fig. 2a) revealed a different pattern from that recorded for batches I and III (Figs. 2b and c) in that the cell numbers declined over the first three days, followed by a rapid increase and stabilisation after day eight. Batch I showed an increase in cell numbers, especially in the blesbok salami on day 5, followed by stabilisation after day 12 (Fig. 2b). Batch III did not show a drastic increase in cell numbers, although the cell numbers in the blesbok salami increased after the third day. The other species did not show much of an increase and remained between 6.0 x 10^6 and 8.0 x 10^6 cfu/g.

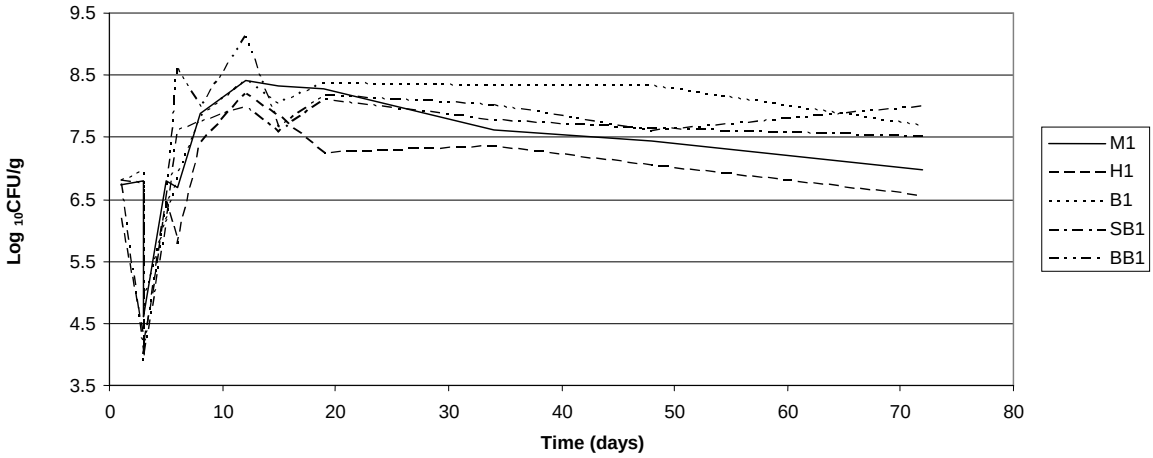

Figure 2a. Dynamics of the microbial population in salami during fermentation and maturation, using starter culture II, *Lactobacillus plantarum* 423

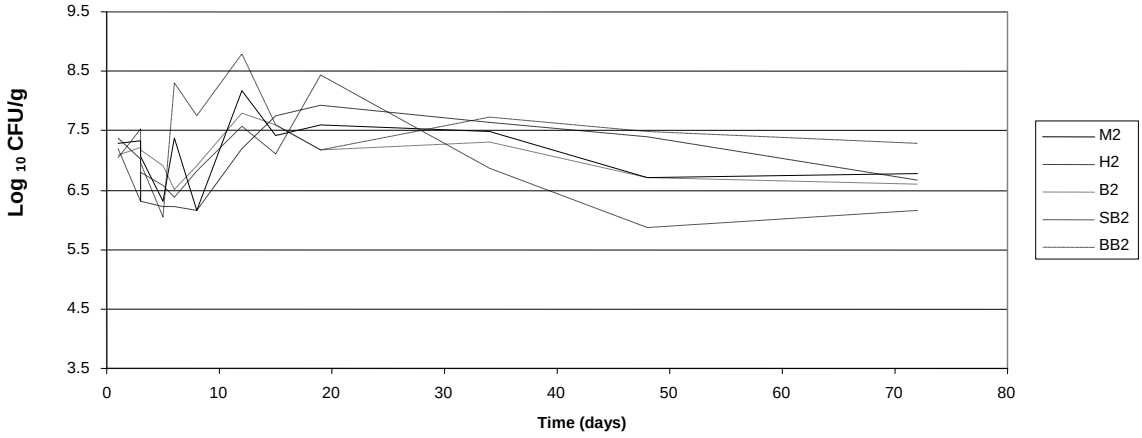

Figure 2b. Dynamics of the microbial population in salami during fermentation and maturation, using starter culture I, *Lactobacillus curvates* DF38

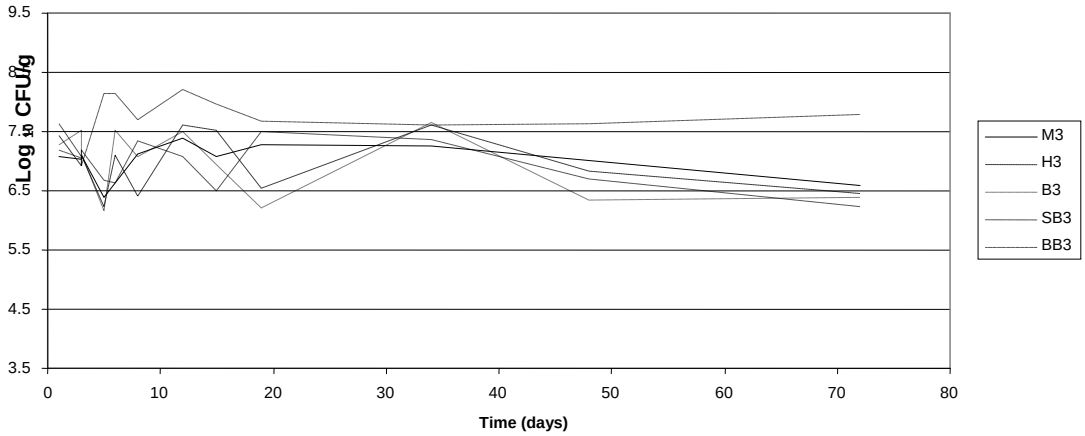

Figure 2c. Dynamics of the microbial population in salami during fermentation and maturation, using starter culture III, *Lactobacillus plantarum* 423m

The percentage of *L. plantarum* 423, compared with the total population of microflora in mutton salami, remained almost the same (80-95% variation) during the entire fermentation and maturation process. In horse salami, *L. plantarum* 423 was present at relatively low cell numbers (55-50% on day 1 and before smoking), but increased to 70% after smoking and stabilised to 70-80% for the remaining fermentation period. In beef salami, strain 423 decreased slightly during the first five days (from 95 to 70%), followed by an increase to 90%. In springbok salami, strain 423 remained fairly stable at 80-90%. In blesbok salami, strain 423 slowly decreased during the first three days from 88% to 70%, increased to 92% after 12 days and stabilised for the rest of the fermentation period (Fig. 3a).

Similar results were recorded for *L. curvatus* DF38. The cell numbers of strain DF38 remained stable in all five salami types (between 75 and 95%). A slow decrease in cell numbers was recorded in mutton, beef and springbok salami at 48 days (68, 70 and 80% respectively), followed by stabilisation (Fig. 3b).

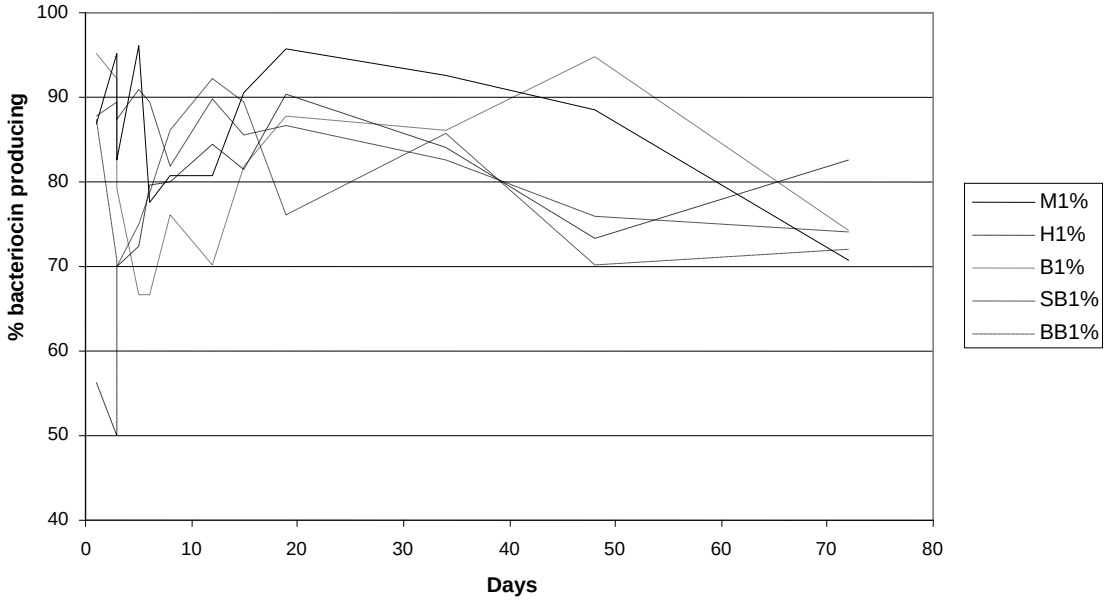

Figure 3a. Percentage of the bacteriocin produced against *L. inocua* F as compared with the total lactic acid microflora in batch II using starter culture *Lactobacillus plantarum* 423

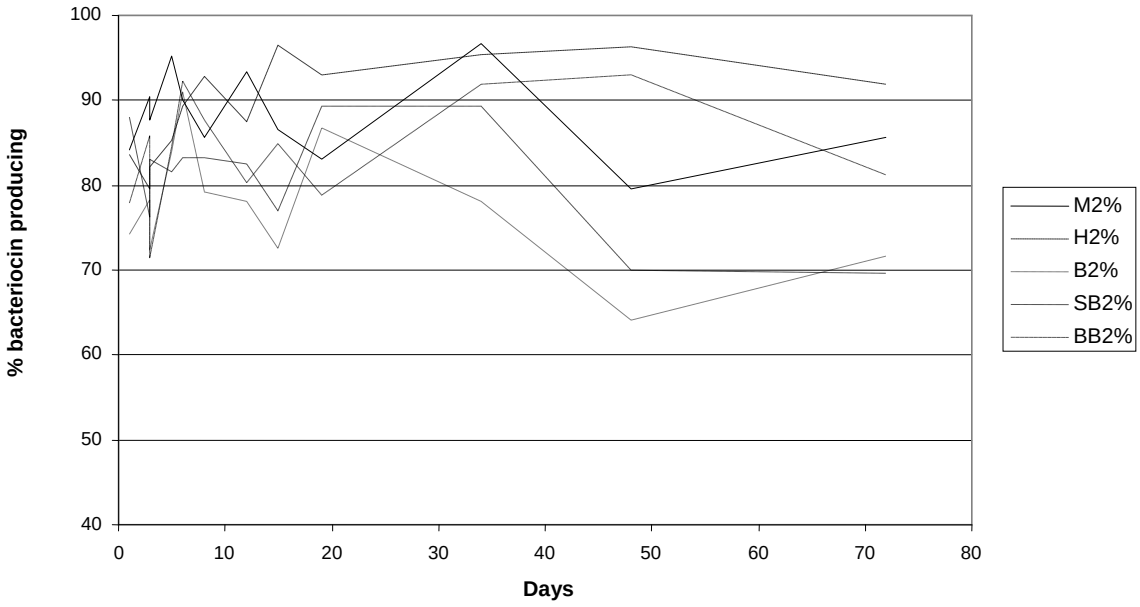

Figure 3b. Percentage of the bacteriocin produced against *L. inocua* F as compared with the total lactic acid microflora in batch I using starter culture *Lactobacillus curvates* DF38

Survival of *Listeria innocua* F in the different types of salami is indicated in Figs. 4a, b and c. Mutton, beef, springbok and blesbok salami fermented with *L. plantarum* 423 inhibited the growth of *Listeria innocua* F. A difference in one log cycle was recorded between the latter salami and a control sample (no bacteriocin present). Results recorded for horse salami did not show a reduction in viable cell numbers of *Listeria innocua* F, when compared with the control sample.

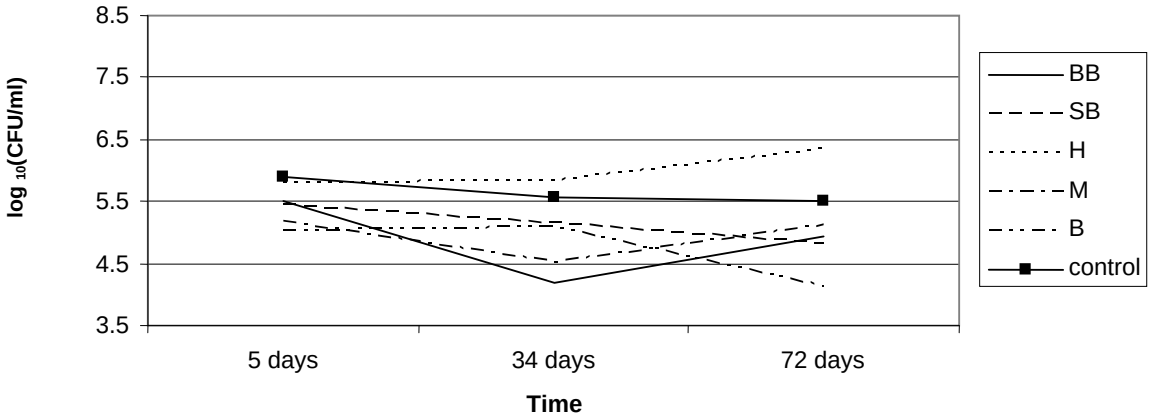

Figure 4a. Survival of *Listeria inocua* F in salami of five species using starter culture *Lactobacillus plantarum* 423 (batch II)

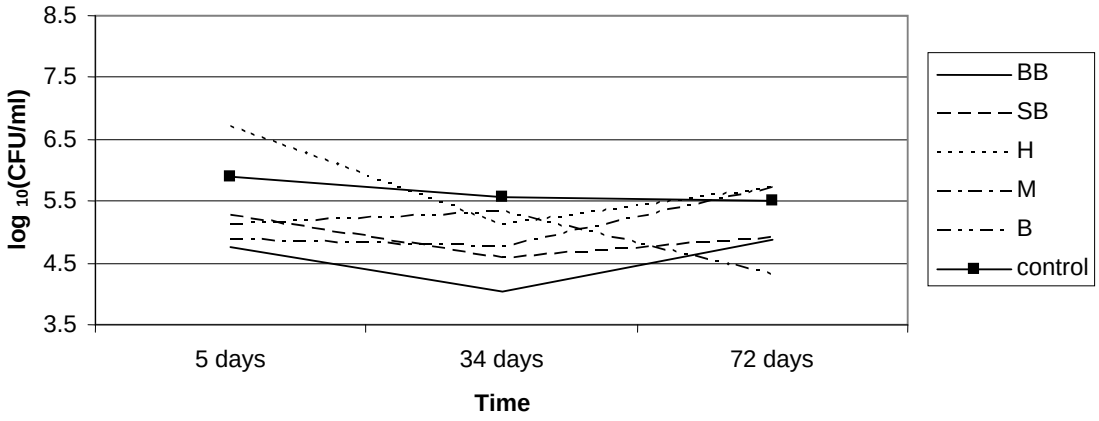

Figure 4b. Survival of *Listeria inocua* F in salami of five species using starter culture *Lactobacillus curvates* DF38 (batch I)

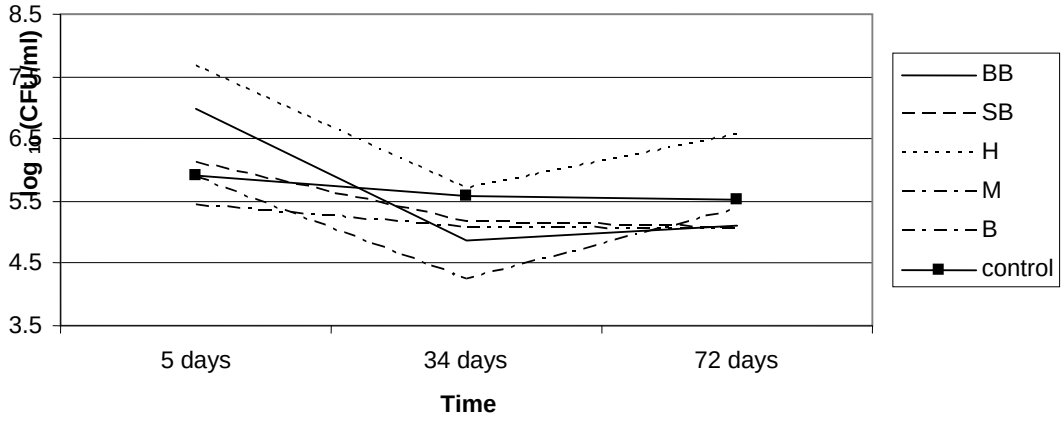

Figure 4c. Survival of *Listeria inocua* F in salami of five species using starter culture *Lactobacillus plantarum* 423m (batch III)

Care should be taken with the incorporation of bacteriocins in meat, since intrinsic characteristics may influence the activity of the bacteriocin. It has been shown that bacteriocins may be inactivated by adsorption to meat and lipid particles (De Martinis & Franco, 1997).

In sausages fermented by bacteriocin-producing *P. acidilactici* JDI-23, *P. acidilactici* PAC 1.0 and *L. plantarum* MSC, the numbers *of Listeria monocytogenes* per gram dry sausages (pH > 5.0) were 1-2 log units lower than in the control sausages (Berry *et al*., 1990; Campanini *et al*., 1993; Foegeding *et al*., 1992). Työppönen *et al*. (2003) reported that *L. monocytogenes* could be reduced by 3 log cfu/g.

However, a reduction of *Listeria innocua* F growth in salami produced with *L. plantarum* 423m suggests that inhibition was not only due to the presence of bacteriocins, but that it is a more complex process, which may include lowering of pH. More research is required into the actual functioning of different organisms when exposed to factors such as smoke, temperature and relative humidity.

Treatment of cell suspensions of *L. plantarum* 423, *L. plantarum* 423m and *L. curvatus* DF38 with smoke yielded a reduction of one log cycle. The controls (without treatment or treated with sterile-filtered air) showed no decrease in bacterial count. The reduction in total bacterial cell numbers after smoking was also one log (Fig. 5). This antimicrobial activity of smoke as been reported previously (Price & Schweigert, 1987).

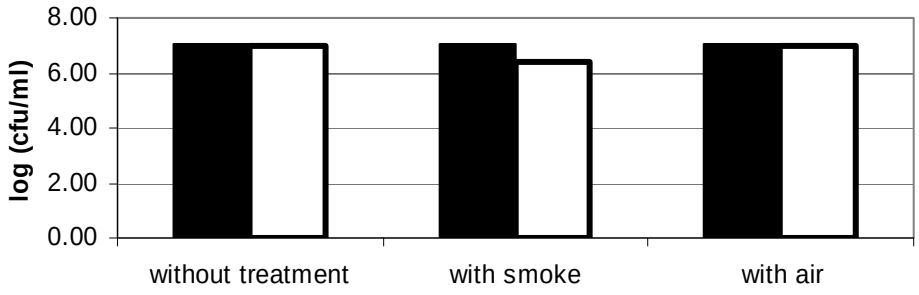

Figure 5a. Dynamics of *Lactobacillus plantarum 423* in the presence of smoke produced by wood chips. The control was untreated and aerated with a 0.22μm filter (solid block = at 0 hours, and open block = at 1 hour).

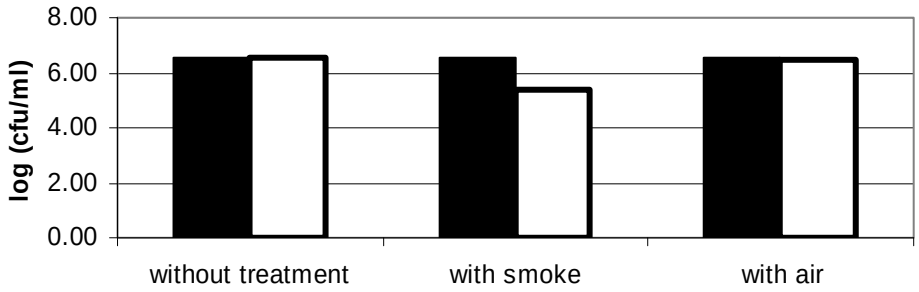

Figure 5b. Dynamics of *Lactobacillus curvatus DF 38* in the presence of smoke produced by wood chips. The control was untreated and aerated with a 0.22μm filter (solid block = at 0 hours, and open block = at 1 hour).

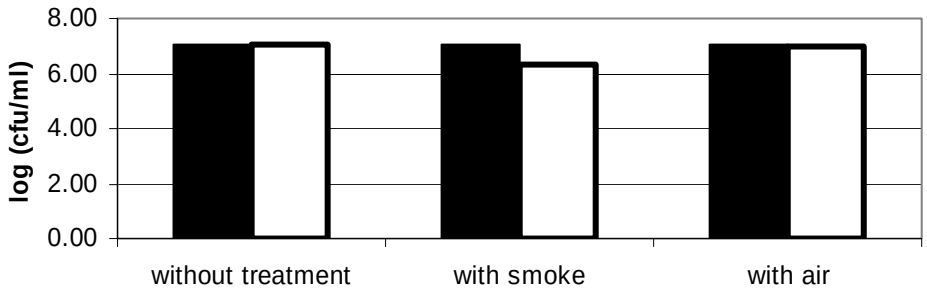

Figure 5c. Dynamics of *Lactobacillus plantarum 423m* in the presence of smoke produced by wood chips. The control was untreated and aerated with a 0.22μm filter (solid block = at 0 hours, and open block = at 1 hour).

6b.5. Conclusion

From this trial it can be seen that the production of salami using these starter cultures is feasible. All three starter cultures yielded similar acceptable results comparable to those of salami produced elsewhere and cited in literature, for instance in the use of ostrich meat and similar starter cultures (Dicks *et al*., 2004). All species used in this trial are acceptable in terms of suitability, with only the blesbok salami meat showing differences when compared to the other species. This might be due to the different chemical composition of this species (see Chapter 6a). Therefore, the manufacture of a product such as salami would satisfy a year-round demand for meat from these species seems viable.

However, more research is needed to determine the exact functioning of the reduction of *Listeria innocua* F growth in salami produced with *L. plantarum* 423m.

6b.6. Acknowledgements

I would hereby like to thank Mr Todorov of the Department Microbiology at the University of Stellenbosch for his assistance in executing the necessary microbiological tests required for this investigation.

6b.7. References

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Chapter 6

Production of salami from beef, mutton, horse, blesbok and springbok
c) Sensory evaluation

6c.1. Abstract

Fifteen batches of traditional salami, comprising five different species: horse, beef, mutton, blesbok and springbok, and using three different starter cultures: *Lactobacillus curvatus* DF38, (batch I), active bacteriocin producing *Lactobacillus plantarum* 423 (batch II) and then a mutant variation of *Lactobacillus plantarum* 423m, which did not produce the bacteriocin (batch III), were made. From analytical sensory evaluations it was concluded that the salami prepared using starter culture resulted in end products with lower sensory qualities. These samples were rated significantly lower ($P \leq 0.05$) for salami aroma, salami flavour and compact structure, and significantly higher for sour meat aroma, sour meat flavour and oily mouth feel, than samples prepared using starter cultures II and III. Salami prepared using horse, beef and springbok meat gave samples with significantly higher mean values for salami aroma and salami flavour, and significantly lower values($P \leq 0.05$) for sour meat aroma, sour meat flavour, venisonlike flavour and muttonlike flavour, whilst salami prepared using blesbok and mutton also resulted in end products with lower sensory qualities and was perceived as significantly lower in salami flavour ($P \leq 0.05$) and higher in venisonlike and muttonlike flavour respectively. The blesbok samples were rated significantly higher ($P \leq 0.05$) in sour meat aroma, sour meat flavour and venisonlike flavour than the rest of the samples. Consumer (n=97) sensory analysis of the salami was tested for acceptability and degree of liking using a nine-point hedonic scale. The colours of the springbok and mutton salami were rated significantly ($P \leq 0.05$) higher than those of the beef and horse salami. The blesbok salami was rated significantly the lowest for colour compared to the rest of the samples. The tastes of the springbok and horse salami were significantly ($P \leq 0.05$) more acceptable than those of the beef and blesbok salami.

Keywords*:* consumer panel, fermented sausage, alternative red meat species, game meat, sensory evaluation

6c.2. Introduction

As the harvesting of game animals such as blesbok and springbok is a seasonal occurrence, products need to be developed that will provide a year-round supply of the meat. Salami is a meat product with a long shelf life. Salami can be defined as a mixture of meat and fat particles, NaCl, curing agents, and spices stuffed into either artificial or natural casings. Starter cultures are often used to aid in the fermentation and maturation of the sausage.

Smoke is applied to many types of semi-dry sausage during the drying stage. Smoke application to meat products affects the product with regards to flavour, colour, antimicrobial, and antioxidant properties (Price & Schweigert, 1987). Although the historical purpose of smoking meat was as a preservative, at present the importance of smoke is to rather provide flavour and colour to the meat and meat products. Smoke is also applied to inhibit mould growth, by drying the surface and by the deposition of antimicrobial phenols, carbonyls and low molecular weight organic acids. Phenolic compounds decrease the extent of fat oxidation Smoking also affects the organoleptic qualities of the sausage. The pyrolysis of cellulose and hemicellulose in the casing of the fermenting sausage produces carbonyls (Price & Schweigert, 1987). These are important in the development of colour of the meat when smoked, as the carbonyls are absorbed into the surface of the sausage. The reaction between carbonyls and amino groups is similar to the Maillard reaction, and is enhanced by the increase in temperature and dryness of the product (Gilbert & Knowles, 1975).

After drying of the salami, the final product may contain up to 50% fat, which makes fat an important ingredient of fermented sausage. This can lead to problems such as rancidity, which has a direct effect on the shelf life of the product. There are many substances that can be added to act as anti-oxidants, one of which is ascorbic acid, which may also act as a curing agent and in stabilising the colour.

The most common culture starter species used in the fermentation of beef and pork sausages are strains of *Lactobacillus, Pediococcus*, non-pathogenic S*taphylococcus* and *Micrococcus* (Lücke, 1986). The organic acids produced by these bacteria prevent the development of pathogenic micro-organisms (Schillinger & Lücke, 1987), aid the cohesion of meat particles (Townsend *et al*., 1980) and contribute to the colour change of fermented sausages. The major role of lactic acid bacteria in the sausage is to produce organic acids, primarily lactic acid, from carbohydrates (Campbell-Platt, 1987). This results in a decrease in pH and contributes to the retardation of the development of undesirable micro-organisms.

Economic and social changes in civilisations have led to nutritional demands being transformed and modified. It is becoming more acceptable to purchase alternative sources of red meat as opposed to that of only the traditional species of meat-producing animals. Nowadays, there is an ever-increasing demand by consumers for foods perceived as natural, fresh-tasting, healthy and more nutritious, which is reflected in the results of a pan-European Union (EU) survey. This study revealed that the five most important factors influencing consumer food choice were: quality/freshness, price, taste, trying to eat healthy and family preferences (Lennarnäs *et al*., 1997). One of the main factors that limit the quality and acceptability of meat and meat products is lipid oxidation.

Increasing consumer interest in meat products (salami) containing non-traditional species such as horse, beef, mutton and certain species of game animals (Blesbok and Springbok) motivated this investigation. The latter two species are not being farmed intensively and are therefore considered as wild game animals. Comparisons need to be drawn between the suitability of these species and the conventional red meat species such as beef and mutton in the production of traditional products such as salami.

6c.3. Materials and methods

The sourcing and processing of the salami prepared for this study are discussed in detail in Chapter 6a and b.

6c.3.1. Sensory analysis by a trained panel

Descriptive sensory analyses were performed on the fifteen samples (AMSA, 1995). A trained, six-member panel evaluated the meat in nine sessions for the following sensory attributes: salami aroma, sour meat aroma, colour, texture, salami flavour, venisonlike flavour, muttonlike flavour, sour meat flavour and oily mouthfeel, and structure, by means of an unstructured 100mm line scale. Table 1 depicts the definitions of the attributes used in the sensory analysis. Roers Italian salami was used as the standard – Roers being the commercial company where the salami for this investigation were smoked and fermented, as well as being the suppliers of the spices used. The panelists were seated in individual booths in a temperature- and light-controlled room, receiving a set of five samples served in a complete randomised order. The samples were served at room temperature on a plate covered with a lid. Crackers, apple slices and distilled water were used to cleanse the palate between samplings.

Table 1

Verbal definitions of sensory attributes evaluated on the salami

Attribute	Verbal definition
Salami aroma	Take a few short sniffs as soon as you remove the lid 0 = no salami aroma, 100 = strong salami aroma
Sour meat aroma	Aroma associated with meat where excessive amount of lactic acid has been produced by bacteria 0 = no sour meat aroma, 100 = strong sour meat aroma
Colour	The colour perceived in comparison with the standard (Roers Italian salami) 0 = light salami red colour, 100 = dark salami red colour
Texture	Evaluate the size of the fat particles by looking at the samples 0 = fine, 100 = coarse
Salami flavour	Taste the samples and evaluate the typical salami flavour 0 = no salami flavour, 100 = strong salami flavour
Venisonlike flavour	Taste the samples and evaluate the flavour as associated with venison 0 = no venisonlike flavour, 100 = prominent venisonlike flavour
Muttonlike flavour	Taste the samples and evaluate the flavour as associated with mutton 0 = no muttonlike flavour, 100 = prominent muttonlike flavour
Sour meat flavour	Taste the samples and evaluate the sour meat flavour as associated with meat where an excessive amount of lactic acid has been produced 0 = no sour meat flavour, 100 = prominent sour meat flavour
Oily mouthfeel	Taste the samples and detect the oiliness as associated with liquid oil after 5 seconds 0 = no oily mouthfeel, 100 = prominent oily mouthfeel
Structure	Taste the samples and evaluate the compactness 0 = Soft, open structure, 100 = solid, compact structure

6c.3.2. Statistical analysis

The experiment consisted of a 3x5 factorial design, with three starter cultures and five animal species as factors. The samples were evaluated in nine sessions (three sessions per starter culture) by six trained panelists, controlling individual starter cultures per session. The data were pooled to test for the main effects of starter culture and animal species. Analysis of variance was performed on the data. The Shapiro-Wilk test was performed to test for non-normality (Shapiro & Wilk, 1965). In some cases, deviations from normality were the cause of one or two outliners, which were removed before the final analysis. Student's t-Least Significant Difference (LSD) was calculated at the 5% significance level to compare treatment means (SAS, 1990).

6c.3.3. Sensory analysis by consumer panel

Consumer sensory analysis was performed on the five samples of salami prepared, using all five species, but using only starter culture II (*Lactobacillus plantarum 423*). A nine-point hedonic scale was used to test for degree of liking (AMSA, 1995). The questionnaire was completed by 97 target consumers (69% female; 31% male) between the ages of 18 and 55. Consumers were

asked to indicate their preference on the colour and the taste of the five samples of salami presented to them. The samples were served at room temperature in glass ramekins coded with a random three-digit code. The five samples were served in a completely randomised order. Due to ethical considerations, the consumers were informed that one of the samples was produced using horsemeat (this species is not readily consumed in South Africa).

The data was subjected to ranks before analysis of variance was performed. The Tukey's Studentized Range (LSD) Test was used to test for significant differences between treatments at the 5% level (SAS, 1990).

6c.4. Results and discussion

As the salamis were all produced under the same environmental conditions, it was assumed that any differences were due to the main effects of starter culture and species. Fermented meat products such as salami have a flavour which is product-specific i.e. derived from the different ingredients used in the production thereof. The formation of aroma and taste is based on enzymatic, as well as non-enzymatic, reactions, both contributing to the wealth of components which all together make up the characteristic flavour of a fermented meat product (Dainty & Blom, 1995). The flavour is composed of volatiles with odour properties and non-volatiles with taste and tactile properties, together with enhancers and synergists (Dwivedi, 1975).

Apart from the contribution of spices employed, flavour components are derived from protein, fat and carbohydrate breakdown, a process dependent on endo- as well as exogenous enzyme activities. Endogenous enzyme activities, together with the lactic acid produced by the starter culture, are believed to be the most important factors in the development of the fermented meat flavour (Blom *et al.*, 1996). However, the contribution from non-lactic acid bacteria both to colour and flavour formation is recognised, as demonstrated by the abundant use of non-acid-producing starter cultures of the family *Micrococcacae* (Hammes & Knauf, 1994).

Salami has a specific image among consumers, related to its sensory attributes (macroscopic appearance, texture, colour, flavour, taste, etc.). Its quality depends on the quality of the raw materials used, as well as the technology of production (Meynier *et al*., 1999).

Formation of heme-containing pigments in fermented sausages follows the same pathways as in other nitrite-containing meat products. However, the low pH-value has a strong influence, initially destabilising myoglobin and increasing the rate of auto-oxidation to metmyoglobin

(Slinde & Nordal, 1978). The heme group is dissociated at the pH value of many fermented sausages and colour is primarily attributed to nitrosomyoglobin (Slinde & Nordal, 1978).

6c.4.1. Sensory analysis by a trained panel

When comparing the starter cultures, significant differences ($P \leq 0.05$) were perceived for the following attributes: salami aroma, sour meat aroma, salami flavour, sour meat flavour, oily mouthfeel and structure. No significant difference ($P > 0.05$) between the three starter cultures was found for the attributes colour and venisonlike flavour. Mean values for the attributes evaluated are given in Table 2.

Table 2

Mean values for the sensory attributes evaluated by starter culture using Tukey's LSD (least significant difference) (data pooled for species)

Attribute	Starter culture			Tukey's LSD (5%)
	I	II	III	
Salami aroma	56.09[b]	64.06[a]	67.73[a]	3.914
Sour meat aroma	10.51[a]	4.25[b]	3.39[b]	2.698
Colour	71.68	70.40	70.60	2.004
Texture	63.94[a]	63.35[a]	58.80[b]	3.844
Salami flavour	53.40[b]	60.98[a]	63.31[a]	3.614
Venisonlike flavour	19.99	17.61	14.93	5.396
Muttonlike flavour	9.82[a]	6.52[b]	8.98[ab]	2.460
Sour meat flavour	8.49[a]	5.47[b]	4.49[b]	2.341
Oily mouthfeel	21.03[a]	15.42[b]	16.07[b]	2.804
Structure	59.22[b]	65.91[a]	67.02[a]	4.367

[a-d] values in the same row with different superscripts differ significantly ($P \leq 0.05$)

The horse salami had the highest mean value for colour, significantly higher than the rest of the samples. This indicated that the horse salami was perceived as being the darkest, while the mutton salami was rated the lowest ($P \leq 0.05$), indicating a light red salami colour. The mean values for the colour of the blesbok and springbok samples were higher than that of the beef sample, indicating that these samples were perceived to be darker than the beef sample. This is correlated to the colour measured in Chapter 6a, where it was measured mechanically, not visually. Thus, it can be assumed that horse and the game species have meat which is higher in myoglobin than that of beef and mutton. This feature could be attributed to the levels of activity the specific animals have experienced in their lives. Animals bred intensively will have lower

levels of activity than wild animals such as game, or sport animals such as a horse. This would influence the muscle type and influence colour, as higher activity levels will require the muscle to be able to carry more oxygen, thus pigment, and, therefore, appear darker.

The textures of the blesbok and beef samples were perceived as being significantly coarser than the rest of the samples, while the mutton salami had the lowest mean value for texture, indicating a finer product ($P \leq 0.05$). This could be due to the fact that mutton has a higher fat content (Chapter 6a) than the other species used, and, therefore, its texture would be smoother and less coarse. The duration of cutting of the raw materials during processing can also influence the size of the meat and fat particles, as length of cutting will influence the size. When evaluating texture, panelists were asked to look at the particle size of the fat in the salami. It was noted that the fat particles in the mutton salami were much smaller and finer than in for instance the beef salami, which was perceived to have a coarser texture. This would also correlate with the chemical proximate composition of the raw meat used and its fat content, which would either facilitate or make it more difficult to reach a specific particle size in the same time, i.e. the higher the fat content in the raw meat, the less resistance there would be during the cutting process, and the particle size would be reduced.

The springbok, horse and beef samples were rated significantly higher ($P \leq 0.05$) for salami flavour than the blesbok and mutton samples were. The blesbok salami was rated significantly higher for venisonlike flavour, as was the mutton sample rated significantly higher for muttonlike flavour than the rest of the samples ($P \leq 0.05$). Consequently, samples which were perceived as being more venisonlike or muttonlike, were rated lower for salami flavour. As salami contains such high amounts of pork fat, it can be assumed that the taste of the fat would dilute the species specific tastes and result in a more evenly distributed taste among the five different species used. As these results show, this was not the case, and definite differences were perceived as to venisonlike and muttonlike flavour, which would have an influence on consumer perception and acceptance of these products.

The blesbok sample had the highest mean value for sour meat flavour, significantly higher than the rest of the samples ($P \leq 0.05$). The beef and horse samples were rated lowest for this attribute ($P \leq 0.05$). The sour meat flavour could be attributed to degree of rancidity in the salami. Aldehydes are often reported to be responsible for the rancid odours of foods (Asghar *et al.*, 1988). Rancidity is also sometimes attributed to 2-methylbutanal (Arctander, 1994b). This compound arises from amino acid catabolism. As the blesbok salami had the highest significant rating for venisonlike flavour, as well as sour meat flavour, there could be a correlation between

the two. As the blesbok had a high protein content in the meat (Chapter 6a), there would be more amino acid catabolism, and thus more 2-methylbutanal produced to give rise to rancidity (Arctander, 1994b).

The panel perceived the horse salami to be significantly oilier than the other samples ($P \leq 0.05$), while the springbok sample was also rated higher for oily mouthfeel than the mutton, blesbok and beef samples. The latter three samples did not differ significantly for this attribute ($P > 0.05$). The beef salami was perceived as being solid and compact, significantly more so than the rest of the samples ($P \leq 0.05$). The horse salami had the lowest mean value for this attribute, indicating that the panel perceived this sample as being less compact and more soft and open. As seen in Chapter 6, the salami containing horse meat did show a different pattern in pH as well as a water activity decline, over the maturation period. This could result in the texture being less compact, and more open and soft. These characteristics can be directly correlated to the fatty acid composition, especially the SFA and UFA components of the different meats types (Chapter 6a). The higher the SFA content, for instance in beef, the less fluid these fats would be compared to those fats are less saturated at the same temperature, resulting in a more open, soft structure. When comparing the means of the attributes of the pooled data (Table 2), it was observed that starter culture I (*L. curvatus*) was rated significantly lower ($P \leq 0.05$) for salami aroma, salami flavour and compact structure and significantly higher ($P \leq 0.05$) for sour meat aroma, sour meat flavour and oily mouthfeel than starter cultures II and III (*L. plantarum* 423 and 423m). The same starter culture also showed different rates of change when observing pH and water activity decline (Chapter 6a and b). Therefore, it can be assumed that the different bacterial action of this starter culture did have an influence on the sensory characteristics of the salami as well.

Table 3

Mean values for the sensory attributes evaluated by species using Tukey's LSD (least significant difference) (data pooled for starter culture)

Attribute	Animal species					Tukey's LSD
	Beef	Blesbok	Horse	Mutton	Springbok	(5%)
Salami aroma	61.54^{b}	63.65^{ab}	67.17^{a}	55.04^{c}	65.48^{ab}	5.054
Sour meat aroma	3.21^{c}	8.90^{a}	5.28^{bc}	5.20^{bc}	7.71^{ab}	3.482
Colour	64.74^{c}	74.02^{b}	83.28^{a}	58.44^{d}	73.54^{b}	2.588
Texture	73.86^{a}	74.40^{a}	57.79^{b}	45.40^{c}	58.60^{b}	4.962
Salami flavour	63.19^{a}	53.43^{b}	64.17^{a}	49.26^{b}	66.26^{a}	4.666
Venisonlike flavour	15.74^{b}	31.53^{a}	10.50^{b}	15.25^{b}	14.49^{b}	6.966
Muttonlike flavour	3.46^{b}	4.20^{b}	1.17^{b}	36.30^{a}	1.54^{b}	3.184
Sour meat flavour	3.42^{c}	12.33^{a}	3.30^{c}	7.23^{b}	4.34^{bc}	3.023
Oily mouthfeel	12.89^{c}	14.11^{c}	27.51^{a}	14.17^{c}	19.14^{b}	3.621
Structure	75.96^{a}	65.96^{bc}	44.28^{d}	69.85^{b}	64.14^{c}	5.639

$^{a-d}$ values in the same row with different superscripts differ significantly ($P \leq 0.05$)

The mean values for the attributes evaluated (between species) are provided in Table 3. The blesbok salami was rated the highest for sour meat aroma, while the beef sample was rated the lowest. These differences were significant ($P \leq 0.05$). No significant difference in sour meat aroma was found between the springbok, horse and mutton salami ($P > 0.05$).

6c.4.2. Sensory analysis by consumer panel

When interpreting the colour of the salami, no correlation between the results of the analytical sensory analysis and the consumer sensory analysis could be found. The analytical panel found the colour of the horse salami to be significantly darker ($P \leq 0.05$) than the rest of the samples and the mutton salami to be significantly lighter ($P \leq 0.05$) than the rest of the samples. This could be attributed to the fact that the horse is not intensively farmed, whereas the mutton was probably from a feedlot. When measured using a colour meter, the same trend was observed between the different species of raw meat samples, as well as the salami produced from each one (Chapter 6a).

Significant differences ($P \leq 0.05$) in the acceptability of the colour of the five samples of salami were found. Consumers found the colour of the springbok and mutton salami to be significantly ($P \leq 0.05$) more acceptable than that of the beef and horse salami, which was again rated significantly ($P \leq 0.05$) higher than the blesbok salami. Table 4 indicates the rank means for the different salamis.

The mean values for the colour of the blesbok and springbok samples were higher than that of the beef sample, indicating that these samples were perceived to be darker than the beef meat. The absence of any correlation between the colour perception of the analytical panel and the consumer panel could indicate that the consumers not only evaluated the colour of the product, but also looked at the size and distribution of the fat particles. As the beef salami had higher L* values (Chapter 6a) than blesbok and springbok, this observation could be substantiated. However, as beef had a higher SFA content than springbok and blesbok, the fat contained in the actual meat component of the salami would be firmer than that of the other two species, and therefore less oily and lighter in colour to the eye of a consumer.

Table 4

Degree of liking of the five species of salami with regards to colour as well as taste as evaluated by the consumer

Treatment	Rank means (with actual means) for total group of consumers			
	Colour		Taste	
Springbok	3.58[a]	(7.23)[a]	3.75[a]	(7.06)[a]
Mutton	3,48[a]	(6.98)[a b]	3.19[b]	(6.37)[a]
Beef	2.93[b]	(6.32)[b c]	2.49[c]	(5.31)[b]
Horse	2.77[b]	(5.80)[c d]	3.57[a b]	(6.89)[a]
Blesbok	2.20[c]	(5.22)[d]	1.97[c]	(4.73)[b]
LSD*	0.54	(0.71)	0.52	(0.75)

*LSD Least significant difference at the 5% level of significance
a-d Values in the same column with different superscripts differ significantly (P≤0.05)

The frequency scores in Tables 5 and 6 and Figs. 1 and 2 give an indication of the distribution of preferences over the nine classes of the hedonic scale. According to Table 5, the colour of the springbok salami was described as “Like moderately”, “Like very much” and “Like extremely” by 75.3% of the total group of consumers, while 72.2% of the consumers used the same positive classes to describe their opinion of the taste of the springbok salami (Table 6).

Table 5
Distribution of preference for colour of the salami of the total group of consumers (N=97)

Hedonic classes	Springbok %	Mutton %	Beef %	Horse %	Blesbok %
Dislike extremely (1)	1.03	0.00	1.03	2.08	6.25
Dislike very much (2)	0.00	0.00	3.09	11.46	9.38
Dislike moderately (3)	3.09	4.17	4.12	7.29	8.33
Dislike slightly (4)	3.09	5.21	9.28	14.58	14.58
Neither like nor dislike (5)	6.19	7.29	9.28	7.29	11.46
Like slightly (6)	11.34	15.63	18.56	8.33	16.67
Like moderately (7)	21.65	21.88	27.84	13.54	13.54
Like very much (8)	34.02	31.25	18.56	25.00	16.67
Like extremely (9)	19.59	14.58	8.25	10.42	3.13

A significant number of consumers found the taste of the springbok and horse salami to be more acceptable than that of the beef and blesbok salami. The results did not show a significant difference between preference for horse and mutton salami. When an analytical panel evaluated the same samples, the springbok, horse and beef samples were rated significantly higher (P≤0.05) for salami flavour than the blesbok and mutton salami. The analytical panel rated the blesbok sample significantly higher (P≤0.05) for venisonlike flavour and for sour meat flavour than the rest of the samples (Table 6).

Table 6

Distribution of preference for taste of the salami of the total group of consumers (N=97)

Hedonic classes	Springbok %	Mutton %	Beef %	Horse %	Blesbok %
Dislike extremely (1)	0.00	1.03	5.15	0.00	7.29
Dislike very much (2)	6.19	7.22	8.25	3.09	14.58
Dislike moderately (3)	0.00	5.15	10.31	3.09	10.42
Dislike slightly (4)	8.25	6.19	19.59	8.25	14.58
Neither like nor dislike (5)	5.15	5.15	4.12	4.12	10.42
Like slightly (6)	8.25	13.40	14.43	14.43	15.63
Like moderately (7)	14.43	28.87	19.59	19.59	17.71
Like very much (8)	35.05	23.71	11.34	30.93	7.29
Like extremely (9)	22.68	9.28	7.22	16.49	2.08

Figs. 1 and 2 indicate that the preference of the consumers was distributed over all nine classes of the hedonic scale when evaluating the colour and taste of the blesbok salami. This shows that 38.6% of the 97 consumers tested, had a negative opinion of the colour (Fig. 1) of the blesbok salami and 46.9% a negative attitude towards the taste (Fig. 2) of the blesbok salami.

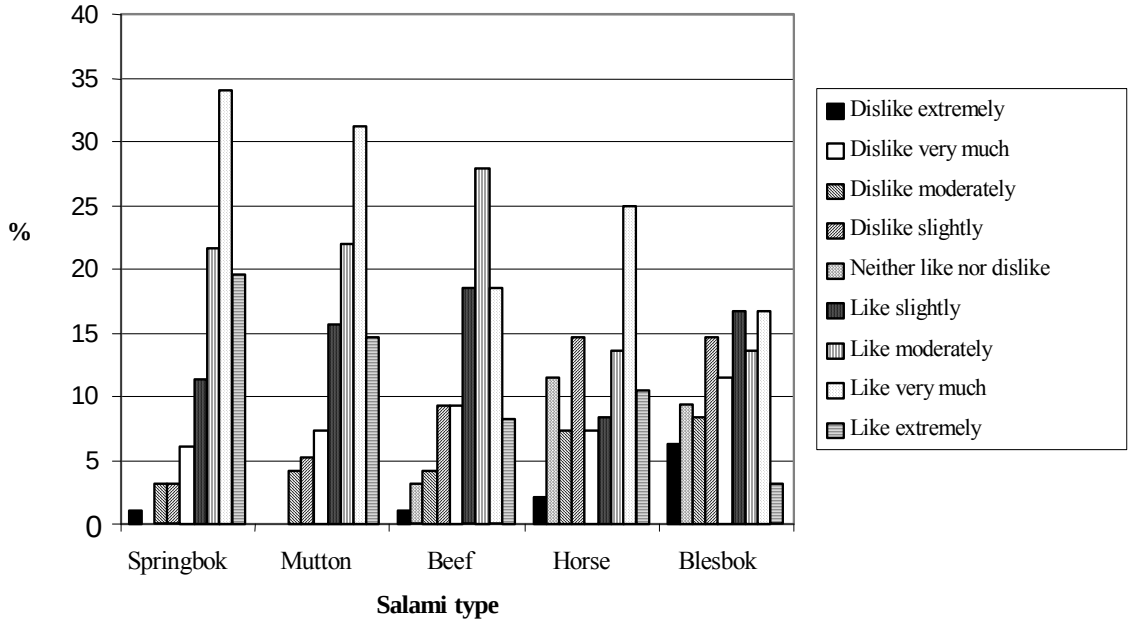

Figure 1. Distribution (%) of consumers preference for the colour of five types of salami

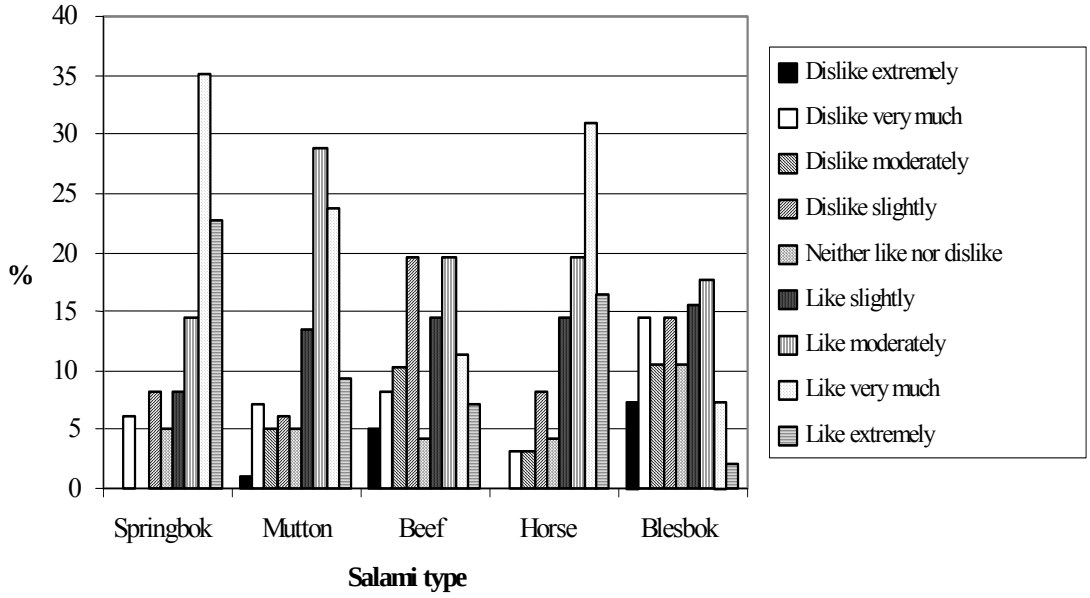

Figure 2. Distribution (%) of consumers preference for the taste of five types of salami

6c.5. Conclusion

When comparing the mean values of the pooled data for the different types of salamis, the samples could be divided into two groups, those acceptable for salami aroma, flavour and those less acceptable for sour meat flavour and aroma. Salami prepared using horse, beef and springbok meat provided samples with high mean values for salami aroma and salami flavour and low values for sour meat aroma, sour meat flavour, and venisonlike flavour and muttonlike flavour. The salami prepared with horsemeat was perceived as dark in colour but also as oily, with a soft and open structure. The beef salami was perceived as having a solid, compact structure.

The salami prepared using meat from beef, horse and springbok resulted in end products with a higher sensory quality than the salami prepared using meat from blesbok and mutton. Although the salami prepared with horsemeat was rated high for salami flavour and aroma, consumers perceived the oily mouthfeel of this product negatively.

When comparing the overall rank means for the consumer evaluation of the salami, it is clear that consumers preferred the colour and taste of the springbok salami. The salami prepared using blesbok meat had the lowest rank means for colour and taste, indicating that this sample was least preferred by this group of consumers. When an analytical panel evaluated the same salami

samples, the blesbok sample was rated highest for sour meat aroma, and sour meat and venison like flavour.

The results from this investigation indicate that salami produced using the meat from alternative species, such as horse, springbok and blesbok is possible and acceptable to the consumer. The blesbok, also being a game animal, was expected to be regarded similarly to the springbok, but salami produced using its meat was not as successful as that from springbok. It would seem as if the blesbok was more rancid than the springbok. Research needs to be done on reducing rancidity in the product using blesbok, as well as possibly using meat from less fat animals, to ensure a leaner product, less inclined to oxidation over time.

6c.6. Acknowledgements

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6c.7. References

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Chapter 7

General Conclusions

The global increase in the human population, resulting in an ever-growing demand for a larger variety of nutritional, high quality food, especially for sources of protein, makes it necessary to research alternatives to traditional red meat, such as aquatic mammals and various game species, and to investigate their potential as alternative, safe food providers. However, knowledge needs to be gained regarding the chemical composition and physical behaviour of these meat types under certain conditions, to investigate whether they could become acceptable sources of protein in the future. In conducting such studies, it is necessary to consider all the conventional, consumer-driven criteria (palatability, safety, purity, affordability, availability) relevant to the production of traditional red meat species, as they are equally valid for game species (Skinner, 1984). To date, however, only scant information is available on the chemical composition, and the viability, of products made from these alternative red meat species in this quest to address the increasing demand for healthy food products (Aidoo & Haworth, 1995). From the information presently available, it is apparent that some red meat types, such as mutton, have a fat content of between 20 and 25%, which is considered to be relatively high, while, game meat generally has a much lower fat content (2-3g per 100 g meat). Certainly, this factor alone already makes game meat an attractive alternative for the health-conscious consumer (Schönfeldt, 1993).

As most game and wild animal species, which are culled annually, are harvested in specifically allocated hunting seasons only, there are times of the year where there is very little or no supply to meet the demand for products made from these particular species. It is, therefore, of fundamental importance to the supplier, as well as the consumer, that a product be made available from such alternative red meats that is shelf-stable (pH <5.0 and a_w <0.9), nutritious and meets food safety standards all year round. This product should be acceptable to the consumer in terms of appearance as well as taste.

Therefore, the primary aim of this study was to investigate the meat of the Cape fur seal, the Grey seal and Minke whale, with regard to their chemical compositions and suitability as potential human food sources. Apart from this, the study investigated the possibility of producing

salami from the meat of these aquatic mammals, using three different starter cultures. The cultures used were primarily *Lactobacillus curvatus* and *Lactobacillus plantarum*. As a means of comparison, the same investigation was repeated, using horse, beef, mutton, blesbok and springbok meat, in order to evaluate the potential of similar products made from these animals.

Chemical analysis showed that both seal and whale meat are highly nutritious in terms of protein, fat and moisture content, especially in terms of the fatty acid content. This can be ascribed to the fact that their diet is primarily of aquatic origin, consisting mainly of fish and crustaceans. As the meat of these animals is high in terms of polyunsaturated fatty acid contents, their predators, in turn, will ingest these properties through their diet. A pilot study was undertaken to assess the possibility of producing seal-only and seal-pork, seal-beef combinations, using various starter cultures. It was found that it was possible to produce a product that conforms to all the conventionally required quality standards mentioned above. Similar findings were made for the meat of the Grey seal and the Minke whale, in products using pork lard and based on three different starter cultures.

With regard to the meat of the terrestrial mammals used in this investigation, i.e. beef, mutton, horse, blesbok and springbok, the horsemeat and meat of the springbok proved to be the healthiest in terms of chemical proximate analysis, as well as fatty acid composition, using P:S and n-6:n-3 ratios.
The production of salami in this study included the use of meat from all the above species. Use was made of pork lard and three primary starter cultures. The two common starter culture strains used in the study were *Lactobacillus plantarum* and *Lactobacillus curvatus*. Results showed that salami made using *Lactobacillus curvatus* consistently had a higher pH after fermentation than salami made from *Lactobacillus plantarum*. Products were also made with two other starter cultures (*Lactobacillus sakei* and a mutant of *Lactobacillus plantarum* 423). These starter cultures were used to test the efficiency of the antioxidant properties of the *L. sakei*, and the effect of a negative-bacteriocin producing strain *L. plantarum* 423m. *L. plantarum* was more suited in the production of traditional salami using the alternative species described above.

As salami is supposed to have a low pH value (<4.5) after fermentation, *L. plantarum* is more reliable in decreasing the pH levels of the sausages to the desired value. As sensory evaluation is such a crucial factor when regarding consumer perception of a product, it is important to note that the sensory testing panel expressed a very definite negative attitude towards the salami made using *L. curvatus*.

In respect of the terrestrial mammal species used in this trial, the horse and springbok were the two species that reacted most favourably throughout, in terms of the various parameters measured, namely: pH, fatty acid analysis and sensory analysis. The salami produced from the meat of the blesbok was deemed unsatisfactory throughout, especially with regard to the sensory analysis. As stated by Smit (2004), there are differences in these parameters within the species with regard to sex, region and age. It is possible, therefore, that the blesbok used for this investigation was not the most suitable in terms of the measured parameters so important in the development of a shelf-stable product meant for human consumption.

In conclusion, it can therefore, be claimed that salami made from a wide range of animals, including non-traditional terrestrial and aquatic meat-producing species, is a product that might well be able to provide a year-round supply of these meat types in the future, irrespective of the fact that most of these alternative red meat species are harvested in specified seasons only. In addition, this study has shown that meat products manufactured from the meat of some non-traditional animals, using particular starter cultures and under certain controlled conditions are able to meet key requirements set by the meat industry and satisfy consumer perceptions and needs.

In order to build on these research findings, and to expand on the knowledge gained in this pilot study, follow-up investigations need to be undertaken, to control for variables, such as age, geographic area and sex, in certain species, and to further fine-tune the process of using particular kinds of meat, with particular starter cultures, under particular salami manufacturing conditions.

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