The assessment of the physico-chemical, microbiological and kinetic parameters of acidulants used in the production of acidified dried sausages made from the meat of blesbok (*Damaliscus pygargus phillipsi*)

by

Mathew Paul van den Honert

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Food Science in the Faculty of AgriSciences at Stellenbosch University

Supervisor: Distinguished Prof L.C. Hoffman
Co-supervisor: Prof P.A. Gouws
Co-supervisor: Dr G. Sigge

December 2015

DECLARATION

By submitting this work electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2015

ACKNOWLEDGEMENTS

First and foremost I would like to thank Prof Louw C Hoffman, who afforded me the opportunity to pursue post graduate studies in the Stellenbosch University Meat Science section. His guidance and support is of much value and highly appreciated in terms of my thesis and the opportunity to experience the excursion and be part of a fun research team has built many memories. This opportunity certainly allowed me to Carpe Diem.

Thanks must also be extended to Dr G Sigge for his willingness to take me on as a student in the Food Science department as well as his co-supervision throughout my academic journey at Stellenbosch University.

The enthusiastic assistance and co-supervision by Prof P Gouws regarding all my chapters and progress of my thesis was invaluable and much appreciated.

I would like to further extend my gratitude to Mr H van Tiddens and Mr E America from Deli Spices (Pty) Ltd, who assisted me with ideas, practical solutions, facilities, equipment and all the functional ingredients for the trial. I could not have completed this thesis without your kindness and enthusiasm.

Thanks also to Blachem Corporation for the supply of the encapsulated acidulants as well as Mr L Dolley from Cape Peninsula University of Technology for the use of their laboratory facilities for my final trial.

Assistance with experimental design and statistical analysis provided by Prof M Kidd and Ms G Jordaan is much appreciated.

Thank you to the National Research Fund (NRF) for the funding of this project and opportunity.

I would like to thank all my fellow Meat Science colleges. Your assistance on hunting trips and in the processing laboratory was vital for the successful completion of this thesis.

Finally, this would not have been possible without the full support from my parents. Mom and Dad, thank you for this opportunity, I am grateful for it and will always be in your debtor, I guess it is time for me to get stuck in the real world now!

NOTES

This thesis is presented in the format prescribed by the Department of Food Science at Stellenbosch University. The structure is in the form of one or more research chapters and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusion. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters, especially within the materials and methods section, is therefore unavoidable.

KEYWORDS

Acidulation – the effect of the addition of an acidulant to reduce the pH of the batter thus acidifying the mixture.

Acidulant – an acid or chemical substance which hydrolyses into an acid in the presence of water which is added in order to reduce the pH.

a_w – Water activity, the availability of water for bacterial growth within the product

DFD – Dark, Firm and Dry meat

Droëwors – Dried sausage, typical product from South Africa

E-lactic acid – Encapsulated Lactic Acid

E-citric acid – Encapsulated Citric acid

E-GdL – Encapsulated Glucono-delta-lactone

GdL – Glucono Delta Lactone

LAB – Lactic acid bacteria

%RH – Relative humidity expressed as a percentage

pH_i – initial pH

pH_f – final pH

pH_u – ultimate pH

PSE – Pale, Soft and Exudate meat

RTE – Ready to Eat

WHC – Water holding capacity

SUMMARY

The physico-chemical, microbiological and kinetic parameters of Blesbok (*Damaliscus pygargus phillipsi*) dry acidified sausage produced by chemical acidification were assessed.

Initially, Blesbok meat dry acidified sausage was manufactured using five different concentrations of Glucono-delta-lactone (GdL), notable 0.5, 1.0, 1.5, 2.0 and 3.0%. All of the treatments pH_u differed ($P<0.05$) from one another, although, only 1.5, 2.0 and 3.0% were below 5.0. Water activity a_W, mass loss and hardness also differed ($P<0.05$) where 2.0 and 3.0% had the lowest a_W, the highest mass loss and highest hardness values. Microbiologically, only 3.0% was safe to consume although 1.5 and 2.0% would suffice if the spice raw material was not contaminated. The choice of 1.5% was considered the optimal concentration in the dry acidified sausage based on the physico-chemical and microbiological results.

Kinetic parameters confirmed that heating at 55°C for 65 min released the acidulant from the encapsulate and fully acidified the dry acidified sausage. A brief comparative study between the probe and sodium iodoacetate method of pH measurement was conducted. Similar regressions were developed however, the simpler probe method had a better fit ($R^2 = 0.9673$) compared to ($R^2 = 0.8944$), resulting in the simpler probe method being chosen for the continuation of the study. Further to this, eight concentrations, one through to eight percent, were produced for each of the three encapsulated acidulants. Upon heating to 55°C for 65 min, the sausage batter was acidified. Measurements for pH enabled linear regressions, E-GdL $y = -16.81 x + 6.0214$ ($R^2 = 0.9694$); E-Lacic acid $y = -13.31x + 5.5864$ ($R^2 = 0.9435$); E-Citric acid $y = -29.536x + 6.0279$ ($R^2 = 0.9844$), to be calculated.

Validation of the trend lines ensued using a 4 x 6 block design where masses of encapsulated acidulant were weighed out according to the value calculated from the trend line regression equations based on a final pH of 4.8. The optimal GdL concentration of 1.5% was used as a control. Only the GdL treatment pH at 4.9 ± 0.06 was close to the target pH of 4.8. All other treatments differed ($P<0.05$) from one another and were lower than the target pH; E-GdL = 3.7 ± 0.02, E-Lactic acid = 4.2 ± 0.04 and E-Citric acid = 3.5 ± 0.08. E-citric acid had an apparent crumbly texture which was confirmed by the Max Force 2 (second compression force) value. All the treatments, although well below the required pH, fell in line with the requirements of dried acidified sausage with mass loss > 30% and moisture loss *ca.* 30%.

OPSOMMING

Die fisiese, chemiese, mikrobiologiese en kinetiese parameters van Blesbok (*Damaliscus pygargus phillipsi*) droë versuurde-wors, wat geproduseer is deur 'n chemiese versuringsmetode, is geëvalueer.

Eerstens was Blesbok droë versuurde-wors vervaardig met die gebruik van vyf verskillende konsentrasies van Glukono-delta-laktoon (GdL) naamlik, 0.5, 1.0, 1.5, 2.0 en 3.0%. Al die behandelings het van mekaar verskil op grond van pH_u ($P<0.05$), maar slegs konsentrasies 1.5, 2.0 en 3.0% het 'n pH_u van laer as 5.0 gehad. Op grond van water aktiwiteit (a_w), massa verlies en hardheid was daar ook verskille ($P<0.05$) tussen die behandelings, met 2.0 en 3.0% wat die laagste a_w, hoogste massa verlies en hoogste hardheid gehad het. Op mikrobiologiese vlak was slegs 3.0% veilig vir verbruikers, maar 1.5 en 2.0% sal ook voldoende wees indien die roumateriale van die speserye nie gekontamineer is nie. Met hierdie inligting in ag geneem, is 1.5% geïdentifiseer as die optimale konsentrasie om droë versuurde-wors te vervaardig.

Kinetiese parameters het bevestig dat verhitting teen 55°C vir 65 min die versuringsmiddel vanuit die kapsule vrystel en die droë versuurde-wors ten volle versuur het. 'n Vergelykende studie is gedoen om die elektrode en natrium iodoasetaat metodes van pH meting te vergelyk. Soortgelyke regressies was saamgestel, maar die eenvoudiger elektrode metode het 'n beter passing getoon met $R^2 = 0.9673$ teenoor $R^2 = 0.8944$ van die natrium iodoasetaat metode. Weens die beter passing op die regressielyn asook die eenvoudiger metode, is die elektrode pH metode gekies vir gebruik vir die res van die studie. Verder is agt konsentrasies (vanaf 1 tot 8 persent) gebruik vir elk van die drie versuringskapsules. Tydens die verhitting van 55°C vir 65 min, was die wors mengsel versuur. Die meting van pH het hom na lineêre regressie verleen: E-GdL $y = -16.81x + 6.0214$ ($R^2 = 0.9694$); E-melksuur $y = -13.31x + 5.5864$ ($R^2 = 0.9435$); E-sitroensuur $y = -29.536x + 6.0279$ ($R^2 = 0.9844$), ter berekening.

Die geldigheid van die tendens lyne is getoets deur die gebruik van 'n 4 x 6 blokontwerp, waar massas van die versuringskapsules geweeg is volgens die waarde wat vanaf die regressie vergelyking se tendens lyn bereken is, met die gebruik van 'n finale pH van 4.8. Die optimale GdL konsentrasie van 1.5% is gebruik as die kontrole. Tydens hierdie eksperiment was slegs die GdL behandeling se pH van 4.9 ± 0.06 naby die vereiste pH van 4.8. Al die behandelings het van mekaar verskil ($P<0.05$) en was laer as die vereiste pH; E-GdL = 3.7 ± 0.02, E-melksuur = 4.2 ± 0.04 en E-sitroensuur = 3.5 ± 0.08. E-sitroensuur het 'n duidelike krummelrige struktuur gehad wat bevestig is deur die Maksimum Krag 2 (tweede kompressie krag) waarde. Al die behandelings (ten spyte van die lae pH) was in lyn met die vereistes van droë versuurde-wors met 'n massa verlies van > 30% en vog verlies ~ 30%.

CONTENTS

CHAPTER 1

Introduction

Since ancient times, drying and salting have been used as preservation modes in the production of sausages by placing chopped salted meats into casings and have similarly been reported by Vandendriessche, 2008 and Toldrá & Hui, 2015. Not until the 1850's, was the concept of micro-organisms and fermentation investigated (Caplice & Fitzgerald, 1999). Traditional dry and semidry sausages were synonymous with their particular regions based on their desired flavour profiles and dehydration state, later linked with the micro-organisms present in the sausage (Vignolo *et al.*, 2010, Toldrá & Hui, 2015). Subsequently, the preservation strategy employed in the dry, salted sausages has incorporated fermentation and the decrease in pH of the sausage batter as an additional preservation hurdle.

Modern science pertaining to microbiology has investigated fermentation thoroughly and identified, not only the micro-organisms responsible for the resultant pH drop, but also by-products which assist in both food preservation and flavour development in the dry acidified sausage (Erkkilä, 2001, Zeuthen, 2007, Fadda *et al.*, 2010, Holck *et al.*, 2011). Further to this, identification, isolation and production of specific micro-organisms and combinations of micro-organisms pertaining to sausage fermentation resulted in the availability of starter cultures (Gilliland, 1985, Hugas & Monfort, 1997, Erkkilä, 2001, Leroy & De Vuyst, 2004, Ammor & Mayo, 2007, Tabanelli *et al.*, 2012, Simion *et al.*, 2014). Much other research into the understanding of fermentation regarding functional ingredients, particularly pertaining to nitrates, salt and fermentable sugars, has been conducted (Skjelkvale & Tjaberg, 1974, Honikel, 2008, Hammes, 2012). The availability of starter cultures and a thorough understanding of fermentation has supported the industrial production of fermented dry sausages facilitating reproducibility during manufacturing, thus supporting the notion of a consistent "quality" product (Kröckel, 2013).

Processing demands cost reduction in the form of ingredients and time. The understanding of fermentation producing lactic acid to decrease the pH spurred on the investigation of acidification by chemical means. Both lactic acid and citric acid have been used as direct acidulants, either singularly or in combination with one another (Desmond & Troy, 2001, Barbut, 2005). An ester, Glucono-delta-lactone (GdL), was developed for the purpose of acidification and is continually being used in large scale processing within South Africa to manufacture dry and semidry acidified sausages (van Tiddens, H., Technical Sales, Deli Spices (Pty) Ltd, personal communication, April 2014). GdL hydrolyses into gluconic acid in the presence of water, resulting is a slightly slower fermentation to that of acids (Leroy & De Vuyst, 2009). Concerns have been raised regarding rapid acidification as the speed of acidification has been associated with denatured proteins resulting in premature drying, insufficient binding and an ultimate crumbly texture (Barbut, 2006).

Newer encapsulation technologies have enabled the development of enrobes acidulants by coating the acidulant in a fat based casing. This fat based casing requires heating to release the acidulant allowing for the salt soluble proteins to form a gel prior to acidification upon heating (Barbut, 2005, Barbut, 2006). Although available, there is not much information regarding these encapsulated acidulants and their effect in the dry acidified sausage processing environment. Furthermore, in South Africa, the industry relies on GdL and has not been exposed to the encapsulated acids in the production of dry and semidry acidified sausages.

Sausages are typically produced from pork although it is not uncommon to use other meat sources such as seal or game meat (Koep, 2005, Todorov *et al.*, 2007, van Schalkwyk *et al.*, 2011). Consumer demands insist on healthier products, including lower saturated fatty acid content as well as the product being defined as “organic” in nature. The availability of game meat in South Africa enables the fulfilment of the organic ideology as they roam in the wild, browse and graze on natural flora resulting in a more favourable fatty acid profile (Hoffman *et al.*, 2005, Hoffman & Wiklund, 2006). Further to this, the predominant products from game meat are primal meat portions, wet fresh sausage (boerewors) or either dehydrated, salted meat (biltong) or dehydrated sausage (droëwors).

The objective of this study was thus to utilise game meat in order to produce dry acidified sausage based on current South African Technology utilising GdL at varying concentrations to develop a recommended, optimal concentration based on physico-chemical and microbiological parameters. Once defined, the kinetics of the encapsulated acidulants were investigated in order to understand their concentration and temperature requirements to acidify the sausage batter. Finally, the kinetics of the encapsulated acids were validated by physico-chemical analysis in order to assess whether the final sausage product met the requirements of dried acidified sausage as well as a physico-chemical comparison with the current GdL technology employed in South Africa.

References

Ammor, M.S. & Mayo, B. (2007). Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: An update. *Meat Science,* **76**, 138-146.

Barbut, S. (2005). Effects of chemical acidification and microbial fermentation on the rheological properties of meat products. *Meat Science,* **71**, 397-401.

Barbut, S. (2006). Fermentation and chemical acidification of salami-type products - effect on yield, texture and microstructure. *Journal of Muscle Foods,* **17**, 34-42.

Caplice, E. & Fitzgerald, G.F. (1999). Food fermentations: role of micro-organisms in food production and preservation. *International Journal of Food Microbiology,* **50**, 131-149.

Desmond, E. & Troy, D. (2001). Effect of lactic and citric acid on low-value beef used for emulsion-type meat products. *Food Science and Technology,* **34**, 374-379.

Erkkilä, S. (2001). Bioprotective and probiotic meat starter cultures for the fermentation of dry sausages. Academic Dissertation, *Department of Food Technology.* University of Helsinki, Helsinki.

Fadda, S., Lopez, C. & Vignolo, G. (2010). Role of lactic acid bacteria during meat conditioning and fermentation: peptides generated as sensorial and hygienic biomarkers. *Meat Science,* **86**, 66-79.

Gilliland, S.E. (1985). *Bacterial Starter Cultures for Foods*. Boca Raton, FL, USA: CRC Press.

Hammes, W.P. (2012). Metabolism of nitrate in fermented meats: the characteristic feature of a specific group of fermented foods. *Food Microbiology,* **29**, 151-156.

Hoffman, L., Muller, M., Schutte, D.W., Calitz, F. & Crafford, K. (2005). Consumer expectations, perceptions and purchasing of South African game meat. *South African Journal of Wildlife Research,* **35**, p. 33-42.

Hoffman, L.C. & Wiklund, E. (2006). Game and venison - meat for the modern consumer. *Meat Science,* **74**, 197-208.

Holck, A.L., Axelsson, L., Rode, T.M., Hoy, M., Mage, I., Alvseike, O., L'Abee-Lund, T.M., Omer, M.K., Granum, P.E. & Heir, E. (2011). Reduction of verotoxigenic *Escherichia coli* in production of fermented sausages. *Meat Science,* **89**, 286-295.

Honikel, K.O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science,* **78**, 68-76.

Hugas, M. & Monfort, J.M. (1997). Bacterial starter cultures for meat fermentation. *Food Chemistry,* **59**, 547-554.

Koep, K.S.C. (2005). Production of salami from meat of aquatic and terrestrial mammals. MSc thesis. *Department of Animal Science.* Stellenbosch University, *Stellenbosch, South Africa*.

Kröckel, L. (2013). The role of lactic acid bacteria in safety and flavour development of meat and meat products. INTECH Open Access Publisher. [Internet document].URL http://cdn.intechopen.com/pdfs/42316/InTech-The_role_of_lactic_acid_bacteria_in_safety_and_flavour_development_of_meat_and_meat_products.pdf. Accessed 23/09/2015.

Leroy, F. & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology,* **15**, 67-78.

Leroy, F. & De Vuyst, L. (2009). Fermentation and acidification ingredients. In: *Ingredients in Meat Mroducts: properties, functionality and applications,* (edited by R. Tarte). Pp. 227-252. New York, USA: Springer.

Simion, A.M.C., Vizireanu, C., Alexe, P., Franco, I. & Carballo, J. (2014). Effect of the use of selected starter cultures on some quality, safety and sensorial properties of Dacia sausage, a traditional Romanian dry-sausage variety. *Food Control,* **35**, 123-131.

Skjelkvale, R. & Tjaberg, T.B. (1974). Comparison of salami sausage produced with and without addition of sodium nitrite and sodium nitrate. *Journal of Food Science,* **39**, 520-524.

Tabanelli, G., Coloretti, F., Chiavari, C., Grazia, L., Lanciotti, R. & Gardini, F. (2012). Effects of starter cultures and fermentation climate on the properties of two types of typical Italian dry fermented sausages produced under industrial conditions. *Food Control,* **26**, 416-426.

Todorov, S.D., Koep, K.S., Van Reenen, C.A., Hoffman, L.C., Slinde, E. & Dicks, L.M. (2007). Production of salami from beef, horse, mutton, blesbok (Damaliscus dorcas phillipsi) and springbok (Antidorcas marsupialis) with bacteriocinogenic strains of *Lactobacillus plantarum* and *Lactobacillus curvatus*. *Meat Science,* **77**, 405-412.

Toldrá, F. & Hui, Y.H. (2015). Dry-Fermented Sausages and Ripened Meats: An Overview. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá, Y.H. Hui, I. Astiasaran, J.G. Sebranek & R. Talon). Pp. 3-6. New Jersey, USA: John Whiley & Sons, Ltd.

van Schalkwyk, D.L., McMillin, K.W., Booyse, M., Witthuhn, R.C. & Hoffman, L.C. (2011). Physico-chemical, microbiological, textural and sensory attributes of matured game salami produced from springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*) and zebra (*Equus burchelli*) harvested in Namibia. *Meat Science,* **88**, 36-44.

Vandendriessche, F. (2008). Meat products in the past, today and in the future. *Meat Science,* **78**, 104-113.

Vignolo, G., Fontana, C. & Fadda, S. (2010). Semidry and dry fermented sausages. In: *Handbook of Meat Processing,* (edited by F. Toldrá). Pp. 379-398. Ames, Iowa, USA.: John Wiley & Sons, Ltd.

Zeuthen, P. (2007). A historical perspective of meat fermentation. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá, Y.H. Hui, I. Astiasaran, W.K. Nip, J.G. sebranek, E.T.F. Silveira, L.H. Stahnke & R. Talon). Pp. 1-8. Ames, Iowa, USA: Blackwell Publishing.

CHAPTER 2

Literature review

2.1 Introduction

History and archaeology have indicated that the use of salt, natural fermentation and drying in the preservation of meat has been present since ancient times (Toldrá & Hui, 2015). The ability to ferment and dehydrate foods have given rise to many different kinds of products which have become synonymous to the regions they are produced in, the likes of which include Italian, Genoa and Milano Salami, Spanish Chorizo, American Pepperoni and many other varieties throughout the Mediterranean and Europe. Cultures have made products from natural resources resulting in many products from many species of animals including pork, beef, horse, seals, whales, deer and game Feiner, 2006e).

South Africa traditionally consumes much meat in the form of wet meat (primal cuts), boerewors (farmer sausage – a fresh sausage requiring cooking), biltong (salted and dried meat) and droëwors (dry sausage), commonly produced from beef and local game animals including but not limited to Springbok, Blesbok, Kudu, Impala, Eland and Zebra (Koep, 2005, Todorov *et al.*, 2007, van Schalkwyk *et al.*, 2011).

Droëwors is produced with fat, meat and spices, often including vinegar which ultimately classifies the product as a dry acidified sausage, although, commonly the classification is considered purely a dry sausage as the vinegar is added for a sensory effect as opposed to relying on the pH for a preservative action (Jones, 2013).

Industry in South Africa primarily produces pork based dry and semi-dry acidified sausages on large scale, typically relying on the use of Glucono-delta-lactone (GdL) to acidify the product as opposed to relying on fermentation (van Tiddens, H., 2015, Technical sales, Deli Spices (pty) Ltd, Epping, South Africa, personal communication, April 2014).

The aim of this literature study was thus to gain an understanding of the South African game industry and game meat as well as where dry acidified sausages fall within sausage classification, dry acidified sausage manufacture. Furthermore, a revision of the understanding of fermentation and chemical acidification of dry-fermented sausages including ingredients, processes, quality testing parameters and food safety requirements with particular reference to food safety management systems, microbiology and chemical safety was investigated.

2.2 Game and the game meat industry

Southern Africa has been known for its wildlife, specifically its variety of migratory antelope (Frost, 2014a). Since ancient times, as depicted on rock paintings, these antelope have been roaming in the wild as well as being hunted. Upon colonisation in Southern Africa, colonialists put up fences

and started farming the barren lands. Although these farming practises focussed more on livestock, even at this point, antelope were raised in a farming environment (Frost, 2014a). The early colonialists participated in much hunting both as a sport and a source of food which resulted in the depletion of the Southern African antelope numbers, particularly those in the wild (Els, 2010, Frost, 2014a). Since the early 20th century, farmers have been implementing wildlife management practises to manage their stocks of antelope in order to initially build up the antelope stocks and then manage these stocks for future profits (Els, 2010).

Fences are put up on farms to control populations of wildlife in terms of movement and ownership. Once this control is in place, the farmer is required to ensure that the animals are cared for appropriately. With reference to animal welfare, stock holding capacity needs to be managed, particularly pertaining to sufficient food sources being available for the populations of animals contained within the boundaries of the farming area (Bothma, 2010a). Ultimately, as breeding progresses, the populations are required to be controlled in terms of numbers of animals in a confined space. This control can come in two forms, namely, control by selling and control by culling (Bothma, 2010b).

Efficiency in this game breeding industry has led to breeders optimising their herds in terms of productivity by selling their males at an early stage in order to have as many females as possible to maximise their productivity on their finite lands. Once fully stocked, selling of animals often involves the selling of breeding herds. These breeding herds often are sold in the form of numerous females with one male as particularly seen in the current game breeding fraternity in South Africa (Smith, D.H., Dodds, N. and van den Honert, L.H., 2015, Nukamma Wildlife, Jansenville, South Africa, personal communication, 16 June 2015.). The fundamentals of selling rely on a demand for the product being sold. Should demand of either breeding herds or of bachelor herds not be great enough, culling would have to be considered as the next viable option of population control in a contained area (Bothma, 2010b). Consequently, the by-product of culling antelope results in the availability of meat.

Other sources of meat include the hunting community, whereby either outfitters from abroad have brought in hunters specifically for the purpose of hunting for trophy animals, or the local biltong hunters who are able to hunt for pleasure and use the meat from the animals as a food source (Bothma & Sartorius von Bach, 2012). Lastly, antelope can be bred particularly for the meat industry whereby the sole purpose for breeding the animal is for the eventual slaughter of the animal and processing into meat products to satisfy venison meat demand (Bothma, 2010c).

Recent global trends have seen an increase in the demand for both organic foods and leaner meat products, as they are considered healthier and of a better quality (Smolin & Grosvenor, 2003). There have been concerns raised regarding the nutritional value of meat (Troy & Kerry, 2010). Particularly, concerns raised by the consumer regarding products with a high fat content, leading to an increased risk in the development of cardio vascular disease, obesity and diabetes have resulted

in a preference for leaner meat alternatives (Schutte, 2008). It is on this premise that the ostrich industry has seen such growth in the last 20 years, particularly in the export markets pertaining to ostrich meat products principally due to the leaner nature of the ostrich meat (Gillespie & Schupp, 2000, NAMC, 2003, Schutte, 2008).

2.3 Definition of meat

Meat is defined by the European Union Directive 2001/101/EU, as skeletal muscle of mammalian and bird species recognised as fit for human consumption with naturally included or adherent tissue, where the total fat and connective tissue content does not exceed specified values (European Union 26 November 2001). However, Warriss (2010a) points out that this definition can be interpreted by other industries, locations and cultures as including lean muscle, fat, connective tissues and other tissues which incorporates non-skeletal muscle and offal. Meat as required in the processed meat industry typically requires lean muscle protein without connective tissues as well as fat which is predominantly saturated as the level of unsaturation may affect the rancidity and sensory qualities of the final product (Marianski & Marianski, 2012). Meat, due to the process of anaerobic glycolysis post-slaughter of the animal, goes into rigor mortis and acidifies (Lawrie & Ledward, 2006a). The final pH post-acidification is vital for the final quality of the meat (Lawrie & Ledward, 2006a, Warriss, 2010b,c). The nature of the stress of the animal prior and during the slaughter process is essential to manage in order to ensure the meat post-acidification is not compromised in any way (Feiner, 2006a). Meat which is designated on either side of normal, whether it be DFD (Dark, Firm, Dry) meat or PSE (Pale, Soft, Exudate) meat due to the decrease in pH during the acidification period, will define the process required, whether it be unprocessed meat, acidified meat, dried meat, brined meat or emulsified products (Feiner, 2006a, Marianski & Marianski, 2012). Game meat is not excluded from this definition and as such is considered a meat source, albeit with a slightly different nutritional profile, none the less is capable of being slaughtered, acidified and depending on the status of the animal pre-slaughter, susceptible to the outcomes of DFD or PSE (Feiner, 2006b).

Currently in South Africa, unlike beef, pork and lamb, the typical cuts of meat have not been defines for game meat however, due to the size of the carcass, designations similar to the lamb cuts have been utilised for small game processing and cuts similar to beef have designated for bigger game animals (Browning, 2008, SAMIC, 2015). Primal cuts on smaller game carcasses often refer to the hind and fore quarter as well as the fillet and loin. The shins and the neck are often processed into stewing meat and the rib meat as well as any offcuts are produced into sausage (Browning, 2008).

2.4 Value added meat

Currently in the South African venison market, game animals, typically Springbok, Blesbok, Kudu and Eland are slaughtered and processed into primal cuts of meat for both the local and export markets (Hoffman & Wiklund, 2006, Mostert, 2007). The processing of primal cuts leads to much waste in the system as a result of offcuts. In order for the producer to maximise profits, the producer

should consider the addition of value to these lower grades of meat. This addition in value may come in many forms (Deogade *et al.*, 2008, Dare *et al.*, 2013). Essentially the goal is to make meat products more appealing to the consumer in order to sell the product at a premium. This could be in the form of improving lower grade meat cuts as well as offcuts (Deogade *et al.*, 2008). This transformation allows the utilisation of more of the carcass thus increasing the production yields and ultimately assisting in maximising the profits for the producer (Dare *et al.*, 2013). Although adding value to a product resolves the utilisation and efficiency of the raw material conundrum experienced in industry, this growth requires an increase in labour and a subsequent potential increase in food safety risks due to the additional handling of raw material and the addition of new raw materials.

One overarching benefit of the addition of value to venison would be the provision of many other products to the market which appeal to consumers and can lead to the growth of the industry as a whole, as well as a potential interest in the game meat and meat product industry (Deogade *et al.*, 2008, Dare *et al.*, 2013). A study conducted by Hoffman *et al.* (2004) highlighted that butcheries within the Western Cape region primarily sold steaks and legs and that wet sausage was 10th on their list. There was no indication as to any other value added products on the market at that stage (Hoffman *et al.*, 2004). In terms of current retail trends, much game meat biltong and droëwors are sold, typically through designated biltong outlets although beef biltong is still predominant in terms of quantity. Although demand is great for game meat biltong and droëwors, there is no standard slaughtering protocol, which compromises safety, the supply of the game meat is not consistent due to seasonality and there is an inability to produce Halaal game meat due to slaughtering practises and other products (Nell, M., 2015, Owner and Managing Director, Cape Deli (Pty) Ltd, Blackheath, Western Cape South Africa, personal communication, 22/09/2015). Further to this, there is currently no other value added type products offered in retail such as polonies, viennas, and salamis however, biltong hunters on a low scale personal level have ventured into producing pies, salamis and other products (Nell, M., 2015, Owner and Managing Director, Cape Deli (Pty) Ltd, Blackheath, Western Cape South Africa, personal communication, 22/09/2015).

2.5 Comparison of various game meat species and other red meat

Game meat is considered healthier than beef particularly due to the diet as game meat typically roams on farms as opposed to being raised in a feedlot environment, where the former relies on browsing and grazing and the latter relies on feed formulations to optimise growth and minimise cost input (Hoffman, 2007). Notably, there is a trend in consumer interest for free range, organic and naturally produced products, of which game meat complies, thus appealing to the market (Steenkamp, 1997). Table 2.1 reveals a comparison of game animals to that of beef, all of which are considered red meat species.

Table 2.1 Comparison of proximate data, Fatty acids and collagen content in red meat species

	Beef[2,6,8,11]	Ostrich[6,9,10]	Kudu[2,]	Springbok[7,5]	Blesbok[1,4]
Proximate Data					
Moisture	71.6	76.24 ± 0.53	75.66 ± 0.31	73.10 ± 0.46	62.7 ±0.7
Protein	20.94	21.0 ± 0.58	22.77 ± 0.34	20.82 ± 0.30	23.3 ± 1.5
Fat	6.33	0.92 ± 0.23	1.48 ± 0.2	1.07 ± 0.14	4.5 ± 0.7
Ash	1.03	1.03 ± 0.13	1.22 ± 0.06	1.20 ± 0.10	6.1 ± 0.6
Lipid analysis					
SFA	43.43	39.73 ± 0.77	43.06	41.11 ± 2.11	43.59 ± 6.39
MUFA	47.89	27.27 ± 1.13	20.08	20.99 ± 2.10	17.53 ± 7.77
PUFA	8.68	32.99 ± 1.22	36.86	36.34 ± 3.37	38.87 ± 8.36
Cholesterol mg/100g	89.58	57 - 83	72.62 ±1.86	59.34	52.76 ± 8.55

References
1-(Van Zyl & Ferreira, 2004)
2-(Hoffman *et al.*, 2009)
3-(Kroucamp, 2004)
4-(Hoffman *et al.*, 2008)
5-(North *et al.*, 2015)
6-(Sales & Hayes, 1996)
7-(Hoffman *et al.*, 2007)
8-(Raes *et al.*, 2003)
9-(Poławska *et al.*, 2011)
10-(Hoffman *et al.*, 2005)
11-(Daley *et al.*, 2010)

For the purpose of this table, only values for males were presented.

Proximate composition values as denoted in Table 2.1 are similar to one another, although the fat content of beef is highest when compared to the other four game species. When comparing the fatty acid profiles, the value for PUFA's is lowest in beef. When considering healthy nutrition, the PUFA:SFA (P:S) ratio is important to consider and based on the values in Table 2.1, game meat of all four species have a lower P:S ratio than beef thus rendering the game meat a preferred source of PUFA's (Hoffman & Wiklund, 2006, McAfee *et al.*, 2010, Scollan *et al.*, 2014). This is particularly due to the fatty acid profile where, due to the grazing or browsing diet of the game animals, as opposed to the feedlot environment of the beef, feeding on grain.

2.6 Blesbok (*Damaliscus pygargus phillipsi*)

As reviewed by Neethling (2012), Blesbok are commonly present in South Africa. Historically, Blesbok (*Damaliscus pygargus phillipsi*) are found in the central and northern parts of South Africa and some private owners have acquired Blesbok in neighbouring Botswana and Zimbabwe (Lloyd & David, 2008, Estes, 2012, Frost, 2014b). Another bastard hartebeest species closely related to the Blesbok is the Bontebok (*Damaliscus pygargus pygargus*) which is found historically in the South West of the Western Cape region (Frost, 2014b). There have been populations of Blesbok farmed in the Southern Cape and other regions within South Africa. From a conservation point of view, Cape Nature has drafted and is enforcing that the historical Bontebok be introduced and managed in their historical areas and that the Blesbok get managed in such a way that they do not pose a genetic integrity risk to both the Blesbok and Bontebok (Birss *et al.*, 2014, Frost, 2014b).

The population of Blesbok as of 2014 is estimated at *ca.* 250 000 animals and is not considered endangered of which *ca.* 90% is found on private land (Lloyd & David, 2008, Frost, 2014b). Blesbok are considered grazers and typically graze on short grass (Estes, 2012). Due to the

deterioration of the nutritional quality of the grass, the Blesbok tend to lose mass in the winter months (Frost, 2014b). Blesbok are considered medium sized herbivores, with a live mass of *ca.* 55 kg although, some research has reported higher live mass values between 59.5 kg and 95.3 kg (Von La Chevallerie, 1970, Neethling, 2012). They have a reported dressing percentage of between 49.5 and 53.7% (Van Zyl & Ferreira, 2004, Hoffman *et al.*, 2008, Neethling, 2012).

Due to the need for population control, both on farms and the requirement to control the populations to their specified areas, the need for relocation and/or culling is justified. Due to the availability of meat, some research has been conducted on the quality aspects of Blesbok meat both as a pure source of meat protein as well as an enhanced process product (Smit, 2004, Van Zyl & Ferreira, 2004, Du Buisson, 2006, Todorov *et al.*, 2007, Hoffman *et al.*, 2008, Neethling *et al.*, 2014). Meat quality data indicates proximate values of moisture 75.5%, protein 23.5% and fat 1.7% (Von La Chevallerie, 1972). Du Buisson (2006) investigated the use of the injection of inorganic salts into Blesbok meat muscle primarily to assist in the tenderisation of the muscle. Attempts to manufacture salami from Blesbok meat have been reported by Torodov *et al.* (2007) whereby fermentation was employed and a comparison of pH and sensory data were compared.

2.7 Sausage classification

Sausages are produced in many forms from around the world, of which the first accounts of sausages have suggested to be produced by the ancient Greeks and Romans (Zeuthen, 2007, Marianski & Marianski, 2012, Toldrá & Hui, 2015). Sausages are defined as an emulsion of meat and fat particles and including salt (NaCl), curing agents, spices, etc., which have been stuffed into a casing (Varnam & Sutherland, 1995a). Simply, an American based system defining sausage types highlighted in Figure 2.1, demonstrates the classification of sausage based on their processing requirements in terms of being fresh, cooked, or fermented (Anonymous, 2015a).

Fresh sausage is preserved by refrigeration, has a short shelf-life and is required to be cooked prior to consumption (Feiner, 2006c). Often fresh sausage can be frozen to extend the shelf-life however from a quality perspective, it is best to not freeze and rather refrigerate between 0 – 4°C and consume within the designated shelf-life (Feiner, 2006c). Advances in packaging, hygiene and processing technologies, including vacuum packaging, modified atmosphere packaging, UV sterilisation and package permeability have enabled the shelf-life to be extended on fresh sausages while still maintaining the freshness of the sausage (Lerasle *et al.*, 2014, Gammariello *et al.*, 2015).

Cooked sausages on the other hand, are prepared typically as an emulsion, although this is not a requirement and then cooked to a core temperature of 85°C although if kept for a longer time, between *ca.* 69°C and 77°C is required provided safety requirements are met (Varnam & Sutherland, 1995b, Feiner, 2006d). This cooking step assists in protein gelation thus solidifying the sausage as well as cooking the sausage in order to inhibit the pathogenic micro-organisms. Heating to reach the core temperature of *ca.* 72°C, rapid cooling after heating and post cooking hygiene as well as

packaging technologies are essential to ensure the safety of the product and extend the shelf-life of the product (Puolanne, 2010, Feng *et al.*, 2013). Typically these cooked products are required to be refrigerated for the duration of its shelf-life and may be eaten cold although, from a sensory point of view, the heating up of the sausages may improve the experience for the consumer.

Lastly, fermented sausages are categorised as either being dry, semi-dry or spreadable (Anonymous, 2015a). Their typical acidic note is as a result of the fermentation process (Kröckel, 2013). The pH decrease due to the lactic acid bacteria (LAB) fermentation has an effect on the pathogenic micro-organisms of the sausage however, only the dry sausages are deemed shelf stable at ambient temperatures due to their dehydration and resultant decrease in a_w (King, 2014). Semi-dry and spreadable sausages may be hot smoked, cooked, cold smoked, or a combination thereof. The pH along with cooking or smoking has an inhibitory effect on the pathogenic micro-organisms thus rendering a safe product although the product is typically still required to be refrigerated to maintain its shelf-life (Ockerman & Basu, 2015).

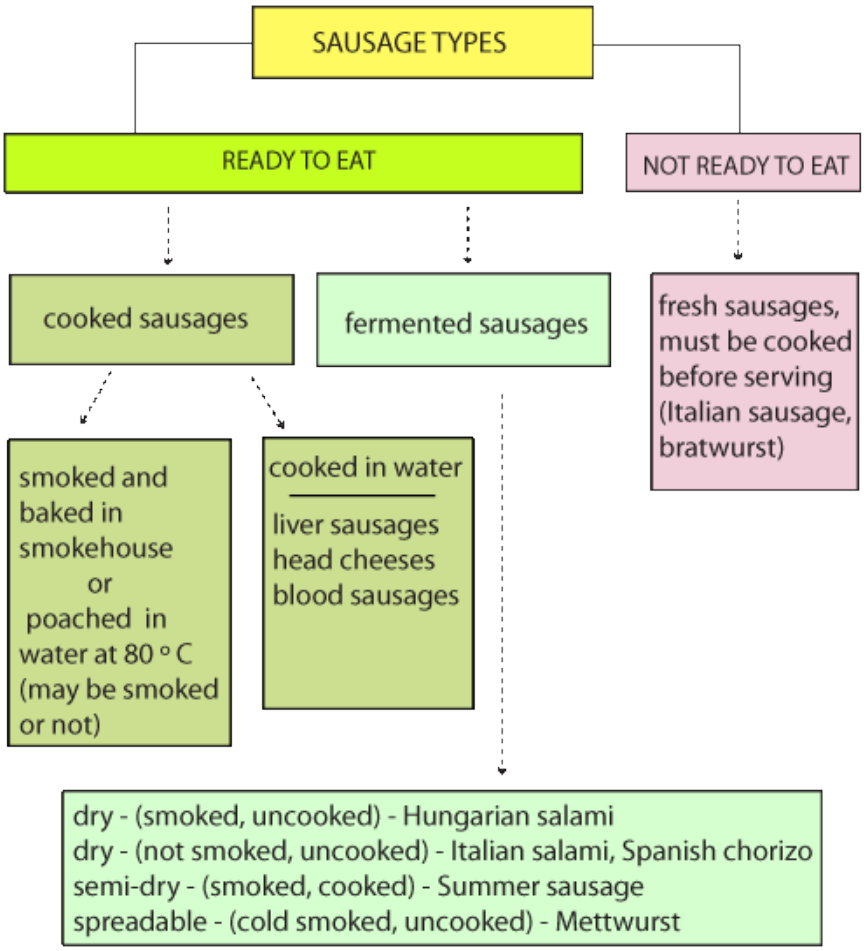

Figure 2.1 An American based system defining types of sausages (Anonymous, 2015a).

As seen in Table 2.2, sausages come in many forms. American Summer sausage is often chemically acidified or rapidly acidified with added sugar to a final pH of *ca.* 4.5, providing a more tangy acidic taste (Feiner, 2006e). Typically, the American summer sausage requires a heating step to inactivate and render the product safe to eat from a microbiological stand point as pH and water activity alone are not considered enough of a measure to ensure food safety from an American legal perspective, particularly referring to a 5 log reduction in *Escherichia coli* O157:H7 (Leroy & De Vuyst, 2009, Roberts & Getty, 2011). Milano Italian salami is reliant on fermentation to create the characteristic flavour profile as well as the hurdles of bacterial antagonism, pH decrease and low water activity in order to be safe to consume, where moisture content classifies this product a dry acidified sausage (Casiraghi *et al.*, 1996, Jay *et al.*, 2005a). Location of production often determines the name given to a sausage product, for example, Pepperoni (North America), Sucuk (Turkey), Milano salami (Italy) and Chorizo (Spain) are all considered dry fermented sausages but the spices used, typical flavours and natural microflora of the region where it is produced differ and thus differentiates between the sensorial characteristics of the particular sausage (Action & Dick, 1976, Ziegler *et al.*, 1987, Casiraghi *et al.*, 1996, Soyer *et al.*, 2005, Ercoşkun & Özkal, 2011, Toldrá & Hui, 2015).

Table 2.2 Examples of sausages from around the world and their classifications

Name	Place	Classification	Further Processing
Boerewors[12]	South Africa	Fresh	To be cooked further
Pork Sausage[12]	Great Britain	Fresh	To be cooked further
Summer sausage[8,13]	North America	Dry-acidified	Ready To Eat
Milano Salami[1,4,7,13]	Italy	Dry-fermented	Ready To Eat
Chorizo[11,13]	Spain	Dry-fermented	Ready To Eat
Pepperoni[1,5,8,9,13]	North America	Dry-fermented	Ready To Eat
Sucuk[1,2,3,13]	Turkey	Dry-fermented	Ready To Eat
Salchichón[6]	Spain	Dry-fermented	Ready To Eat
Cervelat[16]	Italy	Semi-Dry	Ready To Eat
Lebanon bologna[17]	America	Semi-Dry	Ready To Eat
Teewurst[15]	Austria/Germany	Spreadable	Ready To Eat
Frankfurter[14]	Germany	Cooked	Ready To Eat
Droëwors[10]	South Africa	Dry	Ready To Eat

Superscripts define references as detailed below.

1-(Vignolo *et al.*, 2010)
2-(Bajovic *et al.*, 2012)
3-(Soyer *et al.*, 2005)
4-(Casiraghi *et al.*, 1996)
5-(Action & Dick, 1976)
6-(Fernández-López *et al.*, 2008)
7-(Papa *et al.*, 1995)
8-(Palumbo *et al.*, 1976)
9-(Ziegler *et al.*, 1987)
10-(Jones, 2013)
11-(Leroy *et al.*, 2015)
12-(Feiner, 2006f).
13-(Feiner, 2006e)
14-(Feiner, 2006g)
15-(Feiner, 2006h)
16-(Marianski & Marianski, 2012)
17-(Vignolo *et al.*, 2010)
18-(Holck *et al.*, 2011)

Semi-dry and dry fermented sausage are ready to eat products as they do not need further heat processing prior to consumption. The main reason for this is due to the combined hurdles of salt concentration, nitrate concentration, antagonism of lactic acid bacteria, low pH and low a_W (Vignolo *et al.*, 2010).

In terms of the characteristics of acidified sausages, Table 2.3 highlights the predominant product characteristics as well as processing parameters pertaining to dry, semi-dry and moist fermented sausages as highlighted by Ockerman and Basu (2015).

Table 2.3 Characteristics of different types of fermented sausage (Ockerman & Basu, 2015)

Type of Sausage	Characteristics
Dry, long ripening	Chopped and ground meat
	Commercial starter culture or back inoculum
e.g. dry or hard	US fermentation temperature 15 - 35 ◦C for 1 - 5 days
salami, saucission,	Not smoked or lightly smoked
pepperoni.	US bacterial action reduces pH to 4.7 - 5.3 (0.5 - 1.0% lactic acid, total
	acidity 1.3%, which facilitates drying by denaturing protein, resulting in a
Shelf-stable	firm texture; MP < 2.3 : 1, moisture loss 25–50%; moisture level < 35%)
	European bacterial action reduces pH to 5.3–5.6 for a more mild taste than
	US; processing time 12 - 14 weeks
	Dried to remove 20 - 50% of moisture; contains 20 - 45% moisture, 39% fat,
	21% protein, 4.2% salt; a_w 0.85 - 0.86, yield 64%
	MP no greater than 2.3 : 1.0
	Less tangy taste than semidry
Semidry, sliceable	Chopped or ground meat
	Bacterial action reduces pH to 4.7 - 5.3 (lactic acid 0.5 - 1.3%, total acidity
e.g. summer	1%), processing time 1 - 4 weeks
sausage, Holsteiner,	Dried to remove 8–30% of moisture by heat; contains 30 - 50% moisture,
cervelat (zervelat),	24% fat, 21% protein, 3.5% salt; a_w 0.92 - 0.94, yield 90%
tuhringer, chorizos.	Usually packaged after fermentation/heating
	Generally smoked during fermentation
Refrigerate	No mould
	MP no greater than 2.3–3.7 : 1.0
Moist, undried,	Contains 34 - 60% moisture, production time 3 - 5 days
spreadable	Weight loss *ca*.10%, a_w 0.95 - 096
	Usually smoked
e.g. teewurst,	No mould
mettwurst, frishe	Highly perishable, refrigerate, consume in 1 - 2 days
braunschweiger	

Source: Modified from American Meat Institute (1982), Campbell-Platt & Cook (1995), Doyle *et al.* (1997), Farnworth (2003), Gilliland (1985).

2.8 Dry acidified sausage

Dry acidified sausages implies that the sausage batter is acidified resulting in a decrease in pH and subsequent dehydration (Marianski & Marianski, 2012, Ockerman & Basu, 2015). This acidification can take place employing two methods, namely bacterial fermentation by lactic acid bacteria or by direct acidification via the inclusion of chemical acidulants (Puolanne & Petaja, 2015, Roncales, 2015).

Historically, fermentation along with the addition of salt and drying have been the preservation techniques employed (Varnam & Sutherland, 1995c, Puolanne & Petaja, 2015, Toldrá & Hui, 2015). Early processes, prior to the understanding of microbiology relied on the natural micro-organisms often present in the immediate vicinity of the sausage processing area to multiply and provide the characteristic preservation and flavour profile. As the knowledge of fermentation was expanded upon, caution was applied to the processing conditions of time and temperature as to ensure consistent good quality product was produced. Production of fermented sausage still makes use of this technique, often optimising the growth of the favourable lactic acid bacteria by providing some reducing carbohydrates to encourage the sustained growth of the natural lactic acid bacteria (Feiner, 2006b, Ockerman & Basu, 2015). Advances in microbiology allowed for the culturing, identification and the ultimate development and continuous supply of specific micro-organisms required for the fermentation of sausages in the form of starter cultures which are attributed to shorter incubation times (Jay *et al.*, 2005a, Vignolo *et al.*, 2010, Bañón *et al.*, 2014, Çiçek *et al.*, 2014, Palavecino Prpich *et al.*, 2015). The use of these starter cultures, often involving lactic acid bacteria (LAB), *Micrococcaceae*, *Staphylococcus* or *Kocuria* species with the addition of a fermentable carbohydrate, under specific time and temperature parameters, provides a controlled repeatable fermentation to the sausage batter (Bañón *et al.*, 2014, Leroy *et al.*, 2015). The addition of a specified concentration of carbohydrate, typically monosaccharides or disaccharides encourage fermentation and enable a lower desired end point pH to be reached as the LAB are not relying solely on the glycogen from the muscle (Marianski & Marianski, 2012, Ruiz & Perez-Palacios, 2015). This reduced pH aids in the role of drying the product and directly influences the safety of the product based water activity (a_W) and moisture parameters (Ockerman & Basu, 2015).

Furthermore, preservation of dry fermented sausages relies on the addition of sodium nitrite and sodium nitrate (Pegg & Honikel, 2015). The addition of the sodium nitrite also has the ability to enhance colour development often attributed to the darker red colour in the sausage (Jay *et al.*, 2005a, Marianski & Marianski, 2012). In some geographical regions, typically in the northern parts of Europe, the sausages can be smoked providing a characteristic flavour profile for the sausage as well as a preserving effect (Toldrá & Hui, 2015). Geographical regions, like smoking, have become synonymous with certain flavour profiles due to the ingredients added to the sausage batter formulation. Of the multitude of ingredients used in sausage production, some may have an

antimicrobial effect, such as the addition of garlic, which provides bacteriostatic properties, assisting in the preservation of the sausage (Feiner, 2006i).

Although the nomenclature of the sausages differ from region to region, in terms of product characteristics, dry fermented sausages have been allowed to rest and ferment at between 20°C and 40°C for up to 72 h after which the drying and consequently maturation process ensues, ultimately reaching a moisture content of 30 – 40 %, water activity values of *ca.* 0.88 and final pH decreases from *ca.* 5.8 to *ca.* 4.8 (Jay *et al.*, 2005a, Vignolo *et al.*, 2010, Ockerman & Basu, 2015). The method of chemical acidification may be additional to or completely replace fermentation. Chemical acidification combines, predominantly lactic acid, citric acid or Glucono-delta-lactone (GdL) to chemically acidify the sausage batter to a pre-determined pH of *ca.* 4.8, and ensures that moisture loss and water activity remains in the region of 30 – 40 % and 0.88 respectively.

2.9 Fermented sausage vs acidulated sausage

The finished product parameters for dry acidified sausages are defined by the sausage classification and are thus the same in terms of moisture content, pH and water activity for both fermented and directly acidified sausages (Ockerman & Basu, 2015). The combination of fermentation and chemical acidification is also possible. The main difference between the two acidification techniques is the rate at which the pH drops. Fermentation requires an energy source in the form of a fermentable carbohydrate as well as an optimal temperature in order for the lactic acid bacteria to grow and produce the required volume of lactic acid to decrease the pH to the required endpoint (Puolanne & Petaja, 2015). This acidification process has been reported to take up to 72 h within the optimum temperature range of *ca*. 20 - 30°C (Puolanne & Petaja, 2015). The by-products of the fermentation process have been connected with the unique flavour profile of the dry fermented sausage, typical of the organism used in the fermentation process (Ockerman & Basu, 2015). These by-products have also been associated with bacteriocins which have been reported to reduce the growth of pathogenic bacteria (Jay *et al.*, 2005b). Along with bacteriocins, the lactic acid bacteria have an antagonistic effect on the microflora in the sausage resulting in lower numbers of pathogens being able to overcome the lag phase of their growth, thus the bacterial pathogens are reduced in numbers (Jay *et al.*, 2005b).

Acidification by chemical means involves the use of acids, either in their pure form or in an encapsulated form, or chemical compounds such as GdL, in either a pure or encapsulated form, which upon hydrolysis forms gluconic acid thus reducing the pH of the sausage batter (Leroy & De Vuyst, 2009). The addition of a chemical acidulant is rapid in the pure acid form, that is, the sausage batter, when exposed to the acid, decreases in pH to the predetermined point quickly (Sebranek, 2004, Roncales, 2015). The mixing of the batter and temperature of the batter will determine how quickly the acid shall evenly distribute across the entire batter. Observations involving direct acidification, particularly pertaining to the addition of citric acid has revealed a crumbly texture, speculated to be as a result of protein denaturation. Although this denaturation could account for

some loss in water holding capacity, the texture of the finished product has been deemed unsuitable from a sausage quality point of view (Barbut, 2006). The development of acids in their encapsulated form has assisted in the acidification control as the materials used in the encapsulated acids are designed to release the acids upon heating to a core temperature greater than 55°C, the melting point of the encapsulated acids (Anonymous, 2013a, Anonymous, 2013b, Anonymous, 2013c). This, in theory, allows the sausage emulsion to bind and only be acidified after the temperature is raised some time later. The speed at which the acid is released from the encapsulating material is thus reduced and has been shown to have less of a detrimental effect on the texture of the sausage (Leroy & De Vuyst, 2009). Consequently, the addition of an acidulant, whether encapsulated or not, reduces the time of processing the product as the resting period is for the most part mitigated and the drying process, due to the denaturation of sarcoplasmic and myofibrillar proteins, can be quicker (Barbut, 2005). This process is however disadvantaged in that the characteristic flavours from the fermentation process are not able to develop in the chemically acidulated sausage.

2.10 Role of pH

As highlighted earlier, the reduction in pH from that of post rigor meat (*ca.* pH 5.8) to the final pH of the dry acidified sausage (*ca.* pH 4.8 - 4.6) is critical to the product (Puolanne & Petaja, 2015). This reduction provides the characteristic sensory profile and ensures food safety, both due to the direct effect of pH on the pathogens as well as assisting the products dehydration. Water holding capacity (WHC) is defined as the ability of post mortem muscle to retain water when exposed to external forces (Ruiz & Perez-Palacios, 2015). The WHC is influenced by the net charge of the myofibrillar proteins of the post mortem muscle. During rigor mortis, acidification reduces the net charge of the proteins closer to the isoelectric point of the muscle protein (Pisula & Tyburcy, 1996, Ruiz & Perez-Palacios, 2015). The LAB fermentation or addition of a chemical acidulant to a sausage batter, further reduces the pH of the myofibrillar proteins, even closer to the isoelectric point of the protein. The isoelectric point is the point at which the net charges on the myofibrillar proteins in neutral and consequently, the maximum volume of water is released from the muscle (Bouton *et al.*, 1971, Lawrie & Ledward, 2006a). The closer the pH to the isoelectric point of the meat protein, the greater the release of water and thus the easier the removal of moisture during dehydration which is reported to be in the range of between 5.0 and 5.5 (Bouton *et al.*, 1971, Offer & Knight, 1988, Hoffman *et al.*, 2009). The pH thus is a critical parameter in the production of dry acidified sausage as to ensure sufficient dehydration. The pH may be lowered to below the isoelectric point which often gives characteristic acidic tastes to the product. Typically, European fermented sausage with their preferred mild tastes having pH values of 5.0 – 5.2 are not uncommon. The American summer sausage however is more acidic so a pH drop to *ca.* 4.6 is more typical (Marianski & Marianski, 2012).

2.11 Role of drying

The sole purpose of drying is to reduce the water activity of the product by removing unbound water from the salami. The mechanical action on the proteins during processing along with the reduction in pH allows for a reduction in the water holding capacity of the meat protein thus making unbound water available to be removed by the drying process (Warriss, 2010c). This is achieved by balancing the humidity, temperature and air speed in an atmosphere controlled chamber to enable the removal of moisture from the surface of the sausage (Demeyer & Stahnke, 2002, Toldrá, 2012). The ideal balance is gained by matching the rate of diffusion of moisture from the centre towards the surface of the product to the rate of evaporation of moisture from surface of the product (Baldini *et al.*, 2000, Marianski & Marianski, 2012, Toldrá, 2012). Once the balance is achieved, moisture migrates to the surface of the sausage and is removed thus creating the desired dehydrating effect. Drying time is affected by the type of product, its fat content, the diameter of the sausage and the degree of drying (Toldrá, 2012). Typically when drying, the airflow is not recommended to be more than 1 m/s and the a_W of the product and the a_W of the drying air needs to be controlled in order to prevent removal of excessive water from the surface of the sausage or else case hardening may come into effect (Toldrá, 2012, Grau *et al.*, 2015).

2.12 Typical ingredients in the production of dry acidified sausages

2.12.1 Meat

Production of dry acidified sausages primarily relies on the use of pork meat although it is not uncommon to utilise horse, beef, venison, game and occasionally, seal meat (Koep, 2005, Todorov *et al.*, 2007). Typically, whole muscles are preferred specifically from the hind quarter and the lean meat is required to be trimmed of excess sinew and fat (Marianski & Marianski, 2012). The parameters pH and WHC are very important parameters to consider (Ockerman & Basu, 2015). If the pH is too high, often > 6.0 particularly in pork meat, the meat is considered DFD (Dark, Firm and Dry) consequently, the WHC is strong, resulting in tightly bound water and ultimately a higher likelihood of spoiling (Addis, 2015). DFD meat is often as a result of chronic stress and reduced glycogen levels in the animal prior to slaughter (Lawrie & Ledward, 2006a, Lawrie & Ledward, 2006b, Warriss, 2010b, Addis, 2015). PSE (Pale, Soft, and Exudate) meat shall also be avoided. This meat has a pH_u which is lower than normal, causing excessively fast water loss and subsequent reduction in colour which is undesired in the production of dry acidified sausages. Preference for slightly older animals due to their more intense red colour is recommended which producing a high quality dry acidified sausage (Toldrá, 2012).

2.12.2 Fat

Dry acidified sausage is known for the visual appearance of white fat particles (Girolami *et al.*, 2014). Due to this requirement, the importance of fat to retain its white colour, to not oxidise and to remain solid at ambient temperature is required. As a result, limited quantities of poly unsaturated fatty acids (PUFA's) are required as an increase in PUFA leads to a decrease in oxidative stability (Cluff, 2013).

Increased levels of PUFA's have also been linked to inferior dehydration, poorer texture and a negative influence on micro-organisms (Ruiz & Perez-Palacios, 2015). Traditionally, pork subcutaneous adipose tissue is desired due to its saturated nature (Ruiz & Perez-Palacios, 2015). Pork fat comprises on average, 47% mono unsaturated fatty acids (MUFA's), 45% saturated fatty acids (SFA's) and 10% PUFA's (Warnants *et al.*, 1998). Although beef and lamb fat have been utilised in formulations, care should be taken to minimise the PUFA's as well as the lowest possible level of oxidation in order to reduce rancidity of the fat, thus pork is preferred (Toldrá, 2012). Conversely, the flavour compounds in dry acidified sausages have been linked to the oxidation of PUFA's, however, limited PUFA's is still recommended (Leroy *et al.*, 2013). Fat inclusion into dry-acidified sausages vary depending on the region of production so no limits per se exist. Processing, either by mincing or bowl cutting should commence at *ca.* -5°C to -7°C in order to prevent fat smearing as to ensure defined particles in the final product (Demeyer & Stahnke, 2002, Incze, 2010).

2.12.3 Acidulants

Glucono-delta-lactone (GdL)

GdL is a derivative of glucose, an ester of gluconic acid (Feiner, 2006b). In meat applications, GdL is used as an acidity regulator due to its hydrolysis reaction in the presence of water to form gluconic acid (Ricke & Keeton, 1997). Practically, GdL is added to salami at between 3 – 12 g per kg of salami, where 1 g GdL lowers the pH by roughly 0.1 units in 1 kg salami (Feiner, 2006i). Often, GdL is added in lower concentrations as a colour enhancer as the reduction in pH leads to more nitric oxide formation (Feiner, 2006j). When acidifying a salami, 8 – 10 g GdL per kg salami can reduce the pH from 5.6 to between 4.5 – 4.7. In this acidification process, gluconic acid is produced as well as some residual acetic acid often as a result of lactobacilli (Ricke & Keeton, 1997). Caution must be applied when manufacturing with GdL as too much may cause a distinct bitter taste in the final sausage product (Feiner, 2006b).

Acids, commonly lactic acid, citric acid or a combination of the two may be added to the sausage batter formulation, either for the purpose of pure chemical acidification or in addition to fermentation (Varnam & Sutherland, 1995a, Feiner, 2006b). The form of these acids may be either pure or encapsulated (Feiner, 2006b). Chemical acidification is often utilised for the purpose of reducing the acidification time (Sebranek, 2004). The use of encapsulated acids have been shown to render a product similar in texture to that of LAB fermented product as opposed to the more crumbly product when liquid lactic acid was used (Barbut, 2006). Salami sticks using lactic and citric acid were successfully developed resulting in a functional product being able to be produced although the sensory appeal preferred the pH of 5.2 as opposed to 4.6 (Quinton *et al.*, 1997). Preliminary studies are required in order to identify the concentration of acidulant required in order to realise a specific final pH (Barbut, 2005).

2.12.4 Functional ingredients

Sugars

Glucose is utilised by LAB which results in the production of by-products of which, lactic acid is of fundamental importance (Jay *et al.*, 2005a, Jay *et al.*, 2005c, Paramithiotis *et al.*, 2010). Biochemical pathways enable LAB to ferment a sausage batter without any additional carbohydrates to a pH ca. 5.0, depending on the initial pH prior to fermentation. The addition of glucose, a monosaccharide, or the more complex disaccharide, sucrose, will enable the pH to decrease further than a pH of 5.0 (Paramithiotis *et al.*, 2010, Puolanne & Petaja, 2015). The speed of fermentation is a result of the temperature of the fermentation, the sugar merely becomes the limiting factor as to the pH endpoint of the fermentation (Varnam & Sutherland, 1995a). Consideration as to the type of sugar added is required to be assessed as an overly rapid fermentation should be avoided. Typically, carbohydrates are added at 0.4% - 0.8%, however, sometimes glucose has been included up to 2% (Varnam & Sutherland, 1995a).

Salt

Salt, specifically sodium chloride, is the oldest additive used in food processing and particularly meat preservation with historical records going back to ancient civilisations (Toldrá, 2012). Salt provides three main functions in meat processing: 1 – it reduces water activity (a_W) due to its hygroscopic nature, 2 – salt provides a characteristic salty taste, often influenced by the minerals in the particular salt and 3 – salt increases the solubility of the myofibrillar proteins (Toldrá, 2012). Furthermore, salt assists in increasing water retention, facilitates the solubilising of the proteins, reduces enzyme activity and enhances the occurrence of the oxidative process (Ruiz & Perez-Palacios, 2015). Often the inclusion of salt into meat products is around 2 - 4% and a concentration of 2.5 - 3.0% may reduce the initial a_W to *ca.* 0.96 (Varnam & Sutherland, 1995a). The combination of salt and nitrates assist as major hurdles in the inhibition of pathogen micro-organisms ensuring the safety of dry acidified sausages (Varnam & Sutherland, 1995a).

Sodium Nitrate and Nitrite

Nitrite and nitrate, most commonly formed as a salt with sodium provides two crucial parameters in the manufacture of dry acidified sausage. Firstly, nitrate and nitrite have an inhibitory effect on pathogen micro-organisms and secondly, an effect on colour stability (Jay *et al.*, 2005b, Parthasarathy & Bryan, 2012). During fermentation, nitrate is used by the bacteria and reduces to nitrite. Additionally, nitrite can also be added although in fermentation the requirement for the bacteria to reduce nitrate cannot be overemphasised. The nitrite ion is both a reducing and oxidising ion which, in an acid environment, can ionise into nitrous acid and subsequently decompose into nitric oxide (Jay *et al.*, 2005b, Parthasarathy & Bryan, 2012, Toldrá, 2012, Pegg & Honikel, 2015*).* Often, an antioxidant can be added to the formulation. The primary role of the addition of an antioxidant in the form of either erythrobate or ascorbate is to reduce nitric dioxide to nitric oxide in order to ensure the maximum quantity of nitric oxide is produced and available (Jay *et al.*, 2005b,

Pegg & Honikel, 2015). Nitric oxide reacts with myoglobin to produce nitroso-myoglobin, the red pigment typical of cured products. Nitrite has been proven to be effective against *C. botulinum* and *S. aureus*, where a lethal dose of 80 - 140 ppm is required. Nitrate is, however, somewhat ineffective against *Enterobacteriaceae* and LAB (Jay *et al.*, 2005b, Feiner, 2006j). This would be preferred as LAB would be able to flourish in the environment. In South Africa, nitrate in the final product is limited to below 160 ppm residual nitrate (Anonymous, 1977).

Lactic Acid Bacteria (LAB)

When considering bacteria in the fermentation of dry fermented sausages, traditional processes primarily rely on the natural microflora although modern processors encourage the use of starter cultures (Ricke & Keeton, 1997). Developments in pure strains of starter cultures have been developed and more recently blends of various micro-organisms have been provided as starter cultures to assist in fermentation, hygiene and flavour development (Leroy *et al.*, 2015). Typical starter cultures include strains of *Lactobacillus plantarum*, *Pediococcus cerevisiae* or *Pediococcus acidilactici* (Ricke & Keeton, 1997, Jay *et al.*, 2005a). Some *Micrococcus* and *Staphylococcus* species, along with *Lactococcus* is a common practice in Europe (Jay *et al.*, 2005a). These starter cultures are often added with some form of sugar, normally glucose and sucrose in order to ferment to a specific endpoint (Leroy *et al.*, 2015). The importance of the addition of lactobacilli cannot be understated and have been linked to both the safety and specific flavours of dry acidifier sausages (Kröckel, 2013).

2.12.5 Flavour components

Flavour of dry acidified sausages comes from three sources, firstly, fermentation and enzymatic degradation provide volatile and non-volatile compounds which contribute to the flavour of the product (Toldrá, 2012). Secondly, the acid, whether a by-product of LAB fermentation or from additional chemical acidulants provide an apparent acid taste and is dependent on the concentration of the acid included (Kröckel, 2013). Chemical acidulants such as GdL has been known to provide a bitter taste when used in excess, the same can be said for citric and lactic acid (Jafari & Emam-Djomeh, 2007). Finally, the addition of spices and smoking contribute considerably to the flavours in dry acidified sausages. It is not uncommon, specifically in the more traditional artisanal products to include red wine, garlic, chilli, mustard seeds, black pepper and paprika, which all contribute to the unique taste of the sausage product (Feiner, 2006i, Toldrá, 2012, Chi & Wu, 2015). One important aspect to note is that spices can be highly contaminated with pathogen micro-organisms. Typically in processing, the spices used should be sterilised either by steam, irradiation, UV light, chlorine, hydrogen peroxide or high pressure processing in order to ensure that minimal contamination of pathogens is exposed to the product (Feiner, 2006i, Chi & Wu, 2015).

2.13 Processing parameters of dry acidified sausage

In order for dry acidified sausages to be produced, particular environmental parameters are required to be utilised as to ensure the acidification and drying process performs correctly. For

chemically acidified and more importantly fermented sausage production, these controls are crucial in order to, in the case of fermented sausages, support the growth of lactic acid bacteria colonies. Post acidification, these parameters are crucial to ensure efficient drying on the sausage without case hardening. Case hardening is a phenomena created when the rate of moisture removed from the outer surface of the sausage is greater than the rate of moisture migration from the core of the sausage (Varnam & Sutherland, 1995a, Mikac *et al.*, 2008). This in effect dehydrates the an outer layer on the sausage which results is sausage shrinkage and more importantly entraps the moisture within the centre of the sausage providing conditions for spoilage and pathogen microorganism growth (Mikac *et al.*, 2008).

In terms of dry acidified sausage production, of initial importance is the parameter of temperature. Temperature forms part of the cold chain and in instrumental in order to control the safety of the product (Raab *et al.*, 2011). The control of pathogen numbers prior to production of the sausage assist in minimising the growth of pathogens during the acidification period. Of concern is the meat and fat being stored at refrigerated temperatures below 4°C prior to processing (Feiner, 2006b). Once the meat and fat is cubed for processing, the norm is to freeze the meat to between -10°C and -5°C prior to making the sausage batter (Feiner, 2006b). Ideally, whether chopping or grinding the meat, the fat is required to be kept at or below freezing otherwise it is prone to smearing which is not ideal in terms of final sausage quality (Feiner, 2006b). When fermenting the sausage, often temperatures are controlled anywhere between 20 – 40°C (Puolanne & Petaja, 2015). If encapsulated acids are used, the melting point of the acid is required to be reached which is about 55°C (Anonymous, 2013a, Anonymous, 2013b, Anonymous, 2013c). In general, cooler temperatures are preferred when drying the product although lactic acid growth is encouraged so temperatures between *ca.* 15 – 35°C are employed.

Relative Humidity (RH) is a parameter which is crucial in terms of moisture removal from the sausage. Initial relative humidity is preferred to be high as to assist lactic acid bacteria growth (Grau *et al.*, 2015). Once acidified, the relative humidity is required to be reduced in order to facilitate the removal of moisture, where the RH is constantly maintained lower that the a_W of the salami (Feiner, 2006b). A fine balance is required between the air flow and the humidity of the chamber as this interaction needs to match the rate of water diffusion from the sausage as to ensure moisture is removed but there is no case hardening (Kottke *et al.*, 1996, Feiner, 2006b, Grau *et al.*, 2015). At the driest point in the production process, the RH is set to between 75% and 72%.

Time, as a parameter is crucial in the production of dry acidified sausages as it must allow for the development of the lactic acid bacteria, acidification resulting in the reduction in pH as well as the drying process. Typical fermentation may last anywhere from 12 h up to several days, 72 h is not uncommon, after which the dehydration can commence (Varnam & Sutherland, 1995a, Puolanne

& Petaja, 2015). Dehydration and maturation may commence for 3 – 6 weeks in the case of fermented sausages under ideal conditions (Puolanne & Petaja, 2015). The diameter of the sausage influences the dehydration time substantially, whereby the smaller the diameter, the faster the drying time of the sausage (Varnam & Sutherland, 1995a). Time of fermentation can be drastically reduced when chemically acidifying the product as there is an instant drop in pH and no requirement for lactic acid bacteria to reproduce in order to decrease the pH (Sebranek, 2004, Leroy & De Vuyst, 2009).

The primary mode of action in the acidification process by homo-fermentative lactic acid bacteria is the reduction of pH, a well understood process (Jay *et al.*, 2005a). The aim in the processing of dry acidified sausages is to reduce the pH from that of post rigor meat *ca.* 5.8 to a pH of < 5.2 (Feiner, 2006b). Ockerman and Basu (2015) indicated that the American method of fermentation is based on a preference of a more tangy product and as such, the required pH is normally within the pH range of 5.3 – 4.6, in contrast to the European style which is considered more mild with a pH value between 5.6 – 5.3. Feiner (2006j) indicates that the pH should be reduced to below 5.2 within 48 h.

Mass, as a processing parameter is a good indication of moisture loss from the system. The requirement of dry acidified sausages is to loose moisture, typically in the region of > 30% weight loss, equating to between 20 - 50% moisture loss in order to be shelf stable with around 30 - 40% moisture present (Jay *et al.*, 2005a, Ockerman & Basu, 2015). The aim thus is to regulate the moisture loss until it has lost 30% moisture at which point the product parameters of pH and a_W can be confirmed and the product can be consumed.

2.14 Physico-chemical quality parameters of dry – acidified sausage

The summary below is a revision of common physico-chemical quality parameters used in the definition and assessment of the quality of dry-acidified sausages. Publications on quality of dry–acidified sausage often comprise of both chemical and physical tests. These tests for the most part include pH and water activity (a_w), as well as proximate analysis which include the measurement of moisture content, total protein content, fat content and total ash content. Other measures which are present but are not as frequent throughout publications is texture profile analysis, sodium chloride determination, soluble nitrogen indicating levels of proteolysis, colour measurements and collagen (Berry *et al.*, 1979, Casiraghi *et al.*, 1996, Soyer *et al.*, 2005, Ercoşkun & Özkal, 2011, van Schalkwyk *et al.*, 2011, Cluff, 2013, Ciuciu Simion *et al.*, 2014).

Quality is a concept which defines if the product is of a specified standard which; 1 – defines the product and 2 – meets the requirements of the consumer. Improvements in quality thus ensure the product is conforming to the specification which has been developed to meet the consumers need (Arnold & Chapman, 2001). In the case of dry-acidified sausage, the main parameters of concern is that the pH and water activity reach the desired levels to ensure the product is safe, that is a pH *ca.* 4.8 and a_W *ca.* 0.88, respectively (Ockerman & Basu, 2015). Of secondary concern, the

sausage must lose moisture, between 25% and 30% in order to be within the predefined safety limits but must be maintained within moisture limits in order to ensure the product does not become too dry and consequently to hard (Ockerman & Basu, 2015).

2.14.1 pH

The measurement of pH is considered crucial as a quality parameter, particularly due to its influence on the water holding capacity resulting to the effect on the drying of the product and the effect the lower pH has on the pathogenic micro-organisms (Bouton *et al.*, 1971). Method of pH measurements differ, Casiraghi *et al.* (1996) inserted the pH probe directly into the sausage to record pH, whereas Soyer *et al.* (2005) mixed 10 g of sample with 90 mL distilled water, and allowed for the solution to equilibrate for 10 min before measuring the pH. Ercoskun and Ozkal (2011) mixed 10 g of sample with 100 mL distilled water after which the solution was homogenised and the reading of pH was taken. The direct measurement of pH in the sausage is a common practise as described by various authors (Houben & van 't Hooft, 2005, van Schalkwyk *et al.*, 2011). Muscle pH measurements in some meat science research, mix meat samples with sodium iodoacetate solution (Jeacocke, 1977). When considering the measurement of pH, two areas of concern are required to be addressed, namely the calibration of the equipment and the temperature of measurement and calibration (Jansen, 2001, Mettler-Toledo, 2007). The basis of pH measurements is the Nernst equation, a mathematical representation of the hydrogen ion activity in the solution (Walczak *et al.*, 1997). Temperature plays a crucial role in the Nernst equation (Walczak *et al.*, 1997). Some probes have built-in temperature sensor which can allow for the calculation of the corrected pH values. Other probes do not have this sensor and some of the meters are not built to compensate for the differential in temperature but rather rely on the experiment to be conducted at a specific range (Mettler-Toledo, 2007, Thermo-Scientific, 2013). Due to the nature of the pH values, the further one deviates from the median of pH 7, the greater the effect the temperature will have on the actual pH reading as observed in the differences in slope values when calibrating at a differing temperatures (Thermo-Scientific, 2013).

2.14.2 Water activity (a_w)

Water activity (a_W) is defined by physics as a ratio of the vapour pressure of a food substrate to the vapour pressure of water at the same temperature (Jay *et al.*, 2005d). As products dry, they lose moisture, as such, the vapour pressure of the food substrate decreases (Toldrá, 2012). Meat has a a_W of *ca.*0.98 however, the addition of salt, spices, acid and subsequent dehydration, decreases the a_W to below 0.90, often as low as 0.82 (Jay *et al.*, 2005d, Feiner, 2006a, Toldrá, 2012). Jay *et al*. (2005d) suggest that *Staphylococcus aureus* is one of the hardiest bacteria when considering a_W and can grow as low as 0.86 whereas *Clostridium botulinum* cannot grow below 0.94. Pure water has an a_W of 1.00 but when a pure water is made into a solution with 22% NaCl, the a_W decreases to 0.86, thus salt has a direct impact of the a_W of the food substrate (Jay *et al.*, 2005d). In terms of meat application, the a_W value indicates how much water is unbound and free for the microflora to

utilise (Feiner, 2006a). Most notably, the a_W parameter in dry acidified sausages is one of the hurdles assisting in the preservation of the product which, with salt, and pH combine to produce a shelf stable safe product (Feiner, 2006k).

2.14.3 Moisture loss

The measurement of moisture loss is a quantitative indication that the product is drying to the correct levels and as such, reaching the correct a_W. Due to the pH, reducing the muscle protein to the isoelectric point of meat, the free water in the protein becomes available to be removed from the product (Mauriello *et al.*, 2004, Ruiz *et al.*, 2014). The air flow and relative humidity facilitates the removal of this unbound water (Toldrá, 2012). Provided there is no case hardening and the sausage dehydrates evenly from its core, the mass loss is assumed to be the moisture lost and the resultant product should in effect be within its moisture and a_W parameters. Dry acidified sausages aim for > 30% mass loss during the drying and ripening process (Toldrá, 2012, Ockerman & Basu, 2015).

2.14.4 Texture analysis

The primary method of quantitative analysis of texture is the use of texture profile analysis (TPA) (Bourne, 1978). This methodology involves cyclic compression tests which mimic the action of chewing. Most TPA has been implemented using the methodology of Bourne (1978), however, many constants have been varied in terms of core diameter, level of compression, height of compression, compression force applied, diameter of the anvil in instances of research (Casiraghi *et al.*, 1996, Spaziani *et al.*, 2009, van Schalkwyk *et al.*, 2011). The parameters often measured and reported are hardness 1 (the first compression), hardness 2 (the second compression), cohesiveness, gumminess, adhesiveness and springiness (Ruiz de Huidobro *et al.*, 2001, Ruiz de Huidobro *et al.*, 2005).

2.15.5 Colour

Perception of meat quality, whether it be fresh or processed, is often attributed to the observed colour (Mancini & Hunt, 2005, Tapp *et al.*, 2011). As reviewed by Tapp, Yancey and Apple (2011), most research is conducted using Minolta (60.0%) over Hunter (31.6%) colorimeters whereby CIE L*, a* and b* colour coordinates are measured, ultimately allowing for the quantification of the colour. Some dry-acidified sausage have used these methodologies to measure the colour of the sausages (Ercoşkun & Özkal, 2011). Colour perceived in the dry acidified sausages is in part stable due to the decrease in pH as well as the inclusion of nitrates (Fadda *et al.*, 2010, Cluff, 2013). Notably, the fat quality and quantity affects the measurement of light particularly when the fat particles are largely visible as in the case for dry-acidified sausages (Irie, 2001).

2.15.6 Proximate analysis

Proximate analysis involves the quantification of moisture, protein, fat and ash content (Warriss, 2010c). The analysis of proximate composition of dry acidified sausages is one of the more common analyses in terms of quality determination as seen in literature (Ikonic *et al.*, 2010, Warriss, 2010c, van Schalkwyk *et al.*, 2011, Lorenzo *et al.*, 2012, Živković *et al.*, 2014). Moisture is of specific value

pertaining to dry acidified sausages as this enables the quantification of moisture and provides the qualification as to whether the sausage falls within the classification of dry fermented sausages or not. The rest of the values become descriptive due to formulations differing in fat content, resulting in reciprocal variation in protein and ash values. Proximate analysis, although indicative, is a crude methodology as many variables are able to be altered which can affect the outcome of the results. Typically, methods used include those described by AOAC (American Association of Analytical Chemists) as well as fatty acid solvent extraction methods as described by Lee *et al.* (1996) are employed to standardise the testing of the samples pertaining to proximate analysis.

2.16 Food safety

Food safety is, in a broad sense, the commitment for producers to ensure that the products they produce is safe for the consumer to consume encompassing the entire food chain from production to consumption (WHO, 2015). This ensures that risks associated with the product being produced are considered prior to manufacture and mitigating procedures are developed to prevent hazards within products thus rendering the product safe to consume. Although it is every person's right to have safe food, methodical systems have been developed and in some cases enforced to ensure that the product produced, conforms to food safety standards. The basis of these systems involve the Hazard Analysis Critical Control Point (HACCP) approach (SABS, 2007, Pearson & Dutson, 2012). The HACCP system has evolved to incorporate Pre-Requisite Programs (PRP's) and Good Manufacturer Practise (GMP's) into a comprehensive Food Safety Management System (FSMS), the likes of which culminate in ISO 22000:2005 along with ISO/TS 22002-1:2009 the technical specification, defining the PRP's required in the FSMS (ISO, 2005, SABS, 2007, ISO, 2009). More recently, the Food Safety Modernisation Act (FSMA), recently published by the American government, puts emphasis on Hazard Analysis and Risk-based Preventive Controls (HARPC), essentially implementing a system which is based on preventing the introduction and proliferation of the hazard prior to manufacture (FDA, 2015)

The HACCP process involved a closed loop approach where by the seven principles of HACCP ensure the development of the FSMS using a risk based approach, the implementation and monitoring of the system and the reassessment and updating of the system which is the final step ensuring that either the system is working or the system requires redesigning, thus closing the loop (SABS, 2007).

When applying the HACCP principles and specifically the hazard analysis, the hazards to be considered fall into three categories namely, physical, chemical and biological (SABS, 2007). When producing meat and meat products, the physical hazards, although important to consider can often be easily eliminated due to carcass washing and factory GMP's which enable all physical hazards, be it glass, metals, pests, dust, etc. to be easily removed thus not present to contaminate the product. Although risks of physical contamination are increased during value added production, more of a concern is the chemical and biological contaminants and specifically from a biological point of view,

the micro-organisms, as these may directly cause harm to the consumer and they could potentially not be visibly seen.

2.16.1 Microbiologically safe

Microbiology involves all micro-organisms, both 'good' and 'bad'. From a food safety perspective, the major concern is that of pathogenic micro-organisms and secondary to that is spoilage organisms (Jackson *et al.*, 1997, Montville & Winkowski, 1997, Nørrung *et al.*, 2009). Pathogens are defined as micro-organisms which are harmful to human health and may cause disease (Jay *et al.*, 2005e). In terms of classification, there are many numbers of pathogens which cannot all be tested. Notably, indicator organisms have been chosen to assist in monitoring the pathogens (Smoot & Pierson, 1997, Jay *et al.*, 2005f). These indicator organisms range in terms of growth parameters and sources. In the case of meat products and specifically dried acidified sausages, the product is exposed to air so it is expected that the predominant micro-organisms present will be aerobic in nature (Smoot & Pierson, 1997). The meat is processed by hand and may be exposed to faecal matter, as such *E. coli*, *Enterobacteriaceae* and *Coliforms*, are good indicators of hygiene or the raw materials as well as the effectiveness of the process to produce the end product (Jay *et al.*, 2005f).

Total aerobic plate count

Total Aerobic Plate Count (TAPC) is a representation of total aerobic mesophilic bacteria on the product and is an indicator of the food quality (Aycicek *et al.*, 2006). Although TAPC does not supply specific counts of pathogen organisms, the total microflora can be analysed whereby high counts of particularly TAPC are indicative of contamination or unsuitable time/temperature storage conditions, i.e. a possible break in the cold chain (Anonymous, 2015b). Some products, the likes of milk and fermented products may naturally have higher TAPC values when compared to cooked and chilled RTE products. South African microbial guidelines have thus recommended a total count of < 200 000 $CFU.g^{-1}$ on cold smoked or fermented meal items such as salami, a dry acidified sausage (Anonymous, 2015b).

Escherichia. coli

Escherichia coli is facultative anaerobic, gram-negative, non-spore forming bacterium, classed within the *Enterobacteriaceae* group of micro-organisms (Doyle *et al.*, 1997b, Lawley *et al.*, 2008). The most common *E. coli* pathogen of concern is *E. coli* O157:H7, an Enterohemorrhagic *E. coli* (EHEC) serotype, a strain of Verotoxic *E. coli* (VTEC) (Riley *et al.*, 1983, Doyle *et al.*, 1997b, Dalzini *et al.*, 2014). *E.coli* O157:H7 has been found in dry salami and apple cider both of which have low pH < 4.5 and pH 3.6 – 4.0 respectively (Doyle *et al.*, 1997b). Dalzini *et al.* (2014) has cited a few incidence involving the presence of VTEC in the presence of dry cured sausage but not limited to, Canadian Genoa salami, Swedish fermented sausages, Italian dry fermented salami and pepperoni (Alexander *et al.*, 1995, Riordan *et al.*, 1998, MacDonald *et al.*, 1999, Williams *et al.*, 2000, Conedera *et al.*, 2007, Sartz *et al.*, 2008). *E. coli* is synonymous with the intestinal tracts of humans and warm blooded animals (Doyle *et al.*, 1997b, Jay *et al.*, 2005g). The prevalence of *E. coli* in food products is an

indication of faecal contamination in the production process (Lawley *et al.*, 2008). Although there are a few different pathogen strains which may lead to foodborne gastroenteritis, typically in food processing, *E. coli* is a good indicator organism pertaining to the hygiene of the product (Jay *et al.*, 2005g, Lawley *et al.*, 2008). *E. coli* in terms of growth conditions for the species, have a temperature range of 7 - 46°C, a pH range of 4.4 - 9.0 although the optimum is 6.0 - 7.0 and a minimum a_W of 0.95 (Anonymous, 1999). The South African guidelines indicate that *E. coli* should not be present in cooked products or smoked fermented meat items although a suggestion of items requiring further processing may have an *E. coli* limit of < 10 CFU.g^{-1} (Anonymous, 2015b).

Coliforms

Coliforms is primarily an indicator of pathogens in water, suggested by Smith, T. in 1895 as a measure of drinking water potability, based on the research by Escherich in 1885 as cited by Jay *et al.* (2005h) (Escherich, 1885, Smith, 1895). Coliforms are gram-negative non-spore forming rods which ferment lactose within 48 h and typically represent four to five genera of the *Enterobacteriaceae* family, of which *E. coli* is one (Jay *et al.*, 2005f). Coliforms have a wide temperature range revealing growth as low as -2°C and as high as 50°C. The reported growth range of coliforms in terms of pH is 4.4 - 9.0 and the fermentation of lactose typically produces gas (Jay *et al.*, 2005f). Due to the ease of growth and the fact that coliforms grow on many mediums, the bacterium is a useful indicator of questionable sanitation and product quality as it could grow on many food products (Smoot & Pierson, 1997, Jay *et al.*, 2005f). South African guidelines recommend < 200 CFU.g^{-1} on acidified meat products (Anonymous, 2015b).

Listeria monocytogenes

Listeria monocytogenes is of concern in the food industry, not because of the source of contamination but due to the fact that it can grow in somewhat hostile environments, particularly at refrigerated temperatures between 2°C and 4°C, it is resistant to low pH ≥ 4.5 and high salt concentration of ≤ 30% NaCl, is micro aerobic and in reported to be psychrotropic surviving freezing at -18°C (Rocourt & Cossart, 1997, Jay *et al.*, 2005h). Recommended specifications for refrigerated RTE foods require either a pH < 5.0 or a_W < 0.92 or a combination of pH between 5.0 – 5.5 and a_W < 0.95 (Lawley *et al.*, 2008). *Listeria* is a gram-positive, non-spore forming, catalase positive rod (Jay *et al.*, 2005h, Lawley *et al.*, 2008). *L. monocytogenes* also has the potential to be present in all forms of raw food and has been found present in dairy, ice-cream, RTE cooked foods and fermented meat products (Lawley *et al.*, 2008). The processing parameters are often employed to reduce the risk of *L. monocytogenes* growth. Heating above 54°C has revealed a reduction in surviving cells however, modified atmosphere packaging has little effect on the growth of *L. monocytogenes* (Rocourt & Cossart, 1997). *L. monocytogenes* has a maximum survival temperature of 45°C and studies indicate that the lowest temperature of survival was 1.1 ± 0.3°C with a range of growth between 0.5°C and 3.0°C (Lawley *et al.*, 2008). From a susceptibility point of view, the immunocompromised, elderly and pregnant are at the greatest risk (Lawley *et al.*, 2008). The present guideline in South Africa required

the *L. monocytogenes* to be < 10 CFU.g^{-1} which is below the infective dose which is considered to be > 10^3 CFU.g^{-1} (Lawley *et al.*, 2008, Anonymous, 2015b).

Staphylococcus aureus

Staphylococcus aureus, the microorganism responsible for staphylococcal food poisoning, is a spherical, gram-positive organism from the *Micrococcaceae* family (Jablonski & Bohach, 1997). The predominant mode of contamination is through human contact and subsequent temperature abuse during processing or during refrigerated storage will encourage the proliferation of the pathogen (Lawley *et al.*, 2008). This results from inadequate hygiene practises including hand washing, particularly on food which is exposed to direct contact with the producers hands (Jablonski & Bohach, 1997). Contamination is also linked with equipment such as meat grinders, cutting blocks saw blades and knives, particularly ones which have not been cleaned effectively (Jablonski & Bohach, 1997). *S. aureus* is considered osmotolerant and has been reported to grow in 3.5 M NaCl and can survive at a water activity of less than 0.86. Regarding pH, the growth range of *S. aureus* is 4.0 – 9.8, however, the optimal growth range is 6.0 – 7.0 (Jablonski & Bohach, 1997, Jay *et al.*, 2005i). *S. aureus* is also considered a mesophyll and has been recorded as being able to grow at a temperature of 6.7°C, however, enterotoxins are known to be produced between 10 and 46°C (Jay *et al.*, 2005i). Typically, the concentration of the enterotoxin producing *S.aureus* is required to be in the region of 10^5 – 10^6 CFU.g^{-1} in order for staphylococcal food poisoning to occur. Often the effect of food poisoning occurs within 0.5 hours to 7 hours of consumption of the toxin (Lawley *et al.*, 2008). Foods commonly associated with outbreaks pertaining to *S. aureus* are dairy based products, hams and cured meats as well as corned beef, bacon, cooked meats, poultry and sausages (Lawley *et al.*, 2008). Although the guidelines specified by the South African Department of Health suggests less than 100 CFU.g^{-1} as a guideline for food safety, an effective dose of greater than 10^5 organisms per gram of food consumed is required in order to produce the Staphylococcal enterotoxin which is required for the food poisoning symptoms (Jablonski & Bohach, 1997, Anonymous, 2015a).

2.16.2 Chemically safe to consume

Chemicals, as for micro-organisms, can be inherent or added and in both cases are required to be controlled as per HACCP requirements (SABS, 2007). Each ingredient used in the production of meat and meat products should be considered for their potential inherent chemical hazard risk as addressed by a thorough risk assessment and hazard analysis (SABS, 2007). These include but are not limited to heavy metals, allergens, chemical residues from preservation methods such as sulphur dioxide or regulated limits on levels of direct chemical preservatives, of which nitrates and nitrites are dominant (Toldrá & Reig, 2012).

In sausage processing, particularly in processed sausages whether heated or fermented, the use of nitrates and nitrites is extensive if not essential (Skjelkvale & Tjaberg, 1974, Sebranek & Bacus, 2007). Although these have a positive effect of food safety as they retard microbiological growth, the concentrations of the chemicals must be considered (Honikel, 2008, Sindelar &

Milkowski, 2012, Pegg & Honikel, 2015). South African national legislation, notably R965/1977 – Regulations – preservatives and antioxidants, regulates this to 160 ppm in the formulation as measured by residual sodium nitrate concentration (Anonymous, 1977).

Ultimately the risk analysis is required to consider all potential risks within the production of the specific product and the risks are required to be mitigated by implementing PRP's, Operational PRP's (OPRP's) and critical control points (CCP's) in order to exclude of control the hazard so as to ensure the safety of the product during production is not compromised (ISO, 2005, SABS, 2007, ISO, 2009).

2.17 Concluding remarks

There is potential to gain an understanding of value added game meat products in South Africa. Although on an artisanal basis the understanding of fermentation is crucial, from an industrial perspective, the use of acidulants in quickly producing a safe, functional product is warranted. There is scope to produce more value added type products with game meat and a requirement for a greater knowledge base on the production of chemically acidified game meat dry acidified sausage. The relationships between the dry acidified product characteristics along with the processing conditions specifically regarding acidulant concentrations in order to create a functional products from a physico-chemical point of view is proposed.

2.18 References

Action, J.C. & Dick, R.L. (1976). A Research note, composition of some commercial dry sausages. J*ournal of Food Science,* **41**, 971-972.

Addis, M. (2015). Major causes of meat spoilage and preservation techniques: a review. *Food Science and Quality Management,* **41**, 101-114.

Alexander, E.R., Boase, J., Davis, M., Kirchner, L., Osaki, C., Tanino, T., Samadpour, M., Tarr, P., Doldoft, M., Lankford, S., Kobyashi, J., Stehr-Green, P., Bradley, P., Hinton, B., Tighe, P., Pearson, B., Flores, G.R., Abbott, S., Bryant, R., Werner, S.B. & Vugia, D.J. (1995). *Escherichia coli* O157: H7 outbreak linked to commercially distributed dry-cured salami. Washington and California. 1994. *Centers for Disease Control Prevention. Morbidity and Mortality Weekly Report,* **44**, 157.

American Meat Institute (1982). Good Manufacturing Practices, Fermented Dry and Semi-Dry Sausages. Washington, DC: AmericanMeat Institute.

Anonymous (1977). R 965. Regulation: Preservatives and Antioxidants. (edited by Department of Health). Pretoria, South Africa.

Anonymous (1999). Microbiological guidelines for ready to eat foods. In: *Food Watch Western Australia Food Monitoring Program*. Australia.

Anonymous (2013a). MeatSure 333 (Encapsulated Citric Acid) Specification and Safety data sheet. Pp. 1-3;1-6. New Hampton, NY, USA: Balchem Corporation.

Anonymous (2013b). MeatSure 496 (Encapsulated Glucono Delta Lactone) Specification and Safety data sheet. Pp. 1-3;1-6. New Hampton, NY, USA: Balchem Corporation.

Anonymous (2013c). MeatSure 509 (Encapsulated Lactic Acid) Specification and Safety data sheet. Pp. 1-3;1-6. New Hampton, NY, USA: Balchem Corporation.

Anonymous (2015a). Sausage Types. [Internet document].URL http://www.meatsandsausages.com/sausage-types. Accessed 13.09.2015

Anonymous (2015b). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. Pretoria, South Africa: Department of Health. [Internet document].URL http://www.health.gov.za/index.php/shortcodes/2015-03-29-10-42-47/2015-04-30-09-10-23/2015-04-30-09-11-35/category/212-guidelines-training-manuals-and-reports. Accessed 20/08/2015.

Arnold, J.R.T. & Chapman, S.N. (2001). Total Quality Management. In: *Introduction to Materials Management*. Pp. 428-455. Columbus, Ohio, USA: Prentice Hall.

Aycicek, H., Oguz, U. & Karci, K. (2006). Determination of total aerobic and indicator bacteria on some raw eaten vegetables from wholesalers in Ankara, Turkey. *International Journal of Hygiene and Environmental Health,* **209**, 197-201.

Bajovic, B., Bolumar, T. & Heinz, V. (2012). Quality considerations with high pressure processing of fresh and value added meat products. *Meat Science,* **92**, 280-289.

Baldini, P., Cantoni, E., Colla, F., Diaferia, C., Gabba, L., Spotti, E., Marchelli, R., Dossena, A., Virgili, E., Sforza, S., Tenca, P., Mangia, A., Jordano, R., Lopez, M.C., Medina, L., Coudurier, S., Oddou, S. & Solignat, G. (2000). Dry sausages ripening: Influence of thermohygrometric conditions on microbiological, chemical and physico-chemical characteristics. *Food Research International,* **33**, 161 - 170.

Bañón, S., Serrano, R. & Bedia, M. (2014). Use of *Micrococcaceae* combined with a low proportion of Lactic Acid Bacteria as a starter culture for salami stuffed in natural casing. *CyTA-Journal of Food,* **12**, 160-165.

Barbut, S. (2005). Effects of chemical acidification and microbial fermentation on the rheological properties of meat products. *Meat Science,* **71**, 397-401.

Barbut, S. (2006). Fermentation and chemical acidification of salami-type products - effect on yield, texture and microstructure. *Journal of Muscle Foods,* **17**, 34-42.

Berry, B.W., Cross, H.R., Joseph, A.L., Wagner, S.B. & Maga, J.A. (1979). Sensory and physical measurements of dry fermented salami prepared with mechanicaly processed beef product and structured soy protein fiber. *Journal of Food Science,* **44**, 465 - 468

Birss, C., van Deventer, J.D., Hignett, D.L., Brown, C., Gildenhuys, P. & Kleinhans, D. (2014). Approval: Bontebok conservation, translocation and utilisation policy (BCTUP). Western Cape, South Africa: Cape Nature. [Internet document].URL http://www.capenature.co.za/wp-content/uploads/2015/07/2014-Bontebok-Policy270714-2.pdf. Accessed 10-09-2015.

Bothma, J., dP. (2010a). Objectives. In: *Game Ranch Management,* 5th ed. (edited by J. Bothma, dP. & J.G. Du Toit). Pp. 27-32. Pretoria, South Africa: Van Schaik.

Bothma, J., dP. (2010b). Harvesting wild animals. In: *Game Ranch Management,* 5th ed. (edited by J. Bothma, dP. & J.G. Du Toit). Pp. 482-485. Pretoria, South Africa: Van Schaik.

Bothma, J., dP. (2010c). Wildlife meat production. In: *Game Ranch Management,* 5th ed. (edited by J. Bothma, dP. & J.G. Du Toit). Pp. 726-758. Pretoria, South Africa: Van Schaik.

Bothma, J., dP. & Sartorius von Bach, H.J. (2012). Economic aspects of extensive wildlife production in southern Africa. In: *Game Ranch Management,* 5th ed. (edited by J. bothma, dP. & J.G. du Toit). Pp. 83-96. Pretoria, South Africa: Van Schaik.

Bourne, M.C. (1978). Texture profile analysis. *Food Technology,* **33**, 62-66

Bouton, P., Harris, P. & Shorthose, W. (1971). Effect of ultimate pH upon the water-holding capacity and tenderness of mutton. *Journal of Food Science,* **36**, 435-439.

Browning, B.B.R. (2008). Butchering of Wild Game. Africahunting.com. [Internet document].URL http://www.africahunting.com/threads/butchering-of-wild-game.14406/. Accessed 13.09. 2015.

Campbell-Platt, G. & PE., C. (1995). *Fermented Meats*. London, UK: Blackie Academic and Professional.

Casiraghi, E., Pompei, C., Dellaglio, S., Parolari, G. & Virgili, R. (1996). Quality attributes of Milano salami, an Italian dry-cured sausage. *Journal of Agricultural and Food Chememistry.,* **44**, 1248−1252.

Chi, S.P. & Wu, Y.C. (2015). Spices and seasonings. In: *Handbook of Fermented Meat and Poultry,* 2nd ed. (edited by F. Toldrá). Pp. 79-89. New Jersey, USA: John Wiley & Sons, Ltd.

Çiçek, Ü., Kolsarici, N. & Candoğan, K. (2014). The sensory properties of fermented turkey sausages: effects of processing methodologies and starter culture. *Journal of Food Processing and Preservation,* doi:10.1111/jfpp.12274.

Ciuciu Simion, A.M., Vizireanu, C., Alexe, P., Franco, I. & Carballo, J. (2014). Effect of the use of selected starter cultures on some quality, safety and sensorial properties of Dacia sausage, a traditional Romanian dry-sausage variety. *Food Control,* **35**, 123-131.

Cluff, M. (2013). The effect of CLA supplimentation on the quality of a cured, fermented pork sausage. MSc in Food Science thesis, University of the Free State, Bloemfontein, South Africa.

Conedera, G., Mattiazzi, E., Russo, F., Chiesa, E., Scorzato, I., Grandesso, S., Bessegato, A., Fioravanti, A. & Caprioli, A. (2007). A family outbreak of *Escherichia coli* O157 haemorrhagic colitis caused by pork meat salami. *Epidemiology and Infection,* **135**, 311-314.

Daley, C.A., Abbott, A., Doyle, P.S., Nader, G.A. & Larson, S. (2010). A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. *Nutrition Journal,* **9**, 10, 1-12.

Dalzini, E., Cosciani-Cunico, E., Monastero, P., Sfameni, C., Pavoni, E., Daminelli, P., Losio, M.-N., Serraino, A. & Varisco, G. (2014). Reduction of *Escherichia coli* O157:H7 during manufacture and ripening of Italian semi-dry salami. *Italian Journal of Food Safety,* **3**. 137-139.

Dare, R., Jönsson, H. & Knutsson, H. (2013). Adding Value in Food Production. INTECH Open Access Publisher. [Internet Document] http://cdn.intechopen.com/pdfs-wm/41692.pdf. Accessed 29/09/2015

Demeyer, D. & Stahnke, L. (2002). Quality control of fermented meat products. In: *Meat Processing, Improving Quality*. Cambridge, England, UK: Woodhead Publiching Limited.

Deogade, A., Zanjad, P. & Raziuddin, M. (2008). Value Added Meat Products. *Veterinary World,* **1**, 88-89.

Doyle, M.P., Deuchat, L.R. & Montville, T.J. (1997a). Food Microbiology. Washington, DC, USA: American Society for Microbiology.

Doyle, M.P., Zhao, T., Meng, J. & Zhao, S. (1997b). *Escherichia coli* O157:H7. In: *Food Microbiology: fundamentals and fronteirs* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 171-191. Washington D.C., USA: American Soceity for Microbiology Press.

Du Buisson, P.M. (2006). Improving the meat quality of blesbok and springbok by including organic salts. MSc in Food Science Thesis, Stellenbosch University, South Africa.

Els, H. (2010). The role of african rural people in their environment. In: *Game Ranch Management,* (edited by J. Bothma, dP. & J.G. Du Toit). Pp. 906-924. Pretoria, South Africa: Van Schaik.

Ercoşkun, H. & Özkal, S.G. (2011). Kinetics of traditional Turkish sausage quality aspects during fermentation. *Food Control,* **22**, 165-172.

Escherich, T. (1885). Die Darmbacterien des Neugeborenen und Sauglings. *Fortschritte der Medizin,* **3**, 515-522, 547-554.

Estes, R.D. (2012). Hartebeests, topi, blesbok, and wildebeests: tribe alcelaphini. In: *The Behavior guide to African Mammals including hoofed mammals, carnivores, primates,* 20th Anniversary ed. Pp. 133-167. Los Angeles, California, USA: University of California Press.

Fadda, S., Lopez, C. & Vignolo, G. (2010). Role of lactic acid bacteria during meat conditioning and fermentation: peptides generated as sensorial and hygienic biomarkers. *Meat Science,* **86**, 66-79.

Farnworth, E.R. (2003). *Handbook of Fermented Functional Foods*. Boca Raton, FL, USA: CRC Press.

FDA (2015). Food Safety Modernization Act. U.S. Food and Drug Administration. [Internet Document].URL http://www.fda.gov/Food/GuidanceRegulation/FSMA/. 23/09/2015

Feiner, G. (2006a). Definitions of terms used in meat science and technology. In: *Meat Products Handbook: practical science and technology.* Pp. 46-71. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006b). Raw Fermented Salami. In: *Meat Products Handbook: practical science and technology.* Pp. 314-375. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006c). Fresh Sausages. In: *Meat Products Handbook: practical science and technology.* 297-309. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006d). Cooked Sausages. In: *Meat Products Handbook: practical science and technology.* Pp. 239-286. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006e). Typical raw fermented salami products from around the world. In: *Meat Products Handbook: practical science and technology.* Pp. 376-382. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006f). Typical fresh sausage products from around the world. In: *Meat Products Handbook: practical science and technology.* Pp. 310-313. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006g). Typical cooked sausage products from around the world. In: *Meat Products Handbook: practical science and technology,* 1st ed. Pp. 287-296. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006h). Typical raw fermented sausage products from around the world. In: *Meat Products Handbook: practical science and technology*. Pp. 414-416. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006i). Additives: proteins, carbohydrates, fillers and other additives. In: *Meat Products Handbook: practical science and technology*. Pp. 89-141. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006j). Colour in fresh meats and cured products. In: *Meat Products Handbook: practical science and technology.* Pp. 142-157. Cambridge, England, UK: Woodhead Publishing Limited.

Feiner, G. (2006k). Introduction to the microbiology of meat and meat products. In: *Meat Products Handbook: practical science and technology.* Pp. 574-594. Cambridge, England, UK: Woodhead Publishing Linited.

Feng, C.-H., Sun, D.-W., Martín, J.F.G. & Zhang, Z.-H. (2013). Effects of different cooling methods on shelf-life of cooked jumbo plain sausages. *LWT-Food Science and Technology,* **54**, 426-433.

Fernández-López, J., Sendra, E., Sayas-Barberá, E., Navarro, C. & Pérez-Alvarez, J. (2008). Physico-chemical and microbiological profiles of “salchichón” (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Science,* **80**, 410-417.

Frost, W. (2014a). Part 1: About africa and its antelope. In: *The Antelope of Africa,* (edited by T. Carnaby). Pp. 1-46. Auckland Park, South Africa: Jacana.

Frost, W. (2014b). Bastard hartebeest species. In: *The Antelope of Africa,* (edited by T. Carnaby). Pp. 132-149. Auckland Park, South Africa: Jancana (Pty) Ltd.

Gammariello, D., Incoronato, A., Conte, A. & DelNobile, M. (2015). Use of antimicrobial treatments and modified atmosphere to extend the shelf life of fresh sausages. *Journal of Food Processing & Technology,* **6**, 1-7.

Gillespie, J. & Schupp, A. (2000). The role of speculation and information in the early evolution of the United States ostrich industry: An industry case study. . *Review of Agricultural Economics,* **24**, 278-292.

Gilliland, S.E. (1985). *Bacterial Starter Cultures for Foods*. Boca Raton, FL, USA: CRC Press.

Girolami, A., Napolitano, F., Faraone, D., Di Bello, G. & Braghieri, A. (2014). Image analysis with the computer vision system and the consumer test in evaluating the appearance of Lucanian dry sausage. *Meat Science,* **96**, 610-616.

Grau, R., Andres, A. & Barat, J.M. (2015). Principles of drying. In: *Handbook of Fermented Meat and Poultry,* 2nd ed. (edited by F. Toldrá, Y.H. Hui, I. Astiasaran, J. Sebranek & R. Talon). Pp. 31-38. West Sussex, UK: John Wiley & Sons, Ltd.

Hoffman, L. (2007). The meat we eat: are you game? Inaugural address, Stellenbosch University, South Africa.

Hoffman, L., Joubert, M., Brand, T. & Manley, M. (2005). The effect of dietary fish oil rich in n−3 fatty acids on the organoleptic, fatty acid and physicochemical characteristics of ostrich meat. *Meat Science,* **70**, 45-53.

Hoffman, L., Kroucamp, M. & Manley, M. (2007). Meat quality characteristics of springbok (*Antidorcas marsupialis*). 3: Fatty acid composition as influenced by age, gender and production region. *Meat Science,* **76**, 768-773.

Hoffman, L., Muller, M., Schutte, D.W. & Crafford, K. (2004). The retail of South African game meat: current trade and marketing trends. *South African Journal of Wildlife Research,* **34**, p. 123-134.

Hoffman, L.C., Mostert, A.C., Kidd, M. & Laubscher, L.L. (2009). Meat quality of kudu (*Tragelaphus strepsiceros*) and impala (*Aepyceros melampus*): Carcass yield, physical quality and chemical composition of kudu and impala longissimus dorsi muscle as affected by gender and age. *Meat Science,* **83**, 788-795.

Hoffman, L.C., Smit, K. & Muller, N. (2008). Chemical characteristics of blesbok (*Damaliscus dorcas phillipsi*) meat. *Journal of Food Composition and Analysis,* **21**, 315-319.

Hoffman, L.C. & Wiklund, E. (2006). Game and venison - meat for the modern consumer. *Meat Science,* **74**, 197-208.

Holck, A.L., Axelsson, L., Rode, T.M., Hoy, M., Mage, I., Alvseike, O., L'Abee-Lund, T.M., Omer, M.K., Granum, P.E. & Heir, E. (2011). Reduction of verotoxigenic *Escherichia coli* in production of fermented sausages. *Meat Science,* **89**, 286-295.

Honikel, K.O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Science,* **78**, 68-76.

Houben, J.H. & van 't Hooft, B.J. (2005). Variations in product-related parameters during the standardised manufacture of a semi-dry fermented sausage. *Meat Science*, 283–287.

Ikonic, P., Petrovic, L., Tasic, T., Dzinic, N., Jokanovic, M. & Tomovic, V. (2010). Physicochemical, biochemical and sensory properties for the characterization of Petrovská Klobása (traditional fermented sausage). *Acta Periodica Technologica*, 19-31.

Incze, K. (2010). Mould-ripened sausages. In: *Handbook of Meat Processing*. (edited by F. Toldrá). Pp. 363-377. Ames, Iowa, USA: John Wiley & Sons, Inc.

Irie, M. (2001). Optical evaluation of factors affecting appearance of bovine fat. *Meat Science,* **57**, 19-22.

ISO (2005). ISO 22000:2005 - Food safety management systems - Requirements for any organization in the food chain. International Organisation for Standardization.

ISO (2009). ISO/TS 22002-1:2009 Prerequisite programmes on food safety - Part 1: Food manufacturing. International Organisation for Standardization.

Jablonski, L.M. & Bohach, G.A. (1997). *Staphylococcus aureus*. In: *Food Microbiology: fundamentals and frontiers,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 353-375. Washington D.C.: American Society for Microbiology Press.

Jackson, T.C., Acuff, G.R. & Dickson, J.S. (1997). Meat, poultry and seafood. In: *Food Microbiology: fundamentals and frontiers,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 83-100. Washington D.C.: American Society for Microbiology Press.

Jafari, M. & Emam-Djomeh, Z. (2007). Reducing nitrite content in hot dogs by hurdle technology. *Food Control,* **18**, 1488-1493.

Jansen, M.L. (2001). Determination of meat pH–temperature relationship using ISFET and glass electrode instruments. *Meat Science,* **58**, 145-150.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005a). Non dairy fermented foods and products. In: *Modern Food Microbiology,* 7th ed. Pp. 175-195. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, 7th ed. D.A. (2005b). Food protection with chemicals and by biocontrol. In: *Modern Food Microbiology*. Pp. 301-350. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005c). Milk, fermentation, and fermented and nonfermented dairy products. In: *Modern Food Microbiology,* 7th ed. Pp. 149-173. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005d). Intrinsic and extrinsic parameters of foods that affect mirobial growth. In: *Modern Food Microbiology,* 7th ed. Pp. 39-60. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005e). Introduction to foodborne pathogens. In: *Modern Food Microbiology,* 7th ed. Pp. 519-544. New York USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005f). Indicators of food microbial quality and safety. In: *Modern Food Microbiology,* 7th ed. Pp. 473-496. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005g). Foodborne gastroenteritis caused by *Escherichia coli*. In: *Modern Food Microbiology,* 7th ed. Pp. 637-655. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005h). Foodborne listeriosis. In: *Modern Food Microbiology,* 7th ed. Pp. 591-617. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005i). Staphylococcal gastroenteritis. In: *Modern Food Microbiology,* 7th ed. Pp. 545-566. New York, USA: Springer.

Jeacocke, R.E. (1977). Continuous measurements of the pH of beef muscle in intact beef carcasses. *Journal of Food Technology,* **12**, 375-386.

Jones, M. (2013). The addition of rooibos tea extract (*Aspalathus Linearis*) as a natural antioxidant to South African droëwors. MSc in Food Science Thesis. Stellenbosch University, South Africa.

King, A. (2014). Impact of pH and Water Activity on Growth of Staphylococcus aureus in Shelf Stable Ready-to-Eat Snack Sausages, 2014 Annual Meeting Report. *International Association for Food Protection*, 3 - 97.

Koep, K.S.C. (2005). Production of salami from meat of aquatic and terrestrial mammals. MSc in Animal Science Thesis,Stellenbosch University, South Africa.

Kottke, V., Damm, H., Fischer, A. & Leutz, U. (1996). Engineering aspects in fermentation of meat products. *Meat Science,* **43**, 243-255.

Kröckel, L. (2013). The role of lactic acid bacteria in safety and flavour development of meat and meat products. INTECH Open Access Publisher. [Internet document].URL http://cdn.intechopen.com/pdfs/42316/InTech-The_role_of_lactic_acid_bacteria_in_safety_and_flavour_development_of_meat_and_meat_products.pdf. Accessed 23/09/2015.

Kroucamp, M. (2004). Meat quality characteristics of the springbok (*Antidorcas marsupialis*). MSc in Food Science Thesis, Stellenbosch University, South Africa.

Lawley, R., Curtis, L. & Davis, J. (2008). *The Food Safety Hazard Guidebook*. Cambridge, UK: The Royal Society of Chemistry.

Lawrie, R.A. & Ledward, D. (2006a). The conversion of muscle to meat. In: *Lawrie's Meat Science,* 7th ed. Pp. 128-156. Cambridge, England: Woodhead Publishing Limited.

Lawrie, R.A. & Ledward, D. (2006b). The structure and growth of muscles. In: *Lawrie's meat science,* 7th ed. Pp. 41-74. Cambridge, England, UK: Woodhead Publishing Limited.

Lee, C.M., Trevino, B. & Chaiyawat, M. (1996). A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of Association of Official Analytical Chemists International,* **79**, 487–492.

Lerasle, M., Federighi, M., Simonin, H., Anthoine, V., Rezé, S., Chéret, R. & Guillou, S. (2014). Combined use of modified atmosphere packaging and high pressure to extend the shelf-life of raw poultry sausage. *Innovative Food Science & Emerging Technologies,* **23**, 54-60.

Leroy, F. & De Vuyst, L. (2009). Fermentation and acidification ingredients. In: *Ingredients in Meat Products: properties, functionality and applications,* (edited by R. Tarte). Pp. 227-252. New York, USA: Springer.

Leroy, F., Geyzen, A., Janssens, M., De Vuyst, L. & Scholliers, P. (2013). Meat fermentation at the crossroads of innovation and tradition: A historical outlook. *Trends in Food Science & Technology,* **31**, 130-137.

Leroy, S., Lebert, I. & Talon, R. (2015). Micro-organisms in traditional fermented meats. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá). Pp. 99-107. New Jersey, USA: John Wiley & Sons, Ltd.

Lloyd, P. & David, J. (2008). *Damaliscus pygargus*. [Internet document].URL http://www.iucnredlist.org/details/full/30208/0. Accessed 10-09-2015.

Lorenzo, J.M., Temperán, S., Bermúdez, R., Cobas, N. & Purriños, L. (2012). Changes in physico-chemical, microbiological, textural and sensory attributes during ripening of dry-cured foal salchichón. *Meat Science,* **90**, 194-198.

MacDonald, D., Fyfe, M., Paccagnella, A., Trinidad, A., Louie, K. & Patrick, D. (1999). *Escherichia coli* O157: H7 outbreak linked to salami. *British Columbia, Canada*, 283-289.

Mancini, R.A. & Hunt, M.C. (2005). Current research in meat color. *Meat Science,* **71**, 100-121.

Marianski, S. & Marianski, A. (2012). Making fermented sausages. In: *The Art of Making Fermented Sausages,* 2nd ed. Pp. 93-122. Seminole, Florida: Bookmagic LLC.

Mauriello, G., Casaburi, A., Blaiotta, G. & Villani, F. (2004). Isolation and technological properties of coagulase negative staphylococci from fermented sausages of Southern Italy. *Meat Science,* **67**, 149-158.

McAfee, A.J., McSorley, E.M., Cuskelly, G.J., Moss, B.W., Wallace, J.M., Bonham, M.P. & Fearon, A.M. (2010). Red meat consumption: an overview of the risks and benefits. *Meat Science,* **84**, 1-13.

Mettler-Toledo (2007). pH Theory Guide: A guide to pH measurement - the theory and practice of laboratory applications Pp. Schwerzenbach, Switzerland.

Mikac, U., Sepe, A. & Serša, I. (2008). Study of salami drying by MRI. [Internet document]. URL http://www2.matis.is/mrinfood2008/images/stories/posterar/mikac.pdf. Accessed 16.09. 2015

Montville, T.J. & Winkowski, K. (1997). Biologically based prevention systems and probiotic bacteria. In: *Food Microbiology: fundamentals and fronteirs,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 557-578. Washington D.C., USA: American Society for Microbiology Press.

Mostert, A.C. (2007). Meat quality of kudu (*Tragelaphus Strepsiceros*) and impala (*Aepyceros Melampus*). MSc in Animal Science Thesis, Stellenbosch University, Stellenbosch.

National Agricultural Marketing Council N.A.M.C. (2003). Report on the investigation into the effects of deregulation on the South African ostrich industry. [Internet Document].URL http://dms.namc.co.za/published/20040416-1212.pdf. Accessed 17/09/2015.

Neethling, J. (2012). Impact of season on the composition and quality of male and female blesbok (*Damaliscus pygargus phillipsi*) muscles. MSc in Food Science Thesis. Stellenbosch University, Stellenbosch, South Africa.

Neethling, J., Hoffman, L.C. & Britz, T.J. (2014). Impact of season on the chemical composition of male and female blesbok (Damaliscus pygargus phillipsi) muscles. *Journal of the Science of Food and Agriculture,* **94**, 424-431.

Nørrung, B., Andersen, J.K. & Buncic, S. (2009). Main concerns of pathogenic micro-organisms in meat. In: *Safety of Meat and Processed Meat,* (edited by F. Toldrá). Pp. 3-29. New York, NY, USA: Springer.

North, M., Frylinck, L. & Hoffman, L. (2015). The physical and biochemical changes in springbok (*Antidorcas marsupialis*) longissimus thoracis et lumborum and biceps femoris muscle during ageing. *Meat Science,* **110**, 145-152.

Ockerman, H.W. & Basu, L. (2015). Production and consumption of fermented meat products. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá, Y.H. Hui, I. Astiasaran, J. Sebranek & R. Talon). Pp. 7-11. West Sussex, UK: John Wiley & Sons, Ltd.

Offer, G. & Knight, P. (1988). The structural basis of water-holding in meat. part II: drip losses. In: *Developments in Meat Science,* (edited by R.A. Lawrie). Pp. 63-243. London & New York: Elsevier Science Publishers Ltd.

Palavecino Prpich, N.Z., Castro, M.P., Cayré, M.E., Garro, O.A. & Vignolo, G.M. (2015). Indigenous starter cultures to improve quality of artisanal dry fermented sausages from Chaco (Argentina). *International Journal of Food Science,* **2015**, 1 - 9.

Palumbo, S.A., Smith, L.J. & Zaika, L.L. (1976). Sausage drying, factors affecting the percent yield of pepperoni. *Journal of Food Science,* **41**, 1270 - 1272.

Papa, F., Zambonelli, C. & Grazia, L. (1995). Production of milano style salami of good quality and safety. *Food Microbiology*, 9 - 12.

Paramithiotis, S., Drosinos, E.H., Sofos, J.N. & Nychas, G.J.E. (2010). Fermentation: microbiology and biochemistry. In: *Handbook of Meat Processing,* (edited by F. Toldrá). Pp. 185-198. Iowa, USA: John Wiley & Sons, Inc.

Parthasarathy, D.K. & Bryan, N.S. (2012). Sodium nitrite: the "cure" for nitric oxide insufficiency. *Meat Science,* **92**, 274-279.

Pearson, A.M. & Dutson, T.R. (2012). *HACCP in Meat, Poultry, and Fish Processing*. Springer Science & Business Media.

Pegg, R.B. & Honikel, K.O. (2015). Principles of curing. In: *Handbook of Fermented Meat and Poultry,* 2nd ed. (edited by F. Toldrá). Pp. 19-30. New Jersey, USA: John Wiley & Sons, Inc.

Pisula, A. & Tyburcy, A. (1996). Hot processing of meat. *Meat Science,* **43**, 125-134.

Poławska, E., Marchewka, J., Cooper, R., Sartowska, K., Pomianowski, J., Jozwik, A., Strzałkowska, N. & Horbanczuk, J. (2011). The ostrich meat–an updated review II. nutritive value. *Animal Science Papers and Reports,* **29**, 89-97.

Puolanne, E. (2010). Cooked sausages. In: *Hondbook of Meat Processing,* 1st ed. (edited by F. Toldrá). Pp. 313-325. Ames, Iowa, USA: John Wiley & Sons, Inc.

Puolanne, E. & Petaja, -.K., E. (2015). Principles of meat fermentation. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá). Pp. 13-17. New Jersey, USA: John Wiley & Sons, Ltd.

Quinton, R., Cornforth, D.P., Hendricks, D., Brennand, C. & Su, Y. (1997). Acceptability and composition of some acidified meat and vegetable stick products. *Journal of Food Science,* **62**, 1250-1254.

Raab, V., Petersen, B. & Kreyenschmidt, J. (2011). Temperature monitoring in meat supply chains. *British Food Journal,* **113**, 1267-1289.

Raes, K., Balcaen, A., Dirinck, P., De Winne, A., Claeys, E., Demeyer, D. & De Smet, S. (2003). Meat quality, fatty acid composition and flavour analysis in Belgian retail beef. *Meat Science,* **65**, 1237-1246.

Ricke, S.C. & Keeton, J.T. (1997). Fermented meat, poultry and fish products. In: *Food Microbiology fundamentals and frontiers,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 610-628. Washington D.C., USA: American Society for Microbiology Press.

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M. & Hargrett, N.T. (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New England Journal of Medicine,* **308**, 681-685.

Riordan, D.C., Duffy, G., Sheridan, J.J., Eblen, B.S., Whiting, R.C., Blair, I.S. & McDowell, D.A. (1998). Survival of *Escherichia coli* O157: H7 during the manufacture of pepperoni. *Journal of Food Protection,* **61**, 146-151.

Roberts, M.N. & Getty, K.J. (2011). Validation of heating conditions in production of direct acidified venison with beef fat summer sausage for elimination of *Escherichia Coli* 0157:H7. *Journal of Food Safety,* **31**, 480-486.

Rocourt, J. & Cossart, P. (1997). *Listeria monocytogenes*. In: *Food Microbiology: fundamentals and fronteirs,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 337-352. Washington D.C., USA: American Society for Microbiology Press.

Roncales, P. (2015). Additives. In: *Handbook of Fermented Meat and Poultry,* 2nd ed. (edited by F. Toldrá). Pp. 69-77. New Jersey, USA: John Wiley & Sons.

Ruiz de Huidobro, F., Cañeque, V., Lauzurica, S., Velasco, S., Pérez, C. & Onega, E. (2001). Sensory characterization of meat texture in sucking lamb. *Investigación Agraria. Producción y Sanidad Animales,* **16**, 245-256.

Ruiz de Huidobro, F., Miguel, E., Blazquez, B. & Onega, E. (2005). A comparison between two methods (Warner–Bratzler and texture profile analysis) for testing either raw meat or cooked meat. *Meat Science*, 527-536.

Ruiz, J. & Perez-Palacios, T. (2015). Ingredients. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá). Pp. 55-68. New Jersey, USA: John Wiley & Sons, Ltd.

Ruiz, J.N., Villanueva, N.D.M., Favaro-Trindade, C.S. & Contreras-Castillo, C.J. (2014). Physicochemical, microbiological and sensory assessments of Italian salami sausages with probiotic potential. *Scientia Agricola,* **71**, 204-211.

SABS (2007). SANS 10330:2007 - Requirements for a Hazard Analysis and Critical Control Point (HACCP) system. Pretoria, Soth Africa: Standards South Africa.

Sales, J. & Hayes, J. (1996). Proximate, amino acid and mineral composition of ostrich meat. *Food Chemistry,* **56**, 167-170.

South African Meat Industry Company, S.A.M.I.C. (2015). Meat processing charts: Beef, Lamb & Pork. South Africa: [Internet document].URL http://www.samic.co.za/index.php/forms-downloads. Accessed 13.09.2015.

Sartz, L., De Jong, B., Hjertqvist, M., Plym-Forshell, L., Alsterlund, R., Löfdahl, S., Osterman, B., Ståhl, A., Eriksson, E. & Hansson, H.-B. (2008). An outbreak of *Escherichia coli* O157: H7 infection in southern Sweden associated with consumption of fermented sausage; aspects of sausage production that increase the risk of contamination. *Epidemiology and Infection,* **136**, 370-380.

Schutte, S. (2008). Development of value added ostrich (*Struthio Camelus*) meat products. MSc in Food Science Thesis, Stellenbosch University, Stellenbosch.

Scollan, N.D., Dannenberger, D., Nuernberg, K., Richardson, I., MacKintosh, S., Hocquette, J.-F. & Moloney, A.P. (2014). Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Science,* **97**, 384-394.

Sebranek, J. (2004). Semidry fermented sausages. In: *Handbook of Food and Beverage Fermentation Technology,* (edited by Y.H. Hui, L. Meunier-Goddik, J. Josephsen, W.K. Nip & P.S. Stanfield). Pp. 385-396. New York, USA: Marcel Dekker.

Sebranek, J.G. & Bacus, J.N. (2007). Cured meat products without direct addition of nitrate or nitrite: what are the issues? *Meat Science,* **77**, 136-147.

Sindelar, J.J. & Milkowski, A.L. (2012). Human safety controversies surrounding nitrate and nitrite in the diet. *Nitric Oxide,* **26**, 259-266.

Skjelkvale, R. & Tjaberg, T.B. (1974). Comparison of salami sausage produced with and without addition of sodium nitrite and sodium nitrate. *Journal of Food Science,* **39**, 520-524.

Smit, K. (2004). Meat quality characteristics of blesbok (*Damaliscus dorcas phillipsi*) and red hartebeest (*Alcelaphus buselaphus caama*) meat. MSc in Food Science Thesis, Stellenbosch University, South Africa.

Smith, T. (1895). Notes on *Bacillus coli* commune and related forms, together with some suggestions concerning the bacteriological examination of drinking water. *American Journl of Medical Science,* **110**, 283-302.

Smolin, L.A. & Grosvenor, M.B. (2003). How safe is our food supply. In: *Nutrition, science and application,* 4th ed. Pp. 483-515. New Jersey, USA: John Wiley & Sons, Inc.

Smoot, L.M. & Pierson, M.D. (1997). Indicator micro-organisms and microbiological criteria. In: *Food Microbiology: fundamentals and frontiers,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 66-80. Washington D.C., USA: American Society for Microbiology Press.

Soyer, A., Ertas, A.H. & Uzumcuog lu, U. (2005). Effect of processing conditions on the quality of naturally fermented Turkish sausages (sucuks). *Meat Science,* **69**, 135-141.

Spaziani, M., Del Torre, M. & Stecchini, M.L. (2009). Changes of physicochemical, microbiological, and textural properties during ripening of Italian low-acid sausages, proteolysis, sensory and volatile profiles. *Meat Science,* **81**, 77-85.

Steenkamp, J.B.E. (1997). Dynamics in consumer behavior with respect to agricultural and food products. In: *Agricultural marketing and consumer behavior in a changing world*. Pp. 143-188. New York, USA: Springer.

Tapp, W.N., Yancey, J.W. & Apple, J.K. (2011). How is the instrumental color of meat measured? *Meat Science,* **89**, 1-5.

Thermo-Scientific (2013). pH Electrode Selection Handbook. Thermo Fisher Scientific, Inc. [Internet Document].URL https://www.fondriest.com/pdf/thermo_ph_electrode_hb.pdf. Accessed 23/09/20115

Todorov, S.D., Koep, K.S., Van Reenen, C.A., Hoffman, L.C., Slinde, E. & Dicks, L.M. (2007). Production of salami from beef, horse, mutton, blesbok (*Damaliscus dorcas phillipsi*) and

springbok (*Antidorcas marsupialis*) with bacteriocinogenic strains of *Lactobacillus plantarum* and *Lactobacillus curvatus*. *Meat Science,* **77**, 405-412.

Toldrá, F. (2012). Biochemistry of fermented meat. In: *Food Biochemistry and Food Processing,* (edited by B.K. Simpson, L.M. Nollet, F. Toldrá, S. Benjakul, G. Paliyath & Y.H. Hui). Pp. 331-343. Oxford, UK: Johnn wiley & Sons, Inc.

Toldrá, F. & Hui, Y.H. (2015). Dry-fermented sausages and ripened meats: an overview. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá, Y.H. Hui, I. Astiasaran, J.G. Sebranek & R. Talon). Pp. 3-6. New Jersey, USA: John Whiley & Sons, Ltd.

Toldrá, F. & Reig, M. (2012). *Analytical Tools for Assessing the Chemical Safety of Meat and Poultry*. New York, USA: Springer.

Troy, D.J. & Kerry, J.P. (2010). Consumer perception and the role of science in the meat industry. *Meat Science,* **86**, 214-226.

van Schalkwyk, D.L., McMillin, K.W., Booyse, M., Witthuhn, R.C. & Hoffman, L.C. (2011). Physico-chemical, microbiological, textural and sensory attributes of matured game salami produced from springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*) and zebra (*Equus burchelli*) harvested in Namibia. *Meat Science,* **88**, 36-44.

Van Zyl, L. & Ferreira, A.V. (2004). Physical and chemical carcass composition of springbok (*Antidorcas marsupialis*), blesbok (*Damaliscus dorcas phillipsi*) and impala (*Aepyceros melampus*). *Small Ruminant Research,* **53**, 103-109.

Varnam, A. & Sutherland, J.P. (1995a). Fermented sausages. In: *Meat and Meat Products: technology, chemistry and microbiology*. Pp. 314-354. London, UK: Chapman & Hall.

Varnam, A.H. & Sutherland, J.P. (1995b). Cooked Meat and Cooked Meat Products. In: *Meat and Meat Products*. Pp. 224-297. London, UK: Chapman & Hall.

Varnam, A. & Sutherland, J.P. (1995c). *Meat and meat products: technology, chemistry and microbiology*. London, UK: Chapman & Hall.

Vignolo, G., Fontana, C. & Fadda, S. (2010). Semidry and dry fermented sausages. In: *Handbook of Meat Processing*. (edited by F. Toldrá). Pp. 379-398. Iowa, USA.: John Wiley & Sons, Ltd.

Von La Chevallerie, M. (1970). Meat production from wild ungulates. *Proceedings of South African Society for Animal Production,* **9**, 73.

Von La Chevallerie, M. (1972). Meat quality of seven wild ungulate species. *South African Journal of Animal Science,* **2**, 101-103.

Walczak, M.M., Dryer, D.A., Jacobson, D.D., Foss, M.G. & Flynn, N.T. (1997). pH dependent redox couple: an illustration of the Nernst equation. *Journal of Chemical Education,* **74**, 1195.

Warnants, N., van Oeckel, M.J. & Boucque, C.V. (1998). Effect of incoporation of dietary polyunsaturated fatty acids in pork backfat on the quality of salami. *Meat Science,* **49**, 435-445.

Warriss, P. (2010a). The effects of live animal handling on carcass and meat quality. In: *Meat Science: an introductory test,* 2nd ed. Pp. 97-112. Oxfordshire, UK: CAB International.

Warriss, P.D. (2010b). Post-mortem changes in muscle and its conversion into meat. In: *Meat Science: an introductory text,* 2nd ed. Pp. 65-76. Oxfordshire, UK: CAB International.

Warriss, P.D. (2010c). Measuring the composition and physical characteristics of meat. In: *Meat Science: an introductory text,* 2nd ed. Pp. 179-193. Oxfordshire, UK: CAB International.WHO (2015). Food Safety. World Health Organisation. [Internet document].URL http://www.who.int /topics/food_safety/en/. Accessed 23/09/2015

Williams, R.C., Isaacs, S., Decou, M.L., Richardson, E.A., Buffett, M.C., Slinger, R.W., Brodsky, M.H., Ciebin, B.W., Ellis, A. & Hockin, J. (2000). Illness outbreak associated with *Escherichia coli* O157: H7 in Genoa salami. *Canadian Medical Association Journal,* **162**, 1409-1413.

Zeuthen, P. (2007). A historical perspective of meat fermentation. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá, Y.H. Hui, I. Astiasaran, W.K. Nip, J.G. sebranek, E.T.F. Silveira, L.H. Stahnke & R. Talon). Pp. 1-8. Ames, USA: Blackwell Publishing.

Ziegler, G., Rizvi, S. & Acton, J. (1987). Relationship of water content to textural characteristics, water activity, and thermal conductivity of some commercial sausages. *Journal of Food Science,* **52**, 901-905.

Živković, D., Radulović, Z., Aleksić, S., Perunović, M., Stajić, S., Stanišić, N. & Radović, C. (2014). Chemical, sensory and microbiological characteristics of Sremska sausage (traditional dry-fermented Serbian sausage) as affected by pig breed. *African Journal of Biotechnology,* **11**, 3858-3867.

CHAPTER 3

The optimisation of Glucono-delta-lactone in the production of a dry acidified sausage made from Blesbok (*Damaliscus pygargus phillipsi*) meat

Abstract

The objective of this study was to optimise the physico-chemical properties of a dry acidified Blesbok meat sausage with differing concentrations of a chemical acidulant; Glucono-delta-lactone (GdL). Twenty mature Blesbok were harvested for the trial (10 males and 10 females), facilitating the use of a 5 x 5 Latin square design, where each of the 5 blocks comprised of 2 male and 2 female meat sources. There was a significant decrease ($P < 0.05$) in pH and resultant moisture, water activity and mass loss as the concentration of the acidulant was incrementally increased by 0.50% through each of the 5 treatments, beginning at treatment 1 with a 0.50% inclusion. Textural parameters of Maximum Force 1 (N) and Gumminess all significantly increased ($P < 0.05$) as the concentration of the acidulant increased. Lactic acid bacteria, Total aerobic plate count and *Staphylococcus aureus* logarithmically decreased as the pH decreased across the treatments. *Escherichia coli*, although present on the raw material, was reduced to 150 $CFU.g^{-1}$ in the first treatment, 30 $CFU.g^{-1}$ in the second treatment and was not detected in the final three treatments. *Listeria monocytogenes* was not detected in any of the final product treatments. As the pH incrementally decreased, both the water activity and bacteria count similarly decreased, rendering the product safe. An optimal concentration of 1.5% GdL was chosen based of the pH and water activity being sufficient to provide a safe product, provided that the microbial load on the raw materials is controlled.

Keywords: Glucono-delta-lactone; dry acidified sausage; physico-chemical; game meat; venison.

3.1 Introduction

Salami is classified as a dry, fermented sausage, whereby meat and fat are combined with salt (NaCl), curing agents (Nitrates and Nitrites), spices and either natural lactic acid bacteria or the addition of starter cultures or chemical acidifiers. These ingredients are mixed into a batter and then extruded into either a natural or synthetic casing and allowed to acidify and dry (Lücke, 1994, Hugas & Monfort, 1997, Jay *et al.*, 2005a, Koep, 2005, Barbut, 2006).

The traditional manufacture process of producing fermented sausages is commonly associated with long acidification and curing times due to the action of lactic acid bacteria, resulting in the production of a high quality product (Papa *et al.*, 1995). Acidification of salami, results in a

reduction in pH, thereby lowering the water holding capacity of the batter, thus enabling moisture to be removed (Campbell-Platt, 1994, Lawrie & Ledward, 2006). Jay *et al*. (2005b) reports that dry fermented sausage typically contains 30% – 40% moisture.

Since the pre-scientific era (3000BC – 1500BC), the preservation of meat has been associated with the use of salt (Jay *et al.*, 2005b). Later investigations established that under ideal conditions, micro-organisms, notably lactic acid bacteria via the formation of lactic acid as a by-product of the fermentation process, were in part responsible for the preservation of meat (Lücke, 2000, Jay *et al.*, 2005a). Traditional manufacture relied heavily on the natural microflora which enabled unique artisanal fermented sausage products; however, methods for the isolation of micro-organisms providing specific functionality have been developed. This allowed for an inoculum to be produced in order to standardise the fermented sausage product and assist in the potential increase in economies of scale of fermented sausages (Leroy *et al.*, 2006). The primary preservation mode of action of fermentation is the reduction in pH due to the increase in lactic acid, a by-product of the lactic acid bacteria fermentation process (Lücke, 1994, Lücke, 2000). Other by-products of this fermentation process assist in flavour enhancement of the fermented sausages, these by-products, from the different microflora of artisanal sausage production, have a positive impact on the final products perceived quality (Leroy & De Vuyst, 2004, Leroy *et al.*, 2006). Also, the rapid reduction in pH due to the favourable lactic acid bacteria assist in preventing the growth of pathogens thus creating a safe product for the consumer (Lücke, 2000, Jay *et al.*, 2005a, Ammor & Mayo, 2007).

Acidification also assists in the denaturation of the proteins resulting in the lowering of the water binding capacity enabling intrinsic water to be unbound from the protein lattice and free for removal from the sausage as modelled by Offer (1991) and discussed in an extensive review by Puolanne and Halonen (2010). Typically, fermented sausages decrease in pH from 5.8 to 4.8 however, ranges at the end-point of the production of dried sausage, vary between 5.2 and 4.5 (Jay *et al.*, 2005a). One particular study conducted by Barbut (2006), used the base line pH value of 4.66 ± 0.05.

Acidification can be both natural in the case of the action of acidophiles (Lactic acid bacteria) or chemical whereby acids are added to the sausage in order to rapidly acidify the product (Lücke, 2000, Jay *et al.*, 2005a). Glucono-delta-lactone (GdL) is a chemical acidulant which hydrolyses in the presence of water to form gluconic acid in the sausage batter (Barbut, 2006). Depending on the quantity of acid added, the pH can be controlled to drop to a predetermined point as demonstrated by Barbut (2006). Other chemicals frequently used are lactic and citric acids and more recently, encapsulated citric and lactic acids (Barbut, 2006, Barbut, 2010).

Traditional salami has been primarily produced with pork as the meat base; however, this process is not limited to pork. Research involving fermented sausages has also included the use of horse, mutton, whale, seal, beef and venison meat to name a few (Koep, 2005, Todorov *et al.*, 2007, Cluff, 2013). Game meat has been perceived by consumers as a healthier meat source due to the

organic nature of the animals roaming in the wild, when compared to animals raised in a feedlot environment (Harper & Makatouni, 2002). Game meat is reported to have low lipid concentrations and a more desirable fatty acid profile, adding to the health perception of venison meat (Hoffman *et al.*, 2005, Hoffman & Wiklund, 2006).

Traditional processing of venison meat in Southern Africa consists primarily of primal cuts of meat or the manufacture of droëwors (dried sausage) and biltong (salted and dried meat), however there is currently no standard practise in South Africa regarding the cut or quality of game meat (Hoffman *et al.*, 2005, Burnham *et al.*, 2008, Jones, 2013). There is thus, scope for the development of alternative value added game meat products with the added benefits of increasing the range of game meat products and the utilisation of offcuts, ultimately increasing the carcass utilisation.

The game meat product thus developed is a chemically acidified Blesbok meat, dry fermented sausage which is safe to consume according to South African legal requirements. The aim being to optimise the quantity of GdL in order to physically produce acceptable Blesbok meat salami in terms of chemical, physical and microbiological parameters.

3.2 Materials and methods

3.2.1 Experimental design

A 5 x 5 Latin square design was chosen to be implemented where 5 blocks (B1 – B5) were produced, consisting of 5 treatments (T1 – T5) in each block. The Latin square design enabled each treatment within a block to be manufactured in a different time period and over the entire experiment, no one treatment was produced in the same time period allowing the effect of acidulation in the different time periods to be assessed. Table 3.1 indicates the order in which the treatments for each block were processed.

Table 3.1 Latin square experiment design layout

Time Period	Block 1	Block 2	Block 3	Block 4	Block 5
1st	B1T1	B2T2	B3T3	B4T4	B5T5
2nd	B1T2	B2T3	B3T4	B4T5	B5T1
3rd	B1T3	B2T4	B3T5	B4T1	B5T2
4th	B1T4	B2T5	B3T1	B4T2	B5T3
5th	B1T5	B2T1	B3T2	B4T3	B5T4

Time period is defined as the total lead time required to manufacture the product from bowl chopping the frozen meat cubes, through humidification and drying until the mass has been reduced to 75% of the initial wet mass of the sausage.

B - Indicates the block

T- Indicates the treatment.

3.2.2 Acquisition and preparation of ingredients

Blesbok meat was harvested on the Stellenbosch University experimental farm (Brakkekuil, Witsand, Southern Cape, South Africa) on two separate nights with one shot to either the head or the neck after which the animals was exsanguinated (ethical clearance number: SU-ACUM14-001SOP), transported to the farms meat processing facility where it was eviscerated, skinned, washed down with potable water and chilled in a fridge at 4°C for 24 h. Post chilling, the carcasses were deboned, the meat was vacuum packed and labelled with their individual carcass numbers and cut of meat. The meat was then transported to the Stellenbosch University Meat Science laboratory where it was subsequently frozen at -20°C until further processing.

All further processing in the Stellenbosch University Meat Science laboratory was conducted in a manner encompassing Good Manufacturing Practises (GMP) and particular care was taken to ensure utmost hygiene was implemented throughout the manufacture process. This included the use of clean personal protective clothing, hand sanitation program when entering the meat lab and a rinse, wash and sanitation process prior to all manufacture on all equipment utilised in the salami sausage manufacture process.

Upon further processing, the packaged meat was defrosted and prepared by removing excess fat and tissues, then cut into roughly 5 cm^3 cubes. These cubes were subsequently layered in a plastic lug with plastic film separating the layers in order to facilitate easy separation of frozen meat when manufacturing the sausage. Once layered, the lug was placed in the freezer and frozen at -20°C until required for further processing.

The pork back fat was sourced from a commercial pork abattoir based in the Western Cape, South Africa. The pork fat was prepared by removing all meat and rind after which it was cut into approximately 5 cm^3 cubes and layered in plastic lugs with thin plastic film in between the layers and subsequently frozen at -20°C until required in the manufacturing process.

The composite spice pack was separated into three components, namely, the flavour pack consisting of all the required spices to add flavour to the product, the salt pack which included the salt (89%), sodium nitrate (3%) sodium nitrite (6%) and an alkaline base (2%) and finally GdL (0.5 – 3.0%), which was supplied as a single acidifier. These components, supplied individually, allowed for precise control when adding it to the salami sausage batter. All the composite spice pack ingredient components were acquired from Deli Spices (Epping Industria, Western Cape, South Africa). The flavour pack provided, consisted of the flavour components within the Deli Spice Milano Salami pack, a typical commercial salami batch pack used in the South African industry.

The dry acidified sausage batter was filled into pork (hog) intestine casings with a 36 - 38 mm diameter which were also sourced from Deli Spices.

3.2.3 Manufacture of the salami

When manufacturing the salami sausage batter, a 3 kg meat block per treatment was used and all spice batch pack components were calculated as a percentage of the meat block when producing the various treatments. The manufacturing of the salami occurred in blocks as represented in Table 3.1 where each block was manufactured in different time periods. Each block was manufactured from the forequarter of four mature Blesbok, two males and two females. Each block required 11.25 kg of meat, *ca.* 2.82 kg from each animal's forequarter, thus keeping the meat ratio between different animals and different genders constant throughout the treatments and blocks. The pork back fat per block was kept constant at 3.75 kg per block, 750 g per treatment. Table 3.2 indicates the formulations produced for all treatments within a block.

A six blade Manica CM-21(Equipamient os Carnicos, Spain) bowl chopper was used on the slow blade rotation speed and slow bowl rotation speed to produce the salami sausage batter. Initially, the frozen meat was added to the bowl chopper and cut to a coarse texture after which the frozen fat cubes were added to the bowl chopper along with the spice pack and salt pack including the nitrates. The chopping continued until the fat particles were roughly 5 mm^3 after which the GdL was added, the bowl was rotated allowing the meat to be passed through the blades for a further two turns, after which the process was stopped. The sausage batter was subsequently placed in a Talsa hydraulic sausage filler (DMD Foodtech, Epping, South Africa) and filled into 36 - 38 mm size pork intestine casings of roughly ±20 cm in length. The 3 kg batter yielded approximately 14 sausages which were hung in the smoking/drying chamber for further processing. All five of the treatments were produced in the same manner according to the Latin square design presented in Table 3.1, subsequently labelled and weighed in order to quantify the wet sausage mass allowing for the future calculation of the yield. Post weighing, the salami sausages were hung in the smoking/drying chamber. All five treatments per block were manufactured in their designated time period after which they were hung in the chamber.

Table 3.2 Formulation of the 5 treatments, varying in GdL concentration within a block of Blesbok fermented sausage

Ingredient	Treatment 1		Treatment 2		Treatment 3		Treatment 4		Treatment 5	
	%	Mass	%	Mass	%	Mass	%	Mass	%	Mass
Blesbok Meat	75.00	2.25 kg	75.00	2.25 kg	75.00	2.25 kg	75.00	2.25 kg	75.00	2.25 kg
Pork Back Fat	25.00	0.75 kg	25.00	0.75 kg	25.00	0.75 kg	25.00	0.75 kg	25.00	0.75 kg
Meat Block Total	**100**	**3.00 kg**	**100**	**3.00 kg**	**100**	**3.00 kg**	**100**	**3.00 kg**	**100**	**3.00 kg**
Salt & Nitrate	3.00	90.00 g	3.00	90.00 g	3.00	90.00 g	3.00	90.00 g	3.00	90.00 g
GdL	0.50	15.00 g	1.00	30.00 g	1.50	45.00 g	2.00	60.00 g	3.00	90.00 g
Spice	1.00	30.00 g	1.00	30.00 g	1.00	30.00 g	1.00	30.00 g	1.00	30.00 g

All salt, GdL and spice values were added as a percentage of the meat block.

Meat block is defined as the meat and fat portion added together, in this case 3.00 kg.

Once in the chamber, the acidification and slow drying regime commenced. Initially, the Reich Airmaster UKF 2000 BE (Urbach, Germany) chamber parameters were set to 40°C and 99% RH for 20 min after which the chamber switched off and remained closed for a 24 h period. During this 24 h period, the relative humidity remained high and the temperature dropped in the region of 22°C. A LogTag (Recorders SA, Somerset West, South Africa) temperature (TREX-8 with ST1000T-15 probe) and humidity (HAXO-8) recorder was utilised in order to monitor the process as closely as possible. This recorder was set to record the conditions within the chamber every 2 minutes for the duration of the acidification and drying period as reported in Figure 3.2; a second LogTag humidity recorder was stationed outside the chamber to record the environmental temperature and relative humidity parameters as reported in Figure 3.1.

After the first 24 h period, a boiling step, drying step and a repeat function as illustrated in Table 3.3 was followed. The chamber remained closed for the duration of the program. Once the drying regime was finished, the sausages were weighed to record the final mass which enabled the percentage mass loss to be calculated.

$$\%\ mass\ loss = \frac{(Initial\ Mass - Final\ Mass)}{Initial\ Mass} \times \frac{100}{1}$$

The dry acidified sausages were then divided into three batches that were either homogenised and frozen for chemical (proximate) analysis, sampled for direct analysis or vacuum packed in order to preserve the remaining sausages for further analysis.

Table 3.3 Boiling and drying regime in the Reich Airmaster UKF 2000 BE smoker

Processing Step	Regime	Temperature (°C)	Relative Humidity (%RH)	Duration (min)	Repeat	Cumulative time (h)
1	Boil	40	99	20	1	0.33
2	Switch off and remain closed. Temperature decreases to room temperature over a 24 hour period.				1	24.33
3	Boil	20	90	20	6	26.33
3	Dry	20	90	120	6	38.33
4	Boil	20	85	20	6	40.33
4	Dry	20	85	150	6	55.33
5	Boil	20	80	20	6	57.33
5	Dry	20	80	150	6	72.33
6	Boil	20	75	20	6	74.33
6	Dry	20	75	150	6	89.33
7	Boil	20	72	20	6	91.33
7	Dry	20	72	150	6	106.33

3.2.4 Sample plan of the sausage

Each 3 kg treatment was able to produce ±14 sausages. All the sausages post the final drying period were measured for final mass, 5 sausages were measured for water activity and pH. Furthermore, 3 randomly selected sausages were individually homogenised, labelled and frozen for proximate

analysis and lastly, 5 other randomly selected sausages with no penetration from pH or water activity measurements, were placed under vacuum in labelled packets, chilled in the refrigerator for 24 h and then assessed for physical textural parameters using cyclic compression tests via the Instron Universal Testing Machine (UTM), Model 3345 (Instron Corporation, MA, USA). Three sausages from batch three were selected randomly, labelled and packed in a sealed packet under vacuum, placed on ice in a cooler box and sent to University of Stellenbosch Food Science microbiology laboratories for microbial analyses.

3.2.5 Physical analysis

All samples were weighed on a Digi DS-673 (Teraoka Seilko Co. Ltd, Japan) scale prior to hanging in the chamber and post drying. This enabled the calculation of percentage mass loss.

In terms of pH monitoring, a Crison pH 25+ (Spain) meter connected to a Crison PT1000 5053T pH (Spain) probe was used. Post a two point calibration (pH 4.01 and pH 7.00) of the pH meter, the probe was inserted into the core of the sausage, parallel to the length of the sausage representing the pH in the middle of the sausage. The pH readings were conducted on 5 of the 14 sausages as described in section 3.2.4. The 5 sausages were cut perpendicular to the length of the sausage in the middle of the sausage. The probe was inserted in the middle of both halves approximately 3 cm into the core of the sausage allowing for two pH measurements per sausage.

Water activity was measured using an Aqualab Pawkit (Decagon Devices Inc, Washington, USA) portable water activity meter. The sausage was sliced into a 3 mm thick portion, a third of the way down from one end. The sliced portion was placed into the plastic vial designed for the meter. It was ensured that the sausage slice was parallel to the meter reading and that there was sausage covering all areas of the vial. The water activity meter was calibrated on two points, notably a_W = 0.76 standard and a_W = 0.92 standard. Once calibrated, the sample was placed under the meter to record the water activity reading. The water activity was conducted on samples which had equilibrated to room temperature to get the most accurate reading. Five of the fifteen sausages as described in section 3.2.4 were tested for water activity for each treatment. The water activity meter reading was recorded along with the temperature at measurement.

Texture analysis involved three randomly selected sausages from each treatment in each batch as described in section 3.2.4. Once the pork casing had been removed, the sausage was sliced into 20 mm lengths, perpendicular to the length of the sausage. An Instron Universal Testing Machine (Model 3345, Instron Corporation, MA, USA) was set up using the Bluehill 2 TPA (Instron Corporation, MA, USA) software platform, with a 5 kN load cell and a cross hair speed of 5.0 cm/min in an air conditioned room at 23°C to measure a cyclic compression test with a 60% compression from a final anvil height of 23 mm using the 20 mm diameter anvil (Bourne, 1978). Each 20 mm length sample was placed meticulously on the base of the Instron Universal Testing Machine with the length of the sample parallel to the action of the anvil to ensure that the anvil pressed down squarely on the centre of the sample. The values recorded were, 1st cycle hardness 1 (N), energy to

maximum load 1st cycle (J), 2nd cycle hardness 2 (N), energy to maximum load 2nd cycle (J), Gumminess (N), Cohesiveness (Ratio with no units), Adhesiveness (N) and Springiness (mm)(Ruiz de Huidobro *et al.*, 2001, Ruiz de Huidobro *et al.*, 2005).

3.2.6 Proximate analysis

Proximate analysis was determined to assist with the description of the product analysed. The assessment focussed on physical and chemical methods to determine moisture content using method 934.01 (AOAC, 2002a), fat content using a solvent extraction method (Lee *et al.*, 1996), protein content using method 992.15 (AOAC, 2002b) and ash content using method 942.05 (AOAC, 2002c).

3.2.7 Microbiology

Microbiological assessment was conducted on batch number 3, to provide a snap shot picture of the microbial activity of the dried salami sausage product in order to validate whether the hurdles of salt content, pH and a_W were able to control the microbial load within the product. For this purpose, the micro-organisms chosen for analysis were Total Aerobic Plate Count *(TAPC),* Lactic Acid Bacteria *(LAB), E. coli, S. aureus* and *L. monocytogenes* which was conducted both on raw materials and finished product.

Microbial safety was assessed on the spice packs, blesbok meat and pork back fat in order to assess the complete microbial load of the raw materials prior to processing. When sampling the blesbok meat, four 25 g samples, one from each blesbok forequarter included in the block, was pooled together, homogenised and stored in a refrigerated environment below 4°C. Pork back fat was sampled by pooling, homogenising and refrigerating four 25 g samples which were selected randomly from the mass of fat received from the processor. A 5 g spice sample was drawn aseptically from the batch pack used and also stored in the refrigerator at 4°C until testing.

Three sausages from each of the five treatments were randomly selected for microbial analysis (as described in section 3.2.4). The sausages were peeled from their casing and *ca.* 8 g of each sausage was pooled together to form a 25 g sample of each treatment which was subsequently sampled for microbial analysis.

From the 100 g raw meat and fat samples as well as 25 g from the five treatments, 25 g of each individual sample was diluted in 225 mL Buffered peptone water solution and stomached for 60 seconds. The 5 g spice solution was diluted into 45 mL Buffered peptone water solution and also stomached for 60 seconds. Once stomached, the samples were serially diluted to 10^{-5} dilution by diluting 1 mL in 9 ml 0.85% salt (NaCl) solution. Each spread plate was inoculated with 1 mL, in duplicate, for each dilution from 10^{-1} to 10^{-5} and subsequently incubated for 24 h at 36°C after which the plates were read. The plates were prepared for Total Aerobic Plate Count *(TAPC),* lactic acid bacteria *(LAB), E. coli* and *S. aureus*. *L. monocytogenes* was outsourced to Microchem laboratories (176 Sir Lowry Road, Cape Town, South Africa) where it was analysed via multiplex PCR

methodology, in order to confirm whether the selected pathogen was present or not (Gouws & Liedemann, 2005, Rip & Gouws, 2009). Table 3.4 indicates the selective media used to grow the specific organisms in question and the concentrations required to produce the media. All the media were sterilised in an autoclave for 20 min at 121°C. Confirmation of this process was indicated by autoclave tape which turns black after the sterilisation temperature and time is achieved.

Table 3.4 Microbiological test performed and media used for the test

Test	Media Used	Supplier	Concentration (g/L)
Dilution of material	Buffered peptone water (BPW)	Biolab (Merck, South Africa)	20 $g.L^{-1}$
Serial dilution	Salt (NaCl) Solution	(Merck, South Africa)	0.85 $g.L^{-1}$
Total aerobic plate count	Tryptone soy agar (TSA)	Oxoid, Hampshire, England	40 $g.L^{-1}$
Lactic acid bacteria	de Man, Rogosa and Sharp (MRS)	Biolab (Merck, South Africa)	62 $g.L^{-1}$
Escherichia coli	Violet red bile agar (VRB)	Biolab (Merck, South Africa)	39.5 $g.L^{-1}$
Staphylococcus aureus	Mannitol Salt Agar (MSA)	Biolab (Merck, South Africa)	120 $g.L^{-1}$

Media preparation for NaCl solution, BPW solution, TSA, MRS, VRB and MSA were conducted as follows to produce sufficient media for both the raw materials and finished product tested.

3.2.7.1 Salt solution

Merck Sodium Chloride (South Africa) solution was made up at a concentration of 0.85% in bulk and subsequently filled into McCartney glass vials, each vial containing 9 mL of 0.85% NaCl solution. Once filled the vials were sterilised in the autoclave at 121°C for 20 min and allowed to equilibrate to room temperature before use.

3.2.7.2 Other media (BPW, TSA, MRS, VRB and MSA)

The media was diluted with distilled water in a Scott jar according to the concentrations represented in Table 3.4. Subsequently the media was placed in the autoclave and sterilised at 121°C for 20 min after which the media was placed in the water bath and allowed to cool to a temperature between 45°C and 55°C, at which point the media was poured into labelled plates under laminar flow conditions. Once the media was solidified in the plates, the plates were stacked and stored in a refrigerator at 4°C until required.

3.2.8 Statistical analysis

All the statistical analyses were computed using Statistica version 12, Dell Inc. (2015), using a mixed model repeated measures ANOVA, incorporating standard principle component biplots. Post hoc testing employed Fischer's LSD test with a confidence level of 0.95.

3.3 Results

Data for proximate analysis include moisture, fat, protein and ash. All the results are reported in Table 3.5 using $g.100g^{-1}$ meat as a unit of measure on a wet matter basis. Only the moisture content

showed significant difference ($P < 0.05$) between the five treatments where treatment 1 (0.5% GdL) and treatment 2 (1.0% GdL) had the highest moisture content of 38.9 ± 1.63% and 38.7 ± 1.71%, respectively. Treatment 4 (2.0%) and treatment 5 (3.0%) had the lowest moisture content of 35.0 ± 2.09% and 33.6 ± 1.60%, respectively. Treatment 3 (1.5%) differed ($P < 0 .05$) to treatment 1 and treatment 2, as well as treatment 4 and treatment 5 with a value of 36.7 ± 1.72%.

Both the initial pH and the final pH values showed significant differences ($P<0.05$) between the treatments (Table 3.5). The minimum initial pH was 5.59 ± 0.06 and the maximum initial pH was 5.66 ± 0.04. Although statistically significant, from a biological point of view, the range of 0.07 pH points becomes marginal and as a result is considered uniform for the sake of the trial. Blesbok has a reported ultimate pH of between 5.54 – 5.60 (Neethling, 2012). Final pH differed significantly ($P < 0.01$) between each of the treatments, where Treatment 1 was the highest (5.2 ± 0.10) and treatment 5 was the lowest (4.3 ± 0.09).

Water activity was recorded at a specific temperature and differed ($P < 0.05$) across the five treatments, although these differences were marginal. The highest water activity was recorded for treatment T1 at 0.93 ± 0.03 and the lowest water activity was for treatment T5 at 0.90 ± 0.01 (Table 3.5). The percentage mass loss also differed ($P < 0.05$) across the five treatments (Table 3.5). Mass loss ranged from the lowest being 32.3 ± 1.43% for treatment T1 and the highest being 37.2 ± 0.91% for treatment T5.

In terms of texture (Table 3.5), two of the three texture parameters, namely Max Force 1 (N) ($P = 0.005$) and Gumminess ($P = 0.001$), differed across the five treatments, where by treatment 1 and 2 were the lowest values for both parameters and differed significantly ($P < 0.05$) from treatment 3, 4 and 5 in both parameters. Cohesiveness did not differ ($P > 0.05$) across the treatments. Adhesiveness and Springiness were inconclusive due to readings which gave both positive and negative results and in some instances did not record a result. Due to this inconsistent variation, both Adhesiveness and Springiness were removed from the analysed data set.

Bacterial profiling was conducted on the raw material and final product of batch three (Table 3.6). Initial counts on raw materials were established for the spice, meat and fat used for lactic acid bacteria, Total aerobic plate count, *S. aureus, E. coli* and *L. monocytogenes*. In terms of the final product, logarithmic reductions were observed with the lactic acid bacteria, Total aerobic plate count and *S. aureus*. *L. monocytogenes* was not present in any of the raw materials or finished product. In terms of *E. coli*, treatment 1 and treatment 2 had counts of 150 $CFU.g^{-1}$ and 30 $CFU.g^{-1}$, respectively, whilst in the other three treatments no *E. coli* was detected.

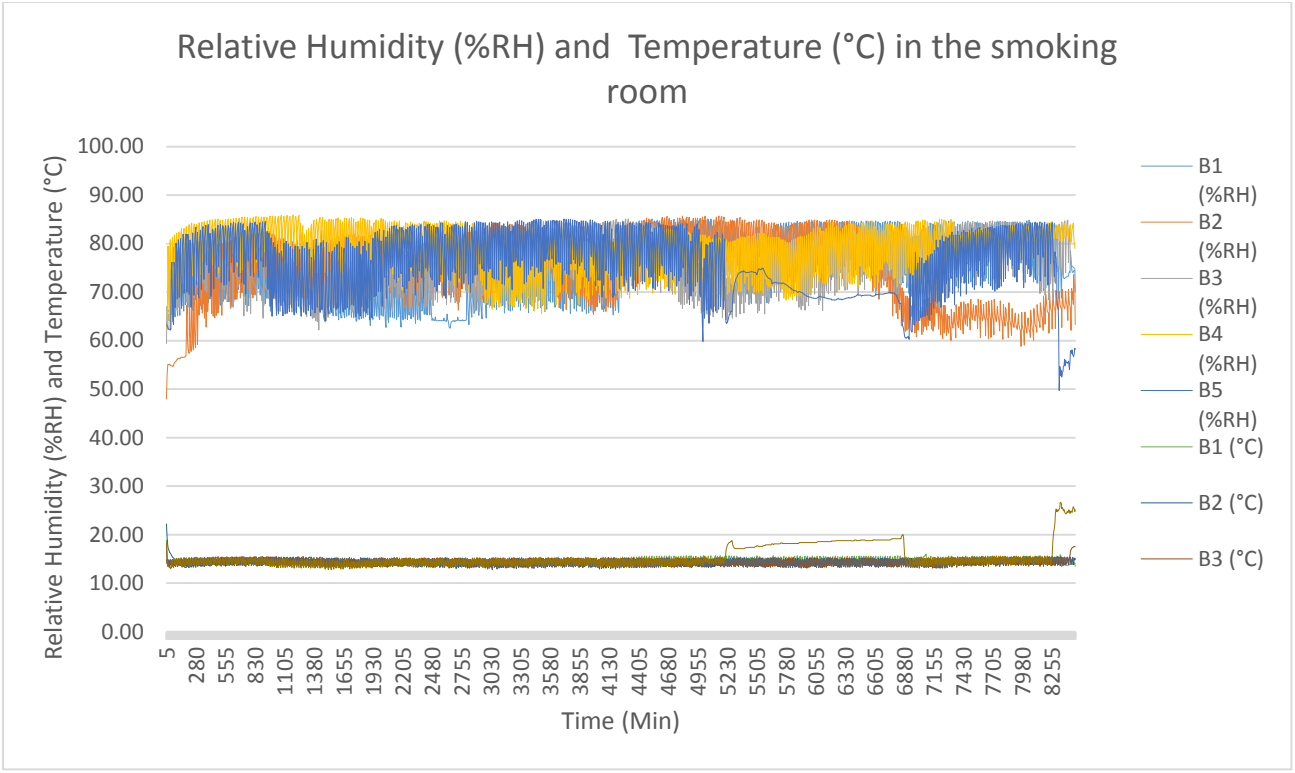

Figure 3.1 Log of the temperature and Relative Humidity in the smoking room during the production of the five batches of fermented sausages.

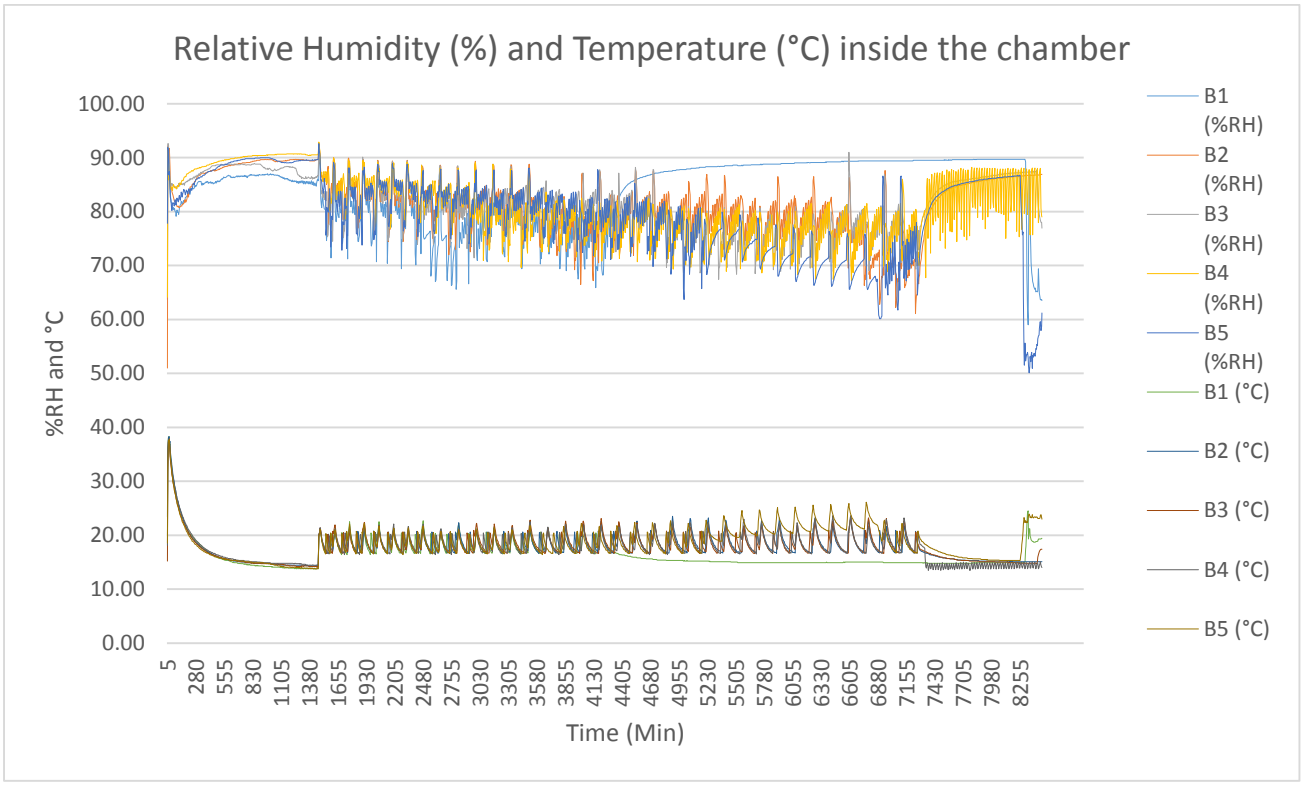

Figure 3.2 Log of the Temperature and Relative Humidity inside the maturation chamber during the production of the five batches of fermented sausages.

Table 3.5 Means and standard deviation for proximate composition analysis and physical analysis on the five treatments of Blesbok fermented sausage

	Treatments					
Measurement	**T1**	**T2**	**T3**	**T4**	**T5**	**P value**
Moisture g.100g^{-1}	38.9^{a} ± 1.63	38.7^{a} ± 1.71	36.7^{b} ± 1.72	35.0^{c} ± 2.09	33.6^{c} ± 1.60	P < 0.001
Fat g.100g^{-1}	32.0 ± 4.26	32.3 ± 4.52	34.4 ± 5.24	31.5 ± 3.96	30.4 ± 5.57	P = 0.740
Protein g.100g^{-1}	20.8 ± 2.99	20.9 ± 2.73	21.1 ± 2.96	23.9 ± 2.48	24.4 ± 3.86	P = 0.209
Ash g.100g^{-1}	5.6 ± 0.24	5.6 ± 0.27	5.5 ± 0.30	5.6 ± 0.32	6.0 ± 0.19	P = 0.128
pH initial	5.7^{a} ± 0.04	5.7^{a} ± 0.04	5.6^{ab} ± 0.05	5.6^{b} ± 0.06	5.6^{b} ± 0.04	P = 0.012
pH Final	5.2^{a} ± 0.10	5.1^{b} ± 0.02	4.9^{c} ± 0.10	4.6^{d} ± 0.04	4.3^{e} ± 0.09	P < 0.001
Water activity	0.93^{a} ± 0.03	0.93^{a} ± 0.02	0.92^{ab} ± 0.02	0.90^{b} ± 0.02	0.90^{b} ± 0.01	P = 0.005
Temperature °C	17.5^{c} ± 1.2	17.6^{cb} ± 1.3	17.8^{ab} ± 1.4	17.8^{ab} ± 1.4	17.9^{a} ± 1.4	P = 0.027
Mass loss %	32.3^{c} ± 1.43	32.8^{c} ± 2.51	34.4^{b} ± 1.93	36.6^{a} ± 1.93	37.2^{a} ± 0.91	P < 0.001
Max force 1 (N)	74.0^{b} ± 27.92	77.3^{b} ± 13.25	106.5^{ab} ± 30.40	135.6^{a} ± 39.04	118.5^{a} ± 25.11	P = 0.005
Gumminess	14.4^{c} ± 2.35	17.2^{cb} ± 2.25	21.5^{a} ± 4.06	24.0^{a} ± 5.27	20.8^{ab} ± 4.51	P = 0.001
Cohesiveness	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.03	0.2 ± 0.02	P = 0.077

$^{a.b.c.d.e}$ Means within a row with different superscripts are significantly different (P < 0.05)

Table 3.6 Results of the microbiological study on raw materials and treatments in batch 3 for pathogens and total aerobic plate count reported in CFU.g^{-1}

Sample	MRS (Lactic bcid bacteria)	TSA (Total aerobic count)	MSA (*S. aureus*)	VRB (*E. coli)*	Multiplex PCR (*L. monocytogenes)*
RM – Spice	1 140	565	475	ND	ND
RM – Meat	22 600	6 060 000	< 10^2	6 550 000	ND
RM – Fat	835 000	51 400 000	ND	1 540 000	ND
FP – B3T1	2 375 000	441 000	206 000	150	ND
FP – B3T2	266 000	> 10^5	27 600	30	ND
FP – B3T3	14 250	3 150	1 550	ND	ND
FP – B3T4	1 135	1 600	505	ND	ND
FP – B3T5	64	41	<10	ND	ND

ND – Not Detected; RM – Raw Material; FP – Final product; B3 – Batch 3; T1 – T5 – Treatments.

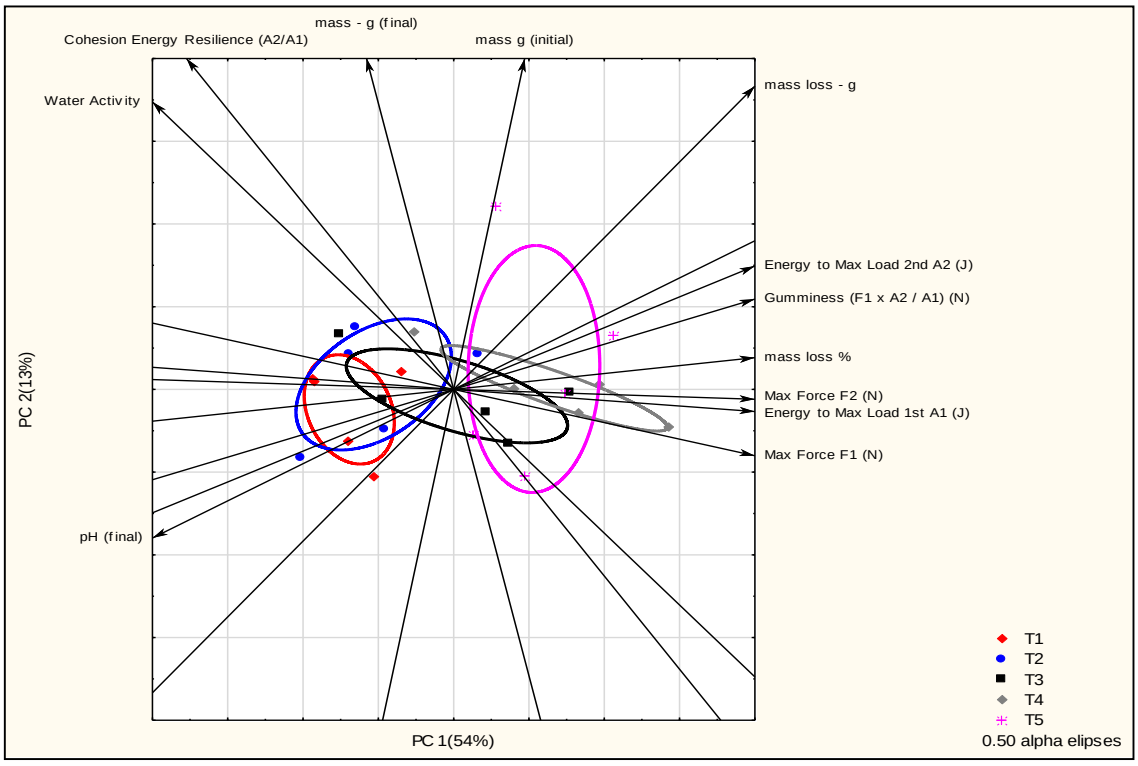

Figure 3.3 Principal component analysis bi-plot of physical analysis data incorporating the five treatments.

3.4 Discussion

The five treatments differ in the concentration of GdL added in the fermented sausage batter (Table 3.2). The hydrolysis of GdL in the presence of water into gluconic acid results in an increase in gluconic acid concentration and subsequent decrease in pH within the sausage (Barbut, 2005). This reduction in pH was confirmed by the final pH values reported (Table 3.5) where the pH was decreasing ($P < 0.001$) between each treatment. In terms of proximate analysis, there was a decrease in moisture content of the sausage treatments as GdL concentration increased (Table 3.5). This decrease in moisture content is expected as a decrease in pH is commonly associated with the reduction of water holding capacity which enables residual 'free moisture' to be removed from the product as the pH approaches the isoelectric point of the muscle protein (Huff-Lonergan & Lonergan, 2005, Feiner, 2006a, Feiner, 2006b, Lawrie & Ledward, 2006).

Fat, protein and ash did not differ significantly ($P > 0.05$) between any of the treatments. The lack of significant differences could be attributed to inter batch variation in terms of external environmental conditions where Batch 1 B1 was interrupted by a power failure (Figure 3.2) and Batch 5 B5 was exposed to extreme temperature (Figure 3.1) causing the chamber conditions to have greater unexpected variation. These differences could have created a product with greater than expected variation thus affecting both the standard deviation and P values of the three parameters (protein, fat and ash). Although great care was taken to disperse the ingredients evenly within the bowl chopper, there could have been slight variation with the GdL dispersion across the sausage. The homogenisation and sampling plan as described in the methodology should have assisted in mitigating this variation but the possibility of slight inter-sausage variation of acid dispersion may have caused the inter-batch variation to be wider than anticipated resulting in no significant differences of the three above mentioned parameters.

The expectation is that as water is removed from the product, there will be less free water available in the product, resulting in a lower water activity. When considering the water activity results, Table 3.5 indicates a decreasing trend in water activity across the treatments. Significant differences ($P = 0.005$) observed for the water activity parameter follow a similar decreasing trend between treatments as the trend in moisture content. This indicates that the trend in decreasing water activity moderately correlates ($r = 0.6999$) with the decreasing trend in moisture content across the treatments.

There is a measured increasing trend in percentage mass loss as the moisture and water activity decrease between the treatments, resulting from the decreasing pH of the treatments (Table 3.5). The principle component analysis bi-plot (Figure 3.3) further illustrates the relationship between pH, water activity and percentage mass loss whereby a decrease in water activity and pH results in an increase in mass loss.

Texture profile analysis incorporated cyclic compression tests of which only three measured results showed significant differences, namely Maximum Force 1 (P=0.005), Maximum Force 2 (P=0.003) and Gumminess (P=0.001). The results confirm that as moisture decreases, the perceived hardness, represented by the force required to compress the sample, increases. This holds true for both first and second compression forces (Table 3.5) with an increase in force required to compress the sample as the concentration of acid increases between the different treatments. Studies conducted on previous research corroborate this as there were slight increases in the hardness of the sausage when the water activity decreased; claimed to be caused by drying of the meat being proportional to the water loss in the sausage, ultimately as a result of a decrease in pH (Spaziani *et al.*, 2009, Lorenzo *et al.*, 2012). Gumminess also showed significant differences (P=0.001; Table 3.5), where it increases as the pH decreases. This is a similar trend observed by Lorenzo *et al.* (2012) where a pH decrease resulted in both gumminess and chewiness values increasing. Cohesiveness was not significant (P=0.077) between the treatments and as such is not considered as a descriptor of the sample tested although, the value is very close to 0.05 and as such more samples could reveal a slightly different result. Adhesiveness and Springiness were not included in the results section as the values obtained when testing had many null results as well as both positive and negative results. This meant that values obtained were not a true reflection of the actual value of the fermented sausage and as such were removed. Previous studies on salami have also not included these two parameters (van Schalkwyk *et al.*, 2011). Lorenzo *et al*. (2012) indicates that sausage, as it is drying loses its springiness. Suggestions for future studies could be that when using cyclic compression tests, one would ensure that the anvil applying the force onto the sausage would have a diameter greater than that of the diameter of sausage being tested and that the compression used is between 60% and 70%.

Microbiological assessment on batch 3 was conducted on the raw materials and each of the treatments post processing as reported in Table 3.6. The microbiological data provided useful information on the safety of the product and validated how the process parameters affected the treatments. Pertinent pathogenic indicator organisms were assessed as well as the effect the different treatments have of the microbial load on the product. Indicator pathogen organisms considered were *S. aureus*. *E. coli* and *L. monocytogenes*. *L. monocytogenes* was not present in any of the raw material and was not found in any of the final product treatments rendering the product safe to consume in terms of *L. monocytogenes*. In terms of *E. coli*, none were detected on the spices, however, a logarithmic value of 6 was found on both the meat and fat raw materials. This is indicative of contamination of the raw materials most probably due to the slaughter process and hygiene surrounding the slaughter process (Borch *et al.*, 1996). Although these values were rather high, the raw materials were still used in the production of the acidified sausage. Treatment 1, containing the lowest concentration of GdL, had a final *E. coli* count of 150 CFU.g^{-1} (log 3 reduction) and Treatment 2, the second lowest concentration of GdL, had a final *E. coli* count of 30 CFU.g^{-1} (log 4 reduction). The rest of the treatments had no *E. coli* detected. South Africa does not currently have specific

legislation defining the microbiological requirements for processed meat products, specifically pertaining to cooked or fermented meat products, however, there are guidelines recommending specifications which should be adhered too when processing cooked or fermented meat products released by the Department of Health (Anonymous, 2015). Based on these guidelines, it is suggested that *E. coli* not be present in the product (Anonymous, 2015). Furthermore, in order for the enterotoxin producing *E. coli* strains to produce toxins, the consumer must consume product which contains more that 10^6 - 10^{10} viable cells per gram, which requires an optimal pH (7.5 - 8.5) to be maintained for toxin production (Mundell *et al.*, 1976, Burgess *et al.*, 1978, Jay *et al.*, 2005c). Based on this information, Treatment 3, 4 and 5 are considered safe to consume in terms of *E. coli*. Treatment 1 and 2 although above the suggested limits, are well within the levels required to be consumed in order for the enterotoxin to be harmful to a consumer but caution must still be exercised even though the pH of the sausages are well below the optimal pH range for the enterotoxin producing *E. coli*.

Raw material testing revealed that *S. aureus* was detected on the meat with less than 100 CFU.g^{-1} and on the spice with 475 CFU.g^{-1}. Furthermore, *S. aureus* was not detected in the fat. Food safety is best maintained by minimising the contamination prior to processing (Borch *et al.*, 1996). In order to do this, hygienic processing and quality control of raw materials needs to be enforced in order to minimise the potential gross contamination of the final product according to GMP, PRP and HACCP principles (Borch *et al.*, 1996, Ramoneda *et al.*, 2013). South African Department of Health guidelines for processed meat stipulates that the desired level of contamination of *S. aureus* post processing should be below 100 CFU.g^{-1} (Anonymous, 2015). Once extruded into sausages, the subsequent processing exposed the sausages to high humidity and a temperature range oscillating between 15°C and 20°C (Figure 3.2). These processing steps resulted in the provision of parameters which promote the growth of *S. aureus,* notable a temperature range of 10 - 46°C, pH range of 4.0 - 9.83, and a_W of 0.86 and above (Jay *et al.*, 2005d). It is thus expected that *S. aureus* will grow in the sausages unless the limiting factors of decreasing pH, decreasing water activity and decreasing moisture inactivates the bacteria thereby reducing the growth of *S. aureus* to differing endpoints within the range of treatments. The results in the final product across the treatments (Table 3.6) indicate a logarithmic reduction of *S. aureus* from 2.06 × 10^5 CFU.g^{-1} (Treatment 1) down to < 10 CFU.g^{-1} (Treatment 5). From the results, the only acceptable treatments in terms of concentration of *S. aureus* based on the South African Department of Health guideline, would be treatment 5 however, due to the initial contamination being relatively high, one may consider treatment 3 and treatment 4 as suitable options (Anonymous, 2015). South African legislation stipulates in regulation R.692, the Regulations Governing Microbiological Standards for Foodstuffs and Related Matters, under the Foodstuffs, Cosmetic and Disinfectants Act No4 of 1972 that *S. aureus* is required to not be present in 20 g of spice samples tested (Anonymous, 1997). High levels (>100 000 CFU.g^{-1}) of *S. aureus* growing optimally between 40°C and 45°C are required in order to produce enterotoxins thus rendering treatment 3 to be considered safe on the recommendation that prior stringent quality

control is required when receiving spices used in production of acidified dry sausages (Jay *et al.*, 2005d).

Total aerobic plate count is a general assessment of total aerobic activity, which is employed in the food industry as an indication of general hygiene (Smoot & Pierson, 1997, Jay *et al.*, 2005e). If found on the product it is indicative of potential cross contamination from the process to the product or, an abuse in the cold chain which enabled growth on the product (Jay *et al.*, 2005e). Limits are usually set in order manage the processing facilities and ultimate shelf-life of the product (Anonymous, 1999, Jay *et al.*, 2005e, Anonymous, 2015). The spice, meat and fat raw materials initial microbial load were 5.65×10^2 CFU.g^{-1}, 6.06×10^6 CFU.g^{-1} and 51.4×10^6 CFU.g^{-1}, respectively (Table 3.6). The meat and fat had high total aerobic plate counts which could be attributed to both the hygienic manner of processing the meat from the source through the value chain or potential abuse within the cold chain (Smoot & Pierson, 1997). Logarithmic reductions were observed throughout treatment 1 to treatment 5. South African Department of Health guidelines for processed meat suggest a total aerobic count of less than 200 000 CFU.g^{-1} in finished products (Anonymous, 2015). All the treatments fall within the South African guidelines for processed meats and as such all of the treatments can be considered as a safe option based on the total aerobic microflora in the final fermented sausage products.

Lactic acid bacteria are considered to have a probiotic effect and to the lay individual could be classified as “good” bacteria (Jensen *et al.*, 2012). These bacteria are responsible for the fermentation of reducing sugars and ultimate production of lactic acid resulting in a decrease in ultimate pH (Ricke & Keeton, 1997, Jay *et al.*, 2005a, Jay *et al.*, 2005f). Ideally, in fermented sausage production one would want increased numbers of lactic acid bacteria, primarily due to lactic acid bacteria antagonism (Jay *et al.*, 2005g). The evidence reported in Table 3.6 regarding the finished products’ lactic acid bacteria count indicates that as the pH decreased in the product between treatments, the numbers of lactic acid bacteria also logarithmically decreased. Due to this acidified product not being reliant on lactic acid bacteria, it is not a requirement for a certain number of lactic acid bacteria to be present, as such any of the treatments may be chosen when optimising the GdL concentration.

3.5 Conclusions

Based on the focus of the research required to determine the optimal concentration of GdL, the following factors from a quality perspective of the acidified dry sausage product are pertinent. The microbial safety of the product is directly affected by a combination of the pH and water activity of the product. The limits chosen as acceptable were below a pH of 5.0 and below a water activity of 0.93. From a manufacturer’s point of view, provided the pH and water activity were within specification, the moisture value should be as high as possible and the textural parameters must describe the product so as to ensure repeatability of the textural parameters upon reproduction of the product. In order to validate that the hurdles of pH and water activity are suitable, microbiological

testing was used. The microbiological specifications met South African Department of Health guidelines for uncooked acidified processed meats. Treatment 3, 4 and 5 were suitable to be chosen as acceptable from a safety point of view. The texture and moisture although descriptive, provided information which could assist in differentiating the treatments. Treatment 3 was ultimately chosen as the optimal concentration of GdL. This was due, primarily, to the fact that it met the pH, mass loss and water activity parameters desired and that it was in the centre of the texture parameters. The microbiological validation activity confirmed that the parameters used in the study provided product which was safe to consume.

3.6 References

Ammor, M.S. & Mayo, B. (2007). Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production: an update. *Meat Science,* **76**, 138-146.

Anonymous (1997). Regulations governing microbiologal standards for foodstuffs and related matters. In: GNR 692/1997, Johannesburg, South Africa: Department of Health.

Anonymous (1999). Microbiological guidelines for ready to eat foods. In: Food Watch Western Australia Food Monitoring Program. Australia.

Anonymous (2015). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. Pretoria, South Africa: Department of Health. [Internet Document].URL http://www.health.gov.za/index.php/shortcodes/2015-03-29-10-42-47/2015-04-30-09-10-23/2015-04-30-09-11-35/category/212-guidelines-training-manuals-and-reports. Accessed 20/08/2015

AOAC International (2002a). Loss on drying (moisture) at 95-100°C for feed. AOAC Official Method 934.01. In: *Official Method of Analysis,* 17th ed. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

AOAC International (2002b). Dumas combustion method. AOAC Official Method 992.15. In: *Official Method of Analysis,* 17th ed. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

AOAC International (2002c). Ash of Animal Feed. AOAC Official Method 942.05. In: *Official Method of Analysis,* 17th ed. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

Barbut, S. (2005). Effects of chemical acidification and microbial fermentation on the rheological properties of meat products. *Meat Science,* **71**, 397-401.

Barbut, S. (2006). Fermentation and chemical acidification of salami-type products - effect on yield, texture and microstructure. *Journal of Muscle Foods,* **17**, 34-42.

Barbut, S. (2010). Colour development during natural fermentation and chemical acidification of salami-type products. *Journal of Muscle Foods,* **21** 499–508.

Borch, E., Nesbakken, T. & Christensen, H. (1996). Hazard identification in swine slaughter with respect to foodborne bacteria. *International Journal of Food Microbiology,* **30**, 9-25.

Bourne, M.C. (1978). Texture profile analysis. *Food Technology,* **33**, 62-66

Burgess, M.N., Bywater, R.J., Cowley, C.M., Mullan, N.A. & Newsome, P.M. (1978). Biological evaluation of a methanol-soluble, heat-stable *Escherichia coli* enterotoxin in infant mice, pigs, rabbits, and calves. *Infection and Immunity,* **21**, 526-531.

Burnham, G.M., Hanson, D.J., Koshick, C.M. & Ingham, S.C. (2008). Death of Salmonella serovars, *Escherichia coli* O157 : H7, *Staphylococcus aureus* and *Listeria monocytogenes* during the drying of meat: a case study using biltong and droëwors. *Journal of Food Safety,* **28**, 198–209.

Campbell-Platt, G. (1994). Fermented foods - a world perspective. *Food Research International,* **27**, 253-257.

Cluff, M. (2013). The effect of CLA supplimentation on the quality of a cured, fermented pork sausage. MSc in Food Science Thesis. University of the Free State, Bloemfontein, South Africa.

Feiner, G. (2006a). The protein and fat content of meat. In: *Meat Products Handbook: practical science and technology*. Pp. 3-32. Cambridge, England: Woodhead Publishing Limited.

Feiner, G. (2006b). Raw Fermented Salami. In: *Meat Products Hanbook: practical science and technology*. Pp. 314-375. Cambridge, England: Woodhead Publishing Limited.

Gouws, P.A. & Liedemann, I. (2005). Evaluation of diagnostic PCR for the detection of *Listeria monocytogenes* in food products. *Food Technology and Biotechnology*, **43**, 201 – 205.

Harper, G.C. & Makatouni, A. (2002). Consumer perception of organic food production and farm animal welfare. *British Food Journal,* **104**, 287-299.

Hoffman, L., Muller, M., Schutte, D.W., Calitz, F. & Crafford, K. (2005). Consumer expectations, perceptions and purchasing of South African game meat. *South African Journal of Wildlife Research,* **35**, 33-42.

Hoffman, L.C. & Wiklund, E. (2006). Game and venison - meat for the modern consumer. *Meat Science,* **74**, 197-208.

Huff-Lonergan, E. & Lonergan, S.M. (2005). Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. *Meat Science,* **71**, 194-204.

Hugas, M. & Monfort, J.M. (1997). Bacterial starter cultures for meat fermentation. *Food Chemistry,* **59**, 547-554.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005a). Non dairy fermented foods and products. In: *Modern Food Microbiology,* 7th ed. Pp. 175-195. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005b). History of micro-organisms in food. In: *Modern Food Microbiology,* 7th. Pp. 3-9. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005c). Foodborne gastroenteritis caused by *Escherichia coli*. In: *Modern Food Microbiology,* 7th ed. Pp. 637-655. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005d). Staphylococcal gastroenteritis. In: *Modern Food Microbiology,* 7th ed. Pp. 545-566. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005e). The HACCP and FSO systems for food safety. In: *Modern Food Microbiology*. Pp. 497-515. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005f). Milk, fermentation, and fermented and nonfermented dairy products. In: *Modern Food Microbiology*. Pp. 149-173. New Yory, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005g). Food protection with chemicals and by biocontrol. In: *Modern Food Microbiology*. Pp. 301-350. New York, USA: Springer.

Jensen, H., Grimmer, S., Naterstad, K. & Axelsson, L. (2012). In vitro testing of commercial and potential probiotic lactic acid bacteria. *International Journal of Food Microbiology,* **153**, 216-222.

Jones, M. (2013). The addition of rooibos tea extract (Aspalathus Linearis) as a natural antioxidant to South African droëwors. MSc in Food Science Thesis, Stellenbosch University, South Africa.

Koep, K.S.C. (2005). Production of salami from meat of aquatic and terrestrial mammals. MSc in Animal Science Thesis, Stellenbosch University, South Africa.

Lawrie, R.A. & Ledward, D. (2006). The conversion of muscle to meat. In: *Lawrie's Meat Science,* 7th ed. Pp. 128-156. Cambridge, England: Woodhead Publishing Limited.

Lee, C.M., Trevino, B. & Chaiyawat, M. (1996). A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of Association of Official Analytical Chemists International,* **79**, 487–492.

Leroy, F. & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology,* **15**, 67-78.

Leroy, F., Verluyten, J. & De Vuyst, L. (2006). Functional meat starter cultures for improved sausage fermentation. *International Journal of Food Microbiology,* **106**, 270-285.

Lorenzo, J.M., Temperán, S., Bermúdez, R., Cobas, N. & Purriños, L. (2012). Changes in physico-chemical, microbiological, textural and sensory attributes during ripening of dry-cured foal salchichón. *Meat Science,* **90**, 194-198.

Lücke, F.-K. (2000). Utilization of microbes to process and preserve meat. *Meat Science,* **56**, 105-115.

Lücke, F. (1994). Fermented Meat Products. *Food Research International,* **27**, 299 - 307.

Mundell, D.H., Anselmo, C.R. & Wishnow, R.M. (1976). Factors influencing heat labile *Escherichia coli* enterotoxin activity. *Infection and Immunity,* **14**, 383-388.

Neethling, J. (2012). Impact of season on the composition and quality of male and female blesbok (*Damaliscus pygargus phillipsi*) muscles. MSc in Food Science Thesis, Stellenbosch University, South Africa.

Offer, G. (1991). Modelling of the formation of pale, soft and exudative meat: effects of chilling regime and rate and extent of glycolysis. *Meat Science,* **30**, 157-184.

Papa, F., Zambonelli, C. & Grazia, L. (1995). Production of Milano style salami of good quality and safety. *Food Microbiology*, 9 - 12.

Puolanne, E. & Halonen, M. (2010). Theoretical aspects of water-holding in meat. *Meat Science,* **86**, 151-165.

Ramoneda, M., Foncuberta, M., Simón, M., Sabaté, S., Ferrer, M., Herrera, S., Landa, B., Musté, N., Martí, R. & Trabado, V. (2013). Prevalence of verotoxigenic *Escherichia coli* O157 (VTEC O157) and compliance with microbiological safety standards in bovine carcasses from an industrial beef slaughter plant. *Letters in Applied Microbiology,* **56**, 408-413.

Ricke, S.C. & Keeton, J.T. (1997). Fermented meat, poultry and fish products. In: *Food Microbiology: fundamentals and frontiers,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 610-628. Washington D.C., USA: American Society for Microbiology Press.

Rip, D. & Gouws, P.A. (2009). Development of internal amplification control using multiplex PCR for the detection of *Listeria monocytogenes* in food products. *Food and Analytical Methods,* **2**, 190-196.

Ruiz de Huidobro, F., Cañeque, V., Lauzurica, S., Velasco, S., Pérez, C. & Onega, E. (2001). Sensory characterization of meat texture in sucking lamb: Methodology. *Investigación agraria. Producción y sanidad animales,* **16**, 245-256.

Ruiz de Huidobro, F., Miguel, E., Blazquez, B. & Onega, E. (2005). A comparison between two methods (Warner–Bratzler and texture profile analysis) for testing either raw meat or cooked meat. *Meat Science*, 527-536.

Smoot, L.M. & Pierson, M.D. (1997). Indicator Micro-organisms and Microbiological Criteria. In: *Food Microbiology Fundamentals and Frontiers,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 66-80. Washington D.C., USA: ASM Press.

Spaziani, M., Del Torre, M. & Stecchini, M.L. (2009). Changes of physicochemical, microbiological, and textural properties during ripening of Italian low-acid sausages. Proteolysis, sensory and volatile profiles. *Meat Science,* **81**, 77-85.

Todorov, S.D., Koep, K.S., Van Reenen, C.A., Hoffman, L.C., Slinde, E. & Dicks, L.M. (2007). Production of salami from beef, horse, mutton, blesbok (*Damaliscus dorcas phillipsi*) and springbok (*Antidorcas marsupialis*) with bacteriocinogenic strains of *Lactobacillus plantarum* and *Lactobacillus curvatus*. *Meat Science,* **77**, 405-412.

van Schalkwyk, D.L., McMillin, K.W., Booyse, M., Witthuhn, R.C. & Hoffman, L.C. (2011). Physico-chemical, microbiological, textural and sensory attributes of matured game salami produced from springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*) and zebra (*Equus burchelli*) harvested in Namibia. *Meat Science,* **88**, 36-44.

CHAPTER 4

Investigation of the temperature profile required to release an encapsulated acidulant and comparison of three concentrations of encapsulated commercial acidulants in a dry acidified sausage batter made from blesbok (*Damaliscus pygargus phillipsi*) meat

Abstract

The objective of this study was to define the kinetics of encapsulated acidulants. This involved the quantification of the temperature regime required to release the acidulant from the encapsulating material as well as the description of reduction in pH at an increasing concentration of three commercially available encapsulated acidulants. Trial 1 involved three treatments (T1 = 43.8 °C ± 0.18; T2 = 54.1 °C ± 0.37 and T3 = 64.4 °C ± 0.91), each treatment repeated three times in duplicate. Temperature regime T1 was not able to melt the encapsulate and release the acid within 170 min, however, both T2 and T3 were able to melt the encapsulate and release the acid after 60 min. A brief comparison of two methods of measuring pH (Sodium Iodoacetate and a simple probe method) was conducted. This comparison affirmed that using the probe directly in the centre of the sausage to measure pH was sufficient to measure sausage pH. In the second trial, three commercial encapsulated acidulants were mixed into Blesbok meat sausage batter at eight different concentrations ranging from 1.0 % to 8.0 % increasing by increments of 1.0 %. This was repeated in triplicate with duplicate measurements. Three linear regression equations were derived for the three encapsulated acids, notably, encapsulated GdL $y = -16.81x + 6.0214$ ($r^2 = 0.9694$); encapsulated lactic acid $y = -13.31x + 5.5864$ ($r^2 = 0.9435$) and encapsulated citric acid $y = -29.536x + 6.0273$ ($r^2 = 0.9844$). The equations provided a model to assist in determining the required concentration of encapsulated acidulant to realise a specific pH which is validated in Chapter 5, the final research chapter.

Keywords: encapsulated acidulant, temperature, concentration

4.1 Introduction

Traditional acidification of dry fermented sausages involves the reduction of simple sugars by homo-fermentative lactic acid bacteria resulting in the primary by-product of lactic acid ultimately reducing the pH of the meat (Montville, 1997, Ricke & Keeton, 1997, Jay *et al.*, 2005). Typically this fermentation process requires a period of between 18 – 72 h in order to fully acidify the product, where additional sugar is added to the batter to enable an endpoint pH of between 4.7 and 4.8 (Ricke & Keeton, 1997). Chemical acidification on the other hand, relies on the addition of either acids or other chemical acidulants which hydrolyse in the presence of water to form an acid, Glucono-delta-

lactone (GdL) being an example of the latter (Ricke & Keeton, 1997, Leroy & De Vuyst, 2009). The benefit of acidulant use is the realisation of a reduced processing time (Roberts & Getty, 2011). The acids, whether derived from chemical or traditional fermentation means, enable the denaturation of the protein network and consequently reduces the water holding capacity of the batter at the iso-electric point of the protein, thus enabling the ideal conditions to remove moisture from the product (Feiner, 2006a, Feiner, 2006b, Lawrie & Ledward, 2006, Warriss, 2010a).

These chemical acidulants come in various forms ranging from liquids to powders and can consist of either pure acids or blends of acids (Barbut, 2006, Leroy & De Vuyst, 2009, Anonymous, 2013a, Anonymous, 2013b, Anonymous, 2013c). The most commonly used chemical acidulants are lactic acid, citric acid and GdL (Leroy & De Vuyst, 2009). Modern technology has enabled encapsulated acids to be produced using a thin film coating of hydrogenated oil which has a melting point in the range of 55 – 75 °C (Sebranek, 2004, Anonymous, 2013a, Anonymous, 2013b, Anonymous, 2013c). The benefit of these encapsulated acids is the controlled release of the acid at a temperature near the melting point of the encapsulate as well as a slower rate of release of acid when compared to the non-encapsulated forms (Sebranek, 2004). The inclusion of acidulants is a function of concentration which, depending on the acidity of the acid at a specific inclusion level, realise a specific pH value as explained by Sebranek (2004).

Quality control parameters which are deemed necessary for both product quality and food safety pertaining to dried acidified sausages are pH and water activity along with moisture content (Ricke & Keeton, 1997, Ockerman & Basu, 2015). Sausages are classified by their moisture content, notably dry, semi-dry and moist sausages, which are verified by the water activity, a value expressing the availability of water in the product (Ricke & Keeton, 1997, Ockerman & Basu, 2015). Thus, in the case of chemical acidification, the sausage batter experiences a rapid reduction in pH which serves as the main parameter to assist in the maturation and drying of the final product.

Typically, encapsulated acidulants are used in American style summer sausages which require the acid purely to provide a typical tangy acidic taste and rely on a further heating step to inactivate pathogenic micro-organisms (Roberts & Getty, 2011). However, the purpose of this research was to utilise the chemical acidulants as a replacement for lactic acid bacteria in the production of a dried acidified sausage. The aim was thus to utilise a stepwise approach to determine a temperature regime, involving a temperature and time combination, in order to release encapsulated acidulants at varying concentrations in order to determine the concentration of acidulant required to realise a pre-determined pH in the production of a dried acidified sausage.

4.2 Materials and methods

There are two sections in Chapter 4; the first section (Trial 1) investigated the effect three different temperature regimes had on the release of GdL from its encapsulated state and the subsequent rate of pH decline. The second section (Trial 2) compared three different encapsulated acidulants namely

GdL; Lactic acid and Citric acid, at eight different concentrations, utilising the temperature regime which was able to yield the lowest and quickest pH drop in Trial 1. This data enabled the description of pH decline in terms of a linear regression and an indicative model for a required concentration to acidify sausage batter to a pre-determined endpoint. An interim investigation in the method of measuring pH was also conducted in order to substantiate the ultimate method chosen.

4.2.1 Acquisition and preparation of ingredients

A standard formula was used for the duration of the trial one and trial two. The formula was based on the formula used in Chapter 3. The salt pack and chemical acidulants masses were calculated as a percentage of the mass of the meat block.

Blesbok Meat and Fat

Seven mature male Blesbok were harvested on the Stellenbosch University experimental farm (Brakkekuil, Witsand, Western Cape) for the purpose of this trial. Care was taken to minimise stress by night culling with one shot to the head or neck of the antelope. Following the shot, the animal was timeously exsanguinated, transported to the farms meat processing facility where it was rinsed, skinned, eviscerated, rinsed again, in both cases with potable water and subsequently chilled in a refrigerator at 4°C for 24 h. After 24 h, the carcass was deboned and the meat was vacuum packed, labelled, transported back to the Stellenbosch University Meat Science laboratory and frozen at -20°C. The meat was subsequently defrosted, to remove all excess fat and sinew, cut into 5 cm^3 cubes then frozen at -20°C.

Subcutaneous pork fat was purchased from a local commercial pork producer in the Western Cape, South Africa and was trimmed to remove all meat and rind, cubed into 5 cm^3 blocks after which it was frozen at -20°C until required for processing.

When required, the meat and fat cubes were allowed to slightly defrost at ambient temperatures for 20 min after which, they were minced separately using a Bizerba mincer (Model TE 22, Germany) setup with the 4.2 mm mincing plate. After mincing, the meat and fat were separately stored in plastic bags within the refrigerator set at 4°C.

Salt and Nitrate/Nitrite blend

The salt pack was a 5 kg commercial mix acquired from Freddy Hirsch (Pty) Ltd, a South African spice supplier situated in Maitland, Western Cape. The formulation listed in Table 4.1, denotes the composition of the salt pack used.

Table 4.1 Ingredient composition of the Freddy Hirsch commercial salt pack used in the sausage batter formulation

Ingredients	Percentage (%)	Mass (g)
Salt	91.00	4550.00
Sodium Nitrite	8.00	400.00
Sodium Carbonate	1.00	50.00

Salt Pack Total	**100.00**	**5000.00**

Acidulants

The chosen encapsulated acidulants, namely meat-sure 496 (GdL), 333 (Citric acid) and 509 (Lactic acid) were sourced from Balchem Corporation, Slate Hill, New York (Anonymous, 2013a, Anonymous, 2013b, Anonymous, 2013c). These all came as pure ingredients and were weighed out according to the concentrations denoted below in Table 4.2. Since industry uses between 0.5 and 3.0% GdL and when gathering data for a linear regression, 8 points with 1.0% increments was chosen to ensure ease of measuring of the acidulants as well as sufficient points were present for the linear regression lines.

Table 4.2 Concentration of encapsulated acidulants required for the two trials investigating temperature, time and concentrations required for acidification of sausage batter

Study	E-GdL (%)	E-Citric acid (%)	E- Lactic acid (%)
Trial 1	2.50	-	-
Trial 2	1.00	1.00	1.00
Trial 2	2.00	2.00	2.00
Trial 2	3.00	3.00	3.00
Trial 2	4.00	4.00	4.00
Trial 2	5.00	5.00	5.00
Trial 2	6.00	6.00	6.00
Trial 2	8.00	8.00	8.00

4.2.2 Trial 1 – Comparison of three temperature regimes

The experimental design consisted of three temperature regimes, repeated three times in duplicate resulting in a total of 18 measurements as laid out in Table 4.3. The three temperatures were 45, 55 and 65°C, respectively. Each repeat was a different mixture of raw material, mixed in a different time period. Duplicate readings of the repeats were monitored with two different pH meters and temperature loggers within the same time period.

Table 4.3 Experimental design of the temperature regime assessment including temperature regime, repeats and duplicate measures

	T1		T2		T3	
R1	D1	D2	D1	D2	D1	D2
R2	D1	D2	D1	D2	D1	D2
R3	D1	D2	D1	D2	D1	D2

T – Temperature regime (45, 55 and 65 °C respectively).
R – Repeats.
D1 – Duplicate reading 1.
D2 – Duplicate reading 2.

Encapsulated GdL was the only acidulant used in the first section of this trial. The concentration chosen was 2.50%. This concentration was chosen as similar research had been conducted with this ingredient indicating that 2.07% resulted in an ultimate pH of 4.66 ± 0.05 (Barbut, 2006). Although the concentration is based on the above-mentioned research, a value greater than the previous researched value was selected. The end-point of the experiment was considered to be attained once the pH value had stabilised between two consecutive readings.

The minced meat, minced fat, salt pack and encapsulated GdL were weighed out according to the formulation stipulated in Table 4.4. All four ingredients were thoroughly mixed together for one minute by hand ensuring that the ingredients were homogenously dispersed. Once mixed, the sausage batter was subdivided into 35 g portions and placed into the 35 mm diameter glass test-tubes and compressed with a spoon to remove as many air pockets as possible.

Table 4.4 Mass (g) and percentage (%) of ingredients required for Trial 1 to determine the temperature regime required to release the encapsulated acidulants

	Percentage (%)	**Mass (g)**	**Mass (g) for 9 treatments**
Meat	75	82,5	742,5
Fat	25	27,5	247,5
Total	100	110	990
salt pack	3	3,30	29,7
E-GdL	2,50	2,75	24,75

The water bath (Memmert W 600, Schwabach, West Germany) was filled with distilled water and set at the required temperatures, 45, 55 and 65 °C, respectively. The temperature was validated by a calibrated glass alcohol thermometer. Once at the required temperature, the calibrated Crison

PH25 (Spain) pH meter connected to an ICRI5054 TC probe (Spain) and LogTag Recorders SA temperature logger TREX-8 with a ST1000T-15 probe (Somerset West, South Africa) were placed into the core of the sausage batter and switched on to ensure continual monitoring of the sausage batter during the heating process. The prepared test-tubes were allowed to stabilise for a minute after which the test tubes were placed into the water bath in their stand, the water bath lid was closed and the first reading of pH and time was recorded. Every 5 min the pH was recorded as well as the glass thermometers value and the water bath temperature setting until the pH had stabilised for two consecutive recordings. Once each treatment had been repeated three times, the data was collected and analysed.

4.2.3 Comparison of two methods to measure pH (probe in sample or Na-iodoacetate)

Due to variation of ± 0.29 pH points in the first trial, fat was removed from the formulation for this comparative study. Sausage research has utilised various methodologies in the measurement of pH including inter alia the simple probe method and the Na-iodoacetate method (Jeacocke, 1977, Houben & van 't Hooft, 2005, González-Fernández *et al.*, 2006). The comparison of these two methods of pH measurement were conducted in order to choose a method of analysis for further research of pH devolution in order to minimise variation within the pH measurement of the sausage. The premise of pH measurement post a 12 h resting period was employed to assist in the homogenisation of the acid throughout the sausage in order to minimise the possibility of localised low pH regions. Devolution pH data was analysed firstly, by inserting a probe directly into the sausage batter post-heating, as well as post-cooling in the refrigerator at 4°C for 12 h. The second method involved the chilled sausage batter and employed the Na-iodoacetate method of homogenisation and measurement of the pH of the slurry with the same probe as in the first method. The specifics of the Na-iodoacetate method involved homogenising 30 mL solution of 5 mM Na-iodoacetate and 150 mM KCl solution with 3 g of sausage sample, after which the pH probe was used to measure the pH of the solution (Jeacocke, 1977). In both cases, the probe was calibrated at room temperature using the standard two point calibration of 4.01 and 7.00. The temperature of calibration and temperature of pH measurement was recorded. The corrected pH value incorporating the temperature of the measurement was calculated using the formula:

$$pH_{corrected} = \left[(pH_{measured} - 7) x \frac{(273.15 + T_{calibration})}{(273.15 + T_{measured})} \right] + 7$$

$T_{calibration}$ – Temperature in degrees Celsius at which the probe was calibrated.

$T_{measured}$ – Temperature in degrees Celsius at which the actual pH measurement was conducted.

$pH_{measured}$ – The reading of pH of the sample measured.

$pH_{corrected}$ – The corrected pH measurement taking into account the effect of temperature of the sample.

Once corrected, the values were compared to make an informed choice of pH measurement methodology going forward.

4.2.4 Comparison of different concentrations of acidulants

Three chemical acidulants, namely, encapsulated lactic acid, encapsulated citric acid and encapsulated GdL were incorporated into the sausage batter at eight different concentrations. These concentrations were standardised at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 % m/m for all three chemical acidulant treatments. The assessment was repeated three times for each concentration and each replicate was measured in duplicate. The parameters of pH and temperature were recorded for both the initial and final pH values. The batter was mixed according to the formulation laid out in Table 5, where the acid was calculated on a mass basis as a percentage of the meat block. The sausage batter, once mixed and placed in the test tube, was placed into the water bath controlled at 55°C for 65 minutes after which the pH and temperature were measured via the probe method and recorded. The pH probe was calibrated using a two point calibration at the standard 4.01 and 7.00 points and the temperature at which the probe was calibrated was noted. All pH measurements were corrected for temperature using the formula stated in 4.2.3.

Table 4.5 Percentage and mass required for the production of the sausage batter in Trial 2

	Percentage (%)	**Mass (g)**
Meat	75	26.25
Fat	25	8.75
Total	100	35.00
Salt cure pack	3	1.05
Acid	1 – 8*	

* The concentration of 1 through to 8 percent was calculated using the meat block total value on a mass per mass basis.

4.2.5 Data analysis

Data analysis for Trial 1, the comparison of pH measurement, and Trial 2 were computed on Microsoft Excel 2013 (California, USA) where mean and standard deviation values were calculated and plotted on a linear regression in order to be analysed. Best fit trend lines with their R^2 values were reported.

4.3 Results

Tabulated and graphical results are presented and fully discussed in the following Discussion section of this thesis.

Table 4.6 Mean pH and core temperature values for the three treatments observing the rate of pH decline until a constant pH was obtained

Time	T1 – 45°C		T2 – 55°C		T3 – 65°C	
	pH	Core Temp (°C)	pH	Core Temp (°C)	pH	Core Temp (°C)
0	5.8 ± 0.04	23.8 ± 3.74	5.8 ± 0.04	35.6 ± 6.87	5.9 ± 0.13	20.1 ± 0.69
5	5.7 ± 0.06	33.0 ± 4.28	5.7 ± 0.00	48.0 ± 1.90	5.7 ± 0.09	48.6 ± 0.80
10	5.7 ± 0.03	39.7 ± 1.68	5.5 ± 0.08	51.7 ± 0.68	5.6 ± 0.07	58.7 ± 0.38
15	5.7 ± 0.03	42.3 ± 0.73	5.3 ± 0.20	53.1 ± 0.20	5.3 ± 0.03	62.3 ± 0.25
20	5.7 ± 0.07	43.3 ± 0.29	5.1 ± 0.25	53.8 ± 0.12	5.0 ± 0.08	63.8 ± 0.34
25	5.7 ± 0.09	43.6 ± 0.28	5.0 ± 0.28	54.1 ± 0.11	4.9 ± 0.07	64.4 ± 0.12
30	5.6 ± 0.11	43.8 ± 0.24	5.0 ± 0.29	54.2 ± 0.13	4.8 ± 0.03	64.8 ± 0.04
35	5.6 ± 0.13	43.9 ± 0.15	4.8 ± 0.22	54.1 ± 0.16	4.7 ± 0.12	64.9 ± 0.19
40	5.5 ± 0.15	43.9 ± 0.11	4.8 ± 0.10	54.2 ± 0.08	4.7 ± 0.11	64.9 ± 0.20
45	5.5 ± 0.20	43.9 ± 0.08	4.8 ± 0.17	54.3 ± 0.11	4.6 ± 0.06	64.9 ± 0.05
50	5.4 ± 0.20	44.0 ± 0.11	4.7 ± 0.18	54.0 ± 0.27	4.8 ± 0.18	63.8 ± 1.19
55	5.4 ± 0.17	44.0 ± 0.15	4.7 ± 0.21	53.7 ± 0.64	4.6 ± 0.10	64.4 ± 0.53
60	5.4 ± 0.10	44.0 ± 0.18	4.6 ± 0.16	54.0 ± 0.14	4.6 ± 0.12	64.6 ± 0.17
65	5.4 ± 0.11	43.9 ± 0.06	4.6 ± 0.15	54.2 ± 0.08	-	-
70	5.2 ± 0.09	43.9 ± 0.12	4.7 ± 0.16	54.3 ± 0.10	-	-
75	5.2 ± 0.10	43.9 ± 0.06	-	-	-	-
80	5.2 ± 0.09	43.9 ± 0.09	-	-	-	-
85	5.1 ± 0.08	43.9 ± 0.04	-	-	-	-
90	5.1 ± 0.10	43.9 ± 0.06	-	-	-	-
95	5.1 ± 0.07	43.9 ± 0.06	-	-	-	-
100	5.2 ± 0.15	43.8 ± 0.02	-	-	-	-
105	5.2 ± 0.16	43.8 ± 0.13	-	-	-	-
110	5.2 ± 0.14	43.8 ± 0.11	-	-	-	-
115	5.1 ± 0.07	43.8 ± 0.14	-	-	-	-
120	5.1 ± 0.08	43.7 ± 0.19	-	-	-	-
125	5.0 ± 0.09	43.8 ± 0.15	-	-	-	-
130	5.0 ± 0.07	43.8 ± 0.12	-	-	-	-
135	5.0 ± 0.09	43.7 ± 0.10	-	-	-	-
140	5.0 ± 0.10	43.8 ± 0.13	-	-	-	-
145	5.0 ± 0.09	43.7 ± 0.12	-	-	-	-
150	5.0 ± 0.08	43.8 ± 0.15	-	-	-	-
155	5.0 ± 0.08	43.8 ± 0.04	-	-	-	-
160	4.9 ± 0.00	44.0 ± 0.00	-	-	-	-
165	4.8 ± 0.00	43.9 ± 0.00	-	-	-	-
170	4.8 ± 0.00	44.0 ± 0.00	-	-	-	-
175	4.8 ± 0.00	43.9 ± 0.00	-	-	-	-

Table 4.6 depicts the values of pH and temperature recorded for the duration of Trial 1 of the three different temperature regimes. Treatment T1 did not fully acidify in the allotted time period and as such the trial was stopped after 175 min. Treatments T2 and T3 acidified more quickly than T1, resulting in the trail being halted after 70 min in the case of T2 and 60 min in the case of T3 at final acidification values of 4.7 ± 0.16 and 4.6 ± 0.12 respectively.

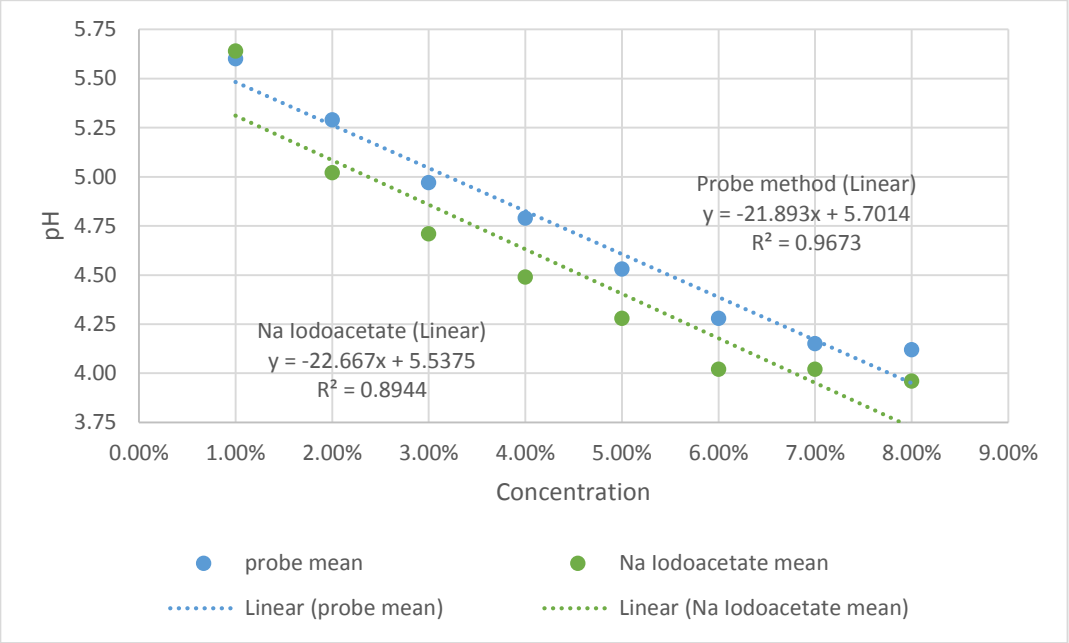

Figure 4.1 Linear regression lines comparing the probe and Na-iodoacetate methods of pH measurement in acidified sausage batter**.**

Figure 4.1 represents the comparison of the two pH measuring methodologies. Linear regressions were fit to the data resulting in the probe method and Na-iodoacetate method having R squared values of 0.9673 and 0.8944, respectively.

Figure 4.2 depicts the linear regressions of the three different encapsulated acids at eight different concentrations all being exposed to the same temperature regime of 55°C for 65 min. E-GdL had an R^2 value of 0.9694, E-lactic acid had and R^2 value of 0.9435 and lastly, E-citric acid had an R^2 value of 0.9844. The gradient in all three regressions (E-GdL, -16.81; E-Lactic acid, -13.31 and E-Citric acid, -29.54) were negative.

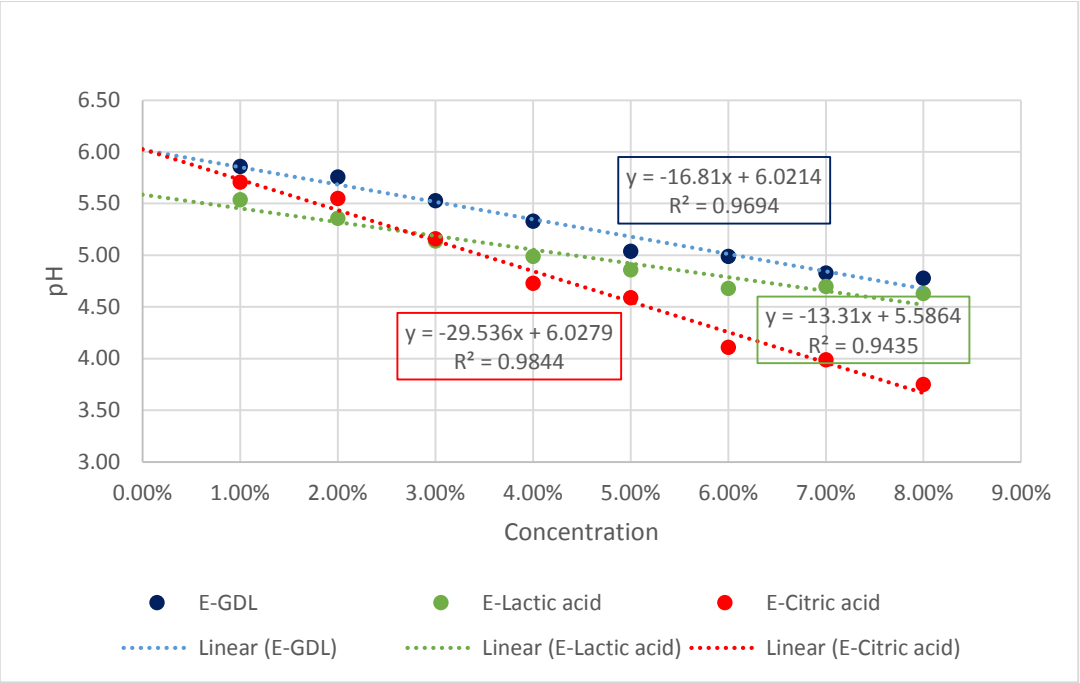

Figure 4.2 Representation of the three pH devolution linear regression lines for the three encapsulated acidulants after being exposed to a temperature regime of 55°C for 60 minutes.

Table 4.7 Derived formula of the linear regression for the different chemical acidulants

Chemical Acidulant	Equation	R^2 Values
Encapsulated GdL	$y = -16.81x + 6.0214$	0.9694
Encapsulated Lactic acid	$y = -13{,}31x + 5{,}5864$	0.9435
Encapsulated Citric acid	$y = -29.536x + 6.0279$	0.9844

x – The pH desired in the final batter allowing for the calculation of y, the concentration of acidulant needed to reach the desired pH.

4.4 Discussion

4.4.1 Trial one – Effect of the degradation of pH over time at three different temperatures

The premise of Trial 1 is based on the assumption that heat melts the fat based encapsulate in order to release the chemical acidulant within. Higher heat will increase the rate at which the heat is transferred to the sausage and in turn, increase the rate at which the fat encapsulate melt. This allows a gradual reduction in pH until the melting point of the fat encapsulate is reached at which point there is a rapid release of acidulant. Notably, the acidulant chosen was GdL which will hydrolyse in water to form gluconic acid. This hydrolysis reaction will in turn slow down the rate of pH reduction post melting of the encapsulate material.

The product specification of MeatSure 496 (encapsulated GdL) declares that the encapsulate comprises of hydrogenated vegetable oil with a melting point of 57 – 71°C (Anonymous, 2013b).

Although the water bath was set to a specified temperature (45, 55 and 65°C) and controlled to ensure a set temperature with the glass bulb thermometer, core temperature in the product was in all three cases slightly lower than the expected temperature. The reason for this is due to equipment variation where the log tag devices have an error of 0.5°C and there is a possible error of parallax when reading the glass bulb thermometer. None the less, the mean temperatures were: T1 = 43.8 ± 0.18°C; T2 = 54.1 ± 0.37°C and T3 = 64.4 ± 0.91°C. The core temperatures for all of the treatments were reached after 25 min. The end of the experiment was confirmed when there was a stabilisation of pH in the sausage that is when the pH did not change between two consecutive pH readings. This point was deemed to be reached after 65 min in the case of T2 – 55°C where the final pH reached was 4.6 ± 0.15 and after 60 min in the case of T3 – 65°C where the final pH reached was 4.6 ± 0.12. Treatment T1 – 45°C although slowly decreasing, did not acidify below a pH of 5.0 as seen between 125 and 150 min. The experiment was stopped at this point. The data confirmed that the melting point of the encapsulating material was required to be reached in order to fully release the acid and to subsequently realise a rapid reduction in pH.

Industrial applications require a cost saving in terms of time and energy used, as well as ingredient efficiency. Based on the time required to acidify treatment T1, the rate of pH decline is too slow and the period to get to the desired pH is too long when compared to T2 and T3. The temperature of T1 was not sufficient to melt the encapsulated material so the acid at no point in the experiment was fully released. Speculation suggests that the reduction in pH experienced in treatment T1 during the investigation could be due to the presence of moisture, hydrolysing residual acidulant which may not have been entirely coated, as well as natural microflora exposed to the inflated temperature which could encourage the growth of Lactic acid bacteria resulting in an increase in Lactic acid concentration.

The product specification indicated that the encapsulated GdL, when diluted with water at a 1% solution, realised a pH of 4 (Anonymous, 2013b). The end points observed were 4.6 ± 0.15 (T2), 4.6 ± 0.12 (T3) and 4.8 ± 0.00 (T1), respectively based on a 2.5% mass-based inclusion of the encapsulated GdL. The values observed were higher than the value of 4.0 reported in the specification. This can be attributed to buffering capacity, a function of the effect of meat, fat, moisture, salt, nitrates and minerals, on the acidification of the product. Since both T2 and T3 were able to fully acidify between 60 and 65 min, the choice of T2 at 55 °C for 65 min was chosen as the optimal conditions for release of 2.50% encapsulate GdL.

The measurement of pH, is based on measuring the difference in potential, an electrical measurement which is based on the hydrogen ion content of the solution (Mettler-Toledo, 2007). Meat contains both water and protein, both of which have an effect on the charge and hence provide a buffering capacity when measuring pH (Warriss, 2010b). The methodologies employed in Trial 1 resulted in standard deviations of pH up to ± 0.29. This variation could be explained by both the temperature at which the pH is measured and the lack of homogenisation of fat particles within the

sausage batter. Further research in this chapter was required to describe the kinetics of the decline in pH with an increase in acidulant concentration. This further research was conducted on lean meat and the pH and temperature values were closely recorded, allowing for future pH measurements to be analysed using the pH value which has been compensated for temperature.

Although the determination of heating requirements for acidulant release were achieved in the laboratory, the important parameter to consider is that the core temperature is reached within the sausage batter. This core temperature point is speculated to be predetermined by the diameter of the product being heated. Further research could quantify the differences in time to reach a predetermined core temperature as influenced by different sausage diameters.

4.4.2 Comparison of two methods to measure pH

The study on the methodology of pH measurement was conducted and reported in Figure 4.1. The linear trend lines for the probe method (R^2 = 0.9673) and for the Na-iodoacetate method (R^2 = 0.8944) had a Y-intercept value of 5.7014 and 5.5375, respectively. Reported Blesbok meat pH, 24 h post mortem is 5.60 ± 0.02 which lies in the middle of the two intercepts reported (Neethling, 2012). Since the probe method was the more simple method to use, had a higher R^2 value and the standard deviation in pH measurements were 0.05 for the probe method and 0.04 for the Na-iodoacetate method, the choice in method to continue the project was the simpler probe method.

Ultimately, the understanding of the variation experienced was not sufficiently explained, thus warranting further research to understand the individual ingredient components and the effect they have on ultimate sausage pH. A comparative study of the migration of pH within the sausage diameter over time would assist in the understanding of the acidification within the sausage. This, along with an in-depth literary and practical review study of different pH measuring methods would assist greatly in defining standard pH measuring methodology.

4.4.3 Trial two – pH devolution at 55°C for 65 min

All three chemical acidulants were fitted with linear regression trend lines as presented in Figure 4.2. As expected, all three lines had negative gradients, thus pH is inversely proportional to the concentration of chemical acidulant indicating an increase in concentration decreased the pH. The three formula represented in Table 4.7, for the three different encapsulated acids, were derived from the linear regression lines which enable the calculation of the concentration of chemical acidulant required to realise a final pH in the sausage batter post acidification.

From the equations, one can deduce that less encapsulated citric acid would be required to drop the pH of the sausage batter to a predetermined end point than that of encapsulated GdL and encapsulated lactic acid. Encapsulated GdL will require more mass than encapsulate lactic acid in order to reach the same pre-determined pH end point in the sausage batter. Although in terms of quantity, a decision based on the choice of acid would be easy to make. GdL has been used in industry as it requires time to hydrolyse into gluconic acid as opposed to both citric or lactic acid

which tend to acidify more quickly (Barbut, 2006). Also, the pure acid forms, due to their rapid acidification have been associated with a crumbly texture of the final sausage product (Barbut, 2006). The encapsulated form of GdL, lactic and citric acid should in effect mitigate this time requirement as it requires heating in order to release the acid. Thus validation based primarily on textural measurements would be able to further differentiate between the encapsulated acidulants and assist in determining the optimal acid.

4.5 Conclusions

When Blesbok meat sausage batter is raised to a core temperature of 55°C after placing the batter in an environment of 55°C for 65 min as represented in trial 1, treatment 2, the encapsulated material was able to fully release the GdL from its encapsulate. In terms of pH measurements, observations indicated variation within the sausage batter. An attempt to compare methods was conducted resulting that for the duration of the experiments required for this thesis, the simple method of placing the probe in the core was deemed sufficient for pH analysis.

Using the probe method of pH measurement with the 55°C for 65 min heating regime, the comparison of pH and the concentration of different encapsulated acidulants was conducted. The comparison defined three linear regression equations which may assist in the calculation of the concentration of acidulant required to realise a predetermined ultimate pH in the dry acidified sausage. The validation of these formula are the basis of Chapter 5.

4.6 References

Anonymous (2013a). MeatSure 333 (Encapsulated citric acid) specification and safety data sheet. Pp. 1-3;1-6. New Hampton, NY, USA: Balchem Corporation.

Anonymous (2013b). MeatSure 496 (Encapsulated Glucono Delta Lactone) specification and safety data sheet. Pp. 1-3;1-6. New Hampton, NY, USA: Balchem Corporation.

Anonymous (2013c). MeatSure 509 (Encapsulated Lactic Acid) specification and safety data sheet. Pp. 1-3;1-6. New Hampton, NY, USA: Balchem Corporation.

Barbut, S. (2006). Fermentation and chemical acidification of salami-type products - effect on yield, texture and microstructure. *Journal of Muscle Foods,* **17**, 34-42.

Feiner, G. (2006a). The protein and fat content of meat. In: *Meat Products Handbook: practical science and technology*. Pp. 3-32. Cambridge, England: Woodhead Publishing Limited.

Feiner, G. (2006b). Raw fermented salami. In: *Meat Products Handbook: practical science and technology,* 1st ed. Pp. 314-375. Cambridge, England, UK: Woodhead Publishing Limited.

González-Fernández, C., Santos, E.M., Rovira, J. & Jaime, I. (2006). The effect of sugar concentration and starter culture on instrumental and sensory textural properties of chorizo-Spanish dry-cured sausage. *Meat Science,* **74**, 467-475.

Houben, J.H. & van 't Hooft, B.J. (2005). Variations in product-related parameters during the standardised manufacture of a semi-dry fermented sausage. *Meat Science*, 283–287.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005). Non dairy fermented foods and products. In: *Modern Food Microbiology,* 7th ed. Pp. 175-195. New York, USA: Springer.

Jeacocke, R.E. (1977). Continuous measurements of the pH of beef muscle in intact beef carcasses. *Journal of Food Technology,* **12**, 375-386.

Lawrie, R.A. & Ledward, D. (2006). The conversion of muscle to meat. In: *Lawrie's Meat Science,* 7th ed. Pp. 128-156. Cambridge, England: Woodhead Publishing Limited.

Leroy, F. & De Vuyst, L. (2009). Fermentation and acidification ingredients. In: *Ingredients in Meat Products: properties, functionality and applications,* (edited by R. Tarte). Pp. 227-252. New York, USA: Springer.

Mettler-Toledo (2007). pH Theory Guide: a guide to pH measurement - the theory and practice of laboratory applications Pp. Schwerzenbach, Switzerland: Mettler-Toledo.

Montville, T.J. (1997). Princliples which influence microbial growth, survival, and death in foods. In: *Food Microbiology: fundamentals and frontiers*. (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 13-29. Washington D.C., USA: American Society for Microbiology Press.

Neethling, J. (2012). Impact of season on the composition and quality of male and female blesbok (*Damaliscus pygargus phillipsi*) muscles. MSc in Food Science Thesis, Stellenbosch University, South Africa.

Ockerman, H.W. & Basu, L. (2015). Production and consumption of fermented meat products. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá, Y.H. Hui, I. Astiasaran, J. Sebranek & R. Talon). Pp. 7-11. West Sussex, UK: John Wiley & Sons, Ltd.

Ricke, S.C. & Keeton, J.T. (1997). Fermented meat, poultry and fish products. In: *Food Microbiology: fundamentals and frontiers,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 610-628. Washington D.C., USA: American Society for Microbiology Press.

Roberts, M.N. & Getty, K.J. (2011). Validation of heating conditions in production of direct acidified venison with beef fat summer sausage for elimination of *Escherichia Coli* 0157:H7. *Journal of Food Safety,* **31**, 480-486.

Sebranek, J. (2004). Semidry fermented sausages. In: *Handbook of food and beverage fermentation technology,* (edited by Y.H. Hui, L. Meunier-Goddik, J. Josephsen, W.K. Nip & P.S. Stanfield). Pp. 385-396. New York, USA: Marcel Dekker.

Warriss, P.D. (2010a). Post-mortem changes in muscle and its conversion into meat. In: *Meat Science: an introductory text,* 2nd ed. Pp. 65-76. Wallingford, UK: CABI Publishing.

Warriss, P.D. (2010b). The chemical composition and structure of meat. In: *Meat Science: an introductory text,* 2nd ed. Pp. 26-47. Wallingford, Cambridge: CABI Publishing.

CHAPTER 5

Physico-chemical validation of four encapsulated acidulant concentrations used in the production of Blesbok (*Damaliscus pygargus phillipsi*) meat acidified dry sausage

Abstract

The objective of this chapter was to validate four linear regression models which were used to predict predetermined end point pH values in Blesbok meat dry acidified sausage. The validation study focussed on the pH, water activity (a_w) and mass loss values, as well as providing descriptions of textural and proximate parameters. The target pH of the three encapsulated acids was 4.80. The respective final pH values were, GdL (4.9 ± 0.06), E-GdL (3.7 ± 0.02), E-lactic acid (4.2 ± 0.04) and E-citric acid (3.5 ± 0.08). In terms of target water activity, the requirement was for water activity to drop below 0.86. The respective water activity values were GdL (0.84 ± 0.02), E-GdL (0.82 ± 0.01), E-lactic acid (0.80 ± 0.01) and E-citric acid (0.84 ± 0.01). Mass loss values as reported in percentage were GdL (38.5 ± 1.47), E-GdL (37.8 ± 0.83), E-lactic acid (39.0 ± 0.91) and E-citric acid (40.7 ± 0.46). Subsequent proximate data including moisture content, protein content, fat content and ash content were quantified along with texture profile analysis including first compression hardness, second compression hardness, gumminess and cohesiveness. Although completed, the validation study did not reach final pH values close enough to the pre-determined end point. The resulting data is merely descriptive and calls for a more thorough understanding of the effect specific ingredients have on the decrease in pH of acidified dry sausages, a more precise kinetic model of the pH devolution and revisiting the evaluation of pH in terms of both theory and specific methodology.

Keywords: Validation; encapsulated acid; acidulant; dry sausage

5.1. Introduction

Examples of the concept of validation exercises can be found in food safety management systems whereby processes are required to be validated; an example would be in ISO 22000:2005 (ISO, 2005). It is a twofold process whereby firstly, the food safety parameters are required to be assessed and proven to be in place, and secondly, that the product parameters are assessed to ensure that the specification of the product are defined and fit in with the general classification of the product type (ISO, 2005).

When observing a dried acidified sausage from a microbiological perspective, all of the bacteria and groups of bacteria have optimal growth conditions (Jay *et al.*, 2005a). The goal of producing an acidified dry sausage is to minimise the parameters to below that which can sustain growth of the organisms concerned in order to prevent the pathogens from proliferating (Jay *et al.*,

2005b). In the case of dry acidified sausage, this is achieved by reducing the pH and reducing the moisture content, serving as physical food safety parameters (Barbut, 2005, Jay *et al.*, 2005b, Barbut, 2006, Danquah *et al.*, 2012).

The pH and water activity are the parameters which are most critically required in order to produce an acidified dry sausage (Ricke & Keeton, 1997). The pH is required to drop to the isoelectric point of the meat which reduces the water binding capacity, due to the reduced charge on the protein molecule, allowing for moisture to be removed from the product during the drying phase (Chieh, 2012). As a result, the water is not available for the bacteria and there is a resultant decrease in water activity during the drying process (Chieh, 2012, Ockerman & Basu, 2015). Jay *et al*., (2005b) alludes to the fact that a typical Italian or dry acidified sausage has a moisture content between 30 - 40% and a pH dropping from 5.8 to 4.8.

Fernandez-Lopez *et al.* (2008) indicates that physico-chemical analysis typically focusses on pH, water activity and proximate analysis when analysing dry acidified sausages. Other researchers include colour, residual nitrate and acid contents as well as free fatty acids, sodium chloride concentration, mineral composition, texture profile analysis into their physico-chemical analysis profile (Pérez-Alvarez *et al.*, 1999, Zanardi *et al.*, 2010, van Schalkwyk *et al.*, 2011).

The addition of acidulants as opposed to the use of fermentation is used to reduce the processing time of the product (Barbut, 2005, Barbut, 2006). The acidulants used in the production of a dry-acidified sausage come in either a pure or an encapsulated form (Leroy & De Vuyst, 2009). The common commercial acidulants available for use in the meat industry are encapsulated forms of lactic acid, citric acid and Glucono-delta-lactone (GdL) (Barbut, 2005, Barbut, 2006, Anonymous, 2013a, Anonymous, 2013b, Anonymous, 2013c). Technology has enabled acidulants to be encapsulated in fat based compounds which have a specific melting point (Gibbs *et al.*, 1999). Once the melting point is reached, the acid can be released into the product thus allowing for a precise control of acid release (Gibbs *et al.*, 1999). This control is reported to be beneficial in that it prevents the crumbling of the product as well as a brittle texture and fat separation (Barbut, 2005, Barbut, 2006).

The aim thus was to produce four variants of dry acidified sausage using four different available commercial acidulants whilst validating that they reach a pre-determined pH and water activity endpoint and furthermore, provide a comparison based on typical physico-chemical quality parameters involving specifically pH, water activity, mass loss, texture analysis and proximate analysis.

5.2. Materials and methods

The production of dry acidified sausage was based on a 4 x 6 block design whereby 4 treatments (T1 – T4) were repeated 6 times (R1 – R6). Each repeat was produced with a different Blesbok. One

Blesbok was mixed and split into four groups of meat, in order that each treatment was produced using the same animal, this was then repeated six times.

Six mature Blesbok (*Damaliscus pygargus phillipsi)* were harvested on the Stellenbosch University experimental farm (Brakkekuil, Southern Cape, South Africa). Ethical clearance was granted prior to the harvesting (ethical clearance number: SU-ACUM14-001SOP). The harvesting operation involved night time cropping with one shot to the head or neck with a 30 calibre rifle for each of the Blesbok. Subsequent to the shot, the Blesbok was exsanguinated and transported to the meat processing area where they were skinned, dressed, rinsed and hung in a cold room for *ca.* 24 h at *ca.* 4°C. Post rigor mortis and chilling, the carcass was deboned on a cleaned surface following GMP protocols with the use of rinse, alkaline based detergent scrub, rinse, chlorine based sanitiser and a final rinse step. The meat, once deboned was vacuum packed in labelled packages and transported back to the meat laboratory at Stellenbosch University. Once in the meat laboratory the packed meat was stored in the freezer at *ca.* -20°C until required for manufacture of the sausage batter.

Subcutaneous pork fat was purchased from Joostenberg Butchery (Stellenbosch, South Africa). The pork fat was cut into 5 cm^3 pieces (the rind had been removed) and placed in plastic bags and frozen at *ca.* -20°C until required for manufacture.

The majority of the dry ingredients were acquired from Deli Spices (pty) Ltd. (Epping, Western Cape, South Africa). These ingredients were: fine sea salt, sodium nitrite, sodium carbonate and Glucono-delta-lactone (GdL). The encapsulated acids, namely encapsulated GdL (MeatSure 496), encapsulated lactic acid (MeatSure 509) and encapsulated citric acid (MeatSure 333) were acquired from Balchem Corporation (Slate Hill, NY, USA).

5.2.1 Producing dry acidified sausage

The ingredients were weighed out precisely as defined in Table 5.1 using a Digi DS-673 (Teraoka Seilko Co. Ltd, Japan) scale. The premise is that the bowl cutter used in the production of the product requires 3 kg or more to efficiently cut the product in order to produce consistent quality product. The sodium nitrite according to South African regulations is required to be included at 160 ppm or below (Anonymous, 1977). Thus, the concentration of sodium nitrite was calculated at 160 ppm of the meat block to ensure compliance with the legal regulated limit. For ease of calculation, a 3 kg meat block was used. The concentrations of the encapsulated acidulants were calculated using the formula derived from Chapter 4 as summarised in Table 5.2. The concentration of GdL was the optimal concentration derived from Chapter 3.

Table 5.1 Ingredient list and concentrations required to produce the dry-acidified sausage

	Ingredient	Concentration (%)	Grams (g) per treatment	Grams (g) per repeat	Grams (g) Total*
Meat Block	Meat	75	2250	9000	54000
	Fat	25	750	3000	18000
	Total mass	100	3000	12000	72000
Salt Pack	Fine Salt	98.47	88.62	354.48	2126.88
	Sodium Nitrite	0.53	0.48	1.92	11.52
	Sodium Carbonate	1.00	0.9	3.6	21.6
	Total mass	100.00	90.00	360.00	2160.00
Acidulants	E-GdL	0.0727	218.1	872.4	5234.4
	E-Lactic	0.0591	177.3	709.2	4255.2
	E-Citric	0.0416	124.8	499.2	2995.2
	GdL	0.015	45	180	1080

* Grams (g) total is the amount required for 6 repeats.

In terms of processing the dry acidified sausage, the frozen meat was defrosted and cubed into *ca.* 5 cm^3 blocks the day before processing. One animal was cubed at a time. The cubes were mixed together and split into four equal portions determined by mass. Each portion was greater than 2.250 kg of meat, the mass required in the formulation of the sausage. Once weighed out, the meat was placed into labelled packets and frozen.

Table 5.2 Linear regression formula required to calculate the concentration of acid required to realise a pH of 4.8 in the dry-acidified sausage

Treatment	Formula	[acidulant] to realise pH 4.8
E-GdL	y = -16.81x + 6.0214	7.27%
E-Lactic	y = -13.31x + 5.5864	5.91%
E-Citric	y = -29.536x + 6.0279	4.16%

On the day of processing the meat and fat were allowed to slightly defrost. The temperature of the meat and fat was maintained below 2°C. Batch processing ensued with the six blade Manica CM-21 (Equipamient os Carnicos, Spain) bowl chopper. After the bowl chopper was thoroughly cleaned, sanitised and rinsed, prior to processing each batch, the meat was added and chopped on slow speed until a fine texture was obtained. Thereafter, the salt, along with the sodium nitrite and sodium carbonate was spread on top of the meat. The bowl chopper was rotated three times and then the frozen previously weighed fat cubes were added and chopped until the desired particle size of *ca.* 1 – 2 mm. Lastly the acidulant was added and the bowl was rotated three times on slow blade speed and slow rotation speed in order to incorporate the acidulant into the sausage batter. All sausage batter was then transferred to the previously cleaned, sanitised and rinsed, Talsa hydraulic

sausage filler (DMD Food tech, Epping, South Africa) where pH measurement and filling of the sausages occurred. The pH of the sausage batter was measured using a previously calibrated (two point calibration, pH = 4.01 and 7.00 standard buffers) Crison PH25+ pH meter (Spain) with a Crison PT 1000 5053T pH probe (Spain) attached. Five pH values were recorded and the sausage batter was filled into the *ca.* 36 – 38 mm sausage casings. The filled sausage casings were twisted into *ca.* 20 cm lengths, labelled with their treatment and repeat number, allowed to rest in the refrigerator for 48 h after which they were placed into the Reich Airmaster UKF 2000 BE (Urbach, Germany) in order to acidify and dry according to the program defined in Chapter 3 (Table 3.3).

After the drying program had finished, the dry acidified sausages were weighed in order to quantify the mass loss and subsequently sampled. Each treatment for each repeat produced *ca.* 13 sausages. Nine sausages were required for further testing; chemical proximate analysis, pH and water activity determination, and Instron evaluation each required 3 sausages. The Instron Universal Testing Machine (UTM) evaluation, pH and water activity samples were vacuum packed and stored in the refrigerator at *ca.* 4°C. The 3 sausages sampled for proximate analysis were peeled from the outer casing, sliced and homogenised after which the homogenised material was placed in plastic bags, vacuum sealed and also placed into the refrigerator at *ca.* 4°C.

5.2.2 Monitoring dry acidified sausage

Mass loss was calculated as a percentage of the mass of the filled dry acidified sausage sausages and the mass of the same sausages post-drying. The difference being the water lost during the drying process. All mass values were weighed out on the Digi DS-673 (Teraoka Seilko Co. Ltd, Japan) scale and recorded to three decimal points.

The pH of the dry acidified sausage post-drying involved cutting the sausage at the midpoint perpendicular to the length of the sausage after which a knife was used to produce a hole, parallel to the length of the sausage in order to insert the probe into the sausage. This was performed on both halves of the sausage for all three sausages per treatment per repeat. Both the pH and the temperature of the measurement were recorded and the corrected pH value was calculated according to the formula below:

$$pH_{corrected} = \left[(pH_{measured} - 7) x \frac{(273.15 + T_{calibration})}{(273.15 + T_{measured})} \right] + 7$$

$T_{calibration}$ - Temperature in degrees Celsius at which the probe was calibrated.

$T_{measured}$ - Temperature in degrees Celsius at which the actual pH measurement was conducted.

$pH_{measured}$ - The reading of pH of the sample measured.

$pH_{corrected}$ - The corrected pH measurement taking into account the effect of temperature of the sample.

After the pH was measured, two dry acidified sausage slices *ca.* 3 mm thick were placed as tightly as possible into the manufacture's plastic tubs with tight fitting lids for determination of the a_W. The Hygrolab model C1 water activity meter (Rotronic, Switzerland) was calibrated using two standards, namely a_W = 0.90 and a_W = 0.75. After calibration, the tubs individually had their lids removed and the sample was placed into the water activity meter. Both the water activity reading and the temperature of the reading were recorded. The room where the water activity was measured, was set at 21°C.

Proximate composition was determined in the same manner as in Chapter 3: moisture content was quantified using method 934.01 (AOAC, 2002a), fat content using a solvent extraction method (Lee *et al.*, 1996), protein content using method 992.15 (AOAC, 2002b) and ash content using method 942.05 (AOAC, 2002c).

Cyclic compression texture profile analysis was used to analyse the samples for which an Instron Universal Texture Machine (UTM), model 3344 (Instron Corporation, MA, USA) with the Bluehill 2 TPA (Instron Corporation, MA, USA) software program was used. The Instron UTM was setup with a 2 kN load cell with a cross head speed of 200 mm/min, in a laboratory set at 20°C. The Instron UTM was set to a 60% compression with an anvil height of 23 mm and an anvil diameter of 35 mm. The sample plan ensured nine measurements were conducted per repeat, whereby three sausage samples were each cut into three 20 mm lengths, perpendicular to the length of the sausage. The methodology follows those described by Bourne (1978) where values were recorded for 1st cycle hardness 1 (N), Gumminess (N), Cohesiveness (N), Adhesiveness and Springiness (mm). Previous research indicate that the most common values pertinent to sausages, particularly dry sausages are 1st cycle hardness, springiness, cohesiveness (Ruiz de Huidobro *et al.*, 2001, Ruiz de Huidobro *et al.*, 2005).

Statistical analysis of the data involved a Oneway ANOVA with the Fischer LSD post-hoc test at a confidence level of 95%.

5.3. Results

All the physico-chemical results are summarised in Table 5.3.

The initial pH of the sausage batter showed a difference ($P < 0.05$) between the treatments; the GdL and E-GdL treatments recorded a value of 5.6 ± 0.02 and 5.7 ± 0.04, respectively whereas the E-Lactic acid and E-Citric acid reported a lower initial pH of 5.5 ± 0.09 and 5.5 ± 0.06, respectively. GdL and E-GdL did not differ ($P > 0.05$) from one another, and E-lactic acid and E-citric acid also did not differ ($P > 0.05$) from one another, however, the GdL and E-GdL differed ($P < 0.05$) from E-lactic acid and E-citric acid.

Final pH values all differed ($P < 0.05$) between the treatments (Table 5.3). Although the predicted model aimed for a pH of 4.8, three of the four treatments exhibited lower pH values, the

lowest being 3.5 ± 0.08 for E-citric acid. GdL, the highest pH value at 4.9 ± 0.06 was the only treatment which was close to the target pH.

The next crucial parameter measured, particularly from a food safety point of view and the nature of dried sausages, was that of a_W. There was no difference ($P > 0.05$) in a_W between GdL and E-citric acid (0.84 ± 0.01 and 0.84 ± 0.02, respectively). Similarly there was no difference ($P > 0.05$) in a_W between GdL and E-GdL where the value for E-GdL was 0.82 ± 0.01. There was however recorded observations where the a_W of E-citric acid differed ($P < 0.05$) from both E-GdL and E-lactic acid (0.80 ± 0.01).

E-citric acid, with a value of 40.7 ± 0.46%, had a mass loss which differed ($P < 0.05$) from the other three treatments. All the other treatments had similar ($P > 0.05$) mass losses. The E-citric acid mass loss was *ca.* 1% higher than the other treatments.

Texture analysis confirmed, in terms of hardness (maximum force 1), that the E-lactic acid treatment was the hardest dry acidified sausage with a value of 318.0 ± 57.92 N. This was higher ($P < 0.05$) than the second hardest dry acidified sausage of E-citric acid which was recorded at a mean value of 235.10 ± 37.17 N. E-GdL and GdL were similar ($P > 0.05$) to one another but both differed ($P < 0.05$) from E-lactic acid and E-citric acid with respective values of 185.4 ± 23.00 N and 167.9 ± 39.20 N, representing the least hard dry acidified sausage. Gumminess did not differ ($P = 0.116$) across the treatments. The final textural parameter of Cohesiveness recorded very low values (0.09 – 0.13): GdL did not differ ($P < 0.05$) from E-GdL. GdL was also similar ($P < 0.05$) to E-lactic acid and E-citric acid. E-GdL however, differed ($P < 0.05$) from E-lactic acid and E-citric acid.

In terms of proximate analysis, all four proximate parameters showed significant differences ($P < 0.05$) between the treatments. GdL had the highest moisture value (31.5 ± 1.89%) which differed ($P < 0.05$) from the other three treatments. The E-citric acid treatment was the highest in terms of fat content at a value of 34.1 ± 1.79%. The rest of the treatments, although different ($P < 0.05$) and lower than E-citric acid, were not significantly different ($P > 0.05$) from one another. The GdL treatment had the highest protein content at 27.7 ± 1.25% which differed significantly ($P < 0.05$) from E-GdL and E-citric acid but not differ significantly ($P > 0.05$) from E-lactic acid. E- GdL had the lowest protein content at 24.3 ± 2.31 which did not differ significantly ($P > 0.05$) from E-citric acid but did differ significantly ($P < 0.05$) from both E-lactic acid and GdL. The highest value for ash content was E-lactic acid with a value of 6.8 ± 0.18% which was significantly different ($P < 0.05$) to all the rest of the values. The second highest ash content was GdL at 5.8 ± 0.21% which was also significantly different ($P < 0.05$) from all the other ash values. The lowest value was E-GdL (5.5 ± 0.30%) and E-citric acid (5.6 ± 0.14%) which were not significantly different ($P > 0.05$) between one another.

Table 5.3 Presentation of physico-chemical results for the dry acidified sausage manufactured with four different commercial acidulants

Parameter measured	Acidulants				P values
	GdL	E-GdL	E-Lactic acid	E-Citric acid	
$pH_{initial}$	$5.6^{a} \pm 0.02$	$5.7^{a} \pm 0.04$	$5.5^{b} \pm 0.09$	$5.5^{b} \pm 0.06$	< 0.001
pH_{final}	$4.9^{a} \pm 0.06$	$3.7^{c} \pm 0.02$	$4.2^{b} \pm 0.04$	$3.5^{d} \pm 0.08$	< 0.001
a_W	$0.84^{ab} \pm 0.02$	$0.82^{b} \pm 0.01$	$0.80^{c} \pm 0.01$	$0.84^{a} \pm 0.01$	< 0.001
Temperature of a_W °C	$24.1^{a} \pm 0.61$	$22.6^{b} \pm 0.63$	$23.7^{a} \pm 0.74$	$24.18^{a} \pm 0.40$	0.001
Mass loss %	$38.5^{b} \pm 1.47$	$37.8^{b} \pm 0.83$	$39.0^{b} \pm 0.91$	$40.7^{a} \pm 0.46$	< 0.001
Max force 1 (N)	$167.9^{c} \pm 39.20$	$185.4^{c} \pm 23.00$	$318.0^{a} \pm 57.92$	$235.10^{b} \pm 37.17$	< 0.001
Gumminess	21.2 ± 0.87	24.9 ± 5.40	27.4 ± 6.30	21.5 ± 4.85	0.116
Cohesion energy	$0.13^{ab} \pm 0.033$	$0.13^{a} \pm 0.012$	$0.09^{b} \pm 0.005$	$0.09^{b} \pm 0.006$	< 0.001
Moisture %	$31.5^{a} \pm 1.89$	$29.7^{b} \pm 0.40$	$29.4^{b} \pm 0.80$	$28.4^{b} \pm 0.70$	0.001
Fat %	$30.3^{b} \pm 1.19$	$30.8^{b} \pm 3.67$	$29.6^{b} \pm 2.32$	$34.1^{a} \pm 1.79$	0.020
Protein %	$27.7^{a} \pm 1.25$	$24.3^{c} \pm 2.31$	$26.4^{ab} \pm 1.88$	$24.7^{cb} \pm 0.90$	0.008
Ash %	$5.8^{b} \pm 0.21$	$5.5^{c} \pm 0.30$	$6.8^{a} \pm 0.18$	$5.6^{c} \pm 0.14$	< 0.001

^{a,b,c,d,e} Means within a row with different superscripts are significantly different ($P < 0.05$)

5.4. Discussion

The goal of this study was to validate that the ultimate pH, after acidification, was able to decrease the initial pH to the predetermined end point based on the linear regressions developed in Chapter 3. From the results, the goal of a final pH of 4.8 was not reached. The closest treatment to the target pH was GdL, the control using the optimised concentration of 1.5% from Chapter 2. None of the encapsulate acids stopped at the desired pH endpoint, rather, they decreased well below the desired pH endpoint.

The encapsulation process is a bulk spray drying process which enrobes the acidulants in a fat encapsulate. However, this enrobing may not entirely coat the acidulant. Both lactic and citric acid have a direct acidifying effect unlike GdL and E-GdL which only release an acid post hydration. This means that a slight instant acidification can be expected in the initial sausage batter from the E-Lactic acid and E-Citric acid causing a slight decrease in batter pH (Barbut, 2006). It must be noted that the initial sausage pH was measured within 2 min of mixing the acid into the batter and at below 4°C, thus minimising the hydration and ultimate acidification of the E-GdL and GdL treatments. This observation confirms that there is some instant acidification when the acid is released and that it takes some time for GdL to hydrolyse and reduce the pH, as confirmed by previous studies (Barbut, 2006)

When considering final pH values, the main reason for the encapsulated acids being well below the desired pH is that the concentration of acidulant added was in excess of what was required to bring the pH down to the required level. Reasons for this could be either due to the lack of fit of the respective linear regressions which did not accurately represent the actual values in the sausage, or there is a great discrepancy even within a bag of encapsulated acid, in the ratio of acidulant to encapsulate. As seen in the specifications, E-citric acid has only 70.0 – 74.0% citric acid, E-lactic acid has between 28.0 – 32.0% lactic acid and E-GdL has between 68.0 – 72.0% GdL (Anonymous, 2013a, Anonymous, 2013b, Anonymous, 2013c).

In the case of all four treatments, the a_W was reduced to below 0.86 which is desired from a food safety point of view due to the hurdle of low pH and low a_W on the activity of pathogen micro-organisms, particularly *S. aureus* (Jablonski & Bohach, 1997, Jay *et al.*, 2005c). This can be attributed to the low pH which is able to denature proteins and allow intrinsic water to become free and thus removed from the product.

Mass loss was in the range for dry acidified sausage as it sat between 30 - 40% for all the treatments (Jay *et al.*, 2005b). One would expect that since the mass loss was significantly ($P < 0.05$) greater for the E-citric acid than the rest of the treatments, the moisture content would follow suit and also be significantly lower, however, this was not the case as the GdL treatment was the only treatment to be significantly ($P < 0.05$) higher (*ca.* 1.5%) in moisture content compared to the rest of the treatments. The sausages were all exposed to the same process and as a result it would

merely be the citric acid inclusion which could affect the difference in mass loss. This observation suggests that the extremely low pH of 3.5 ± 0.08 could well interact with the protein and facilitate greater protein denaturation resulting in a lower water holding capacity and more moisture loss under the same processing conditions. The water activity consequently would be expected to be higher due to this protein denaturation which is the case when comparing the E-citric acid to the E-GdL and E-lactic acid treatments.

The observation of the final pH being significantly different between each of the treatments would suggest that there could be significant differences in moisture content and water activity however, it seems the type of acid and other factors are affecting the final water activity, moisture loss and moisture content results. One may speculate that various factors could be at play but it would seem that there is scope for further research required to assist in identifying the potential factors.

In terms of product description, the differences in first compression hardness values do not follow the same declining trend in significant difference when being compared to the pH of the sausage. This suggests that the hardness is not determined by the pH alone but by other factors such as the type of acid, concentration of acid and the degree of protein denaturation. This was observed in the E-citric acid treatment which was crumbly. Spaziani *et al*. (2009) demonstrated that dry acidified sausage has a low cohesiveness value which is consistent with the values observed in this trial.

In terms of proximate analysis, the loss of moisture would have a definite concentrating effect on the proximate components of the final product. The GdL treatment with the highest pH had the highest moisture content, was the least hard sample and had the highest water activity. The formula added *ca.* 25% by weight of fat which had concentrated within the region of *ca.* 30% in the final product. The moisture loss in the E-citric acid treatment explains the lower value of moisture content and the highest concentration of fat. The significant difference in mass loss is comparable to that of fat content. The protein value would be provided by the meat. Any addition of ingredients as well as differences in mass loss would influence the final protein value. The highest protein content was for GdL, which consequently had the lowest mass of acidulant added to the treatment.

5.5. Conclusions

The results of the validation study of the four chemical acidulants using physico-chemical parameters showed that the specified predetermined pH end point was not able to be reached. However, the study enabled the description of the dry acidified sausage in terms of proximate values and indicated differences in pH, water activity, mass loss and hardness. The result of this study called into question the method of pH measurement employed in the dry acidified sausage as well as the individual effect of the ingredients on the pH in the sausage batter formulation. The linear regression which was used to estimate the required concentration was not able to correctly predict the required concentration of

acidulant. From this information, suggestions into a more detailed structured approach should be initiated to fully understand; 1 - the kinetic modelling of the decrease in pH by the acidulants, 2 - the effect the individual ingredients have on pH as well as 3 - the method of pH measurement.

5.6 References

Anonymous (1977). R 965. Regulation: Preservatives and Antioxidants. Department of Health: Pretoria, South Africa.

Anonymous (2013a). MeatSure 333 (Encapsulated citric acid) Specification and Safety data sheet. Pp. 1-3;1-6. New Hampton, NY, USA: Balchem Corporation.

Anonymous (2013b). MeatSure 496 (Encapsulated Glucono Delta Lactone) specification and safety data sheet. Pp. 1-3;1-6. New Hampton, NY, USA: Balchem Corporation.

Anonymous (2013c). MeatSure 509 (Encapsulated Lactic Acid) specification and safety data sheet. Pp. 1-3;1-6. New Hampton, NY, USA: Balchem Corporation.

AOAC (2002a). Loss on drying (moisture) at 95-100°C for feed. AOAC Official Method 934.01. In: *Official Method of Analysis,* 17th ed. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

AOAC (2002b). Dumas combustion method. AOAC Official Method 992.15. In: *Official Method of Analysis,* 17th ed. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

AOAC (2002c). Ash of Animal Feed. AOAC Official Method 942.05. In: *Official methods of analysis* 17th ed. Arlington, Virginia, USA: Association of Official Analytical Chemists Inc.

Barbut, S. (2005). Effects of chemical acidification and microbial fermentation on the rheological properties of meat products. *Meat Science,* **71**, 397-401.

Barbut, S. (2006). Fermentation and chemical acidification of salami-type products - effect on yield, texture and microstructure. *Journal of Muscle Foods,* **17**, 34-42.

Bourne, M.C. (1978). Texture profile analysis. *Food Technology,* **33**, 62-66

Chieh, C. (2012). Water chemistry and biochemistry. In: *Food Biochemistry and Food Processing,* (edited by B.K. Simpson, L.M. Nollet, F. Toldrá, S. Benjakul, G. Paliyath & Y.H. Hui). Pp. 84-106. Oxford, UK: John Wiley & Sons, Inc.

Danquah, A.O., Benjakul, S. & Simpson, B.K. (2012). Biogenic amines in food. In: *Food Biochemistry and Food Processing,* 2nd ed. (edited by B.K. Simpson, L.M. Nollet, F. Toldrá, S. Benjakul, G. Paliyath & Y.H. Hui). Pp. 820-832. Oxford, UK: John Wiley & Sons, Inc.

Fernández-López, J., Sendra, E., Sayas-Barberá, E., Navarro, C. & Pérez-Alvarez, J. (2008). Physico-chemical and microbiological profiles of “salchichón” (Spanish dry-fermented sausage) enriched with orange fiber. *Meat Science,* **80**, 410-417.

Gibbs, B.F., Kermasha, S., Alli, I. & Mulligan, C.N. (1999). Encapsulation in the food industry, A review. *International Journal of Food Sciences and Nutrition,* **50**, 213 - 224.

ISO (2005). ISO 22000:2005 - Food safety management systems - Requirements for any organization in the food chain. Geneva, Switzerland: International Organisation for Standardization.

Jablonski, L.M. & Bohach, G.A. (1997). *Staphylococcus aureus*. In: *Food Microbiology: fundamentals and frontiers,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 353-375. Washington D.C., USA: American Society for Microbiology Press.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005a). Intrinsic and extrinsic parameters of foods that affect mirobial growth. In: *Modern Food Microbiology,* 7th ed. Pp. 39-60. New York, NY, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005b). Non dairy fermented foods and products. In: *Modern Food Microbiology,* 7th ed. Pp. 175-195. New York, USA: Springer.

Jay, J.M., Loessner, M.J. & Golden, D.A. (2005c). Staphylococcal gastroenteritis. In: *Modern Food Microbiology,* 7th ed. Pp. 545-566. New York, NY, USA: Springer.

Lee, C.M., Trevino, B. & Chaiyawat, M. (1996). A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of Association of Official Analytical Chemists International,* **79**, 487–492.

Leroy, F. & De Vuyst, L. (2009). Fermentation and acidification ingredients. In: *Ingredients in Meat Products: properties, functionality and applications,* (edited by R. Tarte). Pp. 227-252. New York, USA: Springer.

Ockerman, H.W. & Basu, L. (2015). Production and consumption of fermented meat products. In: *Handbook of Fermented Meat and Poultry,* (edited by F. Toldrá, Y.H. Hui, I. Astiasaran, J. Sebranek & R. Talon). Pp. 7-11. West Sussex, UK: John Wiley & Sons, Ltd.

Pérez-Alvarez, J.A., Sayas-Barberá, M.A.E., Fernández-López, J. & Aranda-Catalá, V. (1999). Physicochemical characteristics of Spanish-type dry-cured sausage. *Food Research International,* **32**, 599-607.

Ricke, S.C. & Keeton, J.T. (1997). Fermented meat, poultry and fish products. In: *Food Microbiology: fundamentals and frontiers,* (edited by M.P. Doyle, L.R. Beuchat & T.J. Montville). Pp. 610-628. Washington D.C., USA: American Society for Microbiology Press.

Ruiz de Huidobro, F., Cañeque, V., Lauzurica, S., Velasco, S., Pérez, C. & Onega, E. (2001). Sensory characterization of meat texture in sucking lamb: methodology. *Investigación Agraria. Producción y Sanidad Animales,* **16**, 245-256.

Ruiz de Huidobro, F., Miguel, E., Blazquez, B. & Onega, E. (2005). A comparison between two methods (Warner–Bratzler and texture profile analysis) for testing either raw meat or cooked meat. *Meat Science*, 527-536.

Spaziani, M., Del Torre, M. & Stecchini, M.L. (2009). Changes of physicochemical, microbiological, and textural properties during ripening of Italian low-acid sausages. proteolysis, sensory and volatile profiles. *Meat Science,* **81**, 77-85.

van Schalkwyk, D.L., McMillin, K.W., Booyse, M., Witthuhn, R.C. & Hoffman, L.C. (2011). Physico-chemical, microbiological, textural and sensory attributes of matured game salami produced from springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*) and zebra (*Equus burchelli*) harvested in Namibia. *Meat Science,* **88**, 36-44.

Zanardi, E., Ghidini, S., Conter, M. & Ianieri, A. (2010). Mineral composition of Italian salami and effect of NaCl partial replacement on compositional, physico-chemical and sensory parameters. *Meat Science,* **86**, 742-747.

CHAPTER 6

General conclusions and recommendations

The study encompassed three sections, whereby Blesbok (*Damaliscus pygargus phillipsi*) meat was manufactured into dry acidified sausage utilising chemical acidification for the primary reason of understanding the kinetics of the acidulants.

The stepwise approach initially involved the optimisation of Glucono-delta-lactone (GdL) in terms of concentration based on physico-chemical and microbiological assessment, as denoted in chapter 1. The optimal concentration of 1.5% was chosen due to the fact that the pH was below 5.0, the a_W was below 0.93, the mass loss was >30% as required for a dry acidified sausage and the texture was in the middle of the hardness range of treatments. Although, the only acceptable treatment from a microbiological stand point was treatment 5, the spice ingredient was grossly contaminated with *S. aureus* and was responsible for cross contamination of treatment 3 and 4. Should control on the spice be sufficient in terms of microbial load, both treatment 3 and 4 could be considered as safe to consume.

The second chapter investigated the kinetics of encapsulated acidulants. Initial assessments, trial 1, focussed on the heating requirements required to fully release the encapsulated acidulant from the encapsulating material and the rate of acidification until a stable end point. Trial 2, assessed three different encapsulated acidulants which were mixed using incremental concentrations, subjected to the heating regime from the initial assessment which was deemed sufficient to release the acidulant and allowed the sausage batter to acidify to the final end point. This enabled the development of linear regression trend lines which could be used to indicate the required concentration of acidulant necessary in order to decrease the sausage batter to a final pre-determined endpoint.

Heating confirmed that the encapsulated acidulant is required to be heated up until the melting point of the encapsulating material (*ca.* 55°C) in order to release the acidulant. Full acidification was deemed complete within 65 min. When observing the pH measurements, the variation in pH values justified an investigation into the pH measurement methodology. The decision to continue utilising a direct measurement by inserting the probe into the sausage batter was deemed suitable for the duration of the trials however, a structured review and study into the comparison of different pH methodologies in order to standardise pH measurements in sausages is strongly recommended.

The final investigation as reported in chapter 3, validated the kinetic assessment defined in chapter 2 by producing dry acidified sausages using the trend lines developed in the kinetic study whereby concentrations were calculated to reach the target pH of 4.8. Simultaneous to this, the optimal concentration of pure GdL at a concentration of 1.5% was manufactured facilitating a

comparison of the GdL and three encapsulated acidulants based on physico-chemical parameters. All of the encapsulated acidulants acidified further than the target pH of 4.8. As a result, a comparative study between the different physico-chemical properties could not be conducted. The only interpretation of the physico-chemical parameters is the description of the differently treated dry acidified sausages.

Ultimately, game meat was used successfully to produce dry acidified sausages which satisfied the characteristics of dry acidified sausages and was described by physico-chemical evaluation, as well as confirmation of microbiological parameters. The kinetics, although defined, were not as comprehensive as initially anticipated rendering the validation study more of a product description exercise.

A more complete analysis of the devolution of pH and buffering capacity of the sausage mixture is recommended. The research design should incorporate the individual effects of temperatures, different acidulants, fat, meat and individual functional ingredients including salt, nitrates, nitrites and erythrobate. The interactions of the functional ingredients at varying concentrations should also be investigated allowing for a comprehensive model to be developed in order to indicate the concentration of acidulant required to decrease a sausage to a predetermined end point.

Following on from this, once accurate pH measurements and suitable concentrations as defined by physico-chemical analysis have been identified, focus may shift onto the sensory aspects of the dry acidified sausage including flavour and textural development with a focus on both consumer liking and in depth comparison via a trained sensory panel to facilitate the completion of a product which is understood both functionally as well as commercially.