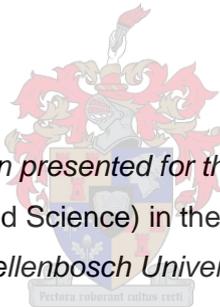


Profiling of traditional South African biltong in terms of processing, physicochemical properties and microbial stability during storage

by

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Dissertation presented for the degree of
Doctor of Philosophy (Food Science) in the Faculty of AgriSciences at
Stellenbosch University



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March 2017

Declaration

By submitting this dissertation 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.

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Date: March 2017

Acknowledgments

Professor Louw Hoffman (Supervisor) for his guidance and support throughout this study. I appreciate all the criticism, encouragement and support you have given over the last five years. I am forever grateful for the opportunities you have given me and your reassurance to always follow my instincts.

Professor Pieter Gouws (Co-supervisor) for his guidance in all things microbiology. Your guidance and kindness I will always appreciate. Thank you for always putting a smile on my face and making sure that the work I put forward is something I would be proud of.

Dr Elodie Arnaud (Co-supervisor) for what seemed like your never-ending comments and questions. Without you I would not have made it to this point, thank you for all your guidance. You have made me a stronger researcher and your involvement in this research will always be appreciated.

Professor Martin Kidd at the Centre for Statistical Consultancy for assisting me in the statistical analysis of the data. The staff at the Food Science Department, I thank you for all your assistance and support; and the staff members at the Department of Animal Sciences, especially Lisa Uys, Janine Booyse and Michael Mlambo for their assistance during my laboratory analyses as well as the smiles and friendship we have shared over my post graduate career.

I wish to thank the following people and institutions for their assistance and contributions to this research: Mr Herman Fourie and staff at Closwa biltong (Okahandja, Namibia); Dr Jean-Christophe Meile, Professor Fabienne Remize and Ms Gwenn Atheaux (CIRAD, Reunion Island); Professor Antoine Collignan (CIRAD, France) and Mr Marius Nel at Cape Deli (Blackheath, Cape Town).

The *National Research Foundation* (NRF), for the financial assistance (The opinions expressed and conclusions arrived at in this study are those of the author and are not necessarily to be attributed to the NRF).

My fellow students thank you for assisting and encouraging me. These last few years would not have been the same without you and you have made these some of my best years. I cannot imagine going through this journey without you, thank you.

A huge thank you to my family, George, June and Devin, I could not have done this without you. For always being my proud parents and brother who show me endless support and encouragement, far too much love and for being the ears that have heard all the laughter, the complaints and the nonsensical nonsense. You are my rocks and I hope I have made you proud. I cannot say thank you enough.

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 (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter with general conclusion and recommendations. 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 has, therefore, been unavoidable.

Results from this dissertation have been presented as posters at the following conferences:

Jones, M., Arnaud, E., Collignan, A., Gouws, P., Atheaux, G. & Hoffman, L.C. (2015). Effect of ultrasonic-brining on the salting and drying kinetics in biltong processing. *International Congress of Meat Science and Technology*, August 23-28, 2015, Clermont-Ferrand, France. Poster presentation.

Jones, M., Arnaud, E., Gouws, P. & Hoffman, L.C. (2016). Effects of the addition of vinegar and degree of drying on the physicochemical properties of traditional South African biltong. *International Congress of Meat Science and Technology*, August 15-19, 2016, Bangkok, Thailand. Poster presentation – awarded prize for second best poster presentation.

SUMMARY

In South Africa, there are no processing guidelines for biltong production and therefore the industry uses different processing parameters which results in variation in the product. The same process was used throughout this study and drying was done using constant parameters – temperature $25 \pm 2^\circ\text{C}$, relative humidity $30 \pm 5\%$, air velocity 2 ± 0.2 m/s.

An initial study investigating the influence of vinegar addition during the production of beef biltong showed that vinegar addition does not influence its drying kinetics. The biltong reached a 50% weight loss after 66 hours and a 65% weight loss after 96 hours. The use of different meat muscles (topside, *semimembranosus* and silverside, *biceps femoris*), beef with subcutaneous fat and gemsbok (*Oryx gazelle*) showed differences ($p \leq 0.05$) in drying rates when dried to a targeted weight loss of 65%. The two lean beef muscles both dried in 96 hours. The gemsbok topside took only 78 hours with a drying pattern similar to the lean beef topside. The fatty beef topside took 118 hours to dry.

The microbiological profile of beef biltong over a three month shelf-life storage were studied. Final weight loss during drying and packaging method (modified atmospheric packaging and vacuum packaging) did not have an effect ($p > 0.05$) on the microbiological profile. Total viable counts and coliforms were only reduced in biltong with vinegar added. After drying, yeasts and moulds were already present at high levels (~ 2.5 log cfu.g⁻¹) but not visible. After six weeks, yeasts and moulds became visible. *Staphylococcus aureus* was present at less than 20 log cfu.g⁻¹ while *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* were not present during the three month storage period.

Yeast and mould growth on biltong is a problem and therefore a challenge study was included. Beef biltong produced without and with vinegar and dried to a 50% or 65% weight loss were inoculated with yeasts and moulds. No yeast and mould growth was seen on biltong with vinegar but 1.8 – 2.5 log cfu.g⁻¹ was detected after 34 days. Biltong without vinegar showed yeast and mould growth after 10 days with levels of 2.8 – 3.1 log cfu.g⁻¹. *Saccharomyces* spp. (yeast) and *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. (moulds) were the most common yeast and moulds.

A small-scale study using ultrasound in the salting step of beef biltong processing showed that ultrasonic-brining did not have an effect on either the salting or drying kinetics contrary to what was expected.

Throughout the study the physicochemical properties of the beef biltong gave consistent results. An approximate 50% and 65% weight loss produced biltong with a moisture content of 50% and 30%, respectively and water activity of 0.74 – 0.78 and 0.81 and 0.86, respectively. Weight loss or the addition of vinegar did not play a role in the salt content (dry basis). Beef biltong without vinegar had a pH 5.56 – 5.75 while the addition of vinegar to biltong lowered the pH of biltong to 4.89 – 4.93.

It is recommended that the biltong industry should standardise their drying parameters to avoid variation in quality and for a more microbial stable product. Vinegar could be added as it has an effect on the yeast and mould growth. Biltong with water activity ranging from 0.74 to 0.83 does not have a shelf-life of more than three months when using modified atmosphere packaging or vacuum packaging.

The data generated in this study serves as a base-line for future studies focused on optimising and standardising the drying procedures applicable to biltong.

OPSOMMING

Daar is geen wetlike prosesseringsriglyne vir die produksie van biltong in Suid Afrika nie en dus maak die industrie gebruik van verskillende prosesseringsparameters wat lei tot variasie in die produk. Dieselfde proses en die droging was uitgevoer in hierdie studie volgens konstante parameters – temperatuur $25 \pm 2^\circ\text{C}$, relatiewe humiditeit $30 \pm 5\%$, lugspoed 2 ± 0.2 m/s.

'n Aanvanklike ondersoek het bepaal dat die byvoeg van asyn gedurende die produksie van beesbiltong nie die droogkinetika van die biltong beïnvloed nie. Die biltong het 'n gewigsverlies van 50% na 66 uur en 65% na 96 uur gehad. Verskillende vleis spiere (binneboud, *semimembranosus* en *biceps femoris*), bees met onderhuidse vet en gemsbok (*Oryx gazelle*), gedroog tot 'n teikengewigverlies van 65% het verskille ($p \leq 0.05$) gehad in terme van droogtempo en -tyd. Die twee verskillende maer beespriere was droog na 96 uur. Die gemsbokbinneboud was droog na slegs 78 uur en het dieselfde droogpatroon as die maer beesbinneboud gevolg. Die vetryke beesbinneboud het 118 uur geneem om te droog.

Die mikrobiese profiel van die beesbiltong oor 'n stoorperiode van drie maande is ondersoek. Die finale gewigsverlies en verpakkingsmetode (*modified atmospheric packaging* en vakuumpverpakking) het nie die mikrobiese profiel geaffekteer nie ($p > 0.05$). Die totale lewensvatbare tellings en *coliforms* was laer in die biltong waar asyn bygevoeg is. Gisse en swamme was reeds teen hōe vlakke ($\sim 2.5 \log \text{cfu.g}^{-1}$) na droging teenwoordig maar was nie sigbaar met die blote oog nie. Beide gisse en swamme was na ses weke met die blote oog sigbaar. Gedurende die stoorperiode was *Staphylococcus aureus* teenwoordig by vlakke minder as $20 \log \text{cfu.g}^{-1}$ terwyl *Listeria monocytogenes*, *Salmonella* spp. en *Escherichia coli* nie teenwoordig was nie.

Die groei van gisse en swamme op biltong is 'n probleem en daarom is 'n uitdagingsstudie (*challenge study*) ingesluit. Biltong geproduseer met en sonder asyn, gedroog tot 50% en 65% gewigsverlies was geïnkuleer met gisse en swamme. Daar was geen sigbare groei van gisse en swamme op die biltong met asyn nie, maar na 34 dae is wel groeivlakke van $1.8 - 2.5 \log \text{cfu.g}^{-1}$ gevind. Gisse en swamme het op die biltong sonder asyn na 10 dae teen vlakke van $2.8 - 3.1 \log \text{cfu.g}^{-1}$ gegroei. Die algemeenste gisse en swamme op die biltong was *Saccharomyces* spp. (gis) en *Aspergillus* spp., *Fusarium* spp. en *Penicillium* spp. (swamme).

'n Kleinskaalse studie op die gebruik van ultraklank gedurende die soutproses van beesbiltong het getoon dat die metode geen effek op die sout of droogkinetika van die biltong gehad het nie, wat teenstrydig was met die verwagte resultate.

Die fisiochemiese eienskappe van die beesbiltong het deurgaans in die studie konstante resultate gelewer. Biltong met 'n gewigsverlies van 50% en 65% het biltong met 'n voginhoud van onderskeidelik 50% en 30% geproduseer, met 'n wateraktiwiteit van onderskeidelik $0.74 - 0.78$ en 0.81 en 0.86 . Die gewigsverlies of byvoeging van asyn het nie 'n rol gespeel ten opsigte van die totale soutinhoud nie (gebaseer op droëbasis). Die beesbiltong sonder asyn het 'n pH van $5.56 - 5.75$ gehad terwyl die biltong met asyn bygevoeg 'n laer pH van $4.89 - 4.93$ gehad het.

Dit word aanbeveel dat die biltongindustrie drogingsparameters standardiseer om sodoende 'n groot variasie in die eindproduk te vermy en 'n meer mikrobies stabiele produk te verseker. Asyn kan

bygevoeg word omdat dit die groei van gisse en swamme beïnvloed. Biltong met 'n wateraktiwiteit van 0.74 to 0.83 het nie 'n rakleef tyd van meer as drie maande nie ongeag die verpakkingsmetode (*modified atmosphere packaging* of vakuumpverpakking). Die data van hierdie studie kan gebruik word as 'n basispunt vir toekomstige studies wat fokus op die optimisering en standardisering van die droogmetodes wat gebruik word in die maak van biltong.

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CHAPTER 1

INTRODUCTION

Biltong is a ready-to-eat, dried meat snack food from South Africa. Not only is it enjoyed by South Africans but also by consumers worldwide. Comparison is often made with other speciality dried meat products such as beef jerky (North America) and charqui (Brazil) but biltong differs in its unique production process, end-product characteristics and taste. The biltong production process is simple and whether produced on a small-scale such as in butcheries and consumer households or on a large-scale for the industry (Strydom & Zondagh, 2014), the steps are the same. Although, on an industrial scale, producers often use more advanced technologies such as vacuum tumbling and temperature-controlled drying chambers.

Biltong can be made using different meat cuts from different species such as beef, game and ostrich (Van Wyk, 2007; Naidoo & Lindsay, 2010a; Strydom & Zondagh, 2014) and lately, pork. The meat is cut into strips of desired sizes, then salted/spiced before being dried. Salting is done using either a dry salt/spice rub or a vinegar-salt/spice mix (Taylor, 1976; Van der Riet, 1982; CSIR, 2001; Naidoo & Lindsay, 2010b). Thereafter the spiced strips are stored in the refrigerator or tumbled before hanging for drying. Under commercial conditions, vacuum tumblers are often used in the salting/spicing step. Tumbling has shown accelerate salt impregnation and diffusion (Krause *et al.*, 1978; Bedinghaus *et al.*, 1992) on other meat products. The salted/spiced meat is then hung and dried at moderate temperatures for up to four days. Previous scientific studies have used drying temperatures between 20 – 35°C (Taylor, 1976; Nortje *et al.*, 2005; Burnham *et al.*, 2008; Naidoo & Lindsay, 2010b).

Salt is essential in biltong production acting as a preservative (Van den Heever, 1970; Van der Riet, 1982; Naidoo & Lindsay, 2010a; Naidoo & Lindsay, 2010b; Strydom & Zondagh, 2014) whilst also contributing to the flavour. Spices which are often added for additional flavour include black pepper, coriander, brown sugar and vinegar. Pre-mixed spice bags sourced from commercial spice companies are often used. The addition of vinegar is common (Van den Heever, 1970; Van der Riet, 1982; Strydom & Zondagh, 2014) and as with salt, its function is to inhibit microbial growth (Naidoo & Lindsay, 2010a; Naidoo & Lindsay, 2010b) and add to the flavour. The increase in consumer knowledge on food products must be acknowledged. Biltong is a lean meat, high protein snack food but when spice packs are used they usually contain some preservatives. This could also explain why commercial biltong has a longer shelf-life than biltong produced at home when made without using these spice packs.

Sufficient drying is based on the weight loss of the biltong. It is usually dried to a weight loss \leq 50%. This however is dependent on consumer preferences and biltong is often described as being either “moist” or “dry.” It has been shown that commercial biltong can have a moisture content ranging from 10.6% to 48.8% (wet basis) (Van der Riet, 1976a; Osterhoff & Leistner, 1984; Petit *et al.*, 2014). This wide range of moisture contents poses the question, what is considered “moist” biltong and what is “dry” biltong? A study by Nortje and others (2005) suggest that “moist” biltong has a moisture content greater than 40% and “dry” biltong a moisture content less than 40%. The level of drying is also of importance to biltong producers as biltong is sold on a weight-basis.

The shelf-life of biltong is dependent on many factors, including but not limited to, the end-product characteristics and packaging of the final product. Water activity, moisture and salt content and pH of the final product will influence the shelf-life. Salt decreases the water activity of biltong and this inhibits microbial growth (Van der Riet, 1976a). Research suggests water activity to be lowered to ≤ 0.90 (Leistner, 1987; CSIR, 2001). After drying, the biltong is sold in butcheries and shops as whole pieces which are displayed openly in a “hanging space” and then typically placed into brown paper bags when sold. When produced on an industrial scale, the biltong is packaged, most commonly using either modified atmospheric packaging or vacuum packaging. It has been suggested that biltong has a shelf-life between three to six months however, there is no scientific literature to support this.

The South African National Standards have set out legal microbiological requirements for biltong, SANS 885:2011, which state a limit for total viable counts ($< 6 \log \text{ cfu.g}^{-1}$) and for minimal presence of pathogenic microorganisms (*Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli*). Limits of coliforms and yeast and moulds are not stated in these legislation but in a committee draft. A factor to consider when discussing the microbial stability of biltong is the common problem of yeast and mould growth. Mould growth is undesirable to consumers (Van der Riet, 1976b) and results in economic losses for biltong producers and suppliers (Van der Riet, 1982). Yeast and mould growth in biltong has been reported in literature from as early as 1976. This literature suggests that yeasts are more predominant than moulds (Van der Riet, 1976b; Osterhoff & Leistner, 1984; Wolter *et al.*, 2000; Petit *et al.*, 2014). It is important to identify the common types of yeasts and moulds found in biltong for safety reasons (some moulds can produce mycotoxins) as well as to investigate possible prevention strategies along the biltong production process.

Using the knowledge and literature at hand, processing guidelines and shelf-life are not the objectives of these studies and therefore more information and research is necessary for recommendations to be made.

Research on South African biltong and the process involved is very limited and there is no definitive legislation which is topically based on the biltong we know today. As it is a wide research topic, this research allows for a good overview that future research will be able to use extensively. By determining the drying kinetics of biltong at constant drying parameters, this will enable biltong producers to produce a more consistent product (in terms of its physicochemical properties) and have the ability to predict drying times based on scientific findings. Once this has been established, the microbial stability of biltong produced with these drying parameters, its shelf-life and the development of yeasts and moulds must be investigated so that the drying parameters chosen can be shown (supported with scientific evidence) to produce biltong safe for consumption.

The objective of this study was therefore to establish a profile for traditional South African biltong in terms of processing, physicochemical properties and microbial stability during storage. This research specifically investigated the physicochemical properties (moisture and salt content, water activity, pH) and drying kinetics of lean beef biltong whereby the effect of vinegar and two weight losses (50% and 65%) were tested when using controlled drying parameters (temperature, relative humidity, air velocity). Separately, the effect of different meat types (lean beef topside, *semimembranosus*; lean beef silverside, *biceps femoris*; fatty beef topside and lean gemsbok topside, *Oryx gazelle*) on the

physicochemical properties and drying kinetics when using the same controlled drying parameters was studied. This was followed by an investigation into the shelf-life of lean beef biltong when produced using different processing factors (use of vinegar; 50% and 65% weight losses; and two packaging methods, vacuum packaging and modified atmosphere packaging). A challenge test was conducted to determine the ability of beef biltong to support yeast and mould growth during storage. Lean beef topside was used for all studies as this is the most commonly used meat in biltong production. Other meat types used in the industry were also explored in a smaller study. As salt is essential in biltong production and the industry uses a standard amount, there was more interest from the industry towards the use of vinegar and its effects on drying and product quality. The drying levels/weight losses were based on consumer preferences and the drying parameters were based on information generated from industrial practices. This profile could serve as preliminary guidelines for biltong producers selling for the local market as well as for exporting of biltong, as scientific evidence is needed to support the already established production process and assumed shelf-life of biltong.

Due to the use of more advanced technologies in the industry, a pilot study was done which investigated the effect of ultrasonic-brining on the salting and drying kinetics of beef biltong. Ultrasound was chosen as it is a novel innovative technology that has been studied for its effects on meat brining (Cárcel *et al.*, 2007; Siró *et al.*, 2009; Ozuna *et al.*, 2013; McDonnell *et al.*, 2014). It has also been found to be less energy-intensive than other techniques (environmentally friendly and cost-efficient) (Piyasena *et al.*, 2003; Cárcel *et al.*, 2007). Apart from its potential to improve salting/spicing and drying of biltong, it could also possibly improve microbial stability.

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CHAPTER 2

LITERATURE REVIEW

INTRODUCTION

Biltong, a popular ready-to-eat salted and dried meat product from South Africa, is growing in popularity all over the world. Ready-to-eat (RTE) products are foods that require no further processing (Levine *et al.*, 2001). The preservation of meat through curing and drying is not a new concept. Centuries ago, seafarers would salt/pickle meat in wooden barrels so they would have food during the long months at sea. It has been said that the indigenous tribes of Africa had their own methods for meat preservation, even before the first settlers came to southern Africa; this involved salting and drying of meat. Once on land, it is said the first settlers adopted this process having a great need for food preservation. While game (wild animals/ungulates) was abundant in Southern Africa, they had no means of storing the meat that they hunted. They took to drying it in the sun like the indigenous tribes but added vinegar and spices that were available to them. The spices used were those of abundance from the then Cape Colony and the vinegars were made from the grapes of the French Huguenots. And so, drying became an accepted means for meat preservation. The Voortrekkers (early pioneers) would cure and dry the meat for up to two weeks and wrap it in cloth for transportation before setting out on their travels across the continent. The word “biltong” is derived from the Dutch word “bil” meaning round or buttock, and “tong” related to tongue as biltong is made from strips of meat (Strydom & Zondag, 2014).

Nowadays, biltong is produced on either a small scale (households, butcheries) or larger scale (industry), mainly from beef and using several recipes and processes so as to accommodate for the demand of biltong. Many countries have a speciality dried meat product as shown in the first part of this review. Beef jerky (North America), charqui, carne seca and carne do sol (South America) are the most frequently compared to biltong.

This review gives an overview of the general and scientific knowledge on biltong and its characteristics, as well as consumer trends and the biltong market. The processing methods commonly used as well as the factors to consider which influence the quality of the final product are discussed. The microbiology and shelf-life of biltong is also highlighted. Ultrasound as a novel technology in food processing, its use in the meat industry and possible application to biltong production is also briefly discussed. Evaluating the literature available, this review allows for an understanding of the research which is lacking and necessary for increasing biltong quality and consistency for both the local market and possible exportation of biltong. The latter is dependant on existing regulations and legislation which is also discussed in relation to biltong.

DRIED MEAT PRODUCTS

Various countries around the world produce dried meat products for which they are known. Table 2.1 shows the most popular dried meat products made from ruminant animals (Leistner, 1987; Hui, 2012; Toldrá, 2015; Pintado *et al.*, 2016).

Table 2.1 Dried meat products from ruminant animals from selected countries around the world (Leistner, 1987; Hui, 2012; Toldrá, 2015; Pintado *et al.*, 2016)

Dried meat product	Country of origin	Description	Meat used
Biltong	South Africa	Salted, dried meat strips	Beef or game
Bündnerfleisch/ Bindenfleisch/ Grisons meat	Switzerland	Salted, dried meat - "deli-style"	Beef
Bresaola	Northern Italy	Salted, dried meat - "deli-style"	Beef
Carne-de-sol	Brazil	Salted, dried meat strips	Usually beef
Carne seca	Mexico	Salted, dried meat	Beef
Cecina	Cuba, Mexico, Northwestern Spain,	Salted, dried, lightly smoked meat strips	Spain: Beef or horse Mexico: Beef or pork
Charque/Charqui	Brazil	Salted, dried meat strips	Llama or alpaca Beef or horse
Droëwors	South Africa	Salted, dried sausage	Beef or game
Jerky	North America	Salted, dried meat strips, sometimes smoked	Usually beef
Kaddid/Qadid	Northern Afghanistan, Northern Africa, Pakistan	Salted, dried meat strips	Often lamb
Kilishi	Sahel African countries	Dried, roasted strips of meat	Beef, sheep or goat
Kitoza	Madagascar	Salted, dried meat strips, sometimes smoked	Beef or pork
Mipku	Northern Canada	Dried strips of meat	Caribou or reindeer
Nikku	Canadian Arctic	Dried strips of meat	Caribou
Pastirma	Armenia, Egypt, Turkey	Salted, dried meat - "deli-style"	Beef
Sucuk	Turkey	Salted, dried, fermented sausage	Beef or buffalo
Suho meso	Bosnia	Salted, dried, smoked meat strips	Beef
Taicang	China	Dried, shredded meat	Pork or beef
Tasajo	Cuba	Salted, dried meat strips	Beef or horse

"Deli-style" – meat produced using whole muscles

Biltong is similar in its shape to the products listed in Table 2.1 made from meat strips (not made as a whole muscle) but, differs as it is a ready-to-eat product, meaning it is eaten as a raw product with no preparation (such as rehydration/desalting/cooking) required before consumption. Charqui, for example, has a higher salt content (12 – 15%) and needs to be desalted before consumption (Santchurn *et al.*, 2012). Jerky is most commonly compared with biltong but jerky is dried at more elevated temperatures (Burnham *et al.*, 2008), for example at 60°C (Carr *et al.*, 1997) and therefore the process and the end product differ from biltong. Another unique attribute of biltong is that on many occasions vinegar is used together in the salting step and this is not seen for any of the other products mentioned in Table 2.1.

BILTONG PHYSICOCHEMICAL CHARACTERISTICS

Biltong can be produced using recipes with different spices/ingredients, amount of salt and can be more or less dried as reflected by the wide range of characteristics reported in the literature. The final physicochemical composition of biltong is important as it influences the sensory properties of the products as well as being an indication of a shelf-stable product. Factors to consider for the final composition of biltong is the moisture and salt content and the water activity (a_w) (these factors usually correlate with one another) as well as pH.

Scientific studies on the physicochemical characteristics of commercial biltong are mostly from the 1970's – 1980's (Van den Heever, 1970; Van der Riet, 1976a; Osterhoff & Leistner, 1984) except for the recent study of Petit and others (2014) which was conducted on 11 samples. Moisture content is dependent on the consumer's preference and were reported to range between 10 – 50% with a a_w between 0.54 – 0.89, one sample being reported at 0.93 (Van der Riet, 1976a; Osterhoff & Leistner, 1984; Petit *et al.*, 2014). There are many reports referring to moist and dry biltong but there is no evidence as which values of moisture content or a_w classifies biltong as "moist" or "dry." In the 1970s, the commercial industry believed the moisture content should be 30% or more whilst a moisture content of 20 – 30% was reported to be ideal in the 1940's (Van den Heever, 1970). Nortje and others (2005) defined moist biltong to have a moisture content higher than 40% and from the data on commercial products, to have a a_w range between 0.84 and 0.93 (Van der Riet, 1976a; Osterhoff & Leistner, 1984; Petit *et al.*, 2014). Petit and others (2014) distinguished two groups of biltong: those with a moisture content between 21.5 – 25.3% and a_w between 0.65 – 0.68 they classified as "dry" and those with a moisture content between 35.1 – 42.8% and a_w between 0.85 – 0.89 they classified as "moist". It is evident that the higher the moisture content, the shorter the potential shelf-life (due to microbial and fungal spoilage/growth) but the higher the yield per unit and the higher the profit. Salt is the main spice used in the production of biltong and is considered to be a "curing agent". The final salt content of biltong varies depending on the amount of salt used initially, as well as the level of drying as the salt concentration increases due to the reduction in moisture. Salt contents can range from 2 – 11%, with most biltong commonly having a final salt content of 4 – 8% (Van der Riet, 1976a; Osterhoff & Leistner, 1984; Petit *et al.*, 2014). The pH of biltong mostly ranges between 4.8 – 5.9 (Van der Riet, 1976a;

Osterhoff & Leistner, 1984; Petit *et al.*, 2014). Biltong is considered an intermediate-moisture food for shelf-life stability classification as most biltong falls in the range of 20 – 50% moisture content and a a_w of 0.60 – 0.90 (Leistner, 1987).

There is no legal definition for biltong contrary to charqui which should, according to the Brazilian legislation contain 40 – 50% moisture and 10 – 20% salt (Brasil, 1997 cited by Lara *et al.* 2003). The derivative meat product, jerked beef, is also officially defined by the Brazilian legislation as having maximum 55% moisture, 50 ppm sodium nitrite, 18.3% ash, a final a_w value of 0.78 and should be vacuum packed (Brasil, 2000 cited by Pinto *et al.* 2002). The United States Department of Agriculture (USDA) guideline suggests a maximum a_w of 0.85 for jerky.

CONSUMER TRENDS AND MARKET SIZE

Over the years, consumers have increased their consumption of snack foods due to convenience. Researchers have noted that the food industry needs to take advantage of these consumption trends and develop and expand product lines to meet the current needs of the average consumer, a situation that is still prevalent today (Miller *et al.*, 1988; Carr *et al.*, 1997; Fuller, 2011). Biltong has become the popular choice for consumers as the “go-to” snack food as it is considered healthy and convenient. Consumer trends show preference towards the higher moisture biltong products (Nortje *et al.*, 2005; Dzimba *et al.*, 2007).

There is currently no official estimation of the annual biltong production in South Africa. In 1982, Van der Riet, stated that several producers were producing over 100 tonnes of biltong annually in South Africa with an estimation of the total biltong production closer to the order of thousands of tonnes. In 2003, 20 years later, Gull Foods (one of the companies producing biltong in South Africa) was producing 80 000 – 90 000 units, from 80 to 180 g, per month (Attwell, 2003) which corresponds to 6 to 16 tonnes per month. In 2015, Closwa Biltong, the largest manufacturer of biltong products in Namibia was producing up to 660 tonnes of product per annum (H. Fourie, 2015, Closwa Biltong, Namibia, personal communication, 18 August) whilst a local Cape Town manufacturer, Cape Deli, produces 480 tonnes per annum (M. Nel, 2016, Cape Deli, Cape Town, personal communication, 23 November).

Biltong has gained popularity in the international markets – Namibia, United Kingdom (UK), Australia, New Zealand, United States of America, Canada and a few countries in Europe (Denmark, Netherlands, Switzerland to name a few). Some of which are beginning to sell biltong, through internet sites and stores which supply traditional South African products. In the UK, Attwell (2003) stated that only one biltong factory has been established (in 1982) as an EU-accredited factory that produces biltong.

Biltong producers in South Africa have difficulty exporting products due to the “virtually non-existent opportunities for export without an EU and HACCP-certified factory” (Attwell, 2003). The high cost of raw meat and the consumer demand for quality and consistency is a problem for both South African and international markets alike. The large commercial biltong factories in South Africa are in

the process of EU-certification so that they can begin to export their product (M. Nel, 2016, Cape Deli, Cape Town, personal communication, 23 November).

BILTONG PRODUCTION PROCESS

The production of biltong involves a series of steps including meat preparation, salting/spicing and drying. Salting is often assisted by tumbling at large scale manufacturing. An overview will be given regarding techniques used in biltong production and their mechanisms.

Meat selection and preparation

Beef, ostrich, game and even chicken meat are commonly used when making biltong (Van Wyk, 2007; Naidoo & Lindsay, 2010b, Strydom & Zondagh, 2014). Lately there has been an increase in the production of pork biltong although rancidity is still of concern amongst processors. Both fresh and thawed meat can be used. The most popular muscles to use for biltong are the silverside (*biceps femoris*), topside (*semimembranosus*), thick flank (*rectus abdominus*), eye of round (*semitendinosus*) and fillets (*longissimus dorsi*), with topside being the preferred muscle choice (Van Tonder & Van Heerden, 1992; CSIR, 2001; Van Wyk, 2007; Strydom & Zondagh, 2014).

When cutting the meat, the connective tissue is removed and the resulting meat is cut into long strips. Although this is commonly done by hand, modern large volume processors typically use mechanical demembrating machines as well as specially designed rotating circular blades (Fig. 2.1) to cut the biltong into the desired strips. The dimensions depend on the muscle type and personal preference; suggestions include a width/thickness of 2.5 – 5 cm and 25 – 40 cm length (Van Tonder & Van Heerden, 1992; CSIR, 2001), the thicker the strips the longer the drying period. Traditionally the meat is cut parallel to the meat fibres (CSIR, 2001) as it is thought to give the most efficient salt and spice absorption as well as best quality (texture) although there is no scientific evidence to support this; this is an aspect that warrants further research. It has also been suggested to cut diagonally across the grain as it is believed to give a better eating quality and appearance to the meat. Biltong can either be lean (no fat on the outside) or fatty (a layer of fat on a surface), both which are popular amongst consumers. It is however recommended that excess fat be trimmed from the meat as it may cause rancidity (Strydom & Zondagh, 2014) and takes longer to absorb salt (Van Tonder & Van Heerden, 1992, Heinz & Hautzinger, 2007). Fat can also increase water diffusivity which was seen in a study on the drying of pepperoni where a lower fat content resulted in increased water diffusivity (Palumbo *et al.* (1977). Beef biltong may contain some fat whilst ostrich and game species seldom have excess fat stored in fat depots such as the subcutaneous fat and therefore produce lean biltong. The muscle is cut into preferred dimensions and kept in the refrigerator (4 – 8°C) until salting/spicing.

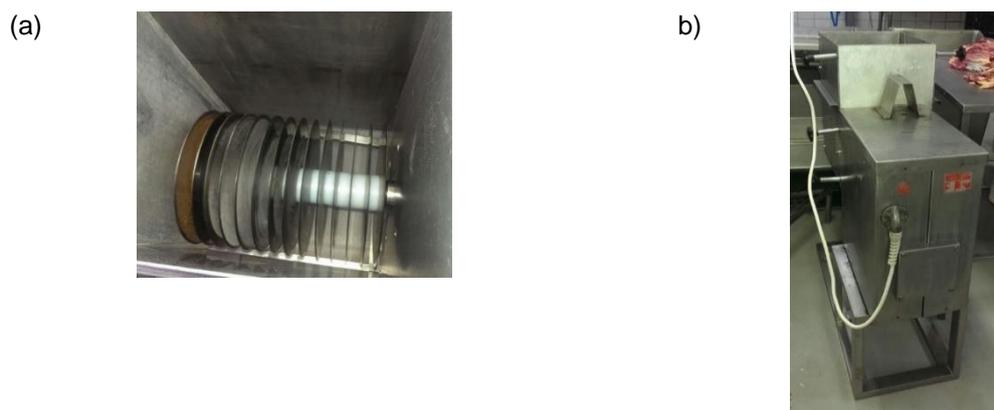


Figure 2.1 Rotation circular blade cutter used at Closwa Biltong, Namibia; (a) above, (b) side view.

Salting/spicing and tumbling

There are several methods used when salting and mixing biltong with other ingredients (spices and sometimes vinegar). The meat can either be dipped in dry spices (Taylor, 1976; Van der Riet, 1976b; CSIR, 2001), dipped in dry spices then a hot acidic liquid (vinegar) (Leistner, 1987), dipped in an acidic liquid, drained, and then dipped in dry spices (Naidoo & Lindsay, 2010c) or dipped in an acidic liquid/spice mix (Naidoo & Lindsay, 2010c). Then the strips are stored, for example, for 18 – 20 hours at 4°C (Naidoo & Lindsay, 2010c). The traditional method is layering the biltong strips and spicing each layer with the ingredients and then storing at ambient temperatures. Nowadays the layered spiced biltong strips is stored in a cold room (4 – 8°C). After 6 – 12 hours, the biltong strips are turned over and left for a further 6 – 12 hours before it is hung to dry (Van Tonder & Van Heerden, 1992).

Salt has been added to biltong (dry salting) at a level of 2.5 – 4% (Taylor, 1976; Van der Riet, 1976b, Van der Riet, 1982). A number of studies use spice mixes containing salt in which salt content is not mentioned and not always determined on the end product (Nortje *et al.*, 2005; Dzimba *et al.*, 2007; Naidoo & Lindsay, 2010c). It has been reported that 2.5% of salt in the formulation gives an acceptable taste over a wide range of moisture contents but can be increased up to 4% (Van der Riet, 1982). Dry salting, even though it can result in an uneven distribution of salt, is an easier process and more economical than brining (submerging meat in a saturated salt brine) (Van der Riet, 1982).

Salting is a method of preservation which causes changes in meat (muscle proteins), generating changes in texture (by modifying myofibrillar proteins solubility), swelling of muscle fibres which relates to an increase in the water binding- and water holding capacities at the salt concentration and pH usually encountered in processed meat products (Offer & Trinick, 1983; Offer *et al.*, 1989; Toldrá, 2010; Oliveira *et al.*, 2012). Salt preservation also acts by decreasing water activity so there is less available for microbial growth which in turn leads to an increased shelf-life. Swelling occurs due to the chloride ions that bind to the protein filaments which increases the electrostatic repulsive force between them causing the protein structure to unfold and swell (Cheng & Sun, 2008). Myofibrillar proteins may also swell due to cellular disruption which is caused by tumbling (Dolata *et al.*, 2004; Siró *et al.*, 2009).

Vinegar may be added during the salting/spicing step (Van den Heever, 1970; Van der Riet, 1982; Naidoo & Lindsay, 2010b; Naidoo & Lindsay, 2010c; Strydom & Zondagh, 2014) which plays a role in inhibiting microbial growth as well as adding to the flavour. Depending in the salt content and pH, the vinegar will also modify the water binding capacity. Brown spirit vinegar and apple cider vinegar are the most commonly used in biltong production (Naidoo & Lindsay, 2010b; Strydom & Zondagh, 2014). Previously, vinegar has been added to biltong at a level of ~3% (Van den Heever, 1970) and ~6% (Naidoo & Lindsay, 2010c).

Other ingredients commonly used include black pepper, coriander, brown sugar and vinegar. Preservatives have been permitted for use in biltong production as stated under the Foodstuff, Cosmetics and Disinfectants Act 54 (1972) (Table 2.2).

Table 2.2 Regulations pertaining to food preservatives and quantities permitted for use in biltong (Foodstuff, Cosmetics and Disinfectants Act 54, 1972)

Preservative	Quantity permitted (mg.kg ⁻¹ or mg.L ⁻¹)
Pimaricin	6
Potassium and sodium nitrates	200 total nitrate, expressed as sodium nitrate
Potassium and sodium nitrites	160 total nitrite, expressed as sodium nitrite
Sorbic acid	2000

Other preservatives mentioned on labelling of commercial biltong include sodium bisulphite and sodium benzoate. Pimaricin and potassium sorbate are the most commonly used preservatives and are in most spice packs used for salting/spicing biltong. The use of 1000 ppm of sorbic acid was effective in retarding the development of the growth of total microorganisms (Taylor, 1976) and moulds (Van den Heever, 1972 and Van der Riet, 1981 cited by Van der Riet, 1982). Sodium nitrate/nitrites have been reported to be added to prevent microbial spoilage but also for a red colour (CSIR, 2001; Strydom & Zondagh, 2014). In one study nitrate was measured between 10 – 860 ppm (Osterhoff & Leistner, 1984). However they are said to no longer be used on commercial biltong. There is research lacking on the level of preservatives found in commercial biltong and whether they do fall within the legislation limits. The consumer acceptance of adding preservatives to such a product has also not been investigated/recorded.

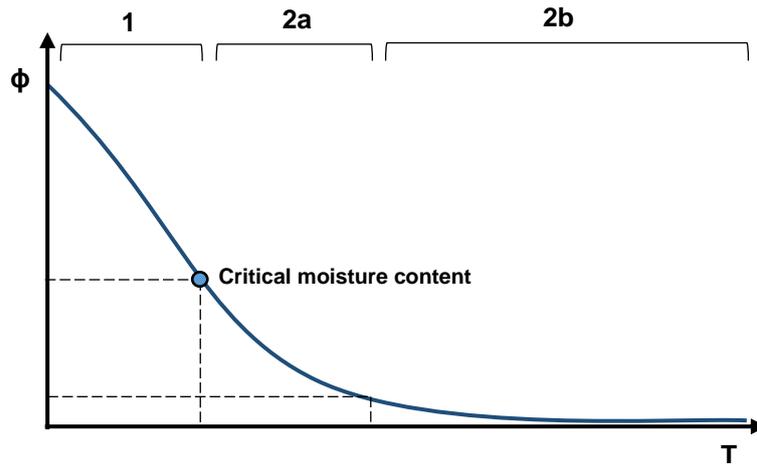
At large scale biltong manufacturing, tumbling is used to help with the mixing of ingredients. Time is substantially reduced compared to the other techniques mentioned, particularly when it is done under a low vacuum. Tumbling is used as a method to accelerate food production processes, commonly in meat production as a post treatment to brining (Toldrá *et al.*, 2010; Hui, 2012). It accelerates salt diffusion (migration), enhances tenderness and juiciness, as well as increases product yields. During this mechanical operation, meat pieces fall and hit paddles in a rotating drum causing cellular disruption of the meat tissue allowing a more even distribution of the salt. It must be done at a relatively low speed as if too fast, it could lead to heat production, mechanical damage and poor salt distribution resulting in lower/poor quality products (Toldrá *et al.*, 2010). This occurs under a vacuum to offset the potential problem of incorporating air into protein exudates and either as a continuous or

intermittent treatment. Brine injection and tumbling have also been compared to dry salting in pastirma processing (Guner *et al.*, 2008). An advantage of using tumbling in the biltong production, from an industrial viewpoint, is the limited handling and hand contact of large quantities of meat, reduced processing time (when waiting over a 24 hour period is the norm as for traditional processing of biltong), costs and increased output yields. Tumbling could also promote the distribution of ingredients (spices and vinegar) more quickly and uniformly within the meat. However, the diffusion of salt in biltong during salting/spicing using the traditional way or when the meat is tumbled with or without vacuum and during its subsequent drying and storage has not been studied.

Drying

The traditional drying of biltong is done by hanging the meat strips outside for one to two weeks depending on ambient conditions (Van der Riet, 1982). The meat strips are hung by hooks (wire or plastic) in shady areas, usually in winter as temperature, humidity and wind conditions are considered ideal. These days, biltong is still made at a household level using this method or by using a small box equipped with a fan and a light. As the demand for biltong increased, these traditional methods were adapted for commercial purposes. At the commercial level, a variety of drying equipment has been used in biltong production from a basic room with fans and heaters to controlled drying chamber units (Van der Riet, 1982). In industry, the most frequently used dryer is only temperature controlled but some dryers can also measure/manipulate the relative humidity. Drying of biltong is usually done at low temperatures (25 – 30°C). It is most commonly dried to a 50% weight loss (Strydom & Zondagh, 2014) or further to accommodate other consumer preferences. There is no data available on the relative humidity and air velocity conditions in the industry. There is however, a study on a research scale which followed the moisture content of biltong during six days of drying at 35°C temperature, 30% relative humidity and 3 m/s air velocity, which was said to replicate industrial conditions (Taylor, 1976). Large biltong producers would like to use drying chambers where the temperature and relative humidity are controlled independently.

Mass transfer during drying of meat products is the migration of water to the environment (external) and the continuation of distribution of salt and other compounds in the product itself (internal) (Comaposada Beringues, 1999). Drying curves illustrate the water transfer phenomena during drying. They are called drying kinetics when the decrease in moisture content is plotted against drying time. They can be drawn from the following of the weight during drying knowing the initial or final moisture content. Three periods can be distinguished on a typical drying curve as represented in Figure 2.2. As the product dries, the water content and activity is reduced and the rate of drying falls.



Φ = moisture content (dry basis), T = drying time

1 – Constant-drying rate period

- Water evaporation not dependent on solid, evaporates as if there is no solid present, externally controlled
- Loosely bound and free water inside the solid diffuses very quickly from the core of the solid to the surface

2 – Falling-drying rate period

- 2a – Wet surface areas become dry due to tightly bound water in the center, internally controlled
- 2b – Surface is dry, evaporation continues moving towards the centre of the solid, internally controlled

Figure 2.2 Ideal drying kinetics curve (Adapted from Comaposada Beringues, 1999).

When studying the drying curve of any food type it is important to consider the water sorption isotherm. This shows the relationship between moisture content and water activity and can be useful to predict the water activity (a_w) knowing the moisture content of the food product. The water sorption isotherm, type II showing the moisture isotherm (sigma-shaped curve) for most products (cereals, dry products, meats, cheese etc.) is depicted in Figure 2.3.

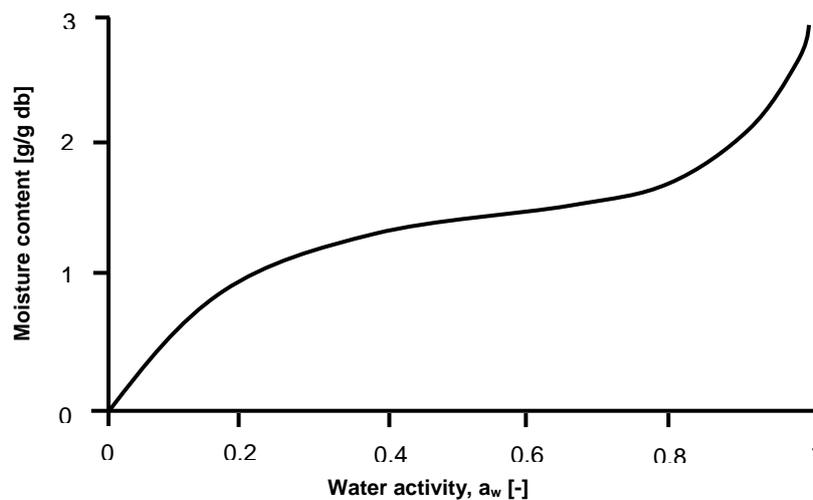


Figure 2.3 Water sorption isotherms, BET (Branauer-Emmett-Teller) classification, type II.

What needs to be taken into account, which is relevant in the case of biltong, is the addition of salt to the product, which influences the water activity (Van der Riet, 1976b). These compounds form chemical bonds with the available water and prevent it from being used by the microorganisms (Betts & Everis, 2008). Figure 2.4 is adapted from Van der Riet (1976b) which shows the difference in water sorption isotherms of biltong (the author represented the moisture content on a wet basis) with different amount of salt used for curing. An increase in added salt results in an increase in the moisture content.

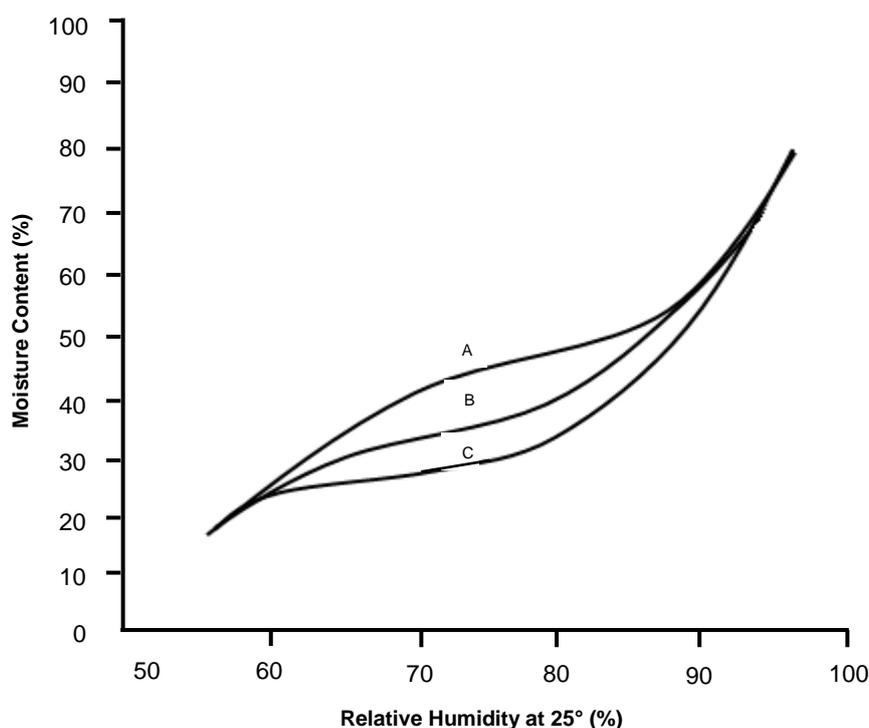


Figure 2.4 Water sorption isotherms for biltong produced with 4% (A), 2.5% (B), and 1% (C) salt (Adapted from Van der Riet, 1976b).

The external mass transfer is dependent on air flow conditions (namely air temperature, relative humidity and air velocity) and product shape/dimensions. It is known that there will be a high rate of drying if there is a large difference between the vapour pressure at the meat surface and water in the air (Comaposada Beringues, 1999). In an only temperature controlled dryer, the air temperature influences the relative humidity of the drying chamber and hence the rate of drying. A decrease in the relative humidity increases the evaporating capability of the air. By raising the air temperature, the relative humidity will lower and the rate of drying will improve. However, if the air temperature rises too high (>50°C), then the biltong starts developing a cooked flavour (CSIR, 2001). Air speed is an important factor which is seldom considered when optimising drying procedures. Air velocity affects the rate of drying through the mass transfer coefficient (Saravacos & Kostaropoulos, 2002). The mass transfer coefficient moves hot air towards the meat, removing moisture from the meat surface and the drying equipment, and influencing the drying rate. Therefore it can be stated that lower speeds will reduce the drying rate whilst higher speed will increase the drying rate. Some studies have been

conducted on the drying kinetics of meat (Trujillo *et al.*, 2007; Clemente *et al.*, 2009; Chabbouh *et al.*, 2011; Hii *et al.*, 2014; Ahmat *et al.*, 2015; Kucerova *et al.*, 2015; Petrova *et al.*, 2015). These covered the effect of drying parameters (temperature, relative humidity and air velocity), meat thicknesses and also the modelling of these kinetics. Regarding dimensions, biltong products range from traditional whole slabs, sliced biltong, thin snack sticks, biltong crisps, small biltong nuggets/bites and even flat biltong wheels/discs; each having a differing surface to volume ratio. A large biltong slab will take longer to dry than a thin biltong stick as the volume is much larger and the water losses will be greater even with a larger surface area. This is based on the basic principle that the larger the product (volume), the more water it will need to lose to reach desired characteristics and the smaller the product, the less water to be removed and therefore less time will be needed for drying. This in conjunction with the surface area will dictate the drying time as the less contact the product has with the drying air the longer the drying process will take, or in a product with an increased surface area, the drying will be accelerated.

Among the changes that occur due to the drying process, surface crust formation (Bellagha *et al.*, 2007) and shrinkage (Duan *et al.*, 2011) may be of concern for biltong drying. “Case-hardening” (surface crust formation) is when the meat surface is dry and hard but the inside of the meat is still moist. This can occur if there are high drying temperatures, and/or a high air velocity and low relative humidity, as this results in a shorter drying time but causes a high rate of drying, therefore a high moisture loss from the meat surface. If the meat surface is too dry, the moisture inside the meat cannot move out quickly enough (Comaposada Beringues, 1999; Serra *et al.*, 2005). Shrinkage is caused by loss of water which causes stress in the cellular structure of foods and is affected by the volume of removed water, mobility of the solid matrix and the drying rate (Mayor & Sereno, 2004). Presence of collagen can also influence shrinkage because it tends to dry faster than the meat. On application of heat the collagen is transformed to soluble gelatin and upon prolonged heating and drying the soluble gelatin binds the muscle fibres together forming an intact structure which causes the meat to bend which in turn, results in increased toughness of the meat (Huang & Nip, 2001). If the collagen dries faster than the meat this will cause shrinkage which could result in biltong to become undesirable and difficult to eat (tough). Research on vegetables, has shown that the shape/size of the samples have a minor effect on the volumetric shrinkage (Hatamipour & Mowla, 2003; Mulet *et al.*, 2000). Clemente and others (2009) studied the shrinkage of pork meat during drying and concluded that when using moderate drying temperatures (25°C) and air speeds (0.6 – 2.8 m/s) the volume shrinkage and water losses that occur are linearly related. Whilst Okos and others (1992) state that in dried meat products, 40 – 50% of the shrinkage may occur in the early drying stages.

Packaging and storage

Biltong is packaged and stored in different ways. Most butcheries in South Africa sell biltong in loose paper bags or over-wrapped trays, dependent on the type of biltong that is being sold (e.g. slabs, sliced, sticks, etc.). Consumers would store biltong in brown bags or closed plastic containers when making biltong at home. Industrially produced products are sold however in nitrogen-

flushed/vacuumed-packed packaging so as to try to give it a longer shelf-life (when unopened) (Van der Riet, 1982; Strydom & Zondagh, 2014). Vacuum packaging is also still used but is not suitable for high moisture biltong as it causes the biltong to stick together and lose its visual appeal. Biltong is sometimes packaged in a sealed bag using none of the above-mentioned technologies but this is not common.

There are three main packaging technologies which are used in the food industry when the aim of the packaging is more than a just barrier to the external environment.

1. Modified atmosphere packaging (MAP) / controlled atmosphere packaging (CAP) – the atmosphere inside the packaging is adapted so the composition is other than that of air. Once sealed, the atmosphere inside the packaging cannot be controlled (Day, 2008).
2. Vacuum packaging (VP) – the air from the packaging is removed before being sealed. It is similar to a low oxygen MAP but the air is not replaced by a combination of gases as with MAP/CAP.
3. Active packaging – the addition of additives into the packaging material or within a packaging container. These additives are capable of scavenging/releasing oxygen and carbon dioxide (Suppakul *et al.* 2003).

There are two methods used to apply MAP technologies depending on the function of the packaging – gas flushing and compensated vacuum gas flushing (Mullen & McDowell, 2011). Gas flushing is a continuous stream of gas that flushes the air out of a package before sealing. With this method a residual oxygen level of 2 – 5% still present inside the package is expected but this can be higher depending on the gas combination desired. Therefore making it unsuitable for foods sensitive to oxygen or with aerobic microorganisms that may be present. Compensated vacuum gas flushing, more commonly used in the food industry, is a two-stage process. Firstly, a vacuum removes the air from the package (dependent on how dry the food product is, the lower the achievable vacuum). This allows for lower residual oxygen levels to exist inside the package after gas flushing. This is followed by the gas flushing stage whereby the package is flushed with the modified gas composition. This method is more suitable for oxygen-sensitive food products. Both of these methods are commonly used in the biltong industry.

Packaging is a method that allows the use of modified atmospheres/vacuum as an extrinsic factor in hurdle technology (Betts & Everis, 2008). It is commonly known that microbial growth is dependent on oxygen availability. Microorganisms can be classified as aerobic (e.g. moulds), anaerobic (e.g. *Clostridium* spp.) or facultative anaerobes (e.g. *Salmonella* spp., *Staphylococcus aureus*, yeasts) (Day, 2008; Mullen & McDowell, 2011). Nitrogen (N) and carbon dioxide (CO₂) are frequently used in MAP to manipulate the environment as a means to control bacterial growth. Nitrogen replaces oxygen in the packaging and therefore inhibits the growth of strict aerobic microorganisms such as moulds whilst CO₂ exerts an antimicrobial effect on spoilage microorganisms and pathogens which are aerobic, and thereby can reduce or prevent the growth of these microorganisms (Day, 2008). Vacuum packaging results in a low oxygen environment and therefore would inhibit growth of aerobic microorganisms such as with MAP. This type of packaging however has higher residual oxygen than MAP. Research on dry-cured hams showed that there wasn't a significant decrease in mesophilic aerobic colonies in MAP in comparison to VP indicating no differences in microbial quality between

differing packaging conditions (MAP and VP) (García-Estaban *et al.*, 2004; Parra *et al.*, 2010). A report by Aas and others (2010) states that modified atmospheric packaging (oxygen-reduced) inhibits halophilic bacteria in salted cod. Halophilic bacteria are strictly aerobic (Elzari-Volcani, 1957) and therefore MAP would have a growth-inhibiting effect. Theoretically, MAP should be the best packaging for biltong as it has a high salt content and low water activity so would help inhibit microbial growth as well as keep the quality characteristics (texture, preferred moisture and visual appeal) expected by consumers.

There is no research to show the impact that packaging has on the quality and shelf-life of biltong. It is known however that unpackaged biltong should not be stored in moist, warm environments as the product may reabsorb moisture and become more susceptible to microbial growth (Burfoot *et al.*, 2010). Studies on salted cod have reported that it is important to maintain an equilibrium between the water activity in the product and the relative humidity in the surrounding air. A higher relative humidity than product water activity will result in weight gain as the product will absorb the available moisture. The opposite occurs when the relative humidity is lower than the product water activity (Doe *et al.*, 1982). The same principle should apply to salted and dried meat products. When biltong is stored by hanging in the open air the storage temperature is also important to consider as this could affect the colour, texture, moisture content and microbial stability of the product. Therefore by monitoring the storage conditions (temperature and relative humidity, packaging), this will allow for better control of product stability – no research on biltong has been conducted to confirm this.

RELATION BETWEEN pH, SALT, WATER HOLDING CAPACITY AND DRYING

The pH of meat can range between 5.2 and 7.0. The pH for fresh meat with a desirable eating quality is between 5.4 and 5.8. In biltong pH ranges from 4.8 to 5.9, as stated previously. This pH is influenced by the type of meat used (beef, game, ostrich) and the stress the animal experienced ante mortem. The final pH of biltong can also be influenced by the addition of an acid in the salting/spicing step. The low pH values of some biltong (4.8 – 5.0) reported in previous studies (Osterhoff & Leistner, 1984, Petit *et al.*, 2014) could be due to vinegar addition but no information is available on their processing methods to verify this. The relationship between pH and the water holding capacity (Fig. 2.5) is important as it affects the drying kinetics.

The water holding capacity is the lowest at the isoelectric point (positive and negative charged groups are equal) of the proteins, therefore there will be no charge to hold the bound and immobilised water. Figure 2.5 also shows that salt increases water holding capacity for a pH higher than the isoelectric point (as discussed in the paragraph dealing with salting) and decreases the isoelectric pH of meat.

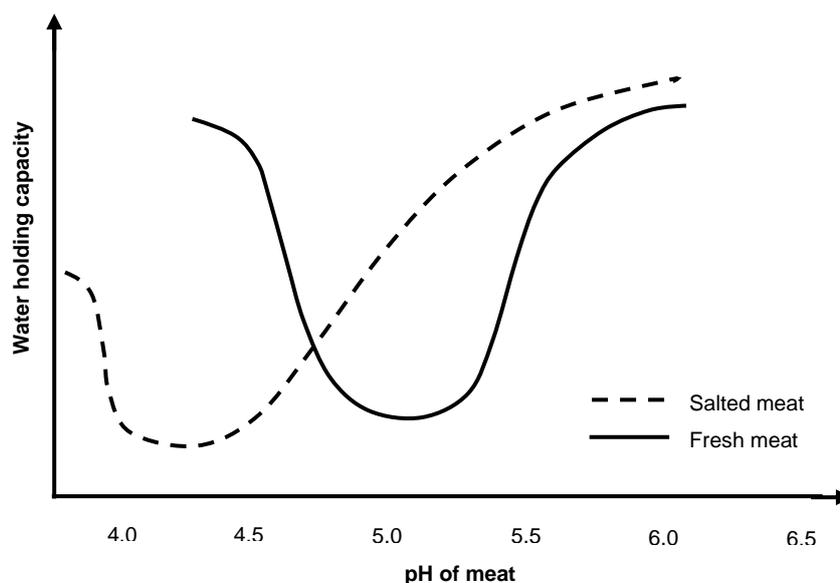


Figure 2.5 The relationship between water holding capacity and pH in meat (Adapted from Xiong, 2004).

When making beef biltong, a high pH (> 6) of the raw material is not common but when working with game meat this is a regular problem due to the *ante mortem* stress experienced by the animal (Hoffman & Wiklund, 2006; Hoffman *et al.*, 2007). The accumulation of lactic acid in the muscle of the animal lowers the pH and results in good eating quality. If an animal is stressed at time of death, the animal depletes muscle glycogen reserves and the pH does not decline noticeably. A high pH leads to an increased water holding capacity resulting in what is known as dark firm dry (DFD) meat. The water in the meat is very tightly bound with the muscle structure. When using DFD meat in biltong production it is hypothesised that as the water is tightly bound within the meat, it may take longer for the removal of the water and therefore delay the drying process; however this hypothesis needs to be clarified. The effect of this increased water holding capacity (and high pH) on the microbial spoilage also needs further research (see next section). It has also been stated that low pH-values improve a decrease in moisture in cured-raw meats as the low water holding capacity allows for an adequate release of water during the proceeding phases (Heinz & Hautzinger, 2007). Further research in this area of study would be beneficial to the game biltong industry as currently drying parameters and time are the same irrespective of whether beef or game is processed.

MICROBIAL QUALITY OF BILTONG

Biltong is considered a safe product, however concerns regarding its microbial profile have been identified. Studies have assessed the levels of naturally present organisms in a variety of biltong that has been bought from different stores throughout South Africa (Van der Riet, 1976a; Wolter *et al.*, 2000; Mhlambi *et al.*, 2010; Naidoo & Lindsay, 2010a; Naidoo & Lindsay, 2010b; Petit *et al.*, 2014) and in Botswana (Matsheka *et al.*, 2010). Burfoot and others (2010) have compiled a summary of results

which conclude that commercial biltong has total viable counts (TVC) up to 7 log cfu.g⁻¹; Enterobacteriaceae and coliforms up to 4 log cfu.g⁻¹; lactic acid bacteria up to 8 log cfu.g⁻¹; yeasts up to 7 log cfu.g⁻¹; moulds up to 5 log cfu.g⁻¹; and Staphylococci up to 8.5 log cfu.g⁻¹.

According to the Food Safety and Inspection Services (FSIS), major concerns in ready-to-eat (RTE) meat products are *Salmonella* spp., *Listeria monocytogenes* and *Escherichia coli* O157:H7 (Levine *et al.*, 2001). Due to the elevated demand for enhanced food safety, modifications are being made in the processing and production of meat products (Jensen *et al.*, 1998). Spoilage microorganisms are those that commonly affect the quality of biltong and its shelf-life. These include coliforms, lactic acid bacteria, yeasts and moulds (Taylor, 1976; Naidoo & Lindsay, 2010b).

Yeast and mould growth commonly occurs on biltong (Van der Riet, 1976a; Osterhoff & Leistner, 1984; Wolter *et al.*, 2000; Mhlambi *et al.*, 2010; Matsheka *et al.*, 2014; Petit *et al.*, 2014). Once present, yeast and moulds will increase over time and eventually dominate the spoilage microorganism's profile of a meat product (Cook, 1995). This occurs in most dried meat products as yeasts and moulds have the ability to survive in a wide range of water activities (> 0.61; Betts & Everis, 2008). *Candida*, *Torulopsis* and *Debaryomyces* are the most dominant yeasts to be isolated in meat and meat products (Cook, 1995; Jay *et al.*, 2005). These yeasts can grow at low temperatures (10°C) which increases the risk of their growth in meat and meat products due to the production factors (such as chill storage and temperature drops). Yeasts are substantially represented in the total biltong ecology and has been seen to range between 2.0 – 7.0 log cfu.g⁻¹ (Wolter *et al.*, 2000). *Debaryomyces hansenii* has been seen to be the most common yeast species in dried-cured products (Jessen, 1995; Núñez *et al.*, 1996) and biltong (Wolter *et al.*, 2000). This could be due to its ability to alter its fatty acid composition in response to temperature changes resulting in higher polyunsaturated fatty acid levels which protect them from membrane damage (Dillon, 1998). This ability in addition to its survival in low to intermediate water activities results in its abundant growth in meat products. In earlier studies, Van den Heever (1970) did not distinguish between yeasts and moulds suggesting both yeasts and moulds are considered as undesirable however, later studies showed that yeasts are predominant compared to mould (Van der Riet, 1976a; Osterhoff & Leistner, 1984; Petit *et al.*, 2014). One study showed that yeasts were present in 19/20 samples ranging between 1.0 – 5.5 log cfu.g⁻¹ while moulds were only present in 6/20 samples ranging between 1.0 – 5.3 log cfu.g⁻¹ (Van der Riet, 1976a). Matsheka and others (2014) found 55 different yeast and mould species that belong to more than nine different genera, on biltong. It must be noted however that in this study the biltong was produced under various different processes to that traditionally used in South Africa. For example, the biltong in their study did not involve soaking the meat in cider (vinegar) and the meat was also dried in the open air (as was done traditionally). This therefore has implications on the microbial stability of the product. *Candida* yeasts and *Aspergillus* and *Penicillium* filamentous moulds were found to be the most common. Other genera found on the biltong include *Pichia*, *Eurotium*, *Fusarium*, *Cladosporium* and *Debaryomyces* (Matsheka *et al.*, 2014).

Moulds are a problem in the biltong industry as mould growth is undesirable to the consumer and may produce mycotoxins (Van der Riet, 1976a; Matsheka *et al.*, 2014). *Aspergillus*, *Penicillium*, *Fusarium*, *Eurotium* and *Mucor* are frequently found on dried-cured meat products (Cook, 1995; Asefa

et al., 2009; Sonjak *et al.*, 2011). These are also commonly found on biltong (Van der Riet, 1976a; Van der Riet, 1982; Wolter *et al.*, 2000). Various studies have been conducted on the presence of yeast and moulds and the aflatoxins they produce in meat products (Bullerman *et al.*, 1969; Aziz & Youssef, 1991; Markov *et al.*, 2013). A study of Van der Riet (1976a) focussed on biltong and concluded that *Aspergillus flavus*, which can produce aflatoxins, was the most frequently isolated from commercial biltong samples being present on 60% (12/20) of the samples. Other yeast and moulds that were isolated from the commercial samples tested are known to produce mycotoxins however the study did not investigate these. The *Aspergillus glaucus* genera is also often present but does not produce mycotoxins, this is often misidentified as *Aspergillus flavus* which is an aflatoxin producer (Van der Riet, 1976a, Van der Riet, 1976b; Van der Riet, 1982). The production facilities of dry-cured meat products have been stated to be linked to the growth of *Penicillium* spp (Núñez *et al.*, 1996; Palmas & Meloni, 1997; Asefa *et al.*, 2009) whilst *Fusarium* has been linked to production/air contamination in the facilities (Núñez *et al.*, 1996). Contamination of biltong by these moulds could be linked to the production facilities as seen with dried-cured meat products. Both yeasts and moulds induce economic losses for the biltong industry (Van der Riet, 1982) and it is a recurring problem in the industry.

Pathogenic bacteria are also a concern with biltong as these too have been found on occasion, to be present on commercial biltong and as they can cause illnesses in consumers who eat contaminated biltong. Moreover, pathogenic microorganisms survive for prolonged periods in food products and are therefore important to identify. Studies have indicated the presence of the following pathogenic microorganisms: *Salmonella* spp. detected in 7/47 samples (Prior & Badenhorst, 1974) and 2/60 samples (Van den Heever, 1970) and *Listeria monocytogenes* in 2/150 samples (Naidoo & Lindsay, 2010b). Among *Staphylococcus* strains, coagulase-negative strains such as *S. pasteurii*, *S. saprophyticus*, and *S. arlettae* are the most predominant of the *Staphylococcus* spp. (Naidoo & Lindsay, 2010b; Matsheka *et al.*, 2014). *S. aureus* was detected only in the study of Naidoo & Lindsay (2010b) with three strains of the 159 *Staphylococcus* isolates confirmed as *S. aureus*. Another study showed that in most biltong sampled in this study had *S. aureus* counts above the infective dose limit (10^5 cfu.g⁻¹) and the South African National Standards (Table 2.3) (Shale & Malebo, 2011).

In South Africa the commercial biltong industry uses the microbiological requirements as stated in the South African National Standards (SANS) 885. These standards show the maximum permitted microbial levels at the end of the indicated shelf-life of the product. These standards do not include microbiological requirements for coliforms and yeasts and moulds but these have however been mentioned in a committee draft. Table 2.3 gives a summary of the microbiological specifications for biltong and biltong-like products according to SANS 885 and its corresponding committee draft.

Table 2.3 Microbiological specifications for dried meat products (SANS 885:2011) for total viable counts (TVC), coliforms (CF), yeasts and mould (YM), *Escherichia coli* (*E. Coli*), *Salmonella* spp., *Staphylococcus aureus* (*S. aureus*) and *Listeria monocytogenes* (*L. monocytogenes*)

Category*	Microbiological requirements (log cfu.g ⁻¹)						SANS 885 (Committee draft)#	
	TVC	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>S. aureus</i>	<i>L. monocytogenes</i>	CF	YM	
Class 3	< 6	< 1	0 / 25 g	< 1.3	< 2	< 2	< 3	
Class 5	< 6	< 1	0 / 25 g	< 1.3	< 2	< 2	< 3	
Class 8	< 6	< 1	0 / 25 g	< 1.3	< 2	< 2	< 3	

*Class 3: Whole muscle, uncured, no/partial heat treated, dried products (uncured biltong); Class 5: Whole muscle, uncured, no/partial heat treated, dried products (cured biltong); Class 8: Comminuted, uncured, no/partial heat treated, dried products (droëwors, biltong wheels or discs)

#Committee draft: A draft of the legislation with possible amendments that can be made to the current legislation

HURDLE TECHNOLOGY IN THE BILTONG PROCESS

Biltong is produced under several microbial growth-limiting steps namely, salting/spicing, the addition of vinegar, refrigeration and drying (Notermans *et al.*, 1995; Wolter *et al.*, 2000; Collignan *et al.*, 2001). Reduction of microorganisms can be achieved during processing through: a) control of the temperature; b) the processing method used such as salting with vinegar, dry salting or tumbling; c) the use of salt and organic acids; d) the use of preservatives such as ascorbic acid/potassium sorbate, which is dependent on pH as this may prevent mould growth but not bacterial growth; and/or e) drying, whereby the final moisture content and water activity play an important role (Taylor, 1976; Van den Heever, 1972 and Van der Riet, 1981 cited by Van der Riet, 1982). These factors however do not necessarily prevent microbial growth when presented as individual factors, a combination/cascade (hurdle technology) would be better.

Hurdle technology is the control of two or more factors to inhibit microbial growth in food products. Factors that influence microbial growth can be intrinsic (properties of the food product itself) or extrinsic (properties of the environment in which the food is exposed) (Betts & Everis, 2008). Intrinsic factors include pH (acidity measurement), water activity (moisture and salt content) and/or preservative content (natural/added if used). Extrinsic factors that can be controlled include temperature during processing and storage, the use of packaging with modified atmospheres, heat (sterilisation/pasteurisation) and light, to name a few. The combination of these factors (hurdles) results in a synergistic effect which does not necessarily result in cell death but rather slows down microbial growth. The main factors that microorganisms (commonly found in biltong) need to grow are given in Table 2.4.

Table 2.4 Minimum* growth factors for food-borne microorganisms (Adapted from Betts & Everis, 2008)

Microorganism	pH	a _w	Anaerobic growth	Temperature °C
<i>Salmonella</i> spp.	3.8	0.92 – 0.95	Yes	4 – 5.2
<i>Staphylococcus aureus</i>	4.0	0.83	Yes	7
<i>Bacillus cereus</i>	4.9	0.93 – 0.95	No	4
<i>Listeria monocytogenes</i>	4.3	0.92	Yes	-0.4
<i>Escherichia coli</i>	4.4	0.93	Yes	7 – 8
<i>Clostridium perfringens</i>	4.5	0.93 – 0.95	Yes	12
Lactic acid bacteria	3.5	0.90	Yes	4
Yeasts	2.0	0.62	Yes	10
Moulds	2.0	0.61	No	10

*Factors minimum needed for growth of the microorganisms when all other conditions are optimum for growth

The biltong process takes place at different temperatures; meat preparation and curing at 4 – 10°C, drying at 20 – 35°C and storage at temperatures up to 25°C. Naidoo (2010) and Naidoo and Lindsay (2010b) studied the in vitro growth at different temperature of strains of *Listeria monocytogenes*, *Staphylococcus aureus*, *Staphylococcus pasteurii* and *Salmonella* spp., some of them being isolated from biltong. As usually observed, *Listeria monocytogenes* could grow at refrigeration temperatures of 4°C and drying temperatures (25 – 30°C) whilst *Staphylococcus aureus*, *Staphylococcus pasteurii* spp. and *Salmonella* spp. grow better at 25 – 37°C. Yeasts and moulds thrive in a wide range of temperatures with the ability to proliferate during low temperature storage (Cook, 1995). Therefore depending on the storage conditions, this will result in a varying microbial profile.

Regarding salt, Naidoo (2010) and Naidoo and Lindsay (2010b) indicate that, as not unexpected, some *S. aureus* strains showed growth at more than 20% even if at salt concentrations of ≥ 3% their production of enterotoxin is drastically reduced. Previous research showed that several *Staphylococcus* strains survive in high salt environments of ≥ 15% NaCl (Chesneau *et al.*, 1993; Ingham *et al.*, 2005) while McLean and others (1968) state that elevated salt concentrations (> 10%) may support the growth of enterotoxin-producing *Staphylococcus* strains but it may not favour the production of the enterotoxin as production is drastically reduced at salt concentrations of ≥ 3%.

Organic acids (brown spirit vinegar and apple cider vinegar) used in the biltong production process was inadequate for growth inhibition of all the strains even though acetic acid in vinegars are known to retain their bacteriostatic and bacteriocidal properties even at minimal concentrations (Entani *et al.*, 1998). These bacterio-static/ -cidal properties of vinegar are normally associated with the acetic acid concentration, NaCl content, temperature and number of viable cells present (Entani *et al.*, 1998; Marshall, 2003; Johnston & Gaas, 2006).

Literature has dealt much less with the microbial changes throughout the production process (Taylor, 1976) than the survival of inoculated pathogens after the biltong process. In an experimental work by Burnham and others (2008) whereby biltong was produced on a small scale, inoculated with pathogenic bacteria and dried for 17 – 26 days (20 - 22°C, 38 – 6% relative humidity), reductions of *Salmonella* between 2.0 – 4.2 log; *E. coli* between 2.0 – 4.4 log; *L. monocytogenes* between 1.0 – 4.0

log; and *S. aureus* between 0.9 – 2.6 log were seen. This study was conducted in order to see to what extent the biltong process would be in accordance to the standards set for jerky from the United States Department of Agriculture (USDA). The USDA requires processes to achieve 5 – 6.5 log reduction of *Salmonella serovars* and 2 – 5 log reduction in *E. coli* O157:H7 depending on the category of the product considered (cooked or shelf stable beef product). It is also recommended that the process includes a heating step (~ 71°C). Another study similar to this but with drying occurring for only 4 days at a temperature of 25°C resulted in a reduction of *L. monocytogenes* up to 4.6 log; and *S. aureus* up to 2.0 log (Naidoo & Lindsay, 2010c) from an initial final cell concentration of 7 log cfu.mL⁻¹.

SHELF-LIFE OF BILTONG

The microbiology of dried-cured meat products has been extensively studied (Rubio *et al.*, 2007). Dried meats, such as beef jerky (North America), charqui (South America) and Rou Gan (China), are all considered microbiologically stable snack foods at ambient temperatures due to their having a water activity of less than 0.90 (Leistner, 1987). The water activity limit of a meat product can however be increased up to 0.95 if the pH < 5.2 (Robertson, 2006). It is also important to try to maintain the water activity at ≤ 0.61 as to avoid yeast and mould growth (Betts & Everis, 2008).

Studies available explore the possibility of adding an antioxidant or preservative to the product in order to test whether it improves the shelf-life (Aksu & Kaya, 2005; Aksu *et al.*, 2005; Rubio *et al.*, 2007). However, for these studies, before the addition of the preservative/antioxidant within the products (pastirma and salchicon, dried salted meat products) and storing in modified atmosphere packaging, the products themselves have an inherent estimated shelf-life of six months, questioning the reason for adding antioxidants and/or preservatives (Aksu & Kaya, 2005; Aksu *et al.*, 2005; Rubio *et al.*, 2007). This also makes the comparison of these types of products with biltong difficult as there is no research reported for biltong shelf-life stability for periods of six months or longer. According to literature, there is no shelf-life period that has been given to biltong. Biltong companies in South Africa suggest/predict a three month shelf-life for biltong packages that have been unopened with a recommendation to store at refrigeration temperatures and consume within three days once opened (H. Fourie, 2015, Closwa Biltong, Namibia, personal communication, 18 August).

No study assesses the microbial growth during storage of biltong except a study in which ground biltong challenged with moulds isolated on commercial biltong did not allow for mould development during two months of storage at 25°C when it's a_w was 0.70 compared to ones having a_w of 0.80, 0.78 and 0.72 (Van der Riet, 1976b, Van der Riet, 1981 cited by Van der Riet, 1982). Unfortunately, the report of Van der Riet (1976b) does not state the salt content of the product or whether or not vinegar had been used.

Regarding the fairly high levels of spoilage microorganisms that biltong can contain, it would be of great interest to assess their growth throughout the production process and shelf-life on a commercial level. Moreover, despite the fact that they could influence this growth, the effect of various

factors, such as the extent of drying, salting/spicing technique used and the type of packaging as well as novel innovative technologies such as ultrasound is still an area lacking in research.

ULTRASOUND IN FOOD PROCESSING

The use of innovative technologies in food processing is becoming more widely used in the food industry for both economic and food safety reasons. Ultrasound is amongst a list of novel processing technologies that have been used in the food industry to improve food production processes and food quality (physicochemical and microbiological properties). These innovative technologies include high pressure technologies, thermal processing, pulsed electric fields and microwave technology. These have been used for processing, quality assessment and prolonged shelf-life/stability (Ashokkumar, 2015).

Ultrasound is one of the emerging technologies that are currently being investigated as a method of food processing. Many studies have been conducted on the use of ultrasound in the processing of fruits, vegetables and milk. Ultrasound techniques in food processing have mainly been used for quality assessment by the use of high frequency low power (Dolatowski *et al.*, 2007). Low- and high-intensity ultrasound has shown great potential as an application for the modification and characterisation of food properties in the food industry (Dolatowski *et al.*, 2007). The ultrasound process as a novel technology for the food industry is less energy-intensive than other techniques and technologies and therefore more environmentally friendly and cost-efficient (Piyasena *et al.*, 2003; Cárcel *et al.*, 2007). Ultrasonic analysis is based on the relationship between the physicochemical properties (composition, structure, physical state) and measurable ultrasonic properties (velocity, attenuation coefficient, impedance) of the food material (McClements, 1995).

Uses for ultrasound in the meat industry

Ultrasound in the meat industry could be useful for tenderisation, salting/curing of meats, drying and inactivation of microorganisms to name a few.

A combination of ultrasound with other common techniques used for meat tenderisation has been researched resulting in using ultrasound with a brining solution (Dolatowski, 1988) and ultrasound with meat tumbling (Dolatowski *et al.*, 1995), both of which caused a loss in myofibrillar structure via fragmentation and disintegration of cellular components, resulting in an improvement of yield, juiciness and tenderness of the meat. Mass transfer can be improved with ultrasound as it allows for greater penetration of solvent into cellular materials (Mason *et al.*, 1996; Dolatowski *et al.*, 2007), this is further assisted by microstreaming (Dolatowski *et al.*, 2007). A combination of ultrasound and drying (at low temperatures) could reduce oxidation and degradation of food materials. Research by Ensminger (1988) has shown that by using ultrasound in the drying process, “the heat transfer between a solid-heated surface and a liquid, is increased by approximately 30-60%”. Other research has indicated that by coupling non-thermal technologies (power ultrasound) with convective drying (low-temperature

drying) this could achieve higher yield, lower energy usage, a high product quality and processing safety (Garcia-Perez *et al.*, 2012).

Ultrasound in recent research is being used as a method of acceleration in food production, mainly in meat products. Studies have found that the use of ultrasound instead of injection and/or tumbling, speeds up the process of brining of pork (Cárcel *et al.*, 2007; Ozuna *et al.*, 2013; McDonnell *et al.*, 2014). Using ultrasound as a pre-treatment could have the same effect on the meat matrix as tumbling by distributing the salt evenly, enhancing salt diffusion as well as a tenderising effect (Mulet *et al.*, 2003; Cárcel *et al.*, 2007; Siró *et al.*, 2009). Cellular disruption would occur through cavitation resulting from high-shear force, pressure and temperature (Siró *et al.*, 2009). Research by Siró and others (2009) shows that the thickness of the muscle fibres increase significantly after ultrasound treatment (4 W.cm⁻²; 90 min), which can be attributed to the fact that sodium chloride (salt) causes swelling of muscle fibres which relates to the water binding- and water holding capacities of the meat (Offer & Trinick, 1983). Myofibrillar proteins may also swell due to cellular disruption which is caused by tumbling and ultrasonic treatment (Siró *et al.*, 2009). Ultrasonic treatment also influences the tissue microstructure due to cavitation. Cavitation disrupts the nebulin network resulting in altering of the protein membranes of myofibrils (Siró *et al.*, 2009). These theories support the notion that an ultrasonic pre-treatment could increase salt diffusion in meat and meat products.

In terms of salt diffusion, it was also proved that salt diffusion into porcine meat tissue followed an exponential pattern for static brining ultrasonic treatment and tumbling. According to Siró and others (2009), salt concentration increased in the first 30 min, due to the large concentration gradient between the meat tissue and brine, thereafter the diffusion rate decreasing. Ultrasound had increased salt diffusion compared to static brining but did not reach a salt equilibrium (which occurred in the samples tumbled for 180 min). Studies by Cárcel and others (2007) and Siró and others (2009) show that enhancement of salt diffusion is dependent on ultrasonic intensity and frequency. Ultrasound also influences mass transfer. Mechanisms involved in ultrasonic mass transfer include the asymmetric implosions of cavitation bubbles close to the meat surface creating microjets that disturb the boundary layer and therefore affect mass transfer and the creation of microchannels causing increased distance between muscle fibres (microstructural changes) supporting salt diffusion (Siró *et al.*, 2009). Even though these effects can be seen in the mentioned studies, it is important to note that meat has different characteristics and changes its physical properties during the curing process and therefore results could differ between studies. As mentioned, studies on ultrasound have been conducted on fresh meat (salting) but there have been no studies that have investigated the effect it may have on drying of this brined meat which would be of interest in the case of the biltong industry. A recent review on power ultrasound in meat processing (Alarcon-Rojo *et al.*, 2015) highlights that using ultrasound during the brining of meat is feasible. There is no mention of its effect on drying of brined meat as it has not yet been explored.

Microorganisms are inactivated/killed due to the thinning of cell membranes, localised heating and free radical production (Fellows, 2000) caused by power ultrasound cavitation and changes in pressures (Piyasena *et al.*, 2003). Research has shown that the effectiveness of ultrasound for microbial inactivation is dependent on the “targeted” microorganism (Piyasena *et al.*, 2003). For this

reason, it has been suggested that ultrasound be used in combination with either heat (thermosonication), pressure (manosonication) or both (manothermosonication), as this should result in more mechanical disruption of cells, in other words greater killing of the microorganisms (McClements, 1995). The effectiveness of the ultrasound treatment is also influenced by other factors such as amplitude of ultrasonic waves, exposure time, volume and composition of food and the treatment time and temperature (Piyasena *et al.*, 2003). *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. are some of the microorganisms which have been used/tested for inactivation by ultrasound. These microorganisms are found in meat and meat products so this could be a viable method for microbial inactivation. From a more general view, research by Alliger (1975) and Hülsen (1999) showed gram-positive (coccus-shaped) bacteria to be less susceptible than gram-negative (rod-shaped) bacteria. This could be due to the difference in morphology, with gram-positive bacteria having a thicker and more adherent layer of peptidoglycan than gram-negative bacteria influencing the mechanical disruption of the cells by ultrasound (Piyasena *et al.*, 2003). Research by Scherba and others (1991) however, showed there to be no difference in their resistance to ultrasound, proposing that the target of ultrasound might be the inner cytoplasmic membrane (consisting the lipobilayer) rather than the peptidoglycan layer. Interest lies in the use of ultrasound to inactivate microorganisms in the meat industry. Microorganisms of concern in meat and meat products, due to spoilage, include *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella* spp. which have been listed in the SANS 885 as pathogenic microorganisms that need to be tested for in biltong as they may be present.

With multiple studies being conducted on meat brining this could be a possible application in the biltong industry. It can be speculated from the results seen in previous studies that ultrasonic brining will assist in salting by replacing the tumbling technique, decreasing salting time and distribute salt more evenly. In addition, the use of ultrasound in this step could ultimately affect the subsequent drying step and the microbial stability after drying, however this avenue of research has not been reflected in literature.

REGULATIONS PERTAINING TO BILTONG AND EXPORT OPPORTUNITIES

The consumption of meat and meat products is common in nearly all cultures worldwide. Meat products have been developed all over the world by using various techniques and flavours which are typical to a country. Many countries produce meat products that have undergone a method of preservation (cured and dried) (Table 2.1). Biltong is no different in that it is a dried meat product that has an extended shelf-life due to its processing methods. The salt content and level of dryness are two factors which influence the characteristic/unique taste of biltong and its shelf-life. In 2015, Prof Melville Saayman conducted an exploratory research project whose aim was to determine the popularity and value of biltong within the South African economy which concluded that biltong contributes more than R2.5 billion to the South African economy (Cloete, 2015). The exporting of biltong would therefore be beneficial for the South African economy. Another step forward for the

biltong industry would be for it to be trademarked, which was done for the popular *Rooibos* tea in 2014. As biltong is a well-known, sought-after, South African commodity, this would be an appropriate next step.

Biltong is increasing in popularity internationally. The biltong industry has been trying to export biltong for many years but due to strict importing regulations this has been a bigger challenge than expected. Biltong is exported to a few countries (e.g. Namibia, Tanzania, Dubai, Abu Dhabi and Indian Ocean islands), which is just the beginning for the biltong industry (M. Nel, 2016, Cape Deli, Cape Town, personal communication, 23 November). However, major markets (United States of America, European Union and Australia) have still not approved the exportation of biltong. In recent years, import and export control has become stricter due to the prevalence of animal related diseases such as foot and mouth (FMD) and avian flu. Import and export control measures are enforced to ensure that health, environmental, safety and technical standards from domestic and international agreements are met. These controls differ according to the product, department and severity of risk of the product. This paper will describe the current regulations of biltong production in South Africa that are applicable in order for biltong to be approved for exporting, the export conditions and requirements for the major markets as a way forward for biltong producers and suppliers.

Current South African regulations for biltong

Biltong industry responsibilities

Biltong factories use sufficient drying systems however these are only temperature-controlled units and therefore this results in varying end-product characteristics. Challenges the biltong industry faces include determining the processing guidelines and the microbiological stability of the product as evidence of its shelf stability. There is legislation for the safety of the product which follows the regulations as laid out in South African National Standards (SANS) 885 for processed meat and meat products. This legislation dictates that it should have few spoilage microorganisms and minimal presence of pathogenic microorganisms. These regulations are used as a guideline for the estimated shelf-life however, with no research to support these. From an exporting view, biltong is considered a concern for other countries as biltong is a dried meat product that hasn't undergone any other form of processing (heating/cooking, smoking) to destroy microorganisms. In South Africa, biltong after drying is seen as a low risk product as the end product ideally has a low water activity and pH, and a high salt content. Apart from this, every country has their own guidelines for exported products and before approval of a product, these need to follow the standards as set by that country. All food product manufacturers in South Africa are advised to have a Hazard Analysis and Critical Control Points (HACCP) system in place. The development of a Food Safety System such as HACCP entails knowledge of the Codex Alimentarius; SANS 10330: 2007, Code of Practice for HACCP; SANS 10049:2012, Code of practice for Food Hygiene Management; and the Global Food Safety Initiative Guidance Document. As this is an international standard this HACCP plan should be in accordance to the other countries HACCP certifications. It has been suggested that factories have Food Safety System Certification 22000 (FSSC 22000) which is an internationally accepted certification scheme

(ISO-based), for auditing and certification of food safety in the whole supply chain. This certification includes that the prerequisite programs ISO 22000, PAS 220, and GMP's are in place. If biltong factories have these two certifications they are one step closer to being able to export their products. Therefore the product itself and the way it is produced is an explanation of why exporting is so difficult.

South African government responsibilities

The application for an export permit is challenging and requires much documentation. The export of meat products from South Africa follows a similar procedure as fresh meat products. The establishment who wants to export meat must be placed on the list of approved export establishments. This is done after an inspection of the establishment by the Department of Agriculture, Forestry and Fisheries, South Africa (DAFF) to make sure it complies with the necessary legislation. An import permit will then be granted with specifications for the exporter regarding evidence of animal health and public health specifications for the raw meat used and certifications of the factory. After which an acceptable health certificate and product specifications is negotiated between the countries (National Department of Agriculture & Directorate of Veterinary Public Health, 1997). Exportation will only be officially certified for establishments that comply with national legislation as well as all the requirements set by the importing country. A DAFF-certified factory must be audited annually so that their export permit may be renewed.

Major market import regulations

Most countries, to enable exporting of particular products, such as biltong, require specific certifications to be held by producers to make sure that the product standards are met and that the product is safe for consumption and shelf-stable. The country of export is responsible for meeting the conditions and requirements associated with the export of meat and meat products of the importing country. These standards differ between countries. The importing regulations for major markets will be briefly discussed.

United States of America importing guidelines

The biltong industry has had difficulty with importing to the United States as there are many steps in the process and the process validation tests become very expensive. In the United States it is illegal to produce and distribute meat products which have not been United States Department of Agriculture (USDA) approved. This approval states that a product is hygienic and safe for consumption. The process of biltong makes this approval difficult and in response, consumers need to make it themselves. Over the years, there has been an increase in the number of family-owned businesses whom produce biltong and sell it online, few of these have been USDA-approved. This not only occurs in USA but all around the world. However, biltong made and sold in other countries is not considered the same as traditional biltong produced in South Africa. It would therefore be beneficial for biltong manufacturers to proceed with this process for approval to export.

The United States acquires a valid meat inspection certificate, issued by an authorised official of the national government from South Africa, to allow for any importation of cured and dried meats

from South Africa. This certificate must accompany the product imported into the United States and include the country of origin; that the meat contains no bones, the meat was fresh (unfrozen, stored between 2 – 7°C) for at least three days immediately following the slaughter of the animals; the meat has been fully dried so that it is shelf stable without refrigeration and that the water-to-protein ratio of the product does not exceed 2.25 to 1 (USDA-FSIS, 2016).

The USDA, Food Safety Inspection Services (FSIS) are in charge of approving the food safety systems of food factories providing food products in the United States. According to their guidelines, any product that is considered high risk, the producer must provide a validation study that shows that the process used for their product (in this case drying) must be able to achieve an appropriate reduction of pathogens (a required \log_{10} reduction of the pathogen) throughout the product. This validation can be designed based on science and supported by experimental data, information from literature that is scientifically valid and/or by comparing different methods used from established/validated factories (USDA-FSIS, 2014).

This is also expected from imported products that are considered “high risk” which is the case for biltong (according to the United States). Biltong however only has a drying step (no heat) and this contributes to the flavour of traditional biltong. Therefore it is the responsibility of the biltong industry to conduct these validation studies to prove that the process used to produce biltong can result in this expected \log_{10} reduction. Before an import permit will be granted, along with these validation studies, the USDA will look at the processing conditions (temperature, relative humidity, time), list of ingredients (salt, spices and preservatives) and the critical product end-characteristics (water activity, pH, salt content and fat content). A record of the microbial stability of the shelf-life is also required. As biltong is often compared with beef jerky it would be suggested that biltong also follow the guideline of a water activity less than 0.85 as suggested by the USDA-FSIS (2014). This water activity is achievable as was seen in the studies of this dissertation that with a 50% weight loss, biltong had a water activity less than 0.85. The packaging and labelling regulations should also be followed according to the USDA guidelines. Once approved, biltong would be accepted for importation into the United States.

European Union importing guidelines

Exporting to Europe is considered a taxing process but once approved for the European Union (EU) this opens up the opportunities for export. Before applying for import certifications, the biltong factory must be DAFF-certified and a permit application must be approved by the South African authorities. For exporting to the EU a factory must be FSSC 2200-certified (in compliance with ISO 22000:2005 and PAS 220:2008) as previously mentioned.

The formal steps towards approval and eligibility for exporting meat and meat products from South Africa to the European Union include (FSSC, 2010):

1. The South African authority for exporting must submit a formal request to the Directorate General for Health and Consumer Protection of the European Commission to export meat products (biltong) to the EU which contains confirmation that the authority can fulfil all relevant legal provisions to satisfy EU requirements.

2. A questionnaire compiled by the Directorate-General for Health and Consumer Protection must be completed and returned and the residue monitoring plan of the exporting country must be submitted and approved.
3. After evaluation (and acceptance), an inspection by the Food and Veterinary Office is carried out to assess the facilities.
4. After the results of the inspection, the Directorate General for Health and Consumer Protection proposes the listing of the country, the specific conditions under which imports from that country will be authorised and the list of approved establishments in the country to the Member States.
5. If the Member States accept this proposal, the European Commission adopts the specific import conditions and an import permit is granted.

This process can take a few years but would be beneficial to the biltong industry due to its popularity in Europe. No formal requirements for the product itself such as a specified water activity or protein content could be sourced but this would be specified on the import permit.

Australian importing guidelines

Australian Export Control Regulations of the Export Control Act 1982 – Export Commodity Orders (Export Control Meat and Meat Products Order 2005) is applicable for export into Australia (DAFF, 2006). The regulations clearly set out the conditions and requirements for each product type. Food product exports must comply with the applicable food standard as set out in the Australia New Zealand Food Standards Code. For meat and meat products, Standard 2.2.1 states that dried meat must comply with a water activity of less than 0.85 and have no less than 160 g/kg meat protein on a fat free basis. This also includes Standard 4.2.3 (production and processing standard for meat) which incorporates the use of the Australian Standard AS-4696-2002, which is the standard for “Hygienic Production and Transportation of Meat and Meat Products for Human Consumption” and Standard 1.2.3 (labelling and naming of ingredients and compound ingredients) which lays out the requirement labelling regulations. Documentation of compliance with these regulations will allow for negotiation of the product to be imported by Australia (DAFF, 2014; ANZFS, 2015).

The South African markets are highly influenced by the international markets which includes the import and export of agricultural goods. Exporting products from South Africa plays a role in the profitability and competitiveness of the agriculture sector. The infrastructure of the biltong industry in South Africa will allow for these conditions and requirements to be met however the other challenge is negotiating opportunities with major market countries and meeting the evolving importing standards and certifications. One of the major constraints in exporting to the USA would seem to be providing evidence of sufficient log reduction of the bacteria whilst for the EU, the residue analyses would be a major cost (for the government) and seems to be the reason why South Africa’s authorities seem reluctant to explore these export markets.

CONCLUSION

The biltong industry have become a very economically important sector in the South African meat industry and any contribution toward the understanding of biltong production process can help to improve this sector. Limited research on South African biltong opens up various pathways for future research. Gaps in research/knowledge/legislation show that there is no legal definition for biltong and no guidance for the manufacturing of biltong (with specific defined properties in terms of drying parameters and times, final moisture contents and water activity and spice combinations to mention a few). This leads to a large variety of production processes and a wide spectrum of end-product characteristics impacting sensory properties and microbial stability while shelf-life has not been assessed even on a standard recipe.

The drive is always for consistency in the quality of biltong. Factors that seem to influence this include meat quality, processing (addition of vinegar, tumbling and overall handling of the meat, drying (parameters and time) and packaging. The drying parameters such as effective temperature, relative humidity and air velocity and time to dry the products efficiently and effectively is an area of research that would be valuable to biltong producers. These results could allow for a scientific profile (moisture content, water activity, drying yields, etc.) to be determined as to when the products could be removed from the driers for good quality and consistency. The addition of vinegar, due to its effect on pH, could be of major influence on the drying rate and shelf-life. The use of preservatives in the industry and their consumer acceptance can also be investigated. Other factors that could be considered include the use of fresh/frozen meat, salt and vinegar penetration (homogeneity) and the use of different muscles. Packaging is another area which is limited and research could involve its impact on the stability and shelf-life of biltong. These areas of research also apply to the use of game in biltong production with special attention to their lower fat content, higher pH and microbial load. Another area of interest would be to apply the use of ultrasound (a novel innovative technology) in order to improve biltong processing in terms of salting/drying time and microbial inactivation. An increase in the scientific knowledge on biltong will allow the industry to improve on its processes, be more informed on it's safety as well as to explore exporting opportunities

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CHAPTER 3

EFFECTS OF RAW MEAT TYPE AND THE ADDITION OF VINEGAR ON THE PHYSICOCHEMICAL PROPERTIES AND DRYING KINETICS OF BILTONG

ABSTRACT

The study objectives were to determine the influence of added vinegar during the production of beef biltong on the drying kinetics as well as to investigate the drying of biltong using different beef muscles, beef with subcutaneous fat and different meat species. Drying throughout the study was done using constant parameters – temperature $25 \pm 2^\circ\text{C}$, relative humidity $30 \pm 5\%$, air velocity 2 ± 0.2 m/s. Initially, the experimental drying of biltong was determined for different formulations (without or with vinegar) and final weight loss (50% and 65%). The addition of vinegar resulted in a higher weight gain (5.0 – 7.3%) during tumbling and a lower end product pH than samples with no added vinegar, but this did not influence the drying kinetics of the biltong and the decrease of water activity over the drying period. Regarding the effect of meat, the physicochemical results (moisture content, salt content, water activity and pH) showed that the fatty beef topside differed from the other meat treatments. The drying time also varied between species/muscles with the gemsbok meat having the highest drying rates and subsequently the shortest drying time to reach the targeted 65% weight loss. The beef topside with a layer of fat on the surface had a similar drying pattern to lean beef topside but the longest drying time due to its higher initial moisture content. Comparing the drying of the two beef muscles used (topside and silverside), these had different drying rates before 24 hours after which no differences were seen and both reached 65% weight loss after 96 hours. These results are an indication of the variation in drying times that can occur when different meats are used in biltong production.

INTRODUCTION

Biltong, a salted and dried meat snack food from South Africa, is often compared with other speciality dried meat products such as beef jerky (North America) and charqui (Brazil). It is made on a commercial level as well as by consumers in their own homes (Strydom & Zondagh, 2014). Biltong production is a very simple process. Meat is cut into desired sizes (traditionally long strips), salted/spiced, using a dry-salting method or acidic-liquid spice mix (Taylor, 1976; Van der Riet, 1982; CSIR, 2001; Naidoo & Lindsay, 2010a) and dried using moderate drying parameters – temperature between $20 - 30^\circ\text{C}$. Biltong can be made using a variety of meat sources such as beef, ostrich and game (Van Wyk, 2007; Naidoo & Lindsay, 2010b; Strydom & Zondagh, 2014). Muscles commonly used for biltong production include topside (*semimembranosus*), silverside (*biceps femoris*), thick flank (*rectus abdominus*), eye of round (*semitendinosus*) and fillets (*longissimus dorsi*). Topside is the preferred muscle choice (CSIR, 2001; Van Tonder & Van Heerden, 1992; Van Wyk, 2007) but there

has been no research to indicate if this is the best muscle. The effect that muscle choice has on the salting/spicing and drying of biltong would be of interest to the industry and consumers alike due to the intrinsic differences (fibre type, fibre diameters, etc.) in the physical and chemical composition between muscles within an animal. Dependent on consumer preference, biltong can either be lean (no fat on the outside) or fatty (a layer of fat on a surface) (Van der Riet, 1982). It has been stated that the meat be trimmed of excess fat as it may cause rancidity (Strydom & Zondagh, 2014) and may take longer to absorb salt (Van Tonder & Van Heerden, 1992, Heinz & Hautzinger, 2007); however, there is no scientific evidence to support this. There are a number of consumers who seem to prefer biltong with a layer of fat. Game and ostrich biltong are regularly used for lean biltong production as these meats seldom have subcutaneous fat. Salt is standard in biltong production, usually added at a 2.5% concentration in the formulation. Research has shown that this amount of salt results in an acceptable taste over a wide range of final moisture levels (Taylor, 1976; Van der Riet, 1982). Other spices added may include black pepper, coriander, brown sugar and vinegar (Van den Heever, 1970; Van der Riet, 1982; Strydom & Zondagh, 2014). Spice packs from biltong suppliers are also commonly used. The addition of vinegar is common at a commercial level, contributing to flavour as well as functioning to inhibit microbial growth. Studies have shown vinegar to be added at a level of ~3% (Van den Heever, 1970) and ~6% (Naidoo & Lindsay, 2010b). The salting/spicing step frequently involves the use of vacuum tumblers under commercial conditions. Tumbling under a low vacuum has been shown to accelerate salt diffusion (Krause *et al.*, 1978; Bedinghaus *et al.*, 1992) thus reducing the salting/spicing time substantially. It has been reported that drying rooms/cabinets with heating elements and electric blowers have been used for large-scale biltong production (Van der Riet, 1982). Nowadays, on an industrial level, temperature controlled drying chambers are used. Large biltong factories are interested in employing the use of temperature- and relative humidity-controlled drying chambers. Air temperature, air humidity and air velocity are important to consider when drying biltong (CSIR, 2001; Azam Ali, 2008; Ahmat *et al.* 2015). Drying studies have been conducted on the drying of meat and processed meat products (Trujillo *et al.*, 2007; Clemente *et al.*, 2009; Chabbouh *et al.*, 2011; Hii *et al.*, 2014; Ahmat *et al.*, 2015; Kucerova *et al.*, 2015; Petrova *et al.*, 2015) but on meat products with a smaller thickness than biltong, making the comparison difficult. The weight loss of biltong during drying is important to both the biltong producer (profits) and consumer (preferences). It is recognised by biltong producers that consumers have different preferences and therefore to accommodate for this, biltong is dried to between a 50% weight loss (moisture content ~50%) and ~70% weight loss (moisture content ~16%). Biltong can be characterised as being moist or dry depending on its weight loss during drying. Research has suggested that biltong with a moisture content greater than 40% is considered moist and biltong with a moisture content less than 40% is considered dry biltong (Nortje *et al.*, 2005).

In South Africa, there are no legal processing guidelines for biltong production and therefore the industry uses different parameters which results in variation in the product. This variability in biltong is due to the lack of information on its drying kinetics. Drying kinetics is influenced by various factors such as meat type, spices used, air conditions and level of drying to name a few. The aims of this study were to investigate the effect of the addition of vinegar and two weight losses (50% and 65%) on the physicochemical properties (moisture and salt content, water activity and pH) and drying kinetics of

beef biltong when dried in a controlled drying chamber with controlled air conditions (temperature, relative humidity, air velocity). A secondary study was conducted to investigate the effect of two beef muscles (topside, *semimembranosus* and silverside, *biceps femoris*), the presence of subcutaneous fat on beef and the use of different meat species (beef and *Oryx gazelle*) on the physicochemical properties and drying kinetics of biltong using the same controlled drying conditions.

MATERIALS AND METHODS

The study was divided into two trials, one for each aim. *Trial 1* involved producing lean beef biltong (topside) without and with vinegar in the salting/spicing formulation and drying to a 50% and 65% weight loss. In *Trial 2*, the biltong was produced using lean beef topside and silverside, beef topside with subcutaneous fat and lean gemsbok topside with the addition of vinegar and drying to a 65% weight loss. Each trial was conducted on three separate occasions (replicates). Table 3.1 indicates the meat used in *Trial 1* and the division of the meat treatment groups for *Trial 2*.

Table 3.1 Meat used for each treatment (*Trial 1* – beef biltong produced without and with vinegar and dried to 50% and 65% weight loss; *Trial 2* – biltong produced using different meats, with vinegar and dried to 65% weight loss)

Treatment	Meat 1	Meat 2
Trial 1	Lean beef topside	
Trial 2, Treatment 1	Lean beef topside	Lean beef silverside
Trial 2, Treatment 2	Lean beef topside	Fatty beef topside
Trial 2, Treatment 3	Lean beef topside	Lean gemsbok topside

Raw material

The raw material used in both trials included frozen lean beef topside (*semimembranosus*), frozen lean beef silverside (*biceps femoris*) and frozen beef topside with ~ 1.1 cm subcutaneous fat which were supplied by a local meat supplier (Beefcor Meat Suppliers, Okahandja, Namibia). Frozen lean gemsbok (*Oryx gazelle*) topside was supplied from a local game farmer (Okahandja, Namibia).

Biltong production process

Trial 1 – Beef biltong produced without and with vinegar and dried to 50% and 65% weight loss

Pieces were cut from 24 beef topside muscles. The 44 pieces per replicate weighed approximately 172.6 g (± 21.1) each. Four separate treatments (11 pieces each; ± 1.9 kg) were made – two treatments without vinegar (C = control, no vinegar) and two treatments with vinegar (V = vinegar). One treatment from each (C, V) was dried for a targeted 50% weight loss (WL 50), and the other for a targeted 65% weight loss (WL 65).

Trial 2 – Biltong produced using different meats, with vinegar and dried to 65% weight loss

Pieces were cut from 15 beef topside muscles and 3 muscles each for beef silverside, fatty beef topside and gemsbok topside. The 42 pieces per replicate weighed approximately 108.4 g (± 4.3) each. Each treatment (Table 3.1) was processed at the same time. All meat treatments (7 pieces each; ± 758.8 g) were made with vinegar and dried for a targeted 65% weight loss (WL 65).

Production process

The selected meat, depending of the trial, was thawed at 4°C for 48 hours before use. Per replicate, the meat was cut parallel to the fibres, approximately 20 mm x 20 mm thick and 260 mm long. After which each piece was individually labelled and weighed. For each treatment (described above), the meat pieces were mixed with a formulation of 2% fine salt (Country Spices, Windhoek, Namibia), 0.2% course black pepper (Freddy Hirsch, Maitland, Cape Town) and if vinegar was to be added, 5% brown spirit vinegar of a 10% (w/v) acidity (Freddy Hirsch, Maitland, Cape Town) and tumbled in a vacuum-tumbler (Freddy Hirsch Vacuum Tumbler, Freddy Hirsch, Maitland, Cape Town) with 912 mbar vacuum for 20 min at 15 rpm. After tumbling, each piece was weighed and hung in a controlled drying chamber (Closwa Biltong, Okahandja, Namibia). The drying chamber parameters were maintained at a temperature of $25 \pm 2^\circ\text{C}$, a relative humidity of $30 \pm 5\%$ and an air velocity of 2 ± 0.2 m/s. The biltong pieces were dried until the targeted weight loss (based on the weights after tumbling) were reached. Each individual meat piece from each treatment was weighed intermittently during drying (due to access limitations of the factory in which this trial was conducted, weighing was done every 6 hours during the day and after 12 hours overnight), one piece of biltong being designated for the measuring of water activity for *Trial 1* (see section on *Water activity determination*). After drying, each piece was weighed and stored individually under vacuum in double-layered polyethylene terephthalate (PET) packages until analysis.

Sample preparation for analyses

For the raw material, a sub-sample was taken from off cuts of the leftover of each of the whole muscles and used for moisture content analysis. The raw meat sample taken for the fatty beef topside included the fat, with a 2:1 meat to fat ratio as this was deemed to be representative of the ratio in the meat pieces. For *Trial 1*, the piece of biltong designated for water activity determination during drying was sampled by cutting a piece from the bottom of the biltong piece. For both *Trial 1* and *Trial 2*, within 30 min after drying was complete, the rest of the biltong was sampled for further analyses. Each sample was sliced and sub-samples were taken for determination of moisture and salt content and these sub-samples were kept frozen at -18°C until analyses, after being thawed at 4°C for ± 2 hours. The remaining sample was immediately tested for water activity and pH determination. Before analyses the samples were homogenised in a Knifetec™ 1095 Mill (FOSS, Höganäs, Sweden) for 90 sec to ensure a representative homogenous sample was analysed for all analyses.

Moisture content analysis

Moisture content was determined using Method 934.01 according to the AOAC (2002). All analyses were performed in duplicate.

Salt content determination

Salt content was determined as described by Goli *et al.* (2012) by measuring the chloride concentration (mg Cl-/L) using a Model 926 chloride analyser (Sherwood Scientific, Cambridge, UK) after extraction of chloride ions in 0.3M nitric acid for 2 hours. All analyses were performed in duplicate.

Water activity determination

The water activity was measured using an Aqualab pa_wkit, portable water activity measurement system (Decagon Devices Inc., Washington, USA) at ambient temperature (around 25°C). All analyses were performed in duplicate.

pH determination

The pH measurement was taken using a calibrated portable Crison PH25 pH meter with a glass electrode (with an automatic paired temperature reading) (Lasec (Pty) Ltd, Cape Town, South Africa) after homogenisation of 3 g sample in 27 ml distilled water. Measurements were taken in duplicate.

Drying curves

The moisture content dry basis (MC_{db}) as a function of time was calculated from the final moisture content and the weight of the meat pieces followed during drying. For each time, equation (1) was used to calculate the drying rates.

$$(1) \frac{dMC}{dt}(t) = \frac{MC_{(t-1)} - MC_t}{dt}$$

Drying curves, $MC_{db} = f(t)$ were fitted using an exponential decay rate function (2);

$$(2) MC_{db} = b_0 + (b_1 - b_0) \times (1 - \text{decay rate constant})^t$$

The b_0 and b_1 are the lower and upper parameter estimates, *decay rate constant* is a dimensionless number which indicates the steepness of the decline of the data and t is the drying time in hours (x-axis).

Statistical analysis

Means (weight gains during tumbling between treatments, raw meat moisture contents, moisture contents and drying rates of different meats for each drying time and for the different drying times for

the same meat in *Trial 2*) were compared using one way ANOVA. To test the effects of treatment combination [*Trial 1*: formulation (C = control, no vinegar; V = vinegar) and weight loss; *Trial 2*: lean beef topside, lean beef silverside, fatty beef topside, lean gemsbok topside] on the various measurements, mixed model repeated measures ANOVA were used. The stage effect was the within subject repeated effect. For the mixed model, treatments were treated as fixed effects and the batches to which the treatments (formulation and weight loss) were applied, as a random effect. The Fisher LSD post hoc test was used to further analyse significant differences when the main effects/interaction effects were significant ($p \leq 0.05$). A two-way ANOVA was also used to test the effects of formulation and drying time on moisture contents and drying rates. The Fisher LSD post hoc test was used to further analyse significant differences when the main effects/interaction effects were significant ($p \leq 0.05$). Statistical analyses were done using the VEPAC module for mixed models and ANOVA of *Statistica 64*. One way ANOVA of XLSTAT® statistical software (Version 2014.2.03, Addinssoft, New York, USA) were also used.

RESULTS

The objective for *Trial 1* was to determine whether the addition of vinegar to beef during salting influenced its drying kinetics. In *Trial 2* the objective was to determine the drying kinetics of different meats. For *Trial 2*, the lean beef topside results for each treatment and replicate were pooled as they show no significant differences between the treatments.

Weight gain during tumbling

Analysis of the weight gain during tumbling (Table 3.2) gives an indication of the salt and/or vinegar uptake by the meat.

Table 3.2 Means (\pm SD) for the weight of meat pieces before processing (raw), after tumbling and the weight gain for each treatment (*Trial 1* – beef biltong, without and with vinegar and dried to 50% and 65% weight loss; *Trial 2* – biltong using different meats, with vinegar and dried to 65% weight loss)

Treatment	Meat pieces weight before processing (g)	Meat pieces weight after tumbling (g)	Weight gain (%)
<i>Trial 1</i>			
WL 50, No Vinegar (n = 33)	174.2 \pm 22.33	177.5 \pm 22.50	1.9 ^b \pm 0.72
WL 65, No Vinegar (n = 33)	177.8 \pm 21.37	180.4 \pm 21.60	1.5 ^b \pm 0.97
WL 50, Vinegar (n = 33)	162.8 \pm 22.01	169.9 \pm 23.81	4.2 ^a \pm 1.85
WL 65, Vinegar (n = 33)	175.9 \pm 18.62	182.8 \pm 19.07	3.8 ^a \pm 1.47
<i>Trial 2</i>			
Lean beef topside (n = 63)	98.8 \pm 1.45	105.1 \pm 1.54	6.0 ^{ab} \pm 1.68
Lean beef silverside (n = 21)	107.8 \pm 8.37	113.5 \pm 8.03	5.0 ^b \pm 1.68
Fatty beef topside (n = 21)	131.1 \pm 4.76	141.3 \pm 6.58	7.3 ^a \pm 3.40
Lean gemsbok topside (n = 21)	95.9 \pm 2.70	103.3 \pm 2.58	7.2 ^a \pm 2.88

^{a,b,c} Means for each trial, within a column with different superscripts differ significantly ($p \leq 0.05$)

As expected, the samples with the vinegar had a higher ($p < 0.0001$) weight gain (~4%) than the ~2% weight gain for the samples without in *Trial 1*. The silverside muscle (*Trial 2*) showed the lowest weight gain (5.0%) although it did not differ ($p = 0.214$) from the weight gain of the lean beef topside (6.0%). The weight gain for the lean gemsbok topside (7.2%), fatty beef topside (7.3%) and lean beef topside (6.0%) were not significantly different ($p > 0.05$) from each other.

Raw meat physicochemical properties

The raw meat used for biltong production was measured for moisture content before mixing with salt and other ingredients (Table 3.3). These results indicate any differences in the initial raw material which could influence the drying.

Table 3.3 Moisture content (means \pm SD) of raw meat

Treatment	MC _{wb} (g/100 g)
<i>Trial 1</i>	
Lean beef topside (n = 15)	73.8 ^{ab} \pm 2.28
<i>Trial 2</i>	
Lean beef topside (n = 9)	72.8 ^b \pm 1.76
Lean beef silverside (n = 3)	71.3 ^{bc} \pm 2.00
Fatty beef topside (n = 3)	70.8 ^c \pm 1.52
Lean gemsbok topside (n = 3)	74.9 ^a \pm 1.10

wb = wet, as is basis, MC = moisture content

^{a, b, c} Means with different letters within a column differ significantly ($p \leq 0.05$)

The raw lean beef topside used in *Trial 1* had an average moisture content of 73.8 g/100 g (\pm 2.28) which was similar ($p = 0.765$) to the lean beef topside used in *Trial 2* (72.8 \pm 1.76 g/100 g). The fatty beef topside had the lowest moisture content (70.8 g/100 g). The lean gemsbok topside (74.9 g/100 g) had a higher ($p \leq 0.05$) moisture content than the lean beef muscles (in both *Trial 1* and 2). The lean beef muscles did not differ ($p > 0.05$) from each other as pertaining to initial moisture content.

Biltong physicochemical properties after drying

The physicochemical profile of biltong was determined after drying. The biltong was taken out the drying chamber after each treatment had reached the targeted weight loss. Table 3.4 shows the means of the moisture and salt content, water activity and pH for each treatment after drying for *Trial 1* and *Trial 2*.

Table 3.4 Physicochemical properties (means \pm SD) of biltong after drying (*Trial 1* – beef biltong, without and with vinegar and dried to 50% and 65% weight loss; *Trial 2* – biltong using different meats, with vinegar and dried to 65% weight loss)

Treatment	MC _{wb} (g/100 g)	SC _{db} (g/100 g)	a _w	pH
<i>Trial 1</i>				
WL 50, No Vinegar (n = 33)	50.7 ^a \pm 1.02	7.59 ^a \pm 0.37	0.86 ^a \pm 0.03	5.57 ^a \pm 0.04
WL 65, No Vinegar (n= 33)	32.2 ^b \pm 1.81	7.70 ^a \pm 0.41	0.77 ^b \pm 0.04	5.56 ^a \pm 0.05
WL 50, Vinegar (n= 33)	51.2 ^a \pm 1.54	6.25 ^b \pm 0.27	0.85 ^a \pm 0.03	4.89 ^b \pm 0.05
WL 65, Vinegar (n = 33)	32.0 ^b \pm 1.41	6.38 ^b \pm 0.48	0.78 ^b \pm 0.03	4.89 ^b \pm 0.05
<i>Trial 2</i>				
Lean beef topside (n = 63)	31.2 ^b \pm 0.46	5.94 ^a \pm 0.05	0.77 ^b \pm 0.02	4.94 ^b \pm 0.04
Lean beef silverside (n = 21)	31.5 ^b \pm 0.46	4.27 ^c \pm 0.07	0.79 ^b \pm 0.01	4.96 ^b \pm 0.01
Fatty beef topside (n = 21)	33.5 ^a \pm 0.20	4.66 ^b \pm 0.06	0.82 ^a \pm 0.01	5.00 ^{ab} \pm 0.01
Lean gemsbok topside (n = 21)	31.5 ^b \pm 0.24	4.55 ^b \pm 0.14	0.78 ^b \pm 0.02	5.05 ^a \pm 0.04

wb = wet, as is basis, db = dry matter basis;

MC = moisture content, SC = salt content, a_w = water activity, WL = weight loss

^{a,b,c} Means for each trial, with different letters within a column differ significantly ($p \leq 0.05$)

Results of *Trial 1* regarding the effects of the addition of vinegar and the degree of drying on physicochemical properties of biltong after drying will be discussed in detail in the following chapter (Chapter 4) as the effects are the same. It should however be noted that contrary to Chapter 4 in which it was noted that salt content expressed in dry basis was higher when vinegar was added, but only for the biltong dried to 65% weight loss, it seems here that salt content is lower when vinegar is added.

In *Trial 2*, biltong dried to a 65% weight loss resulted in a 30% moisture content as in *Trial 1* except the fatty beef topside which had a higher moisture content (33.5 g/100 g) than the other meats, although its initial moisture content was lower (70.8 g/100 g; Table 3.3). The lean beef topside had the highest salt content (5.94 g/100 g) while the lean beef silverside had the lowest (4.27 g/100 g). There is no difference ($p = 0.412$) between the salt content of the fatty beef topside (4.66 g/100 g) and lean gemsbok topside (4.55 g/100 g). The biltong dried to a weight loss of 65%, as in *Trial 1*, had a water activity ≤ 0.79 except for the fatty beef topside ($a_w = 0.82$) which was significantly higher ($p < 0.0001$). Lean beef muscles had a pH ≤ 4.96 , a value similar to the biltong made with vinegar in *Trial 1*. It was observed that the lean gemsbok topside had the highest pH (5.05), although it was not significantly different ($p < 0.0001$) from that of the fatty beef topside.

Experimental drying kinetics

Trial 1 – Beef biltong produced without and with vinegar

The objective of *Trial 1* was to determine the effect of adding vinegar on the drying kinetics of beef biltong. The evolution of the weight loss (WL) and water activity (a_w) of beef biltong during drying when produced without and with vinegar are seen in Figure 3.1.

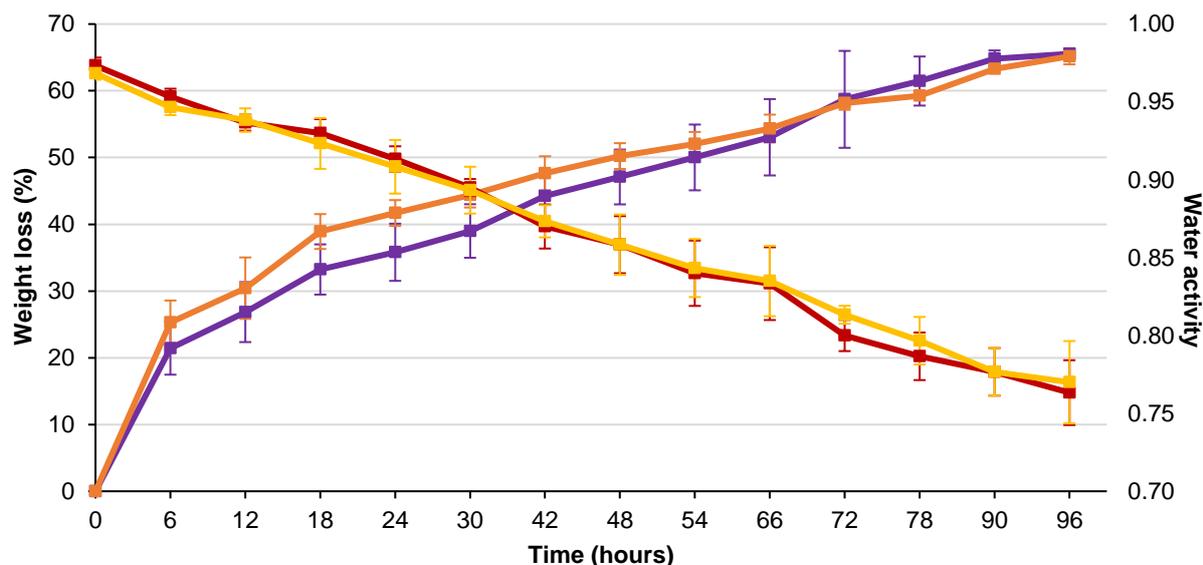


Figure 3.1 Experimental weight loss for each formulation (no vinegar and vinegar) (50% n = 60; 65% n = 30) of beef biltong and its corresponding water activity (50% n = 6, 65% n = 3) over a 96 hour drying time.

—■— WL No Vinegar —■— WL Vinegar —■— aW No Vinegar —■— aW Vinegar

The weight loss curves show that the targeted 50% and 65% weight loss were reached at the same time regardless of whether vinegar was used in the formulation or not. The water activities of the biltong which decreased steadily during drying showed no differences ($p > 0.05$) whether vinegar was added or not.

The p-values for the effects of treatments (formulation/meats), time and their interaction on the moisture content (g/g dry matter) of *Trial 1* are shown in Table 3.5. The interaction noted is expected due to the nature of the experimental design where time was one of the main effects.

Table 3.5 p-values for effects of treatment (*Trial 1* – without and with vinegar; *Trial 2* – different meats) and time (0 – 96 hours) on the moisture content (dry basis, db)

	<i>Trial 1</i>	<i>Trial 2</i>
Treatment	0.103	0.000
Time	0.000	0.000
Treatment*Time	0.000	0.000

Figures 3.2 and 3.3 show the drying curves of the moisture content (g/g dry matter) and the drying rate (dMC/dt) respectively as a function of the drying time for both formulations (no vinegar, vinegar). As the interaction between formulation and drying time was significant (Table 3.5), drying curves for each formulation (no vinegar, vinegar) are represented.

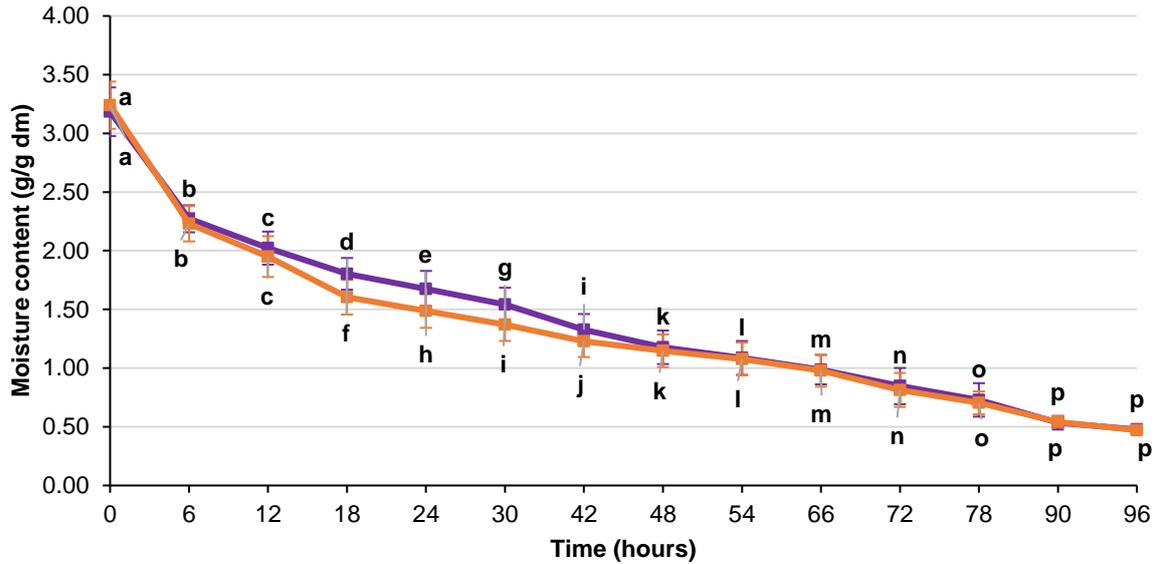


Figure 3.2 Drying curve representing the moisture content on a dry basis (dm) as a function of time for beef biltong produced without and with vinegar.

—■— No Vinegar —■— Vinegar

^{a-p} Means with different letters are significantly different ($p \leq 0.05$)

Initially the moisture contents (db) of the biltong with different treatments (no vinegar, vinegar) did not differ significantly ($p = 0.985$), however differences were seen between 18 and 42 hours of drying ($p \leq 0.05$). For both formulations, after 90 hours the moisture content remains equal to around 0.5 g/g dm (33% wb) indicating that drying could have been stopped at this time point.

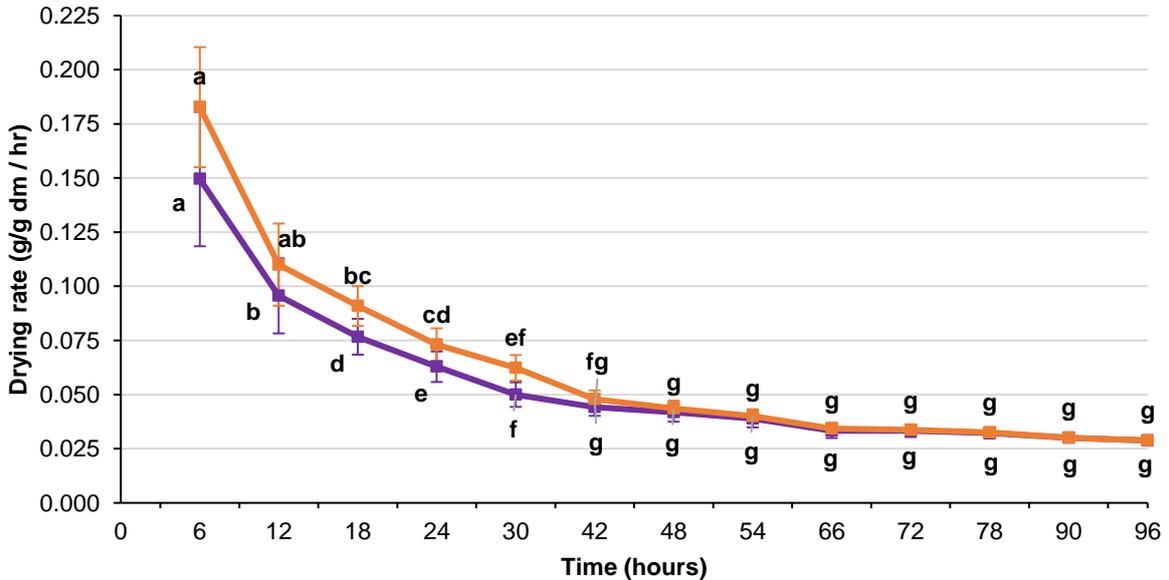


Figure 3.3 Drying curve representing the drying rate as a function of time for beef biltong produced without and with vinegar.

—■— No Vinegar —■— Vinegar

^{a-g} Means with different letters are significantly different ($p \leq 0.05$)

Drying rates decreased from the beginning and were not significantly different ($p > 0.05$) per time point between treatments except between 18 – 30 hours, where the drying rates were higher when vinegar was added which is in accordance with the lower moisture content of the biltong at the same times (Fig. 3.2).

Trial 2 – Biltong produced using different meats

The objective of *Trial 2* was to determine the effect of different meat treatments (lean beef topside, lean beef silverside, fatty beef topside and lean gemsbok topside) on the drying kinetics of biltong. Figure 3.4 shows the evolution of the weight loss (WL) of the biltong made with different meats during drying under similar conditions.

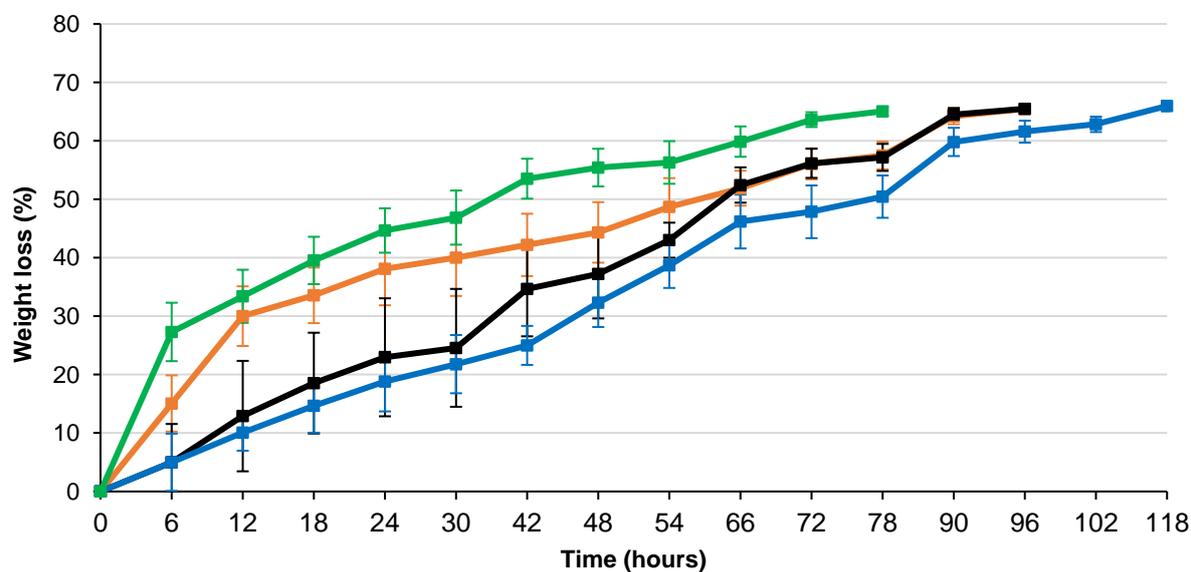


Figure 3.4 Experimental weight loss of biltong produced using different meat muscles, beef with subcutaneous fat and different species ($p < 0.0001$).

— Lean beef topside (n = 63) — Lean beef silverside (n = 21) — Fatty beef topside (n = 21) — Lean gemsbok topside (n = 21)

The weight losses show that each meat treatment reached the targeted 65% weight loss at different times. The lean gemsbok topside took the shortest time (± 78 hours) to dry to this weight loss whilst the lean beef topside and lean beef silverside reach a 65% weight loss at the same time (± 96 hours). The fatty beef topside took the longest time (± 118 hours) to reach the 65% targeted weight loss.

The meat effect ($p = 0.000$) and interaction between meat and time ($p = 0.000$) in *Trial 2* were significant (Table 3.5) so drying curves for each meat were represented and the meat effect at each time point was considered. Figures 3.5 and 3.6 show the drying kinetics for the different meats plotting moisture content (g/g dry matter) and drying rate respectively, against drying time. Lean beef topside showed the same evolution of weight loss during drying (Fig. 3.1) and drying curves (Fig. 3.2 and 3.3) as seen in *Trial 1*.

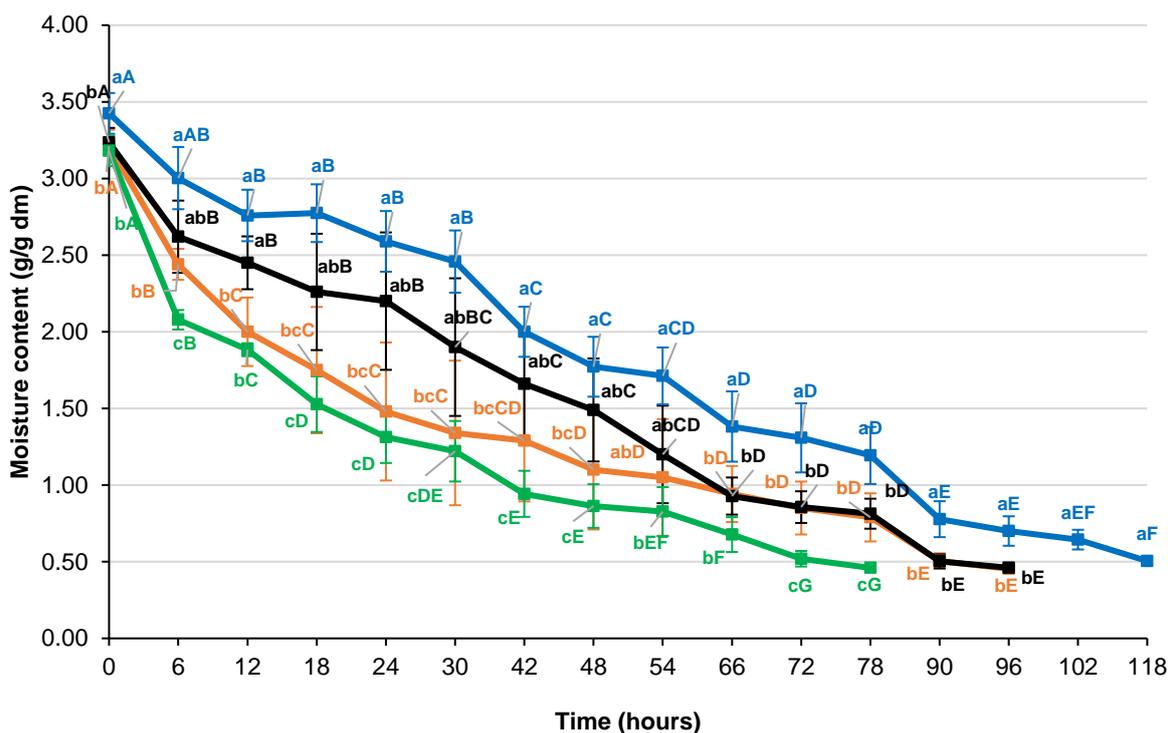


Figure 3.5 Drying curve representing moisture content on a dry basis (dm) as a function of time for biltong produced using different meats.

— Lean beef topside — Lean beef silverside — Fatty beef topside — Lean gemsbok topside

a, b, c Means with different lowercase letters differ significantly ($p \leq 0.05$) between meat treatments at that specific time point

A–F Means with different capital letters differ significantly ($p \leq 0.05$) within a meat treatment over drying period (time)

The fatty beef topside had a significantly higher ($p \leq 0.05$) initial moisture content (3.48 g/g dry matter) than the other meat treatments which is not in accordance with its raw meat moisture content (70.8 g/100 g; Table 3.2) and taking into account that its weight gain (7.3%) was the same as for gemsbok (7.2%) (Table 3.3). Lean beef topside and silverside reached the same moisture content (1.0 g/g dm; 50% moisture content, wet basis) after 66 hours ($p = 0.990$). It was also seen that after 90 hours the moisture content (0.50 g/g dm; 33% moisture content, wet basis) did not change for the topside and silverside muscles and from 72 hours for the gemsbok topside showing that the drying could have been terminated at these respective time points. The moisture content of lean gemsbok topside differed significantly from fatty beef topside for each of the sampling times ($p \leq 0.05$) during the drying. The drying curve of fatty beef topside and beef silverside do not differ significantly.

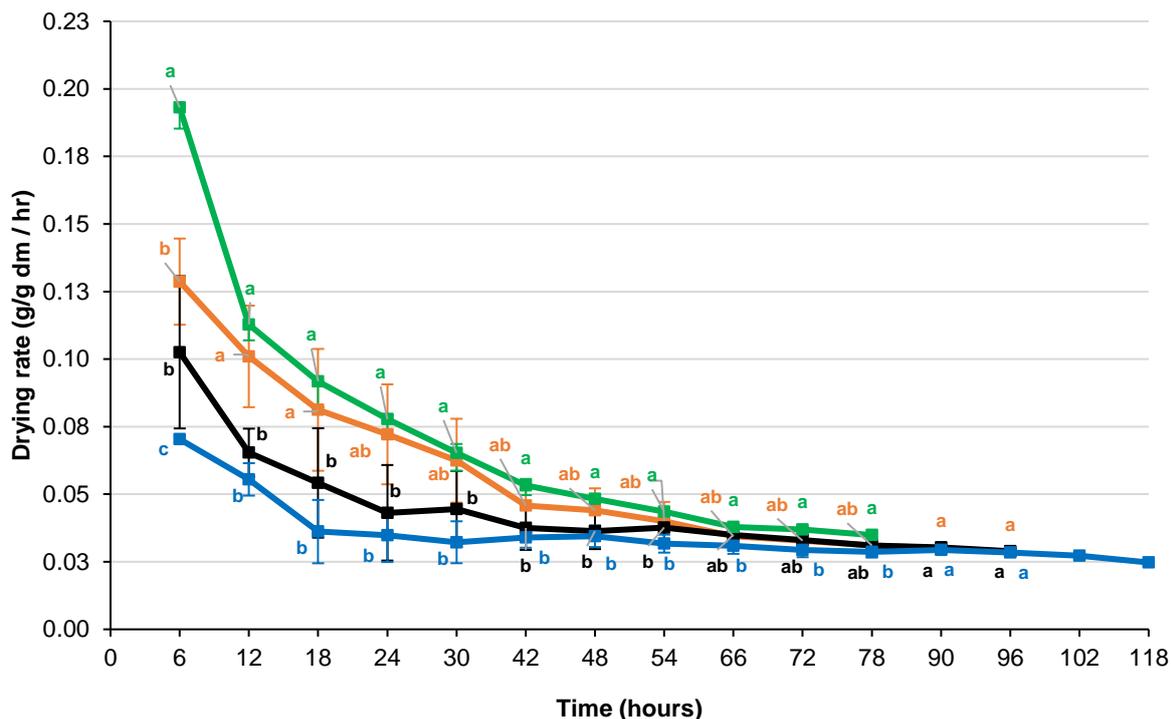


Figure 3.6 Drying curve representing the drying rate as a function of time for biltong produced using different meats.

— Lean beef topside — Lean beef silverside — Fatty beef topside — Lean gemsbok topside

a, b, c Means with different lowercase letters differ significantly ($p \leq 0.05$) between meat treatments at that specific time point

For the drying rates (Fig. 3.6), only the differences between meats at a time are shown. The lean gemsbok topside had a significantly higher ($p < 0.0001$) initial drying rate than the other meats but slows to the same drying rates as the lean beef topside after the first 12 hours. Lean beef topside shows similar drying rates to lean beef silverside from 24 hours onwards. Fatty beef topside initially has a slower drying rate which is relatively constant and similar to lean beef silverside from 12 hours and both lean beef silverside and beef lean topside from 24 hours.

Drying curve fitting

The moisture content (g/g dry matter) as a function of time data were fitted to equation 2. Table 3.6 summarises the estimated parameters of the equation. The coefficient of determination (R^2) represents the quality and accuracy of the fitted model.

In *Trial 1*, both the lower (b_0) and upper (b_1) parameter estimates did not differ ($p > 0.05$) from one another. The upper parameter estimates and the decay rate constant indicate a difference ($p \leq 0.05$) between the fatty beef topside and the lean gemsbok topside. The fatty beef topside and lean beef silverside do not have different parameters.

Table 3.6 Parameters of the exponential decay function equation fitted to the drying curves (moisture content) (*Trial 1* – beef biltong produced without and with vinegar and dried to 50% and 65% weight loss; *Trial 2* – biltong produced using different meats, with vinegar and dried to 65% weight loss)

Treatment	b₀, lower parameter (g/g dm)	b₁, upper parameter (g/g dm)	Decay rate constant	R²
<i>Trial 1</i>				
No Vinegar	0.48 ^a	3.18 ^a	0.042 ^a	0.97
Vinegar	0.52 ^a	3.24 ^a	0.048 ^a	0.97
<i>Trial 2</i>				
Lean beef topside	0.45 ^a	3.21 ^b	0.056 ^{ab}	0.90
Lean beef silverside	0.46 ^a	3.24 ^b	0.045 ^{bc}	0.86
Fatty beef topside	0.49 ^a	3.42 ^a	0.031 ^c	0.74
Lean gemsbok topside	0.46 ^a	3.18 ^b	0.072 ^a	0.97

b₀ = lower parameters is the moisture content when the drying reaches a plateau (begins to slow/stop)

b₁ = upper parameter estimates represent the upper parameters (initial moisture content)

^{a, b, c} Means for each trial, within a column with different superscripts differ significantly ($p \leq 0.05$)

DISCUSSION

Weight gain during tumbling

The weight gain indicates the salt/spice and/or vinegar uptake (Table 3.2). In *Trial 1* the weight gain is higher in treatments with the addition of vinegar. It can be speculated this higher weight gain is due to the vinegar (acid), which denatures the surface proteins of the meat allowing the vinegar to penetrate the meat faster (Capaccioni *et al.*, 2011) subsequently adding weight. The vinegar also lowers the pH which decreases the water holding capacity (Ruiz & Pérez-Palacios, 2015) resulting in less salt uptake. However, this theory could not be confirmed as the salt content was not determined after tumbling and the level of salt that had penetrated the meat could not be determined. Further analyses would be necessary. In *Trial 1*, the addition of vinegar to lean beef topside resulted in a weight gain of ~ 4%. The average weight gain for *Trial 2*, was ~ 6.3% indicating a higher absorption of the vinegar and spices. This could be due to the fact that there were less pieces tumbled at a time in *Trial 2*, which could affect the penetration of salt and vinegar. The fatty beef and lean gemsbok were both made using the topside muscle and no differences between these treatments and lean beef topside is observed therefore the muscle characteristic (lean, fat or species) does not play a role as pertaining to weight gain when limited volumes of spices and vinegar are added.

Physicochemical properties

The moisture content of the raw lean beef topside used in *Trial 1* was similar to the lean beef topside used in *Trial 2* (~ 73 g/100 g) (Table 3.3). The low moisture content of the fatty beef topside (Table 3.3)

is due to the presence of fat as the more fat in a sample, the lower the initial moisture content. However, the moisture content after drying is not in accordance with this result and this could be due to the accuracy of the sampling and therefore not representative of the fatty beef topside used. This could be explained by the difference in moisture content between muscle (dried or wet) and that found in fat and the assumption that the rates of moisture loss will differ between these two components. The latter warrants further research in an attempt to test this hypothesis.

The moisture contents of biltong (Table 3.4) after drying of *Trial 1* with two different weight losses resulted in biltong with moisture content of ~ 50% and ~ 30%, respectively. In *Trial 2*, all treatments were dried to a 65% weight loss and therefore, as expected, had a final dried moisture content of approximately 30%, similar to that seen in *Trial 1*. The low standard deviations of the moisture contents indicate that the drying to a targeted weight loss was relatively constant between pieces and replicates as expected due to the use of a drying chamber and the fact that the dimensions (surface area to volume ratio) of the various pieces were similar. It is observed in *Trial 1* that the salt content (dry basis), was influenced by the addition of vinegar. This could be attributed to the high weight gain during tumbling (Table 3.2). However, analyses of salt content after tumbling is needed to confirm this as previously mentioned. In *Trial 2*, the salt content was highest in the lean beef topside and differed from the other treatments. This suggests that the salt uptake varies between muscle type, species and presence of fat. The large difference between the salt content of the different muscles could be due to the meat structure which influences the penetration of salt. Further investigation research needs to be conducted to confirm this. However, the rate of salt penetration into a muscle is regulated by the dissolution of salt on the meat surface, this is dependent on the muscle or meat type (Sørheim and Gumpen 1986). Research on dried and salted ham products indicates that the salt uptake was affected by muscle structure. Zochowska-Kujawska (2016) showed the *biceps femoris*, *semimembranosus* and *longissimus lumborum* to have varying salt contents after curing in relations to their percentage of red and white muscles fibres, whilst a study by Gou *et al.* (2002) indicated differences in salt uptake to be due to fibre direction. The salt content in the fatty beef topside is lower than the lean beef topside which indicates that the fat may affect the salt absorption as has been previously noted (Van Tonder & Van Heerden, 1992, Heinz & Hautzinger, 2007). Fat influences the absorption of salt is lower due to the decrease water diffusivity. This result was seen in an early study on pepperoni whereby the presence of fat resulted in a lower salt content due to the water diffusivity of the product (Palumbo *et al.* (1977).

The water activity of a selection of commercial samples has been recorded to vary between 0.54 – 0.93 (Van der Riet, 1976; Van der Riet, 1982; Osterhoff & Leistner, 1984; Petit *et al.*, 2014) in accordance with the values of this study. The different water activities between the two weight losses of the biltong show that the addition of vinegar (tested in *Trial 1*) did not influence the water activity. Between the different meat treatments (*Trial 2*), there were no differences between the water activity of lean beef topside, lean beef silverside and lean gemsbok topside. There is however a difference between lean beef topside and fatty beef topside which relates to the higher final moisture content of the fatty beef topside after drying. This could affect the microbial stability (spoilage and pathogenic

microorganisms) of biltong and therefore its shelf-life. Across both trials, the biltong with no added vinegar had a higher pH than the biltong with added vinegar.

A lower pH decreases the water holding capacity of the meat (Ruiz & Pérez-Palacios, 2015) which could result in faster moisture loss and therefore influence the drying rate (increased). The pH of the lean beef topside used in *Trial 2* was within the same range as the pH of the biltong with added vinegar in *Trial 1*. There was no difference in pH between the different beef muscles (topside and silverside). The pH of biltong made from fatty beef topside is no different from any of the other treatments. This suggests that the vinegar uptake during tumbling did not differ between treatments and the fat does not play a role in vinegar absorption as it may with salt absorption as observed. Further research is necessary to confirm this theory. Lean gemsbok biltong has a pH of 5.05 which is higher than the pH of the different muscles. This is due to the higher initial pH (5.96) of the gemsbok meat and therefore the difference was expected.

Experimental drying kinetics

No research has been previously conducted on how the addition of vinegar may affect the drying of biltong and the corresponding decrease in moisture content and water activity over the drying time. As the drying conditions were maintained constant throughout the drying of the biltong, the addition of vinegar was the only factor that may have influenced the drying dynamics. The addition of vinegar did not have an impact on the drying time of beef topside biltong as it took ± 66 hours for both treatments to reach a 50% weight loss and 96 hours for both treatments to reach a 65% weight loss (Fig. 3.2). The initial drying rates between the biltong (no vinegar, vinegar) do not differ (Fig. 3.3). Differences in drying rates are seen between 18 and 30 hours and this could explain why differences in the moisture content are seen between 18 and 42 hours (Fig. 3.2) as these two are correlated. After 42 hours, the drying rates are the same and this suggests that the beef biltong could take the same time to reach a desired weight loss.

The water activity decreases from an initial 0.97 to ~ 0.85 (for a weight loss of 50%) and ~ 0.77 (for a weight loss of 65%) (Fig. 3.1). Water activity is influenced by the dehydration of the product and the addition of the salt. Therefore discrepancies in the water activity over time could be explained by the salt content but this was not tested for at each corresponding interval. The water activity of the biltong was not measured in *Trial 2* during the drying but as the water activity was the same for the end products a similar decrease in water activity over the drying period can be assumed.

Lean beef topside and lean beef silverside was dried for 96 hours to reach an approximate 65% weight loss ($65.5 \pm 0.78\%$) as for the lean beef topside in *Trial 1*. Lean gemsbok biltong reached $65.1\% \pm 0.82$ after 78 hours and fatty beef biltong took 118 hours to reach $66.0\% \pm 0.88$ weight loss. After 90 hours for lean beef topside and lean beef silverside and 72 hours for gemsbok as the moisture content stayed the same, this suggests that for a 65% weight loss the biltong could be taken out earlier for the same results.

Initially, drying is relatively quick, after which the drying became more constant. Water is typically found in meat in three phases; bound, loose and free water (Okos *et al.*, 1992; Vaclavik & Christian, 2014). The free and loosely bound water in the meat will be removed easily allowing for an

initial high drying rate. As the moisture content decreases and the remaining water (liquid) is more strongly bound, the drying rates begin to decrease (Okos *et al.*, 1992). In *Trial 1*, an approximate moisture content of 2.0 ± 0.05 g/g dm (~ 35% weight loss) is reached within the initial 12 hours for lean beef topside (Fig. 3.2). The lean beef topside from *Trial 2* can be compared with the results of the beef biltong with added vinegar from *Trial 1*. In Figure 3.5, the lean beef topside corresponds with the above results as it reaches a moisture content of 2.0 ± 0.22 g/g dm after the first 12 hours of drying showing the same trends. They both reached a 50% weight loss after 66 hours and 65% weight loss after 96 hours (Figures 3.2 and 3.5) and also show a slow constant drying rate after 42 hours (Figures 3.3 and 3.6).

The drying conditions/parameters used allowed for a constant drying of the biltong and the air velocity chosen resulted in no case-hardening of biltong implying moisture loss occurred evenly resulting in a drying rate that was consistent throughout the drying of the biltong. These experimental drying curves as well as the decay rate function (Table 3.6) can also be used to predict drying times for a targeted weight loss, moisture content and or water activity content under the same drying parameters and meat surface area to volume ration.

The different meat treatments are the main influencing factor during the drying of this biltong. Although the biltong was dried to a 65% weight loss, each treatment had a different drying rate due to its physicochemical properties. The lean beef topside and silverside had similar drying rates after 24 hours. The muscle composition (fibre type, chemical composition, etc.) may have influenced the initial drying rates during these first 24 hours which could explain these differences and warrants further research. It could be argued that the effect of the drying environment had minimised these effects after 24 hours of drying as once the free water has been removed, the bound water is slowly removed and drying rates start to become similar.

Biltong produced with fatty beef topside took the longest to dry with the moisture loss being slightly slower and therefore resulting in the slowest drying rate. This is due to the subcutaneous fat causing a barrier effect on water transfer, therefore the higher the fat content, the lower the drying rate (Santchurn *et al.*, 2012) as well as the smaller surface area of the meat. The drying rate of the final 96 hours of the drying process for fatty biltong was relatively constant indicating that the moisture loss of the free water had most probably occurred by this point. Therefore the drying of biltong can be said to be influenced by using meat with subcutaneous fat as this resulted in slower drying. Lean gemsbok biltong had the shortest drying period and highest initial drying rate when compared with the other treatments. Lean gemsbok biltong correlates best with lean beef biltong as after 12 hours the drying rates follow the same trend. There was less intramuscular fat (fat ~1.9%) in the lean gemsbok topside than the lean beef topside (~2.4%) although it is argued that this is inconsequential in the drying. This conclusion was also noted by Kucerova and others (2015) where a study comparing the drying kinetics of beef and eland jerky found the drying behaviours between the two species to be insignificant. Therefore the intramuscular fat content of the muscles could be an influencing factor between the drying of different species. On the other hand, the difference between lean beef silverside and lean gemsbok topside could be suggested to be primarily due to the inherent muscle type differences.

The fitting of the drying curve reiterate the results that the lean beef silverside and fatty beef topside have the same decay rate constant but, the fatty beef topside differs from the other meat treatment for both, this could be attributed to inherent muscle type differences and/or inherent differences in drying properties between muscle and fat tissue.

CONCLUSIONS

In this study, the drying kinetics of biltong were determined at a temperature of 25°C, a relative humidity of 30% and an air velocity of 2 m/s providing useful tools to predict drying times for a targeted weight loss/moisture content. The effect of the addition of vinegar during processing and the effect of the raw material (muscle, fat content, species beef/game) was also studied. The results showed that the addition of vinegar resulted in a lower pH in the end product and a higher weight gain after tumbling but the addition of vinegar did not influence the drying kinetics of the beef biltong. Therefore the use of an acid such as vinegar (traditionally added to inhibit microbial growth and contribute to the flavour) can be added without altering the drying parameters or decreasing the drying time. Using the same formulation, differences were seen for the drying kinetics of different meats for the same final weight loss of 65%. These results revealed that lean gemsbok topside only took 72 hours, lean beef topside and silverside took 90 hours and the fatty beef topside took 118 hours to reach the targeted weight loss (65%). Drying rates were calculated with lean gemsbok topside having the highest drying rates and fatty beef topside having the lowest drying rates all along the drying. However, the drying curves indicated that fatty beef topside and lean beef silverside had similar drying patterns. Therefore it can be concluded that the meat type/composition does play an important role in the drying of biltong. Based on these findings, industry using these constant parameters could use the exponential decay rate function to predict drying time depending on the moisture content of the raw meat used. Using these constant drying parameters results in a consistent final product (physicochemical properties) with minimal variability.

Further research could entail optimising the drying parameters when producing biltong using these different meats which would be beneficial to the industrial biltong processing. It would also be important to investigate the effects of fibre types, chemical composition, etc. of different muscles on the drying kinetics.

As these drying parameters result in a consistent product and drying time, it would be interesting to evaluate the effect of the vinegar addition on the microbial profile of biltong after drying and its shelf-life over a suggested storage period. This would also ensure the safety of the biltong produced in this manner is safe for consumption.

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CHAPTER 4

SHELF-LIFE STUDIES OF BEEF BILTONG – EFFECTS OF THE ADDITION OF VINEGAR, WEIGHT LOSS AND PACKAGING METHOD ON THE PHYSICOCHEMICAL PROPERTIES AND MICROBIOLOGICAL PROFILE

ABSTRACT

This study investigated the effect of the addition of vinegar during salting, the weight loss during drying and packaging method on the physicochemical properties and microbiological profile of beef biltong (salted/dried meat) over a three month shelf-life storage. An approximate 50% and 65% weight loss produced biltong with a moisture content of 50% and 30% (wet basis) with a salt content of 7.49 and 7.14 g/100 g resulting in water activity above 0.81 and below 0.78 respectively. The packaging method (modified atmospheric packaging and vacuum packaging) did not influence the physicochemical properties of the biltong over the shelf-life period. The addition of vinegar decreased ($p < 0.0001$) the pH of the biltong from 5.64 to 4.91. The weight loss and packaging method did not have an effect on the microbiological profile. The addition of vinegar only delayed the growth of total viable counts and reduced the coliforms count, both of which remain within the acceptable limits as set out by the South African National Standards 885:2011. Yeast and mould counts are not included in these regulations but as per the committee draft should not exceed 3 log cfu.g⁻¹. Yeasts and moulds were already present at high level (2.5 log cfu.g⁻¹) just after drying and exceeded the committee draft requirements after six weeks as well as being visible to the naked eye. *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* were not present at any stage of the storage period. *Staphylococcus aureus* was present at less than 20 cfu.g⁻¹. A shelf-life of no more than three months would be recommended for traditional South African biltong with water activity ranging from 0.74 to 0.83 when using either modified atmospheric packaging or vacuum packaging.

INTRODUCTION

The South African biltong industry has grown substantially over the last decades due to its increasing popularity amongst consumers. Biltong is a traditional salted/dried meat product from South Africa. Biltong is produced whereby meat strips are spiced using either a salt/spice dry rub (black pepper, coriander) or a mixture of salt/spice and vinegar (Worcestershire sauce is also sometimes added) before being dried. It is made at the house hold, butcher and industry level following the same procedure (Strydom & Zondagh, 2014), although vacuum tumbling and drying in temperature controlled units are common practices for large scale manufacturing. Drying temperatures between 20 – 35°C have been used in previous scientific studies to make biltong (Taylor, 1976; Nortje *et al.*, 2005; Burnham *et al.*, 2008; Naidoo & Lindsay, 2010a). In industry, biltong is dried at 20 – 25°C and moderate relative

humidity (30 – 65%) up to 4 days but it has not been reported in the scientific literature yet. The biltong process can result in biltong with high microbial counts (Burfoot *et al.*, 2010) and unevenly dried products. The shelf-life for biltong produced by the industry has been assumed to be between three to six months but there is no evidence to support this. There are various factors which can influence the shelf-life including the amount of salt and spices used, whether an acid is used during the salting/spicing step, the degree of drying and the type of packaging used, to mention a few.

Salt is essential when making biltong and is used as the “curing” agent which preserves the meat. Salting can result in changes in the muscle proteins and texture. It also modifies the water holding capacity (Ruiz & Pérez-Palacios, 2015) and ultimately the weight of the end product therefore influencing the effectiveness of the drying period. Salt decreases the water activity of the product thereby inhibiting microbial growth (Van der Riet, 1976). A 2.5% salt addition has been said to be added to the wet biltong as this results in an acceptable taste over a wide range of moisture levels in the final product (Taylor, 1976; Van der Riet, 1982). Vinegar is sometimes added (Van den Heever, 1970; Van der Riet, 1982; Strydom & Zondagh, 2014) with its main function being to inhibit microbial growth and its secondary function being for flavour. It is well-known that the use of salt and/or an organic acid (traditionally vinegar in the biltong process) can be used as a hurdle for the potential exclusion of pathogens and the inhibition of microbial growth in biltong (Naidoo & Lindsay, 2010b). The degree of drying (weight loss) is also an important hurdle. In the industry, biltong is most commonly dried to a 50% weight loss (resulting in a product with a moisture content of ~ 50%), but to accommodate for other consumer preferences, can be dried up to a weight loss ~ 70% (moisture content ~ 16%). Varying drying procedures are utilised as reflected by the wide range of moisture contents and subsequent water activities noted in the literature. The level of moisture loss will influence the price of the biltong for the producer as biltong is sold on a weight-basis. The moisture content of commercial biltong has been recorded to range between 10.6% and 48.8% with water activity (a_w) ranges between 0.54 and 0.93 (Van der Riet, 1976; Osterhoff & Leistner, 1984; Petit *et al.*, 2014). Microbial growth is inhibited by a low water activity. It has been suggested by Leistner (1987) that to avoid microbial spoilage, in particularly moulds, the water activity of a product should be ≤ 0.77 . Other research suggests the a_w could be reduced to 0.75 (CSIR, 2001; Burfoot *et al.*, 2010). Moulds however cannot grow at a $a_w \leq 0.61$ (Betts & Everis, 2008) but this is rarely achieved by dried meat products.

Biltong is typically sold all over South Africa where the products are either sold as whole pieces hanging or packaged under various conditions utilising different types of packaging methods. Industrially produced biltong is commonly sold under modified atmospheric packaging (MAP) or under vacuum (VP). In “informal stores” and butcheries the biltong is displayed for consumers in a “hanging space” whereby they may select the desired piece, which is then removed from this hanging space and sold in brown paper bags. This usually does not have a stated shelf-life as after a while, the exposure to air will affect its shelf-life stability and therefore not last as long as when packaged properly.

The microbiological requirements for biltong are based on the South African National Standards (SANS) 885:2011 which are the regulations for processed meat products, with biltong being classified under Class 3 subsections. These regulations state that the biltong should not exceed a total viable

count $> 6 \log \text{cfu.g}^{-1}$, and minimal presence of pathogenic microorganisms (*Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*).

For this study, the aim was therefore to investigate the effect of vinegar, weight loss (50% and 65%) and the method of packaging (vacuum packaging, modified atmosphere packaging) on the physicochemical properties and microbial shelf-life of beef biltong over a three month storage period.

MATERIALS AND METHODS

Biltong production

Frozen lean beef topside (*M. semimembranosus*) was sourced from a local supplier (Beefcor Meat Suppliers, Okahandja, Namibia). The meat was thawed at 4°C for 48 hours before use. Per replicate, the meat was cut parallel to the fibres into 192 pieces: 20 mm x 20 mm thick and 260 mm long. Each piece was individually labelled and weighed approximately 93 g (± 8.93). Four separate treatments (48 pieces each; $\pm 4.5 \text{ kg}$) were made. Two treatments were made without (C = control, no vinegar) and two with vinegar (V = vinegar), with one treatment of each being dried for a targeted 50% weight loss (WL 50), and the other for a targeted 65% weight loss (WL 65). The meat pieces were mixed together with a formulation of 2% (356 g) fine salt (Country Spice, Windhoek, Namibia), 0.2% (35 g) course black pepper (Freddy Hirsch, Maitland, Cape Town) and if vinegar was to be added, 5% (890 mL) brown spirit vinegar of a 10% (w/v) acidity (Freddy Hirsch, Maitland, Cape Town). After the meat was mixed with the ingredients, it was tumbled in a vacuum-tumbler (Freddy Hirsch Vacuum Tumbler, Freddy Hirsch, Maitland, Cape Town) with 912 mbar vacuum for 20 min at 15 rpm. Each piece was weighed again and hung in a controlled drying chamber (Closwa Biltong, Okahandja, Namibia). The drying chamber parameters were maintained at a temperature of $25 \pm 2^\circ\text{C}$, a relative humidity of $30 \pm 5\%$ and an air velocity of $2 \pm 0.2 \text{ m/s}$. The samples were dried until a weight loss of 50% (± 1.25) and 65% (± 1.92) which were achieved after ± 66 hours and ± 96 hours respectively. Weight loss was based on the weights after tumbling. After drying, the pieces were packaged in groups of three pieces into double-layered polyethylene terephthalate (PET) packages. For each of the four treatments, each was divided in two, whereby one half was packaged and sealed after nitrogen (N) gas-flushing (650 mbar) (MAP) and the other half was vacuum sealed (VP). The samples were stored in a temperature controlled room at an ambient temperature of $25 \pm 2^\circ\text{C}$ for the shelf-life period. Sampling of the packets were done at week 0, 1, 2, 4, 6, 8, 10 and 12. The division of the treatments for each of the three replicates is depicted in Figure 4.1.

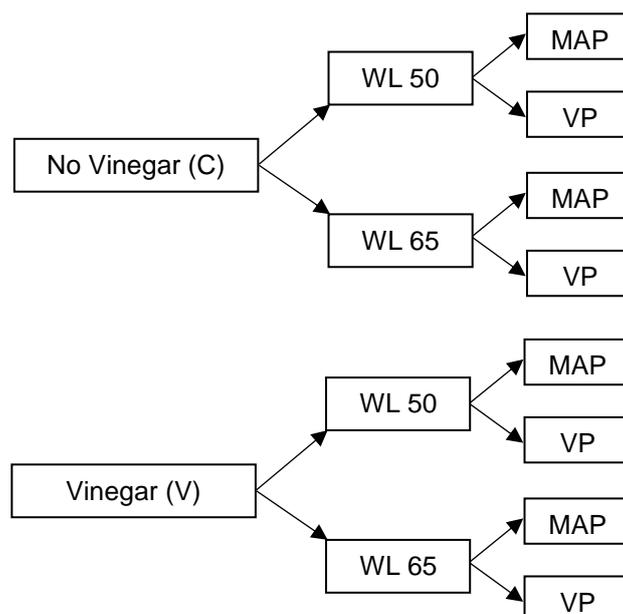


Figure 4.1 Division of treatments in each replicate (x3) according to formulation (C = control, no vinegar; V = vinegar), weight loss (WL = weight loss 50%, 65%) and packaging method (MAP = modified atmospheric packaging, VP = vacuum packaging).

Sample preparation for analyses

Sub-samples ($n = 12$) of the raw beef meat was taken. At each sampling time, the three pieces of biltong of a packet were sliced and pooled. Sub-samples were taken for determination of moisture and salt content, water activity and pH. Sub-samples were kept frozen at -18°C until the analyses, when each sample was thawed at 4°C for ± 2 hours and homogenised using a Knifetec™ 1095 Mill (FOSS, Höganäs, Sweden) for 90 sec or a Retsch Grindomix GM200 (Retsch, Haan, Germany) for 60 sec to ensure a representative homogenous sample was analysed. The rest of the sample was prepared aseptically for microbial analyses.

Moisture content analysis

The samples at week 0, 1, 2 and 12 were analysed to determine the moisture content (Method 934.01) according to the AOAC (2002). All analyses were performed in duplicate.

Salt content determination

The samples at week 0, 1, 2 and 12 were analysed for salt content. Salt content was determined by measuring the chloride concentration ($\text{mg Cl}^{-}/\text{L}$) using a Model 926 chloride analyser (Sherwood Scientific, Cambridge, UK) after extraction of meat in 0.3M nitric acid for 2 hours as described by Goli *et al.* (2012). All analyses were performed in duplicate.

Water activity determination

The water activity was measured using an Aqualab Series 4 water activity measurement system (Decagon Devices Inc., Washington, USA) at 25°C. Measurements were taken after sample preparation at each time period in duplicate.

pH determination

The pH measurement was taken using a calibrated portable Crison PH25 pH meter with a glass electrode (with an automatic paired temperature reading) (Lasec (Pty) Ltd, Cape Town, South Africa) after homogenisation of 3 g of sample in 27 ml distilled water. Measurements were taken after sample preparation in duplicate.

Microbiological analyses

Each sample was prepared by homogenising a 25 g biltong sample in 225 mL diluent (Ringers Solution) after which a 10⁻⁴ dilution was prepared. Standard methods were used for the microbial analysis (Table 4.1).

Table 4.1 International Standards Organisation (ISO)-approved methods for microbiological analyses

	Microorganisms	ISO-approved method	Media used (Merck, Germany)
Spoilage microorganisms	Total viable counts (TVC)	ISO 4833	Plate Count Agar (PCA)
	Coliforms	ISO 4832	Violet Red Bile Lactose Agar (VRBL)
	Yeasts and moulds	ISO 21527-2	Sabouraud Dextrose Agar (SDA) with Chloramphenicol
Pathogenic microorganisms	<i>Staphylococcus aureus</i>	ISO 6888-1/A1	Baird-Parker Agar (BPA) Brain-Heart Infusion (BHI)
	<i>Salmonella</i> spp.	ISO 6579/A1	Buffered Peptone Water (BPS) Rappaport Vassiliadis Soy (RVS) Broth Xylose Lysine Deoxycholate (XLD)
	<i>Listeria monocytogenes</i>	ISO 11290/A1	Fraser ½ Broth Agar Listeria Ottaviani & Agosti (ALOA)
	Presumptive <i>Escherichia coli</i>		Eosin Methylene Blue Agar (EMB)

Statistical analysis

The 24 biltong pieces per treatment were divided and packaged as three pieces per time treatment (8 time points).

To test the effects of treatment combination – formulation (C = control, no vinegar; V = vinegar), weight loss and packaging – on the various measurements, mixed model repeated measures ANOVA were used. The stage effect was the within subject repeated effect. For the mixed model, formulation,

weight loss, packaging and week were treated as fixed effects and the batches to which the treatments (formulation, weight loss, and packaging) were applied as a random effect. The individual weeks were analysed using factorial ANOVA. The Fisher LSD post hoc test was used to further analyse significant differences when the main effects/interaction effects were significant ($p \leq 0.05$). Statistical analyses were done using the VEPAC module for mixed models and GLM for factorial ANOVA of *Statistica 64*. A 5% significance level was used as guideline for determining significant differences.

RESULTS AND DISCUSSION

Weight increase after tumbling

The addition of vinegar in the formulation causes an increase in the initial weight of the meat sample before drying (Table 4.2) although this does not affect the drying time of the biltong (refer to Chapter 3).

Table 4.2 Means (\pm SD) for the weight of meat pieces before formulation (raw), after tumbling and the weight gain for each treatment group (n = 24)

Treatment group	Meat pieces weight before formulation (g)	Meat pieces weight after tumbling (g)	Weight gain (%)
WL 50, No Vinegar	93.2 \pm 6.69	95.1 \pm 6.66	2.0 ^b \pm 0.78
WL 65, No Vinegar	91.1 \pm 10.47	93.4 \pm 10.24	2.5 ^b \pm 0.82
WL 50, Vinegar	93.8 \pm 9.83	98.6 \pm 11.15	5.1 ^a \pm 1.67
WL 65, Vinegar	93.5 \pm 10.51	97.8 \pm 10.67	4.6 ^a \pm 1.05

WL = weight loss

^{a,b} Means within a column with different superscripts differ significantly ($p \leq 0.05$)

The significant (substantial) increase in the weight is due to the uptake of both salt and vinegar. The samples with added vinegar show a weight gain almost 2 times higher than those samples without vinegar. The theoretical weight losses after drying were determined on the sample weights after tumbling.

Physicochemical properties

Theoretically the individual biltong pieces were to be taken out of the dryer after reaching a desired weight loss of either 50% or 65% so as to represent the typical weight loss used in the industry. Table 4.3 shows the p-values of the effect of the addition of vinegar and weight loss on the physicochemical properties of biltong after drying (week 0) and their average for each treatment.

Table 4.3 p-values for effects of formulation (no vinegar, vinegar) and weight loss (50%, 65%) on the physicochemical properties of biltong after drying (week 0) and means (\pm SD) for each treatment group (n = 24)

	MC _{wb} (g/100 g)	SC _{db} (g/100 g)	SC _{wb} (g/100 g)	a _w	pH
Effects			p-values		
Formulation	0.988	0.012	0.012	0.124	<0.0001
WL	< 0.0001	0.197	< 0.0001	0.006	0.449
Formulation*WL	< 0.0001	0.046	< 0.0001	0.024	<0.0001
Treatment group			Means (\pm SD)		
WL 50, No Vinegar	51.8 ^a \pm 1.02	7.15 ^{ab} \pm 0.26	3.44 ^c \pm 0.12	0.81 ^a \pm 0.02	5.66 ^a \pm 0.04
WL 65, No Vinegar	32.4 ^b \pm 0.58	6.68 ^b \pm 0.23	4.51 ^b \pm 0.12	0.74 ^c \pm 0.03	5.62 ^a \pm 0.03
WL 50, Vinegar	52.5 ^a \pm 1.37	7.83 ^a \pm 0.37	3.72 ^c \pm 0.07	0.83 ^a \pm 0.03	4.93 ^b \pm 0.13
WL 65, Vinegar	31.7 ^b \pm 0.35	7.60 ^a \pm 0.71	5.19 ^a \pm 0.47	0.78 ^b \pm 0.02	4.89 ^b \pm 0.12

wb = wet, as is basis (moisture content is taken into consideration)

db = dry matter basis (the actual moisture content of the biltong is excluded)

MC = moisture content, SC = salt content, a_w = water activity, WL = weight loss

a, b, c Means with different letters within a column differ significantly (p \leq 0.05)

The moisture content of the biltong within the same weight loss (WL 50 or WL 65) did not differ significantly from each other regardless of the addition or no addition of vinegar. As expected, the biltong dried to a WL 50 resulted in a final moisture content (wet, as is basis) of \pm 50% and biltong dried to a WL 65 had a final moisture content (wet, as is basis) of \pm 30%. The low standard deviations is an indication that the biltong production process and drying were as expected, relatively constant.

Salt content is calculated on both a wet, as is basis and on a dry matter. The final salt content depends on the initial amount of salt used with the most common salt content of biltong ranging between 4 – 8% (wet basis) in traditional biltong (Osterhoff & Leistner, 1984; Petit *et al.*, 2014). With this study the amount of salt was kept constant for all treatments so the differences seen can be explained by the influence of vinegar and/or the degree of drying on salt content as well as the meat itself. The salt content of biltong, expressed on a wet as is basis, is expected to vary amongst the different degree of drying as when there is less moisture present, the more concentrated the salt in the biltong. Theoretically, tumbling allows for accelerated salt penetration in meat products through structural muscle changes (Krause *et al.*, 1978; Ghavimi *et al.*, 1986) but salt penetration was not assessed in this study. On a dry matter basis, the salt content do not differ between the “WL 50, No Vinegar” and “WL 65, No Vinegar” biltong samples and between the “WL 50, Vinegar” and “WL 65, Vinegar” biltong samples as the amount of salt added was the same between the treatments, as mentioned. This also shows that salting is fairly homogenous in each meat piece of a treatment group after tumbling.

Water activity (a_w) correlates with both the moisture and salt content of a product. Nortje and others (2005) defined moist biltong to have moisture content higher than 40%. It is also commonly acknowledged among biltong producers. According to research conducted on commercial biltong

samples, biltong with a moisture content greater than 40% has a water activity ranging between 0.84 – 0.92 (Van der Riet, 1976; Osterhoff & Leistner, 1984; Petit *et al.*, 2014). Therefore a dry biltong could be classified as having a moisture content less than 40% with a water activity below 0.84. The results from this study show that there was a difference ($p = 0.006$) in a_w between the different weight losses (WL 50 and WL 65); the samples that underwent a higher weight loss having a lower water activity due to their lower moisture content and higher salt content. The biltong samples dried to a 50% weight loss had a a_w ranging between 0.81 – 0.83 while the biltong samples dried to a 65% weight loss had a a_w ranging between 0.74 – 0.78, falling in the range of dry biltong as discussed.

The addition of vinegar was expected to alter the pH of the meat, as the surface of the meat pieces become denatured by the acid. The interaction ($p < 0.0001$) for pH noted between the main effect of formulation and weight loss is expected as added vinegar is an acid. The pH, as expected, was higher in the biltong with no added vinegar (5.64 and 4.91 respectively without and with vinegar). It has been published that biltong can have a pH ranging between 4.81 and 5.83 (Osterhoff & Leistner, 1984; Petit *et al.*, 2014), which is in accordance to the data in Table 4.3. A few samples with a pH below 5 (1/11 and 1/20 respectively) were reported; this can be assumed to be due to the addition of an acid which is common practice in biltong production. Biltong produced without added vinegar had a lower salt content but it was shown on the samples dried to a weight loss of 65% only; so this singularity would need to be confirmed with further studies.

The physicochemical properties after the 12 week storage period were also tested. As a change in the salt content during storage could only be due to a change in the moisture content this was not tested. Table 4.4 shows the p-values of the formulation, weight loss and week effects on the moisture content, water activity and pH between week 0 and week 12. Second- and third-order interactions were not included in the table as no significance differences between week 0 and 12 were seen between these interactions and therefore will not be discussed.

Table 4.4 p-values for effects of formulation (no vinegar, vinegar), weight loss (50%, 65%), packaging (MAP, VP) and week (0, 12) on the physicochemical properties of biltong ($n = 12$)

Effects	MC _{wb}	a_w	pH
Formulation	0.512	0.001	< 0.0001
WL	< 0.0001	< 0.0001	0.386
Packaging	0.825	0.653	0.939
Week	0.949	0.122	0.806
Formulation*WL	0.008	0.124	0.892
Formulation*Packaging	0.756	0.492	0.463
Formulation*Week	0.338	0.404	0.026
WL*Packaging	1.000	0.976	0.988
WL*Week	0.106	0.056	0.548
Packaging*Week	0.595	0.458	0.604

wb: wet, as is basis (moisture content is taken into consideration)

MC = moisture content, a_w = water activity, WL = weight loss

From these results it can be seen that moisture content, and thus salt content, did not change during the 12 week storage no matter the packaging used, as effects of packaging and week on moisture content were not significant.

The significant p-value of the formulation main effect indicates that the water activity was affected over the storage period. At week 0 (Table 4.3) there was no significant relationship between the formulation and water activity but after the 12 week storage a difference can be seen (Table 4.4) which could indicate the formulation influenced the water activity over time.

The effects of formulation and weight loss on the pH of the biltong samples during the 12 week storage have similar results to that explained previously (week 0). Even though the samples with added vinegar had a significantly lower ($p < 0.0001$) pH than the samples without added vinegar, this was stable over the storage period ($p = 0.806$). The consistency of the physicochemical results over the storage results allows for better interpretation of microbiological results. It also suggests that the flavour and aesthetic profile of biltong could stay constant over a three month shelf-life period when packaged in MAP or under vacuum, further investigation into these profiles would be necessary for confirmation.

Microbiological profile

The shelf-life of biltong is directly linked to the pH, water activity and salt content of the final biltong product as well as the composition of the meat itself and the biltong preparation (food handlers and/or equipment used).

It is important to note that all tests were not done on the same piece of biltong but rather on the same batch of biltong so that the shelf-life study could be conducted accurately. Therefore possible inconsistencies of the microbial counts between time treatments could be due to biological variation in the meat itself and/or contamination during the production process. All factors (main effects and interactions) were taken into account when investigating the microbial results as seen in Table 4.5.

The commercial shelf-life of biltong is assumed to be between three and six months. Over the three month (12 week) shelf-life period, it was seen that the microbial counts change as shown by the week effects (Table 4.5).

Both modified atmosphere packaging (MAP) and vacuum packaging (VP) are used in the industry as methods to enhance shelf-life and were therefore evaluated in this study. The results indicate that there were no significant differences (Table 4.5) in the microbiological counts between MAP and VP. The elimination of oxygen from the package and addition of nitrogen inhibits growth of strict aerobic microorganisms (moulds). Studies on products similar to biltong (salted and dried), have also noted no microbial differences between MAP and VP (García-Esteban *et al.*, 2004; Rubio *et al.*, 2007). Therefore the effects of packaging are not included in the figures (Fig. 4.2 and Fig. 4.3).

Microbial counts increased significantly during storage (data not shown for coliforms and *Staphylococcus aureus*) as expected as there was initial microbiological growth at week 0 after drying and biltong has a favourable environment (moisture, salt, pH and storage temperature). The South African regulations for biltong fall under SANS 885:2011 Processed Meat Products Class 5, which indicates the limits at the time of the estimated shelf-life. Throughout the 12 week shelf-life period, all the microorganisms (excluding yeast and moulds) were within the acceptable limits. Total viable counts

should not exceed 6 log cfu.g⁻¹ as per these regulations. Yeasts and moulds are not included in the current SANS regulations but as per the committee draft should not exceed 3 log cfu.g⁻¹. The stability of the microorganisms over the weeks could be due to the theory that anoxic (low oxygen) environments delay microbial growth and spoilage as any anaerobic bacteria that may be present grow slowly (Martínez *et al.*, 2006). Yeasts and moulds however can grow in oxygen concentrations $\leq 0.1\%$ and therefore could grow under vacuum or in gas mixtures (Suppakul *et al.*, 2003). It was also noted that the product became less visually appealing under VP but this was not quantified in this study and requires further research.

In this study, it is seen that for all the tested microorganisms, the weight loss did not play a role (Table 4.5). Differences in the results however could be better explained due to water activity as it is more relevant to microbiological growth than the moisture content and related salt content. Even though the water activities between the biltong dried to 50% weight loss and 65% weight loss were different ($p = 0.024$), it would seem as if the differences (~ 0.82 and ~ 0.76 respectively) were not large enough to influence the microbiological results as each treatment group was still within the favourable range for (limited) microbial growth ($a_w > 0.75$) (CSIR, 2001; Burfoot *et al.*, 2010). The biltong with added vinegar and those without added vinegar showed differences in spoilage microorganisms' growth (total viable counts and coliforms). This was expected as the vinegar decreased the pH of the product and therefore have a more unfavourable environment for microbial growth.

Table 4.5 p-values for effects of formulation (no vinegar, vinegar), weight loss (50%, 65%), packaging (MAP, VP) and week (0, 1, 2, 4, 6, 8, 10, 12) on the microbial profile of biltong

Effects	TVC	Coliforms	Y & M	<i>S. aureus</i>
Formulation	< 0.0001	0.026	0.448	0.616
WL	0.460	0.657	0.557	0.229
Packaging	0.577	0.667	0.459	0.160
Week	< 0.0001	0.005	< 0.0001	< 0.0001
Formulation*WL	0.023	0.482	0.145	0.566
Formulation*Packaging	0.256	0.577	0.621	0.636
Formulation*Week	< 0.0001	0.115	0.673	0.002
WL*Packaging	0.030	0.189	0.878	0.360
WL*Week	< 0.0001	0.801	0.323	0.003
Packaging*Week	0.005	0.997	0.385	0.464
Formulation*WL*Packaging	0.002	0.780	0.546	0.212
Formulation*Packaging*Week	0.579	0.582	0.623	0.016
Formulation*WL*Week	< 0.0001	0.359	0.497	0.491
WL*Packaging*Week	0.641	0.857	0.851	0.108
Formulation*WL*Packaging*Week	0.117	0.331	0.726	0.256

TVC = Total viable counts; Y & M = Yeasts and moulds; *S. aureus* = *Staphylococcus aureus*, WL = weight loss

Total viable counts (TVC) is the quantitative measure of the presence of microorganisms. This gives a good indication of the product quality and to a certain extent, contamination. With pathogens usually being the focus in related studies (Burnham *et al.*, 2008; Naidoo & Lindsay, 2010b; Nortje *et al.*,

2005), it is important to evaluate the TVC to clarify whether there are problems associated with the product and its production process as well as to assess the products shelf-life. Figure 4.2 shows the increase in total viable counts over the 12 week storage period. The dotted line at 6 log cfu.g⁻¹ indicates the limit of TVC for commercial biltong (SANS 885:2011).

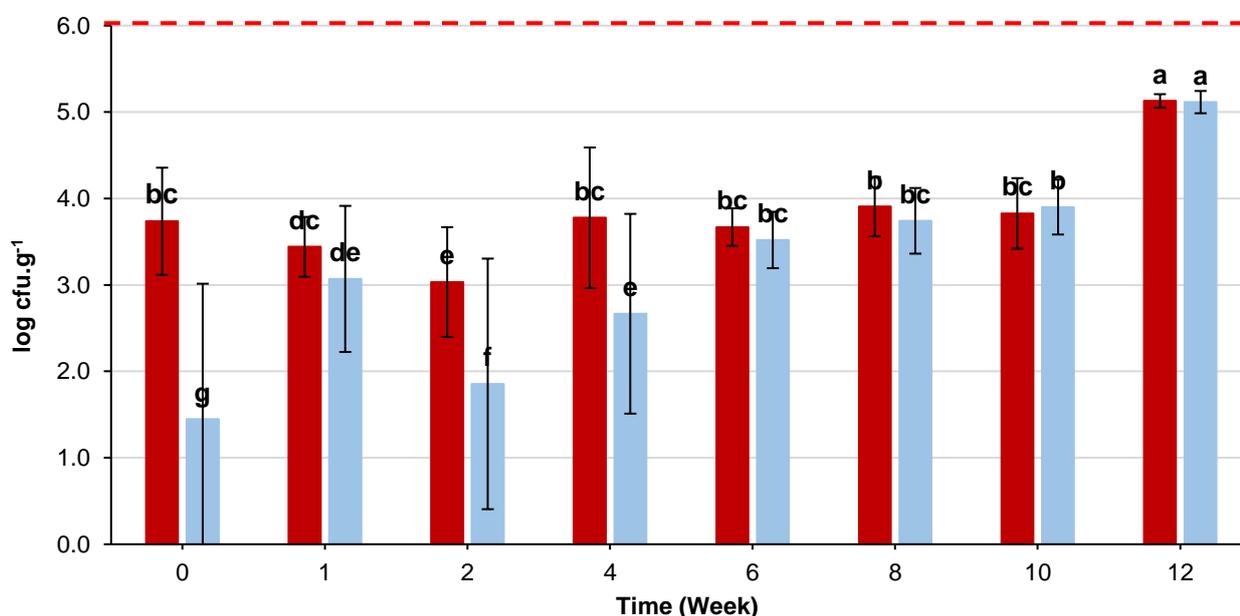


Figure 4.2 Total viable counts log cfu.g⁻¹ of biltong for different formulation (no vinegar, vinegar) over a 12 week storage period (n = 3).

a, b, c, d, e Means with different letters within a column are significantly different ($p \leq 0.05$)

■ No Vinegar ■ Vinegar

The TVC in the “Vinegar” biltong samples are lower ($p < 0.0001$) than the “No Vinegar” biltong samples initially but after week 4, the results become similar and differences are insignificant. The addition vinegar causes a drop in the pH (Table 4.3) wherein some microorganisms cannot grow whilst other microorganisms’ growth is delayed. These results also indicate that the preparation of the biltong was done under hygienic conditions. Other research on commercial biltong has indicated that TVC has been found between 2 – 9 log cfu.g⁻¹ (Mhlambi *et al.*, 2010; Naidoo & Lindsay, 2010b; Shale & Malebo, 2011; Matsheka *et al.*, 2014; Petit *et al.*, 2014). These samples were bought from retail stores and therefore it is difficult to trace back to see whether the high microbial counts sometimes obtained were due to raw materials, preparation hygiene and contamination or even the process parameters including whether preservatives or an acid (such as vinegar) were used. The maximum TVC in both “Vinegar” and “No Vinegar” samples was in accordance with research by Shale and Malebo (2011) and Matsheka and others (2014) who recorded TVC not higher than 6 log cfu.g⁻¹ and 5 log cfu.g⁻¹ respectively. It could be suggested, that TVC could include fermentative bacteria such as lactic acid and this could explain the high counts found in the commercial samples tested and therefore this would be of interest in further studies. Biltong is not commonly tested for lactic acid bacteria (LAB) as it is not a fermented product and has a short drying period as opposed to other fermented products. Petit and others (2014) found that the lactic acid content was high in relation to the total viable count with a LAB/TVC ratio ranging

from 0.90 – 0.99 (in the moist biltong that was analysed). Theory has indicated that TVC, to a certain extent, could also indicate deviations and contamination that has occurred. Coliforms are a better indication of this however and in this study are relatively low for this type of product; research compiled by Burfoot and others (2010) has shown that coliforms can be present up to 4 log cfu.g⁻¹ in biltong. In this study, the coliform counts were below the acceptable limit of < 2 log cfu.g⁻¹ (SANS 885:2011), with the “No Vinegar” samples averaging 0.075 ± 0.082 log cfu.g⁻¹ and the “Vinegar” biltong samples averaging 0.18 ± 0.30 log cfu.g⁻¹ over the storage period. Therefore, as with the total viable counts, the coliforms are lower ($p = 0.026$) in the biltong with vinegar than the biltong without vinegar showing an effect of pH on coliform growth.

Mould growth in biltong is common and is one of the most prominent problems in the biltong industry. With a water activity (≥ 0.74) and a salt content (wet basis) between 3.4 – 5.2% it could be predicted that there will be mould growth, albeit minimal in vinegar samples, as the a_w in these biltong samples is not low enough to inhibit mould growth ($a_w \geq 0.61$); biltong is also known to be susceptible to mould growth. Vinegar could however have an effect on moulds due to the decline in pH (5.64 and 4.91 without and with vinegar respectively). The average growth of yeast and mould in biltong over a 12 week storage period is depicted in Figure 4.3.

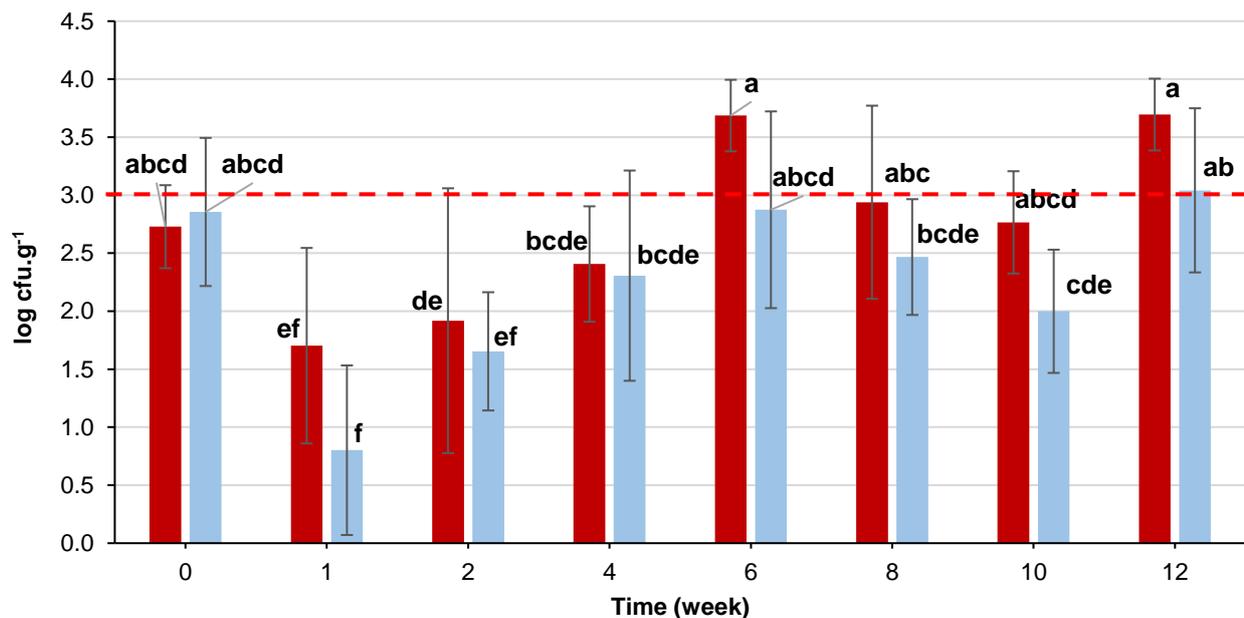


Figure 4.3 Yeast and mould growth log cfu.g⁻¹ of biltong for different formulations (no vinegar, vinegar) over 12 week storage (n = 3).

a, b, c, d, e Means with different letters within a column are significantly different ($p \leq 0$).

■ No Vinegar ■ Vinegar

The major problem with mould is that it is visually unappealing to South African consumers and they will not purchase the biltong if mould is seen (no matter the amount or species) or tend to discard the biltong when it appears. Mould could be a sign of contamination during biltong production, either from the raw material or during the drying process (due to incorrect air flow, relative humidity and

temperature) as well as the biltong not having the correct end properties (appropriate moisture and salt content and a_w).

The results in Figure 4.3 indicate that yeast and moulds were present directly after the drying process, which could be due to an unsuitable drying rate of the biltong which is influenced by the relative humidity and temperature in the commercial chamber and their fluctuations. There is an increase in yeast and mould growth from week 1 over the 12 week storage period, with the yeast and mould counts exceeding $3 \log \text{ cfu.g}^{-1}$ at week 6 and week 12. As mentioned, there are no regulations for yeast and moulds but the committee draft suggests a limit of $< 3 \log \text{ cfu.g}^{-1}$. As this is common in biltong it would be suggested to add a limit to the current SANS regulations. From week 6, the mould becomes visible on the samples. There was no difference ($p = 0.448$) between the samples with vinegar or without vinegar added in spite of its decrease on pH. It is important to note that the visibility of mould on the samples without added vinegar was more prominent. Even though the size of the mould colonies are not of concern until week 12, the yeast and mould exceed the specifications at week 6 which is unacceptable. Even when the mould growth is seen to be below the limit, once present it proliferates rapidly if moisture is present. The high yeast and mould counts at week 0 could be the result of spores being present as there was no visible mould at week 0 as with the proceeding weeks. As previously mentioned, yeasts and moulds can grow in oxygen concentrations $\leq 0.1\%$ and therefore can grow under vacuum or in gas mixtures (Suppakul *et al.*, 2003); moulds however are less visible in vacuum packaging. Taylor (1976) investigated the microbial population in biltong during processing and a short storage period but this did not include yeasts and moulds. As moulds are becoming an increasing concern in the biltong industry, a more in-depth study, to identify the moulds and their development over time, would be of interest.

A qualitative (absence/presence) analyses of pathogenic microorganisms was conducted whereby *Listeria monocytogenes*, *Salmonella* spp. and *Escherichia coli* were not present at any stage of the 12 week testing period. This can be attributed to the fact that the raw meat used in the biltong production did not contain any of these bacteria. It is still important to test for these microorganisms as they can be harmful and also cause food poisoning in humans when digested.

Positive *Staphylococcus aureus* results detected $< 1 \log \text{ cfu.g}^{-1}$ over the shelf-life period and this was still within the acceptable limits ($< 1.3 \log \text{ cfu.g}^{-1}$). This however must be monitored carefully as it is considered a pathogenic microorganism but is spread through the mishandling of the raw meat. It can be postulated that *S. aureus* growth would possibly exceed the regulations after a couple more weeks of storage. Once present in the meat, *S. aureus* can survive and proliferate as the drying conditions and biltong itself create a favourable environment. In biltong, *Staphylococcus* strains are likely to be present as they can grow at intermediate water activity levels (≥ 0.83) and high salt contents. *S. aureus* in response to a low a_w environment, accumulates several compounds in its bacterial cell, which lowers the intracellular a_w to match the external a_w allowing it to survive (Montville and Matthews, 2008). It is important to note however that *S. aureus* does not produce toxins at a $a_w < 0.83$ and this desired a_w is achieved by biltong dried to a 50% and 65% weight loss. Previous research has shown that *Staphylococcus* spp. can survive at high salt concentrations between 10 – 20 % (McLean *et al.*, 1968; Ingham *et al.*, 2005) and that the use of a vinegar in biltong production is not effective for inhibition

of pathogenic microorganisms and *Staphylococcus* strains (Naidoo & Lindsay, 2010b) as it has the ability to grow under pH stress (Montville and Matthews, 2008). There is no trend in the effect of formulation, weight loss and packaging in the growth of *S. aureus* but a difference ($p < 0.0001$) can be seen between the weeks. *Staphylococcus aureus* is a facultative anaerobe that can grow under aerobic and anaerobic conditions but growth occurs at a much slower rate under anaerobic conditions (Stewart, 2003). Therefore, as expected, there was no difference ($p = 0.160$) in *S. aureus* growth between the different packaging methods (MAP and VP). Environmental, human and animal contamination are the most common sources of *S. aureus* in food and therefore it is always tested for as high concentrations thereof may cause food poisoning (FDA, 2012). This could also explain any fluctuations between the weeks due to the human handling of the biltong during production.

CONCLUSIONS

The moisture content, salt content, water activity and pH are characteristics of biltong that play a role in consumer perception as well as influencing the shelf-life of biltong. A longer drying time resulted in a higher weight loss and a higher salt content, and a reduction in moisture and water activity, which are important for the shelf-life of biltong. The addition of vinegar may have had an effect on the salting. It would be of interest to study this aspect further by studying the penetration and diffusion of salt in meat and its drying thereafter (throughout the process from raw material to dried material). The addition of vinegar decreased the pH of biltong.

The microbiological results in conjunction with the physicochemical results, could be used as a recommendation to determine the actual shelf-life of biltong and evidence for the legal regulations in place. The shelf-life of traditional biltong can be said to be three months (12 weeks) as the results indicate it to be under the legal limits. The yeast and mould growth exceeded acceptable limits and therefore further studies regarding the development of yeast and moulds need to be conducted. As mould is an increasing problem in the industry, a study involving identification of the moulds and growth patterns would help the industry. Tracking mould growth will also allow for estimations to be made in terms of when and why mould growth is occurring.

It can be speculated that the biltong shelf-life is shorter than has been assumed by industry and consumers alike (up to 6 months) as the microbial results (total viable counts and *Staphylococcus aureus*) were just under the acceptable limits after three months storage. Weight loss between 50% and 65% did not play a role in the microbial profile but this could be due to the narrow range seen in the water activity (ranging from 0.74 – 0.83) therefore suggesting that the water activity could play a lesser role in determining the shelf-life of beef biltong in this range. Addition of vinegar may play the most important role whilst packaging method is of minor importance with no difference seen throughout the study. The two packaging methods used, vacuum packaging and nitrogen gas-flushed packaging (modified atmosphere packaging) would both therefore be deemed acceptable. The moisture content of biltong is a personal preference for consumers and therefore the industry should still produce biltong of varying moisture content but utilising water activity as the best parameter for the shelf-life determination. The addition of vinegar allowed for a delay in total count growth and therefore can also

be recommended as an addition to the biltong production process but this may change the flavour profile and therefore a sensory panel (consumer) should be conducted to determine preference amongst consumers.

These results indicate the problem of yeast and moulds in the industry. Producing beef biltong using the same drying parameters as this study and studying the development of yeast and moulds over time would help to assess the types of yeasts and moulds found in this biltong and in turn the main areas of concern in regards to contamination. It could be of interest to extend the study to six months and improve the study with a more in-depth investigation into the correlation between the microbial results with the physical profile of the biltong. Also, the storage of biltong at different environmental conditions (particularly temperature and relative humidity) will also be of interest. Additionally, studying the biltong once opened for the recommended three days could be helpful for microbiological guidelines but also for the interest of the consumer. It is recommended that these results be used as a guideline to determine the actual shelf-life of biltong and as evidence to support the legal regulations in place.

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CHAPTER 5

EVALUATION OF THE ABILITY OF BEEF BILTONG TO SUPPORT YEAST AND MOULD GROWTH DURING STORAGE EVALUATED BY MICROBIAL CHALLENGE TESTING

ABSTRACT

The growth potential of yeasts and mould in beef biltong during storage was assessed using a challenge test protocol. The biltong was produced without and with vinegar and dried to a 50% and 65% weight loss. A mixed inoculum of yeasts and mould found on naturally contaminated biltong pieces was used. An inoculum level of 2.3×10^{-4} cfu.g⁻¹ was used to inoculate biltong after production. The visual appearance of the yeast and mould growth was monitored throughout 34 days of storage, and the yeast and mould counts were determined at day 0 (day of inoculation), day 10 (colonies visible) and at day 34 (end of storage). On biltong without added vinegar, yeasts and mould started to become visible after 10 days with yeast and mould growth exceeding the acceptable limit of 3 log cfu.g⁻¹ at this point. No yeast and mould growth was seen on biltong with vinegar for the whole testing period (34 days) with counts < 2.5 log cfu.g⁻¹. The non-inoculated (control) samples, without vinegar and with vinegar, showed natural yeast and mould growth after 34 days with ≤ 3.0 log cfu.g⁻¹ and ≤ 1.7 log cfu.g⁻¹ respectively. Genera of *Saccharomyces* (yeast) and *Aspergillus*, *Fusarium* and *Penicillium* (moulds) were isolated and identified as the most common yeast and moulds found on the biltong after completion of the challenge test.

INTRODUCTION

Biltong is a South African delicacy enjoyed by both local and international consumers. Biltong is a dried meat snack produced by drying salted/spiced meat strips. Different meats (beef, game and ostrich) (Van Wyk, 2007; Naidoo & Lindsay, 2010b; Strydom & Zondagh, 2014) are used and different formulations (combinations of salt and spices like black pepper and coriander, brown sugar and vinegar). Salt is essential in biltong production as it acts as a preservative as well as contributes to the flavour. Vinegar is often added to inhibit microbial growth (Van den Heever, 1970; Van der Riet, 1982; Naidoo & Lindsay, 2010a; Naidoo & Lindsay, 2010b; Strydom & Zondagh, 2014) as well as for flavour. Biltong is usually dried to a weight loss $\leq 50\%$ or further to accommodate other consumer preferences according to industry and some literature (Strydom & Zondagh, 2014). The moisture content and corresponding water activity of biltong play an important role in the microbial stability of a product.

Biltong is considered to be shelf-stable between three to six months. However, biltong is very susceptible to mould growth which is a common problem in the commercial biltong industry. Mould growth, on the surface of biltong, is unappealing to consumers (Van der Riet, 1976a) and results in economic losses for biltong producers and suppliers (Van der Riet, 1982). The majority of yeasts and moulds are considered obligate aerobes (they require oxygen for growth) and can survive in a broad

acid/alkaline (pH 2 – 9) and temperature (10 – 35°C) spectrum (McLandsborough, 2004). Foodborne moulds can grow at a water activity ≤ 0.85 while yeasts may require a higher water activity (McLandsborough, 2004). Yeasts and moulds grow slowly at a low a_w of 0.62 (Wolter *et al.*, 2000). Therefore yeasts and moulds are considered to have relatively adaptable environmental growth requirements. Yeast and mould growth commonly occurs on beef biltong (Van der Riet, 1976a; Osterhoff & Leistner, 1984; Wolter *et al.*, 2000; Mhlambi *et al.*, 2010; Matsheka *et al.*, 2014; Petit *et al.*, 2014) as it has a favourable growth environment.

It has been noted that yeasts are more predominant than moulds on biltong (Van der Riet, 1976a; Osterhoff & Leistner, 1984; Wolter *et al.*, 2000; Petit *et al.*, 2014). An early study tested 20 biltong samples where yeast was present in 19/20 samples (1.0 – 5.5 log cfu.g⁻¹) and moulds were present in 6/20 samples (1.0 – 5.3 log cfu.g⁻¹) (Van der Riet, 1976a).

Yeasts and moulds are not deemed a food safety issue. However, this should be dependent on the yeast and mould count as well as the strain/species that is present as some moulds are more dangerous than others as they can produce mycotoxins. Yeast and mould regulations are not included in the South African legislation (SANS 885:2011) pertaining to biltong but has been suggested in the committee draft to have a collective count < 3 log cfu.g⁻¹ after shelf-life. Yeast and mould identification should also be considered in the drafts. Yeast and mould growth is due to contamination and end-product characteristics (moisture and salt content, water activity and pH) of the biltong. Contamination can occur during production (processing and packaging) and/or storage. It has been speculated by the industry that the mould contamination occurs during drying due to the drying chamber setup (temperature, relative humidity and air flow) and the cleanliness of the dryer. The progression of mould growth during processing and/or storage is not known and therefore it cannot be predicted how it spreads/grows and the implications thereafter. Storage and distribution of biltong is usually at room temperature. As mentioned, yeasts and moulds grow in a wide range of temperatures, and have the ability to proliferate during low temperature storage if present (Wolter *et al.*, 2000). The growth of yeasts and moulds under these conditions should be tested. By performing challenge tests this can be assessed. Critical factors to consider when designing a challenge test include choice of test strain, preparation of inoculum, inoculum level and inoculation procedure. The storage conditions (temperature and the relative humidity) and product characteristics should also be considered.

With this increasing concern of yeasts and moulds in biltong the study aims were therefore to conduct a challenge test on beef biltong produced without and with added vinegar and dried to a 50% and 65% weight loss when dried using constant drying parameters (temperature, relative humidity, air velocity) to assess the growth of yeasts and moulds after production during storage at 25°C in sealed packaging. The predominant yeasts and moulds present were also identified on a genus level.

MATERIALS AND METHODS

Biltong production

Lean beef topside (*semimembranosus* muscle) was used. The biltong was produced as described in Chapter 3. Four separate treatments (8 pieces each; ± 776 g) were made, two of these treatments without vinegar and the other two treatments with vinegar. One treatment from each was dried for a targeted 50% weight loss (WL 50), and the other for a targeted 65% weight loss (WL 65). Each treatment was done in triplicate. After salting/spicing and drying, the biltong was packaged in double-layered polyethylene terephthalate (PET) packages and sealed after nitrogen (N) gas-flushing (650 mbar) (MAP). The samples were stored in a temperature controlled room at a low temperature ($5 \pm 2^\circ\text{C}$) until inoculation.

Inoculum preparation

The inoculum was sourced from biltong pieces which was made using the same production process. These samples had been contaminated during the production process and/or during storage (sealed in PET packages at room temperature, $\sim 25^\circ\text{C}$) resulting in mixed yeast and mould growth.

Inoculum was prepared by scraping the visible moulds (62.5 g) off the contaminated biltong pieces and adding it to 250 mL sterile Peptone Physiological Saline solution (PPS; 8.5/L NaCl). This served as the inoculum for the challenge test with a low inoculum concentration of 2.3×10^{-4} cfu/mL, determined using a hemocytometer (Malassez method).

Biltong inoculation

Inoculation of yeasts and moulds occurred three weeks after production. Each piece of biltong was cut in half (two separate pieces approximately 20 mm x 20 mm thick and 13 mm long) in a laminar flow safety cabinet (Faster-air, Cornaredo, Italy) at room temperature ($\pm 25^\circ\text{C}$) and aseptic conditions. This resulted in 16 biltong pieces per treatment, per replicate. The one half was used as the control (C) and the other half was used for the yeast-mould inoculum (YM). Surface inoculation of one side of each piece of biltong was performed by placing 10 μL of either sterile distilled water (C) or inoculum (YM) at three separate staggered points on the biltong surface. It was allowed to dry (~ 15 min) before being sealed in PET packages (no vacuum and no gas flushing). All samples were stored in a climatic chamber (BIA Climatic, Conflans Sainte Honorine, France) at 25°C during 34 days. The samples were rotated in the chamber after each inspection.

Visual inspection

After inoculation and every 4 days for 34 days, each biltong piece was inspected for yeast and mould growth via visual inspection with assistance from an experienced microbiologist. Colony size, colour, description, position of colony on sample and coverage were noted. Any irregularities seen were also noted and photographed.

Sample preparation for analyses

Samples were taken at day 0 (just before inoculation), day 10 and at day 34 (end of storage) for physicochemical and microbiological analyses (n = 3, per treatment, for both control and inoculated samples for physicochemical and microbial analysis at day 0 and day 10 and for physicochemical analysis at day 34; n = 18 per treatment, for microbial analysis at day 34). Each sample was aseptically sliced and then homogenised in a Retsch Grindomix GM200 (Retsch, Haan, Germany) for 60 sec to ensure that a representative homogenous sample was analysed.

Physicochemical analysis

Moisture content was determined according to the AOAC (2002), method 934.01. Salt content was measured using a Model 926 chloride analyser (Sherwood Scientific, Cambridge, UK) after extraction of chloride ions in 0.3M nitric acid for 2 hours as described by Goli *et al.* (2012). For water activity determination an Aqualab Series 4 water activity measurement system (Decagon Devices Inc., Washington, USA) was used at 25°C. The pH measurement was taken using a calibrated portable Crison PH25 pH meter with a glass electrode (with an automatic paired temperature reading) (Lasec (Pty) Ltd, Cape Town, South Africa) after the sample was prepared by mixing/homogenising 3 g sample in 27 ml distilled water. All analyses was performed in duplicate.

Yeasts and moulds enumeration

The biltong sample (25 g) was homogenised in 225 mL diluent (Ringers Solution) after which a dilution series was prepared. The standard ISO 21527-2 method for detection of yeasts and moulds was used. Sabouraud Dextrose Agar (SDA) with chloramphenicol was prepared and poured into petri dishes. A 0.1 mL samples was transferred and spread onto the agar. Plates were incubated in an inverted position at 25°C for four days.

Yeast and mould identification

Yeasts and moulds that appeared prominently on biltong samples were identified. They were transferred using a sterile loop onto Potato Dextrose Agar (PDA), pH \pm 5.6 and Sabouraud Dextrose Agar with chloramphenicol (SDA), pH \pm 5.6 and incubated at 25°C for four days. Yeasts and moulds were isolated and characterized for macroscopic examination of their morphological and physiological traits (Barnett *et al.*, 2000).

Statistical analyses were done using a one way ANOVA of XLSTAT® statistical software (Version 2014.2.03, Addinsoft, New York, USA). The Fisher LSD post hoc test was used to further analyse significant differences with 5% significance level ($p \leq 0.05$) as a guideline for determining significant differences.

RESULTS AND DISCUSSION

Physicochemical analyses

The physicochemical properties of biltong with different treatments is given in Table 5.1. These results show the end-product characteristics of the biltong which would have influenced the yeast and mould growth. As no significant differences were shown between day 0, day 10 and day 34 they were averaged.

Table 5.1 Physicochemical properties of biltong for each treatment (means \pm SD, n = 9)

	MC _{wb} (g/100 g)	SC _{db} (g/100 g)	SC _{wb} (g/100 g)	a _w	pH
WL 50, No Vinegar	50.2 ^a \pm 0.96	7.64 ^a \pm 0.31	3.80 ^c \pm 0.13	0.82 ^a \pm 0.03	5.74 ^a \pm 0.05
WL 65, No Vinegar	31.7 ^b \pm 1.47	7.79 ^a \pm 0.35	5.33 ^a \pm 0.25	0.74 ^b \pm 0.06	5.75 ^a \pm 0.03
WL 50, Vinegar	51.1 ^a \pm 1.21	6.26 ^b \pm 0.23	3.03 ^d \pm 0.09	0.83 ^a \pm 0.02	4.90 ^b \pm 0.05
WL 65, Vinegar	31.8 ^b \pm 1.51	6.33 ^b \pm 0.59	4.32 ^b \pm 0.40	0.75 ^b \pm 0.03	4.91 ^b \pm 0.07

wb = wet, as is basis; db = dry matter basis

MC = moisture content, SC = salt content, a_w = water activity, WL = weight loss

^{a, b, c} Means with different letters within a column differ significantly (p < 0.05)

The moisture content of the biltong were in accordance with the targeted and measured weight loss at the end of the drying. A 50% weight loss resulted in an averaged 50.7% moisture content and a weight loss of 65% resulted in an averaged 31.8% moisture content. The difference (p < 0.0001) between weight losses indicates that the biltong can be classified as two separate moisture groups which could therefore influence yeast and mould growth. The salt content being expressed in both dry and wet basis differs between formulations (p < 0.0001 and p < 0.0001 respectively) with salt content lower when vinegar was used. This could be due to the vinegar penetrating the meat faster than the salt (Capaccioni *et al.*, 2011). As expected the salt content in dry basis did not differ between weight losses (p = 0.262) showing salt impregnation in the same salting condition (with or without vinegar) is repeatable. The biltong dried to 65% weight loss have a higher salt content (as showed by the values in wet basis) than the ones dried to 50% weight loss due to the concentration of salt when further dried. An earlier study on biltong indicated no correlation between total yeast and mould counts and salt content but also no correlation with the moisture content and water activity (Van der Riet, 1976a). As a consequence of the moisture and salt contents, the water activity results show a difference (p < 0.0001) between different weight losses (WL 50 and WL 65) – biltong dried to a 50% weight loss had a a_w ~ 0.83 while biltong dried to a 65% weight loss had a a_w ~ 0.74, although the a_w did not differ with or without vinegar within the two weight loss treatments. A significantly higher pH was seen in the biltong with no added vinegar (~ 5.75) than biltong with added vinegar (~ 4.91). Their range corresponds to pH values of commercial samples (4.81 and 5.83) (Osterhoff & Leistner, 1984; Petit *et al.*, 2014). All biltong samples fall in the growth requirements for yeast and moulds.

Yeast and mould growth

As noted, the same biltong piece was used for both the control and yeast-mould inoculated samples to take into account the natural growth that may have occurred in the samples during storage. Table 5.2 shows the range of yeast and mould colonies in the biltong. The biltong was tested before inoculation (day 0), once colonies became visible (day 10) and at the end of the storage period (day 34). Due to the wide ranges noted in the log counts, it was not deemed feasible to conduct any statistical analyses on the data. The differences between the biltong produced without vinegar and with vinegar can clearly be seen therefore these will be discussed separately.

Table 5.2 Ranges for the yeast and mould counts log cfu.g⁻¹ at day 0, day 10 and day 34 of the control and inoculated biltong samples produced without and with vinegar and dried to a 50% and 65% weight loss

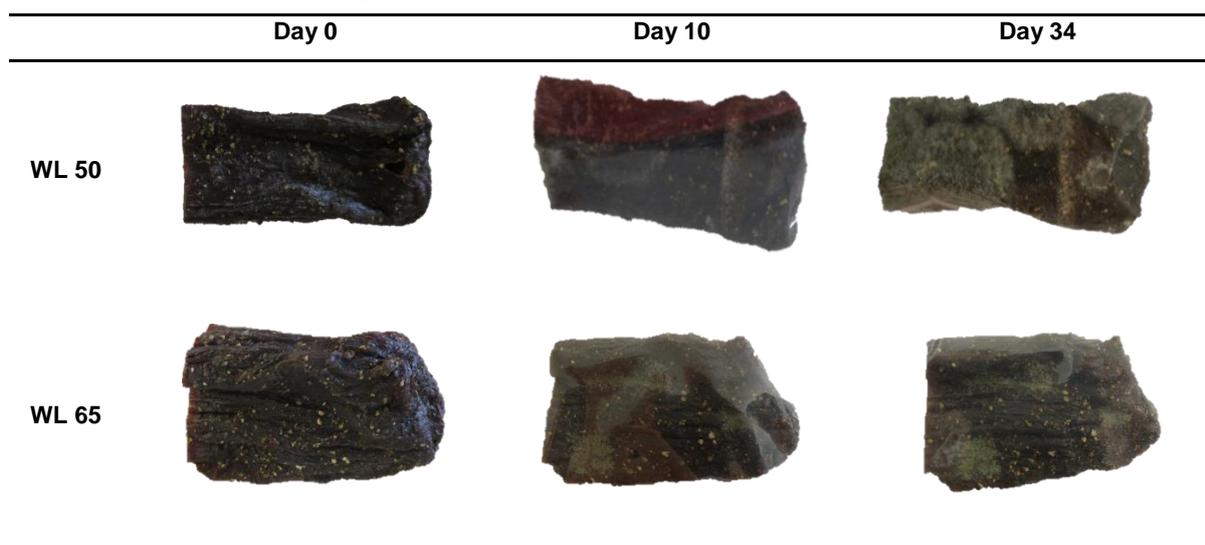
	Day 0 (n = 3)		Day 10 (n = 3)		Day 34 (n = 18)	
	C	YM	C	YM	C	YM
WL 50, No Vinegar	1.5 – 1.8	1.2 – 1.8	2.0 – 2.4	2.8 – 3.1	2.6 – 3.0	5.2 – 8.2
WL 65, No Vinegar	< 1.0 – 1.6	< 1.0 – 1.2	1.4 – 2.1	2.8 – 3.1	2.1 – 2.5	4.0 – 6.0
WL 50, Vinegar	< 1.0	< 1.0	< 1.0 – 1.1	< 1.0 – 2.1	1.4 – 1.7	2.0 – 2.5
WL 65, Vinegar	< 1.0	< 1.0	< 1.0 – 1.1	< 1.0 – 2.0	1.0 – 1.3	1.8 – 2.2

C = control; YM = yeast-mould inoculated; WL = weight loss

Regarding the difference between the biltong dried to 50% and 65% weight loss made without vinegar, on the day of inoculation (day 0), both the control and inoculated biltong samples had some growth, with low yeast and mould counts between < 1.0 – 1.8 log cfu.g⁻¹; the higher counts tended to be on the more moist and less salted biltong (WL 50). This growth was not visible and therefore could be due to the presence of yeast and mould spores. For the three weeks after production before the inoculation, the biltong was stored at low temperatures (4 ± 1°C) however some yeasts and moulds if present can still proliferate at these low temperatures (Cook, 1995), this is also dependent on the relative humidity of the storage environment, therefore the yeast and mould growth seen could be suggested to be due to the raw material and/or process contamination as the biltong samples were packaged. Moulds started to appear in some samples (WL 50, 17/21 samples; WL 65, 12/21 samples) 10 days after inoculation but this was faint. At day 10, the inoculated biltong had yeast and mould counts of 2.8 – 3.1 log cfu.g⁻¹. This is higher than at day 0 and it can be speculated that the increase is mainly due to the inoculated yeast and moulds as the growth was seen in biltong dried to a 50% and 65% weight loss while the control samples had a lower colony count ranging between 1.4 – 2.4 log cfu.g⁻¹. On day 34, the final day of the challenge test, the yeasts and moulds were seen on all the samples. The inoculated samples had a very high unacceptable count of 4.0 – 8.2 log cfu.g⁻¹ (recommended < 3 log cfu.g⁻¹) whilst the control samples had counts of 2.1 – 3.0 log cfu.g⁻¹, which would be described as within the recommended limits. Once yeasts and moulds are present they proliferate quickly if a_w not too low. A summary report by Burfoot and others (2010) concludes that commercial biltong has been found to

have up to 7 log cfu.g⁻¹ yeasts and 5 log cfu.g⁻¹ moulds. Another report found yeasts in biltong between 2 – 7 log cfu.g⁻¹ (Wolter *et al.*, 2000). These results correspond with what was found on the challenged biltong samples. A recent study on commercial biltong showed that yeast counts were observed to be high (≥ 7 log cfu.g⁻¹) in samples with high moisture content (> 35%), low salt content (3.5 – 5.6 g/100g) and high water activity (0.85 – 0.89) when compared with those (≤ 5 log cfu.g⁻¹) with low moisture content (< 25%), higher salt content (5.5 – 7.9 g/100g) and low a_w (≤ 0.68) (Petit *et al.*, 2014). The counts of yeast and mould growth in the biltong dried to a 65% weight loss (Table 5.2) is less than in the 50% weight loss biltong even though after inoculation at day 10 they were within the same range. This suggests that yeast and mould growth slows at the lower water activity resulting from the lower moisture content and higher salt concentration. Research on ground biltong challenged with moulds showed mould development to only be inhibited at a water activity < 0.70 and a moisture content of 24% (Van der Riet, 1976a, Van der Riet, 1981 cited by Van der Riet, 1982). Table 5.3 shows the growth of yeast and moulds (in the form of photos taken after visual inspection) on pieces of inoculated biltong produced without vinegar and dried to a weight loss of 50% and 65%.

Table 5.3 An example of the development of yeast and mould (day 0, day 10 and day 34) on inoculated biltong produced without vinegar and dried to a 50% and 65% weight loss



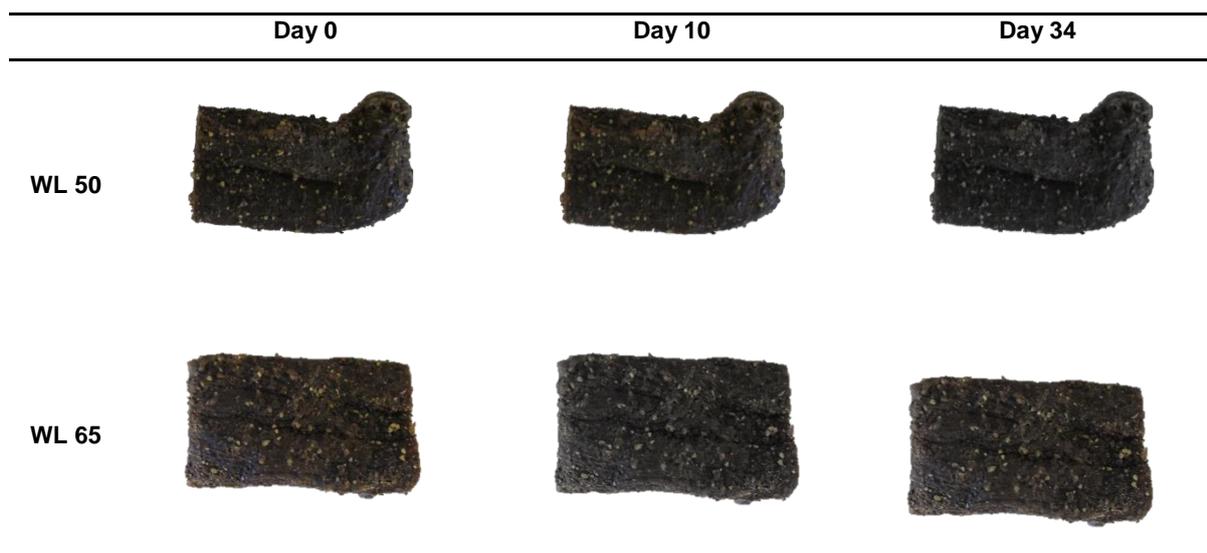
WL = weight loss

It can be seen that there is less growth in the biltong with a higher weight loss (lower a_w) which supports the results seen in Table 5.2. There are three distinctive colonies which can be seen in these examples.

Biltong with vinegar had no growth at day 0 with a small increase (< 10 – 2.1 log cfu.g⁻¹) at day 10 of the challenge test. The yeast and mould count did not change considerably as the yeast and mould count was between 1.8 – 2.5 log cfu.g⁻¹ for inoculated samples at day 34 and no yeasts and moulds were visible. The minimal yeast and mould growth is due to the use of vinegar which lowered the pH (~4.9) in spite of their lower salt content (3 – 4 g/100g) (Table 5.1) which most probably acted in combination with the moisture and salt to inhibit growth; a classical example of hurdle technology. No specific research on the effect of vinegar (lower pH) on yeast and mould growth in biltong could be

sourced. The yeast and mould counts (Table 5.2) for biltong made with vinegar are below the suggested specification of $< 3 \log \text{cfu.g}^{-1}$ and these biltong samples would therefore be deemed acceptable. This study indicates that a $\text{pH} \leq 4.91$ (and $a_w \leq 0.83$) could inhibit the growth of yeasts and moulds in beef biltong when stored in sealed packages. Table 5.4 shows the lack of yeast and mould growth on a piece of biltong produced with vinegar and dried to a 50% and 65% weight loss (photos).

Table 5.4 An example of the (lack of) development of yeast and mould (day 0, day 10 and day 34) on inoculated biltong produced with vinegar and dried to a 50% and 65% weight loss



Unlike the biltong produced without vinegar, there is minimal yeast and mould growth in biltong with vinegar and no visual signs are seen through the whole challenge test period for both the control and inoculated samples. As there was no yeast and mould counts at day 0, any growth seen was due to the yeast and mould inoculation. Therefore, a preliminary conclusion can be made that the mould growth and progression in dried biltong is more dependent on incorrect end physicochemical properties than process contamination however this still is dependent on the hygienic handling of the biltong. Surface yeast and mould growth on dry-cured meat products have been reported to be due to the raw materials quality, temperature of drying and water activity of the product (Pitt & Hocking, 1999; Mizakova *et al.*, 2002; Asefa *et al.*, 2010). It has been noted that salt is a major influence on the type of yeast and moulds that will grow on dried-cured meat products (Núñez *et al.*, 1996a; Mizakova *et al.*, 2002; Cocolin *et al.*, 2006) in addition to its water activity, production process and hygienic quality of the production environment (Mizakova *et al.*, 2002, Asefa *et al.*, 2010).

The visual inspection showed some interesting trends between treatments on the inoculated samples. Besides showing more mould growth than biltong dried to a 65% weight loss, biltong dried to a 50% weight loss had bigger colonies, this could be explained by the higher water activity of the latter. Visually, from samples in this study, green-coloured moulds were the most commonly found on the samples and tended to grow rapidly and be denser on biltong dried to a 50% weight loss. These type of moulds are unappealing to South African consumers. Small white colonies were also seen on most samples. Initially these moulds are not as unappealing as the green-coloured moulds but soon begin

to grow and become visually unacceptable. From day 14, the mould growth became prominent at the three inoculation points with colony sizes ranging between 5 – 30 mm without the colonies touching each other. Between day 18 and 20, the colonies begin to merge and started to cover the one side of the biltong over the remaining inspection period. By day 34, the majority of the surface area of the biltong was covered with mould. It is important to note that some yeasts and moulds did not grow or spread further after day 22. This occurred in the biltong dried to a 65% weight loss and could be because yeast and mould growth slowed at a low water activity. Some biltong pieces had full yeast and mould coverage over the storage period giving a good representation of the types of yeast and mould present on the beef biltong while other samples had minimal growth. Figure 5.1 and 5.2 give a few examples of biltong showing different yeast and mould growth.

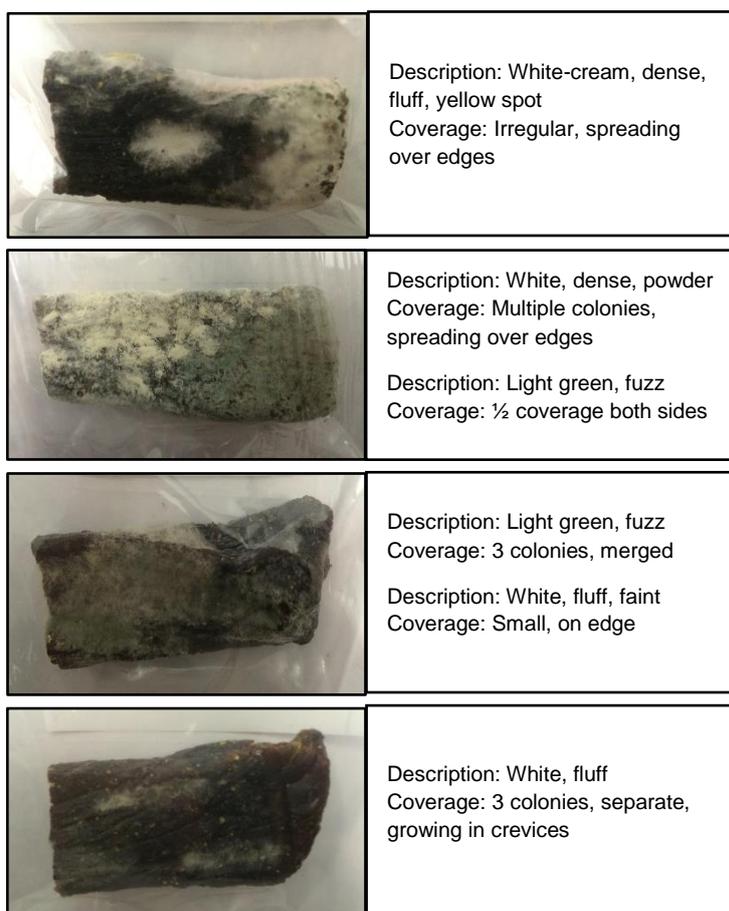


Figure 5.1 Examples of beef biltong produced without vinegar and dried to a 50% weight loss at the end of the challenge test (day 34) – photo (left) and short description (right).



Figure 5.2 Examples of beef biltong produced without vinegar and dried to a 65% weight loss at the end of the challenge test (day 34) – photo (left) and short description (right).

Once the moulds became noticeably visible (two weeks after inoculation), the moulds grew rapidly. Therefore, taking into consideration the period before inoculation, the biltong was produced 5 weeks prior to this yeast and mould growth. A couple assumptions can be made at this point. If biltong is contaminated during production (processing/drying/packaging), mould growth will only be seen after ± 14 days and the product will either already be in shops or purchased by the consumer. Another assumption is that if the biltong is tested after production and has yeast and mould counts, it will only be visible after the first 14 days even if faint which has a negative implication for the biltong producers as yeast and mould growth results in increasing production costs and products losses (undesirable economic impact for producers) (Asefa *et al.*, 2010).

The biltong was packaged for the duration of the challenge test which also is a factor in the results seen. The packaging used was double-layered polyethylene terephthalate (PET) packaging which is impermeable to oxygen and moisture. Air has been described as the main source of yeast and mould spores contaminating dry-cured meat products (Battilani *et al.*, 2007; Sorensen *et al.*, 2008, Asefa *et al.*, 2010). Therefore the packaging plays an important role in yeast and mould growth as it creates a barrier between the product and external environment and therefore slows yeast and mould growth and prevents further contamination.

In the whole production process the main areas of concern would be to ensure the raw material is not contaminated, the tumbling (damage to the meat pieces will create crevices which are ideal for yeast and mould growth), drying (controlled parameters and good air flow) and packaging (type of packaging material and method) and handling of the product. It has been observed that yeasts and moulds are commonly found in the environment of the drying chamber and due to the fact that there is

a reasonable air flow created to dry the biltong, this may be a concern for contamination. Similarly, it is important to ensure that the air being blown over the drying biltong pieces is clean (Asefa *et al.*, 2010). It would be of interest to also evaluate/identify the colonies found in the drying chamber environment. Storage is not a source of contamination if packaged and controlled correctly. Any yeasts and moulds seen on biltong after packaging was a result of contamination during the production process. With this information it can be predicted what is responsible for the contamination based on the growth patterns of the yeasts and moulds.

Yeast and mould identification

Yeast and moulds have typically low counts initially but if present, will increase over time and dominate the microbial profile of dried meat products (Cook, 1995). This is because of their ability to survive in a wide range of water activities unlike other spoilage microorganisms. Some yeast and moulds can adapt to their environment and therefore to properly identify what yeast and mould is commonly present in biltong is important, as this could help understand yeast and mould growth in biltong. Earlier studies on commercial biltong have found many different species of yeasts and moulds from the genera *Candida*, *Saccharomyces*, *Debaryomyces*, *Torulaspora*, *Rhodotorula*, *Sporobolomyces*, *Trichosporon* (yeasts) and *Aspergillus*, *Penicillium*, *Eurotium* (moulds) (Van der Riet, 1976a; Van der Riet, 1982; Wolter *et al.*, 2000).

In this study, the moulds are more predominant than yeasts which is contradictory to the previous research conducted on yeast and moulds in biltong. This could be because the biltong was produced under more controlled processing parameters and due to higher water activities and salt contents of the samples (Table 5.1) as water activity and salt influence yeast and mould growth on dried-cured meat products (Núñez *et al.*, 1996a; Mizakova *et al.*, 2002; Asefa *et al.*, 2010). The two yeasts that were isolated were similar in shape, colour and smell and were identified to be from the genus *Saccharomyces*. Other studies have found *Debaryomyces hansenii* (species of the yeast genera *Saccharomyces*) and *Candida zeylanoides* (species of the yeast genera *Candida*) to be prominent in biltong (Van der Riet, 1976a; Van der Riet, 1982; Matsheka *et al.*, 2014). *Debaryomyces hansenii* has been seen to be the most common yeast species in dried-cured products (Jessen, 1995; Núñez *et al.*, 1996b). This is due to its ability to survive in low to intermediate water activities (Dillon, 1998). This has also been found abundantly on biltong (Wolter *et al.*, 2000).

Dried-cured products often have visible mould growth on the surface of the product with *Aspergillus*, *Penicillium*, *Fusarium*, *Eurotium* and *Mucor* being frequently found on these products (Cook, 1995; Asefa *et al.*, 2009; Sonjak *et al.*, 2011). Some moulds may produce mycotoxins (Van der Riet, 1976a) and therefore the identification of moulds is important. In the current study, the moulds identified belong to the genera *Aspergillus*, *Penicillium* and *Fusarium*. Studies have shown that *Aspergillus* spp. is the predominant mould with the second most favourable being *Penicillium* spp. found in biltong (Van der Riet, 1976a; Van der Riet, 1982). These moulds have also been predominantly found dried-cured hams (Núñez *et al.*, 1996a; Wang *et al.*, 2006; Asefa *et al.*, 2009). This was also seen in this investigation. *Aspergillus glaucus* is often present and does not produce mycotoxins and therefore not considered dangerous to consume. However it is often misidentified as *Aspergillus flavus*

which is an aflatoxin producer which is toxic to humans (Van der Riet, 1976a, Van der Riet, 1976b; Van der Riet, 1982). *Penicillium* spp. are frequently isolated from meat surfaces as well as environmental samples (Asefa *et al.* 2010). Certain strains of this mould are not toxic and at low levels can be consumed. Many studies have described a relationship between *Penicillium* spp. and production facilities on dry-cured meat products (Núñez *et al.*, 1996a; Palmas & Meloni, 1997; Asefa *et al.*, 2009) and with similarities between dry-cured meat products and biltong, this relationship could also exist between *Penicillium* spp. and the production facilities of biltong. Another genera of mould identified was *Fusarium* (a white-cream colour, with a fluff/wool-like texture which spread rapidly). This mould is linked to production/air contamination and grows rapidly in both dry and moist conditions. A white mould was also isolated but could not be identified. However, a second opinion from an expert regarding these identifications would be advisable. Further analyses (molecular microbiology such as DNA sequencing) for identification of species would be necessary for yeasts and moulds with moulds having a high possibility of mycotoxin production.

CONCLUSIONS

Many factors influence the growth of yeast and moulds in a food product. This study highlights the ability of biltong produced with different formulation and dried to different weight loss to support yeast and mould growth with artificial inoculation imitating contamination. The yeasts and moulds multiply rapidly which was seen particularly in the biltong without added vinegar. The water activity and salt content was also seen to have an influence on yeast and mould growth. This information is useful to the industry for if yeast and mould contamination does occur, their development could be estimated. It would be of interest to look at yeast and mould growth after opening of the product as the packaging in this study was sealed until the completion of the challenge test. The use of DNA sequencing would enable a better conclusion regarding these yeasts and moulds as this method can identify yeasts and moulds on a species level rather than just the genera as was done in this investigation.

The biltong produced was acceptable for the first two weeks after inoculation after which the moulds dominated the surface of the biltong samples. The occurrence of yeasts and moulds in high counts after 34 days indicates possible risks associated with biltong consumption. This study proposes the dominant genera of yeasts and moulds present in biltong after production due to contamination during packaging and storage. It could be suggested to use other alternative strategies/hurdle technologies to avoid this contamination, however further investigation is needed.

The use of novel innovative technologies is becoming popular in the industry to improve processes such as salt diffusion/curing (Siró *et al.*, 2009; McDonnell *et al.*, 2014), drying (Başlar *et al.*, 2014) and microbial inactivation (Piyasena *et al.*, 2003) which would be beneficial for the biltong industry. By using ultrasound as a hurdle to inhibit microbial growth (particularly yeast and moulds) this could also reduce biltong processing time.

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CHAPTER 6

PILOT STUDY: EFFECT OF ULTRASOUND-BRINING ON THE SALTING AND DRYING KINETICS IN BEEF BILTONG PROCESSING

ABSTRACT

This study investigated the effect of ultrasound on the salting and drying kinetics during the processing of beef biltong. Biltong is a salted/dried South African meat product. Biltong was produced by using brining instead of the usual dry-salting of meat strips followed by drying. Ultrasound, used in the meat industry to enhance salt penetration amongst others, was applied during the brining phase. Its impact on mass transfer during both salting of beef and its subsequent drying were studied. Contrary to what was expected, the ultrasonic-brining did not have an effect on either the salting or drying kinetics.

INTRODUCTION

Biltong is a South African salted and dried meat product traditionally produced by salting/spicing beef meat strips followed by air drying in ambient conditions/environment for up to two weeks (Van der Riet, 1982). As methods have developed over the years and biltong is produced on an industrial level, temperature-controlled drying chambers (20 – 35°C) are now used and this allows to reduce processing time to a few days. This method is still time consuming for the industry and can result in an unevenly dried product.

Ultrasound (US) is one of the emerging technologies that are currently being investigated as a method of food processing. It has been used in food research as a tool to both accelerate mass transfer and reduce microbial growth (Davies *et al.*, 1959; Piyasena *et al.*, 2003; Awad *et al.*, 2012; Alarcon-Rojo *et al.*, 2015; Caraveo *et al.*, 2015). The ultrasound process as a novel technology for the food industry is less energy-intensive than other techniques and therefore more environmentally friendly and cost-efficient (Cárcel *et al.*, 2007a). Studies have been conducted on the use of ultrasound in the processing of fruits and vegetables by assisting air drying or as a pre-treatment in liquid in order to reduce air drying time (Cárcel *et al.*, 2007b; Sabarez *et al.*, 2012). It has also been used in assisting the brining process of cured meat products to accelerate salt uptake and diffusion (Cárcel *et al.*, 2007a; García-Perez *et al.*, 2007; McDonnell *et al.*, 2014a; Siró *et al.*, 2009).

Ultrasound mechanisms are most pronounced at the solid to liquid interface, but also have effects within the meat matrix as ultrasound can affect liquid-uptake due to protein and myosin denaturation. Pressure fluctuations that occur due to soundwave cycles cause mechanical squeezing and releasing of the sample. In meat brining, this may lead, during salting, to better impregnation of the brine within the meat. Ultrasound has also been shown to cause increased inter-myofibrillar spacing

in post-rigor processed meat which could aid mass transfer (McDonnel *et al.*, 2014a; McDonnel *et al.*, 2014b).

Salting of biltong is usually done using a dry-salting technique or using an acidic-liquid spice mix (Taylor, 1976; Van der Riet, 1982; CSIR, 2001; Naidoo & Lindsay, 2010) often helped by tumbling at the industrial scale. For adequate salt penetration and spicing of the biltong these techniques are labour-intensive and time-consuming. Brining could be considered as a method of salting for biltong as it is commonly used in curing of meat products. Therefore it would be of interest to assess the efficacy of the ultrasound to improve biltong processing as it could accelerate mass transfer during brining and the meat product matrix, thus affecting the drying time.

With this pilot study, this innovative technology will allow for preliminary results to be given regarding the processing (salting and drying) of biltong. If this proves successful further investigation could be done on microbial inactivation in biltong using this technology. Therefore the aims of this pilot study was to evaluate the effect of ultrasound on brining of beef meat in comparison with static brining and to determine whether ultrasound had an impact on drying thereafter.

MATERIALS AND METHODS

Raw meat preparation

Beef meat (*M. semimembranosus*) was cut parallel to the muscle fibre into approximate 40 mm x 30 mm x 50 mm pieces. A combined total of 54 samples, each weighing 60.0 g (± 6.26) were used. The pH of raw meat muscle was on average 5.55 (± 0.06). Each meat piece was blotted to remove excess moisture and weighed before each treatment.

Salting kinetics

This method was adapted for this study as described in McDonnel *et al.*, (2014a). All equipment, such as the brining containers, solutions and ultrasound apparatus were kept at 5°C. The salt kinetics trial investigated six treatment groups for each brining technique (static- and ultrasound-brining), namely 10 min, 20 min, 30 min, 40 min, 60 min and 90 min. Each treatment group consisted of three replicates for each technique. For the brining treatments, meat pieces were placed in individual 2.5 L plastic containers using a plastic grid support for the meat piece. These meat pieces were then left for the allotted time period. For US treatments, a high intensity ultrasonic processor (VCX 750; Sonics & Materials. Inc., Newtown, CT, U.S.A) was used at a set frequency of 20 kHz with an electrical power of 750W. All treatments were applied at 5°C, using an ice-waterbath to keep the temperature constant. Temperature was monitored using a thermometer probe. A 200 g/L water salt solution was used as the brining solution at a 1:15 solution/meat mass ratio accordingly. After each brining treatment, the sample was rinsed in water and blotted dry with absorbent paper before being weighed again.

Drying kinetics

Brining was conducted at 10°C with the same salt solution (200 g/L) and ratio (1:15) using 60 min with or without ultrasound, using the same procedures as with the previous trial except for the ultrasound apparatus (REUS, Contes, France). The ultrasonic frequency was at a set 24 kHz with an electrical power of 200W. Each treatment group consisted of three replicates for drying and one meat piece which was used for the determination of moisture content and pH after brining (before drying). Salt content was determined after drying and was used to calculate salt content after brining. All treatments were conducted on the same day.

The drying occurred 12 hours after completion of the brining treatments to allow for the salt to settle. All meat pieces were placed in a controlled drying chamber at parameters of 30% relative humidity at 30°C until a 50% weight loss was reached as most commonly done in the industry. The pieces were each weighed before and during drying every hour.

Analytical methods

After brining or drying, meat pieces were minced in a blender and samples were taken for analysis. Moisture content was determined according to the AOAC Method 934.01 (2002). Salt content was determined using a Model 926 chloride analyser (Sherwood Scientific, Cambridge, UK) after extraction of chloride ions in in 0.3M nitric acid for 2 hours.

Data analyses

The following calculations were used to determine the weight loss (WL), salt gain (SG) and water loss (WaL) during brining on a g per 100 g initial meat mass.

$$WL = \frac{m_{initial} - m_{final}}{m_{initial}} \times 100$$

$$SG = \left(SC_{final} \frac{m_{final}}{m_{initial}} \right) - SC_{initial}$$

$$WaL = MC_{fresh\ meat} - MC_{final} \frac{m_{final}}{m_{initial}}$$

The m indicates the mass of the sample (g), SC is the salt content and the MC is the moisture content expressed in g/100 g, wet basis. Initial describes values before brining, and final describes values after brining.

The drying process was monitored every hour calculating the moisture content (MC) expressed as g per g dry matter.

Statistical analyses were done using a one way ANOVA of XLSTAT® statistical software (Version 2014.2.03, Addinsoft, New York, USA). The Fisher LSD post hoc test was used to further

analyse significant differences. A 5% significance level ($p \leq 0.05$) was used as guideline for determining significant differences.

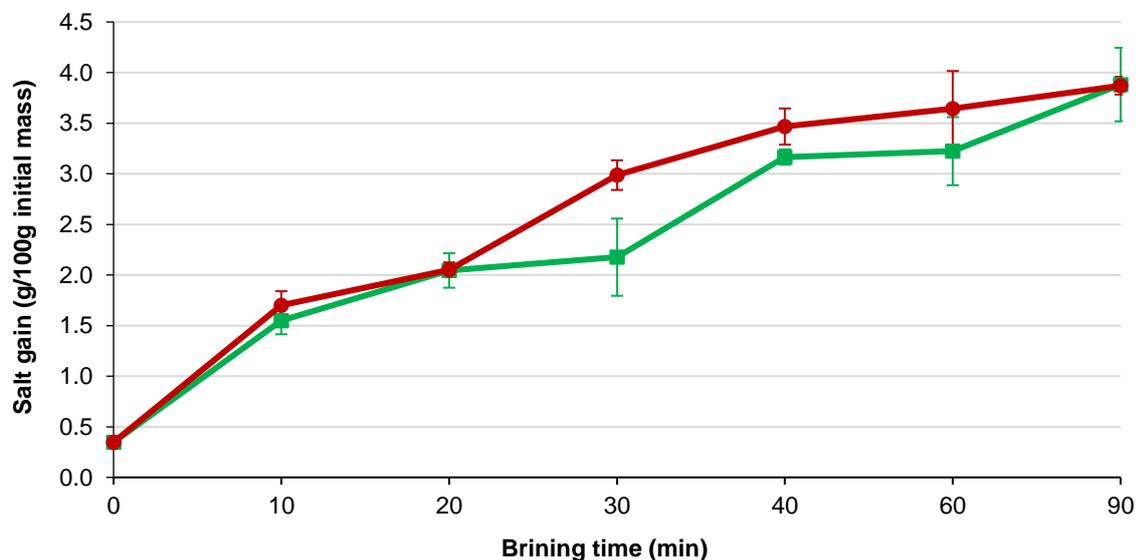


Figure 6.1 Salt gain in beef meat over time (n=3) using static- and ultrasonic-brining.

—■— without US —●— with US

RESULTS AND DISCUSSION

Impact of US-brining on salting kinetics

The salting kinetics during brining of the beef is depicted in Figure 6.1. There is only a significant effect of US on salt gain at time 30 min and 40 min which have a higher salt gain than the static-brining treatment but this is not confirmed by water loss and weight loss. In fact, no significant differences between static and ultrasonic-brining were seen in the weight loss and water loss results (not shown), they do however correspond with the salt gain results. Both static- and ultrasonic-brining treatments reached a salt gain of 3.9 g/100 g initial mass after 90 min. Biltong usually has a salt content ranging between 4 – 8% (wet basis, wb) (Osterhoff & Leistner, 1984; Petit et al., 2014). Therefore, with these parameters, a 60 to 90 min treatment would be sufficient for biltong as the salt gain before drying, which is close to 3.5 – 4%, will allow for an approximate salt content after a 50% weight loss drying to be within the usual range of biltong's salt content. Further brining time would result in a higher final salt content after drying.

Impact of US-brining on drying kinetics

A 60 min brining treatment was used when investigating the impact of US treatment on drying. The salt content before drying for each treatment confirmed there was no impact of US on salt uptake during brining – 3.4 g/100 g \pm 0.24 (wb) with ultrasonic-brining and 3.1 g/100 g \pm 0.28 (wb) without US. These values are slightly lower than with the previous trial which is probably due to differences in raw material.

The drying kinetics of beef biltong after brining are depicted in Figure 6.2.

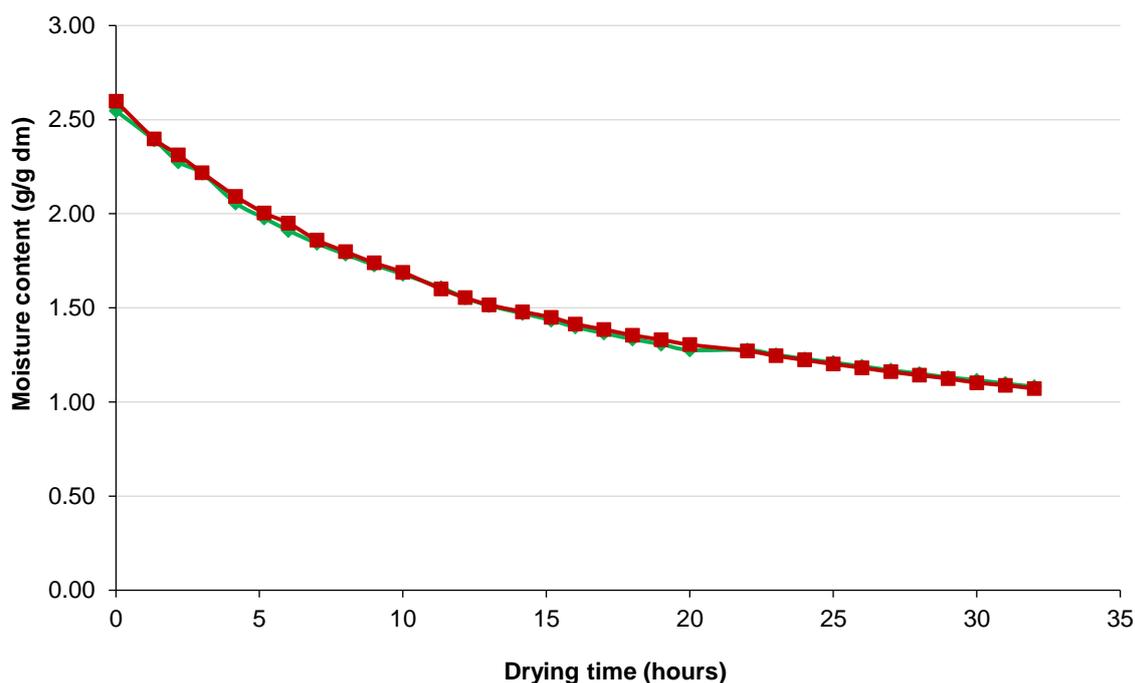


Figure 6.2 Moisture content (dry basis) of beef over drying time (n= 3) treated using static- and ultrasonic-brining.

—♦— without US —■— with US

Drying kinetics showed that the brining process of the meat before drying does not influence the moisture content consistency with a moisture content (wb) decreasing from an initial ± 72.0 g/100 g wb to ± 47.8 g/100 g wb for both treatments. Therefore, the ultrasound treatment of the meat before drying does not influence the drying time as there were no differences between the samples. Both static brining and ultrasound treated samples took 32 hours to reach a 50% final moisture content.

CONCLUSION

Ultrasound had a no impact on mass transfers during brining in our conditions contrary to what was showed in other studies. However, it could be interesting to study the impact of ultrasound on homogeneity of salt distribution compared to both static brining and usual dry-salting methods. Furthermore ultrasound could influence the microbial load of meat and thus the shelf-life (microbial stability). It has also been shown to tenderise meat and therefore ultrasound could also be used to assist in adding flavour. The penetration of spices added in the ultrasound bath could also be improved which could influence the flavour and microbial stability of the final dried product. With increasing popularity of biltong and the increase of production, the use of novel techniques such as ultrasound (brining-assisted or not) to reduce the salting and drying time and to still produce a good quality product, should be explored. Therefore further experiments as mentioned should be undertaken in this field.

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CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The biltong industry has become an economically important sector in the South African meat industry and has the potential for further growth. With increasing popularity both locally and worldwide, the demand for biltong is high. This research contributed to the current knowledge of biltong processing with the potential for future exportation. Quality and consistency in biltong is important to both the consumer and the industry. There is no research on the effect of different processing methods on the physicochemical characteristics and microbial stability and therefore this study undertook a few common processing methods to determine this.

There is limited research on the process and/or quality of this unique southern African product. This dissertation contributes to the small library of literature focussed on traditional South African biltong. Much of the current literature at hand focusses on a collection of commercial samples and their profile thereafter, or on the inoculation of biltong with pathogenic organisms to discuss the possible inhibition of these due to the biltong process. The research in this dissertation looked at the biltong which was produced using specific factors and parameters and the effect of these on the drying kinetics, physicochemical and microbiological profile of biltong so as to bridge the gaps of knowledge regarding the production and shelf-life of South African biltong. The results will also assist industrial biltong manufacturers as they can use this information to evaluate their systems as well as form the basis for future research in biltong production. Therefore, results obtained will benefit both academia as well as the food industry on a commercial level.

Before the physicochemical and microbiological properties could be determined, the drying of biltong was investigated. The results showed that, using specified drying parameters (temperature, relative humidity, air velocity), the use of vinegar in the salting/spicing step did not influence the drying of beef biltong. This part of the study benefits the industry as using the investigated process parameters, the drying times of biltong can be predicted with confidence of a consistent end-product. An additional trial was also conducted whereby the different meat and muscle types (lean beef topside, lean beef silverside, fatty beef topside, lean gemsbok topside) were used, this showed that fatty beef biltong and lean gemsbok biltong have different drying kinetics and that lean beef topside closely resembled the drying kinetics of lean gemsbok topside. This additional study should be repeated for different game species and muscles using a larger number of samples to establish whether there is a difference between the varying game species commonly used.

The second study involved using the same processing and drying procedures as in the first trial. This study showed the effect of vinegar addition, weight loss (50%, 65%) after drying and packaging method (vacuum packaging, modified atmospheric packaging) on the physicochemical properties and growth of microorganisms over a three month shelf-life. From this study, it was confirmed that using moderate drying parameters resulted in biltong with consistent physicochemical properties (moisture and salt content, water activity and pH). It was also seen that the microbial growth had reached the acceptable limits as stated in the South African National Standards (SANS 885:2001) at

the end of the three month storage period independent of weight loss and packaging method. Both spoilage and pathogenic microorganism counts were within the SANS 885:2011 regulations but further studies would need to be conducted to confirm the necessity to lower the limit of yeast and mould counts which was suggested in the committee draft. A more extensive shelf-life assessment, to assess the microbial profile of the biltong after the recommended three month shelf-life, would also be of interest. This will allow for a microbiological baseline to be determined and more intensive studies to be conducted such as methods to improve the shelf-life and if pathogens are present, developing methods to eliminate pathogens in biltong.

The following study involved a challenge test where the development of yeasts and moulds in beef biltong was inspected. The inoculum used had a low concentration which was not representative for the study. This could have been improved as the inoculum should have been more concentrated by adding more of the mixed yeast and moulds from the contaminated biltong. However, the results obtained indicated that if biltong was contaminated during processing, the growth of yeast and moulds is rapid. Yeast and mould growth on samples with vinegar occurred although these organisms were not visible. The yeast and moulds were identified on a genus level under the supervision of an experienced microbiologist. Future research should include identifying the species of yeasts and moulds commonly found in beef biltong through DNA sequencing. This would be beneficial to the industry. A study investigating the mould growth throughout the production process (at various stages) would also allow confirmation of assumptions made in this study. This research applies to beef biltong using the same drying methods as was done previously in this study.

Overall, the use of vinegar has been established to be useful in the biltong production process and therefore should be added to the spice formulation and the drying parameters tested resulted in a consistent product which was safe for consumption.

Even though the use of ultrasound did not result in the expected outcomes (no effect on the salting and drying of beef biltong), further research in this area would be beneficial as no microbiology was done on these samples and if it were to decrease or inhibit microbial growth, this would be useful to the industry.

Limitations

The results from this study will contribute to the scientific evidence that is lacking in these areas of biltong production although there were some limitations which need to be noted. The research trials were conducted in a biltong factory and therefore limited facilities were available and trials could only be conducted at specified times according to the factory's operating hours. This research does not take into account other drying parameters limiting the results to be based on these alone, however, the parameters used in this study were based on what the industry uses. To a certain extent, the microbiological profile could be considered a limitation in this study as only one type of biltong produced in a single factory was assessed however various factors (formulation, weight loss and packaging) were included. Further limitations on this study is the lack of available research on spoilage microorganisms in biltong. The importance of the salt content of biltong is also realised (study limitation), therefore expanding on this in the study could have explained some of the discrepancies seen in the results.

Another limitation, as previously mentioned, is that this study focussed on beef biltong while the biltong industry uses the meat from various species (domesticated and wild). This was briefly touched on in the drying kinetic studies where gemsbok meat was used.

Recommendations

One of the objectives of the study was to assess the process of biltong production. It has been suggested in literature that to minimise risk in biltong it should be produced using the following guidelines (Leistner, 1987):

1. Meat with no *Salmonella* spp. or other organisms that can survive the production process should be used.
2. Use of 0.1% potassium sorbate to inhibit Enterobacteriaceae and mould growth thereby reducing hygienic risk.
3. The water activity should be rapidly decreased to <0.80 by salt addition and the drying process.
4. During processing, good hygiene is essential as biltong is eaten in the raw state.

It would be advised for South African legislation to include processing guidelines such as this for biltong. Recommendations for the suggested guidelines, based on the data obtained from this research, could read as follows:

1. To avoid cross contamination, the factory must be separated into two divisions, the “wet” factory (raw meat preparation and spicing) and the other division being the “dry” factory (post-drying and packaging of the biltong). The drying chamber can act as the gateway between the two divisions as this is frequently found, in meat processing facilities, where hygiene and minimising cross contamination is of utmost importance.
2. Raw meat must be supplied by a certified meat supplier who provides a certificate of analysis proving it is hygienic (containing no pathogenic microorganisms).
3. If vinegar is used in the process, a 5% level (wet basis) would be suggested.
4. If there is a wait period between processes, the meat must be stored at low temperatures ($\leq 5^{\circ}\text{C}$).
5. The temperature and relative humidity must be monitored throughout the drying process.
6. Moisture content and water activity after drying,
 - a. Biltong should not have a water activity > 0.85 .
 - b. An approximate moisture content of 35 – 55% should have a water activity ≤ 0.85 .
 - c. An approximate moisture content of $\leq 35\%$ should have a water activity ≤ 0.78 .
7. The salt content after drying should not exceed 8% (dry basis).
8. The pH of biltong (beef or game) after drying should not exceed pH 6.

Ideally a few extra guidelines could be added which are not recommended from the results obtained from this research but rather from literature and observations made in the industry which include stating that no preservatives are to be used and for biltong to be packaged using modified atmospheric packaging (higher level of nitrogen gas-flushing). These guidelines are a suggestion and based on the results from this study and other literature, but further research to “test” these is recommended.

This research clearly states the typical physicochemical properties of beef biltong and its microbial profile based on these physicochemical properties. As discussed, the assumed shelf-life of biltong is three to six months. Based on these results, it would be suggested that a three month shelf-life limit is realistic. However, this would need to be confirmed with further studies. It would be advisable for every biltong processor who starts producing on a large scale for numerous outlets to have their own in-house testing facility. The setting up of this lab would be beneficial and not considered too cost- or labour-intensive. This was demonstrated when a microbiological laboratory was set up at a large-scale biltong factory (see the Addendum).

In regards to the exporting of biltong to major international markets, these results indicate that biltong is suitable for exporting with specifics to the water activity and the processing methods. Verification studies would still need to be implemented for exporting to the United States of America but the theory and trials which are necessary to conduct those tests can be based on some of the results (spoilage and pathogenic microorganisms) seen in this study.

Future research

There are many avenues which could be explored to increase our knowledge on biltong. Some examples of future research include:

- Salt/brining in biltong – a study could include investigating the penetration of salt into the meat during salting/after tumbling and after drying (this was seen throughout the study to be of importance); the effect of tumbling on salting; the effect of salt form (surface area to volume ratio; fine or coarse) on salt penetration; final salt content when using a dry-salting method versus a salt-vinegar method; and salt precipitation on the biltong surface during storage;
- Qualitative study on consumer preferences;
- The use of preservatives in the industry, actual levels and their consumer acceptances;
- Quality of biltong produced using fresh/frozen meat, different muscles and cutting parallel/against the grain;
- Shelf-life of biltong at different environmental conditions (particularly temperature and relative humidity), packaging and once opened;
- Descriptive sensory analysis and consumer panels on the acceptability of biltong produced using different spice formulations (levels of salt, vinegar and other spices) and the effect of varying drying parameters on the sensory profile of biltong.

These areas of research also apply to game biltong production. Game biltong brings another level of complexity as the pH in the meat depends on animal stress when harvested which could have an effect on the biltong quality in terms of toughness, flavor and drying time. The shelf-life of game biltong is also an issue as the harvesting conditions can play a role on this as could the physical properties of the meat itself.

Other queries raised by some biltong factories were to look into the microbiological counts of wildlife harvested, testing for STEC (Shiga toxin-producing *Escherichia coli*) bacteria, its origin and how it can be destroyed, improving the aesthetic value of biltong, investigating the factors that cause

toughness in biltong and the improvement of the shelf-life of biltong without changing its traditional profile.

Using the knowledge gained from the challenge test it would be interesting to look at the production process in various commercial factories and confirm where these points of contamination for yeasts and moulds are. Then Critical Control Points (CCP's) can be added to the Hazard Analysis and Critical Control Points plan as a point of possible contamination where preventive steps can be taken (preservative/vinegar “spray”, cleaning stations, covering the meat between processes, etc.).

The importance of this research was to increase the scientific knowledge on South African biltong; raise an awareness on the lack of research in this area to academia; and to assist the biltong industry. It also provides an overview for future studies on biltong. This research was not done to prove policies and regulations specific to biltong processing and production but to act as a preliminary guideline with suggestions for improvements that could possibly be implemented. Such suggestions include adding processing guidelines into legislation and adding yeast and mould limits to the existing microbiological guidelines.

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ADDENDUM

IMPLEMENTING A MICROBIOLOGY LABORATORY IN A BILTONG FACTORY

Biltong is increasing in popularity both locally and internationally. With this increasing popularity the industry has expanded its large scale manufacturing. The quality of biltong, both from a physicochemical and microbial view are important to producer and consumer alike. Biltong production involves cutting the meat into strips, salting/spicing and drying to a desired weight loss. Biltong is a wide spectrum product (type, size, spicing, drying, moisture content, packaging, etc.) and many biltong factories produce more than just traditional biltong (whole slabs and sliced) but also other similar products such as thin snack sticks, biltong crisps, small biltong nuggets/bites and flat biltong wheels/discs as well as droëwors (dried sausage). These can be made using beef, ostrich and a variety of game (e.g. Springbok, *Antidorcas marsupialis*; Gemsbok, *Oryx gazelle*; Kudu, *Tragelaphus* and Eland, *Taurotragus oryx*) meat. Another aspect to consider are the range of spices used which include traditional salt, pepper and coriander; barbeque; chilli and chutney. The biltong process is labour and time intensive which will influence the microbial quality and therefore the shelf-life the product.

Biltong is considered a ready to eat (RTE) product that has a long shelf-life of between three to six months. As a RTE product, the microbiological safety of the final product before leaving the factory is crucial as there is no other step that could inhibit/decrease the microbial load before consumption. The biltong industry typically employs the use of independent microbiology laboratories to test for spoilage and pathogenic organisms. Although it is praiseworthy to have an independent evaluation, this can become expensive and thus larger biltong producers are advised to have their own in-house testing facility which will also allow for results to become available more rapidly. This would benefit the biltong producer as results will be direct, tests can be conducted more frequently and the process can be continually analysed.

This report lays out the microbiological specification for biltong, the setting up of a standard microbiology laboratory, sampling and methods used for basic analyses for a laboratory implemented in a biltong factory and the necessary training needed for running a working laboratory.

BILTONG MICROBIOLOGICAL LEGISLATION

Before an in-house microbiology laboratory for biltong producers can be set up the legislation must be noted so as to ensure the microbiology laboratory will be capable of conducting the required analyses. If the factory is a supplier for a food chain, the customer's specifications and regulations must also be taken into account if they differ in any way from the national standards.

The South African National Standards (SANS) 885:2011 outlines the regulations for 'Processed Meat Products'. The legal microbiological requirements for biltong are listed under Class 3 subsections. These regulations state that the biltong should not exceed a total viable count $> 6 \log \text{ cfu.g}^{-1}$, with minimal presence of pathogenic microorganisms, *Staphylococcus aureus*, $< 3 \log \text{ cfu.g}^{-1}$; Salmonella

spp., 0/25 g; *Listeria monocytogenes*, < 2 log cfu.g⁻¹ and *Escherichia coli*, < 1 log cfu.g⁻¹). The committee draft regulations suggest yeast and mould and coliforms to be included at a limit of < 3 log cfu.g⁻¹ and 2 log cfu.g⁻¹ respectively. For these regulations, implementing a basic microbiology laboratory at a fully functioning biltong factory would be appropriate.

MICROBIOLOGY LABORATORY SETUP

The objective of a microbiology laboratory is to estimate the qualitative and quantitative measure of microorganisms present. In this case to ensure the quality and safety of the product and the production process. The following microorganisms are tested:

- Spoilage microorganisms – Total Viable Counts (TVC), coliforms, yeasts and moulds
- Pathogenic microorganisms – *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, presumptive *Escherichia coli* (*E. coli*)

The need to test for specific microorganisms is based on the product type, raw materials and process. Further confirmation can be given by conducting these basic tests in combination with biochemical tests. An independent microbiology laboratory may (and should) still be used if confirmation of results is needed.

The microbiology laboratory should have an area whereby the media is prepared, an area where the testing/analysis of samples is done, an incubator and counting area, a washing area and a storage area. The minimal requirements for a functioning microbiology laboratory will be described. The laboratory should be in close proximity to the factory so that sample collection is quick and cross contamination is limited. The room should be temperature controlled (air-conditioned) and be maintained at 25°C. The workbenches constructed should be approximately 90 cm (height) and 95 cm (width). The sink is to be located at the water point in the far corner of the laboratory. There must sufficient electricity outlets and a gas outlet. The gas tank should be located outside the laboratory but accessible. In a small laboratory such as one in a biltong factory, the storage can be cupboards below the work surfaces with allocations for media, equipment, supplies and tools being made. It must be noted that these guidelines are for a fully functioning biltong factory and therefore some necessities (thermometers, pH meter, water activity meter, etc.) are already available and do not need to be acquired. The layout of a basic microbiology laboratory that has been implemented at a functioning biltong factory is at the end of the Addendum (Figure 1).

Equipment, glassware and consumables

The initial setup of the microbiology laboratory requires an extensive list of equipment and consumables. A reliable equipment supplier with experience in laboratory setups should be used. The quantity indicates the minimum requirement for the daily running of a small microbiology laboratory but will be dependent on the work load of the laboratory. The estimated costs are based on prices from July/August 2015. Calibration certificates and manuals are to be kept for each piece of equipment purchased. A suggested list of equipment, glassware and consumables is given in Table 8.1.

Table 8.1 Equipment, glassware, consumables and other necessities needed for the initial setup and running of a microbiology laboratory with estimated prices and total costs

Equipment	Units	Price per unit	Quantity	Total costs
Autoclave 45 L w/ baskets	1	R 45 500	1	R 45 500
Stomacher 400 Circulator 80 - 400 mL	1	R 67 500	1	R 67 500
Incubator single display w/o fan 53 L	1	R 19 800	1	R 19 800
Waterbath basic w/ lid 29 L	1	R 13 400	1	R 13 400
Precision Balance Scale 750 g to 0.001 g	1	R 8 900	1	R 8 900
Bunsen Burner LP Gas 13 mm	1	R 110	1	R 110
Vortex mixer w/ cup head and flat head	1	R 3 800	1	R 3 800
Pipettes P20G, P200G, and P1000G	1	R 8 200	1	R 8 200
Water distiller 4L/cycle	1	R 5 000	1	R 5 000
Standard refrigerator	1	R 7 000	1	R 7 000
Glassware				
Media bottles 500 mL	10	R 450	1	R 450
Media bottles 1000 mL	10	R 600	1	R 600
McCartney bottles w/ caps 28 mL	144	R 1 600	2	R 3 200
Measuring cylinder graduated 1000 mL	1	R 250	1	R 250
Beaker 250 mL	10	R 200	1	R 200
Dispenser bottle top simplex 5 - 50 mL	1	R 4 000	1	R 4 000
Consumables				
Pipette tips N/F yellow 10-200 UL (with holder)	1	R 600	1	R 600
Pipette tips N/F yellow 10-200 UL	1000	R 150	2	R 300
Pipette tips N/F blue 200-1000 UL (with holder)	1	R 600	1	R 600
Pipette tips N/F blue 200-1000 UL	1000	R 200	2	R 400
Stomacher bags 400 Circulator	500	R 450	1	R 450
Petri dishes - aseptic 90 mm	500	R 725	4	R 2 900
Loop wire insert 10 µL	25	R 550	1	R 550
Scalpel sterile blades	100	R 50	1	R 50
Gloves Latex – medium	100	R 100	1	R 100
Gloves Latex – large	100	R 100	1	R 100
Autoclave tape 12 mm x 50 m	1	R 100	1	R 100
Biohazard bags (autoclavable)	500	R 2 800	1	R 2 800

Table 8.1 continued Equipment, glassware, consumables and other necessities needed for the initial setup and running of a microbiology laboratory with estimated prices and total costs

Other				
Autoclave heat resistant gloves	1	R 2 100	1	R 2 100
Loop wire holder aluminium	1	R 150	1	R 150
"Hockey stick"	1	R 30	2	R 60
Scalpel handle	1	R 20	1	R 20
Metal spatula spoons 210 mm	1	R 80	2	R 160
Weighing boats	250	R 700	1	R 700
Measuring jug 5000 mL	1	R 280	1	R 280
Funnel glass, diameter 100 mm	1	R 80	2	R 160
Spray bottle 500 mL	1	R 60	1	R 60
Bottle cleaning brush	1	R 100	2	R 200
Rack dryer - wall mounted	1	R 2 600	1	R 2 600
Initial setup cost				R 209 300

This list gives the minimum necessary for the running of the microbiology laboratory, although for continual running and to avoid interruptions in testing it would be recommended to evaluate the expected output of the laboratory and alter the quantity accordingly. To ensure accuracy of results, it would be recommended that the microbiology employ the use of a laminar flow cabinet or PCR prep station (estimated cost for 32 inch PCR prep station with UV light, R47 500), this however is optional.

Media and reagents

The media and reagent will depend in the type of food product which will be analysed. Quality assurance certificates from suppliers should be obtained and storage and expiry instructions should be adhered to for reliable results. For biltong, analysis will include total viable counts, coliforms, yeast and moulds, *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes* and presumptive *Escherichia coli* (*E. coli*). Table 8.2 gives a list of media and reagents necessary to conduct these analyses. Media and reagents are running costs for a functioning microbiology laboratory and should be ordered regularly.

Table 8.2 Media and reagents needed for the analysis of microorganisms found in biltong with estimated prices and total costs

Media/Reagents	Units	Price per unit	Quantity	Total costs
Ringers solution, ¼ strength, tablets	100	R 480	2	R 960
Ethanol	2.5 L	R 1 540	1	R 1 540
Plate Count Agar (PCA)	500 g	R 870	2	R 1 740
Violet Red Bile Lactose (VRBL)	500 g	R 730	2	R 1 460
de Man, Rogosa and Sharpe Agar (MRS)	500 g	R 880	2	R 1 760
Sabouraud Dextrose Agar w/ Chloramphenicol	500 g	R 980	2	R 1 960
Baird-Parker Agar (BPA)	500 g	R 1820	1	R 1 820
Brain-Heart Infusion (BHI)	500 g	R 970	1	R 970
Buffered Peptone Water (BPS)	500 g	R 630	1	R 630
Rappaport Vassiliadis Soy (RVS) Broth	500 g	R 1 030	1	R 1 030
Xylose Lysine Deoxycholate (XLD)	500 g	R 740	1	R 740
Fraser ½ Broth	500 g	R 900	1	R 900
Agar Listeria Ottaviani & Agosti (ALOA)	500 g	R 1 710	1	R 1 710
Eosin Methylene Blue Agar (EMB)	500 g	R 400	1	R 400
Media/reagents cost				R17 620

STANDARD OPERATING PROCEDURES

The standard operating procedures for a microbiology laboratory at a biltong factory involves the sampling and analysis of all raw material and the final product (biltong) and the documentation thereafter. These guidelines aim to achieve efficiency, quality and compliance with the industry regulations.

Appointment and training of a microbiology analyst

For the running of a functioning biltong factory with an experienced and competent food safety team, a small microbiology laboratory can be run by an individual with the ability to be the manager and analyst of the laboratory. This individual should have the background knowledge and skills as well as appropriate training before being appointed. Training of the individual should be done internally with a member of the food safety team as well as external training whereby developments in techniques and methods can be learnt.

Sampling and analysis

Microbial growth is irregular and therefore several samples need to be taken for a good representative of the growth of microorganisms on biltong. The sampling procedure needs to be done under aseptic conditions – the analyst will use gloves to collect samples and sterile sampling apparatus (blades,

sampling bags, swabs, etc.). The sampling times will be set out according to the standing Hazard Analysis Critical Control Points (HACCP) plan of the factory. An example of a biological hazard analysis and the corresponding sampling times is given at the end of the Addendum (Table 1). Sampling is done at various steps of the biltong production such as at receiving of the meat, the cutting of the meat strips, after drying (occasionally) and after packaging. Other testing conducted on a weekly basis should include (but is not limited to): Equipment swabs (after daily cleaning and deep cleaning), factory employees (hands and boots) and water and air samples (processing areas and drying chamber). If a sample cannot be tested at the time of sampling, the sample must be correctly sealed in a suitable sterile container and stored at low temperatures (~ 4°C). Testing must be done within 36 hours of sampling.

Both spoilage and pathogenic microorganisms need to be tested to ensure a safe product. Routine analyses are done daily to test for spoilage microorganisms while pathogenic microorganisms are tested on a monthly basis. This however is just a guideline and can be altered if necessary. For microbiological analyses, ISO-approved methods are used as these have been validated and are internationally recognised. Table 8.3 shows the testing methods for the critical microorganisms and what media is to be used. An estimation of media and reagents is previously mentioned in the microbiology laboratory setup (Table 8.2). The preparation of dilutions is done according to ISO 6887-1 method using Ringer's solution (1/4 strength).

Table 8.3 Summary of ISO-approved methods and media used for basic microbiological analyses of biltong

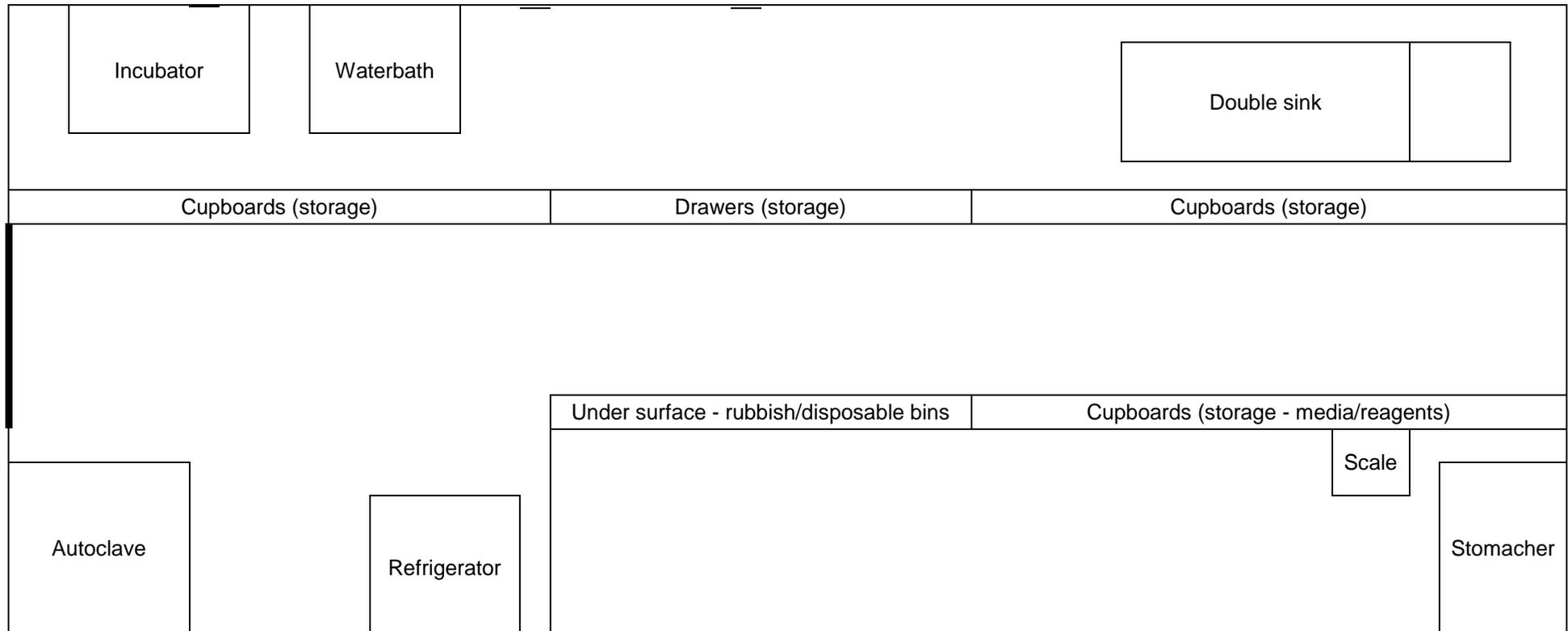
	Microorganisms	ISO-approved method	Media used
Spoilage microorganisms (Daily)	Total viable counts	ISO 4833	Plate Count Agar (PCA)
	Coliforms	ISO 4832	Violet Red Bile Lactose Agar (VRBL)
	Lactic acid	ISO 15214	de Man, Rogosa and Sharpe agar (MRS)
	Yeasts and moulds	ISO 21527-2	Sabouraud Dextrose Agar (SDA) with chloramphenico
Pathogenic microorganisms (Monthly)	<i>Staphylococcus aureus</i>	ISO 6888-1/A1	Baird-Parker Agar (BPA) Brain-Heart Infusion (BHI)
	<i>Salmonella</i> spp.	ISO 6579/A1	Buffered Peptone Water (BPS) Rappaport Vassiliadis Soy (RVS/Broth) Xylose Lysine Deoxycholate (XLD)
	<i>Listeria monocytogenes</i>	ISO 11290/A1	Fraser ½ broth Agar Listeria Ottaviani & Agosti (ALOA)
	Presumptive <i>Escherichia coli</i>		Eosin Methylene Blue Agar (EMB)

Upon completion of analyses, all testing material (petri dishes, media and samples) must be disposed of correctly (autoclaved and incinerated). A test report must be filled out which indicates who

did the analysis, the dates biltong samples were taken, analyses started and completed. The type of sample, its traceability code and purpose for analysis must be recorded. Once analyses are completed, the results in CFU/mL will be noted and if out of specification the action to taken will be recorded. The report is passed along to the food safety manager who will implement the corrective action. These reports will be filled out for both the daily and monthly testing.

CONCLUSION

The analyses conducted test for product safety as well as the cleanliness of the production process and factory. It is important, as with a factory, to carry out internal audits on a regular basis. These audits help to recognise the strengths and weaknesses of a laboratory as well as the competency of the manager/analyst. Trends can also be discussed and preventive/correction actions established to be implemented. These are also necessary for the accreditation process. Setting up an in-house microbiology laboratory does not only save time and money but is beneficial for the producer as if problems arise corrective action can be taken immediately.



- * Cupboard storage - for media and reagents, media bottles, McCartney bottles, beakers, etc.
- * Drawer storage - Pipettes, pipettes tips, loop inoculation sticks, "hockey" stick, gloves, tape, etc.
- * Door (bolded line) - left side of figure between autoclave and incubator, opens inward
- * Electricity points (double-lined)

Figure 1 Layout plan for a basic microbiology laboratory setup for a biltong factory with suggested equipment layout.

Table 1 Biological hazard analyses (including the potential hazard, likelihood, severity and sampling times) for beef and game biltong products

Process step	Potential hazard	Likelihood / Severity of hazard
Step 1: Receiving of meat	Spoilage and pathogenic bacteria growth present	LIKELIHOOD: Medium
	<ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>Listeria monocytogenes</i> absent • <i>E.coli</i> absent 	SEVERITY: High
	Due to contamination with faecal matter, environmental contaminants Hazards: Temp of meat > 5°C, meat with pH ≥ 6	Certificate of Analysis from meat supplier
Step 2: Chilling/freezing of meat	Spoilage and pathogenic bacteria growth	LIKELIHOOD: Medium
	<ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>Listeria monocytogenes</i> absent • <i>E.coli</i> absent 	SEVERITY: High
	Due to temperature of meat rising > 5 °C	
Step 3a: Removal of meat from chiller/freezer	<ul style="list-style-type: none"> • No common hazard 	LIKELIHOOD: Low SEVERITY: Low
Step 3b: Thawing (if meat was frozen)	Spoilage and pathogenic bacteria growth present	LIKELIHOOD: Medium
	<ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>Listeria monocytogenes</i> absent • <i>E.coli</i> absent 	SEVERITY: High
	Due to contamination with faecal matter, environmental contaminants Hazards: Temp of meat > 5°C, meat with pH ≥ 6	SAMPLING (EVERY 3 weeks)

Table 1 continued Biological hazard analyses (including the potential hazard, likelihood, severity and sampling times) for beef and game biltong products

Step 4: Deboning of meat (if applicable)	<p>Spoilage and pathogenic bacteria growth present</p> <ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>Listeria monocytogenes</i> absent • <i>E.coli</i> absent <p>Due to contamination with faecal matter, environmental contaminants</p> <p>Hazards: Temp of meat > 5°C, meat with pH ≥ 6, unhygienic working practices</p>	<p>LIKELIHOOD: High</p> <p>SEVERITY: High</p> <p>SAMPLING (if applicable)</p>
Step 5: Selection and cutting of meat	<p>Spoilage and pathogenic bacteria growth present</p> <ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>Listeria monocytogenes</i> absent • <i>E.coli</i> absent <p>Due to contamination with faecal matter, environmental contaminants</p> <p>Hazards: Temp of meat > 5°C, meat with pH ≥ 6, unhygienic working practices</p>	<p>LIKELIHOOD: High</p> <p>SEVERITY: High</p> <p>SAMPLING (to be taken DAILY)</p>
Step 6: Mixing of meat with spices	<p>Spoilage and pathogenic bacteria growth present</p> <ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>Listeria monocytogenes</i> absent • <i>E.coli</i> absent <p>Due to contamination with faecal matter, environmental contaminants</p> <p>Hazards: Temp of meat > 5°C, meat with pH ≥ 6, unhygienic working practices</p>	<p>LIKELIHOOD: Medium</p> <p>SEVERITY: Medium</p> <p>Certificate of Analysis from spice suppliers</p>

Table 1 continued Biological hazard analyses (including the potential hazard, likelihood, severity and sampling times) for beef and game biltong products

Step 7: Hanging meat	Spoilage and pathogenic bacteria growth present	LIKELIHOOD: Low
	<ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>Listeria monocytogenes</i> absent • <i>E.coli</i> absent <p>Due to contamination with faecal matter, environmental contaminants</p> <p>Hazards: Temp of meat > 5°C, meat with pH ≥ 6, unhygienic working practices</p>	SEVERITY: Low
Step 8: Drying meat	Spoilage and pathogenic bacteria growth present	LIKELIHOOD: Low
	<ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>E.coli</i> absent <p>Due to contamination with faecal matter, environmental contaminants</p> <p>Hazards: Temp of meat > 5°C, meat with pH ≥ 6, unhygienic working practices</p> <p>Mould growth due to temperature out of specification</p>	SEVERITY: Low
Step 9: Cutting product (if applicable)	Spoilage and pathogenic bacteria growth present	LIKELIHOOD: Low
	<ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>E.coli</i> absent <p>Due to contamination with faecal matter, environmental contaminants</p> <p>Hazards: Temp of meat > 5°C, meat with pH ≥ 6, unhygienic working practices</p>	SEVERITY: Low

SAMPLING
(AFTER drying, IF raw
meat sampling was high,
Step 5 and occasionally)

Table 1 continued Biological hazard analyses (including the potential hazard, likelihood, severity and sampling times) for beef and game biltong products

Step 10: Packing product	Spoilage and pathogenic bacteria growth present	LIKELIHOOD: Low
	<ul style="list-style-type: none"> • Total plate counts must be < 1 000 000 cfu.g⁻¹ • Coliforms < 100 cfu.g⁻¹ • Yeasts and Moulds absent • <i>Staphylococcus aureus</i> absent • <i>Salmonella</i> absent • <i>E.coli</i> absent <p>Due to contamination with faecal matter, environmental contaminants</p> <p>Hazards: Temp of meat > 5°C, meat with pH >or= 6, unhygienic working practices</p>	SEVERITY: Low
Step 11: Final packing*	Any spoilage and pathogenic bacteria growth present due to raw material, processing and/or contamination	LIKELIHOOD: Low
	<ul style="list-style-type: none"> • No common hazards 	SEVERITY: Low
SAMPLING (AFTER packaging, stored for retention/shelf-life tests)		
Step 11a: Chilling of final product (small chiller)	<ul style="list-style-type: none"> • No common hazard 	LIKELIHOOD: Low
		SEVERITY: Low
Step 11b: Chilling of final product (large chiller)	<ul style="list-style-type: none"> • No common hazard 	LIKELIHOOD: Low
		SEVERITY: Low
Step 12: Metal scanning of final products	<ul style="list-style-type: none"> • No common hazard 	LIKELIHOOD: Low
		SEVERITY: Low
Step 13: Loading and distribution	<ul style="list-style-type: none"> • No common hazard 	LIKELIHOOD: Low
		SEVERITY: Low