Extending the Shelf life of Packaged Biltong

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Abstract

Biltong is an intermediate moisture ready-to-eat meat snack that originated in South Africa. Low water activity, increased solutes content and low pH help extend biltong's shelf life. A Hamilton company, Safari Biltong, specialises in producing biltong. Its current product is manufactured to a food control plan (FCP) approved by the Ministry of Primary Industries. This FCP allows the biltong to be kept for a maximum of two weeks. The company wants to develop more markets and also to export biltong. This involves researching systems that would allow the product to retain the desirable quality and food safety for up to a year without using chemicals or preservatives. A good quality biltong has dark colouring (black to dark brown), no visible fungal growth, no bacterial populations that exceed allowable limits, water activity below 0.85, a pH around 5.3, little to no rancid flavours and a chewy consistency.

Freshly made biltong was packaged using film with low water, oxygen and carbon dioxide transmission and sealed under full vacuum (VS), partial vacuum (PV) or partial vacuum with an oxygen scavenger (PVS) then stored at 35°C, 20°C and 4°C for 46 weeks. The water activity, pH, appearance and mould spore viability was monitored periodically. Sensory evaluations were also done using an untrained panel. A second, smaller trial investigated the effect of adding a proprietary natural antioxidant on storage quality and lipid oxidation. The national lockdown due to the Covid 19 pandemic prevented some data being collected during the trial.

Water activity of biltong kept at the 35 and 20°C decreased during storage, especially in biltong kept at 35°C, where water activities decreased to as low as 0.4. The water activity of biltong stored at 4°C fluctuated around the initial value of 0.69 over the 46-week storage period. Storing under full vacuum was best for maintaining a higher water activity associated with biltong having an acceptable texture. During storage, none of the biltong had a water activity that exceeded the allowable limit of 0.85. Biltong maintained the best appearance and pH when kept at 4°C in full vacuum packs.

Colour of fresh biltong is black, which fades to a light brown colour with deterioration. This occurred the most in biltong stored at 35ºC, especially in the PV environment, which became unacceptable after 16 weeks. Biltong stored in a VS environment maintained colour best, becoming unacceptable after 42 weeks whilst biltong stored in the PVS environment was unacceptable after 24 weeks. Biltong stored at 20°C and 4°C remained acceptable throughout storage for all packaging environments, with the best colour being maintained in the VS environment.

Bacteria were not isolated from the biltong during storage. No visible mould growth was observed on the packaged biltong during storage, other than one pack with a faulty seal. Viability of mould spores decreased during storage. Viable mould spores were not detected on biltong stored at 35°C after 9 weeks storage or after 15 weeks on biltong stored at 20°C. However, viable spores were isolated from biltong stored 4°C at the end of the 46 week trial.

Sensory analysis after six weeks showed biltong stored at 35°C PV conditions was very unacceptable and that biltong stored at 4°C, regardless of packaging environment, was best. All biltong stored at 20°C was acceptable, with that stored in VS and PVS environments being preferred over biltong stored in the PV environment. Scores for sensory evaluations varied greatly and panellists could not discern differences between biltong stored in different environments.

Including a proprietary rosemary extract in the marinade decreased the rate of lipid oxidation (measured as thiobarbituric acid reactive substances) and development of rancid flavours (measured using a sensory panel). Lipid oxidation, as expected, was highest in biltong stored at 35°C and lowest in biltong stored at 4°C. The greatest oxidation occurred in the VS environment and the least in the PV environment. Further trials on the effect of the packaging environment on lipid oxidation need to be done as data did not agree with that obtained for biltong with no added extract, which showed that oxidation was greater in the PV environment.

Sensory data indicated that the quality of biltong containing the rosemary extract and packed under partial or full vacuum was still acceptable after 14 weeks at 20°C and 4°C. Adding an oxygen scavenger slightly improved quality. The acceptability of biltong stored at 35°C deteriorated quickly but this temperature could be used for accelerated storage of the effect of packaging environments and additives on biltong quality.

Recommendations for further research are given, including further investigating factors that affect lipid oxidation, using a trained panel for sensory evaluations, investigating other packaging environments and storage temperatures, and investigating the reason for variation in water activity and pH measurements.

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Glossary

- ADP: Adenosine diphosphate
- AMSA: American Meat Science Association
- ATP: Adenosine triphosphate
- Aw: Water activity
- DFD: Dark, firm and dry
- ELISA: Enzyme linked immunosorbent assay
- Eh: Redox potential
- FSANZ: Food Standards Australia and New Zealand
- FCP: Food control plan
- FSP: Food safety plan
- FV: Full vacuum
- GMO: Genetically modified organisms
- HACCP: Hazard analysis and critical control points
- IMF: Intermediate moisture food
- IP: Isoelectric point
- MAP: Modified atmosphere packaging
- MPI: Ministry of Primary Industries (NZ)
- NZFS: New Zealand Food Safety
- OTR: Oxygen transmission rate
- PCR: Polymerase chain reaction
- PV: Partial vacuum
- PVS: Partial vacuum with an Oxygen Scavenger
- RH: Relative Humidity
- SANS: South African National Standards
- TBARS: Thiobarbituric acid reactive substances
- USDA: United States Department of Agriculture
- VS: Vacuum seal
- WHC: Water holding capacity

1 Introduction

Anthropological research shows that meat has been a food source for humans for thousands of years. This nutrient-dense food provides protein (amino acids), vitamins (especially vitamin B), iron, zinc, other minerals and energy in small portions. The prime method for obtaining meat was hunting but as cultures settled on productive land, farming made acquiring meat easier and more reliable to obtain. The popularity of red meat in Western culture increased as farming became prominent (Pereira & Vicente, 2013).

Meat has numerous definitions but the most agreed definition, used by the American Meat Science Association (AMSA), is "skeletal muscle and its associated tissues derived from mammalian, avian, reptilian, amphibian, and aquatic species commonly harvested for human consumption" (Boler & Woerner, 2017).

Fresh meat is highly perishable due to its high water content and nutrient availability, which creates ideal conditions for microbial growth and chemical reactions that promote structural, sensory and colour changes. Throughout human history, many cultures have developed various techniques to prolong the shelf life of meat. Before electricity was invented, common preservation techniques included curing, drying, smoking and fermentation (Pal & Devrani, 2018).

Dried food products are shelf stable foods with a low water activity (e.g. less than 0.6) and a moisture content below 15%. It is often brittle and usually is rehydrated before being eaten. Intermediate moisture foods (IMF) are shelf-stable products with water activities of 0.6-0.84 and a moisture content between 15% and 40% (Fig 1.1). They are edible without rehydration (Barbosa-Canovas *et al*., 2003).

IMF meat products such as beef jerky, a well-known preserved processed meat product, rely on removing a lot of the water from fresh meat to obtain a product with a much longer shelf life than fresh meat. Jerky originated in the Americas, where Inca and Native American tribes in the 1500s, would cut deboned and defatted meat into thin strips, make them thinner by pounding, then rub in salt. The meat strips were then dried over a fire or in the sun (Fehrs, 2014). Another variation of dried meat is Borts, which is made in Mongolia where winters are harsh and food is scarce, making long-term food storage essential. Borts is made by hanging thin strips of meat under the roof of a tent. After a month of drying, the meat has shrunk and developed a brown colour, giving it an appearance much like wood (Mischler, 2005). The Nigerian dried meat product Kilishi is a spicy beef jerky made from wafer thin meat slices that have been coated in a spice blend containing ginger, suya pepper and cajun pepper seeds. It is then traditionally dried in the hot sun for about three days (Chinyere, 2020). Biltong, an IMF dried meat product originating from South Africa, is prepared from whole muscle tissue. Dutch settlers moving through South Africa in the 17th century developed the product so they could have an energy rich food when travelling (Jones, 2017).

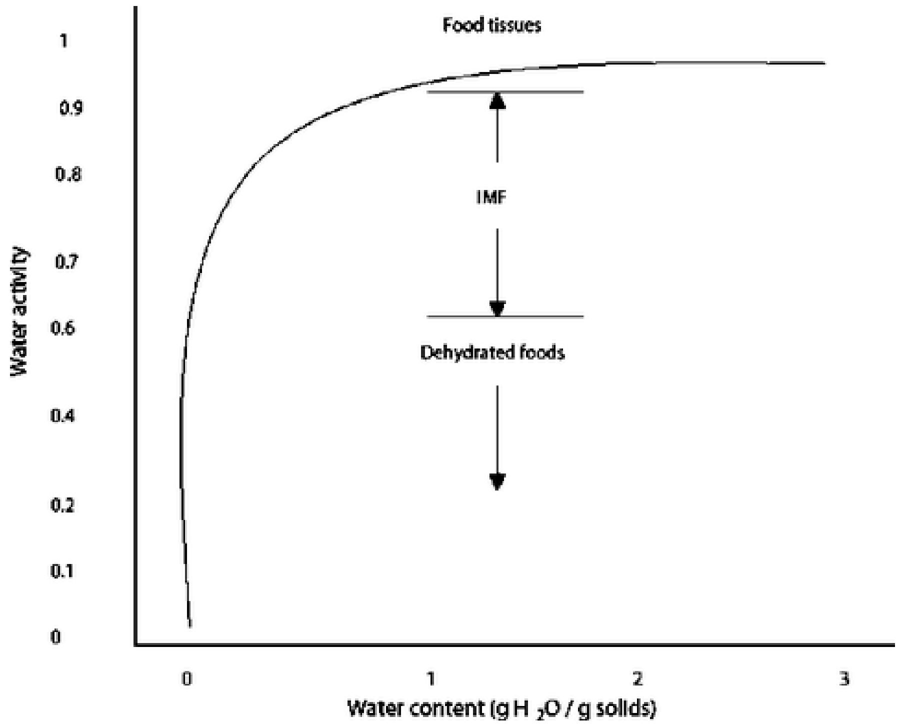

Figure 1.1. Relationship of water activity and moisture content for different classifications of dried food (Barbosa-Canovas *et al.*, 2003).

The ready availability of large-scale refrigeration and freezing means that preserving meat by drying is not as important as previously. However, the popularity of dried meat products has increased in recent times, with biltong and beef jerky being common “snack” foods across the globe. The satiating effect of proteins means small amounts of meat will satisfy hunger more than the same amount of carbohydrates. Dried meats are considered healthy snack options as they do not contain carbohydrates, sugars and are very close to their natural state (Abou-Samra *et al.*, 2011). A study found that 75% of consumers agree protein is important in a healthy diet and that a high protein snack does not compromise weight control quickly (Shaffer, 2019).

Due to the declining economy and increasing crime rates, emigration from South Africa has increased markedly since the mid-1990s. For instance, between April 2018 and April 2019, approximately 8200 South Africans migrated to New Zealand (Stats NZ, 2019), making them the fourth largest group of people New Zealand receives annually (Anon., 2019a). Because these expatriates want to have foods they are used to, traditional South African foods such as biltong are being produced in their new country. The increasing demand for South African food products in New Zealand means local businesses, such as New World, have introduced South African products in an international foods section. New companies are manufacturing the products and small businesses such as Safari Biltong in Hamilton, which specialises in manufacturing and/or selling traditional South African products, are experiencing increased demand as locals are also beginning to favour this introduced snack. Over the past three years, this company has had to expand its facilities, purchase more automated machinery, and hire more employees to meet demands (J. Bouwmann, personal communication, 2019).

Manufacturing, distributing and storing foods have the associated risks of contamination and spoilage, which can affect food safety and human health. Every year about 200,000 New Zealanders suffer from a food related illness, which is a higher rate than most other developed countries (Anon., 2019b). Most cases of food poisoning are due to unsafe handling in homes, which cannot be regulated, but food manufacturing companies use processes to minimize the risks (Holl, 2009).

To ensure food safety, New Zealand food manufacturers making or selling higher risk foods need to have a food control plan (FCP) agreed and registered with the Ministry of Primary Industries (MPI). Individual FCPs specify the processing steps that ensure that any foodstuff manufactured, distributed and sold is safe for consumption. If the strict standards and requirements cannot be met, the foodstuff cannot be sold (MPI, 2020).

Biltong is a ready-to-eat dried food product consisting entirely of meat. The MPI standards for ready-to-eat meat are (NZFS, 2019):

- Meat must be cured in conditions that minimise contamination, inhibit growth of pathogenic and spoilage microorganisms, and facilitate uniform curing (NZFS, 2019).
- The correct amount of salt must be used and spread evenly over the entire surface (NZFS, 2019).
- During curing, the temperature must be low enough to avoid spoilage and growth of pathogens while ingredients equilibrate (NZFS, 2019).
- The drying process and any additional controls must render the product microbiologically safe for its purpose (NZFS, 2019).
- The product must be dried to a water activity below 0.85 (NZFS, 2019).
- The food standards code does not provide microbiological limits for dried meat products. The operator can establish their own limits based on criteria for similar ready-to-eat meat products (NZFS, 2019).

Safari Biltong hangs its product freely in cabinets in the shop. When it is purchased, the biltong is packaged in brown paper bags designed to only contain the biltong while it is transported from the shop to the consumer's place of consumption. Safari Biltong's FCP allows it to sell biltong for up to two weeks after production. Any unsold biltong must then be disposed of. As the expiry date approaches, the price of the biltong is often lowered to encourage sales and decrease wastage. However, selling the product cheaply impacts on profitability. Extending the allowable shelf life would reduce wastage and help maintain profitability. A possible solution to extending the shelf life of biltong further is by using appropriate packaging. As well as allowing a longer time for being able to legally sell the biltong, long term packaging could create opportunities to sell biltong in other centres or even export it (J. Bouwmann, personal communication, 2019).

Chemicals and preservatives are viewed negatively in the western society and therefore, impacts profitability when incorporated. Chemicals and preservatives are often used when manufacturing biltong to help maintain sensory quality and limit microbial growth and establishment (Jones *et al.*, 2017). To increase sales, it would be beneficial to have a product free of chemicals and preservatives while still retaining safety and desired characteristics (Shafie & Rennie, 2012).

There is very little published research on the changes occurring when biltong is stored for extended periods or what occurs when it does not contain chemicals and preservatives (Jones *et al.*, 2017). Storage costs could be minimised if the biltong can be kept at ambient temperature without adversely affecting the biltong.

The aim of the research described in this thesis is to assess the effect of storage temperature and packing environment on biltong quality. The biltong will not contain chemicals or preservatives and the storage trials will be carried out for up to a year under different storage temperatures. It is hypothesised that adverse chemical, sensory and biological changes will occur more slowly in biltong stored at lower temperatures or in environments with a lower oxygen content. Safari Biltong, the company supporting this research, will use the findings of this research to explore the possibility of producing packaged biltong with no chemicals or preservatives, with a shelf life of a year. The recipe and methods developed by Safari Biltong are confidential and therefore not included in this research report.

Chapter 2 of this thesis contains a review of dried meat products, with an emphasis on biltong, and on ways to extend storage life of food products. It also includes methods to assess food product deterioration during storage. The chapter ends with the specific aims/hypothesis of the research. Chapter 3 contains the methods and equipment used and the trials carried out. The results are presented and discussed in Chapter 4 and Chapter 5 contains the conclusions and recommendations for further research.

During the research period of this thesis, the 2020 Covid-19 Level 4 isolation period occurred and access to the University and use of facilities was prohibited. The stored samples could not be accessed so data for the storage trial is incomplete. However, sufficient data was collected to draw conclusions and suggest improvements if this study was repeated or taken further.

2 Literature Review

2.1 Living Muscle

Skeletal muscle tissue of bovine animals is used for commercial biltong production. Muscle is the soft tissue of animals, arranged in bundles of protein fibres (Fig. 2.1) that allow movement.

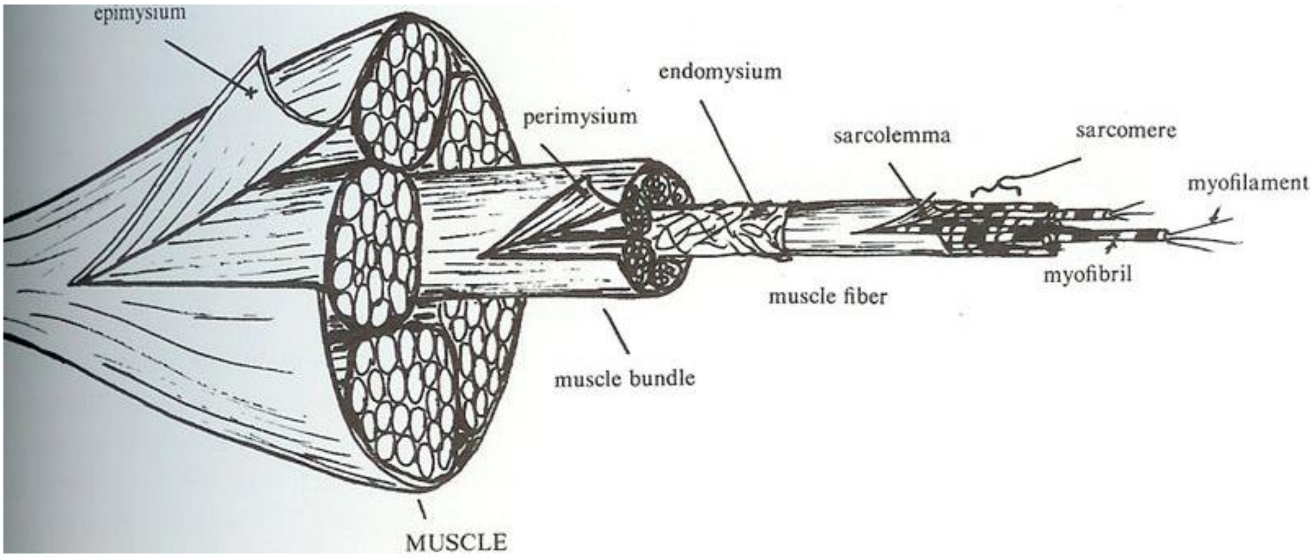

Figure 2.1. Structure of muscle fibre bundles (Baker, 2016).

The repeating sarcomere subunits make up the myofibril, which is enclosed in the sarco-lemma. Repeating myofibrils are enclosed in the sarcoplasmic reticulum creating the fascicle. Fascicles are enclosed in the epimysium creating the muscle that is converted to meat post-mortem (Lieber, 1992).

The sarcomeres contain thick myosin and thin actin proteins that are able to slide past each other (Fig. 2.2). The fibrous myosin molecules have a strong attraction to actin but are separated by tropomyosin. During contraction, calcium is released, which triggers tropomyosin movement, allowing myosin and actin to interact. Myosin ‘heads' bind to the actin filament and metabolise ATP to ADP. The phosphate released provides the energy for the head to contract. Another phosphate release triggers the head to release and bind again. This action shortens the sarcomere causing the muscle to shorten and contract.

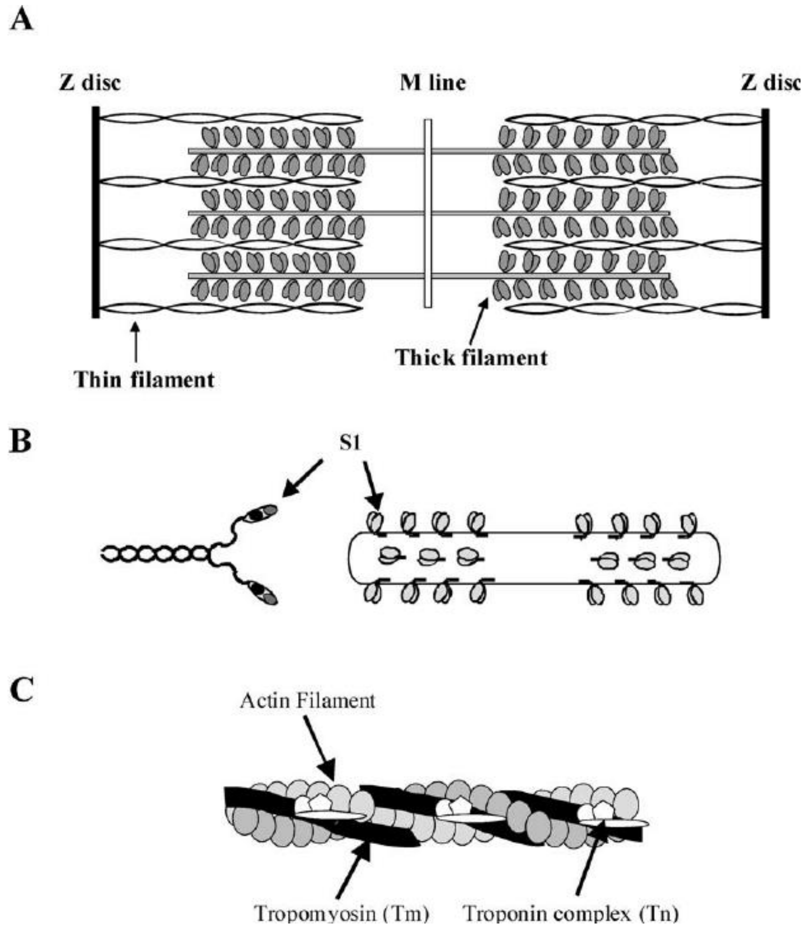

Figure 2.2. Actin and myosin arrangement and morphology (Pollesello *et al.*, 2004).

Water is essential for contraction and function of living muscle. The two mechanisms that retain water in muscle tissue both depend on the amount of space between fibres. The first is dependent on the sarcomeres. Water is held within the sarcomeres, and the longer the sarcomere the more space there is for water. The second depends on muscle pH and the net electrical charge on the muscle proteins. Like charges repel each other, creating space between the fibres. The charge is neutral at the isoelectric point (IP) of the protein. As the pH moves above or below the IP (pH of about 5.5 for most meat proteins), the amount of either negative or positive charge increases, causing greater repulsion. The greater the repulsion the greater the space and therefore the greater the water retention. This is referred to as the water holding capacity (WHC) of the muscle (Hunt *et al.*, 2011).

The pH of normal living muscle tissue is neutral (pH 7) but changes when the animal is slaughtered. After death, oxygen is not delivered to the muscle, ATP is metabolised anaerobically and lactic acid is produced, which lowers the pH. Under normal conditions, the ultimate pH of meat (when all energy reserves have been used up) is pH 5.4 - 5.5. When all the ATP has been metabolised, the actin and myosin heads cannot release and the muscle enters rigor mortis, which results in constant contraction. After several days post-mortem, proteolytic activity in protein fibres degrades the structures resulting in tenderisation (Darrel *et al.*, 2001). Leaving meat attached to the bone for a few days post-slaughter prevents the meat from shrinking. Once the actin and myosin bonds have weakened, the muscle can be removed more easily from the carcass. Electrical stimulation accelerates ATP use, hastening the onset of rigor mortis (Walker *et al.*, 1995). Electrical stimulation, which accelerates biochemical processes, and temperature control will ensure that rigor mortis and the subsequent muscle tenderisation is optimised (Devine *et al.*, 2014).

If the animal experiences stress before death, muscle energy reserves are used up before the post-slaughter biochemical reactions can occur so the pH will not fall as much, creating dry, firm and dark (DFD) meat with a pH of about 6. Modern slaughter operations diminish the possibility of producing DFD meat by reducing stress before slaughter and using appropriate slaughtering processes, such as electrical stunning (Adzitey & Nural, 2011).

2.2 Biltong

Throughout history, many different cultures have used drying as a common method for preserving meat. Jerky, biltong, charque, pastirma, odka, kilishi, qwanta and variations of salami are some types of IMF and dried meat products. These products are made from meat obtained from various animals such as beef, sheep, wild game, camels, etc. and were originally a way of extending the supply of meat before refrigeration was widely available (Domzalski, 2017).

Biltong is an intermediate moisture, ready-to-eat meat snack made originally from antelope and beef. It originated from predominantly Dutch settlers in South Africa, called 'Voortrekkers', in the early 17th century. While at sea, seafarers salted and pickled meat in wooden barrels to supply meat for the months they were unable to replenish their food resources. After arriving, these Dutch settlers found it difficult to build up livestock numbers while they were moving through the land but there were abundant numbers of wild game, which supplied plenty of meat. However, there was no means of preserving the large amounts of meat from a successful hunt. The settlers observed the indigenous peoples drying meat in the sun (Jones *et al.*, 2017).

The Cape region of South Africa had a large spice trade and French settlers produced vinegars from their vineyards. The Dutch settlers adopted the practises and ingredients from the cultures around them and used the ingredients available to develop the recipe for the biltong known today. The name biltong is derived from the Dutch word "bil', referring to the buttock of an animal, and 'tong' meaning tongue, which refers to the strips the meat is cut into (Jones, 2017). The recipe was very successful in extending the shelf life of the meat while maintaining desirable gustatory characteristics. Hence, the recipe has remained largely unchanged from the first developments (Jones *et al.*, 2017). Biltong was particularly useful during the Boer war, where soldiers were supplied with biltong and dried fruits that allowed them to stay in the field for weeks without the need to replenish nutritional resources (Lewis *et al.*, 1956).

The impact of the war and the difficulties Afrikaans (descendants of Dutch settlers) ancestors faced caused the following generations to be extremely patriotic, finding their identity in tradition. Biltong is, amongst other things, considered to be uniquely Afrikaans and an item that helped the survival of the culture. This, along with its desirable organoleptic qualities, retained the love for biltong through centuries (Grundlingh, 2019).

South African citizens migrating to other countries take their culture and traditions to their new home. Countries, such as New Zealand have a high immigrant population, providing an influx of different cultures. Overall, the New Zealand population is open to accepting aspects of the different cultures into their own. This has allowed biltong to become increasingly popular amongst the New Zealand population. For similar reasons the popularity is growing worldwide. The snack is particularly popular amongst people in physically labouring jobs, such as construction, as it provides high amounts of energy in small volumes and does not require refrigeration or cooking on site. The current New Zealand housing crisis has allowed increased numbers of trade staff to meet the demands. As a result, biltong sales are increasing as well (J. Bouwmann, personal communication, 2019).

2.3 Manufacturing Biltong

The South African settlers used ostrich and game such as kudu, springbok and gemsbok to make biltong. Later, beef became the main meat due to its abundance and the desirable characteristics of beef biltong. The most preferred cuts for making biltong are the loins directly behind the kidneys, the dorsal muscles on each side of the spine and the haunches (Lewis *et al.*, 1956).

To manufacture biltong, connective tissue and any excess fat is removed before cutting the meat into long strips parallel to the muscle fibres, which allows better salt and spice infusion. Strip dimensions vary between producers but slices typically are 20 to 40 cm long. Dimensions are extremely important as it affects drying time and biltong characteristics. If the slices are too thick, water removal may take longer than the standard time, increasing production time and cost and decreasing profitability. If the slices are too thin, the meat will dry too much, making an undesirable product. A strip of fat can be left on the edges because many consumers enjoy he fat; however, this can allow rancidity development due to lipid oxidation. Fat also increases the time for salt absorption and decreases water diffusivity (Jones, 2017).

Different manufacturers have different recipes for marinating the biltong. Meat strips are often covered in a low pH marinade containing vinegar and Worcester sauce, then coated with a blend of dry spices high in salt and left to infuse for up to a day. Marinating takes place over 24 hours at 4°C after which the salt content of biltong is between 2 and 13% (Rogers *et al*., 2004). For acceptable taste, salt content is 2.5-4% (Jones *et al.*, 2019). After being marinated, the slices are threaded onto a hook and hung to dry. In the 17th century, biltong would have been hung outside in a windy but shady area to allow moisture removal without the meat undergoing heat-induced changes (Rogers *et al*., 2004).

As biltong demand increased, traditional methods were adapted to modern operations. Airtight containers, such as drying rooms or boxes with dehumidifiers and fans, create an environment of dry circulating air at room temperature. This reduces drying time from two weeks to a few days (Jones *et al.*, 2017) and allows better process control.

Fresh meat has a moisture content of 45-75%, depending on the cut, type of meat and fat content. During the drying process, fresh biltong loses half its weight after an adequate amount of moisture has been removed (Rogers *et al.*, 2004) and will have a moisture content around 20% (Lewis *et al.*, 1956). The water activity is usually below 0.85 (Naidoo & Lindsay, 2010).

Biltong sold in stores can be classified as dry, medium or wet. Consumers prefer 'wet' biltong, which has a slightly higher moisture content and water activity between 0.80 to 0.89, and 'medium' biltong, which has a water activity between 0.7-0.79, rather than 'dry' biltong, which has a water activity of 0.65 to 0.69. Biltong with water activities below 0.65 has a dry, flake-like consistency and would not be commercially acceptable (Petit *et al.*, 2013).

2.4 Drying

2.4.1 The drying process

The drying process involves removing water from a material to produce an end-product with no or low moisture content. Drying food products have two advantages - extending shelf life (preservation) and reducing weight. The water that is removed is usually transferred to the surrounding air but can also be achieved via sublimation (e.g. freeze-drying). The drying of a material is affected by the rate moisture can be transferred from the interior to the exterior, the temperature difference between the heating medium (which usually is hot air) and the material being dried, and the amount of moisture the drying medium (for example, air) can hold. The macro- and microstructure of the material also affects drying rate (Heldman & Hartel, 1997).

There are three stages during drying (Fig. 2.3). In the settling-down period (A), the product equilibrates with the ambient temperature. In the constant-rate period (B), the rate of moisture removal is constant because the moisture being removed from the surface is being replenished by an equal amount of moisture from the interior. In the falling-rate period (C), moisture cannot be transferred from the interior to the surface rapidly enough to balance the moisture being removed from the surface (Heldman & Hartel, 1997).

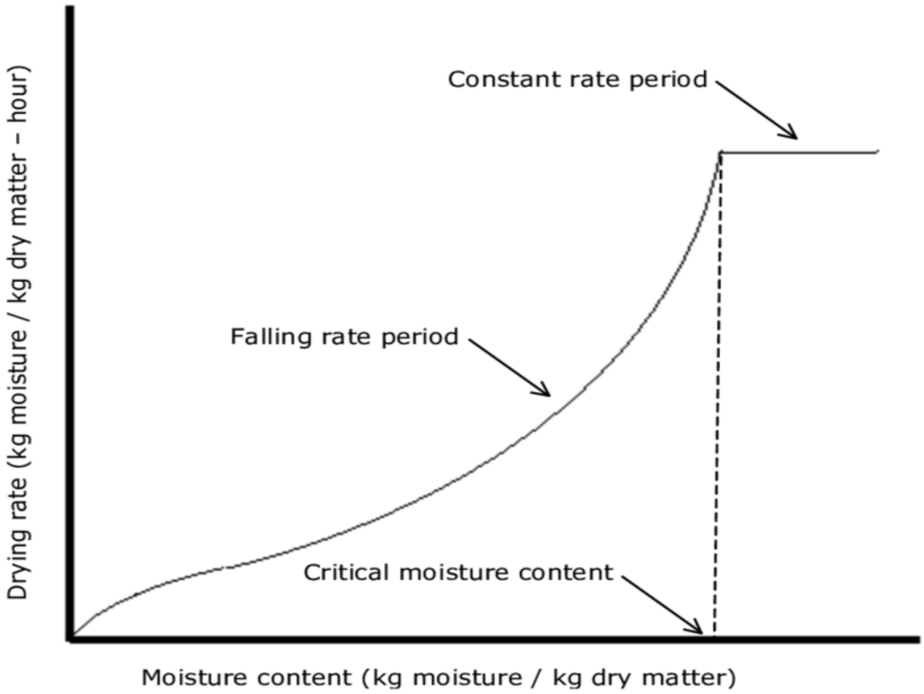

Figure 2.3. Three stages of a typical drying curve (Heldman & Hartel, 1997).

The falling rate period often has two stages and two associated moisture contents. These are the critical moisture content and the equilibrium moisture content. The critical moisture content occurs when the drying rate begins to decline and the equilibrium moisture content occurs when no more water can be removed (or gained) under the prevailing environmental conditions (i.e. drying rate is zero) because the product is in equilibrium with the environment (Diamante *et al.*, 2010).

Water molecules will move from the high concentration areas to low concentration areas. The relative humidity (RH) of the air must be significantly less than the free moisture in the meat for mass transfer to occur effectively. This difference draws free moisture from the product and into the surrounding air. After being removed from the food, the moisture is in the air surrounding the food, reducing the imbalance in RH between the air and the food. If the air is constantly moving, the saturated air is removed and replaced with dry(er) air (Fig 2.4.) (Heldman & Hartel, 1997).

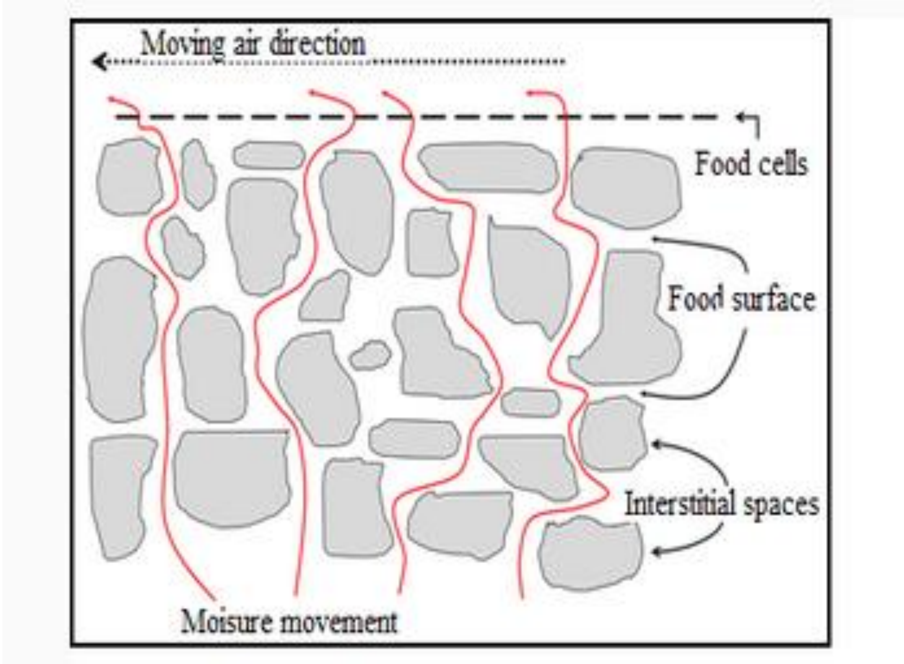

Figure 2.4. Schematic diagram of movement of moisture in the interstitial spaces of food cells during drying food (Anon., 2020).

Drying rate can be increased by increasing the difference between the vapour pressure of the air and the meat surface. Once the surrounding air becomes saturated, drying stops (and can work in reverse if the air contains more water than the meat). Warmer air can hold more moisture than cold air before it becomes saturated so increasing the air temperature will increase drying rate (Potter & Hotchkiss, 1995). Air flowrate also affects drying rate. If it is too low, air surrounding the product will become saturated and drying is inhibited. If air flow is too fast, there is insufficient time to transfer much water, leading to inefficient (and uneconomic) drying (Anon., 2020).

There is an equilibrium relative humidity for every food where the food neither gains nor loses moisture to the environment. When the environment has a higher RH than the dried food, water moves from the air to the food and the food will increase in weight (as long as it is not fully saturated). If the RH of the air is lower than the dried food, the food loses moisture until equilibrium with the immediate environment can be established (Heldman & Hartel, 1997).

2.4.2 Case hardening

If moisture is transferred from the surface of the food to the air faster than it is moving from the interior (such as when the temperature difference between the product and the air is large and/or air flow rate is too fast), the surface can start to harden, which inhibits further drying. This is called case hardening (Heldman & Hartel, 1997) and affects product sensory and microbiological quality. The outside of the product forms a hard surface (crust, leather-like texture, etc.) while the interior remains soft with a high water activity (Fig. 2.5).

Figure 2.5. Case hardened biltong (U/I_EAT_THE_FAT, 2019).

Case hardening is common in nutrient rich foods and when the initial drying temperature is too high. During the initial high temperature, solutes form and deposit on the surface creating a solid layer on the surface and preventing water being removed from the interior (Anon., 2020). Case hardening is deleterious to the texture and, more importantly, can allow microbial spoilage to occur within the product because it has not been dried sufficiently (Gulati & Datta, 2015).

2.4.3 Water in meat

Water has unique properties that enable it to be essential to life. Electrostatic charges between the positive hydrogen atoms and the negative oxygen atoms allows for strong hydrogen bonding. It can dissolve a large number of hydrophilic compounds such as proteins, vitamins and glucose but does not dissolve hydrophobic compounds like lipids. Hydrophobic interactions are crucial for the formation of cell membranes, protein folding, micelles and liposomes. Intracellular processes cannot occur without water (Lorenzo *et al.*, 2019). For these reasons, microbial cells cannot survive without sufficient water.

Meat contains free (easily removed) and bound water. Free water is defined as water in a food that acts as pure water and can be removed during the constant rate period of drying. Bound water, on the other hand, has a low vapour pressure, low mobility and a greatly reduced freezing point. The water activity of meat depends on the bound and unbound water (Table 2.1).

Table 2.1. Water activity correlation with extent of bound water (Anon., 2020).

Extent of bound water	**Water activity (Aw)**
Tightly bound water	<0.3
Moderately bound water	0.3-0.7
Loosely bound water	>0.7
Free water	~1.0

Water molecules, which are dipolar, are attracted to charged species such as proteins. The WHC of meat indicates the ability of meat to trap water within the three-dimensional structure of the muscle. The minimum WHC occurs when proteins are at their IP and carry no charge. The equal number of positive and negative charges maximises the number of bonds thereby decreasing the space for water (Huff-Lonergan, 2010). The IP for meat proteins is pH of 5.0-5.5. This is achieved in biltong production by adding a low pH marinade containing vinegar (Jones *et al.*, 2017).

2.4.4 Water activity (Aw)

Water activity in food is the ratio between vapour pressure of the food in an undisturbed balance with surrounding media, and the vapour pressure of pure water under identical conditions (Anon., 1984). A water activity of 0.80 means the food item has a vapour pressure 80% that of pure water under the same environmental conditions. The water activity affects microbial growth and chemical and enzyme reactions in food associated with browning and lipid oxidation along with particle caking and food texture (Fig. 2.6).

Most bacterial species likely to establish on foods and be a human health concern require a water activity of 0.85 or higher to grow. Some bacterial species, like halophiles, can grow at lower water activities and mould and yeast can proliferate at water activities as low as 0.60 (Jones, 2017). Most food items have a water activity above 0.95, which supports microbial growth (Anon., 1984). A preservation factor that is very effective for extending shelf life of foods is having a low water activity.

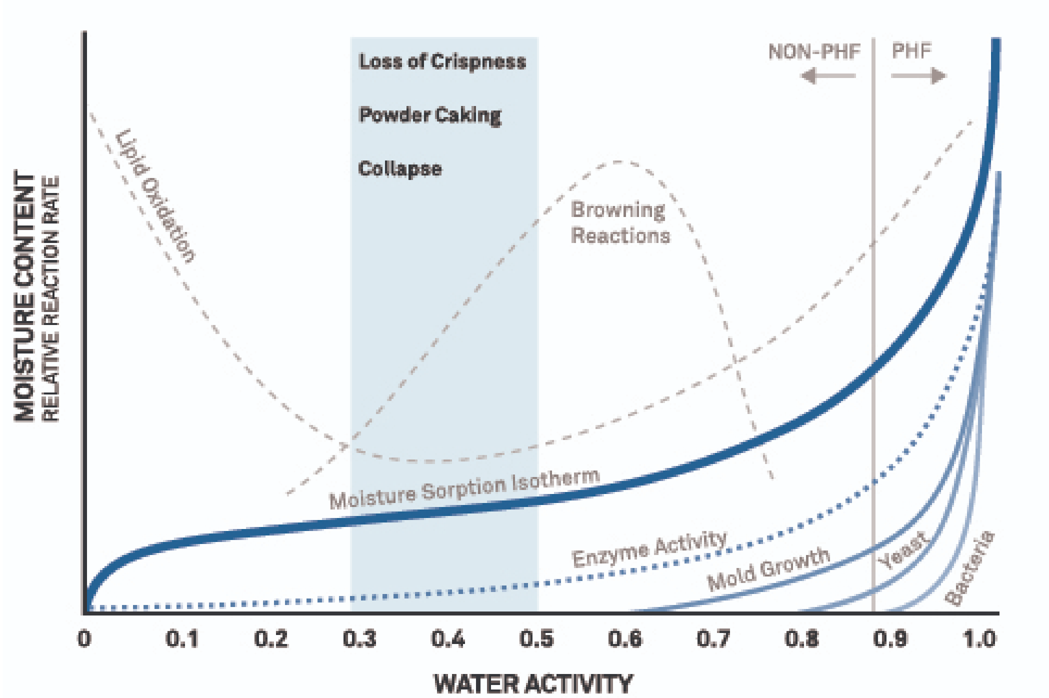

Figure 2.6. Effect of water activity and moisture content on microbial growth limits chemical reactions in foods (Galloway, 2020).

Microbial cells get water from the growth medium through osmosis at the cell surface. For this to happen, there must be a water activity gradient where the moisture outside the cell is greater than inside the cell. If there is more water within the cell than the growth medium, water moves out of the cell to equilibrate the concentration of solids. Under these high solute conditions, the cells become dormant but can germinate once water is re-introduced (Anon., 2017a).

Dried meat product manufacturers produce products with various moisture levels. The legal requirement in many countries is a water activity below 0.85 to ensure product safety. The low water activity makes water re-absorption likely if the surrounding air contains more moisture. If this occurs, microbial populations can re-establish, thereby spoiling the product. Water re-absorption can be prevented by using water impervious, sealed packaging (Jones, 2017).

2.5 Microbial Quality

Microbial metabolism of organic material to recycle nutrients is a naturally occurring process that is essential in the environment. However, when the biological material is important for human/animal use, microbial growth is considered as spoilage. It is estimated that a quarter of the world's food is lost to microbial spoilage (Blackburn, 2006).

Materials high in water content and nutrient availability, like most food products, are susceptible to microbial spoilage. A product is spoiled when qualities of a food item has been changed to an undesirable state and/or the safety of the food has been compromised. Spoilage can be caused by bacteria or fungi. Bacterial spoilage is more common as bacteria tend to outcompete fungi in ideal conditions. However, the diversity of fungi allows them to grow when the environmental conditions inhibit bacterial growth (Odeyemi *et al.*, 2020).

The following describes microbes commonly found on the surface of biltong.

2.5.1 Bacteria

Bacteria are unicellular prokaryotic organisms that are not visible to the naked eye. Pathogenic bacteria can cause illness when consumed, either from their growth or from the toxins they produce. Toxins, which can be formed before the food is consumed or within the body after consuming the bacterial cells, cause adverse reactions within the body or due to the body's immune response to foreign material within the system (Albrecht & Sumner, 1992). The best preventative measure against pathogenic bacteria establishing on food items is to maintain a low water activity.

Listeria monocytogenes (*L. monocytogenes*) is a Gram-positive pathogenic bacteria that causes listeriosis. People particularly at risk are pregnant women and the immunocompromised, with a mortality rate of 24% amongst infected people (Farber & Peterkin, 1991). This non-spore forming bacterium optimally grows at water activities of 0.97 but has been detected at water activities of around 0.9. Cells can also survive for extended periods at water activities around 0.81 and are tolerant to high solute conditions. Survival is temperature dependent and increases at lower temperatures. Under normal conditions, the optimal growth temperatures for *L. monocytogenes* are between 30 and 37°C (i.e. body temperature) but it can grow between -1.5 and 45°C, meaning growth can occur at refrigerated temperatures. It also can grow over a broad pH range of 4.0 to 9.6. Although growth below 4.0 has not been detected, *L. monocytogenes* is thought to be able to tolerate more acidic environments. Growth is best in anaerobic conditions but it is fully capable of aerobic growth (FSANZ, 2013).

Salmonella is a non-spore forming, Gram-negative, facultative anaerobic bacillus that can grow between 5 and 37°C with an optimal range of 35-37°C. It grows best at a pH between 6.5 and 7.5 but can survive between pH 4 and 9. *Salmonella* spp. are sensitive to water activity and cannot establish at levels below 0.94 (Graziani *et al.*, 2017). This microbe infects the body through ingestion and then multiplies in the intestines, where it produces an enterotoxin that causes inflammation and diarrhoea. It can enter the blood and lymphatic system, causing more severe illnesses. Death from *Salmonella* spp. infection is very rare but possible (Bell & Kyriakides, 2009).

Escherichia coli (*E. coli*) is a Gram-negative, non-spore forming, facultative aerobic rod-shaped bacteria commonly found in animal and human intestines. There are no published reports of growth below a water activity of 0.9 (Albrecht & Sumner, 1992). This bacteria is commonly found in undercooked beef and can cause many illnesses such as haemorrhagic colitis and uremic syndrome due to shiga toxin production (Gonthier *et al.*, 2008). Not all strains of *E. coli* are pathogenic but the O157:H7 strain is highly pathogenic. This strain can survive refrigeration and freezing (has a growth range of 4 to 45°C with an optimum of 37°C) and has an unusual tolerance to acidic environments (growing at pH values as low as 3.7).

Staphylococcus aureus (*Staph. aureus*) is a pathogenic Gram-negative, non-spore forming, halotolerant bacteria that is a part of the human body's microbiota in the upper respiratory tract and on the skin. It grows between 7 and 48°C, with an optimum of 37°C, and can grow in salt concentrations up to 15%. Its optimal pH is neutral but growth still occurs between pH 4 and 10. It produces an enterotoxin that causes severe food poisoning. In low water activity conditions, its intracellular concentrations change to match the surrounding environment, which prevents cell rupture from osmotic stress (FSANZ, 2013). This microbe can grow under pH stress so lowering the pH does little to reduce its growth. Although *Staph. aureus* is a facultative aerobe, growth is much slower under anaerobic conditions (Jones *et al.*, 2019). It is a poor competitor species but due to its wide range of growth conditions, *Staph. aureus* is able to flourish unchallenged where other microbial populations are inhibited (FSANZ, 2013).

2.5.2 Fungi

Fungi can be defined as a diverse group of eukaryotic single-celled or multinucleate organisms that absorb organic material in or on which they grow. The group consists of mushrooms, moulds, mildews, smuts, rusts and yeasts (Dictionary.com, 2020). Mould and yeast species typically establish on food items, inducing spoilage because the visible fuzz and white, black, orange, green, red or brown powders or slime makes the food item unpalatable or unsafe (Pitt & Hocking, 1997).

Because fungi can grow at water activities as low as 0.6 as well as over a wide pH and temperature range, it is difficult to inhibit fungal growth. Bacteria usually outcompete fungi, which can limit establishment of fungi. However, when the water activity is below 0.85, bacteria cannot grow fast enough to outcompete fungi, allowing the fungi to thrive (Jones, 2017).

Candida is a polymorphic yeast that can occur as individual oval yeast cells, pseudohyphae or hyphae. It can also form biofilms that strongly adhere to the surface of the growth medium

(Aryal, 2018). This facultative anaerobic opportunistic yeast targets anaerobic environments in the body such as the gastrointestinal tract. The doubling time at 25°C for *C. albican* is 2 hours (Ahrens *et al.*, 1983). The species can grow over a broad pH range, from below 2 to above 10, which increases the chance of a successful opportunistic infection (Dumitru *et al.*, 2004). In restricted nutrient conditions, amino acids are metabolised to ammonia and *Candida* have been recorded to raise the pH from 4 to near pH 7 in 12 hours and induce morphogenesis (Vylkova *et al.*, 2005).

Penicillium species cause food spoilage by producing blue-green powdery mould on the food surface. They form spores that easily contaminate food, equipment, bench surfaces, surrounding air and employee's hands. Many *Penicillium* spp. produce mycotoxins, which can cause severe illness when ingested, but it is not a common species on foods (Munkvold *et al.*, 2019).

Aspergillus can colonize many foods. They thrive, or at least tolerate, a wide range of temperatures and reduced water levels. Their ability to produce mycotoxins such as aflatoxin creates a hazard to consumers. A study in Tunisia, on sorghum, found that isolates grew best at 37°C at a water activity of 0.99. The minimum water activity for growth at 15°C was 0.99. Aflatoxin was not produced below a water activity of 0.91 or 15°C (Lahour *et al.*, 2015).

2.5.3 Microbes on biltong

The water content of biltong is very low, limiting the type of bacteria that can establish on the surface. The interior of the product remains sterile until it comes into contact with air. Commercial biltong purchased from different stores in South Africa and Botswana had total viable counts up to 7 log cfu/g, Enterobacteriaceae and coliforms up to 4 log cfu/g, yeasts up to 7 log cfu/g, moulds up to 5 log cfu/g, lactic acid bacteria up to 8 log cfu/g, and *Staphylococci* up to 8.5 log cfu/g (Jones *et al.*, 2017). The species likely to cause the greatest health risk to people consuming the biltong were *L. monocytogenes*, *Salmonella* spp., *Staph. aureus* and *E. coli* O157:H7 . Yeasts and moulds are more likely to only cause spoilage, and hence affect appearance and acceptability.

Legal requirements for biltong in South Africa include a maximum total viable count of 6 log cfu/g; *E. coli*, *L. monocytogenes* and *Staph. aureus* should be below 1, 1.3 and 2 log cfu/g respectively and *Salmonella* spp must be absent in a 25-g sample (SANS, 2011). These regulations do not specify yeast and mould levels but committee recommendations are that they should not exceed 3 and 2 log cfu/g respectively (Jones *et al.*, 2019).

Because bacteria are very sensitive to low water activity, properly prepared biltong is usually not adversely affected by bacteria (Jones *et al.*, 2017). For instance, *Staph. aureus* enterotoxins are not produced at water activities below 0.83, which is achieved by drying biltong to 50-65% weight loss (FSA, 2013). The other bacterial species are inhibited at water activities below 0.85.

Because biltong has a water activity below 0.85, microbial spoilage is usually due to growth of yeasts and moulds rather than bacteria. Germinating spores form visible mycelium before the product reaches the end of its shelf life. A study in Botswana identified 55 different fungi species on the surface of commercially sold biltong. Yeast of the genus *Candida* were the most prevalent species with *C. catenulata* and *C. pararugosa* being the most dominant. The next most isolated species were the filamentous *Aspergillus* and *Penicillium* moulds, with 17 from the *Aspergillus* genus, eight *Candida* and seven *Penicillium* species. The most prevalent *Aspergillus* species were *A. restrictus* and *A. niger* (Matsheka *et al.*, 2014).

3.5.4 Microbial testing

Common methods for microbiological testing of food include growing on culture media, immunoassays, and polymerase chain reaction tests. Culture media tests are simple, cost effective and do not require advanced equipment (Alam, 2020). This method involves extracting microbial cells from the sample then incubating them at an optimum temperature on a nutrient rich agar. Generic conditions are usually used for isolating unknown species (i.e. growing at 25-35°C on an undefined agar) but the media and growth conditions can be adapted to encourage growth of specific species. Results can be both qualitative and quantitative.

The well-known solid-phase enzyme-linked immunosorbent assay (ELISA) detects the presence of a ligand in a sample by using antibodies for the macromolecule of interest. The antibody-antigen relationship indicates the presence or absence of the macromolecule. The antigen is immobilized on a plate and coated with a non-specific protein to bind unbound sites. An enzyme-bound antibody and a substrate are then added and any unbound antibody-enzyme complexes are then washed off. Enzyme substrate is added, which is converted to a coloured product indicating the presence of antibody-antigen complexes. This method can also be used quantitatively when serial dilutions are graphed in a standard curve. This method is accurate, reasonably simple and cost effective. However, to exploit the antigen-antibody binding, the microbe of interest must be known and the specific antibody/antigens and enzymes for the reaction are needed (Alam, 2020).

Polymerase chain reaction (PCR) detects the concentration of DNA or RNA in a sample belonging to the microbe of interest. The PCR amplifies the DNA of any microbes present. These DNA strands are separated and bound to primers (short specific nucleotide chains) specific for the microbes of interest. The bound complexes are separated by size using gel electrophoresis. The size of the electrophoresis gel bands indicates the size of the DNA fragments which can be matched to microbes thereby, identifying microbes in a sample (Pepper & Gerba, 2020). Although the PCR assay can quickly determine the presence or absence of specific microbes it also does not indicate the viability of the cells or which microbes outcompete other populations after being in storage (Alam, 2020).

Culture media is most appropriate for a study investigating the microbial changes in a product such as biltong during an extended storage time. The method is cheap, requires minimal equipment and resources, does not need an advanced skill level to complete, and can be used to identify unknown microbes and their numbers in a food sample. When the microbes are unknown, undefined media is best as it is nutrient rich and likely to meet the growth requirements of most microbes. Defined media, on the other hand, may lack some nutrients because it is for growing specific microbe species (Alam, 2020).

2.6 Food Safety Standards

Businesses involved in producing processed meats in New Zealand must follow one of the following regimes:

- an approved FSP under Section 8G of the Food Act 1981 (MPI, 2020)
- a registered RMP under the Animal Products Act 1999 (MPI, 2020); or
- the Food Hygiene Regulations 1974 (MPI, 2020) and local authority (Council) registration

Safari Biltong operates under an approved FSP, which is legally required and must be relevant to New Zealand legislation (J. Bouwmann, personal communication, 2019). During cutting, boning and trimming the meat must be checked for visual defects such as abnormal colours, indications of microbial growth (e.g. slime) or physical contaminants. The curing agent must evenly cover the entire surface of the meat to prevent contamination occurring on uncoated surfaces. The correct amount of salt must be used and meat should be uniform in width. During dry curing, the meat must be kept at a temperature that prevents microbial growth (MPI, 2020).

The meat must be evenly spaced in the dryer so slices are not in contact. The result of the drying process must be a microbiologically safe product for its purpose (in this case, for human consumption). Australian and USDA (United States Department of Agriculture) standards require that dried meats (such as jerky) be dried to a water activity below 0.85. Yeast and mould growth is deterred by drying to 0.80 and then vacuum packaging or by maintaining a water activity at 0.7. Most commercially produced jerky in New Zealand has a water activity between 0.75-0.85 (MPI, 2020).

Dried meat that does not undergo a process to kill microbes (such as heating) must be made from pathogen-free meat. The drying equipment and environment must be operated so microbial growth is inhibited during the process. Drying parameters such as temperature and time spent in the dryer, must be recorded for each batch. The drying process must be validated by a suitably skilled person and re-evaluated whenever the process is changed (MPI, 2020).

Packaging requirements include:

- Using only new packets for products
- Having adequate separation between products to prevent contamination and storing packaging materials in a separate room from where packaging occurs
- Dispensing packaging materials so any risk of contamination is minimised
- Checking packaging seals regularly and promptly transferring packed products to an appropriate temperature-controlled environment (MPI, 2020).

The HAACP (Hazard Analysis and Critical Control Point) for manufacturing a dried meat product such as beef jerky or biltong is given in Figure 2.7.

Indicator microbes, which are commonly found microbes that thrive in certain conditions, are used to indicate whether a food product is likely to be contaminated. The presence and concentration of these indicator microbes can indicate the level of contamination from other microbes. Indicator microbes include *E. coli*, *Enterobacteriaceae (Salmonella* and *Shigella)*, *Clostridium perfringens*, *Bacillus cereus* and other *Bacillus* spp, *Vibrio parahaemolyticus*, *Campylobacter* and *L. monocytogenes.*

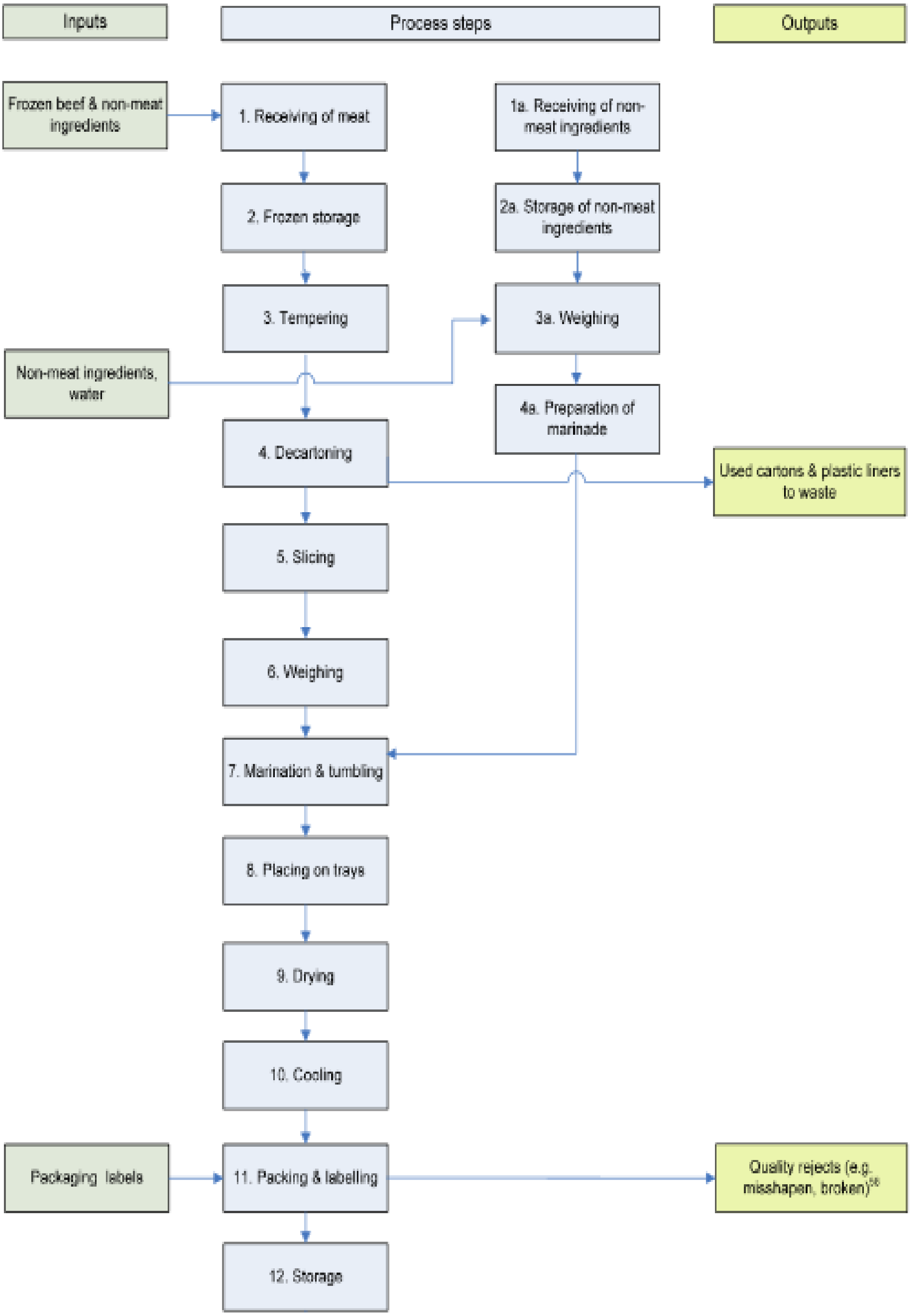

Figure 2.7. Flow diagram of typical dried meat product processing (MPI, 2020).

There are four categories of microbial quality: satisfactory, marginal, unsatisfactory and potentially hazardous (Table 2.2). Microbial levels in the "satisfactory" category are good and no action is required. The marginal category is on the border between acceptable and non-acceptable and hygiene needs to be reviewed. If the status persists, the entire process should be reviewed. If a product is in the "unsatisfactory" category, action is needed to identify and remedy the cause of contamination. If the "potentially hazardous" category is identified, instant remedial action must be taken, including product withdrawal and recall, followed by process investigation (ICMSF, 2001).

Table 2.2. Guidelines for microbial quality status of ready-to-eat foods (ICMSF, 2001).

Test	**Microbiological Quality (CFU/g)**			
	Satisfactory	Marginal	Unsatisfactory	Potentially
Standard Plate Count				
Level 1	$<10^4$	$<10^5$	$\geq10^5$	
Level 2	$<10^6$	$<10^7$	$\geq10^7$	
Level 3	N/A	N/A	N/A	
Indicators				
Enterobacteriaceae	$<10^2$	$<10^2$-$<10^3$	$\geq10^4$	
E.coli	<3	3-100	≥100	
Pathogens				
Coagulase +ve	$<10^2$	10^2-10^3	10^3-10^4	
C. perfringens	$<10^2$	$\geq10^4$	10^3-10^4	
Bacillus cereus and other	$<10^2$	$\geq10^4$	10^3-10^4	$\geq10^4$
Vibrio parahaemolyticus	<3	<3-10^2	10^3-10^4	$\geq10^4$
Camypylobacter spp	Not			Detected
Salmonella spp	Not			Detected
L. monocyotgenes	Not	Detected		Detected

2.7 Factors Affecting Shelf Life of Foods

Spoilage can be defined as any change in a food item that renders the food unfit for consumption. This spoilage can be due to microbial populations, insect infestation, contact with contaminants in the environment, and chemical or biological degradation of the product. Spoilage can result in structural and sensory changes that make the food unattractive and/or unsafe to eat. Using preservation factors will extend the shelf life of fresh foods and increase consumer safety. The common methods for extending the shelf life of foods involve temperature, heat processing, additives, removing water (drying or concentration), using packaging and modifying the packaging environment. New methods include pasteurization, irradiation, freezing, freeze-drying, etc. (Desrosier & Singh, 2018).

2.7.1 Moisture content

The moisture content of a food will largely determine the shelf life of that food. High moisture contents allow microbial cells to establish as well as chemical and biological degradation. High moisture content accelerates chemical and biological degradation by mobilizing reactive components that at low water activities would have been encapsulated within a matrix of nonreactive food components. At low water levels, water acts as an antioxidant through promoting recombination of free radicals and in certain foods promote non-enzymatic browning which produces active antioxidants. Hydroperoxide degradation mechanisms also change, reducing the production of free radicals (Karel & Simic, 1980).

Research has shown the importance of water in lipid oxidation. Lipids were freeze dried from carbohydrate samples to produce lipids in two different states. One lipid state was on the surface of cellulose or localized in small reactive droplets. The second state was trapped in a carbohydrate matrix and was not accessible to atmospheric oxygen. The surface lipid was washed away with hexane and no lipid oxidation was observed. When re-hydrated with water lipid oxidation continued as normal (Karel & Simic, 1980).

The microbiological safety of food also depends on the safety of the ingredients used in the formulation. Drying inactivates bacterial cells but does not inactivate any toxins that have already been produced. Cells can also germinate when moisture content increases. Moulds and yeasts, like *Aspergillus*, pose the greatest risk to shelf life deterioration as they grow at low moisture contents (Odeyemi *et al.*, 2020).

2.7.2 pH

Microbes that grow on food items often have an optimum pH near 7 (neutrophiles). For example, the commonly found food spoilage microbes (*Salmonella, E. coli, Aspergillus, Campylobacter, Staphylococcus,* etc.) are neutrophiles (Gordon, 2019). The further the pH is from neutral, the greater the damage to intracellular macromolecules in these microbes. However, some microbes on foods are fermenting bacteria that can produce acids, which lower the pH of the surroundings. In acidic conditions, the high concentration of hydrogen atoms disrupt the proton motive force so the cell has no energy. Acidic conditions also impair hydrogen bonding and causes changes in molecular folding, resulting in cell death and thereby protecting food from microbial cell deterioration. At an alkaline pH, the hydrogen bonds in DNA begin to break and lipids are hydrolysed (Keenleyside, 2019).

Lowering pH through acid addition is a common way to increase shelf life because it imparts desirable tastes compared to adding a base. Acidified foods are foods with a pH reduced to 4.6 or lower by adding acids such as vinegar (acetic acid) or using beneficial microbial populations that produce lactic acid. This allows the food to have an extended shelf life unrefrigerated and also decreases the noticeability of lipid oxidation (Featherstone, 2015). In addition to maintaining microbial integrity, adding acid also retains desirable qualities of the food for long term storage. Foods like pickles, pudding, cucumbers, cauliflower, artichoke, peppers and fish are examples of foods typically stored in an acid.

2.7.3 Curing

Curing involves adding salt, often with added spices, to prolong the shelf life of otherwise highly perishable food items. Typical curing methods are brine curing, where the meat is immersed in brine, or dry curing, where the salt and spices are rubbed on the surface. Curing mixes generally consist of salt, sugar, baking soda, pepper and coriander. Nitrites and nitrates are also excellent preservatives in meat products as it decreases lipid oxidation and gives a bright red colour (Jones *et al.,* 2017).

The high solute concentrations around the microbial cells draws water through osmosis from the cells into the environment. The low moisture environment inhibits cellular activity and the high salt and sugar concentrations have a toxic effect on internal cell processes, damaging the enzymes and DNA (Koo & Seo, 2019).

2.7.4 Redox potential (Eh)

The redox potential (Eh), which is measured in volts (V) or millivolts (mV), indicates the tendency of a chemical species to acquire or lose electrons from a donor source and thereby be reduced or oxidised respectively. A radical oxygen species has only one rather than two electrons in the outer orbital of the oxygen. This makes it highly unstable and highly reactive as it readily attempts to fill the orbital. The presence of radical species in foods will cause biological and chemical degradation and enable microbial growth (Papuc *et al.*, 2016).

Lipids are made from long chain fatty acids, composed of carbons, and impart desirable sensory characteristics to meat. For example, tenderness, juiciness and aroma is affected by the amount and type of lipid present. Meat product spoilage is often due to oxidation of the fatty acid chains. Unsaturated fatty acids contain double bonds, which create kinks in the chain and prevent the chains from packing tightly and forming a solid at room temperature. These double bonds are a target for free radical reactions.

Oxygen radicals take electrons, break the double bond and create shorter carbon chains such as butyric and propionic acid, resulting in "off" flavours and odours. Phospholipids in cell membranes have unsaturated fatty acid groups highly susceptible to oxidation because they are located where oxygen can easily reach them. Red meat, like beef, is highly susceptible to rancidity as it is high in iron, which catalyses the oxidation reaction. Although lipid oxidation does not affect consumer safety, sensory quality decreases due to the rancid tastes and odours that develop (Amaral *et al.*, 2018).

Radical oxygen species can also react with proteins causing oxidation in both the amino acid side chains and in the protein backbone. Reduction reactions in red meat are particularly high due to the high iron content, which catalyses the reaction. The reactions affect digestibility, decrease nutritional value due to oxidation of amino acids and increase the risk of certain illnesses. For example, many studies have found a link between oxidation in meat products and arteriosclerosis, neurodegenerative diseases and cancer. Packaging can help exclude oxygen from the product and reduce the rate of off-flavour development in biltong (Papuc *et al.*, 2016).

Aerobic bacteria require oxygen for survival, facultative bacteria can establish in aerobic and anaerobic environments while anaerobic bacteria require the absence of oxygen. Microbial species known to contaminate biltong are either aerobic or facultative species. Growth of aerobic and facultative bacteria is lower at low Eh values. A low redox potential prevented aerobic bacteria growing in sausages with a water activity of 0.96 (Rockland & Beuchat, 1987). If the Eh was higher, the water activity had to be below 0.85 to prevent bacterial growth. This demonstrates that if oxygen is present, other preservation factors are needed to prevent microbial growth but having other hurdles is not as necessary in low oxygen environments.

2.7.5 Temperature

Temperature controls the rate of both biological and chemical reactions. Thermal energy is the sum of the kinetic energy of the atoms in a system. Heat is the flow of thermal energy from one system to another system of a different temperature (Kurtus, 2019). This heat provides the energy needed for reactions to occur. The higher the temperature, the faster the reaction rate because molecules are moving faster, creating a greater chance of molecules interacting. As temperatures decline, there is less energy put into the system and therefore fewer interactions between molecules, decreasing reaction rates (Brenann, 2017).

In chemical degradation, such as lipid oxidation, the rate fatty acid chains breaking is faster at higher temperatures. In biological processes, like mould and yeast growth, increased heat increases the rate of enzymes and other intracellular processes. However, above a certain temperature, these proteins and structures begin to denature resulting in cell death and protein denaturation in foods (Keenleyside, 2019).

2.7.6 Colour

Meat colour is one of the most important factors affecting consumer choice. Fresh meat muscle has red pigmentation due to the globular protein myoglobin, which has heme groups. The concentration and oxidation state of this protein determines meat colour. Myoglobin has a higher affinity for oxygen than haemoglobin, allowing it to take the oxygen that haemoglobin has received from the lungs to the muscle (Hunt & King 2012).

Most of the blood, and subsequently haemoglobin, is removed after the animal has been slaughtered, effectively increasing myoglobin concentration. Before slaughter, myoglobin contains only 10% of total iron in the body but post-slaughter this increases to 95%. The iron in the myoglobin is the primary colouring factor. In the presence of oxygen, myoglobin can be reduced to reduced myoglobin, oxymyoglobin and metmyoglobin. The red pigmentation in raw meat comes from the concentration of oxymyoglobin under high oxygen tension. Metmyoglobin, formed by oxygenation of the iron heme in lower oxygen tension conditions, produces brown pigmentation (Seideman *et al.*, 1983).

2.7.7 Preservatives

A preservative is a substance or chemical designed to prolong the natural shelf life of a food product. Preservatives and chemicals, such as pimaricin and potassium sorbate, are commonly added to food to prolong consumer acceptability (Jones *et al.*, 2017). Pimaricin (also known as natamycin; additive 235), an antibiotic agent produced from aerobic fermentation by *Streptomyces natalensis*, inhibits growth of yeast and mould species (Mattia *et al.*, 2015). Potassium sorbate also inhibits fungal growth. It is made by reacting sorbic acid with potassium hydroxide to form crystals and was first patented in 1945 (Robach & Sofos, 1981).

Any food additive must be regarded as safe and most countries have legislation on permitted additives. The levels permitted are dependent on the food type. For meat, sorbic acid (as potassium or sodium sorbate) can be used in cured meat products in New Zealand at levels up to 1500 mg/kg, nitrites (as potassium and sodium salts) up to 125 mg/kg and pimaricin in fermented meats up to 1.2 mg/dm^2 (FSANZ, 2016).

There is increased concern in Western society about use of chemicals and preservatives in food products. Research shows that 'middle class' consumers tend to respond negatively to animal products containing agrochemicals, hormones or medicine and any form of genetic modification. Using artificial additives in fruit and vegetable products is commonly undesired. Products perceived as natural because they do not contain refined chemical compounds are preferred (Shafie & Rennie, 2012).

2.8 Hurdle Theory

Hurdle technology was first proposed by a scientist named Leistner in 1978 (Leistner & Gould, 2002). He proposed that combining existing and novel preservation techniques, each below the level required to limit microbial growth when used alone, created a series of preservation factors (hurdles) that would prolong food shelf life and limit microbial deterioration. The synergistic effect of the hurdles limited that amount of processing required, and hence the changes to food structure and appearance.

The technology can be depicted as a series of hurdles microbes must leap over to obtain the food (Fig. 2.8). By incorporating multiple hurdles, the range and efficiency of preventing microbes establishing improves (McIntyre & Hudson, 2009).

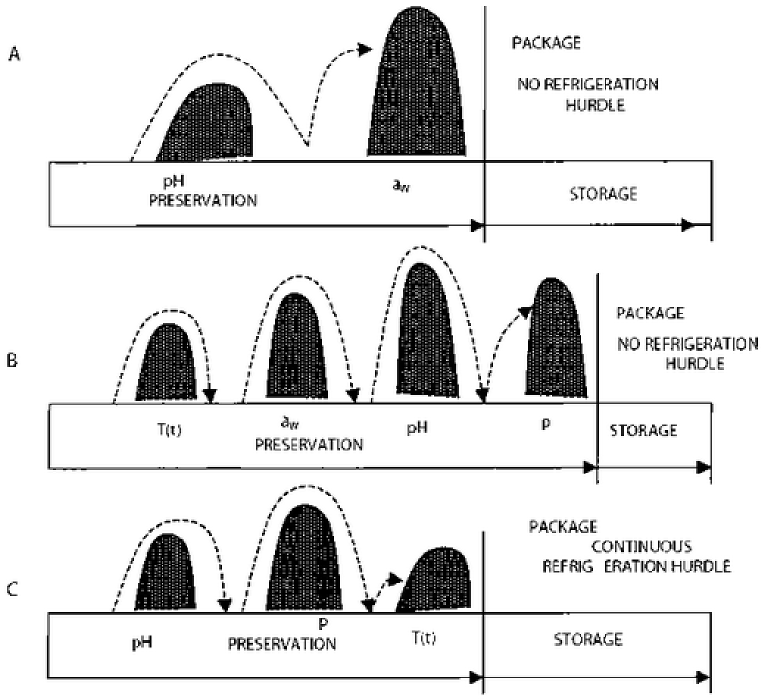

Figure 2.8. Hurdle technology to preserve food items (Leistner & Gould, 2002).

2.9 Packaging

Food packaging maintains the benefits of food processing after the process is complete and enables food to be transported from the place of manufacture to the place of consumption. It prevents external factors (pathogens, fungi, moisture, oxygen, animals and physical contaminants) from deteriorating or accelerating natural deterioration of the food item (Marsh & Bugusu, 2007). Packaging also allows seasonal foods to be available year-round and introduces convenience through heatable and single serve packets (Abdullahi, 2018). As processing technology improved, so has packaging technology. Combining Hurdle factors (such as low pH, low Aw, high solutes, etc.) with packaging allows the product to have a shelf life significantly longer than when it is unprocessed. This helps meet increasing product demand by increasing populations as well as reducing waste and food-related illnesses (Cartmell, 2017).

2.9.1 Packaging film

Packaging creates a barrier between the surface of the food and contaminants in the environment. It can also reduce the effect of biological, chemical and physical factors on product quality between manufacturing and the point of consumption. The effect of packaging on the quality of the packed product depends on the properties of the packing material, including crystallinity, morphology and chemical composition.

The packaging material is chosen for its compatibility with the food. Materials used for packaging foods include paper, paperboard, and various types of plastic film. Metals and glass are commonly used for liquid/semi-liquid foods. Sustainability considerations have resulted in the development of bioplastics and other bio-based materials (Han & Scanlon, 2014). Effective packaging, especially for modified atmosphere packaging (MAP), takes into account gas concentration, product respiration, gas diffusion and optimal storage temperature to maintain film integrity (Farber *et al.*, 2003).

Limiting (or promoting) the amount of gas that can pass through the plastic film (i.e. gas permeability) is an important characteristic. This permeability is affected by storage temperature, partial pressures on both sides of the film and RH of the environment. Oxygen accelerates deterioration of packaged meats but MAP can extend the shelf life of fresh meat by 10-15% if the barrier film's oxygen permeability is below 2 cm^3/m^2/day/atm (Lee, 2010).

Lee's (2010) study on the effects of physically manipulated packaging showed that growth of both aerobic and anaerobic microflora on meat packaged in gas barrier film is reduced. The packaging also improved meat colour and odour during storage. The colour and odour of fresh meat packed in gas barrier film did not change over 32 days storage at 5°C but colour was unacceptable after 5 days and off-odours developed after 4 days when the meat was packaged in permeable film.

Barrier films with permeability of 100 $cm^3/m^2/24$ h/atm (at 23 °C and 0% RH) are generally used for vacuum packing or MAP meat products. Carbon dioxide (CO_2) has a high solubility coefficient so its permeability through plastic film is four to five times greater than oxygen and thirteen times greater than nitrogen. Researchers found vacuum-packed meat had an oxygen concentration consistently above 1% while the CO_2 concentration increased to above 20% (Lee, 2010).

2.9.2 Packaging types

Gas permeability is considered the most important preservative factor for packaging (Hab & Scanlon, 2014). Modified atmosphere, controlled atmosphere and smart packaging have been developed to meet increasing consumer demands and expectations for convenience and portion control. Research in nanotechnology incorporated into packaging could allow for packets that self-repair, has moisture and temperature monitoring and response, and could alert consumers/producers of contamination (Abdullahi, 2018).

Small businesses need to select packaging methods that balance cost effectiveness and product preservation. Nanotechnology packaging would be a highly effective preserving packaging but is not currently cost effective.

Traditional methods like vacuum sealing, effectively extend shelf life while being cost effective as it does not require advanced technology, supplies or training (J. Bouwmann, personal communication, 2019). Vacuum packaging involves complete removal of all air before sealing the pack. The absence of oxygen decreases the rate of microbial growth and lipid oxidation. Another advantage of vacuum packaging is that packet volume and weight is only slightly more than the product itself, thereby, reducing transport costs. The disadvantage of vacuum sealed pouches is the irregularity and the un-aesthetic appeal of the packs (Embleni, 2013).

Vacuum-sealed packaging restricts oxygen reaching the meat surface (oxygen transmission rate (OTR) levels from ~5 to 20 $mL.m^{-2}.day^{-1}$ at 23°C and 90% RH). This reduces the growth of spoilage organisms, which in turn allows fermenting bacteria such as *Lactobacillus* to establish because the competing microbes have been inhibited. Colour and lipid oxidation are maintained at a desired level because oxidation is inhibited (Mazzola & Sarantopoulos, 2019).

In MAP, the original air in the packet is removed and replaced with a specific gas mixture such as nitrogen. As in vacuum packaging, the absence of oxygen decreases the rate the food will deteriorate with the added advantage that packing gas can influence chemical and microbial changes. For example, using inert gas nitrogen to fill the pack will prevent oxygen diffusing in.

Nitrogen has a low solubility in water, prevents packages from collapsing and decreases the amount of outside protection required (Fairchild, 2015).

High oxygen levels have been shown to cause "oxygen shock", inhibiting the growth of both aerobic and anaerobic organisms. Optimal growth conditions are 21% O_2 for aerobic microbes and 0-2% for anaerobes. To be truly effective, oxygen needs to be 100 kPa in combination with 15 kPa CO_2. This is a hazardous condition as this oxygen level is highly flammable. Of the three MAP gases (O_2, N_2 and CO_2), CO_2 is the most effective in retarding microbial growth.

High CO_2 levels and low O_2 levels also decrease lipid oxidation rates (Farber *et al.*, 2003). The MAP tends to help retain the desirable colour in meat products while also having a better shelf-appeal than most other packaging techniques as it has a "full" appearance (Mazzola & Sarantopoulos, 2019).

The South African biltong company "Safari-Vac" uses approaches such as MAP to extend the shelf life of its biltong (Mavuso, 2019). Oxygen in the packs is removed under vacuum until there is only 0.02% oxygen. The pack is then filled with a MAP gas mixture. The packet expands with the gas and then is sealed to retain the gas. The MAP gas can be pure nitrogen blends of nitrogen and CO_2. Biltong with a high moisture content tended to be more acceptable when packed in gas mixtures with higher CO_2 concentrations. However, packaged biltong with moisture contents of 50% or higher were still too unstable and are avoided in packaging (Mavuso, 2019).

2.9.3 Effect of packaging on shelf life

A study on dry cured pork loins (Kim *et al.*, 2014) reported that the pH of vacuum packaged dried pork loins during 90 days storage at 10°C was higher than those packed in MAP because the latter had absorbed CO_2 from the MAP gas. Water activity and moisture content decreased during storage. The change in vacuum-sealed loins was slower than those in MAP, with dried pork loins showing a significant decrease in water activity at around 30 days. Colour stability decreased noticeably after 60 days, regardless of packaging type, but there was no significant difference between packaging types (Kim *et al.*, 2014). Neither packaging excluded oxygen and prevented radical oxygen entry. Lipid oxidation was greater in MAP than in vacuum sealed loins. Sensory evaluation showed that consumer acceptability of dry cured loins decreased over the storage time, with vacuum-packed pork loins retaining the desired characteristics better than MAP samples after 60 days storage (Kim *et al.*, 2014).

Yeast and mould cells and spores are normally present on freshly dried biltong. A study by Jones *et al.* (2019) on the effect of adding vinegar to biltong then storing vacuum and MAP packaged samples at 25°C for 12 weeks showed that irrespective of the packaging environment used, yeast and mould numbers increased, from week 1 and exceeded 3 log cfu/g from week 6 to 12. The study did not identify mould or yeast species but confirmed that removing oxygen inhibited growth of strictly aerobic species such as some moulds. However, some yeasts and moulds can grow at oxygen concentrations as low as <0.1% so could establish in MAP or vacuum sealed packages. No *E. coli*, *Salmonella* spp or *L. monocytogenes* was detected after 12 weeks storage. Although *Staph. aureus* was below the limit of 1.3 log cfu/g, the researches postulated that limits would have been exceeded if storage was extended (Jones *et al.*, 2019).

2.10 Biltong Quality

The main factors consumers consider when selecting a food include visual (colour, shape and size, regularity, etc), gustatory quality (texture, flavour, and aroma), nutritional composition, and product safety. Consumers prefer products that are of high quality and producers want to have efficient cost-effective processes to meet expectations. Therefore, ensuring a long stable shelf ife is important (Mazzola & Sarantopoulos, 2019).

Biltong is an example of a food that is being effectively preserved by incorporating numerous hurdles: it has a low water activity, low pH, high solute concentration and may contain spices with antimicrobial properties. Suitable packaging and storage conditions help extend the effect of these hurdles and therefore help the product retain its quality for long periods.

A Spanish study (Diaz *et al.*, 2002) on the effect of individual and combined preservative techniques such as water activity, pH, temperature, adding antimicrobial agents (sodium chloride, sodium nitrate and potassium nitrate) and spices (oregano, paprika, garlic) on shelf life of chorizo sausage (an intermediate meat product) considered the sausage was spoiled when *Penicillium* species established. Lowering the pH or adding nitrates and nitrites did not significantly inhibit *Penicillium* growth. A combination of low water activity, low temperature and high sodium chloride concentration was the most effective in extending the shelf life.

2.10.1 Moisture content

Producing a ready-to-eat meat product requires a fine balance between making shelf-stable dried meat while still meeting consumer preferences. If biltong is dried to water activities below 0.6, the risk of microbial spoilage is extremely low. However, research indicates that consumers prefer ‘medium’ to ‘wet’ biltong. Water activity values for biltong classifications vary. Biltong with water activities of 0.85-0.93 are classified as wet, those with water activities around 0.8 as medium and those with water activities below 0.7 as dry. Although having a higher water activity increases the microbial risk, consumers tend to prefer the product because it is tender, has a chewy consistency, appears to have a lower salt content (because of the higher moisture content) and is 'juicier' (Petit *et al.*, 2014). However, consumer preference of biltong is under-researched (Jones *et al.*, 2019).

Fresh lean meat has a high water content and a water activity around 0.98. For extended storage, the water activity of the biltong needs to be at least 0.85, which can require a 50% weight reduction. The moisture content of commercial biltong varies from 10.6 g/100 g to 48.8 g/100g with corresponding water activities of 0.54 to 0.93 (Jones *et al.*, 2019). New Zealand MPI standards (MPI, 2020) do not approve food control plans unless the biltong produced has a water activity below 0.85.

Skeletal muscle protein fibres begin to denature at around 40°C so biltong is usually dried using ambient temperature air (25°C) to prevent protein denaturation. As there is a small temperature difference between the meat and the environment, the rate of moisture removal will depend on air velocity, the air's relative humidity (RH) and dimensions of the meat (Heldman & Hartel, 1997). The myosin fibre contraction during denaturation causes the sarcomere to shorten, resulting in dense, tough meat (Earl, 2019). A Kwazulu-Natal study on infrared drying of biltong found that optimum commercial drying conditions are circulating 25°C air at 60% RH. Higher temperatures caused case hardening (Cherono, 2014).

Collagen, the connective tissue in meat, dries more quickly than muscle tissue and can cause shrinkage, which decreases consumer appeal of the resultant product. When heat is applied in a moist environment, collagen solubilizes to gelatine; under prolonged drying periods. This gelatine binds muscle fibres, making the product tough and undesirable (Kerry, 2018).

Additives can affect the drying rate. The low-pH marinades, salt and spices characteristic of biltong affect the water binding ability of muscle fibres and therefore the drying rate (Jones *et al.*, 2017). As water is removed, additive and protein concentration increases, affecting taste and shelf life (J. Bouwmann personal communication, 2019).

2.10.2 pH

Vinegar, which is added at 3-6%, makes biltong unique amongst dried meat products. The main function of vinegar is microbial inhibition and its second function is flavour enhancement (Jones, 2017). The pH of biltong is around 5.5, which can help inhibit microbial growth. Research shows that having a low pH decreases water holding capacity. As mentioned before, normal meat has an ultimate pH of about 5.5, which is the IP of many meat proteins. However, studies show that the amount of water expelled may not be significant in reducing water activity (Huff-Lonergan, 2010).

The pH can affect the drying rate of biltong. Biltong that had been tumbled in vinegar and curing mix gained twice as much weight as biltong that had been tumbled without vinegar (Jones *et al.*, 2019). Although the tumbling ingredients had no significant effect on the weight of the finished product, biltong that had been tumbled with vinegar had a higher salt concentration on both a wet and dry basis. The increased absorption rate accounted for some of the weight gain. Unpackaged biltong with lowered pH and low water activity had greater shelf-stability after three months than biltong at a neutral pH (Jones *et al.*, 2019).

2.10.3 Curing

Curing meat is one of the earliest methods of meat preservation. In addition to flavour enhancement, salt inhibits microbial growth and reduces detection of rancid flavours. Not all biltong recipes contain vinegar, but all recipes contain a curing mix high in sodium chloride and other solutes. Salt, pepper and coriander are the basic ingredients with brown sugar, baking soda, garlic, paprika, chilli flakes etc. also appearing in many recipes. The meat is coated in a dry-curing mix after being dipped in vinegar (J. Bouwmann personal communication, 2019).

Salt, which is present in the curing mix, affects the electrostatic interactions in and between proteins and also disrupts the structure of the water associated with the protein. The chloride ions binding to protein filaments increase electrostatic repulsion, denaturing the proteins. Adding salt to meat modifies myofibrillar protein solubility and causes fibre swelling due to proteins unfolding. A salt concentration, across numerous moisture levels, of 2.5% is acceptable but can be increased to 4%. Applying dry curing mix can result in uneven application but is a more economical method than brining (Jones *et al*., 2017)

Salt concentration can affect the relationship between water activity and moisture content (Fig. 2.9). The moisture content is plotted on a wet basis instead of dry basis to show the initial increase in water sorption following curing. At a constant water activity, increasing the salt concentration increases moisture content due to the increased WHC of the denatured myofibrillar structures (Van der Riet, 1976).

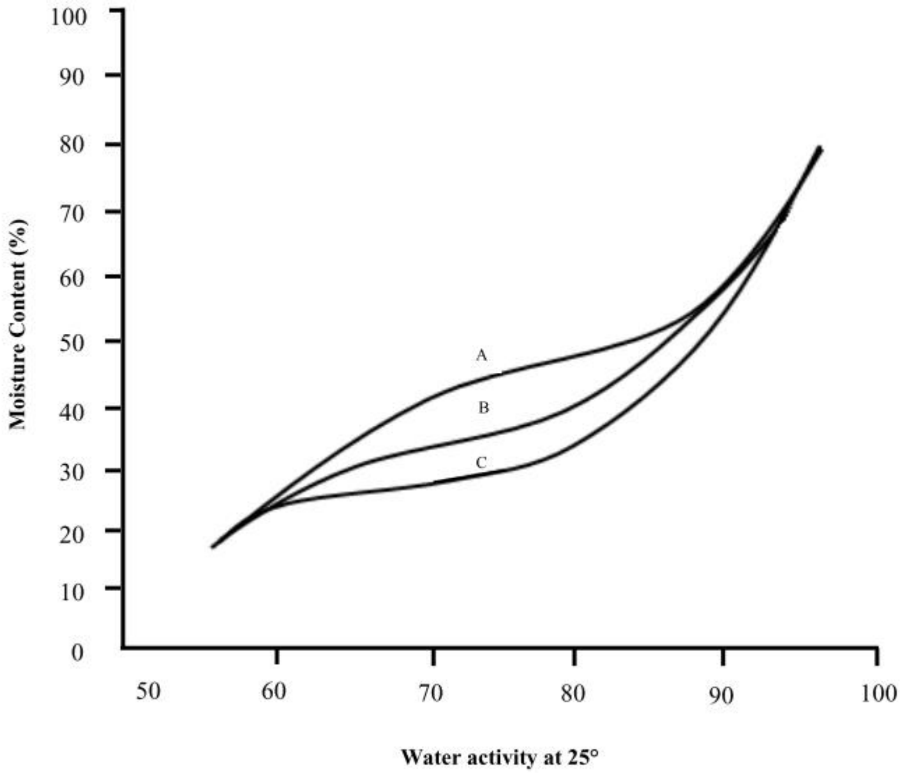

Figure 2.9. Effect of adding 4% (A), 2.5% (B) and 1% (C) salt on moisture content and water activity (aw) of biltong (Van der Riet, 1976).

2.10.4 Redox potential (Eh)

When a meat product, such as biltong, is exposed to an oxic environment, the lipids oxidize, resulting in adverse changes in colour, nutrient quality, taste, texture and smell. Phospholipids are the dominant lipid in cell membranes and account for 80% of lipids in the sarcoplasmic reticulum. The phospholipids have more double bonds (the target of oxidation in unsaturated fatty acids), than other lipids such as triglycerides (Aksu *et al.*, 2017). The cell membranes of bovine meat tissue is high in phospholipids, which is easily affected by atmospheric oxygen.

Muscle type is another factor affecting the rate of oxidative deterioration. Oxidative muscles contain more phospholipids than glycolytic muscles. The oxidative, slow twitch fibres in red bovine muscle tissue also are high iron, which is known to catalyse oxidation reactions (Aksu *et al.*, 2017). There is evidence that interaction between metmyoglobin and hydrogen peroxide forms ferrylmyoglobin, which can initiate the free radical chain reaction. Therefore, oxidation rates are very high in beef meat products (Amaral *et al.*, 2018). Some conditions such as temperature and oxygen level accelerate oxidation whilst low oxygen concentration, low storage temperatures and the presence of antioxidants will deter oxidation (Wazir *et al.*, 2019).

Adding 2% sodium chloride (NaCl) to beef increases the rate of lipid oxidation but adding 3% or higher levels did not significantly increase lipid oxidation from a concentration of 2%

(Amaral *et al.*, 2018). The mechanism is not clearly understood but it is suspected that the salt disrupts cell integrity, allowing oxygen easier access to the phospholipids. Biltong typically has a NaCl concentration of 2.5 to 4%, which is likely to increase the rate of oxidation (Amaral *et al.*, 2018).

A study on unpackaged beef 'droewors' (a dried beef sausage) found no significant changes in lipid oxidation in the first 12 days. However, there was a significant increase in lipid oxidation between days 21-28 (Mukumbo *et al.*, 2018).

Research shows that lipid oxidation in meat products increases with time regardless of packaging, temperature and antioxidant conditions. A study reported dried serunding (a shredded beef product) had less unsaturated fat (7.29%) than raw (51.35%) or cooked (56.26%) serunding. During six-months of storage at 25°C, 40°C and 60°C, the lipid oxidation in serunding steadily increased. Adding natural antioxidants such as ginger, garlic, red chilli and coriander stabilised the fatty acids. Coriander is an important ingredient in biltong curing mix.

Malondialdehyde (MDA) is a relatively stable secondary product of oxidative degradation of polyunsaturated fatty acids. This three-carbon dialdehyde can exist in different forms depending on the pH. The MDA level indicates the level of lipid oxidation when reacted with thiobarbituric acid in the thiobarbituric acid reactive substances (TBARS) test. This method is widely used in scientific research due to the accuracy, simplicity and cost effectiveness (Amaral *et al.*, 2018).

2.10.5 Colour

Meat appearance is important in consumers' perception of product acceptance. Colour does not reflect the meat's age or affect taste but rather indicates its oxidation state. Consumers in taste panels said they preferred fresh beef to have a bright red colour. Purple is still acceptable with brown being perceived as old meat. This is due to the belief in western society that fresh beef that is not a bright red colour is not of good quality (Carpenter *et al.*, 2001). Therefore, it can be assumed consumers will purchase a product when it fits the appearance, and especially if the colour is perceived as being fresh.

Freshly dried biltong has a characteristic black or dark brown colour because it has a higher metmyoglobin content than undried products. The pigment is highly sensitive to light and oxygen and fades to a lighter brown if not protected by proper packaging (Mazzola & Sarantopoulos, 2019). Protein degradation during storage will also affect colour (Listrat *et al.*, 2016). Any fat present will appear white or yellow. Freshly sliced biltong will have a dark red, smooth interior surface. Light brown colours on the surface or interior of the biltong, along with cracked surfaces and frayed edges, make the biltong appear overly dried and old (J. Bouwmann, personal communication, 2019).

2.10.6 Temperature

Food Standards Australia and New Zealand (FSANZ) specify that hazardous foods to be kept below 5°C or above 60°°C during transport, display or storage (FSANZ, 2020). Biltong is not a hazardous food and can be kept at room temperature for short periods without deteriorating as long as other factors such as water activity are not compromised (Jones, 2017). However, the drying process will continue if the biltong is stored at high temperatures (e.g. >30°C), resulting in colour, texture and taste changes. High temperatures also increase the chances of increasing microbial load and lipid oxidation. Storing at cooler temperatures decreases the rate of degradation processes and enables biltong that has been packaged appropriately to remain palatable and safe for consumption for months instead of weeks.

Aksu and Kaya (2005) reported that storage temperature significantly affected pastirma (a dry-cured beef or water buffalo product) quality. Moisture content of pastirma stored at 10°C in MAP (50% CO_2 + 50% N_2) packages decreased significantly after 30 days storage whereas packages stored 4°C had a significant change after 150 days storage. Because enzyme activity is temperature dependent, the pH of samples stored at 10°C was recorded after 30 days but not in samples stored at 4°C. On average, samples stored at 10°C had a higher TBARS value than similar samples stored at 4°C. These researchers concluded that the lower storage temperatures slowed microbial growth but was also necessary to maintain efficacy of the MAP. The solubility of the CO_2 in the MAP environment decreases drastically with high temperatures, allowing microbes to establish. Although most characteristics during storage were better for samples stored at 4°C, colour of samples stored at 10°C for 150 days were more acceptable (Aksu & Kaya, 2005).

2.10.7 Preservatives

Although biltong is a low moisture product with an extended shelf life, the time involved in shipping to overseas markets means the 'sell by' date in the FCP for biltong made in New Zealand often will have expired or only a short time is available for selling the product. Using lower transport temperatures slows chemical and biological processes, allowing product quality to be retained for longer. However, shipping costs are lower if the product is transported at ambient rather than refrigerator temperatures (J. Bouwmann personal communication, 2019).

Another way to extend shelf life of foods is to use preservatives. In 1972 the South African government passed the Foodstuff, Cosmetics and Disinfectants Act 54 (1972), which allowed the use of selected preservatives in biltong (Table 2.3).

Table 2.3. Preservatives and quantities permitted by Foodstuff, Cosmetics and Disinfectants Act 54 (1972) (Jones *et al.*, 2017).

Preservative	**Quantity permitted (mg/kg or mg/L)**
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

Potassium sorbate and pimaricin are the two most commonly used preservatives. They normally are incorporated into the curing mix to allow proper infusion into the meat. At 100 ppm, sorbic acid can retard growth of any microorganism. Sodium nitrates and nitrites are also effective in inhibiting microbial growth and lipid oxidation while also giving the biltong its red colour (Jones *et al.*, 2017).

Many consumers are concerned about genetically modified foods. A study (Anon., 2017b) on the snacking habits of Americans showed that a claim of “no GMOs” (Genetically Modified Organisms) would increase biltong sales in the United States by 18.2%, a claim of “no artificial colours/flavours” would increase sales by 16.2% and a claim for “no/reduced sugar” would increase sales by 11.3%.

Marketing strategies often include claims of a ‘natural’ product due to the concerns of consumers. Surveys regarding products with ‘all natural’ labels found that perceived taste, nutritional value and food safety to increase along with the likelihood of purchase (Dominick *et al.*, 2018). Therefore, the absence of preservatives can greatly increase the sales and consumer contentedness with the packaged biltong.

2.10.8 Novel biltong preservation techniques

With increasing demand for biltong, there is a need to be able to produce high quality and safe products quickly. Innovations allow new ways for biltong shelf life to be extended while maintaining properties, such as high moisture content, that traditionally would render the product unsafe for consumption. The increasing concern about plastic pollution in the environment is making people wary about plastic packaging. Therefore, it would be beneficial to develop techniques that help maintain biltong quality and to use packaging with a low carbon footprint packaging such as paper bags. However, meat is a highly perishable food item and the technologies being developed are still relatively new so costs of alternative techniques are still high.

- **Irradiation**

A relatively new way of extending the shelf life of biltong is gamma irradiation. Irradiation involves passing radiant energy through food material without leaving any residue. Gamma rays produced by cobalt or caesium, or X-rays or electrons can kill bacteria and other pathogens. Using radiation to preserve food is not a new technology. It was first authorised in 1963 in the United States to kill insects that contaminate wheat and flour. A major concern associated with irradiating food is the possible decrease in nutritional value but this has been found to be insignificant (Stanley, 2017).

There were no adverse changes in the organoleptic quality of lean moist biltong (47% moisture, 3.7% NaCl, 1.5% crude fat, water activity 0.92) after exposure to 10k Gy. However, irradiation had to occur under vacuum and be exposed to aerobic conditions to release volatile compounds. Lower gamma irradiation levels are perceived to be more acceptable to consumers. The *Staph. aureus* count on biltong with 53.6% moisture, 1.91% NaCl, and a water activity of 0.98 that had been irradiated with 4 to 5k Gy was still under legal limits even when initial numbers were 10^7 cfu/g. However, using 5k Gy was insufficient for preventing fungal growth if the initial fungal concentration was $>10^3$ cfu/g (Minaar *et al.*, 2009). However, gamma irradiation is not a common practise in biltong production.

- **Fruit extracts**

Concentrated fruit extracts can also exhibit an antimicrobial effect without decreasing nutritional value. Using extracts may overcome the adverse consumer reaction to irradiation or adding preservatives (Hintz & Matthews, 2015). Raisins have high concentrations of phenolic compounds and high antioxidant activity. After 10 weeks at 30°C, the pH of vacuum packaged beef jerky that had been coated with 15% raisin concentrate decreased from 5.5 to 4.5 and its water activity decreased from 0.64 to 0.62. The extract was effective against two bacteria strains (*S. choleraesuis, E. coli O157:H7*) and one yeast strain (*Saccharomyces fermenti*) but had no effect on any moulds. Beef jerky coated with raisin extract had an improved oxidation state of 600% compared to the control, suggesting the extract would be effective in preventing lipid oxidation. The disadvantage of adding raisin extract is that it alters the taste of the product. However, beef jerky tends to have a sweet taste, so the extract compliments the product or is not as noticeable (Bower *et al.*, 2003). Biltong is a very salty product so using a sweet concentrate may be more noticeable.

Rosemary has a long history of being used as a seasoning herb in human diets. Rosemary extracts isolated from the dried rosemary leaves have biological bioactivities including being hepatoprotective, antifungal, insecticidal, and antibactericidal (Berdahl & McKeague, 2015). The extracts have not been used widely in the food industry because of their strong odour and taste. However, new commercial processes involving distilling fresh twigs and leaves of the rosemary plant are now producing extracts that are undetectable to a consumer. The extract contains carnosic acid, epirosmanol, rosmanol, methylcarnosate and isorosmanol, which are effective antioxidant agents. The antioxidant activity of rosemary extract is due to the high isoprenoid quinone content, which chelate reactive oxygen species. Antioxidants also react with lipid and hydroxyl radicals to form stable compounds (Nieto *et al.*, 2018).

2.11 Sensory Evaluation

Sensory evaluation can be defined as a scientific discipline that measures and analyses interprets responses to characteristics of products as perceived by the senses (Gimenez & Ares, 2019). Sensory evaluation test methods provide information on the acceptability of a product to consumers. Products are purchased by consumers making the preferences of consumers vital to economic benefit. Like other quantitative analysis, sensory testing strives to provide accurate and replicable data. However, as testing relies on human judgement, data varies greatly and variables are difficult to control (Anon., 2020).

The following are types of sensory evaluations used in scientific research (Anon., 2013):

- Discriminative testing. Participants are asked to compare similarities or differences of two or more samples,
- Scoring. A number is given for each attribute of a product participants are asked to assign for a sample,
- Ranking. Participants rank samples in order of greatest to least for a certain attribute, for example, sweetness,
- Descriptive analysis. This involves in depth description of perceived attributes for a sample,
- Preference. Participants rank samples in order of most preferred to least.

Consumer and trained panels are the two types of panels of participants used in sensory evaluation. While both panels have the aim of detecting sensory differences between products, participants and their purpose differ. Consumer preferences are tested with consumer panels, which include randomly selected participants. Trained panels are not used to determine consumer preference but focus more on the intensity of specific attributes. All participants receive the same training on specific descriptors involved in the research (Anon., 2018).

2.12 Purpose of this Study

Safari Biltong, which manufactures biltong for the local market, wants to investigate the effect of different packaging on the shelf life of its product while maintaining an "all natural" label. The company would also like to decrease manufacturing cost. The current FCP does not allow product to be kept for longer than two weeks. This research will assess the changes in biltong slices with no added chemicals or preservatives, packaged under different environments and stored at different temperatures for up to a year. The findings will help Safari Biltong design a cost-effective packaging method to extend the storage life of biltong so it maintains its desirable gustatory characteristics. Being able to extend the shelf life will also help reduce costs because larger batches can be made, with reduced time for start-up and cleaning the facilities.

Biltong from the same batch will be packaged in three different environments (vacuum sealed, partially vacuum sealed and partially vacuum sealed with an oxygen scavenger) and stored at a high (35°C) ambient (20°C) and low (4°C) temperature. The high temperature simulates accelerated storage conditions, the ambient replicates the current and preferred storage temperature, while the low temperature simulates chilled storage. All samples will be packed in sachets made from the same packaging film (70 µ thick, and O_2 permeability of 50 cc/m^2/day). Visual observations will be made periodically and triplicate samples tested for water activity, pH, appearance, microbial quality and lipid oxidation. Sensory trials will be done using a panel of participants familiar and unfamiliar with biltong. A small trial will also be done to examine the effect of adding rosemary extract to limit oxidation.

2.13 Testing Methods

During storage, changes in biltong appearance (colour and texture), chemical properties (pH, lipid oxidation and water activity), microbial safety (fungi or bacterial growth) and general consumer acceptability will be assessed. The colour and texture (such as frayed edges, cracked surfaces) of three replicates of each treatment will be taken periodically. A panel of untrained participants will also be asked to rate the appearance of the sample every 6 weeks. Changes in pH may indicate chemical changes in the biltong during extended storage. Monitoring water activity will indicate whether the sample is absorbing water through the packaging, thereby decreasing product safety. Lipid oxidation will be measured using the TBARS assay.

The biggest threat to packaged biltong is fungal growth. Spore tests will be done frequently and bacterial tests done halfway through storage and again at the end.

Sensory evaluation will be done every 6 weeks to determine product acceptability. An untrained panel, with people familiar and unfamiliar with biltong, will be used to assess texture, appearance, taste acceptability, level of “off” flavours, and whether the participant would purchase the product. The pH value can indicate the level of oxidative substances but taste test panels give a further description of the extent of deterioration.

This project will run for an extended time with a lot of repetitive testing and monitoring. The methods chosen are simple, cost effective, accurate and reproducible yet will not compromise the amount of information that can be obtained.

3 Materials and Methods

3.1 Introduction

This chapter outlines the materials and methods used in this research. Detailed methodology of individual methods and timeline of testing are given in the Appendices 1 and 2.

3.2 Materials

3.2.1 Biltong

Safari Biltong provided the biltong for the trials. It was made from beef selected with no attached fat and minimal marbling and produced following a (confidential) standard recipe and preparation procedure that met the FCP. The biltong had been dried to a water activity of 0.53 to minimise product spoilage in the packages. The trials were set up using freshly prepared biltong, immediately after coming from the dryer.

3.2.2 Packaging

The 80-mm x 200-mm vacuum pouches (Contour International Ltd, Tauranga) were made from 70-μ thick film with an oxygen permeability of 50 cc/m^2/day at 23C. The full specification sheet is in Appendix 3.

The iron-based, 50-mm x 50-mm sachets of oxygen scavengers were purchased from ECP Ltd, Auckland. They had an absorptive capacity of 100 cc, typically used in containers of up to 1 L capacity.

3.2.3 Chemicals

AgResearch provided the method for the TBARS test (Appendix 4). The analytical grade trichloroacetic acid (TCA), thiobarbituric acid (TBA), tetraethoxypropane (TEP) and butylated hydroxytoluene were obtained from the University of Waikato (UoW) stores or purchased from Sigma-Aldrich.

OxiKan (Kalsec Inc, Kalamazoo), a rosemary extract, was purchased from Karala, India. The allowable limit is 150 mg/kg final product.

Fungal spore tests were done on dichloran-glycerol (DG18) agar plates. Packs of 20 plates were purchased from Fort Richard Laboratories Ltd., Auckland and stored at 4^{o}C until required. DG18 agar has a low water activity, allowing fungi to outcompete any bacteria present.

Trypticase soy agar (TSA) plates, pre-prepared by a University of Waikato microbiology laboratory technician, were used for from the samples. This agar provides enough nutrients and water for bacterial species to grow uninhibited.

3.2.4 Equipment and facilities

Samples were stored in a 4°C walk-in chiller at Safari Biltong, a cleared office space at the University (ambient, approximately 20°C), or laboratory incubator (35°C) in the Thermophile Research Unit (TRU) at the University.

Safari Biltong provided all the equipment for packaging (vacuum packer, knives, chopping boards), a water activity tester and a pH meter. Equipment and facilities for carrying out the microbial tests (vortex mixer, pipettes, Bunsen burner, culture loops, peptone water, and ethanol) were provided by the TRU.

The TBARS tests were done in the bioengineering laboratory in the School of Engineering at the University. The School provided the spectrophotometers, vortex mixers, centrifuge, water bath, pipettes, dispensers, scales and dispensable items.

3.3 Trials

The first trial involved a partial factorial experiment to investigate the effect of the following variables:

- Packaging environment: Vacuum seal (VS), partial vacuum (PV), or partial vacuum with an oxygen scavenger (PVS)
- Storage temperature: 4°C, 20°C, 35°C
- Storage time: periodically up to one year. Individual treatments were discontinued when product quality had deteriorated too much.

Individual strips, weighing approximately 10 g, of biltong were put into individual packages. Each biltong strip was cut to an approximate 10g weight before packaging. The oxygen scavenger sachets were added if required and then the packages were sealed using a vacuum sealer. During sealing, a vacuum of -14 psi was pulled on the full vacuum packages (VS) and a partial vacuum of -7 psi was pulled on the partial vacuum packages (PV and PVS).

Triplicate samples were taken at known storage times and tested for:

- Visual observations
- Sensory tests
- Water activity
- pH
- TBARS (Trial 2 only)
- Fungal spores
- Bacterial numbers

Due to the work involved, not all tests were done at each sampling time (Appendix 2). The times samples were collected and the reasons for analysing for different attributes is summarised in Table 3.1.

Table 3.1. Trials conducted, purpose of the trials and testing interval.

Attribute	**Purpose**	**Time**
pH	Changes in pH can indicate rancidity, water absorption/loss and microbial activity, which will affect taste and food safety	Fortnightly
Water activity	An increase in water activity will allow microbial growth and promote lipid oxidation; a decrease may adversely affect taste	Fortnightly
Fungal spores	Indicates viability of spores and species present	Fortnightly until no growth for three consecutive tests
Visual observations	Provides an indication of consumer acceptability	Fortnightly
Sensory tests	Measures consumer preference and detects off-flavours	Every 6 weeks
TBARS	Indicates level of lipid oxidation and, consequently, rancidity	After 10 months
Bacterial numbers	Indicates bacterial numbers and which species are establishing or declining	After 6 and 12 months

The second trial investigated the effect of rosemary extract (OxiKan) on lipid oxidation during storage. OxiKan was added to the marinade to give a concentration of 0.255 g per 100 g dried biltong. The calculations are given in Appendix 5. The biltong was then prepared in the same way as the first batch but had a water activity of 0.78 after drying. Samples were cut into 10-g strips, then packed into the same environments and stored for up to eight months at the same temperatures as used in the first trial. Samples were analysed for TBARS and pH at known times.

3.4 Microbial Methods

3.4.1 Fungal spore numbers

Mould and yeast are resilient microbes that establish well in the absence of bacteria. Fungi can grow at lower water activities than bacteria, thereby allowing product spoilage. Because it is very difficult to produce food completely free of fungal spores, it is important to test the germination viability of any fungal spores that may be present. Measuring the viability during storage will indicate whether spores are surviving or dying during storage.

The bottom of a DG 18 agar plate was divided into three sections using a permanent marker. Sterilised scissors were used to aseptically cut two approximately 2-g pieces from randomly selected corners of each triplicate biltong strip. The pieces were then placed singly on a section of an agar plate taking care to ensure pieces were not in contact (Fig. 3.1). After five days incubation at 25°C, observations on fungal colour and morphology were recorded and visual growth was recorded on an arbitrary scale (Table 3.2). Photos were also taken.

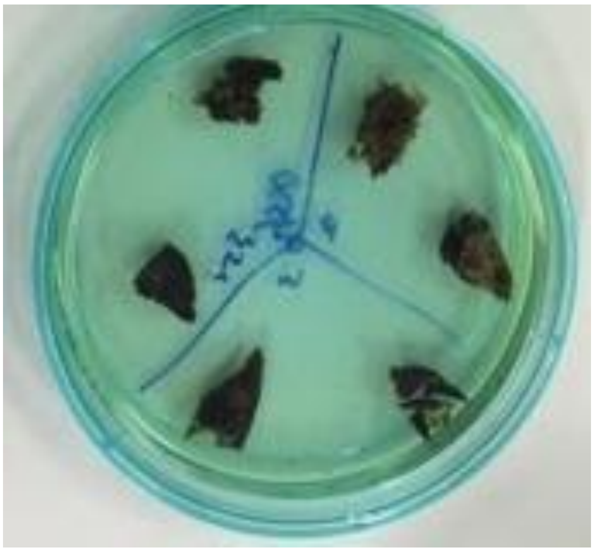

Figure 3.1. Fungal spore plate set-up.

Table 3.2. Scale for fungal spore growth.

Growth	% sample covered
Extensive	>60
Moderate	10-60
Low	<10

3.4.2 Bacterial numbers

Due to its low water activity, bacteria usually do not grow on properly prepared biltong. However, contamination can occur and bacteria can establish under certain environmental conditions. Some bacteria, such as *Staph. aureus,* are more resilient and can tolerate conditions associated with biltong.

Small pieces, sufficient to make a 2.5-g sample, were aseptically cut from the biltong using sterilised surgical scissors, placed in 9 mL of distilled water and left for 30 minutes (10^{-1} dilution). The solution was shaken for 1 minute on a vortex mixer and then a 1-mL aliquot was transferred and vortexed with 9 mL of distilled water (10^{-2} dilution). One-mL aliquots from each dilution was aseptically spread onto separate nutrient agar plates and incubated at 37°C. Photos of the plates were taken at 24, 36 and 96 hours and the number of colonies recorded.

Prior to storage and at the end of the trial, 100 g of each treatment, in its packaging, was sent to a commercial laboratory (Hill Laboratories Ltd., Hamilton) for total plate count (TPC), aerobic plate count, *E. coli, Staph. aureus, Salmonella*, yeast and mould tests.

3.4.3 Microbial characterisation

Samples of the fungal species that had grown on the DG 18 agar plates were aseptically transferred to a new DG 18 agar plate using a sterilised loop and incubated for three days at 25ºC. Photographs were taken and the species were identified by a microbial specialist.

3.5 Sensory Assessments

3.5.1 Ethical approval

As sensory tests involve consuming biological material, ethical approval was required. As the commercial biltong had been prepared in a food grade environment and the sensory tests were carried out at the factory, a low risk ethical approval was completed and approved by a delegated from the University Human Research Ethics Committee. Participants were allocated a code and no personal information was recorded.

3.5.2 Visual observations

Biltong has a very characteristic black to dark brown colouring and smooth surfaces when fresh. As biltong ages the colour fades to a lighter brown that eventually becomes a gold/yellow colour. The surfaces become cracked and frayed giving a visual appearance of dryness. Fungal growth is the greatest microbial threat, and becomes apparent when it produces visible mycelium. Therefore, biltong quality can be initially assessed using visual observations.

At known storage intervals, a sample of biltong was cut crosswise with a scalpel blade. A visual assessment was made of the external and interior colour, presence of salt crystals, and appearance of frayed edges. The texture was assessed from the difficulty in cutting the sample. Photographs were taken at each test time.

3.5.3 Organoleptic testing

Sensory tests can indicate the acceptability of the stored packaged biltong. A panel between 3-10 random participants were asked to participate in the test. The panel did not consist of the same participants each time. A subset of samples was presented to a random group of people of different ages (between 18-70), gender, nationality (New Zealander, European and South African) and familiarity with biltong (i.e. non-consumers to enthusiasts). Three 10-g samples from each storage environment were manually sliced into thin slices and placed into coded containers. A fresh biltong sample was used as the control. Participants scored the biltong for appearance, texture, taste "off taste" and whether they would purchase the sample using a scale of 1-5, where 1 is very unacceptable to 5 very acceptable (Table 3.3). Panellists cleansed their palate between samples by eating a plain popcorn/potato chip and having a drink of water. The average score and standard deviation for each sensory attribute was determined.

Table 3.3. Taste test score sheet.

Texture	Soft 1	2	Chewy 3	4	Brittle 5
Appearance	Unappealing 1	2	3	4	Very appealing 5
Taste	Unacceptable 1	2	3	4	Very acceptable 5
"Off" taste	"Off" 1	2	3	4	None 5
Would you buy this?	Yes		Maybe		No

3.6 Chemical Methods

3.6.1 pH and water activity

As the storage time increases, water can diffuse through the packet, which may change the product's water activity. Biltong has a very low water activity, so water reabsorption is likely and detrimental to the microbial profile of the sample. The sample could also dry further, making it unpalatable. Lipid oxidation decreases pH due to acid production. Product pH, therefore, can be an indicator of the rancidity in the sample as well as of water gain or loss.

The water activity of a thin slice of biltong taken from a random section of the sample was measured using the water activity analyser. The remaining sample was cut into small pieces and homogenized in a blender until it was fine fibres. The homogenized biltong was then mixed with 10 mL of water and left for three minutes before measuring the pH.

3.6.2 TBARS analysis

The sample was cut into 5-g pieces and placed in a 50-mL centrifuge tube. Distilled water (15 mL) was added and each tube was vortexed for 30 seconds then centrifuged at 4000 rpm for 10 minutes. A 1-mL of aliquot was transferred to a new tube and vortexed with 50 uL of BHT, 2 mL of TCA and TBA for 30 seconds. The tubes were incubated at 90^{o}C for 15 minutes then placed in cold water for 10 minutes. The tubes were vortexed for a further 30 seconds then centrifuged at 10 000 rpm for 5 minutes. The absorbance at 531 nm was recorded. The blank consisted of 1 mL distilled water and 2 mL TBA/TCA.

The standard curve was obtained using a series dilution of standard TEP (Fig. A4.1). The TBARS content was expressed as MDA/kg.

3.7 OxiKan Addition

OxiKan (Kalsec Inc.) is an antioxidant food additive made from rosemary extract. The oil stabilises foods high in oil by preventing free radicals from hydrolysing fatty acid chains. The maximum permitted level (MPL) for dried meat products is 150 ppm.

The oil is added to the marinade the biltong is dipped into. To calculate the amount needed in the marinade so the MPL in the dried biltong was not exceeded, a calculation was made of how much marinade remains on the biltong after dipping, taking into account the concentration on the product after drying. Calculations (Appendix 5) indicated the marinade could contain 255 mg/100 mL to ensure the MPL was not exceeded. This amount of OxiKan was added to the marinade and then the biltong was prepared using the standard procedure.

4 Results and Discussion

This chapter discusses the data obtained when investigating the effect of temperature and packaging environment on biltong stored for up to 46 weeks. The water activity, pH, and the ability of samples from the packaged biltong to support microbial growth if appropriate growth conditions are emulated and measured at known intervals. Colour and sensory evaluations on acceptability of the stored biltong are also done. The best conditions that would allow extended storage of biltong are then identified.

4.1 Water Activity

One of the greatest conserving factors for stored foodstuffs is having a low water activity, as this limits chemical and microbial changes. Chemical processes, such as lipid oxidation, and microbial growth occur more rapidly if the water activity is above 0.85 (Fig. 2.6). Most bacteria will not grow if the water activity is below 0.85 and fungal growth is inhibited if the water activity is below 0.6. However, drying products to achieve the low water activity can have adverse effects on consumer acceptability of product colour and texture (as well as the energy and cost of the drying). A change in water activity during storage would indicate the product was not at an equilibrium when first packed and/or moisture was being transferred through the packaging film.

The biltong in the trial was packaged in plastic film with a water transmission rate of 8 g/m^2/day at 39°C and 90% RH, an oxygen transmission rate of 50 cc/m^2/day at 23°C in dry conditions, and a CO_2 transmission rate of 200 cc/m^2/day at 23°C in dry conditions. These levels should help minimise adverse changes during storage.

Safari Biltong reported that the water activity of the biltong it supplied for the trial, before it was packaged, was 0.53. This is substantially lower than the typical value for commercial biltong of 0.85 but Safari Biltong intentionally dried to this value in an attempt to further extend shelf life.

The water activity of triplicate samples from each storage environment after two weeks storage was 0.6 to 0.71 (Figs. 4.1 to 4.3), which was substantially higher than the value reported for the fresh biltong. As it is highly unlikely that the water activity would have changed markedly in airtight packages, it is suspected the initial water activity measured by the company was incorrect. Therefore, the average water activity across all treatments of 0.69 after two weeks storage was assumed indicative of the initial water activity.

The variability of water activity measurements at each sampling time was small, being an average of 2.6% for triplicate samples across all treatments. The observed wide fluctuations water activity values with time may be caused by packing relatively small samples, which may have accentuated any difference in the consistency of biltong composition, failures in some of the packaging, or inaccurate calibration of the water activity meter. The degree of fluctuation appeared to depend on storage temperature, decreasing with increased temperature.

The water activity of biltong stored at 35°C decreased markedly during storage (Fig. 4.1). There was little difference in the water activity values and trends during storage for biltong stored in a partial vacuum (PV) or under a partial vacuum with an oxygen scavenger (PVS), indicating that adding oxygen scavenger packs did not affect the water activity. The water activity of biltong in either environment decreased from 0.69 and plateaued at 0.4 after 30 weeks of storage. The water activity of biltong stored in packs sealed under a full vacuum (VS) was always about 0.1 units higher than biltong stored under a partial vacuum (i.e. 0.72 at week 2, plateauing to 0.51 at week 32). The probable reason for the decrease in water activity during storage is vapour transmission through the packaging film, which is more rapid as the temperature increases. Storing the biltong under full vacuum limited the changes in water activity. Based on water activity values, biltong should be packaged under vacuum to retain a suitable water activity when stored at higher temperatures.

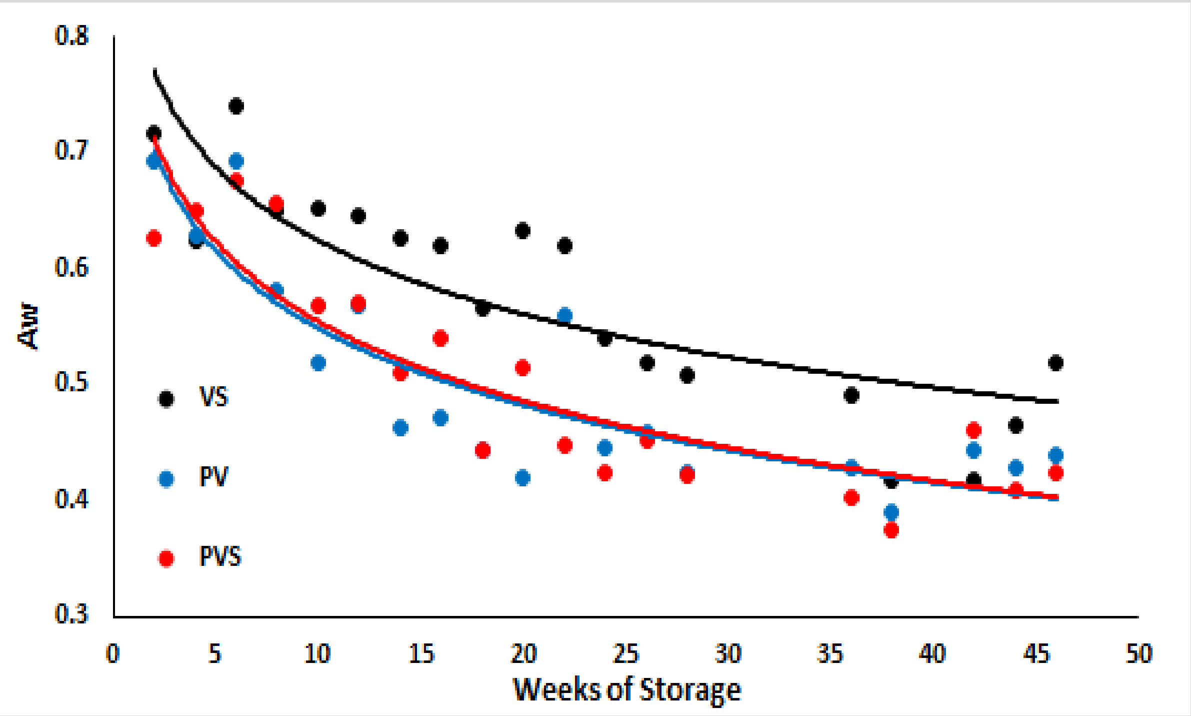

Figure 4.1. Effect of packaging environment on water activity of biltong stored at 35C (n=3).

The water activity of the biltong stored at 20°C, which fluctuated more than for biltong stored at 35°C, decreased during storage at 20°C (Fig. 4.2) but to a lesser extent than biltong stored at 35°C. Packs with a partial vacuum had a water activity of 0.7 at two weeks, which decreased to 0.68 and 0.67 after 46 weeks. VS packs had a water activity of 0.7 at 2 weeks, which decreased to 0.68 after 46 weeks. Again, adding the oxygen scavenger did not affect the values for packs with a partial vacuum. Although the water activity of packs with a full vacuum was higher than for biltong packed under partial vacuum, the difference was smaller than for packs stored at 35°C (0.01 to 0.04 compared with 0.1).

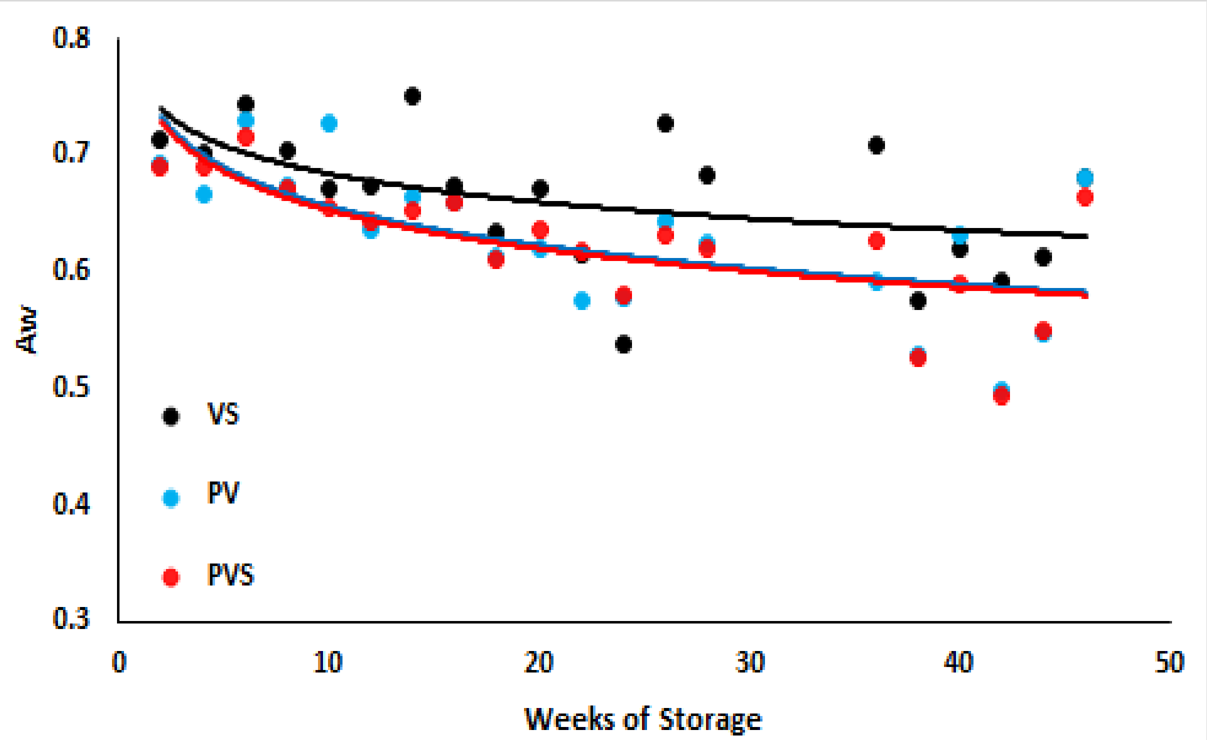

Figure 4.2. Effect of packaging environment on water activity of biltong stored at 20°C (n=3).

The water activity of biltong stored at 4°C fluctuated the widest over the storage time. As the standard deviation of water activity readings on triplicate samples at each sampling time was small (2%), the variation could be due to non-uniformity of the biltong (discussed later in this section). Unlike biltong stored at 20C or 35°C, there was no or minimal decrease in water activity during storage at 4°C (Fig 4.3). Again, the water activity of biltong stored under a full vacuum was higher than biltong stored under a partial vacuum and adding an oxygen scavenger had little or no effect on water activity.

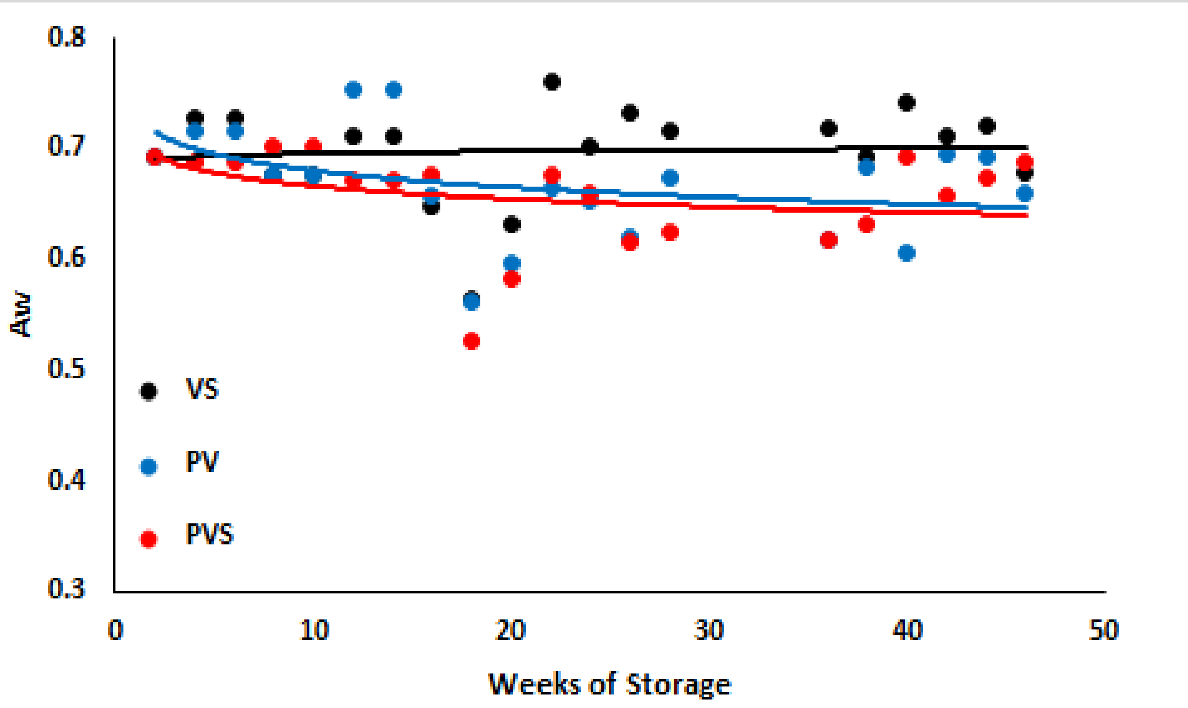

Figure 4.3. Effect of storage environment of the water activity of biltong stored at 4°C (n = 3).

Irrespective of packaging environment or storage temperature, the water activity of the biltong was always lower than required to support growth of bacteria commonly occurring in the manufacturing environment, and well below the 0.85 allowable limit under the FCP. However, biltong with extremely low water activity normally indicates over drying, which has the disadvantages of increased manufacturing cost and lower product yield.

Consumers normally do not like dried meat products with the tougher texture associated with having a low water activity (Petit *et al.*, 2014). They prefer "wet" biltong with a water activity of 0.8-0.89 (Leistner & Osterhoff, 1978) but this is on the limit of having a safe, long shelf life product. The freshly packaged biltong had an initial average water activity of 0.69, which is the upper value of the 'dry' biltong category (0.65-0.69). Biltong stored at 4°C retained a water activity in this range (0.66-0.69). The decrease in water activity of biltong stored at 20°C and 35°C inferred that the surrounding environment had a lower moisture content than the biltong. The packaging film had a water vapour transmission rate of 8 g/m2/day at 39°C and 90% RH so water could move from the biltong to the environment, effectively 'drying' the biltong further. The higher the storage temperature, the greater the transfer, which explains why the water activity of biltong stored at 35°C decreased more than for biltong stored at 20°C or 4°C.

The water activity values fluctuated widely during storage, irrespective of the packaging environment. The first source of experiment error is variation between samples. However, the SD of triplicate samples of each treatment was low (Table 4.1). The second source of experimental error is instrument error due to inaccurate calibration and/or drift while using the water activity meter as well as inexperience in the methodology. The biltong used for the trial came from the same batch and samples from each treatment were collected and measured at the same time but there was no consistency in when fluctuations occurred, reducing the chance it was equipment error. The third source of the error is due to variation of the biltong composition. The reasons for the variability in the data is unknown so further work needs to be done to identify the cause.

Table 4.1. Effect of temperature and storage environment on average water activity ± SD.

Environment	Storage temperature		
	35°C	20°C	4°C
VS	0.57 ± 0.09	0.66 ± 005	0.69 ±0.04
PV	0.49 ± 0.09	0.62 ± 0.05	0.66 ±0.04
PVS	0.50 ± 0.09	0.62 ± 0.04	0.65 ±0.04

Meat is not a uniform material. It contains water, protein, fats and solutes and has a fibrous structure. The biltong is made with added ingredients (salt, spices, vinegar) and does not have a uniform thickness. The small pieces of biltong (approx. 10 g) put into the sample packs probably had a variable composition and therefore varying water activity. These factors, along with water transmission through the packs during storage may affect the water activity of an individual sample. During storage, the biltong will come to equilibrium with the environment. During this trial, this involved water moving out of the product and the water activity decreasing. The values tended to plateau after approximately 30 weeks storage. To maintain the desired 0.65 - 0.7 water activity and minimise product loss will involve identifying a packaging film that limits water transmission.

Decreasing the water content in a food increases solute concentration. If the solution becomes saturated, the solutes will precipitate out. Salt deposits (from the salt in the marinade) tend to give biltong an unappealing taste and appearance.

Salt deposits appeared early on biltong samples stored at 35°C under VS or PV environments but the time it occurred did not seem to be related to any particular factor. For example, salt crystals were observed after 4 weeks storage (water activity of 0.62 for VS and 0.63 for PV environments) and biltong stored in the PVS environment had salt crystals after 6 weeks of storage (water activity of 0.68). Figure 4.4 shows the surface deposits after water activity decreased to 0.43 at 26 weeks.

Figure 4.4. Salt formation on the surface of biltong stored under a partial vacuum (PV) at 35°C for 26 weeks.

The VS and PVS samples stored at 20°C had salt crystals after week 10 (water activity of 0.66 and 0.67 respectively) but no salt crystals had formed on the PV sample stored for the same time (water activity of 0.73). Salt crystals were observed on samples stored under the PVS environment after 13 weeks storage at 4°C (water activity of 0.67). An investigation of the water activity when salt crystals were observed on samples found that, independent of the packaging environment, salt crystals will appear if the water activity is below 0.7 (Table 4.2).

Table 4.2. Water activity and storage time when salt deposits were first observed.

	Storage temperature					
Environment	35°C		20°C		4°C	
VS	4 weeks	0.62	10 weeks	0.68	28 weeks	0.72
PV	4 weeks	0.63	13 weeks	0.66	19 weeks	0.59
PVS	6 weeks	0.68	10 weeks	0.67	13 weeks	0.67

Summary

Regardless of packaging type, the water activity of the biltong decreased during storage at 35°C and 20°C but tended to be constant when stored at 4°C. Water activities values were never high enough to support microbial growth and therefore be a hazard to food safety. The water activity of biltong stored at 35°C decreased to an unacceptable level early in storage, resulting in visible salt deposits appearing. The VS environment was best for retained moisture in the product but water activity was still much lower than consumers find unacceptable after 46 weeks storage. The water activity of samples stored at 20°C decreased during 46 weeks storage but was still acceptable, especially when stored under VS conditions. Biltong stored at 4°C in the three packaging environments underwent minimal change in water activity during storage. The most cost-effective storage temperature would be 20°C, which does not require refrigeration. The SD of triplicate samples was small (1-3.5% of the water activity value) but there were unexplained fluctuations in water activity values over the storage period, which could be due to variability in biltong composition along with only putting small pieces (10 g) in each package. Visible salt deposits appeared when the water activities dropped below 0.7, which probably would decrease consumer appeal for the product. The water activity of all samples fell below 0.7 during storage. The higher the storage temperature, the greater the decrease with samples stored at 35°C decreasing to 0.43.

4.2 pH

The pH has an important role in microbial and chemical changes occurring in foodstuffs. Most microbes associated with food items and food poisoning are neutrophiles, meaning they do not grow well at a pH higher or lower than pH 7. Adding certain acids can decrease the effect of lipid oxidation by encapsulating the fatty acid chains (Abandansarie *et al.,* 2019).

Although biltong recipes may not include an acid, vinegar (acetic acid) it is a popular additive because it enhances flavour and improves microbial inhibition. The isoelectric point of meat proteins is about 5.5. The recipe Safari Biltong uses for its biltong includes malt vinegar, which lowers the initial pH of the biltong to 5.2. The low WHC at this pH further inhibits microbial growth. Product pH can also indicate the level of lipid oxidation occurring. The products of lipid oxidation are short fatty acid chains such as butyric and propionic acid, which give rancid odours and tastes. The associated decrease in pH can adversely change the taste of the biltong. To maintain consumer acceptability, the packaged biltong should maintain a pH similar to that of fresh biltong.

The pH of the fresh biltong provided by Safari Biltong employees was 5.3 and has been included in the data sets. Changes in pH that deviate from 5.3 are considered to indicate deterioration because the starting pH is desirable.

The pH of the biltong tended to stay constant and then decrease (Figs. 4.5 to 4.7). Biltong stored at 35°C decreased markedly around week 22 (Fig. 4.5) to pH 4.8 (VS or PV environments) and pH 4.9 (PVS environment). Values for biltong in PV and VS were similar but the pH of biltong stored with oxygen scavengers (PVS) was consistently 0.1 units higher. This may indicate that the short chain fatty acid produced from lipid breakdown contributes to the decrease in pH. Adding oxygen scavengers helped limit the lipid breakdown and therefore the decrease in pH. The pH of biltong, regardless of packaging environment, had decreased to 5.0 after 35 weeks. The PV environment still contains oxygen, which allows lipid oxidation. The VS condition allows oxygen entry because of the packaging film's oxygen permeability. The VS maintained a higher water activity, which has been shown to contribute to lipid oxidation because compounds can move more freely than in products with lower water activity, where reactive compounds have been immobilized (Karel & Simic, 1980).

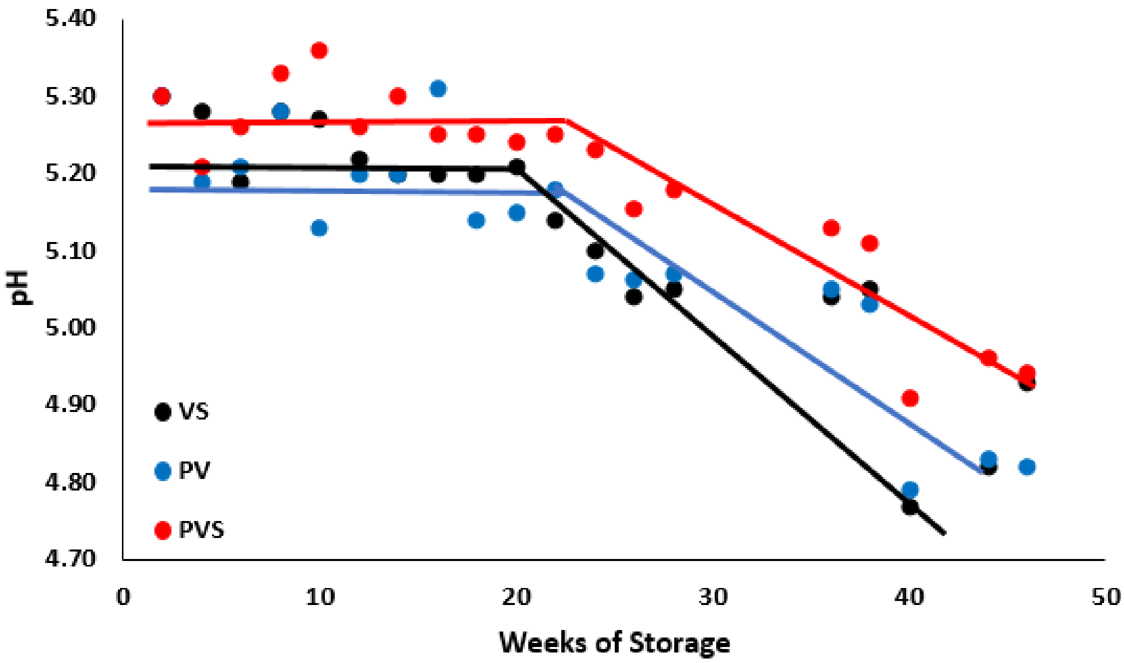

Figure 4.5. Effect of storage environment and temperature on pH of biltong stored at 35°C.

The pH of samples stored at 20°C tended to be constant for about 20 weeks and then decreased (Fig. 4.6). Again, the pH of biltong stored with oxygen scavengers was higher than in the VS or PV environment. Until week 38, all samples stored at 20°C had a pH above 5.0.

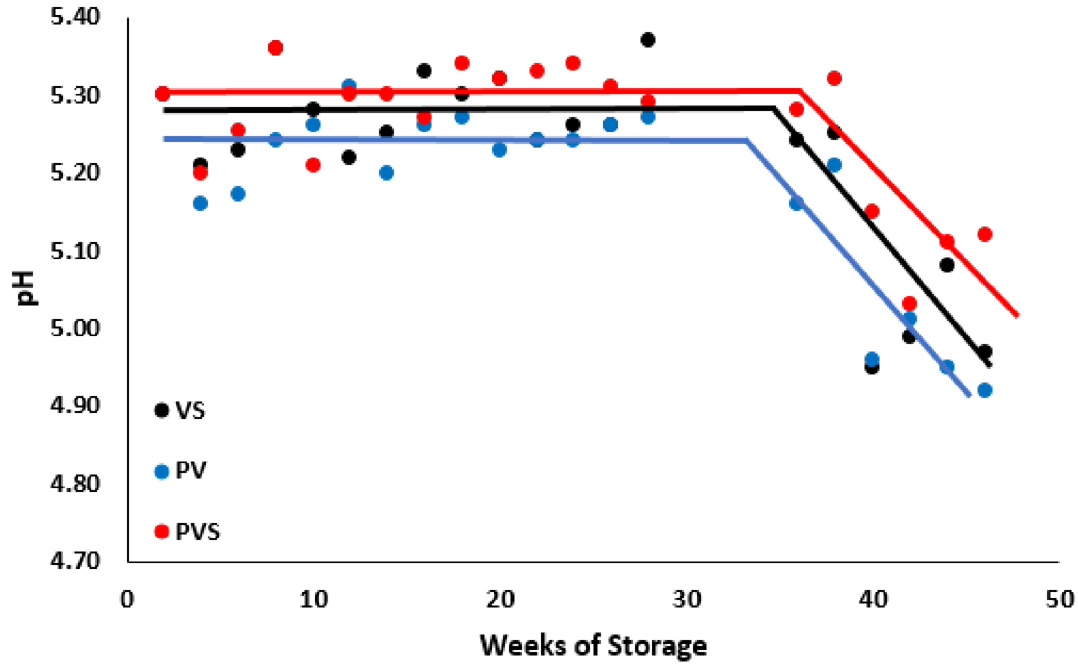

Figure 4.6. Effect of storage environment and temperature on pH of biltong stored at 20°C.

The pH of biltong stored at 4°C showed a similar trend. It was constant until about 30 weeks, followed by a linear decrease (Fig. 4.7). The pH drop indicates lipid oxidation is occurring during chilled storage. Packaging environment had no effect on pH change at 4°C, indicating that including an oxygen scavenger in biltong stored at 4°C does not affect pH. The pH of the biltong did not fall below pH 5 during storage.

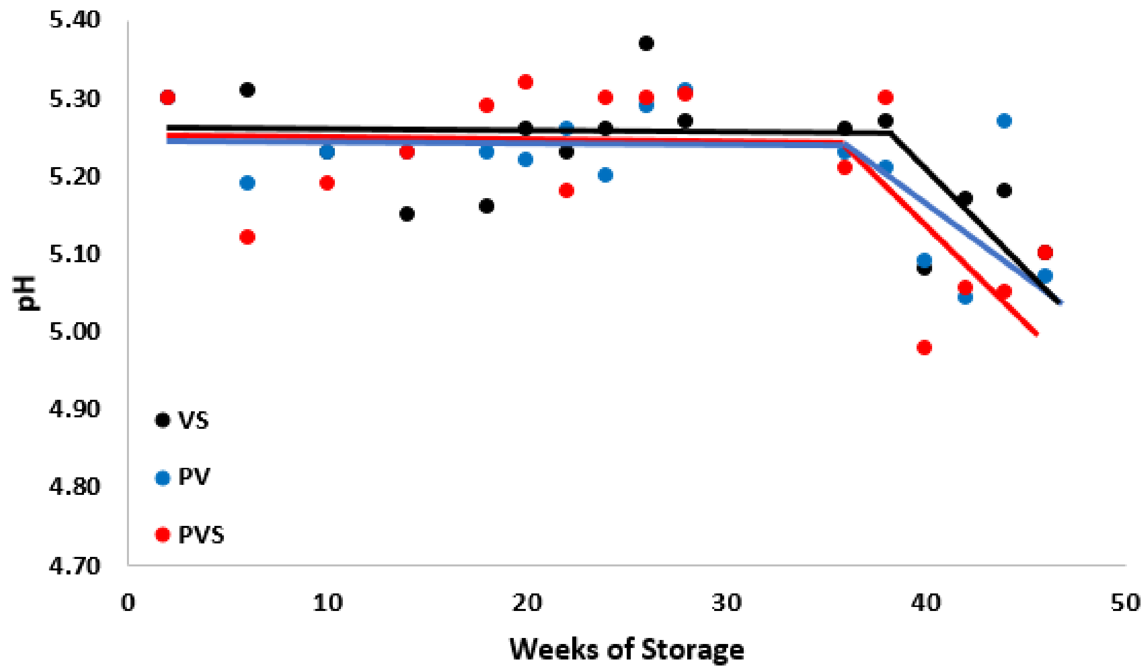

Figure 4.7. Effect of storage environment and temperature on pH of biltong stored at 4°C.

Moisture loss would have contributed to concentration of acidic products. This contributed to the greater pH drop observed in biltong stored at 35°C, which also had a greater decrease in water activity (Fig. 4.1). Biltong stored at 20°C had moderate decrease in water activity and a smaller drop in pH. The biltong stored at 4°C retained its water activity, which would dilute the effect of any acids produced, so the pH remains near the initial value.

Storage temperature also affects biltong pH. It is important that the pH of stored biltong remains similar to that fresh biltong. The pH of biltong stored at 35°C drastically decreased making the product unsatisfactory after 46 weeks of storage. Although the pH of biltong stored at 4°C was constant for 30 weeks compared with 20 weeks for biltong stored at 20°C, the cost of keeping biltong chilled may outweigh the gain of obtaining a longer storage time.

The oxygen scavengers had a capacity of 100 cc, which is typical for containers or packets with a capacity of up to 1 L. Regardless of storage temperature, the pH of biltong packed with scavengers (PVS) began to decrease markedly at week 38. The oxygen scavengers may have become saturated, which allowed lipid oxidation to increase. The data indicates that storage temperature did not affect functionality of the oxygen scavengers.

As with water activity values, the SD of means of triplicate pH values of each treatment at each sampling time was small, indicating the sampling method does not contribute greatly to the variation seen across the storage trial. The reason for the fluctuations in pH value over the storage time were not investigated but could be due to natural variability in biltong and because only small pieces of biltong (10 g) were put into each package. The SD for average pH across the storage time is higher (5.6%) than the SD for water activity (Table 4.3).

Table 4.3. Effect of temperature and storage environment on average pH values ± SD.

	Storage temperature		
Environment	35°C	20°C	4°C
VS	5.10 ± 0.15	5.21 ± 0.12	5.02 ±0.08
PV	5.09 ± 0.16	5.17 ± 0.12	5.99 ±0.09
PVS	5.14 ± 0.17	5.25 ± 0.09	5.19 ±0.10

Summary

The pH of stored biltong was constant for several weeks and then decreased. The storage environment and temperature affected how long the pH was constant. The lower the storage temperature, the smaller the decrease in biltong pH during storage. The storage environment affected the amount, being higher at higher storage temperatures and minimal when biltong was stored at 4°C. In general, including an oxygen scavenger in the packs tended to limit the decrease in pH, especially at higher storage temperatures and therefore, should be included. The changes in pH of packages kept at ambient or chilled temperatures were similar so it would be more cost effective to store biltong under partial vacuum with oxygen scavengers (PVS) or full vacuum (VS) at ambient temperature. An indication of the likely changes in product stored at 4°C or 20°C could be found by using accelerated storage at 35°C, where changes at 20 weeks can indicate the changes at 30-38 weeks at lower storage temperatures.

4.3 Colour

Consumers perceive the colour of meat products as an indicator of 'freshness'. Fresh meat has a bright red colour because the pigment myoglobin is present as oxymyoglobin. As oxygen is used up, the myoglobin becomes the brown pigment metmyoglobin, which may make the meat look old or off. Freshly made biltong is black to a very dark brown because the myoglobin has been concentrated by the drying process. The myoglobin in the biltong is highly sensitive to light and oxygen. As the biltong ages, its colour lightens and fades to a light golden brown. The change in colour is not as quick as fresh meat due to the low water content and the curing agents. However, the colour changes can indicate the age and oxidation state of the biltong.

During the storage trial, biltong colour was periodically graded on a scale of 1 to 5 (Table 4.4), where grades 1 to 3 are still acceptable to sell to consumers but 4 to 5 are unacceptable.

Table 4.4. Colour grading scale.

Colour	**Grade**
Black (highly acceptable)	1
Dark brown	2
Brown	3
Light brown	4
Gold (highly unacceptable	5

When the biltong was first packaged, it had the characteristic black colour, which changed during storage (Fig. 4.8).

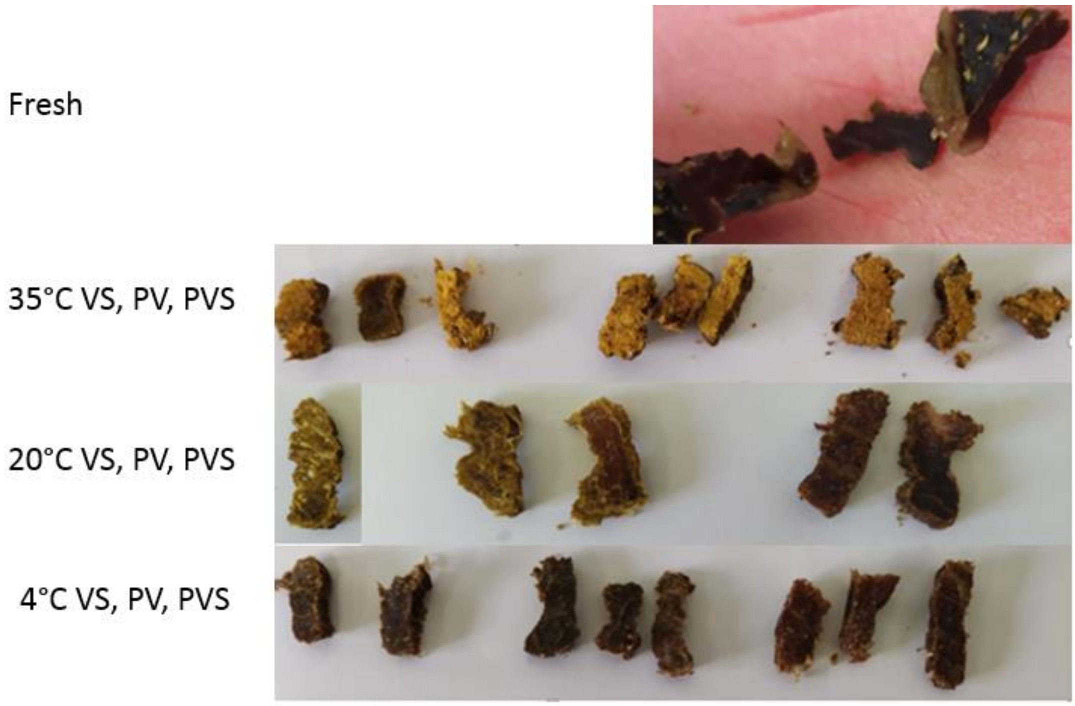

Figure 4.8. Effect of storage environment and temperature on the colour of sliced biltong after 46 weeks of storage.

As expected, the fastest (and greatest) colour change occurred in biltong stored at 35°C (Fig. 4.9), which faded to a golden colour and had a rough surface. Higher storage temperatures accelerate degradation processes such as protein denaturation, lipid oxidation and oxygenation of myoglobin. Biltong stored at 35°C under full vacuum (VS) retained its colour better than biltong stored under partial vacuum, with (PVS) or without (PV) oxygen scavengers. On the basis of colour, the maximum storage life of biltong stored under VS conditions is 42 weeks, that stored under PVS conditions is 24 weeks, and that stored under PV conditions is 16 weeks.

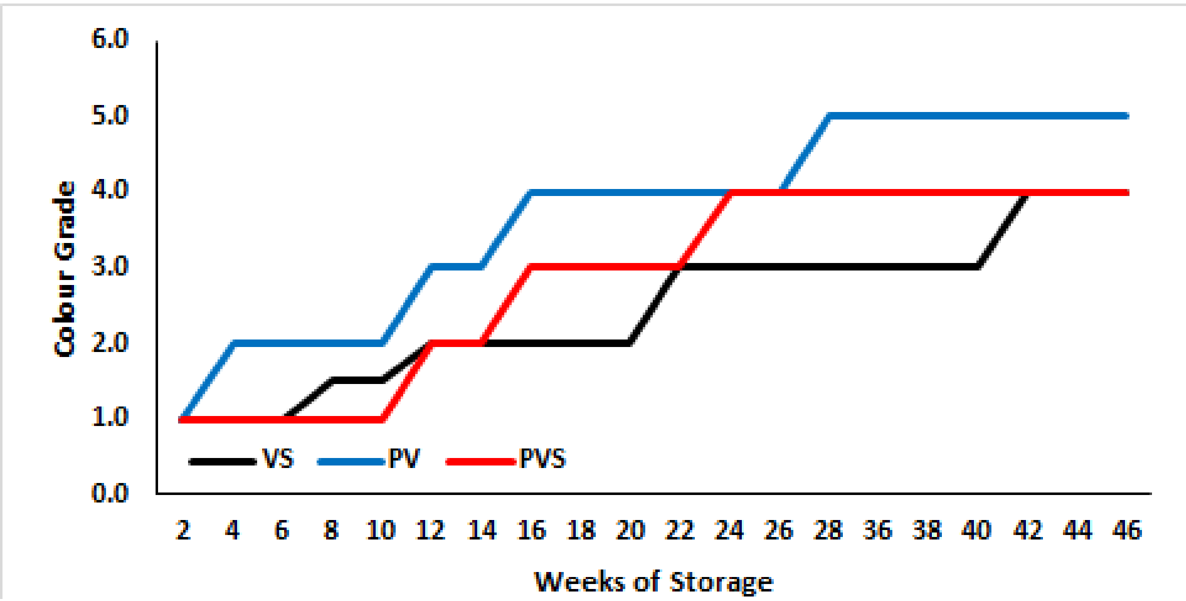

Figure 4.9. Effect of packaging environment on colour grade of biltong stored at 35°C (1 = acceptable to 5 = highly unacceptable).

The colour of biltong stored at 20°C changed less than biltong stored at 35°C (Fig. 4.10) and packaging environment had a smaller effect on colour change. All biltong still had an acceptable colour score of 3 or lower after 46 weeks. Those stored in PVS retained the internal dark colour best. The surface was smoother than biltong stored at 35°C but still had significant cracks and frayed edges compared with fresh biltong. Biltong stored under a PVS environment had the smoothest surface. Biltong stored under VS or PV environments had a similar appearance, which was rougher than biltong stored under PVS.

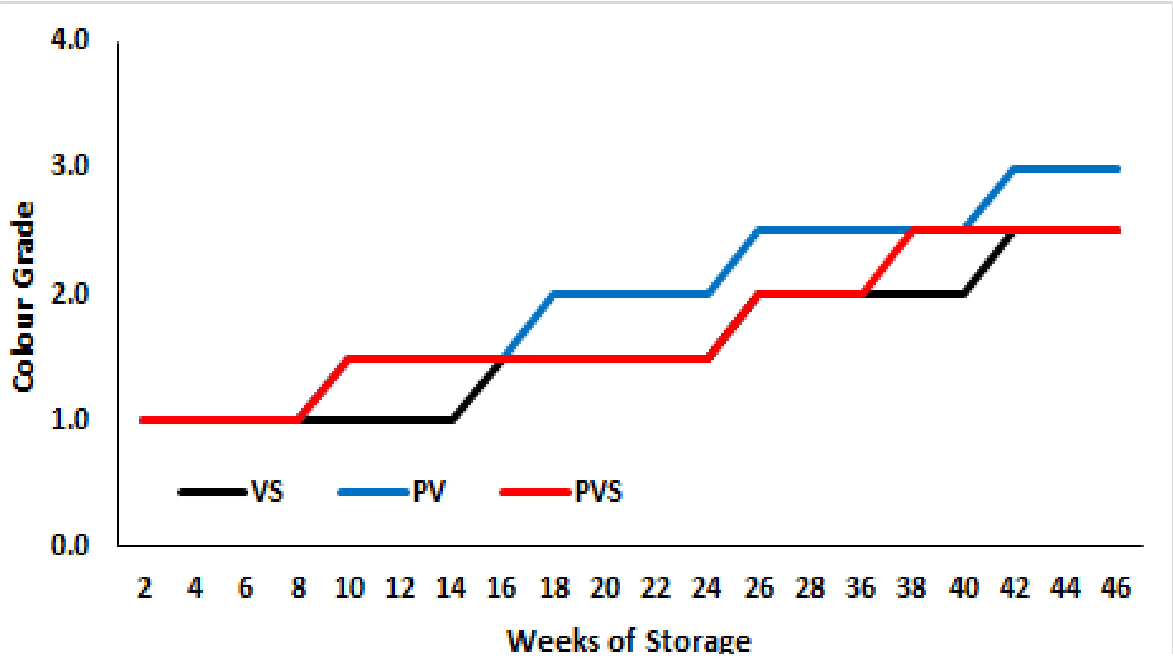

Figure 4.10. Effect of storage environment on colour grade of biltong stored at 20°C (1= highly acceptable to 5 = highly unacceptable).

The colour of biltong stored at 4°C remained very similar to fresh biltong. Irrespective of packaging environment, all had an acceptable colour grade and appearance after 46 weeks storage (Fig. 11). Biltong stored under PVS changed faster than that stored in PV. A colour change in biltong stored in VS was only detected after 26 weeks. Although the surface was the most similar to fresh biltong, cracks and lines of lighter colour did appear during storage.

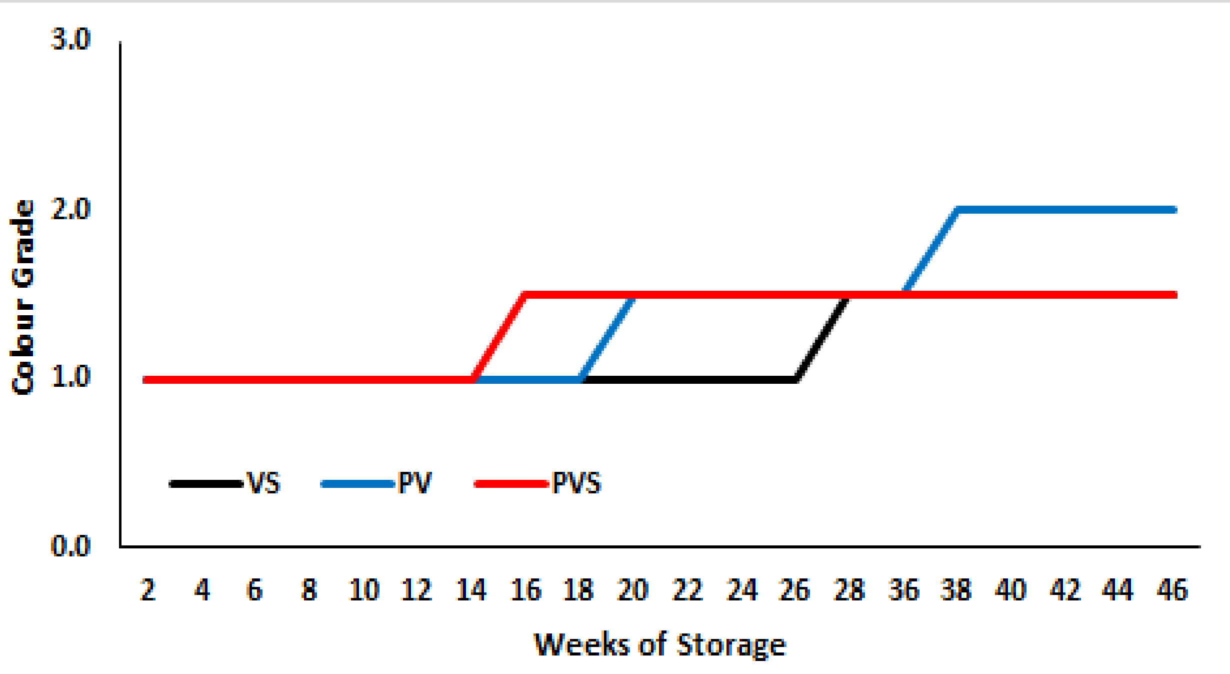

Figure 4.11. Effect of storage environment on colour grade of biltong stored at 4°C (1 = highly acceptable to 5 = highly unacceptable).

Summary

The higher the storage temperature, the greater the colour deterioration. The best packaging environment for surface colour retention was VS. The PV environment was the poorest for maintaining colour, regardless of storage temperature. Storing biltong in a PVS environment was best for maintaining optimum internal colour (Fig. 4.8). As well as producing rancid flavours and odours, lipid oxidation can adversely affect the visual appearance of meat products. Including an oxygen scavenger helped decrease colour deterioration. Accelerated storage at 35°C under PV conditions for 4 to 6 weeks could indicate the colour changes that may occur in biltong stored at 20°C or 4°C at 18 or 36 weeks respectively. As with pH change during storage, storing biltong at ambient temperatures may be more cost effective in terms of colour retention than chilled storage.

4.4 Microbial Quality

Any food item is a target for microbial spoilage as it provides nutrients. Dried foods such as biltong provide a specific habitat for microbes that are better adapted, to low moisture, low pH and high solute concentration environments, than other microbes. Fungal growth presents the greatest threat of microbial spoilage on biltong but bacteria can also grow and/or survive.

The microbial profile of biltong before it was packed was done by an analytical company (Table 4.5). It indicated that bacteria and fungi were present on the freshly made biltong but the levels were not high enough to create a public health concern. However, if the packaging is unacceptable or the storage environment allows growth of the microbes present, microbial loading could increase to unacceptable values and create a health issue. Another profile was to be done after 46 weeks but not completed because the University and industrial laboratories were closed and not accessible during New Zealand's nationwide Covid-19 lockdown.

Table 4.5. Microbial analysis of fresh biltong before being packed (Hills Laboratory Ltd).

Test	**Unit**	**Value**
Aerobic Plate Count	CFU/g	860
E. coli	CFU/g	<5
Enterobacteriaceae	CFU/g	<5
Staph. aureus	CFU/g	<10
Yeasts	CFU/g	<50
Moulds	CFU/g	300
Yeasts and Moulds	CFU/g	300
Salmonella	Per 120 g	None detected

4.4.1 Fungal spore germination

Fungal spores in the environment contaminate surfaces, including processing equipment, in the manufacturing plant and are easily transferred to the biltong surface. Fungi can thrive at water activities as low as 0.6. If conditions are unfavourable, these spores will not germinate and may eventually become unviable (Jones *et al*., 2017). If conditions allow any bacteria present to grow, they will usually outcompete the fungi. However, if the fungi spores germinate, they will produce visible mycelium, thereby spoiling the product. If packaging is compromised or inadequate, any spores present may be able to germinate.

Spore longevity is affected by temperature, conidial moisture content, relative humidity (RH) and oxygen concentration. The effect of temperature and RH are the most studied factors (Smilanick & Mansour, 2007). For most spores, longevity (measured as the spore's ability to germinate with time), decreases with increasing temperature, conidial moisture content and RH. At a fixed RH, spore germination decreases with increase in temperature (below the temperature that kills spores). However, spores can withstand freezing and germinate once conditions are favourable.

Conidial moisture content is the moisture within the spore. Dry proteins survive high temperatures better than wet proteins. The low moisture content of the spore also allows water absorbance from the surroundings by osmotic pressure (Smilanick & Mansour, 2007). The effect RH has on germination depends on the fungi. Most spores can survive at a lower humidity with the optimum being between 7 and 38%. There is a decline in germination at higher RH values but this decline is less than when at 7% RH (Smilanick & Mansour, 2007).

Spore germination was tested by incubating pieces of biltong on DG-18 agar and scoring the growth (Table 4.6) after the plates had been incubated at 25°C for 6 days. Spores were deemed no longer viable when no germination occurred in three consecutive tests (6 weeks).

Table 4.6. Scores for visual growth of fungi on biltong after sampling.

Growth	**Percent sample covered**
Extensive	>60%
Moderate	10-60%
Slight	<10%

Temperature and storage environment affected spore germination (Table 4.7), with germination being inversely affected by temperature. The ability of fungal spores to survive lower temperatures better has also been reported by Smilanick & Mansour (2007). The lower germination at the higher storage temperatures is thought to be due to spores being killed by heat stress and water loss.

At three weeks storage at 35°C, there was moderate fungal growth on biltong stored in VS environments and extensive growth on biltong stored under PV and PVS environments. No growth was observed after five weeks storage under VS and PV environments and after nine weeks storage under PVS environment. The greatest growth occurred in PVS environments, less in PV environments and the least in VS environments. As the water activity values of biltong in the PVS and PV environments were lower than biltong stored in the VS environment, factors other than water activity must have helped the spores retain their viability.

There was extensive fungal growth on all biltong after three weeks storage at 20°C, which decreased to moderate growth after five weeks storage. Although no germination occurred in samples stored in the VS environment at week 11, slight growth was observed at week 13. After week 15, none of the biltong had germinating spores.

Spores on biltong stored at 4°C germinated over the entire storage time. Water activity of all biltong during storage at 4°C (Fig. 4.11) was relatively high (0.66 to 0.71), which may have contributed to the growth observed. The extent of the growth between packaging environments were variable showing packaging environment had little to no effect when stored in chilled conditions.

Table 4.7. Effect of storage environment and temperature on fungal growth.

Storage time, weeks	**Storage conditions**								
	35°C			20°C			4°C		
	VS	PV	PVS	VS	PV	PVS	VS	PV	PVS
3	10-60	>60	>60	>60	>60	>60	NT	NT	NT
5	0	0	<10	10-60	10-60	10-60	>60	>60	>60
9	0	0	0	>60	10-60	>60	10-60	>60	>60
11	0	0	0	0	<10	10-60	NT	NT	NT
13	0	0	0	<10	<10	<10	>60	>60	>60
15	NV	NV	NV	0	0	0	NT	NT	NT
17	NV	NV	NV	0	0	0	<10	<10	<10
19	NV	NV	NV	0	0	0	<10	10-60	10-60
21	NV	NV	NV	NV	NV	NV	<10	10-60	>60
23	NV	NV	NV	NV	NV	NV	<10	<10	10-60
20	NV	NV	NV	NV	NV	NV	<10	<10	10-60
28	NV	NV	NV	NV	NV	NV	<10	>60	>60
30	NV	NV	NV	NV	NV	NV	10-60	10-60	10-60
33	NV	NV	NV	NV	NV	NV	10-60	10-60	>60
36	NV	NV	NV	NV	NV	NV	<10	<10	10-60
39	NV	NV	NV	NV	NV	NV	>60	<10	10-60
42	NV	NV	NV	NV	NV	NV	<10	10-60	>60
44	NV	NV	NV	NV	NV	NV	<10	10-60	<10

Key: NT= Not tested; NV= Not viable

Failures in the packaging change the packaging environment, allowing conditions that may support growth as well as allowing post-packaging contamination. White fungal growth (Fig. 4.12) observed in a single biltong sample stored at 20°C in a PV packaging for 24 weeks was found to have a faulty seal.

Figure 4.12. White fungal growth on improperly sealed biltong stored in a PV environment at 20°C for 24 weeks.

The effect of temperature on spore germination agrees with published research (Smilanick & Mansour, 2007). Spores retain their viability better at lower temperatures (such as 4°C). Another factor may be the RH (not measured) of the package. If all other factors are constant, spores germinate better in environments with higher RH (Pasanen *et al.*, 1991). Environments with the same moisture content have a higher RH at lower temperatures so the RH in packages at 4°C would be higher than if the package was stored at 20°C or 35°C.

In terms of packaging environment, the ability for the spores to germinate was lowest in VS environments, then the PV environment and highest in the PVS environments. Although spore germination was observed on incubated samples taken from biltong that had been stored for extended periods, visible mycelium was not observed in any of the packages during storage other than the package with the faulty seal (Fig. 4.12). This indicates that a combination of the packaging environment and intrinsic properties of the biltong such as low water activity, low pH and ingredients in the marinade helped prevent fungal growth during storage.

4.4.2 Fungal spore identification

Fungal species commonly found on biltong are *Candida, Aspergillus* and *Penicillium* (Jones *et al*., 2017). There were two types of fungi growing on the germination plates for samples taken from the fresh biltong used for the trials (Fig.4.13).

The colour and morphology allowed the indicative genus of the fungi to be identified. The yellow colony is *Aspergillus*. Species in the *Aspergillus* genus produce a variety of surface coloured powders with white to pale yellow undersides. The most common *Aspergillus* species isolated from foodstuffs are *A. fumigatus, A. flavus* and A. *niger*. *Aspergillus fumigatus* forms blue-green to grey colonies, *A. niger* forms black colonies and *A. flavus* produces yellow-green colonies (Micheli, 1809). It is strongly suspected that the yellow colony is *A. flavus* (Fig. 4.14), a weak opportunistic pathogenic fungus that produces secondary metabolites such as aflatoxins, cyclopiazonic acid, aflatrem, aflavinin, kojic acid, aspergillic acid, neoaspergillic acid, β-nitropropionic acid, and paspalinine (Chang *et al.*, 2014). It is one of the most widely reported food-borne fungi and is a dominant species in stored products especially, products with low moisture contents. The pH has little effect on *A. flavus* growth and it can grow at temperatures between 10 and 55°C with an optimum of 33°C (Jackson & Dobson, 2016).

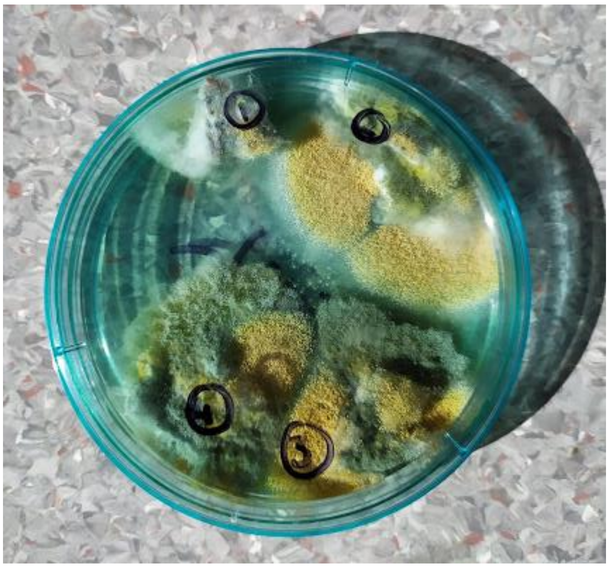

Figure 4.13. Fungal growth at 25°C from spores present on fresh biltong.

Figure 4.14. Microstructure of *A. flavus* (from Noman *et al.*, 2019).

The green colonies could be *Penicillium* or *Cladosporium*. The underside of *Cladosporium* colonies ar*e* black whereas the underside of *Penicillium* colonies is white. Penicillium species are fast growing, often beginning as white before becoming green and have a cotton or wool-like texture (Anon., 2020). The green colonies on the plate grown from the packaged biltong had a white underside indicative of *Penicillium*.

Penicillium is the most common fungi found on foods, including dried meat products (Bullerman, 2003). There are many different *Penicillium* species, some of which are used in food productions, such as the species *P. camemberti* involved in cheese. *P. chrysogenum, P. citrinum, P. janthinellum, P. marneffei,* and *P. purpurogenum* are the most common species infecting mammals and other biological material. Many Penicillium species produce mycotoxins such as the well-known antibiotic penicillin, which may be harmful when consumed (Bullerman, 2003). A Norwegian study (Anon., 2011) isolated 39 fungal species from dry cured sausages, with *Penicillium* being the dominant species. *Penicillium nalgiovense,* which can produce penicillin, was the most frequent species (Anon., 2011).

Penicillium colony colour is not as distinctive as for *Aspergillus* species so colour is not a useful species indicator. Instead, macroscopic morphology and microscopic structures are used for species confirmation (Anon., 2020). Many *Penicillium* species found on foods can grow at refrigeration temperatures and on foods with a variety of water activities.

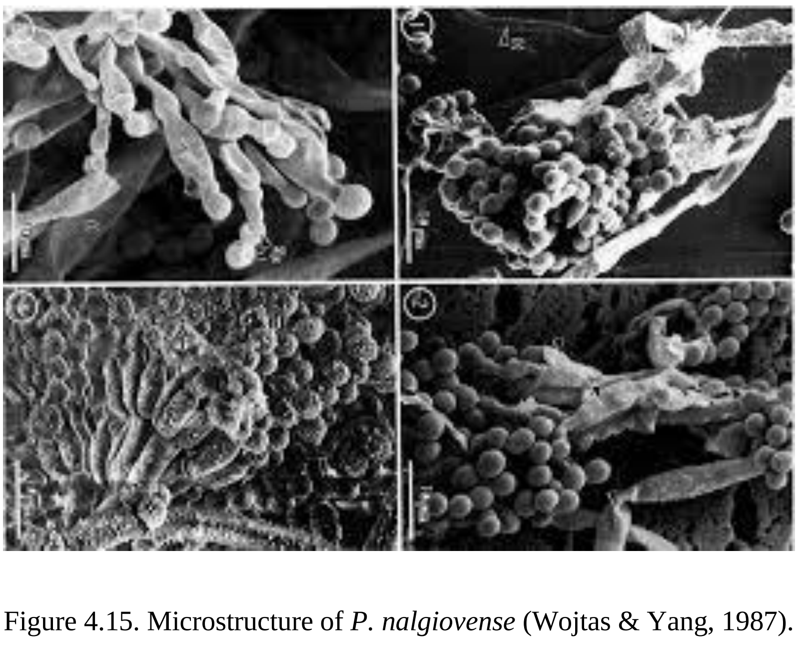

Figure 4.15. Microstructure of *P. nalgiovense* (Wojtas & Yang, 1987).

Due to the Covid-19 lockdown, microscopic examination to identify the cultures could not be done. However, from the appearance of the colonies, it is deduced the green colony is *P. nalgiovense*.

4.4.3 Bacterial growth

Growth of food poisoning bacteria can result in illness if they are consumed. Sterilization of food can only be achieved with intense processing, which alters the chemical, nutritional and textural properties of the food. Using hurdle factors to help maintain the populations of harmful bacteria below levels that would be hazardous to health is often the best action. Biltong incorporates many hurdle factors, making it a microbiologically stable product. However, improper preparation, inadequate packaging, or contamination can still occur. Testing for the types and level of bacteria present will indicate the stability and safety of a food.

The effect of storage temperature and environment on bacterial numbers was investigated by monitoring growth of bacteria on inoculated nutrient agar for up to 96 hours (Table 4.8). A bacterial count of biltong at the beginning of the trial was not done. The bacterial count on freshly made biltong from a different batch to that used for the trial, with a different water activity and which had been stored unpackaged in air was 1000 CFU/mL for all incubation times of the test (Table 4.8).

No bacteria grew on samples of biltong that had been stored under different environments at 35°C or 20°C for 20 weeks, even when the agar plates were incubated for 96 h.

Table 4.8. Effect of storage temperature, packaging environment incubation time on growth (CFU/mL) of bacteria at 35°C on agar plates from biltong stored for 20 weeks.

			Storage conditions								
			35°C			20°C			4°C		
Packaging		Fresh	VS	PV	PVS	VS	PV	PVS	VS	PV	PVS
Incubation time	24 h	1000	0	0	0	0	0	0	60	0	0
	36 h	1000	0	0	0	0	0	0	60	10	0
	96 h	1000	0	0	0	0	0	0	200	65	115

Growth was observed on biltong that had been stored at 4°C for 20 weeks. Bacteria were identified on biltong that had been stored under VS after 24 h incubation, after 36 hours incubation for biltong stored under PV, and after 96 h incubation for biltong stored under PVS. Because microbial growth on biltong stored under the PV environment was lower than biltong that had been stored under full vacuum or partial vacuum with oxygen scavengers, the microbes on the biltong are likely to be aerobic or facultative aerobes.

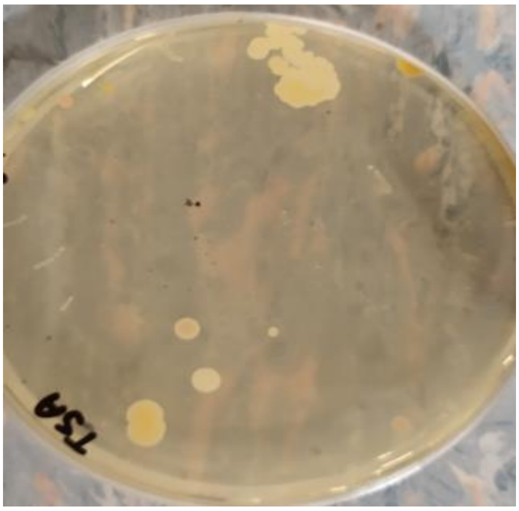

Figure 4.16. Appearance of microbial growth on biltong stored at 4°C for 20 weeks in a VS environment after 96 hour incubation.

Morphology examination indicated the colonies were not bacterial but a genus of *Penicillium*. The overall results from the bacterial investigation indicate that bacteria did not grow in the packaging environment and that fungi represent the greater threat to the shelf life and safety of biltong.

Summary

Moulds and bacteria were detected on fresh biltong. No bacteria growth was detected on biltong that had been stored at various temperatures and packaging environments for 20 weeks, indicating that the combined effect of low water activity, packaging environment and biltong composition inhibited or even killed any bacteria originally present. Germination of any fungal spores present was affected by storage temperature and time but packaging environment had a minimal effect on germination. In general, the higher the storage temperature, the faster spore germination was inhibited. Storage at 4°C was not effective at decreasing fungal spore viability and growth was still able to occur after 46 weeks storage when incubated on DG-18 agar. Fungi outcompete bacteria in low moisture environments, such as biltong, further confirming that fungi is a greater threat to biltong than bacteria.

4.5 Sensory Analysis

Sensory testing is crucial in determining whether the packaged biltong is acceptable to consumers. If the biltong is microbiologically safe and meets all MPI standards but is unpalatable, people will not buy it. A panel of untrained panellists familiar and unfamiliar with biltong were asked to evaluate biltong at 6-week intervals.

Panellists were asked to score texture, appearance, taste and off-taste on a scale of one to five (Fig. 3.2) for each attribute after 12 (Fig. 4.17), 18 (Fig. 4.18), 23 (Fig. 4.19), 28 (Fig 4.20) and

36 (Fig. 4.21) weeks. Further sensory analyses were not done due to the Covid shut down. For appearance and taste, a score of 5 represents desired/appealing and 1 is unappealing. For texture, 1 was 'soft', 3 (the optimum) as chewy and 5 was 'brittle'. A score of 5 for "off-taste" indicated no rancid flavours and a score of 1 indicated a strong rancid taste. Panellists were also asked to indicate if they would purchase the biltong sample.

Due to their lack of awareness of typical biltong characteristics, the scores from panellists unfamiliar with biltong were more variable than scores from panellists familiar with biltong.

In the first sensory tests, done after 12 weeks storage, fresh biltong had a good appearance and taste, no off-tastes and a soft texture (Fig. 4. 17). Panellists scored the texture, taste, off taste and appearance of all biltong stored at 35°C poorly (Fig. 4. 17) and asked that biltong stored at 35°C not be included in future sensory tests.

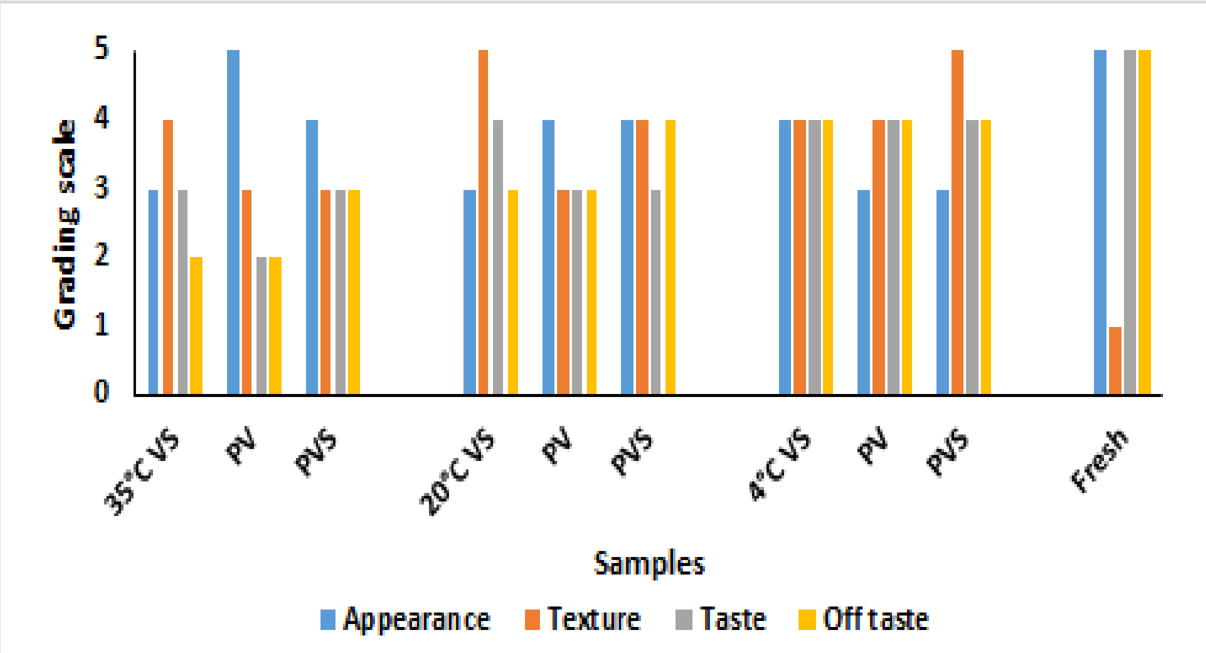

Figure 4.17. Effect of storage temperature and environment on sensory scores of biltong after 12 weeks of storage, where 5 is very acceptable and 1 is very unacceptable (n=9).

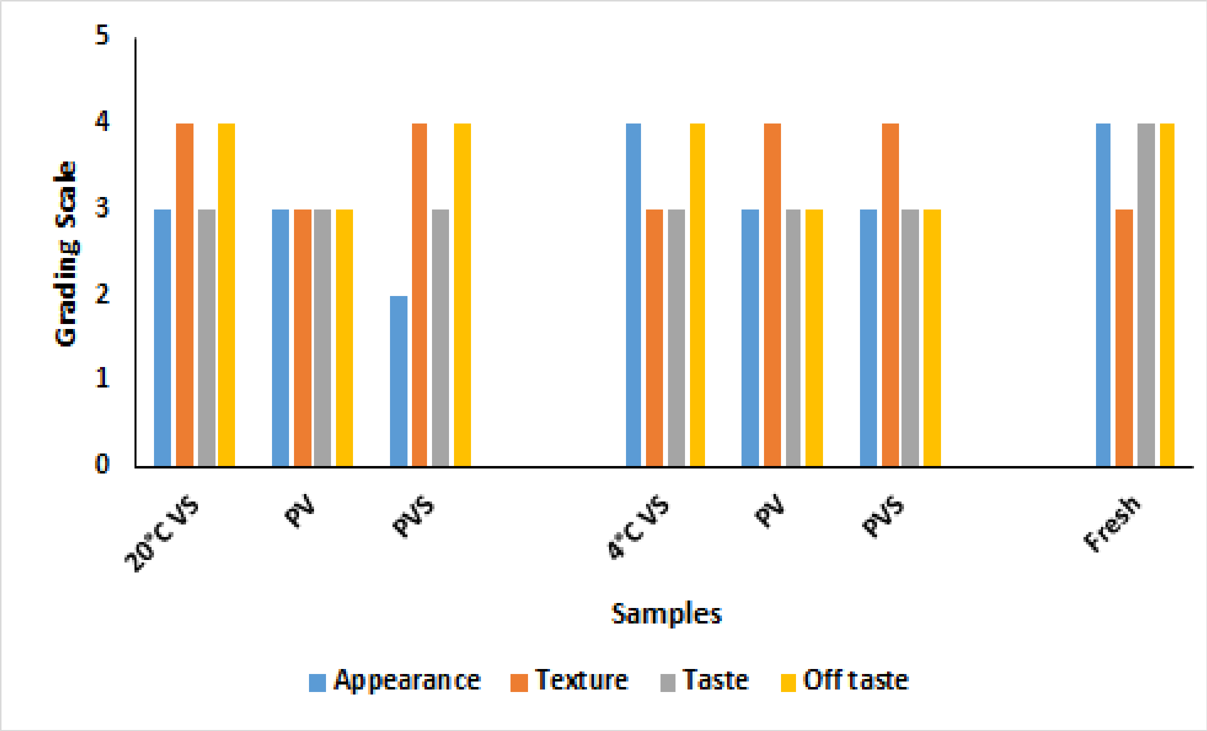

Figure 4.18. Effect of storage temperature and environment on sensory scores of biltong after 18 weeks of storage where 5 is very acceptable and 1 is very unacceptable (n=6).

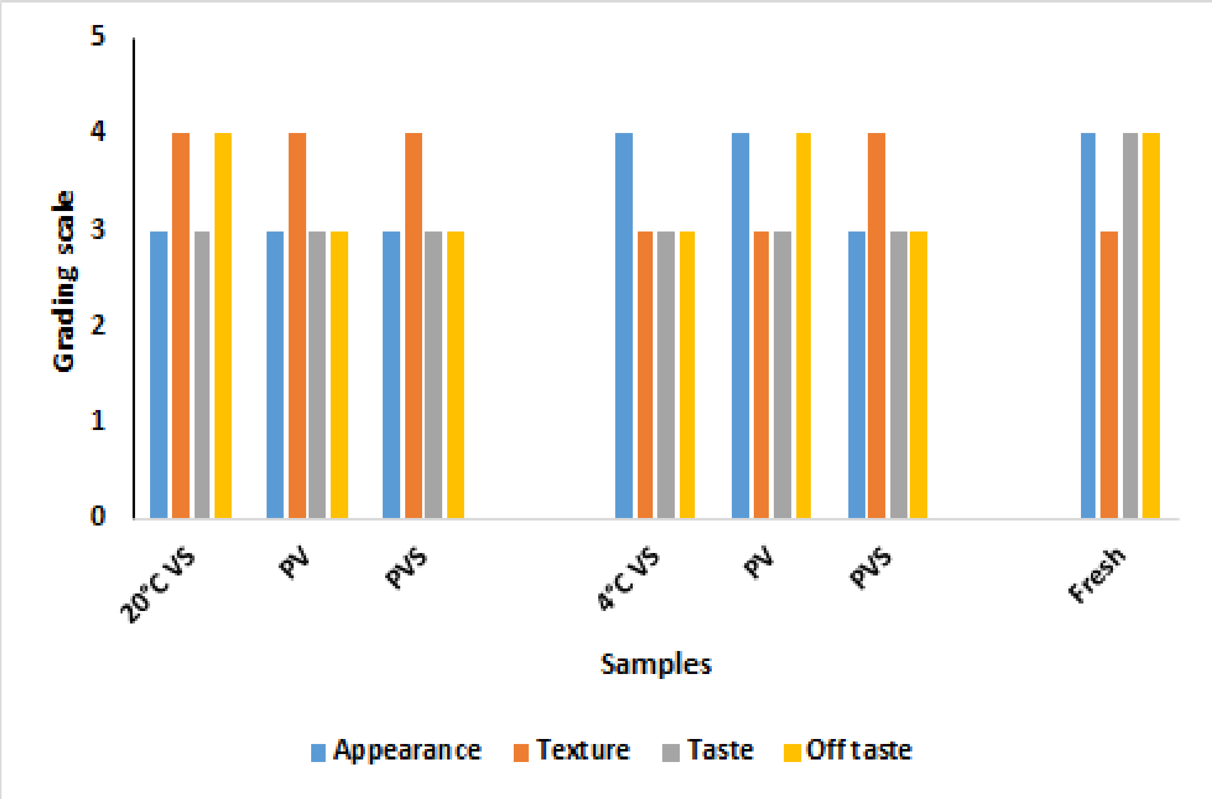

Figure 4.19. Effect of storage temperature and environment on sensory scores of biltong after 23 weeks of storage, where 5 is very acceptable and 1 is very unacceptable (n=6).

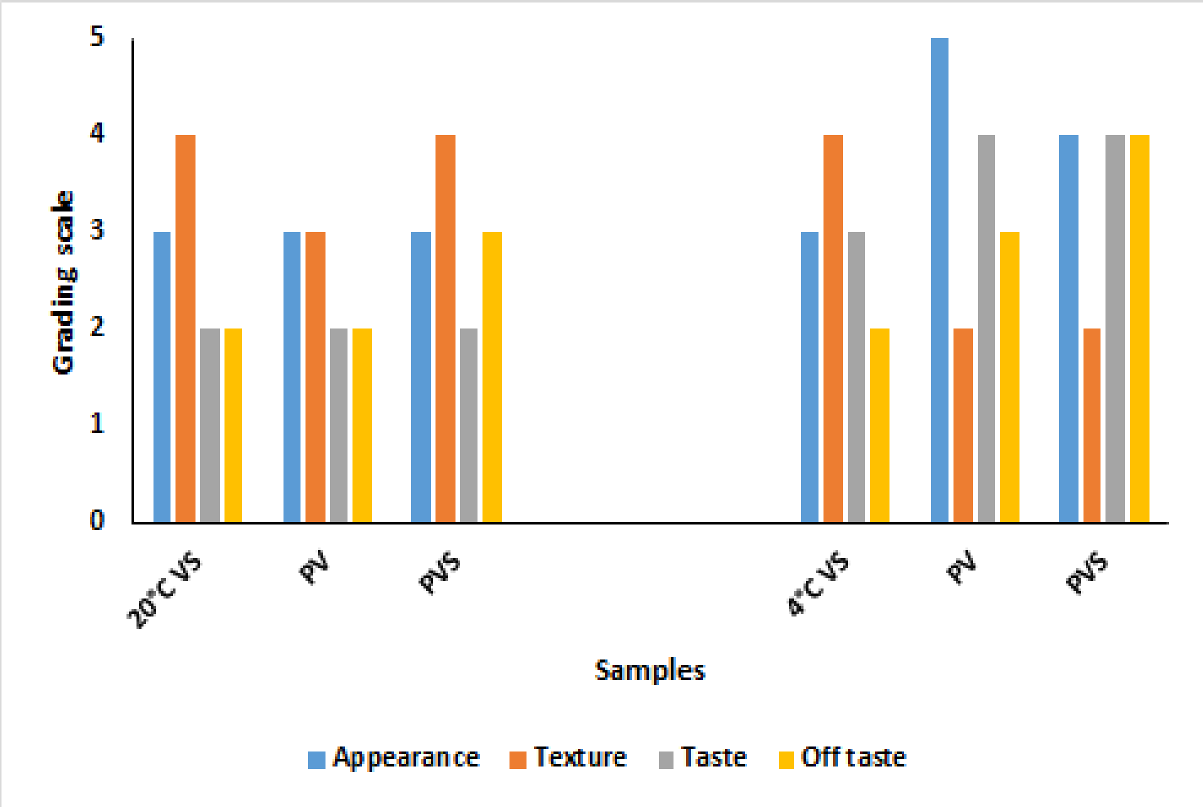

Figure 4.20. Effect of storage temperature and environment on sensory scores of biltong after 28 weeks of storage, where 5 = highly acceptable and 1 = highly unacceptable (n=3).

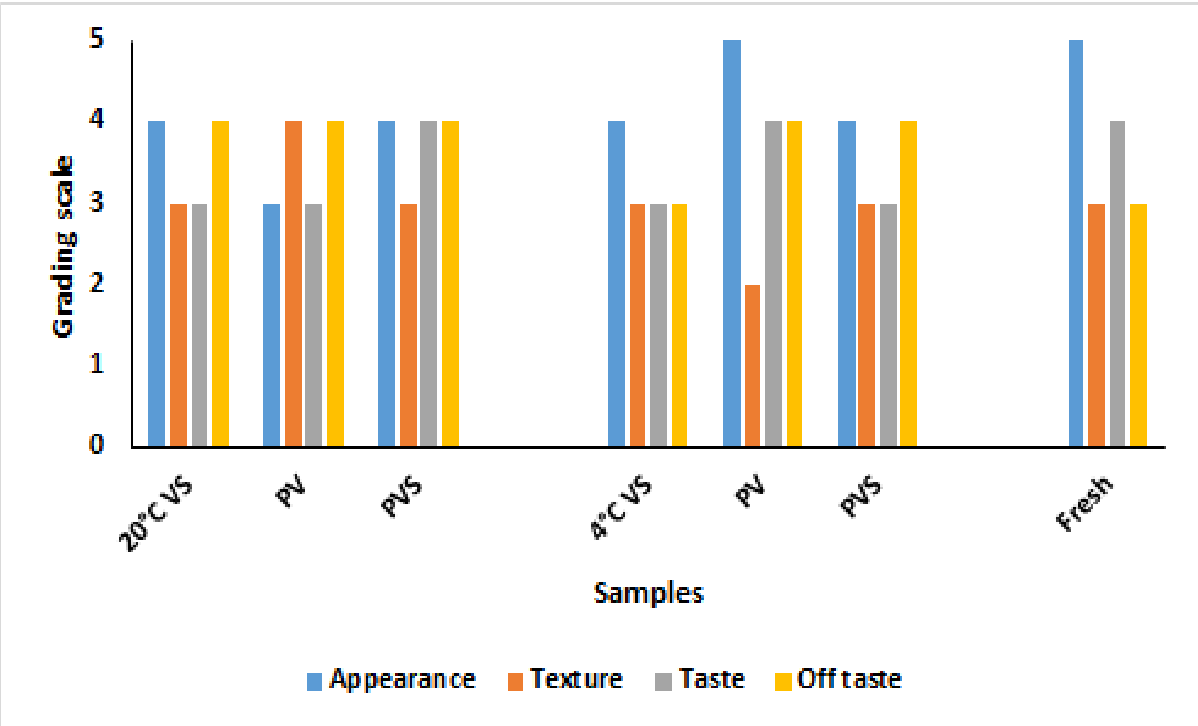

Figure 4.21. Effect of storage temperature and environment on sensory scores of biltong after 36 weeks of storage, where 5 = highly acceptable and 1 = highly unacceptable (n=8).

4.5.1 Appearance

After 12 weeks storage, the appearance of the 35°C samples was still scored as "very acceptable" especially the PV sample, which was equal to the fresh biltong (Fig. 4.17). The appearance of biltong stored in the VS environment was the poorest. The appearance of biltong stored under VS at 20°C was the poorest while the PV and PVS were equal and better than VS. The VS was the most appealing for the 4°C samples PV and PVS were equal and scored lower than VS.

After 18 weeks, appearance scores for biltong stored under VS and PV environments at 20°C had equal scores, which were higher than biltong stored under the PVS environment (Fig. 4.18). The appearance scores of biltong stored at 4°C had not changed over this interval.

At 23 and 28 weeks, all biltong stored at 20°C had the same score of 3 for appearance (Figs. 4.19 and 4.20). After 23 weeks (Fig. 4.19), scores for biltong stored at 4°C remained constant apart from biltong stored in PV, which increased by one. At 28 weeks (Fig. 4.20), the appearance of biltong stored in VS decreased to 3, PVS had increased to 4 and PV to 5 (Fig. 4.20). After 36 weeks (Fig. 4.21), biltong stored at 20°C in VS had the best scores, PVS the second best and that in PV the worst scores. The score for biltong stored at 4^{o}C in PV was equal to the fresh sample (5), while biltong stored in VS or PV both scored 4.

4.5.2 Texture

The water activity of biltong stored at 35°C indicated the biltong should have become brittle early in the storage trials. However, sensory evaluation scores at 12 weeks did not reflect this (Fig. 4.17). It is suspected there was a miscommunication about the scoring of texture as the fresh sample was scored as soft (score of 1) when it was the example of chewy (score of 3). Also, biltong stored at 20°C under VS and biltong stored at 4°C under PVS were both scored as being very brittle whilst biltong stored at 35°C PV and PVS were scored as chewy and biltong stored under VS as more brittle.

Data from the 18-week sensory evaluation (Fig. 4.18) followed the expected trend. The fresh sample, biltong stored at 20°C in PV and biltong stored at 4°C in VS all were scored as “chewy” (score of 3).The remaining samples were scored as somewhat brittle. After 23 weeks storage (Fig. 4.19), texture scores for 20°C samples were more brittle than for biltong stored at 4^{o}C, which were scored as chewy other than the biltong stored at 4C in PVS, which was scored as being slightly more brittle.

After 28 weeks storage (Fig. 4.20), the texture of biltong stored at 20°C, other than that stored in PV, was scored as chewy. Biltong stored at 4°C in VS was scored as being more brittle than biltong stored in PV or PVS, which were scored as soft. After 36 weeks storage (Fig. 4.21), biltong stored at 20°C in VS or PVS were scored as chewy and biltong stored in PVS was scored as being more brittle. Biltong stored at 4°C in VS or PVS were also scored as being chewy while that stored in PV was scored as soft.

4.5.3 Taste

After 12 weeks (Fig. 4.17), biltong stored at 35°C in PV was given a low taste score (2) whilst biltong stored at 35°C in VS or PVS and biltong stored at 20°C in PV or PVS environments had the same taste score of 3. Biltong stored at 20°C in VS and all biltong stored at 4°C had a higher score of 4, only one unit less than fresh biltong.

After 18 (Fig. 4.18) and 23 (Fig. 4.19) weeks, all taste scores were equal (3) and lower than that of fresh biltong (4). After being stored for 28 weeks (Fig. 4.20), the taste scores for all biltong stored at 20°C had decreased to 2, the biltong stored at 4°C in PV and PVS environments had increased to 4, and biltong stored in VS did not change.

After 36 weeks (Fig. 4.21), biltong stored at 20°C in VS and PV environments were scored 3 and that stored in PVS as 4. The 4°C VS and PVS were both scored a 3 while the PVS had a higher score of 4.

4.5.4 Rancidity

Rancidity was detected earliest in the biltong stored at 35°C, especially under VS and PV environments after 12 weeks of storage (Fig. 4.17). Biltong stored at 20°C had moderate scores for off-taste other than biltong stored under PVS, which had little rancidity. All biltong stored at 4°C had low off-taste scores, irrespective of packaging environments.

After 18 weeks (Fig. 4.18), less off-taste was detected for 20°C VS or PVS environments or the 4°C VS. All had the same score as fresh biltong. Biltong stored at 20°C in PV or 4°C in PV or PVS had moderate rancidity (3).

Most biltong had moderate off taste scores after 23 weeks storage (Fig. 4.19). However, after 28 weeks storage, off-tastes become more prominent in biltong stored at 20°C, particularly in VS or PV environments, and also in biltong stored at 4°C in VS. Biltong stored in other environments had low to moderate off-taste scores.

After 36 weeks of storage (Fig. 4.21), off-taste scores were still acceptable (score of 4) except for biltong stored at 4°C in VS environment (score of 3). The fresh sample had moderate levels of rancidity.

An untrained panel was used so inconsistencies in scores were expected because each individual would have personal preferences on tastes, textures and appearances as well as having varying organoleptic sensitivity. Also, the panel consisted of different participants each time with different numbers of panellists. This introduces a great source of variation, which is reflected in the high variability (as SD) of the mean scores (Table 4.9). Overall, biltong stored at 35°C was very unacceptable after 12 weeks of storage but those stored at 20°C or 4°C samples remained acceptable throughout the storage time. Although differences due to temperature and packaging environments where not observed, it appears storing biltong at 4°C under VS environment maintained the characteristics of fresh biltong the best.

Table 4.9. Effect of temperature and environment on average sensory scores (mean ± SD) of biltong during storage.

Storage time, weeks	**Storage conditions**								
	35°C			20°C			4°C		
	VS	PV	PVS	VS	PV	PVS	VS	PV	PVS
12	3.4±0.7	3.0 1.2	3.0±0.9	3.1±1.3	3.0±0.9	3.1±0.7	3.5±1.0	2.8±1.1	3.0±0.9
18	NT	NT	NT	3.2±0.7	3.3±0.8	3.2±0.9	3.5±0.7	3.0±0.6	3.4±0.8
23	NT	NT	NT	3.4±0.7	3.0±0.8	3.4±0.9	3.1±0.6	3.5±0.7	3.1±1.1
28	NT	NT	NT	2.6±0.7	2.8±1.6	2.8±0.8	2.9±1.3	3.6±0.7	3.7±1.1
36	NT	NT	NT	3.4±1.1	3.3±1.1	3.9±0.8	3.3±1.1	3.7±1.2	3.5±1.1

Key: NT = not tested

Summary

The untrained panel was unable to detect major changes in sensory attributes caused by the packaging environment. Even the fresh ‘control’ used at week 36 received an adverse score. The biltong stored at 35°C had become unacceptable after 12 weeks storage and was removed from further sensory trials. The panellists noticed differences in appearance and texture more than changes in taste and off-tastes. Sensory trials should be carried out with a trained and consistent panel to discern whether storage conditions affect sensory characteristics of biltong.

4.6 Rosemary Extract (OxiKan)

4.6.1 TBARS analysis

When lipids are oxidized, they first form unsaturated hydroperoxides. The higher the temperature, the greater the oxidation. The hydroperoxides then further oxidise into aldehydes and ketones such as malondialdehyde (MDA). As MDA is the most abundant aldehyde generated during secondary lipid oxidation, it is used as an oxidation marker. Consuming MDA has been linked to cancer and cardiovascular conditions (Reitznerová *et al.*, 2017).

In a second storage trial, the proprietary rosemary extract (OxiKan) was incorporated into the marinade and the resultant biltong stored for 24 weeks in the same environmental conditions used in the first trial. The control did not have any added OxiKan. Due to space limitations, the control was only stored under full vacuum seal conditions.

Including OxiKan should limit the amount of lipid oxidation. It was predicted that the PV environment would allow the highest lipid oxidation as oxygen present in the packet will supply radical species for breaking lipid chains. Oxygen has been removed in the VS environment but oxygen can still diffuse through the film barrier so this is likely to have a moderate effect on oxidation. The PVS environment is the most effective at reducing oxidation because the oxygen scavenger removes oxygen within the packet or through the film.

The control biltong had the highest level of rancidity (measured as MDA), regardless of storage environment and temperature (Table 4.10). Packaging the biltong in an environment with lower oxygen content (i.e., pulling a vacuum) reduced the oxidation level, with the best conditions being achieved if an oxygen scavenger was included in the package (PVS).

Table 4.10. Effect of storage temperature and packaging environment on the MDA content (mg/kg) of biltong after 18 weeks storage.

Temperature	**Environment**			
	VS	PV	PVS	Control
35°C	0.090	0.078	0.060	0.111
20°C	0.036	0.018	0.024	0.036
4°C	0.018	ND	0.009	0.036

Key: ND = Not detected

Depending on the storage environment, reducing the temperature to 4°C or 20°C reduced the oxidation to 10 to 50% of that at 35°C. The packaging environment had minimal effect over the control when biltong was stored at 20°C (zero to 50% reduction). However, there was a marked effect of the packaging environment on oxidation when the biltong was stored at 4°C (50% to complete reduction). Overall, storing biltong containing OxiKan at lower temperatures, along with including an oxygen scavenger in the package reduced the amount of oxidation.

The effect of the packaging environment on preventing oxidation was not clear. Adding an oxygen scavenger to the partial vacuum environment tended to decrease oxidation (i.e. PVS versus PV). It would be expected that a full vacuum (VS and therefore no oxygen) would have prevented oxidation, which occurred in biltong stored at 20°C and 4°C but not in biltong stored at 35°C. This anomaly may have been due to incorrect analysis or compromised packaging so further testing is required. The highest MDA concentrations occurred in the biltong that had no Oxikan, indicating that adding OxiKan will limit oxygen under the environments and storage temperatures used in the trial.

4.6.2 Sensory analysis

The maximum acceptable level of MDA in meat for human consumption meat is 2 MDA/kg (Abandansarie *et al.*, 2019). Although the MDA concentration in all biltong (Table 4.10) was below the maximum acceptable value, taste panel data did not indicate this (Fig. 4.22).

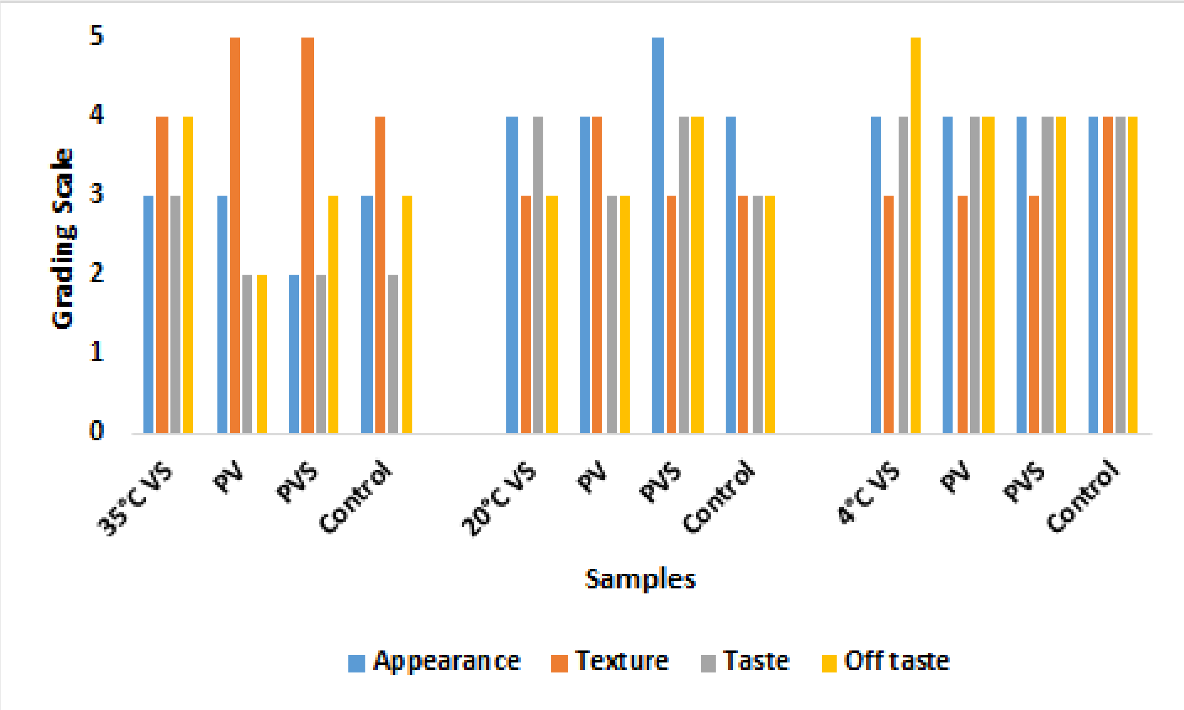

Figure 4.22. Effect of storage temperature and environment on sensory scores for biltong containing rosemary extract after 14 weeks storage. Control has no extract. (n = 3).

Again, there as a high variability in sensory scores, as indicated by the high SD (Table 4.11).

Table 4.11. Standard deviation of sensory scores for biltong with OxiKan after 14 weeks.

35°C				20°C				4°C			
VS	PV	PVS	control	VS	PV	PVS	control	VS	PV	PVS	control
3.5 ±0.6	3.0 ±1.4	3.0 ±1.4	3.0 ±0.8	3.5 ±0.6	3.5 ± 0.6	4.0 ±0.8	3.2 ±0.5	4.0 ±0.8	3.8 ±0.5	3.8 ±0.5	4.0 ±0

The rancid taste was noticed most in biltong stored at 35°C in a PV environment even though this biltong did not have the highest MDA concentration. Biltong stored at 35°C in a VS environment had the highest MDA content but had the same off-taste score as biltong stored at

20°C in PVS, or at 4°C in PV or PVS environments, and control, which had extremely low MDA concentrations. The overall qualities of the sample, such as texture and appearance, likely contributed to the lower acceptability of 35°C samples. Biltong stored at 4°C in VS had a score of 5 (no rancidity), and a low MDA concentration (0.018 mg/kg), which is very low but still higher than biltong stored at 4°C in PV or PVS environments, which had no detectable or 0.009 mg/kg MDA respectively.

The sensory panel could not distinguish clearly which biltong have higher levels of rancidity. Even though the MDA in all biltong were well below the 2 MDA/kg acceptable limit, panellists stated they would not buy any of the 35°C samples, although biltong stored in VS may be considered acceptable. However, panellist would buy biltong stored at 20°C in PVS and the control, might buy biltong stored in VS but not biltong stored in PVS. Panellist would buy any of the biltong stored at 4°C. Other factors, such as appearance and texture, likely played a big role in panellist's preference to purchase biltong stored at 4°C. Biltong stored at 4°C and 20°C had better appearance and texture than biltong stored at 35°C. Rancidity scores for biltong stored at 4°C were consistently higher (more acceptable), while acceptability of biltong stored at other temperatures was affected by packaging environment.

The most acceptable packaging environment in terms of rancidity is PVS, even though this environment did not have the highest score for each temperature.

Summary

Adding OxiKan reduces lipid oxidation in biltong. The controls had higher MDA concentrations than biltong with the proprietary rosemary extract. As expected, the ion degree of oxidation was temperature dependent with biltong stored at 35°C having the highest level of lipid oxidation and biltong stored at 4°C the least. The highest lipid oxidation occurred in the VS packaging environment while the least occurred in the PV environment at 20°C or 4°C. Biltong stored in a PV environment had the lowest lipid oxidation at 35°C and the second lowest at 20 and 4°C. This is unexpected and could be due to operator error, so further testing is required. Although, MDA concentrations were below that typically acceptable to consumers, it was still detected by panellists. Biltong stored at 35°C was least liked. There was no marked differences in acceptability of biltong stored at lower temperatures, regardless of the storage environment. Biltong stored in a PVS environment, along with storing at 4°C was most favoured.

4.7 Chapter 4 Summary

Biltong samples were stored under three different packaging environments and three temperatures for 46 weeks. Periodically, the water activity, pH, colour, microbial quality and sensory quality was assessed. A second trial investigated the effect of adding a proprietary rosemary extract on stored biltong quality.

Testing shows that the quality of biltong stored at 35°C in any of the packaging environment tested will deteriorate well before the expiry date. The most influential measure was consumer acceptability – biltong stored for 12 weeks at 35°C had already reached an unacceptable status. Of the packaging environments tested, the VS was best at retaining biltong quality when stored at 35°C. This would be the environment recommended if biltong was being stored in uncontrolled temperature conditions.

Biltong stored at 20°C was more acceptable after extended storage, especially when kept in VS or PVS environments. The quality of biltong in PV environments usually deteriorated the fastest. The VS and PVS environments affected the various quality factors differently. The VS environment was the best for controlling fungal spore germination and for maintaining colour and water activity. The PVS environment was best for maintaining internal colour, and for limiting pH changes and lipid oxidation.

Over the 46-week storage trial, biltong stored at 4°C remained the most similar to fresh biltong. Packaging environment had minimal effect on the quality when biltong was stored at 4°C so the VS environment is recommended for its cost-effectiveness. The disadvantage of this environment is the unappealing appearance of the package - it seems emptier than biltong in a PVS environment and fat from the biltong often smears the packaging film.

Rosemary extract reduces lipid oxidation. As the VS environment was not as effective as a PVS environment in limiting lipid oxidation, incorporating rosemary extract in the marinade of biltong being stored in VS packaging may be cost effective. Adding an oxygen scavenger will further inhibit rancidity. Further research could be done to investigate the effect of storing packaged biltong at 10°C and in comparing the cost of extended storage at 4 and 10°C.

Although biltong stored at 35°C quickly lost its acceptability, this temperature could be used for accelerated storage tests, allowing the effect of packaging environments to be analysed quickly.

5 Conclusions and Recommendations

5.1 Introduction

Biltong is a ready-to-eat preserved meat product that has increased in popularity due to its flavour, being a concentrated, nutritious protein that is available in small portions that do not need any preparation. New Zealand MPI regulations allow the Hamilton manufacturing company Safari Biltong to keep biltong for a maximum of two weeks before mandatory disposal. The company aim to export the product, which will involve manufacturing biltong with an extended shelf life. Part of Safari Biltong's marketing strategy is to have chemical/ preservative free, packaged biltong that can last for up to a year. Profitability would increase if the biltong can be stored at ambient temperature in cost-effective packaging, maintain consumer acceptability and meet legal requirements.

Samples of freshly manufactured biltong were packaged under full vacuum (VS), partial vacuum (PV) and partial vacuum with and an oxygen scavenger (PVS) for 46 weeks at 4°C, 20°C and 35°C. The water activity and pH were measured fortnightly and microbial analysis and sensory tests done periodically. A second trial investigated the effect of adding a rosemary extract to help limit rancidity development.

5.2 Summary of Findings

5.2.1 Water activity

- During the 46-week trial, the water activity of all biltong remained below the allowable limit of 0.85.
- Water activity of biltong stored at 35°C and 20°C decreased during storage. Although the water activity of biltong stored at 4°C fluctuated widely, irrespective of the packaging atmosphere, the water activity did not change significantly during storage.
- There was no significant difference in water activity of biltong stored at 35°C or 20°C under PV and PVS atmospheres but the water activity of biltong stored under a VS environment was consistently 0.1 units higher when biltong was stored at 35°C and 0.01-0.04 units higher when biltong was stored at 20°C.
- Regardless of the packaging environment, biltong stored at 35°C becomes very brittle and unpalatable during storage. The sensory panel considered the biltong unpalatable after 12 weeks.
- The water activity of biltong stored at 20°C in a VS environment decreased at a slower rate than the PV environment.

- The reason for fluctuations in the water activity during storage is not known and will require further investigation.
- Visible salt deposits formed when water activity decreased below 0.7, which occurred in all biltong irrespective of packaging environment and storage temperature.
- Biltong stored at 4°C in a VS packaging environment retained a higher water activity, which is the product consumers tend to prefer.

5.2.2 pH

- Biltong pH decreased during storage. The extent was temperature dependent. The pH of biltong stored at 35°C decreased the most, indicating rancidity was greater at higher temperatures.
- Storing biltong in a PVS environment limited the change in pH, probably because the oxygen scavenger in the pack helped reduce oxidation and consequent formation of fatty acids. Biltong stored in a PV environment had the greatest pH decrease.
- Biltong stored at 4°C in a PVS environment best maintained consumer acceptability in terms of pH.

5.2.3 Colour

- The higher the storage temperature, the faster the change in biltong colour. The colour of biltong stored at 20°C decreased but remained acceptable for the 46 weeks of the trial. The colour of biltong stored at 4°C changed very little during storage other than having lines of lighter colouring.
- The packaging environment affected colour, especially when stored at 35°C. The colour of biltong stored at 35°C in a VS environment was acceptable for 42 weeks but only for 24 weeks in a PVS environment and 16 weeks in a PV environment.
- Storing biltong at 4°C in a VS packaging environment was the best for maintaining external appearance at a desired quality.

5.2.4 Microbial growth

- Although low levels of bacteria were present on the fresh, unpackaged biltong, no bacterial growth was detected during the 46-week trial.
- During the storage trial, no mycelial growth appeared on any of the biltong, indicating all packaging environments suppressed fungal growth. The one exception was found to have a faulty seal.
- Spores did germinate when samples of stored biltong were incubated at conditions suitable for fungal growth. The ability of spores to germinate on biltong stored at 35°C or 20°C decreased as storage time increased. Spore viability on biltong stored at 35°C

ceased after 9 weeks, with suppression being highest in the VS environment, followed by the PV and least in the PVS environment. Viability of spores on biltong stored at 20°C ceased after 15 weeks storage, with the environmental conditions having same pattern on germination ability (quickest in VS, PV second and lowest in PVS).

- Storing biltong at 4°C did not suppress spore germination over the 46 weeks of the storage trial, regardless of the packaging environment.

5.2.5 Sensory attributes

- Sensory evaluation indicated that biltong stored for 12 weeks at 35°C was unacceptable to consumers, especially biltong stored in a PV environment.
- Members of the untrained sensory panel could not clearly distinguish taste differences or "off-taste" development in biltong stored at 20 and 4°C over the 46 week storage.
- Biltong appearance and texture changed during storage.
- The best storage conditions in terms of sensory quality was a VS or PVS environment at 4°C or PVS environment at 20°C.

5.2.6 Antioxidant use

- Adding a proprietary rosemary extract within the allowable limit decreased lipid oxidation, regardless of storage temperature and environment.
- Biltong stored at 35°C had the highest level of lipid oxidation and biltong stored at 4°C the least.
- The greatest lipid oxidation, in terms of MDA concentration, occurred in a VS environment, was moderate in a PV environment and the least in a PVS environment. However, pH decreased the most in biltong stored in a PV environment, indicating that oxidation is not necessarily directly associated with the decrease in pH.
- Although the MDA concentration was well below the maximum acceptable limit (2 MDA/kg), rancidity (as 'off' taste) was detected in the sensory analysis. It was greatest in biltong stored at 35°C and lowest in biltong stored at 4°C.

5.2.7 General observations

- Although the sensory quality of biltong stored at 35°C had become unacceptable after 12 weeks storage, this temperature could be used for accelerated storage tests for parameters such as colour, pH and water activity. The changes by weeks 12-20 were indicative of changes after storing biltong in the same environment at 4°C or 20°C for 32 weeks.

- The quality attributes of biltong stored at 20°C under various environments were acceptable. The VS environment would be the simplest but including an oxygen scavenger (PVS environment) helped reduce oxidation. Storing at 20°C would be a cost-effective temperature for achieving a longer shelf life than permitted under the current FCP.
- Biltong stored at 4°C maintained high quality. However, unlike products stored at higher temperatures, any mould spores present could germinate once the biltong was exposed to a suitable environment.
- Including a proprietary rosemary extract decreases lipid oxidation, especially at higher storage temperatures.

5.3 Recommendations

If Safari Biltong wishes to market biltong with a longer shelf life, it would be beneficial to gain further detailed and accurate data on the water activity, colour, pH and microbial quality of biltong that has been vacuum sealed with an oxygen scavenger and stored at 20°C.

Other recommendations for further research include:

- Investigating the effect of using other types of packaging film with lower water and oxygen transmission rates.
- Investigating the effect of storing temperatures between 4°C and 10°C.
- Investigating the effect of storing biltong that has different initial water activities.
- Investigating the effect of different additives (e.g. chilli) on sensory qualities.
- Investigating the reasons for the wide fluctuations in water activity and pH values during storage.
- Identifying the microbial species present on the biltong and investigating whether changes in manufacturing practice can decrease the initial microbial loading.
- Further investigating the development of lipid oxidation by measuring TBARS.
- Using a trained panel for sensory evaluations.
- Investigate the effect of using chemicals/preservatives and compare data with that from biltong with no additives.
- Including a fungal inhibitor in the media used for bacterial culture testing.

References

Abandansarie, S.S.R., Ariaii, P., & Langerodi, M.C. (2019). Effects of encapsulated rosemary extract on oxidative and microbiological stability of beef meat during refrigerated storage. *Food Science & Nutrition, 7*(12), 3969-3978.

Abdullahi, N. (2018). Advances in food packaging technology - a review. *Journal of Postharvest Technology, 6*(4), 55-64.

Abou-Samra, R., Keersmaekers, L., Brienza, D., Mukherjee, R., & Macé, K. (2011). Effect of different protein sources on satiation and short-term satiety when consumed as a starter. *Nutrition Journal, 10*. doi.org/10.1186/-2891-10-139.

Adzitey, F., & Nural, H.. (2011). Pale soft exudative (PSE) and dark firm dry (DFD) meats: Causes and measures to reduce these incidences-A mini review. *International Food Research Journal, 18*(1), 11-20.

Ahrens, J.C., Price, M.R., Daneo-Moore, L., & Buckley, H.R. (1983). Effects of culture density in the kinetics of germ tube formation in *Candida albicans*. *Journal of General Microbiology,* (129), 3001-3006. 1475

Aksu, M.I., Dogan, M., & Sirkecioglu, N.A. (2017). Changes in the total lipid, neutral lipid, phospholipid and fatty acid composition of phospholipid fractions during pastirma processing, a dry-cured meat product. *Korean Journal for Food Science of Animal Resources, 37*(1), 18-28.

Aksu, M.I., & Kaya, M. (2005). Effect of storage temperatures and time on shelf-life of sliced and modified atmosphere packaged Pastirma, dried meat product, produced from beef. *Jounal of the Science of Food and Agriculture, 85*(8). https://doi.org/10.1002/jsfa.2118.

Alam, I.I. (2020). *Three common microbiological testing methods for food products*. https://delishably.com/food-industry.

Albrecht, J., & Sumner, S.S. (1992). *Food microbiology/foodborne illness.* University of Nebraska - Lincoln Extension EC 92-2307. https://digitalcommons.unl.edu/extension hist/1572.

Amaral, A.B., da Silva, M.V., & da Silva Lannes, S.C. (2018). Lipid oxidation in meat: mechanisms and protective factors - a review. *Food Science and Technology, 38, supl 1. ISSN 1678-457X.*

Anonymous. (1984). *Water activity (aw) in foods*. Department of Health, Education and Welfare Public Service. Retrieved May 6th, 2020 from https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/water-activity-aw-foods.

Anonymous. (2011, November 29). *New findings about unwanted fungal growth on dry-cured meat products.* Science Daily. https://www.sciencedaily.com/releases /2011 11/ 111129092020.htm.

Anonymous. (2013). *Methods of sensory evaluation. Engineering properties of biological materials and food quality.* Retrieved April 30th, 2020 from http://ecoursesonline. iasri.res.in/mod/page/view.php?id=1029.

Anonymous. (2017a). *Water activity controls microbial growth*. Meter Food. Retrieved April 17th, 2020 from https://www.metergroup.com/food/articles/microbial-growth/.

Anonymous. (2017b). *Individual snacking categories on the rise in the U.S.* Insights. https://www.nielsen.com/us/en/insights/article/2017/individual-snacking-categories-on-the-rise-in-the-us/.

Anonymous. (2018). *Sensory evaluations: Consumer panel vs. trained panel*. The Produce Nerd. Retrrieved May, 13th, 2020 from https://www.theproducenerd.com/2018/08/sensory-evaluations-consumer-panel-vs-trained-panel/.

Anonymous. (2019a). *Meat curing methods*. Morton Salt. Retrieved March 18th, 2020 from https://www.mortonsalt.com/article/meat-curing-methods/.

Anonymous. (2019b). *Tips for food safety*. New Zealand Food Safety. Retrieved April 17th, 2020 from https://www.mpi.govt.nz/food-safety/food-safety-and-suitability-research/human-health-surveillance/foodborne-disease-annual-reports/.

Anonymous. (2020). *Food technology -1. Lessons in drying: Principle, methods and applications.* Retrieved April 17th, 2020 from http://ecoursesonline.iasri.res.in/mod/resource/view.php?id=147595&fbclid=IwAR3DWhkgIbrBECVqsAPvon7LYks0y6HgN5YucEFdpseQdoewWRmD3NOLTUI.

Aryal, S. (2018). *Candida albicans – habitat, morphology, cultural characteristics, life cycle, pathogenesis, lab diagnosis, treatments, prevention and control*. Online Microbiology Notes. Retrieved April 24th, 2020 from https://microbenotes.com/candida-albicans/.

Baker, K. (2016). *Muscle microstructure/ contraction & relaxation muscle* [powerpoint slides] https://slideplayer.com/slide/4452525/?fbclid=iwar1TCl4U0ZBO1fiNH3kOZscyTpRuPbM3sqqZc76DXFOP7xJQ2HToediW7OE.

Barbosa-Canovas, G.V., Fernandez-Molina, J.J., Alzamora, S.M., Tapia, M.S., Lopez-Malo, A., & Chanes, J.W. (2003). Handling and Preservation of Fruits and Vegetables by Combined Methods for Rural Areas. *FAO Agricultural Services Bulletin 149.* http://www.fao.org/3/y4358e/y4358e00.htm.

Bell, C., & Kyriakides, A. (2009). Salmonella. In: C.W. Blackburn & P.J. McClure (Eds.), *Foodborne Pathogens – Hazards, Risk Analysis and Control* (2nd ed., pp. 627-674). CRC Press/Woodhead Publishing

Berdahl, D.R., & McKeague, J. (2015). Rosemary and sage extracts as antioxidants for food preservation. In: *Handbook of Antioxidants for Food Preservation*. DOI: 10.1016/B978-1-78242-089-7.00008-7.

Blackburn, C.W. (Ed.) (2006). *Food Spoilage Microorganisms*. Elsevier.

Boler, D.D., & Woerner, D.R. (2017). What is Meat? A perspective from the American Meat Science Association *Animal Frontiers, 7*(4), 8-11.

Bower, C.K., Schilke, K.F., & Daeschel, M.A. (2003). Antibacterial properties of raisins in beef jerky preservation. *Food Microbiology and Safety, 68*(4). https://doi.org/10.1111/j.1365-2621.2003.tb09671.x.

Brenann, J. (2017). *What happens in exergonic chemical reactions?* Sciencing. Retrieved July 8th, 2020 from https://sciencing.com/happens-exergonic-chemical-reactions-8498370.html.

Bullerman, L.B. (2003). Spoilage: Fungi in Food - An Overview. In: B Cabellero (Ed.), *Encyclopedia of Food Sciences and Nutrition (*2nd ed., pp. 5511-5522).

Carpenter, C.E., Cornforth, D.P., & Whittier, D. (2001). Consumer preferences for beef colour and packaging did not affect eating satisfaction. *Meat Science, 57*(4), 359-363.

Cartmell, N. (2017). *Why is oducts.* Medium. Retreiveved July, 8th, 2020 from https://medium.com/@nan*packaging considered important for food pr*cycartmell/why-is-packaging-considered-important-for-food-products-70570e8844ad?fbclid=IwAR05fKBnouGZCdrbGK0OzCorvi6NrtX6X-KFmJsTZeux2ZNzcOz9LxKZLgY

Chang, P.K., Horn, B.W., Abe, K., & Gomi, K. (2014). Aspergillus. In: *Encyclopedia of Food Microbiology* 2nd ed. pp. 77-82. doi.org/10.1016/B978-0-12-384730-0.00010-0.

Cherono, K. (2014). *Infrared drying of biltong: Effect of pre-treatment and drying conditions on the drying characteristics and product quality.*, University of Kwazulu-Natal https://researchspace.ukzn.ac.za/bitstream/handle/10413/12764/Cherono_Kipchumba_2014.pdf?sequence=1&isAllowed=y.

Chinyere, F. (2020). *Kilishi (Nigerian beef jerky).* All Nigerian Recipes. Retrieved February 12th, 2020 from https://www.allnigerianrecipes.com/snacks/kilishi/.

Darrel, E.G., Boehm, M.L., & Thopson, V.F. (2001). *What Causes Postmortem Tenderization?*Scemantic Scholar. https://www.semanticscholar.org/paper/What-Causes-Postmortem-Tenderization-Goll-BOEHM/4a7908052cace03c5f0861c7ca07dd8b986517d6.

Desrosier, W., & Singh, R. P. (2018). *Food preservation*. Encyclopaedia Britannica. Retrieved May 18th, 2020 from https://www.britannica.com/topic/food-preservation.

Devine, C.E., Hopkins, D.L., Hwang, I.H., Fergsuon, D.M., & Richards, I. (2014). Electrical Stimulation. *Encyclopedia of Meat Sciences*, *2*, 486-496.

Diamante, L., Durand, M., Savage, G., & Vanhanen, L. (2010). Effect of temperature on the drying characteristics, colour and ascorbic acid content of green and gold kiwifruits. *International Food Research Journal, 17*, 441-451.

Diaz, L., Gonzalez, C.J., Moreno, B., & Otero, A. (2002). Effect of temperature, water activity, pH, and some antimicrobials on the growth of *Penicillium olsonii* isolated from the surface of Spanish fermented meat sausage. *Food Microbiology, 19*(1), 1-7.

Dictionary, T.A.H.S. (Ed.) (2020). Definition of Fungi. In *Dictionary.com*. https://www.dictionary.com/browse/fungi.

Dominick, S.R., Fullerton, C., Widmar, N.J., & Wang, H. (2018). Consumer associations with the “All Natural” food label. *Journal of Food Products Marketing, 24*(3), 249-262.

Domzalski, T. (2017). *The 23 Most common types of cured meats, explained*. Spoon University. Retrieved February 8th, 2020 from https://spoonuniversity.com/lifestyle/23-common-types-of-cured-meats.

Dumitru, R., Hornby, J.M., & Nickerson, K.W. (2004). Defined anaerobic growth medium for studying *Candida albicans* basic biology and resistance to eight antifungal drugs. *Antimicrobial Agents and Chemotherapy, 48*(7), 2350-2354.

Earle, M. (2017). *Heat and its effect on muscle fibres in meat*. ThermoBlog. Retrieved February 8th, 2020 from https://blog.thermoworks.com/beef/coming-heat-effects-muscle-fibers-meat/.

Embleni, A. (2013). Modified atmosphere pakaging and oher active packaging systems for food, beverages and other fast-moving onsumer goods. In: *Trends in Packaging of Food, Beverages and Other Fast-Moving Consumer Goods (FMCG), pp.* 22-34. Woodhouse. doi.org/10.1533/9780857098979.22.

Fairchild, D. (2015). *Food packaging with less spoilage & waste and fewer preservatives*. West Air Blog. Retreived Jun, 7th, 2020 from https://www.westairgases.com/blog/map-modified-atmosphere-packaging-gases-applications.

Farber, J.N., Harris, M.E., Beuchat, L.R., Suslow, T.V., Gorney, J.R., Garrett, E.H., & Busta, F.F. (2003). Microbiological safety of controlled and modified atmosphere packaging of fresh and fresh-cut produce. *Comprehensive Reviews in Food Science and Food Safety, 2(1)*. doi.org/10.1111/j.1541-4337.2003.tb00032.

Farber, J.M., & Petterkin, P, M. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbial Reviews*, *55*(3), 476-511.

Featherstone, S. (2015). *A Complete Course in Canning and Related Processe,:Vol. 2: Microbiology, Packaging, HACCP and ngredients* 14th ed. Woodhead..

Fehrs, G. (2014). *History of jerky.* .Hi-Country Snack Foods Inc. Retrieved February 20th, 2020 from https://www.hicountry.com/company/about-us/company-history/.

FSANZ. (2013a). *Listeria monocytogenes*. Food Standards Australia. Retrieved May 23rd, 2020 from https://www.foodstandards.govt.nz/publications/Documents/Listeria%20 monocytogenes.pdf.

FSANZ. (2013b). *Staphylococcus aureus*. Food Standards Australia. Retrieved May 23rd, 2020 from https://www.foodstandards.govt.nz/publications/Documents/Staphylococcus %20aureus.pdf.

FSANZ (2016). *Food Additives and preservatives*. Food Standards Australia New Zealand. Retrieved May 29th 2020 from https://www.foodstandards.govt.nz/consumer/additives/ additiveoverview/Pages/default.aspx.

Galloway, M. (2020). *Water abctivity 101: Master the basics.* Meter Food. Retrieved March 9th from https://www.metergroup.com/food/events/water-activity-101-master-the-basics/.

Gimenez, A., & Ares, G. (2019). Sensory shelf-life estimation. In: *Food Quality and Shelf Life*, pp. 333-357, Academic Press. doi.org/10.1016/B978-0-12-817190-5.00011-2.

Gonthier, A., Guéerin-Fauble, V., Tilly, B., & Delignette-Muller, M.L. (2008). Optimal growth temperature of O157 and non-O157 *Escherichia coli* strains. *Letters in Applied Microbiology, 33*(5). doi./10.1046/j.1472-765X.2001.01010.x.

Gordon, B. (2019). *Most common foodborne pathogens.* Academy of Nutrition and Dietetics. Retreived May 23rd, 2020 from https://www.eatright.org/homefoodsafety/safety-tips/ food-poisoning/most-common-foodborne-pathogens.

Graziani, C., Losasso, C., Luzzi, I., Ricci, A., Scavia, G., & Pasquali, P. (2017). *Salmonella*. *Foodborne Diseases, 3*(133-169).

Grundlingh, A. (2019). The war in twentienth-century Afrikaner consciousness. *The Impact of South African War*,1, 23-37. https://doi.org/10/1057/9780230598294_2.

Gulati, T., & Datta, A. (2015). Mechanistic understanding of case-hardening and texture development during drying of food materials. *Journal of Food Engineering, 166*. 119-138.

Han, H.J., & Scanlon, M.G.(2014). Mass transfer of gas and solute through packaging materials. In: *Innovations in Food Packaging* 2nd ed., pp 37-49. doi.org/10.1016/B978-0-12-394601-0.00003-5.

Health Navigator Editorial Team. (2019). *Salmonella*. Health Navigator New Zealand. Retrieved March 7th, 2020 from https://www.healthnavigator.org.nz/healthy-living/f/food-safety/.

Heldman, D.R., & Hartel, R.W. (1997). *Principles of Food Processing*. Chapman & Hall.

Hintz, T., & Matthews, K.K. (2015). The use of plant antimicrobial compounds for food preservation. Epidemiology, detection, and control of foodborne microbial pathogens. *Biomed Research International,* doi.org/10.1155/2015/245264, 12 pp.

Holl, M. (2009). The Rotten Link in the Food Chain. *Dominion Post*. http://www.stuff.co.nz/life-style/well-good/195029/The-rotten-link-in-the-food-chain.

Hopkins, D. L., Ponnampalam, E., van de Ven, R. J., & Warner, D. R. (2014) The effect of pH decline rate on the meat and eating quality of beef carcasses. *Animal Production Science, 54*(4), 407-413. 10.1071/AN12314.

Huff-Lonergan, E. (2010). *Water-holding capacity of fresh meat.* American Meat Science Association fact sheet. Retrieved April 9th, 2020 from https://porkgateway.org/wp-content/uploads/2015/07/water-holding-capacity-of-fresh-meat1.pdf.

Hunt, M.C., Honikel, K., Puolanne, E., Kapper, C., Klont, R., Young, J.F., & Algers, A. (2011). *Fundamentals in water holding capacity (WHC) of meat*. Aarhus University. Retreived April, 9th, 2020 from http://pure.au.dk/portal/en/persons/jette-f-young(93e1c32a-23c8-4141-8799-56c91ff992a2)/publications/fundamentals-in-water-holding-capacity-whc-of-meat(f5a4d0d2-6861-4dee-b45a-f13036d45742)/export.html.

Hunt, M.C., & King, D.A. (2012). *Meat colour measurement guidelines*. American Meat Association. Retrieved July 7th, 2020 from https://meatscience.org/docs/default-source/publications-resources/hot-topics/2012_12_meat_clr_guide.pdf?sfvrsn=d818b8b3_0.

ICMSF (2001). Guidelines for the Microbiological Examination of Ready-To-Eat Foods. *International Commission on Microbiological Specifications for Foods*.

Jackson, S.A., & Dobson, A.D.W. (2016). Yeast and Molds: *Aspergillus flavus*. Reference Module in Food Science. 10.1016/B978-0-08-100596-5.01086-6.

Jones, M. (2017). *Profiling of Traditional South African Biltong in Terms of Processing, Physicochemical Properties and Microbial Stability During storage.* PhD Thesis Stellenbosch University.

Jones, M., Arnaud, E., Gouws, P., & Hoffman, L. C. (2017). Processing of South African biltong – a review. *South African Journal of Animal Science, 47*(6), 744-755.

Jones, M., Arnaud, E., Gouws, P., & Hoffman, L.C. (2019). Effects of the addition of vinegar, weight loss and packaging method on the physicochemical properties and microbiological profile of biltong. *Meat Science, 156*, 214-221.

Keenleyside, W. (2019). *Microbiology: Canadian Edition*. OpenStax Microbiology Textbooks. Retrived 20th April 2020 from https://ecampusontario.pressbooks.pub/microbio/.

Kerry, A. (2018). *What makes meat juicy and tender?* Science of Cooking. Retrieved February 4th, 2020 from https://www.exploratorium.edu/cooking/meat/INT-what-makes-juicy.html.

Kim, I., Jin, S., Yang, M., Ahn, D., Park, J., & Kang, S. (2014). Effect of packaging method and storage time on physicochemical characteristics of dry-cured pork neck products at 10°C. *Asian-Australas Journal of Animal Sciences, 27*(11), 1623-1629.

Koo, H.B., & Seo, J. (2019). Antimicrobial peptides under clinical investigation. *Peptide Science, 111*(5), doi.org/10.1002/pep2.24122.

Krans, J. L. (2010). The sliding filament theory of muscle contraction. *Nature Education, 3*(9), 66. https://www.nature.com/scitable/topicpage/the-sliding-filament-theory-of-muscle-contraction-14567666/.

Kurtus, R. (2019). *Thermal energy is kinetic energy*. School for Champions. Retrieved June, 7th from https://www.school-for-champions.com/science/thermal_energy.htm.

Lahour, A., Crespo-Sempere, A., Marin, S., Said, S., & Sanchis, V. (2015). Toxigenic molds in Tunisian and Egyptian sorghum for human consumption. *Journal of Stored Products, 63*, 57-62. https://doi.org/10.1016/j.jspr.2015.07.001.

Lee, K.T. (2010). Quality and safety aspects of meat products as affected by various physical manipulations of packaging materials. *Meat Science, 86*(1), 138-150.

Leistner, L., & Gould, G.W. (2002). The hurdel concept. In: *Hurdle Technologies.* Springer doi.org/10.1007/978-1-4615-0743-7_2.

Lewis, H.E., Masterton, J.P., & Ward, P.G. (1956). The food value of biltong (South African dried meat) and its use on expeditions. *British Journal of Nutrition, 11*(1), 5-12.

Lieber, L.R. (1992). *Skeletal Muscle Structure, Function and Plasticity, The Physiology Basis of Rehabilitation* 2nd ed., Lippencott Williams & Wilkins.

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B., &. Bugeon, J. (2016). How muscle structure and composition influence meat and flesh quality. *Scientific World Journal*. doi.org/10.1155/2016/3182746.

Lorenzo, I., Serra-Prat, M., & Yebenes, J.C. (2019). The role of water homeostasis in muscle function and frailty: A review. *Nutrients, 11*, 1857. doi.org/0.3390/nu1108157.

Marsh, K., & Bugusu, B. (2007). Food packaging - roles, materials, and environmental issues. *Journal of Food Science, 72*(3), R39-R56. doi: 10.1111/j.1750-3841.2007.00301.x.

Matsheka, M.I., Mpuchane, S., Gash, B.A., Allotey, J., Khonga, E.B., Coetzee, S.H., & Murindamombe, G. (2014). Microbial quality assessment and predominant microorganism of biltong produced in butcheries in Gaborone, Botswana. *Food and Nutrition Sciences, 5*, 1668-1678. doi.10.4236/fns.2014.417189.

Mattia, A., Cerniglia, C., & Baines, J. (2015). *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 70.

Mavuso, Z. (2019). *Biltong receives gas flush and packaging makeover.* Creamer Media Engineering News. Retrieved June 4th, 2020 from https://www.engineeringnews.co.za/article/biltong-receives-gas-flush-and-packaging-makeover-2019-02-01/rep_id:4136.

Mazzola, N., & Sarantopoulos, C.I.G.L. (2019). Packaging design alternatives for meat products. In: R.A. Marc, A.V. Diaz & G.D.P Izquierdo (Eds.) *Food Processing.* 10.5772/intechopen.88586.

McIntyre, L., & Hudson, J.A. (2009). Something old, something new: Hurdle technology. *Food New Zealand, 9*(1), 15-20.

Micheli, P. (1809). *Aspergillus*. *Magazin der Gesellschaft Naturforschenden Freunde Berlin, 3*(1), 3-42. https://www.aspergillus.org.uk/content/candidus.

Minaar, A., Nortje, K., Kanadu, A.P., & Buys, E. (2009). Use of irradiation to improve the safety and quality of ethnic South African foods. Part I: Combined edible coating and irradiation treatment on sensory and microbiological quality of moist beef biltong, *International Atomic Energy Agency,* pp. 219-229. http://www-pub.iaea.org/MTCD/publications/PDF/Pub1365_web.pdf.

MPI (2020). *New Zealand Food Safety*. Ministry of Primary Industries. Retrieved February 8th, 2020 from https://www.mpi.govt.nz/food-safety/.

Mischler, G. (2005). *All Mongolian recipes*. *The food of the nomads*. Retrieved February 10th, 2020 from https://www.mongolfood.info/en/recipes/borts.html.

Mukumbo, F.E., Arnaud, E., Collignan, A., Hoffman, L.C., Descalzo, A.M., & Muchenje, V. (2018). Physico-chemical composition and oxidative stability of South African beef, game, ostrich and pork droewors. *Journal of Food Science and Technology 55*, 4833-4840.

Munkvold, G.P., Arias, S., Tasch, I., & Gruber-Dorninger, C. (2019). Mycotoxins in corn: occurrence, impacts, and management. In: *Corn* 3rd ed., pp 235-287. doi.org/10.10.1016/B978-0-12-811971-6.00009-7.

Naidoo, K., & Lindsay, D. (2010). Pathogens associated with biltong product and their *in vitro* survival of hurdles used during production. *Food Protection Trends, 30*(9), 532-538.

NZFS (2019). *The MPI Standards for ready-to-eat meat*. New Zealand Food Safety Authority, Wellington. https://www.mpi.govt.nz/food-safety/.

Nieto, G., Ros, G., & Castillo, J. (2018). Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinali*s, L.): A review. *Medicine,s 5*(3), 98; doi.org/10.3390/medicines5030098.

Noman, E., Al-Gheethi, A., Rahman, N.N.N., Hideyuki, N., Talip, B.A, Mohamed, R., & Kadir, M.O.A. (2019). Microstructures of *Aspergillus* spp. and their role in contaminating clinical solid wastes. *Songklanakarin Journal of Science and Technology, 42*(6), 1-10.

Odeyemi, O.A., Alegbeleye, O.O., Strateva, M., & Stratev, D. (2020). Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. *Comprehensive Reviews in Food Science and Food Safety, 19*(2), 311-331.

Pal, M., & Devrani, M. (2018). Application of various techniques for meat preservation. *Journal of Experimental Food Chemistry, 4*(1). 10.4172/2472-0542.1000134.

Papuc, C., Goran, G.V., Predescu, C.N., & Nicorescu, V. (2016). Mechanisms of oxidative processes in meat and toxicity induced by postprandial degradation products: A review. *Comprehensive Reviews in Food Science and Food Safety, 16*(1). doi.org/10.1111/1541-4337.12241.

Pasanen, A.L., Kalliokoski, P., Pasanen, P., Jantunen, M., & Nevalainen, A. (1991). Laboratory studies on the relationship between fungal growth and atmospheric temperature and humidty. *Environmental International, 17*(4), 225-228.

Pepper, I., & Gerba, C. (2020). *Detecting environmental microorganisms with the polymerase chain reaction and gel electrophoresis*. Environmental Microbiology. Retrieved May 6th, 2020 from https://www.jove.com/v/10081/detecting-environmental-microorganisms-with-polymerase-chain-reaction?fbclid=IwAR0ip5kNcvbIIRaY AjXJ4BWOBz3bHMJCNEkoQgoNgnLYD9yYPPFewM2B958.

Pereira, P.M., & Vicente, A.F. (2013). Meat nutrititional composition and nutritive role in the human diet. *Meat Science, 93*(3), 586-592. doi.org/10.1016/j.meatsci.2012.09.018.

Petit, T., Caro, Y., Petit, A.S., Santchurn, S.J., & Collignan, A. (2014). Physicochemical and microbiological characteristics of biltong, a traditional salted dried meat of South Africa. *Meat Science, 96*(3), 1313-1317. https://doi.org/10.1016/j.meatsci.2013.11.003.

Pitt, J.J., & Hocking, A.D. (1997). *Fungi and Food Spoilage*. Springer.

Pollesello, P., Solaro, R.J., & Sorsa, T. (2004). The contractile apparatus as a target for drugs against heart failure: Interaction of levosimendan, a calcium sensitiser, with cadiac troponin C. *Molecular and Cellular Biochemistry, 266*, 87-107.

Potter, N. & Hotchkiss, J.H. (1995). *Food Science*. Chapman & Hall.

Reitznerová, A., Šuleková, M., Nagy, J., Marcinčák. S., Semjon, B., Čertík, M., & Klempová, T. (2017). Lipid peroxidation process in meat and meat products: A comparison study of malondialdehyde determination between modified 2-thiobarbituric acid spectrophotometric method and reverse-phase high-performance liquid chromatography. *Molecules, 27*(11). doi.org/10.3390/molecules22111988.

Robach, M.C., & Sofos, N.,J. (1981). Use of sorbates in meat products, fresh poultry and poultry products: a review. *Journal of Food Protection, 45*(4), 374-383.

Rockland, L.B., & Beuchat, L.B. (1987). *Water Activity: Theory and Applications to Food*. IFT Basic Syposium Series (USA). Dekker.

Rogers, G.B., Carroll, M.P., Serisier, D.J., Hockey, P.M., Jones, G., & Bruce, K.D. (2004). Characterization of bacterial community diversity in cystic fibrosis lung infections by use of 16S ribosomal DNA terminal restriction fragment length polymorphism profiling. *Journal of Clinical Microbiology, 42*(11), 5176–5183.

Seideman, S.C., Cross, H.R., Smith, G.C., & Durland, P.R. (1983). Factors associated with fresh meat color: A review. *Journal of Food Quality,* 5(3). doi.org/10.1111/j.1745-4557.1984.tb00826.x.

Shaffer, E. (2019). *The buzz around biltong*. Food Business News. Retrieved June 2nd, 2020 from https://www.foodbusinessnews.net/articles/13305-the-buzz-around-biltong.

Shafie, F.A., & Rennie, D. (2012). Consumer perceptions towards organic food. *Procedia - Social and Behavioral Sciences, 49,* 360-367.

Simic, M.G., & Karel, M. (Eds.) (1980). *Autoxidation in Food and Biological Systems*. Springer.

Smilanick, J.L., & Mansour, M.F. (2007). Influence of temperature and humidity on survival of *Penicillium digitatum* and *Geotrichum citri-aurantii*. *Plant Disease, 91*(8). doi.org/10.1094/PDIS-91-8-0990.

South African National Standards. (2011). *Processed Meat Products*.Regulations of the Foodstuffs, Cosmetics and Disinfectants. Retrieved April 14th 2020 from https://store. sabs.co.za/pdfpreview.php?hash=e30b09485e8c4d317c2ce80be37730a4ffa9310d&preview=yes.

Stanley, D. (2017). Backgrounder: *Food Irradiation.* Agricultural Research Service. Retrieved June 23rd, 2020 from https://www.ars.usda.gov/news-events/news/research-news/1997/backgrounder-food-irradiation/.

Stats NZ. (2019). *International Migration: April 2019*. StatsNZ. Retrieved April 14th, 2020 from https://www.stats.govt.nz/information-releases/international-migration-april-2019.

U/I_EAT_THE_FAT. (2020). *Is This Case Hardening and Does it Need More Time.* In. REDDIT. Retrieved June 13th, 2020 from https://www.reddit.com/r/Biltong/comments/dqdupl/is_this_case_hardening_and_does_it_need_more_time/.

Van der Riet, W.B. (1976a). Water sorption isotherms of beef biltong and their use in predicting critical moisture contents for biltong storage. *South African Food Review,* 3(December), 93–95.

Vylkova, S., Carman, A.J., Danhof, H.A., Collette, J.R., Zhou, H., & Lorenz, M.C. (2011). The fungal pathogen *Candida albicans* autoinduces hyphal morphogenesis by raising extracellular pH. *American Society for Microbiology. doi: 10.1128/mBio.00055-11.*

Walker, L.T., Shackelford, S.D., Birjhold, S.G., & Sams, A.R. (1995). Biochemical and structural effects of rigor mortis-accelerating treatments in broiler *pectoralis*. *Poultry Science*, 74(1), 176-186.

Wazir, H., Chay, S.Y., Zarei, M., Hussin, F.S., Mustapha, N.A., Ibadullah, W.Z.W., & Saari, N. (2019). Effects of storage time and temperature on lipid oxidation and protein co-oxidation of low-moisture shredded meat products. *Antioxidants*, *8*(10). doi.org/10.3390/antiox8100486.

Webster, J., & Weber, R. W. S. (2007). *Introduction to Fungi*. Cambridge.

Wojtas, P.A., & Yang, A.F. (1987). Solutions to difficulties encountered in low-temperature SEM of some frozen hydrated specimens: Examination of *Penicillium nalgiovense* cultures. *Journal of Electron Microscopy Technique, 6*(4), 325-333.

APPENDICES

Appendix 1. Detailed Methodology

A1.1 Sample preparation

Employees of Safari Biltong cut topside beef steak cuts into strips approximately 20 to 40 cm long and 4 cm wide. The strips for the packaging trials originated from the same batch and contained minimal marbling. . The strips were coated in a marinade consisting of Worcester sauce and malt vinegar before being coated in a Biltong spice mix high in salt (ingredients are confidential). This was left to marinade overnight to meet the requirement of a minimum marinade time of 4 hours. One end of the strip was hooked onto a plastic hook end and then hung in drying rooms for 3-4 days. In normal operation, biltong is dried to a water activity of 0.85. Biltong prepared for this experiment was dried to a water activity of 0.53.

A1.2 Packaging

The dried biltong was cut into pieces of approximately 10g. The vacuum pouches had a 70 µ thickness and had 50 cc/m^2/day permeability to oxygen at 23C. There were 300 sealed packets with a vacuum of -14 psi, 300 packets with a partial vacuum of -7 psi and a further 300 packets with a partially vacuum of -7 psi with a 100-cc oxygen scavenger added.

All packets were evenly divided across three temperature environments: 35^{o}C, 20^{o}C and 4^{o}C. The 4^{o}C samples were stored in a box in a walk in chiller located in the Safari Biltong butchery. The 20^{o}C samples were kept in a box in a temperature controlled office on the University of Waikato Hamilton campus. The 35^{o}C samples were kept in a box in an incubator located in the thermophile research unit located on the University of Waikato Hamilton campus.

A1.3 Water activity

A thin slice of biltong was placed in the Aqua lab PRE water activity analyser (Meter Food, 2365 NE Hopkins Ct. Pullman). Gloves were worn when loading the sample to prevent moisture from being transferred from the operator's hand. Care was also taken to prevent moisture from the operator’s breath getting into the water activity cup. The measurement cup on the meter was dried with a paper towel between individual measurements. The water activity meter was calibrated periodically by an external company. Water activity measurements were taken every fortnight.

A1.4 pH

Biltong samples were weighed then cut into small squares to prevent damaging the blender (F.E.D TS-02 Spice grinder, Penrith NSW). Pieces were then homogenized for approximately 15 seconds or until all the sample was thin fibres. Fibres were transferred to a cup and 10 mL of water added and left for 3 minutes. The pH reading of the fluid was then taken using a standard pH meter (Pocket pH Tester, pH Testra). The probe was cleaned between each sample using water and paper towels. The pH probe was calibrated using standard pH solutions.

A1.5 Mould germination

DG18 agar plates were purchased from Forth Richards. The plate was divided into three equal sectors by drawing lines on the bottom of the plate. Each sector was numbered and identified by sample type and temperature. Biltong samples were assigned a number that corresponded to a sector on the agar plate. The corner of the biltong packet was cut open with scissors sterilised, by dipping in ethanol and flaming over a Bunsen burner, taking care to prevent air entering the packet. Sterilised scissors were then used to cut a randomly elected corner from the biltong without removing the biltong from the packet or handling it inside the plastic packet.

The biltong sample was then placed on the agar surface. The process was repeated for another randomly selected corner so that each sector contained two chunks. The plates were incubated for 5 days at 25C. Growth was recorded using the grading scale (Table 3.2). After two corners had been cut from the biltong, the packet was closed with masking tape, vacuum sealed and stored at 4C until used for the other tests.

A1.6 Sensory tests

An untrained panel of 3 to 8 people familiar and unfamiliar with biltong were used for the sensory tests. Participants varied at each sensory session, depending on their availability. Thin (2-mm) slices of biltong were placed into coded paper cups. Panellists were asked to rate taste, texture, acceptability, “off taste” and whether they would buy the product on a five-point scale on the provided form, where 1 = low value and 5 = high value. Panellists ate a plain potato chip or plain popcorn and had a drink of water to cleanse their palate between samples. They were also advised to taste the 8 - 10 biltong samples in random order. No personal information was not recorded.

A1.7 TBARS method

A 5-g sample was sliced into small pieces, added to 15 mL distilled water and vortexed for 30 seconds in 30-mL centrifuge tubes. The tubes were then centrifuged at 4200 rpm for 10 minutes. A 1-mL aliquot of the supernatant was transferred to a tube containing 2 mL of TCA/TBA solution and 1 mL butylated hydroxytoluene and vortexed for 2 seconds. The tube was then incubated at 90C for 15 minutes then placed in cold water for 10 minutes. The solution was transferred to 2-mL Eppendorf tubes and centrifuged for 5 minutes at 10 000rpm. The absorbance of the supernatant at 531 nm was recorded.

Appendix 2. Testing Schedule

Key: 1 = one 10-g sample
2 = three 10-g samples
VS = full vacuum
PV = partial vacuum, no oxygen scavenger
PVS = partial vacuum, with oxygen scavenger

		Storage Temperature								
Week	Date	4°C			20°C			35°C		
Environment		VS	PV	PVS	VS	PV	PVS	VS	PV	PVS
0	15/5	1	1	1	1	1	1	1	1	1
2	29/5				1	1		1	1	
4	12/6	1	1	1	1	1	1	1	1	1
6	26/6				1	1		1	1	
8	10/7	1	1	1	1	1	1	1	1	1
10	24/7				1	1		1	1	
12	7/8	2	2	2	2	2	2	2	2	2
14	21/8				1	1		1	1	
16	4/9	1	1	1	1	1	1	1	1	1
18	18/9				1	1		1	1	
20	2/10	1	1	1	1	1	1	1	1	1
22	16/10				1	1		1	1	
24	30/10	2	2	2	2	2	2	2	2	2
26	13/11				1	1		1	1	
28	27/11	1	1	1	1	1	1	1	1	1
30	11/12				1	1		1	1	
32	25/12	1	1	1	1	1	1	1	1	1
34	8/1				1	1		1	1	
36	22/1	2	2	2	2	2	2	2	2	2
38	5/2				1	1		1	1	
40	19/2	1	1	1	1	1	1	1	1	1
42	5/3				1	1		1	1	
44	19/3	1	1	1	1	1	1	1	1	1
46	3/4				1	1		1	1	
48	17/4	2	2	2	2	2	2	2	2	2

Appendix 3. Packaging Film Specifications

Property	ASTM	Values
Thickness, µ		70
Tensile strength, N/mm^2 MD	D-882	52
Tensile strength, N/mm^2 TD	D-882	37
Elongation, % MD	D-882	713
Elongation, % TD	D-882	725
Impact, F50 G	D-1709	720
Permeability		
Oxygen cc/m^2/day, (23°C, dry)	D-3985	50
Carbon dioxide cc/m^2/day, (23°C, dry)	D-1434	200
Nitrogen cc/m^2/day, (23°C, dry)	D-1434	12
Water vapour transmission rate, g/m^2/day at 38°C, 90% RH	E-96	8

Appendix 4. TBARS Method

Supplied by Agresearch

TBARS (Thiobarbituric acid reactive substances) protocol

Chemicals	*MW*	*Concentration*
TCA (Trichloroacetic acid)	163.39	15% (w/v) TCA: 15 g TCA + DW to 100 ml
TBA (Thiobarbituric acid)	144.15	20 mM TBA - 15% (w/v) TCA: 2.883 g/l (in 15% TCA)
TEP (tetraethoxypropane)	220.31	
Butylated hydroxytoluene	220.35	7.2% w/v in ethanol: 7.2g + ethanol to 100 ml
Blank		MilliQ water-TBA/TCA solution: 1:2, v/v

Reference:

Originated from Buege and Aust (1978), and modified by Ahn, Olson, Jo, Chen, Wu, and Lee (1998) with minor changes.

Reagents and materials:

1. TCA/TBA stock solution:

   Dissolve 2.94g TBA in warm DDW first, add 150g TCA and then add DW to make up to 1L, or [1.47g TBA in warm DDW, 75g TCA, then up to 500mL], make sure TBA is dissolved well.

2. TEP standard: $1x10^{-4}$ M working solution: 1,1,3,3-tetra-ethoxypropane (Sigma, T9889, MW 220.31, 97% purity, d=0.919 g/mL) in DW.

   a. Stock solution: Dilute 0.1mL TEP + 99.9mL DW: $4.046x10^{-3}$ M

   b. Dilute the resulting solution 2.47mL TEP (stock solution) +97.53 mL DW to obtain working solution $1x10^{-4}$ mol/mL.

Required

- Centrifuge tubes (50 ml)
- 12 ml reagent tubes
- Homogeniser
- Centrifuge
- Cuvette
- Water bath
- Vortex mixer
- May need Eppendorf centrifuge tubes (1.5-2 ml)

Procedures:

Homogenize minced meat (5 g, record the weight) with 15 mL (V1) of MQ Water at 14,000 rpm for 30 s over ice slurry

↓

Centrifuge at 5,000 g for 5 min at 4 °C

↓

Transfer 1 mL (V2) of supernatant to a reagent tube

\+

50 µL of butylated hydroxytoluene (BHT)

\+

2 mL TBA–TCA

[Total volume in the test tube V3 = 3.05 mL]

↓

Vortexed and incubated in a water bath (90 °C) for 15 min to develop colour

↓

Then samples were subjected to cooling on ice for 10 min

↓

Vortexed

↓

Centrifuged at 10,000g at 4°C for 5 min

↓

Supernatant was measured at 531 nm against a blank prepared with 1mL MilliQ water & 2mL TBA/TCA (Zero with Blank)

↓

The amount of TBARS was expressed as mg of malondialdehyde/kg (meat)

Note:

1. Homogenate is not stable after mixing with water, keep chilled and use ASAP.
2. If it is difficult to obtain clear supernatant before reading, then try sampling 1.5-2.0 mL of 'supernatant' into a 2 mL Eppendorf centrifuge tube, centrifuge again at 14,000g for 5 min.

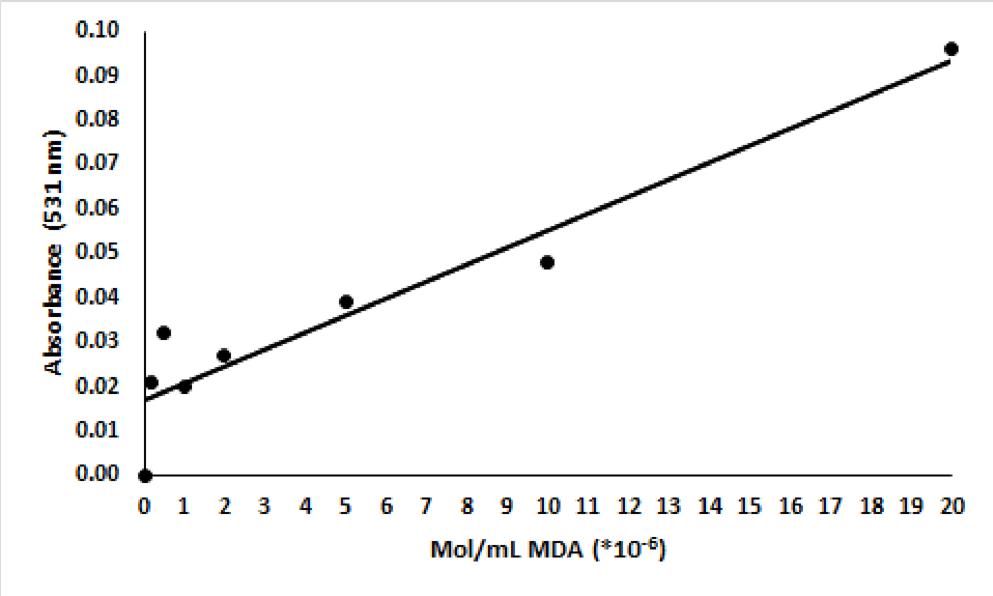

Figure A4.1. TEP standard curve for MDA calculations.

Appendix 5. Calculation of OxiKan in Marinade

Weight loss when drying biltong = 47%
Allowable OxiKan limit on dried meat = 150 mg/kg

Table A5.1 Dipping data

Before (g)	After (g)	Marinade (g)	Gain (g/g)
443	463	16	0.0358
243	249	6	0.0247
278	288	10	0.0360
		Average gain (g/g) =	0.0321

Based on 100 g of meat, 47% weight loss and final OxiKan concentration 150 mg/kg.

Table A5.2 Meat weight with marinade

Meat (g)	With marinade (g)	Added marinade (g)	After drying (g)
100	103.21	3.21	54.74

Therefore: 0.054 kg x 150 mg/kg= 8.2 mg
8.2 g will be in 3.2 L of marinade
Or 2.55 mL/L

Appendix 6. Raw data

Table A6.1. Water activity (for codes, see Appendix 2) (n=3).

Time, weeks	35°C			20°C			4oC		
	VS	PV	PVS	VS	PV	PVS	VS	PV	PVS
2	0.717	0.692	0.626	0.714	0.694	0.691	0.693	0.692	0.694
4	0.624	0.628	0.651	0.702	0.668	0.69	0.729	0.716	0.687
6	0.741	0.693	0.676	0.745	0.731	0.717	0.729	0.716	0.687
8	0.65	0.582	0.657	0.704	0.675	0.672	0.677	0.677	0.703
10	0.652	0.519	0.568	0.672	0.729	0.656	0.677	0.677	0.703
12	0.645	0.568	0.571	0.675	0.636	0.643	0.711	0.753	0.671
14	0.626	0.464	0.51	0.752	0.664	0.654	0.711	0.753	0.671
16	0.619	0.471	0.54	0.673	0.661	0.66	0.649	0.658	0.676
18	0.566	0.444	0.444	0.634	0.613	0.611	0.564	0.561	0.526
20	0.632	0.42	0.515	0.671	0.621	0.636	0.632	0.596	0.583
22	0.621	0.559	0.449	0.616	0.575	0.617	0.761	0.664	0.677
24	0.541	0.445	0.424	0.538	0.577	0.581	0.703	0.652	0.66
26	0.52	0.458	0.452	0.728	0.643	0.631	0.733	0.621	0.616
28	0.509	0.425	0.423	0.683	0.624	0.621	0.717	0.673	0.625
30	0.492	0.428	0.403	0.71	0.592	0.628	0.719	0.618	0.619
36	0.419	0.389	0.375	0.576	0.53	0.526	0.694	0.684	0.631
38	NT	NT	NT	0.62	0.6329	0.5891	0.741	0.6053	0.6926
40	0.4177	0.444	0.4613	0.5929	0.4986	0.4945	0.7118	0.6948	0.6577
42	0.465	0.429	0.409	0.613	0.547	0.55	0.721	0.694	0.674
44	0.518	0.439	0.425	0.682	0.682	0.665	0.678	0.661	0.688

Table A6.2. pH data (n=3).

Time, weeks	35°C			20°C			4°C		
	VS	PV	PVS	VS	PV	PVS	VS	PV	PVS
0	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
2	5.28	5.19	5.21	5.21	5.16	5.2	NT	NT	NT
4	5.188	5.21	5.26	5.23	5.173	5.253	5.31	5.19	5.12
6	5.28	5.28	5.33	5.36	5.24	5.36	NT	NT	NT
8	5.27	5.13	5.36	5.28	5.26	5.21	5.23	5.23	5.19
10	5.22	5.2	5.26	5.22	5.31	5.3	NT	NT	NT
12	5.2	5.2	5.3	5.25	5.2	5.3	5.15	5.23	5.23
14	5.2	5.31	5.25	5.33	5.26	5.27	NT	NT	NT
16	5.2	5.14	5.25	5.3	5.27	5.34	5.16	5.23	5.29
18	5.21	5.15	5.24	5.32	5.23	5.32	5.26	5.22	5.32
20	5.14	5.18	5.25	5.24	5.24	5.33	5.23	5.26	5.18
22	5.1	5.07	5.23	5.26	5.24	5.34	5.26	5.2	5.3
24	5.04	5.063	5.153	5.26	5.26	5.31	5.37	5.29	5.3
26	5.05	5.07	5.18	5.37	5.27	5.29	5.27	5.31	5.306
28	5.04	5.05	5.13	5.24	5.16	5.28	5.26	5.23	5.21
30	5.05	5.03	5.11	5.25	5.21	5.32	5.27	5.21	5.3
36	4.77	4.79	4.91	4.95	4.96	5.15	5.08	5.09	4.98
38	NT	NT	NT	4.99	5.01	5.03	5.17	5.043	5.056
40	4.82	4.83	4.96	5.08	4.95	5.11	5.18	5.27	5.05
42	4.93	4.82	4.94	4.97	4.92	5.12	5.1	5.07	5.1
44	4.88	4.83	4.84	5.07	4.98	5.15	5.04	5.01	5.1

Table A6.3. Spore germination (1 = slight; 2 = moderate; 3 = extensive; NT = not tested) (n=3).

Time, week	35°C			20°C			4°C		
	VS	PV	PVS	VS	PV	PVS	VS	PV	PVS
3	2	3	3	3	3	3			
5	0	0	1	2	2	2	3	3	3
9	0	0	0	3	2	3	2	3	3
11	0	0	0	0	1	2	NT	NT	NT
13	0	0	0	1	1	1	3	3	3
15	0	0	0	0	0	0	NT	NT	NT
17	0	0	0	0	0	0	1	1	1
19	NT	NT	NT	0	0	0	1	2	2
21	NT	NT	NT	NT	NT	NT	1	2	3
23	NT	NT	NT	NT	NT	NT	1	1	2
25	NT	NT	NT	NT	NT	NT	1	1	2
28	NT	NT	NT	NT	NT	NT	1	3	3
30	NT	NT	NT	NT	NT	NT	2	2	2
33	NT	NT	NT	NT	NT	NT	1	2	3
36	NT	NT	NT	NT	NT	NT	3	1	2
39	NT	NT	NT	NT	NT	NT	1	1	2
42	NT	NT	NT	NT	NT	NT	1	2	3
44	NT	NT	NT	NT	NT	NT	1	2	1

Table A.6.4. Colour grade (1=black, 5= golden brown) (n=3).

Time, weeks	35ºC			20ºC			4ºC		
	VS	PV	PVS	VS	PV	PVS	VS	PV	PVS
2	1	1	1	1	1	1	1	1	1
4	1	2	1	1	1	1	1	1	1
6	1	2	1	1	1	1	1	1	1
8	1.5	2	1	1	1	1	1	1	1
10	1.5	2	1	1	1.5	1.5	1	1	1
12	2	3	2	1	1.5	1.5	1	1	1
14	2	3	2	1	1.5	1.5	1	1	1
16	2	4	3	1.5	1.5	1.5	1	1	1.5
18	2	4	3	1.5	2	1.5	1	1	1.5
20	2	4	3	1.5	2	1.5	1	1.5	1.5
22	3	4	3	1.5	2	1.5	1	1.5	1.5
24	3	4	4	1.5	2	1.5	1	1.5	1.5
26	3	4	4	2	2.5	2	1	1.5	1.5
28	3	5	4	2	2.5	2	1.5	1.5	1.5
36	3	5	4	2	2.5	2	1.5	1.5	1.5
38	3	5	4	2	2.5	2.5	1.5	2	1.5
40	3	5	4	2	2.5	2.5	1.5	2	1.5
42	4	5	4	2.5	3	2.5	1.5	2	1.5
44	4	5	4	2.5	3	2.5	1.5	2	1.5
46	4	5	4	2.5	3	2.5	1.5	2	1.5