PROXIMATE AND MICROBIAL ASPECTS OF DONKEY (*EQUUS ASINUS*) BILTONG IN BOTSWANA

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DECLARATION OF INDEPENDENT WORK

I, **Leutlwetse Motsumi**, do hereby declare that this research project submitted to the Central University of Technology, Free State for the degree MASTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH is my own original work and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements of any degree.

SIGNATURE OF STUDENT DATE

SUMMARY

Research on donkey meat and meat products has been overlooked for a long time even though there is considerable global research focused on horse meat. Recent literature is limited to investigations exploring the nutritional value of donkey meat (Aganga *et al.*, 2003), and very little information is available on people's knowledge, attitudes, beliefs and practices (KABP), or on the microbial and chemical contents of donkey meat and meat products. In this study, an overall view on the KABP as well as on the microbial and physico-chemical aspects of donkey meat and biltong was acquired in Kanye, Botswana, within six selected districts where donkey meat is used over other species of meat.

The knowledge of food handlers and meat processors regarding hygiene, food safety and meat legislation, together with opinions on business performance, availability of equipment and facilities in meat processing plants, was assessed. The survey included total of 285 respondents in the Southern, Kweneng and Central districts of Botswana of which 280 were consumers. Proximate analysis of related parameters and profiles of *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Salmonella* spp, total viable counts (TVC), yeasts and moulds on both fresh meat and biltong were assessed. Two donkeys from Banyana farms were slaughtered in a period of two months and from each, two fresh meat samples and one sample for biltong were taken. The highest mean TVC counts recorded were for donkey 1. The mean values of *Staphylococcus aureus* counts detected from donkey 1 samples from the forequarter were $1.6x10^1$, hindquarter 0, and donkey 2 mean values were $3.3X10^{-1}$ (forequarter), $1.5x10^4$ - $8.16x10^3$ (hindquarter) and

2.3x10^2 – 7x10^1 (biltong). In general, meat samples did not show high loads of bacteria; however the biltong showed presence of *Staphylococcus aureus*. The mean microbial load on the fresh meat ranged from 1.0x10^1 to 3.5X10^2 cfu.g^{-1} while loads on the biltong sample ranged between 1.26X10^6 and 6.5X10^3 cfu.g^{-1}. To prevent bacterial contamination, meat and meat products such as biltong must be handled and packed properly in sterilised polyvinyl containers.

On the other hand, total fat, total ash, moisture content and water activity were measured and recorded at 2.8 of fat, 4.38 of ash, 24.65 of moisture and 0.998 (a_w) for the forequarter of donkey 1. The hindquarter of the same donkey recorded 12.26 (fat), 3.81 (ash), 23.72 (moisture) and 0.999 (a_w). Moreover, for donkey biltong from donkey 1, 15.31 (fat), 9.29 (ash), 84.37 (moisture) and 0.654 (a_w) were recorded. The donkey 2's samples were 28.48 (fat), 7.31 (ash), 30.02 (moisture) and 0997 (a_w) for the forequarter, with the hindquarter of this donkey recording 44.6 (fat), 5.29 (ash), 33.5 (moisture) and 0.998 (a_w). Finally, 11.66 (fat), 5.26 (ash), 69.84 (moisture) and 0.668 (a_w) were recorded for biltong product produced from donkey 2. Extensive scope still exists for research into the microbiology and nutritional value of donkey meat especially in the light of great interest in donkey meat in arid areas such as Botswana and other Southern regions. In conclusion donkey meat (both fresh and biltong) is just like other red meats.

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LIST OF ABBREVIATIONS

GMP:	Good Manufacturing Practices
USFDA:	United States Food and Drug Agency
FAO:	Food and Agricultural Organisation
KABP:	Knowledge, attitudes, Beliefs and Practices
TVC:	Total Viable Count
PCA:	Plate Count Agar
BPA:	Baird Parker Agar
BCSA:	*Bacillus cereus* Selective Agar
PDA:	Potato Dextrose Agar
RV:	Rappaport Vassiliadis Medium
XLD:	Xylose Lysine Desoxycholate Agar
SCB:	Selective Cysteine Broth
TTB:	Tetrathionate Broth
HEA:	Heiktoen Enteric Agar
AOAC:	Association of Official Analytical Chemists
SPSS:	Statistical Package for the Social Sciences
NFTRC:	National Food Technology Research Centre

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Chapter 1

Literature review

Proximate and microbial aspects of donkey meat and biltong product in Botswana: a review

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1. GENERAL BACKGROUND

Meat is defined as the fleshy tissue of animals suitable for consumption as food (Ajiboye *et al.*, 2011). The term meat includes all processed and manufactured products prepared from such animal tissue, that is, it may be fresh, cured, dried or otherwise processed (Ajiboye *et al*., 2011). Meat has always been part of the human diet, providing a large portion of the proteins, iron, zinc, and vitamins which are essential nutrients and energy necessary for proper growth and development especially in developing countries (Jimenez-Colmenero *et al.,* 2001). While meat provides man with a valuable source of nourishment, it also spoils rapidly because of microorganisms that utilise specific water activity levels, partial acidity and proteins for their growth and survival. In countries like Botswana, donkey meat has gained preference over beef and lamb due to the price increases of red meat (Blench, 2009).

Donkey, scientifically known as *Equus asinus* is a member of the horse family and has been ignored as a subject for scientific investigation, especially as a potential source of meat (Blench, 2009). Historically, donkeys were used mainly for carrying goods in the United Kingdom and East Midlands, although they were also hunted for their meat and skin (Blench, 2009). In Africa, specifically in rural areas, donkeys are kept for working, breeding, milking and eating, although they are only eaten in few areas (Blench, 2009). Due to its ability to survive, reproduce and supply meat in challenging environmental conditions, the donkey is a vital source of meat and milk in arid and semi-arid areas such as Botswana. Donkey meat is considered to be of very high nutritional value attributable to high levels of unsaturated fatty acids (Polidori *et al.,* 2009).

Moreover, most farmers in Botswana are able to keep donkeys as they do not require complex husbandry practices, they have minimum feeding demands and are known to be tolerant to some tropical diseases and parasites (Blench, 2000). Donkeys therefore are an affordable alternative source of meat. Preservation technologies for this type of meat are however crucial. Preservation of perishable foods is not easy or cheap in Southern Africa, especially in Botswana where very hot and dry conditions prevail. However, these hot and dry conditions in the country explain why many people in Botswana prefer solar drying when processing meat towards biltong production (Blench, 2009; Sofos *et al*., 2003; Polidori *et al.,* 2009). It is also a fact that drying, salting, smoking and fermentation have been used to preserve meat products for thousands of years all over the world (Zakhia *et al.,* 1998). According to Hayward (2011), smoking also serves as a method of keeping away flies and insects thus creating a good opportunity to air dry meat naturally and hygienically.

Drying meat lowers the water content, thus creating a hostile internal environment that appears to be an efficient method for hindering most microbial growth in meat (Wolter *et al.,* 2000; Hayward, 2011). Salting is another preservation method used for biltong and other Intermediate moisture Meat (IMM) production although this has been reported to contribute to the production of enterotoxins by *Staphylococcus aureus* (Gillespie and McLauchlin, 2007). To acquire good quality biltong, the meat used for processing should have low microbial counts to ensure a harmless and wholesome end product. The shelf life of intermediate moisture meat products can be enhanced by hindering the growth of microorganisms by establishing parameters such as appropriate microbe inhibiting pH, lowered water content and a specific water activity (Sofos *et al*., 2003). Despite effective management practices, major food borne microbial pathogens such as *E. coli* 0157:H7, *L. monocytogenes* and *Salmonella* spp amongst others, still continue to spoil meat products if not restricted with target-specific techniques.

1.1 LITERATURE REVIEW

Meat is a vehicle for a significant proportion of food-borne diseases and the variety of meat-borne diseases of public health concern has changed with current production and processing systems. However, specific and problematic meat-borne diseases have been well illustrated in recent years by human surveillance studies of pathogens such as *E. coli* 0157:H7*, Salmonella* spp*, Campylobacter* spp. and *Yersinia enterocolitica* amongst others. To produce a pathogen-free product, it is important to ensure good manufacturing practices (GMP) during meat processing. Highly contaminated fresh meat will ultimately yield a poor and hazardous product with a very short shelf-life. Contamination may be from bacteria (such as coliforms and *E. coli*) originating from the animals which contaminate the carcasses during slaughter (Borch and Arinder, 2002). The contaminating bacteria are subsequently distributed via cuts of meat or raw meat intended for further processing (Borch and Arinder, 2002).

In general, the external surface of animals and agents in the animal environment contribute greatly to contamination of carcasses during slaughter, dressing, chilling, cutting, storage and handling. Contaminants can also be transferred to food through the use of contaminated water, manure (if slaughtered in the kraal) and human handling (Borch and Arinder, 2002). Moreover, microorganisms such as *Salmonella spp, Listeria monocytogenes, Campylobacter spp* and *Escherichia coli* are usually picked up by animals on farms via animal faeces, furthering the spread of microbes during slaughter (Currie, 2004). Unfortunately, microorganisms such as *Salmonella, Listeria monocytogenes* and *E. coli* 0157 can survive on dried meat and are the main causal agent of food borne disease outbreaks (Shan Yu *et al*., 2013). *Listeria monocytogenes* is further known to adhere to food preparation surfaces such as stainless steel surfaces of processing machines, which then serve as reservoirs for cross-contamination (Abong'o and Momba, 2008). During meat processing, sliced meats are a great potential source of *Listeria monocytogenes* as cross-contamination can occur during handling.

Biltong on the other hand, can pose a potential health risk to consumers because of microorganisms that are present as a result of: (1) raw meat from abattoirs that may be significantly contaminated with *S. aureus* amongst other microbes; (2) manufacturers that may not have sufficient or any sanitary and hygienic practices in place (Attwel, 2003); (3) biltong that is still sold unpackaged in most places together with other raw meat products in butcheries, consequently facilitating possible cross-contamination. Moreover, excessive fungal contamination levels (up to 10^6 cfu.g^{-1}) are often encountered on biltong (Wolter *et al*., 2000). Furthermore, spoilage by yeast and mould growth is highly problematic in moist biltong.

1.2 HISTORY OF BILTONG

Biltong is a common ready-to-eat meat product which is often consumed as a snack especially in Southern Africa (Mhlambi *et al.,* 2010; Shale and Malebo, 2011). It can be produced from beef, game, and fillets of ostrich or even donkey meat. Biltong is normally referred to as an intermediate moisture meat (IMM) which derives its name from Dutch words 'bil,' means buttock and 'tong' referring to strip (Mhlambi *et al.,* 2010). The idea of drying meat originated from the pioneering South African settlers who used the sun to dry meat when they travelled across the African subcontinent (Sebastian, 2011). *Voortrekkers* (emigrants during the 1830s and 1840s who left the Cape Colony and moved into the interior of what is now South Africa), required stocks of durable food because they travelled a great deal and needed food that could be preserved from decay and insects. They preserved biltong by sun-drying and salting. The production of IMMs, a heterogeneous group of foods plays a significant role in space travel and other instances where long term stability, tastiness and convenience are critical (Wolter *et al.,* 2000).

1.3 GENERIC AND TRADITIONAL BILTONG PREPARATION

Although there are several methods of producing biltong; the generic and traditional way involves salting and drying it in an open space at ambient temperature for a prolonged period (Mhlambi *et al.,* 2010). Fresh meat of good quality, typically from the hind quarter: is preferred for biltong production. The meat used is kept clean and free from flies, dust and dirt, and at low temperatures to inhibit possible microbial growth. Preparation of biltong on household and industrial scale usually involves the use of spices, herbs and seasonings to enhance flavour, aroma and colour (Mhlambi *et al.,* 2010). Figure 1.1 illustrates the generic steps involved in biltong production from raw meat using donkey as an example in this case.

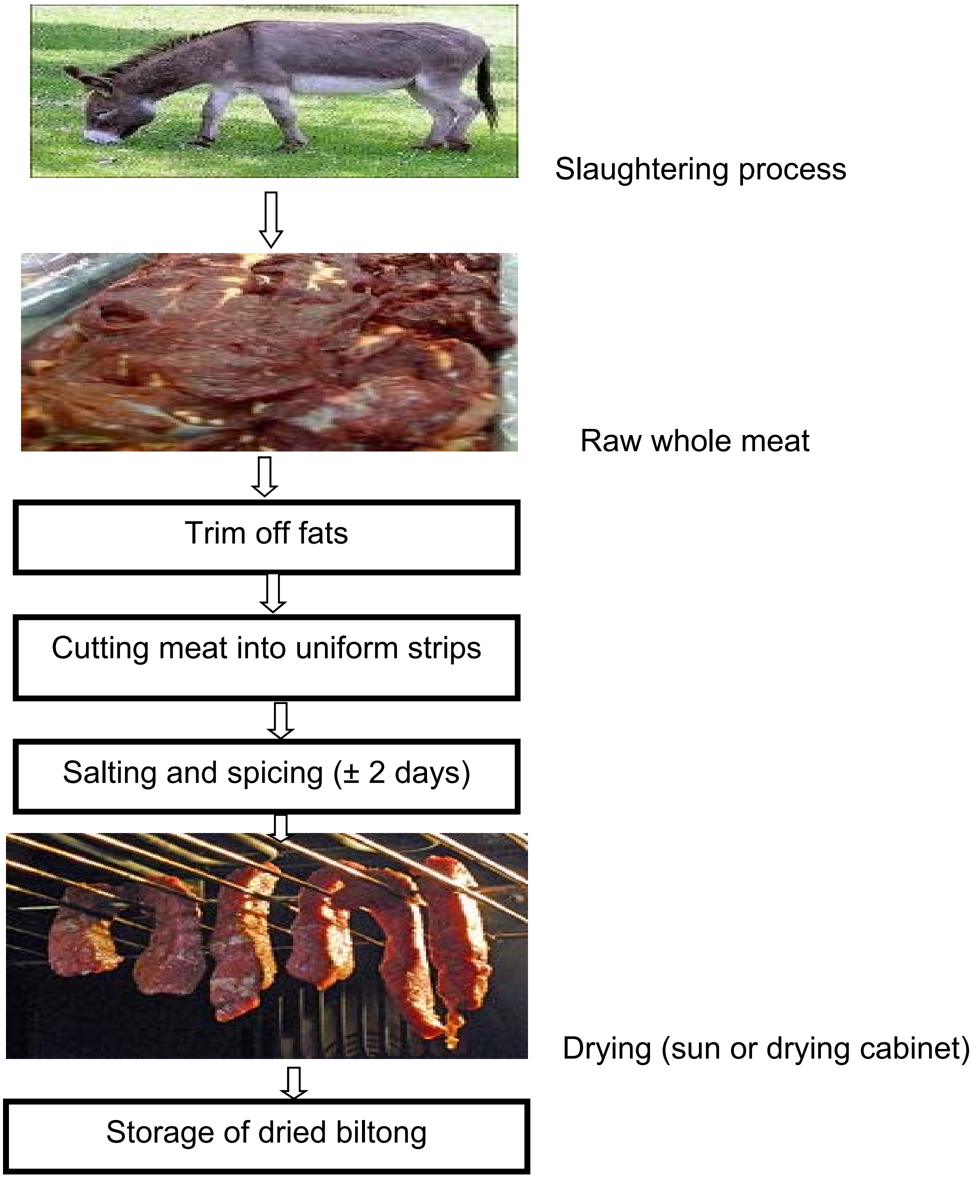

Figure 1.1: Generic steps involved in biltong production from raw meat (Mhlambi *et al.,* 2010); (modified)

1.4 BASIC PRESERVATION ASPECTS FOR CONSIDERATION DURING BILTONG PREPARATION

1.4.1 pH

Together with colour, certain levels of pH indicate the quality of meat (Weglarz, 2010). Before slaughter, animal flesh has a pH value of 7.1, while immediately after slaughter, glycogen in the meat turns into lactic acid lowering the pH to about 5.2. The rate at which the pH drops in a maturing carcass depends on a variety of factors, which include the type of animal, the breed, rearing characteristics and the treatment of the animal before slaughter. Microorganisms can stay alive and grow in a low pH environment provided their cytoplasmic pH is fairly constant at a neutral level. According to the United States Food and Drug Agency (2014), certain pathogens such as *Clostridium botulism* can grow at a pH as low as 4.2. Pathogens such as these may be detrimental to the meat industry as well as to consumers who have an already compromised health status (Polawska *et al.,* 2010).

1.4.2 Moisture content

Moisture content is expressed as the percentage of water in relation to the dry weight of the product. According to the Food Safety Inspection manual (2000), meat naturally contains moisture being made up of approximately 75% water, 20% protein and 5% fat, carbohydrates and minerals. The water content in the food or meat product can be reduced or eliminated using various methods, but care must be taken during these processes to protect the nutritional and organoleptic properties of the meat such as, taste, smell, texture and appearance (Food and Agriculture Organisation, 1991). The production of biltong or dry sausages involves the reduction of moisture content by dehydrating the meat (Wolter *et al*., 2000).

1.4.3 Total fat and protein content

The fat and protein in meat contribute to quality attributes such as tenderness. Polawska *et al.* (2010) report that ostrich meat, for example, has the lowest levels of fat and proteins, ranging between 0.9 and 1.5% and sometimes even as low 0.44%, and is therefore appreciated for its tenderness, texture, easiness to chew and digestibility. High fat content is assumed to mechanically limit the release of water from muscle fibres during heating and chewing (Daszkiewicz *et al.,* 2005). However, McDonagh *et al.* (2004), explain that in some meat products such as sausage, fat plays a vital role in colour, flavour and texture, and adds juiciness to the product because of its water holding capacity.

1.4.4 Total ash

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents and is used to measure the total amount of minerals within a food. Because the mineral-containing analyte can be separated from the matrix, it is possible to quantify the mineral content in food using specific analytical techniques. The most widely used methods are based on the fact that minerals are not destroyed by heating, and have low volatility compared to other food components. The three main types of analytical procedures used to determine the ash content of foods are: dry ashing, wet ashing and low temperature plasma dry ashing. The method chosen for a particular analysis depends on the reason for carrying out the analysis, the type of food to be analyzed and the equipment available. Ashing may also be used as the first step in preparing samples for analysis of specific minerals, by atomic spectroscopy or other traditional methods. Ash contents of fresh foods rarely exceed 5%, although some processed foods can have ash contents as high as 12%, e.g. dried beef (McClements, 2005).

1.4.5 Water activity (a_w)

Water activity is the measure of how efficiently the water can take part in chemical and physical reactions. Water activity is the most important and useful characteristic for articulating the water requisite for microbial growth, enzyme activity and chemical spoilage. This parameter is defined as the ratio of water vapour pressure measured in the product, to the pressure of a saturated water vapour atmosphere at the same temperature. Water activity affects the shelf life, juiciness, tenderness, firmness, appearance, safety, texture, flavour and smell of meat (Pearce *et al*., 2011). Both loss and gain of water content depend on the nature and concentration of water vapour available in the atmosphere. Active bacterial growth depends on the condition of the water present, not just the water content. Water activity is integral in regulating the metabolic

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production or excretion of microorganisms which are proven to affect the aroma or smell of the meat or food product. Food product stability is also dependent on humectants (an ingredient having water binding properties) (Beckett, 1995). Humectants are distributed between the muscles fibres modifying the structure and affecting how light is reflected and absorbed, hence also affecting the visual appearance. The lowest water activity that permits bacterial growth is 0.91. For the growth of yeasts it is 0.88, for moulds 0.80 and for salt-tolerant (halophilic) bacteria it is 0.77. Lowering water activity below these thresholds is an old method used to extend the shelf life of food (FAO 1991). Table 1.1 shows the appropriate minimum water activity values required to limit the growth of a variety of bacteria.

Table 1.1: Approximate minimum a_w values (adapted from Lind *et al*., 2000)

Bacteria	**Minimum a_w**
Aeromonas hydrophilic	0.97
Bacillus cereus	0.93
Bacillus stearothermophilus	0.93
Campylobacter jejuni	0.98
Clostridium botulinum type A	0.94
Clostridium botulinum type B	0.94
Clostridium botulinum type E	0.97
Clostridium perfringens	0.97
Escherichia coli	0.95
Halobacterium halobium	0.75
Lactobacillus helyticus	0.95
Listeria monocytogenes	0.92
Micrococcus halodenitrificans	0.85
Pseudomonas fluorescens	0.97
Salmonella species	0.95
Staphylococcus aureus	0.86
Streptococcus lactis	0.97
Vibrio costicola	0.85
Vibrio parahaemolyticus	0.95
Yersenia enterocolitica	0.95

1.4.6 Salt content

Fresh meat is naturally low in sodium. Most food processes however increase the sodium content of food products by adding salts to improve flavour and functionality. Salt hinders the growth of microorganisms through dehydration which slows spoilage (Liu *et al.*, 2013). Salting should be carried out within five hours of slaughter to control the proliferation of microbes, particularly as it also keeps away insects.

1.4.7 Organic acids

Numerous organic acids are utilised as food stabilisers, additives and/or preservatives and according to Jansons *et al*. (2011), the addition of organic acid additives may inhibit the growth of microorganisms thus, acting as a natural antimicrobial system. Acids such as sorbic acid or potassium sorbate have been shown to effectively hamper yeast and mould growth (Crozier-Dodson *et al.,* 2005). Table 1.2 shows the chemical composition of red meat, including various components such as proteins, fat and water level amongst others.

Table 1.2: The chemical composition of red meat (Jay, 1996)

Component	Value %
Water	75.5
Protein	18.0
Fat	3.0
Amino acids	0.35
Glycogen	0.10
Glucose	0.01
Potassium	0.35
Sodium	0.05
Magnesium	0.02

1.5 POSSIBLE CONTAMINANTS OF BILTONG PRODUCT

1.5.1 Meat contamination during handling

Improper packaging of foods poses a high risk of contamination and spoilage by pathogenic and deteriorative microorganisms (Raybandi-Massilia *et al*., 2009). Contamination can also cause packaging materials to become inflated or deformed (Asefa *et al.,* 2010). Microbial groups known to cause such challenges are normally bacteria, viruses and/or yeasts and moulds. The first group, i.e. yeasts and moulds, is known to cause spoilage in food products (Ledenbach and Marshall*,* 2009) but mainly to cause a bitter taste and a change in the colour and texture of food. Literature shows that the above mentioned organisms are acid tolerant, with yeasts favouring a pH of about 4 and moulds surviving in even lower pH environments. Besides being acid tolerant, yeasts and moulds are also resistant to high salt concentrations and can grow at low water activity levels of 0.60 and above.

Microorganisms other than yeasts and moulds can also spoil dry meat products, especially through contamination from utensils, food handlers and the meat itself. These microorganisms include *Salmonella, Listeria monocytogenes, Chlostridium jejuni* and *Escherichia coli,* known to be contracted by animals on farms through faecal contamination and easily spread during the slaughtering process (Currie, 2004). These bacteria unfortunately survive on dried meat even salt is used in its preservation, and salt is furthermore thought to be contributory to the survival of enterotoxin produced by *Staphylococcus aureus* (Gillespie and McLauchlin, 2007).

In addition to the mentioned parameters, the survival and growth of microorganisms in food is determined by the availability of nutrients and physical factors such as temperature, moisture availability (generally expressed in terms of water activity (a_w), pH, the oxidation-reduction potential and the nature of the gaseous environment to which the organisms are exposed (Wolter *et al*., 2000). Meat has been described as the most perishable of all important foods because its moist, nutritious surface is conducive to the growth of a wide range of spoilage bacteria. The colonization and growth of microorganisms on meat surfaces occurs in stages, the first of which involves the attachment of bacterial cells. This process has been described as a loose and reversible sorption, which may be related to Van der Waals forces or other physico-chemical factors, one of which may be the population of bacteria within the water film present on the surface of the meat. The second and irreversible stage of attachment involves the production of a glycocalyx by the bacterium that consists of an adhesive extracellular polysaccharide layer. The attachment of bacteria to meat surfaces is also affected by surface morphology, temperature, growth phase, motility and other bacteria already present on the meat surface (Narendran, 2003).

A gradual increase in world population and changes in lifestyle have resulted in demands for quality oriented foods of animal origin. The concept of quality has been evolving within the production industry of food of animal origin, following changes in scientific knowledge, in analytical methods and in consumer expectation. Consumers have always played a major role in the various definitions of quality. Although the meat from a healthy animal, with the exception of the lymph nodes, is sterile prior to slaughter, during commercial slaughter of domestic animals it is nearly impossible to guarantee that the carcass surface will not be contaminated with faecal matter. Visual inspection, removal and washing of the carcass can reduce, but not reliably eliminate, contamination. The contamination of beef during the slaughter and processing of carcasses is a major risk for subsequent food-borne infection in humans. Numerous factors interact to affect the level of hide contamination on animals presented for slaughter which can then affect the microbiological content of the resulting carcass.

During de-hiding, there are factors affecting hide-carcass cross contamination: making the initial cuts through the skin (particularly in the brisket area), alternate use of the same hand for handling the hide itself and the carcass surface, and roll-back of the hide during the process. In South Africa, large animals are slaughtered in central abattoirs and distributed in refrigerated trucks to supermarket chains as half carcases or primal cuts where they are further sectioned to retail cuts. Throughout this process, different pathogenic and spoilage organisms may be introduced into the meat, which cause rapid spoilage, great loss of value and may also affect human health. Therefore, it is very important to reduce the initial microbial load in order to increase the acceptability and shelf-life of the meat (Dave and Ghaly, 2011; Polawska *et al.*, 2010).

Many countries have their own traditional meat products and wish to keep their traditions. However, the raw materials (meat) can be a source of different microorganisms; which can contaminate the final products. Depending on their activity, microorganisms induce changes in flavour, nutritional quality, texture, safety and other aspects of meat (Hati *et al*., 2007). Meat comes from warm-blooded animals and, as such, its microbial flora is heterogeneous, consisting of both mesophilic and psychrotrophic bacteria. These bacteria include pathogenic species from the animal itself and from the environment, and bacterial species introduced during slaughter and processing of raw products. Raw meat has a water activity (a_w) of >0.99 and a pH range of 5 to 7, which is an optimum environment for microbial growth. When red meats and poultry are cooked or processed and subsequently refrigerated, the bacterial load from the raw tissue is greatly reduced, leaving only spore formers, enterococci, micrococci, and some lactobacilli. In addition, environmental post processing pathogen contamination can occur and the reduction in competitive bacterial biota may allow for pathogen growth.

According to Sun-Young (2004), spoilage of food is the result of the microbial activity of a variety of microorganisms in food due to contamination. Spoilage can have serious economic implications in the food market. The microorganism that colonises a particular food depends greatly on the characteristics of the product and how it is processed and stored. The parameters affecting proliferation of microorganisms in foods can be categorised in four groups: (i) intrinsic parameters; (ii) extrinsic parameters; (iii) modes of processing and preservation; and (iv), implicit parameters. However, Sun-Young (2004) highlights the fact that any of the above mentioned parameters will influence the effect of the others and the combined effect of parameters is generally more significant than the perceived effect of each individual parameter.

1.5.2. Specific bacteria found in meat and biltong

1.5.2.1. *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, psychrotrophic, facultative anaerobic bacterium that can be isolated from soil, animal feed, water, faeces, and tissues from a variety of invertebrate and vertebrate animals (Farber and Peterkin, 1991). Due to its character the bacteria inevitably results in contamination of numerous food products and can be carried by humans and animals (Farber and Peterkin, 1991). This bacterium has been isolated at every level of the meat processing chain, including slaughter and processing plant environments. *Listeria monocytogenes* can cause an infection after the ingestion of virulent strains and can be present in cooked meat due to cross contamination. Certain people are at high risk of contracting listeriosis, such as pregnant women, the elderly and immune-compromised individuals (e.g., transplant patients, persons with cancer), which can result in serious illness and even death (Farber and Peterkin, 1991).

The detection of *Listeria* spp. in meat is of particular concern in terms of consumer safety. It is capable of growing on both raw and cooked meat at refrigeration temperatures and has been associated with large numbers of pregnant women, children and chronically ill patients. *L. monocytogenes* can be introduced by cross-contamination during transformation processes of raw meat into meat products, the extent of which is determined by factors such as personal and general hygiene practices and the process parameters (Yücel *et al*., 2005). During curing and drying of the meat, the *L. monocytogenes* count tends to decrease substantially because of an environment created by a set of hurdles (low pH, low water activity and high salt concentration).

However, contamination of raw meat and the natural ability of microorganisms to adapt to their environment may result in toxic metabolites. This pathogen is of concern in the production of dried meats Nieto-Lozano *et al.,* (2002), since it is able to grow under both aerobic and anaerobic conditions and can survive dry conditions. It is also salt tolerant and can grow under a broad range of temperature conditions (0.4-45°C). *Listeria spp* do not grow well in acid conditions, but can survive in some instances; the utilisation of bacteriocin should help reduce levels of pathogenic microbes in meat preservation (Nieto-Lozano *et al.,* 2002).

1.5.2.2 Staphylococci

Staphylococcus aureus is a major bacterial pathogen responsible for a broad range of human and animal infections, including toxin-mediated food borne diseases. In many countries, *S. aureus* is considered the second or third most common pathogen after *Salmonella* and *Clostridium perfringens* to cause outbreaks of food poisoning (Ananou *et al*., 2005; Munoz *et al*., 2007). Contamination of meat by pathogens is considered an important issue in terms of food safety. Since animals arriving at abattoirs are frequently carriers of *S. aureus*, the organism is commonly found on carcasses and cuts, and staphylococcal contamination may or may not result from lesions (Rodríguez-Calleja *et al.,* 2006). *Staphylococcus aureus* is a facultative anaerobic Gram-positive bacterium, and a common cause of foodborne illness worldwide (Public Health Agency, 2011). It is an opportunistic pathogen capable of persisting and multiplying in a wide range of environments and causing diseases in both human and animal species (Corpa *et al.,* 2009). Under the microscope they usually appear as grape-like clusters. Furthermore, they can grow at temperatures ranging from 6 to 46°C, pH values ranging from 4.2 to 9.3 and water activity values of between 0.83 and 0.99; however they are fairly heat sensitive (Amuna*,* 2014). *Staphylococcus aureus* enterotoxins pass via the stomach into the intestinal tract where they cause diarrhoea. Symptoms appear 1-6 hours after consuming contaminated food stuffs and may be resolved within 3 days with no treatment. Furthermore, *Staphylococcus aureus* is capable of producing toxins that cause food poisoning or septicaemia (Amuna*,* 2014). Foods which have been implicated in staphylococcal food poisoning include meat and meat products, such as ham, poultry and egg products. *Staphylococcus aureus* usually contaminates the food during the handling stage of ready-to-eat food, raw foods or after cooking (USFDA, 2001).

Although *Staphylococcus aureus* is a ubiquitous organism, the largest reservoir of enterotoxin-producing staphylococci is man. Humans are a predominant source of *Staphylococcus aureus,* so its presence in cooked or processed foods indicates poor hygiene practices amongst food handlers. Animals may also act as a source of *S. aureus*, typically from raw meat, milk and animal skin, thus continuing to be a common problem in dairy farming (USFDA, 2001). It is a characteristic of such heat-stable toxins that even when cooked, food contaminated with *S. aureus* can result in illness when ingested by humans. This organism is salt tolerant but can be destroyed by cooking or inhibited by high salt levels (Harris *et al.,* 2002). In salted meats, where many microorganisms are inhibited, *S. aureus* can flourish in the absence of proper controls. Even though *Staphylococcus aureus* can grow with or without the presence of air, it prefers to grow aerobically and usually occurs at the product surface.

1.5.2.3 *Bacillus cereus*

Bacillus cereus is an aerobic and facultative anaerobic, spore-forming, Gram-positive rod (Drobniewski, 1993) and the most important Bacillus species with respect to food. In addition to food-related illnesses, *B. cereus* may cause non-gastrointestinal diseases like endocarditis and endophthalmitis (Drobniewski, 1993). *Bacillus cereus* is normally found in about 25% of sampled food products but it is limited to cream, pudding, uncooked and cooked meat. Contamination of these products generally occurs prior to cooking (Ayari *et al*., 2011). These microbes can grow and produce enterotoxins over a wide range of temperatures from <5° C to 45°C, although some psychrotrophic strains cannot grow below 10°C (Lawley *et al*., 2008). *Bacillus* sp can grow at pH values of between 4.3 and 9.3 and at water activity values of up to 0.912. Their spores can survive extreme temperatures and when allowed to cool relatively slowly, they will germinate and multiply. Due to the resistance of the endospores to various stresses, these microbes can survive longer under unfavourable conditions (TeGiffel *et al*., 1996; Hariram, 2015).

1.5.2.4. Yeasts and moulds

Moulds are microscopic, multicellular organisms, composed of long filaments called hyphae. Mould hyphae grow over the surface of, and inside nearly all organisms of plant or animal origin. They are related to mushrooms and toadstools, differing only in that their filaments do not unite into large fruiting structures (Rahman, 2007). Fungi are ubiquitous in nature and they commonly contaminate meat and meat products. They may cause spoilage or render meat hazardous by the production of mycotoxins. The air, water, walls and floors of abattoirs are considered the main sources of the fungi which contaminate carcasses (Rahman, 2007). Spoilage by yeasts and moulds can further cause sensory changes. Visual changes include the production of slime, pigmented growth on the product surface, fermentation of sugars to produce acid, gas or alcohol. Other sensory changes may include the development of off-odours and off-flavours (Barth *et al.,* 2009).

Yeasts and moulds can also be present in salted meats and can survive and proliferate in low pH acidic environments. Although yeasts and moulds can grow anaerobically, they both prefer to grow under aerobic conditions at the outer edges and external surfaces of meat products. Some moulds can break down proteins and produce ammonia, which raises the pH on the surface, for instance on sausage. Others may be lipolytic, attacking fats, or cellulytic, attacking sausage casings. Moulds have the ability to produce mycotoxins, although this has not yet been shown to be a problem in sausage production (Barth *et al.,* 2009).

Yeasts and moulds are undesirable, but they can be controlled via fermentation and proper drying. According to Wolter *et al*. (2000), moulds and yeasts are usually present in low numbers on fresh meat, but counts may increase during low temperature storage and eventually dominate the microflora. Species of *Candida, Debaryomyces* and *Torulopsis* are the most frequently isolated genera from meats. Other genera include *Bullera, Cryptococcus, Pichia, Saccharomyces, Schizosaccharomyces, Torulaspora, Trichosporon* and *Williopsisk*. It has been suggested that yeasts play a dual role in the production and ripening of IMM, either by contributing positively by the development of the typical red colour of ripened sausages and an acceptable aroma of the final product, or being detrimental and causing spoilage.

1.6 RATIONALE

1.6.1 Problem statement

Meat has always been part of the human diet and plays an important role in human health as it offers the human body essential nutrients such as proteins, iron, zinc and certain vitamins. Fresh meat is a very perishable food item and because of its rapid spoilage nature, many people convert it into biltong, which is classified as dried meat or intermediate moisture meat (Bender, 1992). Botswana is a landlocked country with abundant numbers of cattle, donkeys, sheep, goats, poultry and game. The country boasts the largest number of wild ostriches in the world. In Botswana processed meat and meat products such as biltong are preferred in homes and at social functions. However, biltong and donkey meat have gained popularity because they are affordable, in spite of a lack of research about donkey meat and the related biltong product. Therefore the main aim of this study is to assess the microbial and chemical contents of donkey meat and biltong, in order to find out whether donkey meat has the potential to achieve the same level of popularity as beef.

1.6.2 Aim

The overall aim of this project was to assess the microbial and physico-chemical characteristics of biltong and fresh meat from donkeys in selected areas of Botswana. The study also aimed to assess the attitudes and practices of the Batswana with regard to the use of donkey meat to make biltong.

1.6.3 OBJECTIVES

- To assess the knowledge, attitude, beliefs and practices of the Batswana on the use of fresh donkey meat and biltong derived from donkey meat;
- To determine the physico-chemical properties (water activity, pH, moisture, fat, ash and protein content) of fresh donkey meat and biltong produced from donkey meat;
- To characterise microbes associated with donkey biltong product and fresh meat.

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Chapter 2

KNOWLEDGE, ATTITUDES, BELIEFS AND PRACTICES OF THE BATSWANA REGARDING DONKEY MEAT AND BILTONG

KNOWLEDGE, ATTITUDES, BELIEFS AND PRACTICES OF THE BATSWANA REGARDING DONKEY MEAT AND BILTONG

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ABSTRACT

A knowledge, attitude, beliefs and practices (KABP) survey was undertaken in three districts as part of the microbial and proximate analysis of donkey meat and biltong produced in Botswana. The objectives were to determine amongst other things, the consumers' knowledge, attitudes, beliefs and practices regarding donkey meat and biltong product, consumer preference for donkey meat, level of operation, food safety and product development. Further objectives were to assess the level of hygiene knowledge of food handlers, including knowledge regarding meat legislation. Opinions on business performance, availability of equipment, facilities and food safety facilities were also ascertained in areas of meat processing. The survey was conducted amongst a total of 285 respondents in the Southern, Kweneng and Central districts of Botswana, of which 280 were consumers, three meat processors and two meat inspectors. A checklist was used to check the available facilities and equipment necessary for a meat processing facility. Consumption of donkey meat is more common and appreciated in the central district and less appreciated in one area of the Southern district. The survey gave a picture of the operations of meat processors and KABP of the Batswana in these areas and it became clear that the majority of people who do not consume donkey meat believe profoundly in myths surrounding the donkey. The main positive finding was with regard to the possible growth of the donkey meat business. This survey was the first of its kind to be carried out in the country on donkey meat and biltong product.

Key words: survey, consumption, knowledge, attitude, belief and practice.

2. 1 INTRODUCTION

Meat forms part of the daily diet of most human beings with the exception of vegetarians and people who are influenced by factors such as religion. The choice of meat is dependent on many elements, e.g. availability, religion, culture, tradition and health issues, amongst others. In Nigeria for example, different communities have different preferences such as goat meat, beef and fish. This is possibly due to a combination of factors including religious beliefs, culture, adaptability, food habits, age, sex, socio-economic class and personal preference. Literature however reports that amongst the Igbos of the East Central states of Nigeria, goat meat is predominantly consumed because its products are readily available and cheap, so that most people can afford them. In the South East, chicken is the meat of choice as there are a number of poultry farms and a big market for chicken (Ajiboye *et al*., 2011).

People use great numbers of animals for the food value of their meat proteins but the effects of these proteins could be perceived in aggression, violence, hatred and moral insensitivity. However, meat can have a negative effect on human behaviour and some people choose to become vegetarians instead. On the other hand, vegetarians have built the foundations for an attitude of tolerance, gentleness, sociability and a spirit of sharing. To be specific, the animals that human beings eat include cattle (oxen, buffalo, bison), deer (including roebuck, fallow deer, reindeer), camels, elk, dromedaries, goats, sheep, donkeys, horses, hares, rabbits, hedgehogs, hippopotamus, kangaroos, swine (pigs, wild boars), flesh of marine vertebrates and aquatic animals (whales, frogs) (Armando, 1997).

Other meats which are enjoyed are meat from different types of birds (poultry, ducks, turkeys, ostriches, various gamebirds). Even though human beings enjoy different kinds of meat, there are some that they don't like and towards which they have certain attitudes.

According to research Asians generally consume more donkey meat than Europeans and Africans. In Southern Africa there are only few tribes that tolerate the consumption of donkey meat. In Botswana it is evident, however, that a number of tribes eat donkey meat, especially in the central districts of the country (Sebastian, 2011). Even though the majority of people still have a negative attitude towards donkey meat, the promotion and sale of donkey meat by some retailers, as well as research done by the National Food Technology Research Centre have been influential in changing people's attitudes, especially once people understand its nutritional value and have had the chance to taste it.

The above clearly indicates that the choice of meat varies according to the influence of a number of factors. High demand for meat by consumers forces the meat industry and all stakeholders to work hard especially in Botswana where the beef sector makes up a large part of the economy. Although beef is the dominant meat upon which the country heavily depends for economic and practical reasons, donkey meat also shows potential to become more popular (Blench, 1999).

The main aim of the survey was to assess the knowledge, attitudes, beliefs and practices of the Batswana regarding donkey meat (particularly in the form of biltong). Further objectives were to assess the general attitude of people concerning the consumption of donkey meat: to investigate the levels of operation of meat processors; to establish the food safety and product development; to determine the role of meat inspectors in the donkey business; and to establish whether the meat processors have all the necessary equipment, facilities and food safety management systems in place.

2.2 Materials and methods

The current survey was done using questionnaires as a data collection tool. The questionnaire was written in English, but for interviewees who were not literate in English, the questions were translated into the local language (Setswana). These questionnaires were administered by the researcher and clarification was given to interviewees where needed.

2.3 Survey coverage and questionnaire

The survey covered three districts of Botswana: the Southern district (Kanye and Metlojane), Kweneng (Metsimotlhabe) district and Central district (Palapye, Serowe and Mahalapye). These three districts were chosen because of the following: in the Central district - the majority of the Batswana consume donkey meat; in the Kweneng district- there is a donkey meat processor and many people in Metsimotlhabe consume donkey meat, although because there are various tribes in this area, there are also non-consumers; and in the Southern district, for people in Kanye the consumption of donkey meat is a new concept while people in Metlojane have a long history of appreciation for donkey meat. The survey covered the KABP of consumers and non-consumers of donkey meat as shown in Table 2.1.

Table 2.1: Coverage of the survey in the three districts

District	*Village*	*Total number of respondents*	*Total number of meat processors*	*Total number of meat inspectors*
Southern	Kanye	120	0	0
	Metlojane	30	0	0
Kweneng	Metsimotlhabe	30	1	2
Central	Serowe	10	1	0
	Palapye	80	0	0
	Mahalapye	10	1	0
Total		**280**	**3**	**2**

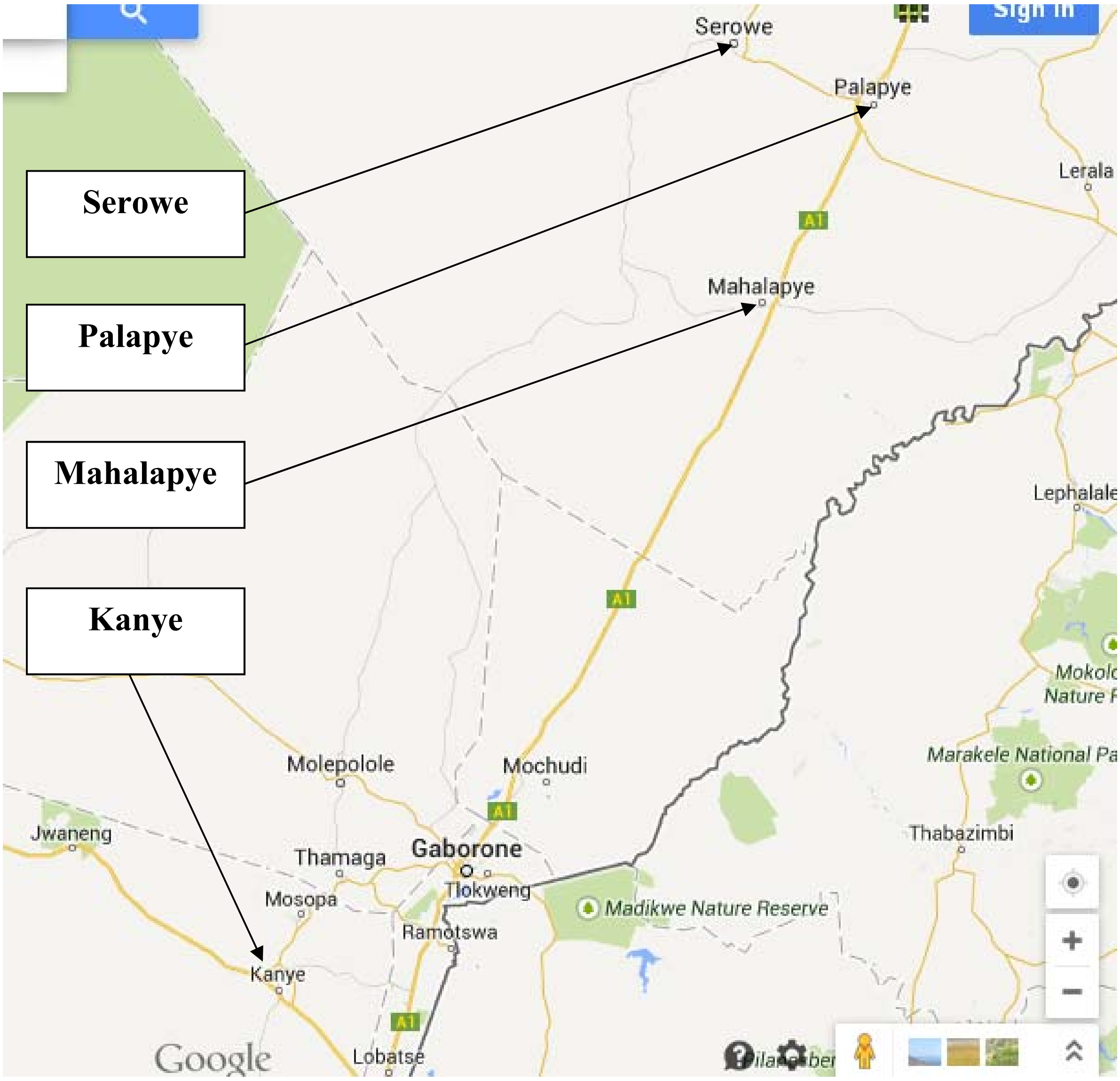

Figure 2.1: Map showing central district areas covered during survey (Maps of the world)

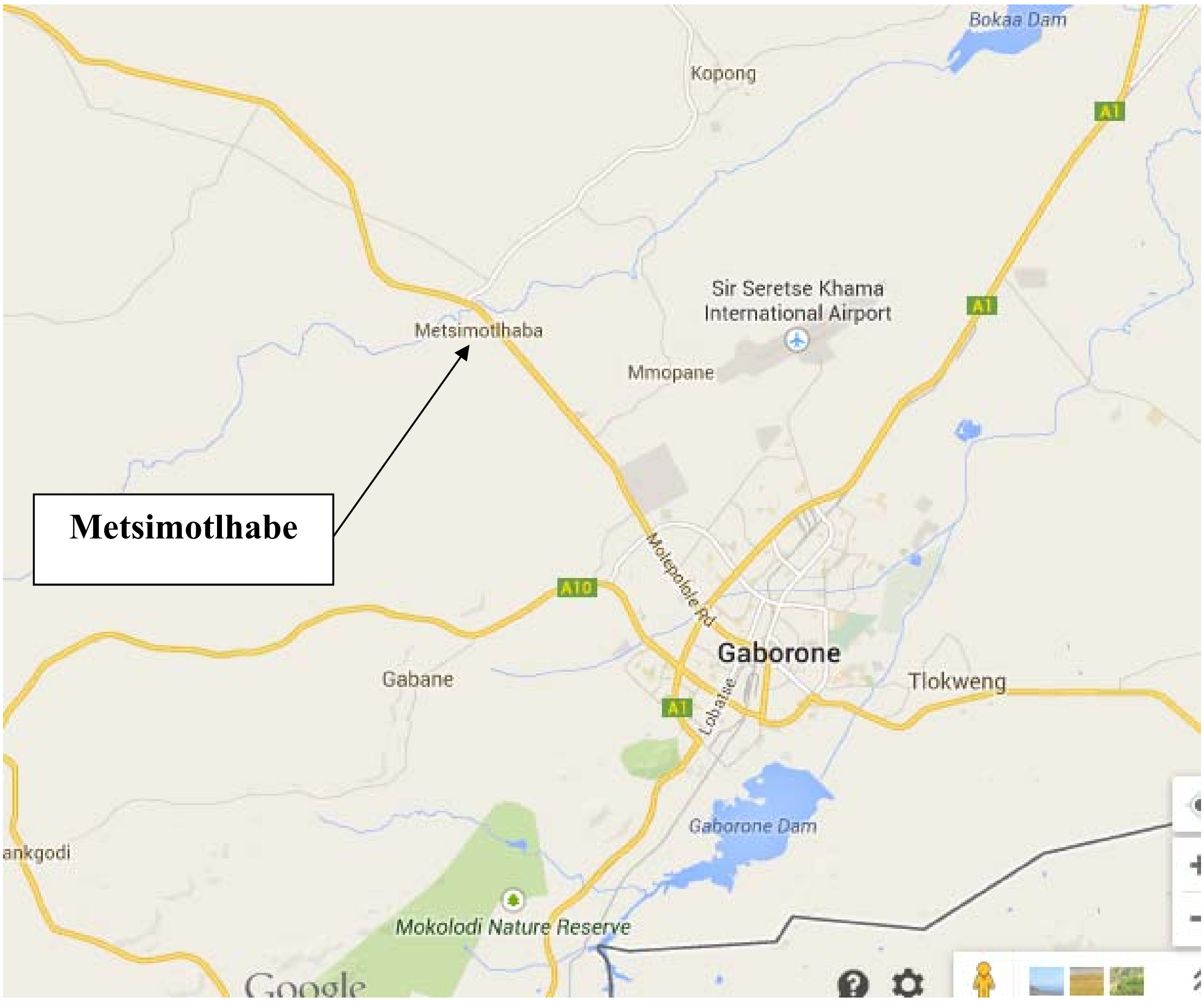

Figure 2.2: Map showing Kweneng district areas covered during survey (Maps of the world)

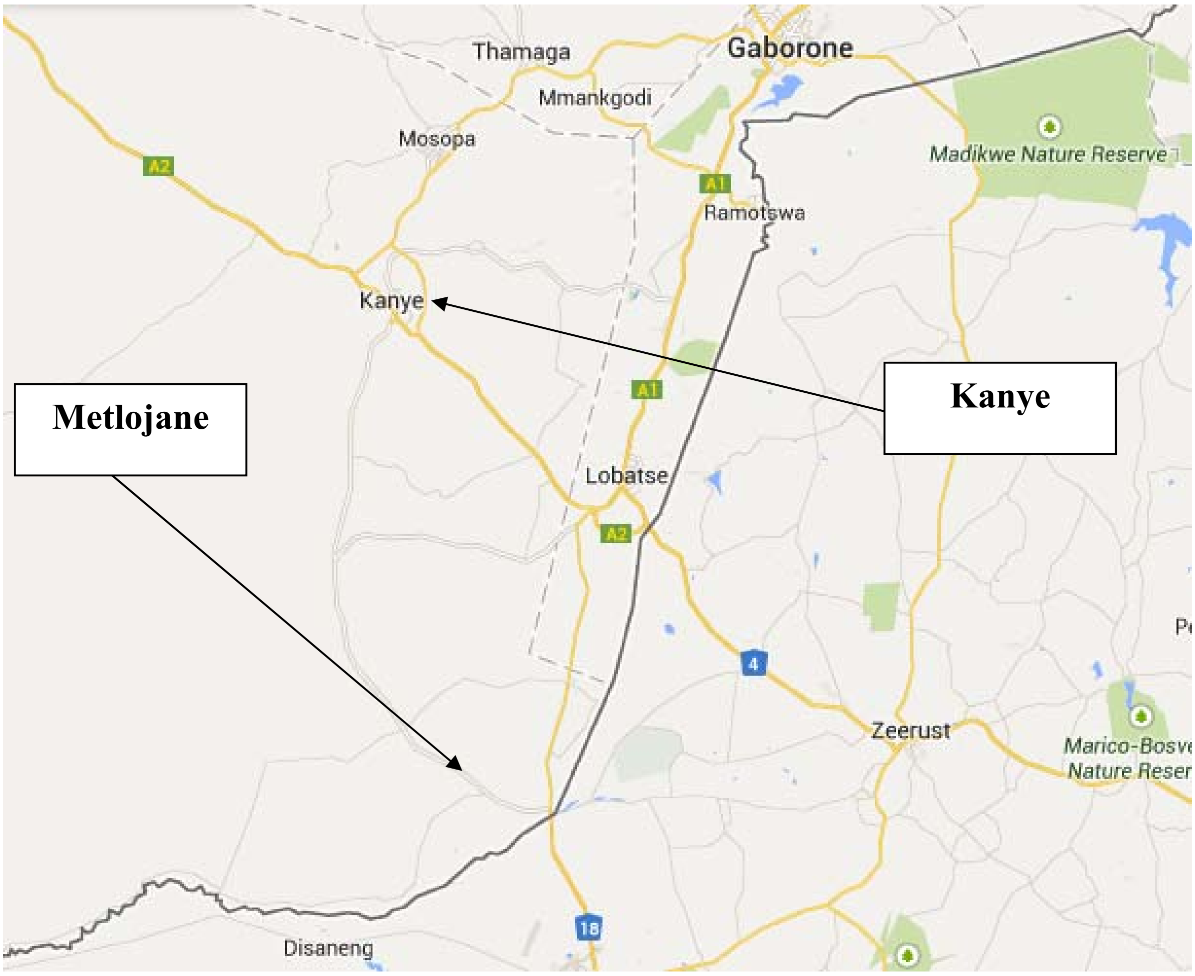

Figure 2.3: Map showing southern district areas covered during survey (Maps of the world)

2.4 Pilot study

A pilot study is identified as a feasibility study, a trial run, pre-testing or a small scale version of the real study (Teijlingen and Hundley, 2001). Before the full scale study of the survey was carried out, a pilot study was done where ten samples of questionnaires were tested. The main aim of this study was to test the adequacy of the research instrument (the questionnaire) to capture the required information and furthermore to check the achievability of the survey, pull together the preliminary data, establish whether sample structure and procedure were operational and finally to identify logistical complications which may occur using the suggested methods (Teijlingen and Hundley, 2001).

Researchers have different opinions and arguments with regard to the conducting and use of data from pilot studies. However, Frankland and Bloor (1999) claim that the number of interviewees can be determined by the population of each area. Piloting gives qualitative researchers a preliminary picture of the study and identifies focal points and projected critical areas for data collection. The data from the pilot surveys completed by the Kanye group were included in the final results since no changes were made to the pilot questionnaires compiled for this cohort. The number of interviewees was determined by the population of each area.

2.5 Results and discussion

Demographic characteristics of respondents

The characteristics selected for demographic profiling of the respondents were age, gender, racial origin, nationality, religion and educational level.

2.5.1 Age of respondents from the study

The results regarding the distribution of respondents according to age are reflected in Figure 2.4. The percentage of respondents decreased with increasing age, where the majority (33.7%) of respondents were young (18-29), 32.6% were middle aged (30-39), 15.09% were between 40 and 49 years, and only 4.2% were above 64 (Figure 2.4). From the respondents it was recorded that respondents between 18 and 39 years old consume a large proportion of donkey meat while individuals older than 50 years of age are less partial to this kind of meat. This could be because donkey meat has only recently become a popular choice for consumers.

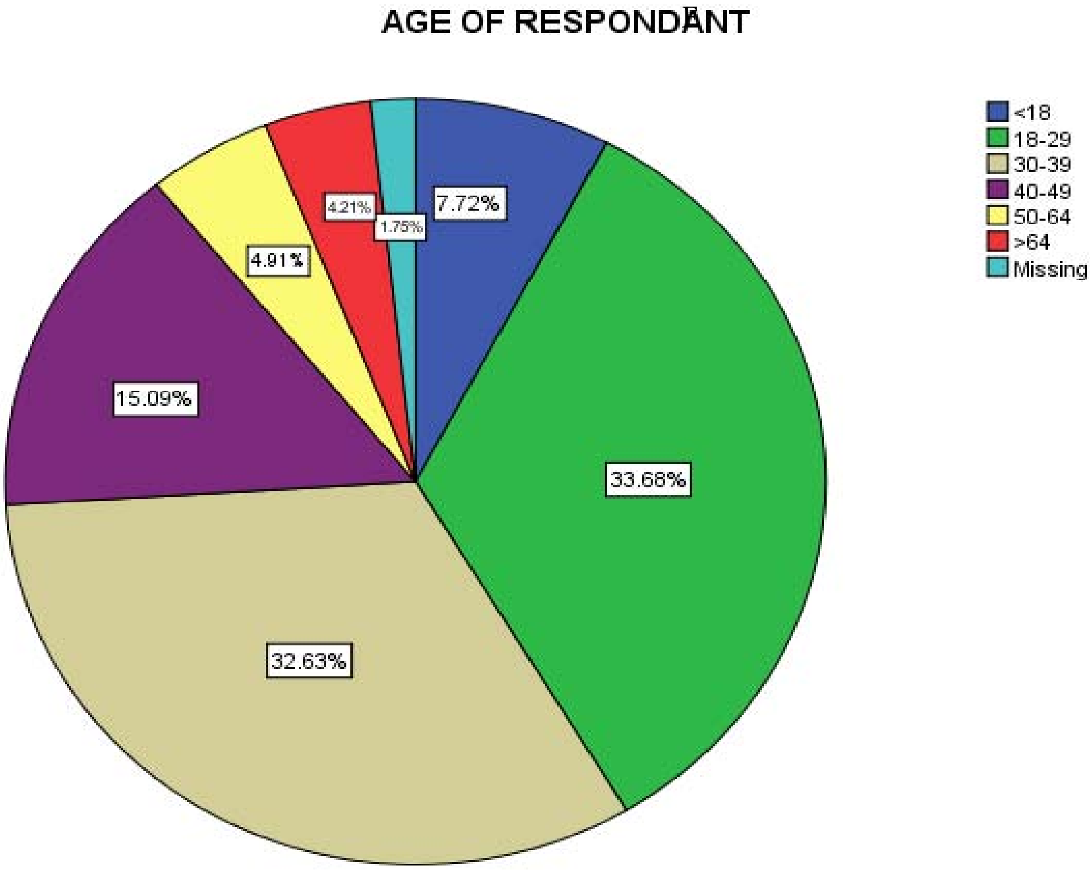

Figure 2.4: Age distribution of the respondents in six selected study areas

2.5.2 Gender of respondents and racial origin

The respondents consisted of 150 females and 120 males. This distribution shows that especially in rural areas, a large number of females serve as house wives and take care of the household chores, hence being available at home to participate in the survey. The 2001 and 2011 national censuses also indicate a higher number of females than males in the population of Botswana. With regard to race, black people made up 97.1% of the respondents, followed by Whites and Asians at 1.1%. Other races were negligible at 0.4%.

2.5.3 Nationality, religion and educational level

The nationalities of the respondents were not intentionally selected but since the survey was undertaken in Botswana, it was not surprising that most of respondents were Batswana. The results show that 96.8% were Batswana, with 1.4% Zimbabwean and 1.8% of other nationalities. Religion tends to influence people's lifestyles and eating habits thus questions about religious affiliation were included in the study. The majority of respondents in this survey were Christians (82.4%) and the minority were Hindu (0.4%). The high percentage of Christians was expected because Christianity has long been established in Botswana while other religions are quite new to the citizens of Botswana.

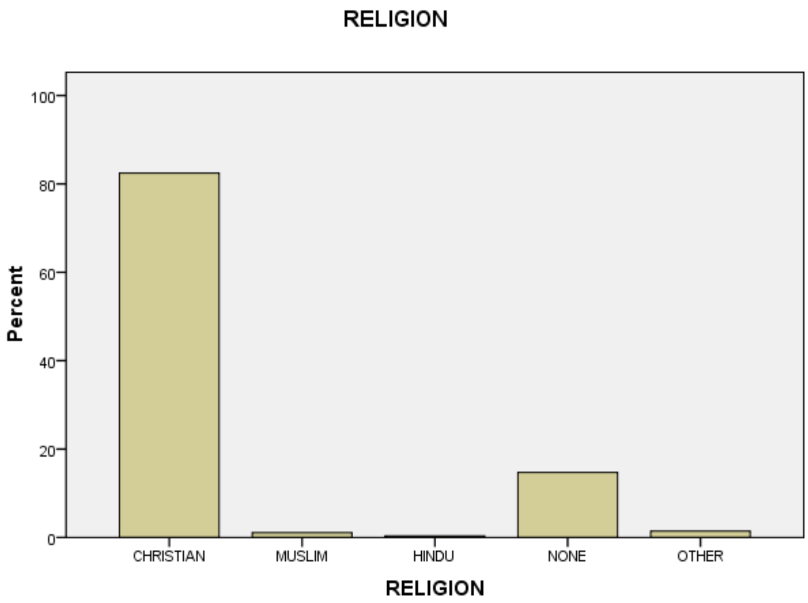

Figure 2.5: Religion of respondents in the six selected areas.

During the survey, people with tertiary education qualifications had no difficulty in answering the majority of the questions as they understood the content and importance of the entire research project, as opposed to those with only primary or no formal education (10.4% and 7.2% respectively).

2.5.4 Type of livestock

It was clear from the 278 respondents that the Batswana still believe in agriculture, especially in keeping domestic livestock, as most of the respondents kept livestock (56.8%). Reasons for keeping livestock were that it is the norm for a Motswana to have livestock because they are a sign of wealth. In some areas good rainfall supports pastures for grazing, making it cheap to maintain the livestock. Batswana from the areas covered by the survey had a dominant interest in rearing cattle. Of the 161 respondents, 129 (80.1%) kept cattle and only four kept donkeys indicating that these people don't have a great interest in breeding donkeys. It is not surprising that such a large majority of respondents breed cattle. Traditionally cattle form the lifeblood of a Motswana man and they are a source of pride for most Batswana. Beef exports to Europe and other parts of the world also contribute significantly to Botswana's economy. Sheep are the least popular type of livestock and are reared by only 0.6% of the respondents.

From the study, it is clear that Botswana livestock are kept for different reasons; however in the six areas visited, 10.6% of respondents kept livestock for commercial purposes and

28% for consumption. Cattle are the preferred livestock primarily because in Botswana the beef industry is a lot more lucrative than the other meat industries.

2.5.5 Most preferred meat

The type of meat preferred could be influenced by various factors such as health, religion, beliefs norms, and availability of the meat or even economic status. The type of livestock kept by most people influences their meat preference and beef is the most preferred meat with 60.1% of respondents preferring this. Mutton is the least preferred (1.8%), and 9.1% prefer other alternatives including donkey meat. According to Blench (2009), donkeys are not traditionally a source of meat and in semi-arid areas communities are critically dependent on these animals for assistance in heavy duty work, transport, carrying of goods and ploughing. These alternative uses of donkeys mean that the consumption of the meat remains low with 61.5% of respondents claiming not to consume it at all and only 19.3% regularly consuming it. Further reasons include religion, beliefs, availability, attitude and knowledge regarding the product. Education is needed for people to understand that donkey meat may be a healthier alternative to beef.

2.6 Donkey meat product preferred

The majority of donkey meat consumers preferred donkey biltong with only 6 out of 149 (4%) individuals having a preference for sausages and fresh meat. Figure 2.6 shows that 51.7% of the consumers of donkey meat prefer biltong due to the absence of the strong characteristic donkey smell in the biltong, which is associated with the fresh meat. Again, the method of preservation which entails the addition of salt and/or spices could make the product more appealing to the consumer.

Just as with any other food products, the taste determines the first choice, which is what 89% of donkey meat consumers like about their products. Only 1.8% indicated they were attracted by the smell of the product. Similar numbers of respondents claimed that they would (47.9%) or wouldn't (46.3%) buy the product. There were 6.7% of the respondents who were non- committal and indicated that they might buy the meat. Biltong is a delicacy and generally consumed as a snack between meals. The results show that 80.2% of people consume donkey biltong as a snack, 12.9% for lunch, 5.9% for supper and 1% as breakfast. In terms of preparation of donkey, 34.9% boil, 23.9% fry, 21.1% grill, 13.8% grind and 6.4% of people dry the meat or use alternative techniques.

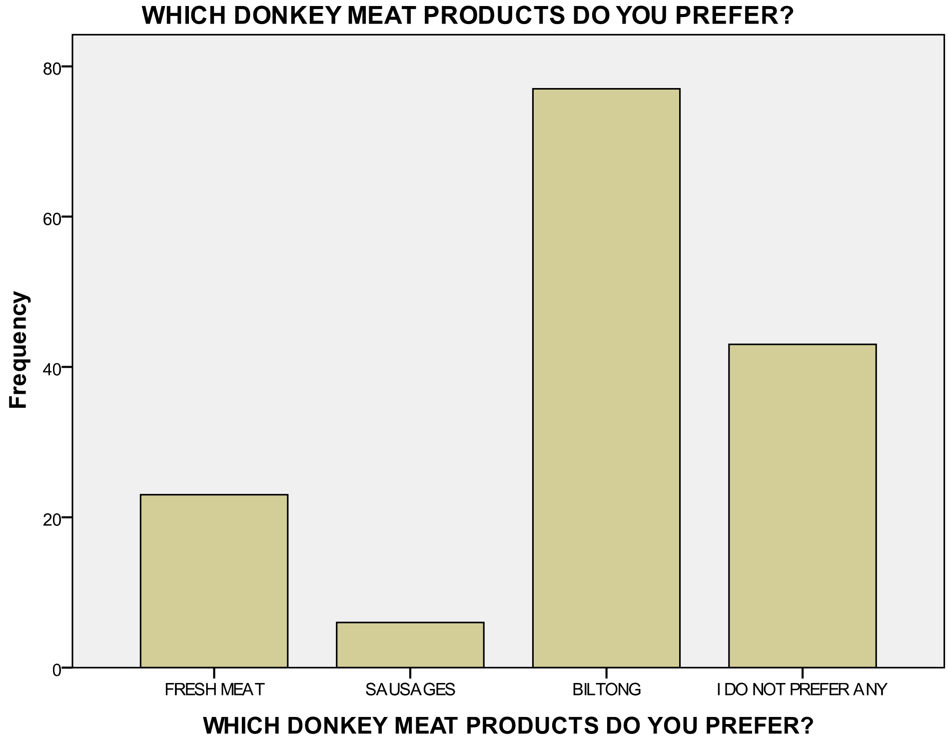

Figure 2.7: Most preferred meat by respondents in six selected areas

Although not everyone consumes donkey biltong, a great number of respondents were willing to consider commercialisation of donkey biltong and other products, as long as there were people consuming it. Only a small percentage of respondents did not agree with selling of donkey meat products (18.8%), mainly because they do not eat it and believe that it is cruel to slaughter donkeys for meat. The survey indicates that donkey meat is not yet consumed by the majority of the Batswana. Butcheries selling this product are rare and few people sell it from their homes. However, one butcher from the areas in the central district sells it alongside beef and other types of meats.

Religion is one of the major role players in donkey meat choice and consumption. In the current results, 82.2% of the respondents indicated that their religions do not prohibit them from eating donkey meat, while 17.8% revealed that they are forbidden to eat it. Despite the low level of religious limitations, 60.4% of respondents declared that donkey meat would never be an alternative to their meat of choice, while 39.6% think it could be. This means that some people who do not eat donkey meat are willing to give it a try. Donkey meat is not commonly consumed during special occasions and 95.7% of respondents who consume donkey meat slaughter a donkey whenever the family wants to eat donkey meat. Conversely, cattle are saved for specific events and slaughtered for funerals, weddings and other special occasions. The study reveals that older donkeys are targeted first followed by foals and then injured animals.

In addition to religion practices in some parts of Africa, including Botswana, it is believed that donkey meat has medicinal value in the treatment of certain illnesses (the same is believed about the milk, on which research is also being done), and 38.2% of respondents agreed that it could solve some medical conditions. However, 7.5% disagreed with this, and 54.3% were not sure. It is clear from the research that people are slowly beginning appreciate donkey meat as 78.1% of respondents think that abattoirs should be set up for hygiene purposes, management and for proper inspection, as is done for cattle. The rest believe that it should not be done because so few people eat donkey meat.

Meat processors in different districts

Apart from the above issues about age, educational level and religion; there are several meat processors of donkey meat who were also engaged in the study. Meat processor A slaughters twice a week, i.e. two donkeys per week; meat processor B slaughters one donkey per week; and meat processor C slaughters 1-5 per day, depending on demand from his clients. Of the three meat processors interviewed, two of them slaughter at the farm and one at the slaughter slab. The first two processors use farms because their businesses are informally run. They sell the meat from their homes with no proper facilities or equipment, and for little income, while the third processor sells from a butcher shop with the necessary equipment and facilities. Meat processor A sells 15% of the meat fresh and the rest as biltong, meat processor B sells 100% fresh meat and meat processor C sells 60% fresh meat and uses 40% for other products such as sausages (boerewors), biltong, minced meat and pounded meat known as ‘seswaa’. Meat processor A sells donkey meat as dog food, but stated that some people buy the meat for personal consumption.

Meat processor B gives meat to labourers involved in the slaughtering process, and meat processor C sells meat as pet foods. Meat processor A sells to individuals in different institutions like government departments and any other private institutions, meat processor B sells to individuals, and meat processor C sells to individuals and other outlets such as depots, bars and trade shows. Meat processors A and C do not have scales so they sell a plastic plate of meat for P5.00, while meat processor B sells meat at P15.00 per kilogram. According to all three meat processors, they have not received any professional training in food hygiene, processing or management skills. Table 2.2 shows the complete lack of training of all three meat processors.

Table 2.2: Areas of training at three meat processing venues.

Area of training	*Meat processing venue A*	*Meat processing venue B*	*Meat processing venue C*
1.Food and safety hygiene (handling)	No	No	No
2.Food processing skills	No	No	No
3.Business management skills	No	No	No
4.Food regulations	No	No	No
5.Government regulations	No	No	No
6.Catering skills	No	No	No

2.7 Conclusion

It is evident from the survey that, for different reasons, most people in Botswana do not consume donkey meat but utilise it mainly for different purposes. Other than religion, preference or level of education there are also other beliefs associated with donkey meat held by some people. Some undocumented beliefs are as follows: it is taboo to consume donkey meat because a donkey was Jesus' transportation (religion point of view); it smells terrible and looks very dark in colour hence is not suitable for consumption; the human body retains the terrible smell after eating the meat; it is meant for domestic work and not for consumption; it is pet food. Though the study exposed myths and beliefs for non-consumption, there is a potential industry in donkey meat as a result of the number of young people who seem to be interested in the use of donkey meat. Even though only three meat processors were interviewed, each of them was processing donkey meat in some or other way. Meat processor B was in a better position in terms of the conditions of the workplace and processing methods, and although meat processors A and C had unprofessional operations, there is nevertheless potential for improvement in production and standards of facilities and equipment used for donkey meat processing.

The relationship between consumer perception of quality and the food industry's drive to please consumer needs is complex and involves a lot of different mechanisms. In donkey meat production, as in other red meat industries, variables include age, gender, nutritional value and so forth. Science and innovation play a major role in equipping the food industry, especially in regard to meat production, to respond positively to consumer concerns and expectations. However, it must be borne in mind that the industry faces other challenges in terms of consumer perceptions, especially in areas relating to the health, animal welfare and convenience.

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Chapter 3

MICROBIAL ASPECTS OF *EQUUS ASINUS* MEAT AND BILTONG PRODUCED IN BOTSWANA

MICROBIAL ASPECTS OF *EQUUS ASINUS* MEAT AND BILTONG PRODUCED IN BOTSWANA

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Abstract

Meat and meat products have been implicated in food borne disease outbreaks due to contamination by various bacteria in most parts of the world. In Botswana a large number of homes consume meat and meat products daily, even though the microbiological quality of these types of food is unknown. In the current study the aim was to evaluate the microbiological profiles of donkey meat and meat products (mainly biltong) by assessing the presence of *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Salmonella* spp, total viable counts (TVC), yeasts and moulds. Two donkeys were slaughtered with a two month interval between slaughtering. From the two trials only six samples were taken (2 fresh meat samples and one biltong sample from each trial), from Banyana farms. The highest mean TVC counts recorded were for donkey 1. The mean values of *Staphylococcus aureus* counts detected from donkey 1 samples from the forequarter were $1.6x10^1$, and hindquarter 0; donkey 2 mean values were $3.3X10^1$ (forequarter), $1.5x10^4$ - $8.16x10^3$ (hindquarter) and $2.3x10^2$ – $7x10^1$ (biltong).In general meat samples did not show high loads of bacteria; however the biltong showed the presence of *Staphylococcus aureus*. The mean microbial load on the fresh meat ranged from $1.0x10^1$ and $3.5X10^2$ $cfu.g^{-1}$ while loads on the biltong sample ranged between $1.26X10^6$ and $6.5X10^3$ $cfu.g^{-1}$. To prevent bacterial contamination, meat and meat products such as biltong must be handled and packed properly in sterilized polyvinyl containers.

Key words: *E. coli*, *Staphylococcus aureus*, biltong, fresh meat, mean microbial load, outbreaks.

3.1 INTRODUCTION

Meat is a common vehicle for food-borne diseases. Though production and processing has improved over the years, recent studies still indicate the presence of food-borne diseases, especially from meat pathogens such as *E. coli* 0157*,* Campylobacter, *S. aureus* and *Y. enterocolitica* (Jiménez-Colmenero *et al*., 2001). Food-borne illnesses are typically caused by bacteria or their metabolites, parasites, viruses or toxins (Datta *et al*., 2012). The living animal carries pathogenic bacteria, while the processing environment harbours them. Bacteria originating from the animal can contaminate the carcass during slaughter and subsequently be distributed via cut or raw meat intended for further processing (Borch and Arinder, 2002). There are substantial human health consequences due to microbial infections ranging, from prolonged illness to death in patients with compromised immune systems. Older people, children and the disabled are at higher risk of contracting food-borne illnesses. The levels of impact depend on the types of foods being consumed, food processing and preparation procedures, handling and storage techniques, and the sensitivity of the population (Datta *et al*., 2012).

Meat is highly perishable and should be stored, processed, packaged and distributed correctly to reduce the risk to consumers due to microbial proliferation (Jiménez-Colmenero *et al*., 2001). This product is not only highly susceptible to spoilage, but also often implicated in spreading food borne illnesses (Bhandare *et al.,* 2007; Podpecan *et al.,* 2007). While general cleanliness and proper sanitation are very effective, other means of controlling microbial growth in meat have also proven useful (Sallam and Samejima, 2004).

Most organisms are transferred onto the carcass during slaughter, through poor hygiene. Common microbes include non-pathogenic spoilage bacteria and indicator microbes like coliforms, *E. coli, L. monocytogenes*, *S. aureus*, *Bacillus,* and *Salmonella* spp. which can proliferate and lead to illness if the contaminated meat is consumed (Abong'o and Momba, 2008). The bacteria of most concern for meat spoilage include *Pseudomonas, Acinetobacter, Moraxella, Aeromonas, Alteromonas putrefaciens, Lactobacillus* and *Brochothrix thermosphacta.* The pathogenic bacteria of greatest concern are *E. coli* 0157:H7, *Salmonella* spp, *L. monocytogenes, Campylobacter, C. botulinum, C. perfringens, S. aureus, A. hydrophylia* and *B. cereus* (Abong'o and Momba, 2008). During the processing of meat products such as biltong, extra care is needed in terms hygiene as the manufacture of these processed products depends heavily on the quality of the raw material; as a result, microbial content of the raw meat just before processing starts is crucial.

Biltong is known as an intermediate moisture meat (IMM), and requires a few stages to achieve a wholesome product which is safe for consumption (Calicioglu *et al*., 2002; Keshia *et al*., 2010; Shale and Malebo 2011). Although people perceive this product as safe, no one can confirm its safety without proper microbial testing (Calicioglu *et al*., 2002). Even though the product has a long shelf life (up to a year in some instances), the steps used during the production of dried meat products may become the source of contamination if not carried out properly (Calicioglu *et al*., 2002).

The meat used for the processing of biltong ought to have low microbial counts to ensure a harmless and more wholesome end product. Parameters such as pH and water content determine the shelf life of intermediate moisture meat products, while dryness and water activity inhibit the growth of microorganisms (Sofos *et al*., 2003). The survival ability of the above mentioned organisms, including halo-tolerant microbiota, depends on the cleanliness of working surfaces and the personal hygiene of handlers. Fungi and certain bacteria can spoil biltong if not restricted. Intermediate moisture meat products such as biltong are expected to have lower counts of pathogenic microorganisms, due to the number of hurdle methods applied.

Despite the fact that the hurdles are applied as measures to preserve the meat or to inhibit the growth of major food-borne pathogens such as *E. coli* 0157:H7, *L. monocytogenes* and *Salmonella* spp., there is always the question regarding the conditions under which the biltong is processed. The survival ability of the above mentioned organisms, including halophiles, and the personal hygiene of handlers all constitute crucial factors. It is known that halophilic and halotolerant microorganisms will grow on biltong as conditions are more favourable for their growth compared to the growth of other microorganisms. The exact strains should however be noted since they may be pathogenic.

Dried foods are shelf-stable (e.g. 0.75-1.00 moisture protein ratio, $a_w < 0.70$) products stored at room temperature and generally consumed without further cooking. Dried products, such as biltong, have been considered one of the safest food groups for humans (Calicioglu *et al*., 2002). Bacterial growth during storage of such products is primarily inhibited by low a_w and other factors such as low pH, preservatives, and reduced oxidation-reduction potential. Although bacterial growth is not expected, at least for major food-borne pathogens such as *E. coli* O157:H7, *L. monocytogenes* and *Salmonella spp*, their survival may vary depending on the severity and variety of antimicrobial hurdles and the history of the bacterial contaminant. For example, acid adaptation of *L. monocytogenes* and other pathogens may enhance their survival in acidic foods and increase cross-protection to sub-lethal stresses associated with other food processing treatments (Francis and O'Beirne, 2001; Ananoua *et al*., 2005). Therefore the aim of the study is to ascertain the microbial content in both fresh meat and biltong.

3.2 Materials and methods

3.2.1 Study area

The study was conducted in Kanye, about 85km from Gaborone, the capital city of Botswana. Samples were collected over a period of two months, namely January and March. The first donkey was slaughtered at the end of January and the second one at the beginning of March. The two donkeys were selected on the basis of having the same age, sex, colour and area of origin.

3.2.2 Slaughter protocol

A humanitarian slaughter technique was used involving stunning and bleeding. The animal was stunned, then allowed to bleed to avoid the unpleasant smell that usually annoys consumers. The carcass was then split laterally and forequarters and hindquarters cut out and hung. Samples from both sections were placed into well labelled sterile plastic bags and stored in a cooler box with ice packs. Samples from the two forequarters were combined to make a composite sample and the process repeated for the hindquarter samples.

3.2.3 Sample collection

Hind quarters of donkey meat were collected as experimental samples. These were collected aseptically into whirl packs and marked with separate codes to differentiate them. These were then placed in cooler boxes loaded with ice packs and were transported to the National Food Technology Research Centre. Upon arrival at the National Food Technology Research Centre laboratory, 10g of each sample was homogenised in 90 ml of sterile peptone buffered water in a stomacher for 2 minutes. Serial dilutions of the liquid portions were performed and aliquots of 0.1ml spread-plated on different solidified media in triplicate and incubated at different temperatures (Davis *et al*., 2005).

3.2.4 Biltong preparation

Meat from the hindquarter and forequarter were trimmed of fats and contaminating spots to ensure quality and shelf life and sliced into strips of about 50cm long and 2cm wide. A mixture of salt and vinegar (50g/80ml) was added to 1419.2g of meat and left over night at a temperature of 8^{o}C. Thereafter the meat is hung to dry. After 4 days of drying the meat was weighed to measure the moisture lost.

3.2.5 Sample preparation

Approximately 10g of each sample was aseptically weighed off, 90ml peptone buffered water added and the samples homogenised for 2 minutes using a stomacher (Stomacher 400 circular lab blender, Fischer) (Fang *et al.*, 2002). A range of microbiological tests were then performed to determine sample quality. Serial dilutions of up to 10^{-6} were made, using MRD, universal bottles and pipettes. Selective media were prepared according to the manufacturer's instructions and the microorganisms present in cultures. Aliquots of 0.1ml were spread-plated on different solid selection media in duplicate (first trial) and triplicate (second trial) and incubated. Organisms were counted using a colony counter.

3.3 Microbial analysis

3.3.1 Total viable counts

For the enumeration of Total Viable Counts (TVC), Plate Count Agar (PCA) plates (Merck, RSA) were incubated at 35°C for 48 hours (Maturin and Peeler, 2001).

3.3.2. *Staphylococcus aureus*

Baird Parker Agar (BPA, Oxoid CM275) containing 50ml egg yolk telluride emulsion (Merck, RSA) was used for the quantification of staphylococci after 48 hours of incubation at 35°C (AOAC, 1990). *Staphylococcus aureus* ATCC 25923 was used as a positive control and a blank BPA plate used as a negative control. Typical *S. aureus* colonies were black with white margins surrounded by clear zones. All *S. aureus* colonies were confirmed using gram staining as well as coagulase tests (Hudson, 2010).

3.3.3 *Bacillus cereus*

To enumerate *Bacillus cereus*, a blank *B. cereus* agar plate was used as a negative control and *B. cereus* ATCC 14579 as positive control. *Bacillus cereus* selective Agar plates (BCSA) (Oxoid) were used for the enumeration of *B. cereus*. The plates were incubated at 30°C for 24 hours and examined for typical *B. cereus* colonies which are characterised by a peacock blue colour, with egg yolk precipitate. Confirmation of colonies was done according to the method of Holbrook and Anderson, (1980) (Tallent *et al*., 2012).

3.3.4 Yeasts and moulds

For the enumeration and isolation of fungal colonies, Potato Dextrose Agar (pH 3.5) (PDA, Merck), was incubated for 5 days at 25°C (Masago *et al*., 1976). All colonies were transferred to Plate Count Agar for microscopic examination using a Nikon phase contrast light microscope (Nikon Eclipse E600, IMP). Fungal identification was based on the phenotypic characteristics of the isolated fungi by using the prescribed identification keys described by Dhama *et al*., (2013).

3.3.5 *Salmonella spp.*

RV (Rappaport-Vassiliadis Medium), XLD Agar (Xylose lysine desoxycholate agar), SC Broth (Selenite cysteine broth), TT Broth (Tetrathionate broth) and HE Agar (Hektoen enteric agar) were used for the isolation of *Salmonella.* API 20E and gram staining were used as confirmation tests.

3.3.6 *E. coli 0157:* H7

3M^{TM} *Petrifilms* for *E. coli and coliforms* were incubated at 35^{o}C for 24 hours.

3.4 Results and discussion

3.4.1 Incidence of microorganisms in donkey meat

Two donkeys were used in the present study and for each donkey, the hindquarter and forequarter were the fresh meat cuts used to determine contamination by different microorganisms. In addition, the donkey meat was dried and made into biltong which was also analysed for contamination by microbes. For both donkeys 1 and 2, *Staphylococcus aureus* was the most prevalent microbe and potentially pathogenic microorganism found in this study. *Bacillus cereus* and *Salmonella* species were completely undetected (Table 3.1). On average the occurrence of *S. aureus* in donkey meat was statistically significantly higher than that of other microbes with 95% confidence ($p<0.05$; $p=0.000$). However, when taking into consideration the absence of other microbes in certain samples, a statistical evaluation was not vital.

Table 3.1: Detection of microorganisms in different samples of donkey meat

Microorganism	**Donkey 1**			**Donkey 2**		
	Forequarter	**Hindquarter**	**Biltong**	**Forequarter**	**Hindquarter**	**Biltong**
S. aureus	√*	ND	√	√	√	√
B. cereus	ND	ND	ND	ND	ND	ND
E. coli	√	ND	√	√	ND	ND
Yeasts and moulds	ND	ND	√	ND	√	ND
Salmonella	ND	ND	ND	ND	ND	ND

√ * indicates that a microbe was detected in the sample. ND = not detected

3.4.2 Microbial counts of donkey meat

Microbial counts were performed on donkey meat to determine the microbial load and subsequently establish if the meat was of acceptable sanitary quality. No counts were recorded for *B. cereus* or *Salmonella* spp (Table 3.2). A very high mean total viable count (TVC) exceeding 10^6 was recorded for donkey 1 biltong. Neither the difference in mean TVC nor the mean counts for the different microorganisms were statistically significant ($p<0.05$; $p=0.000$). Generally, the meat of both donkeys was of acceptable sanitary quality except for the one instance mentioned in paragraph 3.5.1.

Table 3.2: Total viable counts and microbial counts of donkey meat

	Microbial counts (CFU.g^{-1})					
Donkey sample	Total viable count	*Staphylococcus aureus*	*Bacillus cereus*	*Salmonella*	Yeasts and moulds	*E. coli*
Donkey 1						
Forequarter	$1.5X10^3$	$1.6X10^1$	0	0	0	$1.0x10^1$
Hindquarter	$1.0X10^3$	0	0	0	0	0
Biltong	$1.26X10^6$	$6.5X10^3$	0	0	$3.5X10^2$	$3X10^1$
Donkey 2						
Forequarter	$1.8X10^2$	$3.3X10^1$	0	0	0	$1X10^1$
Hindquarter	$1.4X10^4$	$1.16X10^2$	0	0	$3.5X10^2$	0
Biltong	$1.3X10^2$	$2.3X10^2$	0	0	0	0

When looking at the contamination of donkey meat by pathogenic or potentially pathogenic bacteria and fungi, it was found that both donkey carcases were contaminated by *Staphylococcus aureus, Escherichia coli* and moulds and yeasts, with *S. aureus* being the most common. The detection of S*. aureus* in donkey meat is an important public health concern due to the inherent pathogenic capacity, hardiness and multidrug resistance of this organism (Perez-Roth *et al.*, 2004). A previous study in France (Poutrel and Sutra, 1993) detected *S. aureus* in horses, another equine species. It can therefore be reported that staphylococci will most likely be present in any related animal species due to the ubiquitous nature.

Escherichia coli on the other hand is an indicator organism used to reflect the hygienic quality of the food. Its presence in food generally indicates direct or indirect faecal contamination. Although the occurrence of *E. coli* was within acceptable limits according to the FAO manual (1992), there is potential for the presence of other enteric pathogens in the donkey meat and it is important to cook meat properly before consumption so that these pathogens are inactivated. It is interesting to note that *Bacillus cereus* and *Salmonella* species were not detected in the present study. The presence of these genera in the donkey meat can however not be ruled out as the conventional methods used in this study may not be sensitive to all species. In fact, a study conducted on horse carcasses in the United States of America (Anderson and Lee, 1975) detected different *Salmonella* species on horse meat.

3.4.3 Total viable counts and microbial counts of donkey meat

Although pathogenic and potentially pathogenic microorganisms were detected in the present study, the meat was of acceptable sanitary quality as the counts of these pathogens were below the limit of 5.0 Log_{10}CFU/g, as recommended by the Food and Agriculture Organisation (FAO, 1992). Unacceptably high total viable counts (TVC) of more than 10^6 encountered in the biltong sample of donkey 1 exceeded the acceptable limit for red meat as set by the FAO (FAO, 1992) and is therefore a public concern. Although the counts for pathogenic and potentially pathogenic microbes met acceptable limits, this does not completely preclude a potential risk to the consumers, especially when coupled with the fact that biltong is not cooked before consumption.

In this study the high TVC for biltong may be attributed to post-processing contamination from either the physical work environment or the food handlers. All but one of the meat types were colonised by *Staphylococcus aureus*. This is not surprising because donkeys like other equines dissipate heat mainly by sweat and sweat glands are found all over the body (Payne and Wilson, 1999). The sweat contains salts which have been shown to enrich for the growth *Staphylococcus* (McMeekin *et al*., 1987).

From the above mentioned sections and literature it has been noticed that the majority of studies conducted on donkey meat throughout the world have focused on the physico-chemical characterisation, largely ignoring the microbiological sanitary aspects of this alternative red meat. Consequently, the microbiological aspects of donkey meat remain largely unknown. A previous study (Aganga *et. al*., 2003) investigated the mineral and chemical composition of donkey carcases in Botswana and this study is a first attempt to analyse the chemical composition of donkey meat and couple it with the microbiological sanitary quality of the meat.

3.5. Conclusion

The presence of different kinds of bacteria in meat is reported globally (Datta *et al.*, 2012). In the present study an effort was made to quantify and identify the major bacteria in fresh donkey meat and biltong. The results show that some bacteria were present and others completely absent. Those identified were minimal but *S. aureus* was the most prevalent of all the microbes analysed. The presence of *S. aureus* indicates possible contamination from the food handlers especially in the case of biltong, which can cause infections together with *E. coli* which indicates poor hygiene practices. Nevertheless, the microbial counts were low, which means that according to the findings of this study the meat can be consumed. The analyses in this study are however limited and do not guarantee that other organismal species will not be found following more in-depth studies on more animals.

3.7. References

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Chapter 4

PROXIMATE ANALYSIS OF *EQUUS ASINUS* MEAT AND BILTONG IN KANYE, BOTSWANA

PROXIMATE ANALYSIS OF *EQUUS ASINUS* MEAT AND BILTONG IN KANYE, BOTSWANA

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Abstract

The quality of meat is based on its microbiological status; however, the chemical composition also contributes to the meat quality as it regulates the growth of microorganisms. In most African countries, drying, together with salting and/or the addition of vinegar, is a traditional way of meat preservation resulting in a long shelf life product (biltong). Donkey meat (fresh and biltong) was analysed for proximate factors such as ash, fat, moisture content and water activity. Total fat, total ash, moisture content and water activity were found to be 2.8, 4.38, and 24.65, 0.998 (forequarter, donkey 1); 12.26%, 3.81%, 23.72%, 0.999 (hindquarter, donkey 1), 15.31%, 9.29%, 84.37%, 0.654 (biltong, donkey 1); 28.48%, 7.31%, 30.02%, 0997 (forequarter, donkey 2); 44.6%, 5.29%, 33.5%, 0.998 (hindquarter, donkey 2) and 11.66%, 5.26%, 69.84%, 0.668 (biltong, donkey 2). The total fat content was significantly different from the moisture (p-value=0.02) and water activity (p-value=0.003) but did not differ significantly from ash content (p-value=0.322). Surprisingly, analysis showed that the total fat content was high in the forequarter (28.48%) and hindquarter (44.6%) of donkey 2 when compared to the forequarter (12.8%) and hindquarter (12.63%) of donkey 1. In general donkey 2 had higher percentages of all analysed proximates.

Key words: proximate, significant, hindquarter, forequarter, preservation

4. 1 INTRODUCTION

The chemical composition of food items is crucial and one of the major problems faced by the food industry is the preservation of meat and meat products using locally available means. The physical and chemical characteristics of meat are very important to both consumers and researchers (Lebert *et al*., 2005). A number of hygroscopic chemical compounds are presently used or being considered for use as humectants by the food industry. Most of them can be classified into one of four categories: salts (mineral and organic), sugars, polyols, and protein derivatives. Water activity (a_w), total fat, total ash and moisture content are some of the most critical factors in determining the quality and safety of food (Bradley Jr., 2010).

Different methods of quality control such as organoleptic evaluation, physical test methods, chemical analyses and microbiological examination are needed and important (Mons'on *et al*., 2005). The most common and basic quality control methods normally involved in the processing and handling of meat and include water activity, pH and NaCl measurements. The processing involved is not easy as it involves numerous simultaneous and interactive mechanisms such as mass transfer, together with physico-chemical and microbial modification (Deumier *et al*., 1996). Water activity (a_w) is one of the most critical factors in determining the quality and safety of frequently consumed foods, as it affects the shelf life, safety, texture, flavour, and smell of food.

The significance of water in food preservation, and specifically in controlling food quality has long been recognised. Reduction of available water by sun-drying or the addition of sugars and salt is the basis of some of the earliest preservation techniques. Water activity is probably the single most important factor for shelf-stability (as indicated by microbial stability) in most dried meats. Water activity (a_w) is calculated as the vapour pressure of a product divided by the vapour pressure of pure water with values of fresh meats, fruits and vegetables greater than 0.98; semi dry sausages, 0.95-0.97; condensed milk, 0.85-0.93; and honey and chocolate, less than 0.60. pH is another parameter that varies with genetic origin, ante-mortem treatment, and muscle and fibre type, and it affects the water-holding capacity of meat as well as water loss in dry-cured meat, sausages and hams (Gou *et al*., 2002).

It was therefore the aim of this study to assess the total fat, total ash, moisture content and water activity in order to shed light on the physicochemical aspects of donkey meat and related biltong product.

4.2 Materials and methods

4.2.1 Study site

Two donkeys from a government cattle production farm (Banyana Farms) in the Southern district of Botswana were slaughtered at selected intervals. The two donkeys were identified and selected based on age, sex, colour and area of origin. Proximate analyses were performed at the National Food Technology Research Centre (NFTRC) in Kanye at biochemistry laboratories. Total fat, total ash, moisture content and water activity were determined using methods developed by the Association of Official Analytical Chemists (AOAC) as detailed below.

4.2.2 Total fat

Two grams of sample were weighed in a thimble with sand. The thimble and rod were placed in a 50ml beaker and dried in an oven for 6 hours at 100°C or 1.5 hours at 125°C. Total fat calculations were done using the equation shown below;

$$\%\ \text{total fat} = \frac{\text{flask + fat flask - weight}}{\text{Sample weight}} \times 100$$

(AOAC, 1995).

4.2.3 Total ash

Weight crucible and sample were dried in an oven for 6 hours at 200° C.

$$\%\ \text{total ash} = \frac{\text{crucible + sample weight – weight after ashing}}{\text{Weight of sample}} \times 100$$

(AOAC, 1995)

4.2.4 Moisture content

Aluminium dishes were pre-dried 105ºC for 1 hour and weighed to the nearest 0.1 mg on an analytical balance. Biltong slices of 3-5 g were transferred to the aluminium dishes. The samples were then dried at 105ºC for approximately 16 hours and the final weight determined. Moisture content was calculated using the equation:

$$\% \text{ moisture} = \frac{(\text{weight of wet sample} - \text{weight of dry sample}) \times 100}{\text{Weight of a wet sample}}$$

4.2.5 Water activity (rotronic instrument)

Samples were cut and placed in Petri dishes, placed in the rotronic instrument, weighed and results recorded (Rotronic Measurement Solutions, 2015).

Table 4.1: Weights of meat product over four days

Day	Weight (g)
Friday	1419.2
Saturday	543.6
Sunday	467.4
Monday	386.8
Final weight of biltong	**386.8**
Total moisture lost during drying	1032.4/1419.2*100= **72.8%**

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4.3 Results and discussions

4.3.1 Chemical composition of donkey meat

Total ash, total fat, moisture content and water activity of donkey meat were determined. The total fat of the two donkeys varied in the parts sampled, with samples ranging from 2.80% to 44.60% (Table 4.2). Table 4.2 also shows that the total ash ranged from 3.81% to 9.30% in the two donkeys, with the highest moisture content recorded for biltong in donkey 1 at 84.37%. Water activity of donkey meat varied from 0.665 to 0.999. The differences in all these individual chemical factors were, however not statistically significant ($p<0.05$; $p=0.000$). The fresh meat and biltong samples were subjected to chemical analyses using the procedures of Association of Analytical Chemists (AOAC, 1995).

4.3.2 Total fat

The total fat composition of donkey meat ranged from 2.80% to 44.80% in the fresh and dry meat tested. Although statistically insignificant, differences in the total fat content were apparent depending on the meat type and different donkeys sampled. The 2.08% total fat in this study links with the 2.02% measured in a recent donkey meat study in Italy (Polidori *et al.,* 2008).

4.3.3 Total ash

In this investigation the total ash content varied from 3.81% to 9.30% in the meat of the two donkeys sampled. These results are in line with the 5.10% to 8.19% reported in a previous study involving the analysis of donkey meat in Botswana (Aganga *et al.,* 2003). In a study by Polidori *et al.* (2008), the mean content of ash in donkey meat was 1.01% while Libby (1975) measured it at 1.5%. These results are comparatively much lower than those obtained in this study. Disparities in total ash content in different studies may be due to factors such as age differences and differences in weather at the time of analysis.

Table 4.2: Proximate chemical characteristics (means ± s.e.) of the different parts of two donkeys

Donkey part	Chemical factor			
	Total fat (%)	**Total ash (%)**	**Moisture content (%)**	**Water activity**
Donkey 1				
Forequarter	2.80	4.38	23.74	0.998
Hindquarter	12.26	3.81	23.74	0.999
Biltong	15.31	9.30	84.37	0.655
Donkey 2				
Forequarter	28.48	7.31	30.02	0.997
Hindquarter	44.60	5.29	33.5	0.999
Biltong	11.67	5.26	69.84	0.669

4.3.4 Moisture content

As shown in the results in Table 4.2, the moisture content in the donkey meat was between 23.74% and 84.37% and these findings are not in accordance with the moisture content of 70.1% to 77.8% found by Polidori *et al.* (2008) or the moisture content of 68.35% to 74.72% found by Aganga *et al.* (2003) following analysis of donkey carcasses. Even though the above mentioned studies were conducted in the same country, the type of meat and ages of the animals differed, which could account for the vast differences in results.

4.3.5 Water activity

The water activity in the different donkey samples in this study varied from 0.665 to 1. The water activity for the biltong was comparatively lower than that of fresh meat, a result which was not unexpected since drying of food reduces its water activity (Oritz *et al*., 2013). The water activity therefore has a profound effect on the growth of microorganisms (Villa-Rojas *et al*., 2013). Salt (sodium chloride) is one of the most important ingredients used in the manufacture of dried meat products. At high concentrations these solutes have a desiccating effect on both the food and micro-organisms by exerting ionic as well as osmotic effects which inhibit the growth of microorganisms (Ryu *et al*., 1999). Other than suppressing microbial growth, salt exhibits many functions including the reduction of water activity and releasing salt soluble proteins, thus penetrating easily into meats to enhance cure penetration, flavour and showing a pro-oxidant effect (Gou *et al*., 1996). At the same time different solutes may vary in how effectively they inhibit or kill microbial cells.

In conclusion, proximate analysis of the donkey meat and biltong shows that its low in fat and the ranges correspond to previous studies. The rest of the characteristics also do not really differ from what was found by Aganga *et al.* (2003) and Polidori *et al.* (2008).

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Chapter 5

DISCUSSION, RECOMMENDATIONS AND CONCLUSION

5.1 INTRODUCTION

The discipline of food science has reported a great deal of research in the meat industry with the focus mainly on food safety and quality. However, this remains a critical concern world-wide because of the continuing high number of food-borne disease outbreaks which results in economic losses and frequent fatalities (Abee and Kuipers, 2011). Food is considered to be a habitat for microbes that depend on availability of nutrients and general factors such as temperature, moisture availability (generally expressed in terms of water activity (a_w), pH, the oxidation-reduction potential and the nature of the gaseous environment to which the organisms are exposed (Wolter *et al*., 2000). Meat has been described as the most perishable of all important foods because of its moist, nutritious surface which unfortunately makes it a medium conducive to the growth of a wide range of spoilage bacteria. Much research is still required in order to reduce the cases of illness and fatality that occur as a result of food-borne pathogens. This study was based on donkey meat and biltong product; donkey meat is considered a product of very high nutritional value with a high content of unsaturated fatty acids (Polidori *et al.,* 2009). Research should therefore be done on other nutritional values so that farmers in Botswana, who are able to keep donkeys, can do so with the confidence and knowledge that they will benefit from selling them.

5.2 Summary of Chapter 2

This chapter investigated the knowledge, attitudes, beliefs and practices (KABP) of people regarding the consumption of donkey meat and biltong. Data were collected using a questionnaire which was written in Setswana and English to favour both literate and illiterate respondents. Results were analysed using SPSS 20. People typically showed that there is still a stigma attached to the consumption of donkey meat, but there are people who do appreciate it, especially the biltong product. Religion and culture play an integral role in people's choice of meat and these are some of the major reasons for low incidence of donkey meat consumption. It was interesting to note people's willingness to break habits and to try something that has been taboo and still has a lot of stigma attached. It was deduced from the survey that there is great potential for future use of donkey meat and biltong.

5.3 Summary of Chapter 3

Knowledge of the microbiological status of meat is very important in order to show the quality and safety of the meat product. Although pathogenic and potentially pathogenic microorganisms were detected in this present study, the counts of these pathogens were less than 5.0 Log_{10} $CFUg^{-1}$ making it of acceptable sanitary quality as recommended by the Food and Agriculture Organisation (FAO, 1992). The unacceptably high total viable counts of more than 10^6 encountered in the biltong sample of donkey 1 is of public health concern. It is important to note that this figure exceeds the acceptable limit for red meat as set by the FAO (FAO, 1992). Even though some pathogens were not identified due to the method used in this study, there is a need to use other specialised techniques to quantify and identify microbes as this was the first report about donkey meat and its biltong product.

5.4 Summary of Chapter 4

The chemical profile of red meat is defined by water activity, moisture content, ash, pH, fat content and proteins amongst others. In this study only four aspects were analysed from the fresh meat and biltong product. The water activity of the different donkey samples in this study varied from 0.665 to 1. Moisture content on the other hand ranged between 23.74% and 84.37% and these findings were not in accordance with those from related previous studies. The total fat content of donkey meat ranged from 2.80% to 44.80% in the fresh and dry meat tested. Though statistically insignificant, differences in the total fat content were apparent depending on the meat type and different donkeys sampled. This investigation found the total ash content in the meats of the two donkeys sampled, varied from 3.81% to 9.30%.

In conclusion, no grounds were found for negative attitude towards donkey meat (both dry and fresh) as the microbial counts were low and some were not detected at all. The research indicates that the attitude of human beings towards donkey meat is baseless and that people do not have proper knowledge about the product. The research has shown that donkey meat is safe for consumption because of low microbial counts and low fat content.

5.5 Recommendations

- Donkeys should be taken care of in the same way that cattle and goats are cared for as they show great potential economically and health-wise.
- People should change their attitudes and practices towards donkeys and stop using them only for heavy domestic duties such as carrying water for long distances. Rather, if there is potential for donkeys being a source of food, this should be studied thoroughly.
- More research should be done on the nutritional value of donkey meat and also donkey milk.
- Slaughter houses for donkeys are needed to improve quality and reduce contamination that may occur as a result of traditional slaughter methods.
- More education regarding the great business potential is needed.
- Donkey meat retailers should start taking the business seriously by building proper structures like butcheries.

5.6 References

Abee, T. and Kuipers, O.P. (2011). Understanding microbial behaviour within and outside the host to improve food functionality and safety. *Food Biotechnology*, 22:133–135.

Food and Agriculture Organisation (FAO). (1992). *Guidelines for slaughtering meat cutting and further processing.*

Polidori, P., Carallucci, C., Beghelli, D. and Vincenzetti, S. (2009). Physical and chemical characteristics of donkey meat from Martina Franca breed. *Meat Science*, 82(4):469-471.

Wolter, H., Viljoen, C.B. and Laing, E. (2000). Isolation and identification of yeasts associated with intermediate moisture meats. *Food Biotechnology,* 38(1): 69-75.

APPENDIX

Slaughtering process at the farm

Step 1: Selection of the two donkeys

Step 2: Stunning

Step 3: Bleeding

Step 4: Cutting off the head

Step 5: Carcass split laterally and forequarters and hindquarters cut out

Step 6: Samples from both sections cut placed into well labelled sterile plastic bags and stored in cooler box with ice packs. Samples from the two forequarters were combined to make a composite sample and the process repeated for the hindquarter samples.

Step 1

step 2

Step 3

Step 4

Step 5

step 6

Analysis at the laboratories

Example of attributes which can be assessed in donkey meat

Colour	**Cherry red to dark red**
Liking of aroma (odor)	Natural to pronounced off flavour
Strength of aroma	Strong to weak (odourless)
(Odour)	
Liking of flavour	Very good to tasteless
Strength of flavour	Strong to weak
Juiciness	Very moist to very dry
Tenderness	Very tender to very tough
Firmness	Very firm to very soft
Overall	Excellent to very poor

(Aganga *et al*., 2003)

Questionnaire used for the study

National Food Technology Research Centre, Kanye in collaboration with Central University of Technology, Bloemfontein, Free Srate (S.A)

KNOWLEDGE, ATTITUDE, BELIVE AND PRACTICE OF BATSWANA REGARDING DONKEY MEAT (BILTONG)

QUESTIONNAIRE FOR DONKEY BILTONG KABP SURVEY JULY-AUGUST 2011

Start Time: ____________________

Interviewer Introduces Herself/Himself and the organisation - NFTRC

Interviewer Name ____________________

Purpose of Survey

Donkey (Equus asinus) has stimulated a lot of scientific and consumer attention as an alternative to beef. As a source of food, attention has only been limited to its milk which is said to resemble human milk in composition. Recently, donkey meat has brought attention and gained appreciation as an alternative red meat. In Botswana it has been largely preferred as dried meat (biltong) by the consumers. The donkey meat as a trade has been reported in some parts of Botswana. Hence, this survey seeks to establish the donkey meat business and its consumer utilisation, especially the preferred products such as biltong. The survey will be conducted in an area where the donkey meat consumption and business is a new concept as well as area where the use of donkey meat has a long history. The expectation is that both consumers and donkey meat processors would partake in this survey for better understanding of knowledge, attitude, believe and practice of Batswana regarding donkey meat (especially biltong). This information would assist in identifying areas where technical support and education can be rendered, align product development activities to the recent practices as well as industry needs.
The meat industry, especially beef, is regarded as the largest in the food sector. However, the commercial potential of the meat types, such as donkey meat, remain to be tapped. In order for the Masters Project and NFTRC to respond better to the challenges facing commercial utilisation of donkey meat an assessment of people's perception about the meat is necessary as this is a new industry in Botswana.

Consent

You are totally at liberty to participate in this survey, and to answer or refuse to answer any of the questions. Do please insure that the information provided is correct to the best of your knowledge.

If you have any queries about the questionnaire please do not hesitate to contact the Botswana National Food Technology Reseach Centre Personnel. Alternatively, you can call Botswana National Food Technology Research Centre on (267) 544 5520/544 0441

Confidentiallity

All personal information gathered in this survey shall be kept strictly confidential Names and addresses of respondents shall not be included divudge.

The survey consists of 4 pages and should take no longer than 60 minutes to complete.

QUESTIONNAIRE IDENTIFICATION NUMBER [ ]

SECTION A: BACKGROUND INFORMATION OF THE RESPONDENT

A1	**Name of respondent**							
A2	**Contact Address**							
A3	**Contact Telephone number**							
A4	**Age of respondent**	1)<18	2)18-29	3)30-39	4)40-49	5)50--64	6)>64	
A5	**Gender**	*1) Male*	*2) Female*					
A6	**Racial Origin**	1) Black	2) White	*3) Asian*	4) Other (specify)			
A7	**Nationality**	1) Motswana	2) Indian	3) Chinese	4) Koren	5) Zimbabwean	6) other (specify)	
A8	**Religion**	1) Christian	*2) Muslim*	3) Hindu	4) none	5) other (specify)		
A9	**Educational level**	***1)No formal education***	***2)Primary***	***3)Junior secondary***	***4)Secondary***	***5)Tertiary***		

SECTION B: CONSUMER PREFERENCE OF DONKEY MEAT

B1	**Do you keep livestock?**	1) Yes	2) No				
B2	**What type/kind of livestock do you keep?**	1) Cattle	2) Sheep	3) Goats	4) Donkeys	5) Other(specify)	
B3	**What is the reason behind keeping them?**	1) Commercial	2) Consumption	3) Both	4) Other (specify)		
B4	**What is your most prefered meat?**	1) Beef	2) Chicken	3) Mutton	4) Game	5) Other(specify)	
B5	**Do you eat Donkey meat?**	1) Yes, most of the time	2) Occasionally	3) Not at all			
B6	**Which donkey meat products do you prefer?**	1) Fresh meat	2) sausages	3) Biltong	4) I do not prefer any		
B7	**What do you like about your prefered donkey meat product?**	1) Smell	2) Texture	3) Taste	4) Other (specify		

B8 **Would you buy donkey biltong if available in your meat store?** 1) Yes 2) No 3) May be

B9 ***How would you eat your donkey biltong?*** 1) As breakfast 2) For lunch 3) As a snack between meals 4) For supper

B10 **How do you cook it?** 1) Boil 2) Fry 3) Grill 3) Pound/swaa 4) Other (specify)

B11 **Do you think donkey biltong should be commercially sold?** 1) Yes 2) No

B12 **Is there a butchery selling selling donkey meat in your area?** 1) Yes 2) No

B13 **Does your religious belief prohibit you from eating donkey meat?** 1) Yes 2) No

B14 **Do you think donkey meat could be an alternative to your most prefered meat?** 1) Yes 2) No

B15 **When do you slaugher donkey?** 1) At weddings 2) At Funerals 3) Other (specify)

B16 **What type of donkey do you slaughte** 1) Jenny 2) Jack 3) Foal 4) Old donkey 5) Injured donkey 6) Other (specify)

B17 **Donkey meat can solve some medical conditions.** 1) Agree 2) Disagree 3) Not sure

B18 **Do you think there should be an abattoir for donkeys?** 1) Yes 2) No

SECTION C: MEAT PROCESSORS-LEVEL OF OPERATION

C1 **How many donkeys do you slaughter per day?** 1) 1-5 2) 6-10 3)>10

C2 **Where do you slaughter your donkeys?** 1) At the farm 2) At the slaughter slab 3) Other (specify)

C3 **How much donkey meat is sold as fresh meat and how much is used for other products** 1)% sold as fresh meat 2)% sold for processing

C4 **What other meat products do you produce and sell apart from fresh meat?**

1) Mince-meat 2)Salami 3 Biltong 4) Raw sausages (boerewors) 5 (other (specify)

C5 **What happens to the other parts of the donkey e.g. head, tail, intestines, etc?** 1) Thrown away 2) Sold as dogs fee 3) Other (specify)

C6 **Whom do you sell your products to?** 1) Individuals 2) Wholesalers 3) Supermarkets 4) Other (specify)

C8 **What is the price of fresh meat per kilogram (kg)?**

C7 **What are the average monthly sales of donkey meat?**

SECTION D: FOOD SAFETY AND PRODUCT DEVELOPMENT

D1 **Have the personnel responsible for processing gone through meat processing training?** 1)Yes 2) No

D2 **Indicate with a tick in which of the listed areas your employees have received training**

Area of Training	Staff trained
1) Food safety and hygiene (handling)	
2) Food processing skills	
3) Business management skills	
4) Food regulations	
5) Government regulations	
6) Catering skills	

D3 **When was the last training done?** 1) Never trained 2) < 2 years 3) 2-5years 4) over 5 years 5) cant remember

SECTION E: FOOD HANDLERS

E1 **How often do you wash your hands during working periods?**

period of washing hands	Respon se
1) Once when I start work	
2) Every time before handling meat	
3) Every time after handling meat	
4) Every time I leave for home	
5) Any time I ffeel my hands are dirty	
6) Other (specify)	

E2 **Do you think its important to wash hands before handling me** 1) Yes *2) No*

E3 **Do you wash hands after using the toilet?** 1) Yes 2) Sometimes 3) No

E4 ***How many times a day do you clean equipments?*** 1) Once 2) Before and after use 3) Only after use 4) Other (specify)

SECTION F: MEAT LEGISLATION

F1 **Which of the following Government Policies and Regulations are you conversant with and how has it affected your business?**

Policy/regulation	Know	How it has affected you

1) Food Control Act		
2) Meat Act		
3)Trade Act		
4) Land policy		
5)Town and Regional Planning regulations		
6) SMME policy		
7) Industrial development policy		
8) Other; specify		

SECTION G: OPINION ON PERFORMANCE OF THE BUSINESS

G1 **On a scale of 1 to 4, how would you rate the performance of your business?** 1) Excellent 2) Vey good 3) Good 4) Below average

G2 **In your opinion what are the challenges faced by this meat industry?**

a) ____

b) ____

c) ____

G3 **What actions are you taking to address these challenges?**

a) ____

b) ____

c) ____

G4 **How do you think the adopted/suggested intervention could be helpful?**

a) ____

b) ____

c) ____

SECTION H: MEAT INSPECTORS

H1 **Do you think it's a good idea to eat donkey meat?** 1) Yes 2) No

H2 **Donkey Meat should be inspected like other types of meat** 1) Agree 2) Disagree 3) Not sure

H3 **What are the diseases assoscited with donkey meat?**

a) ____

b) ____

c) ____

H4 **How do these diseases in H3 affect human health?**

a) ____

b) ____

c) ____

H5 **What is your role in the donkey meat industry?**

a) ____

b) ____

c) ____

H6 **General comments by the respondent**

a) ____

b) ____

c) ____

H7 General comments by the interviewer

a) ______________________________

b) ______________________________

c) ______________________________

SECTION I: LIST OF EQUIPMENT AND FACILITIES

Below is a list of meat processing equipment in the business. Tick as appropriate

I1	**Display Fridge**	1) Yes	2) No
I2	**Freezer**	1) Yes	2) No
I3	**Cold transport/refrigerated transport**	1) Yes	2) No
I4	**Band saw**	1) Yes	2) No
I5	**Meat Trough/Containers**	1) Yes	2) No
I6	**Bowl Cutter**	1) Yes	2) No
I7	**Stainless steel tables**	1) Yes	2) No
I8	**Sausage filler**	1) Yes	2) No
I9	**Mincer**	1) Yes	2) No
I10	**Scale (weighing balance)**	1) Yes	2) No

SECTION J: FOOD SAFETY FACILITIES

JWS	**Wash room**	1) Yes	2) No
JHWS	**Hand washing sation (sink and soap)**	1) Yes	2) No
JDHP	**Dryer/hand paper towels**	1)Yes	2) No
JPC	**Protective clothing**	1) Yes	2) No
JBSS	**Boots/Safety shoes**	1) Yes	2) No
JHC	**Hairnets/caps**	1) Yes	2) NO

Time Ended ______________