DEVELOPMENT OF BEEF SAUSAGES THROUGH BOVINE BLOOD UTILIZATION AS FAT REPLACER TO REDUCE SLAUGHTERHOUSE BY-PRODUCT LOSSES

By

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND TECHNOLOGY OF THE UNIVERSITY OF NAIROBI.

DEPARTMENT OF FOOD SCIENCE, NUTRITION AND TECHNOLOGY

November, 2016.

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This thesis is my original work and has not been presented for a degree in any other university.

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DEPARTMENT: FOOD SCIENCE NUTRITION AND TECHNOLOGY
COURSE NAME: M.Sc. FOOD SCIENCE AND TECHNOLOGY
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Dedication

This thesis is dedicated to my family for their support, care and encouragement throughout the entire school time.

Acknowledgements

I would like to acknowledge and thank the Federal ministry of Education and Research, Germany (BMBF) for their sponsorship of this study through the project, RELOAD (Reducing losses adding value).

I greatly thank my supervisors, Dr. Catherine N. Kunyanga and Prof. Jasper K. Imungi for their guidance, patience, unending encouragement and invaluable inputs that led to the successful completion of this work.

I wish to express my gratitude and appreciation to all individuals who participated in this study in any way for their unforgettable inputs that highly contributed to the success of this study. Special mention to Mister Josphat Njenga who continuously assisted me in a huge way on how to write scientific works especially when I joined this program as a fresh student. His views were really significant for the eventual being of this study.

My sincere appreciation also goes to the pilot plant, food chemistry and microbiology laboratories staff, Department of Food Science, Nutrition and Technology for their support and assistance throughout the analysis period.

God bless you all.

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Abbreviations and acronyms

MOLD	Ministry Of Livestock Development
FAO	Food and Agricultural Organization of the United Nations
STTP	Sodium Tri polyphosphate
MSG	Mono Sodium Glutamate
SPSS	Statistical Package for the Social Sciences
KEBS	Kenya Bureau of Standards
TVC	Total Viable Counts
ANOVA	Analysis of Variance
CFU	Colony forming units
AOAC	Association of Official Analytical Chemists
XLD	Xylose lysine desoxycholate
BGA	Brilliant Green Agar
VRBG	Violet red bile glucose agar
mCCD	Modified charcoal-cefoperazone-deoxycholate agar
PV	Peroxide vale
meq/Kg	milliequivalent per kilogram

GENERAL ABSTRACT

Slaughterhouse by-product losses after slaying of animals are major problem in Kenya at present. Many slaughterhouse operators and meat traders either lack proper preservation techniques or lack technologies on how to incorporate by-products into commercial products. Value addition is one of the ways that losses can be reduced since new products can greatly improve uptake by-products. This leads to increased income for operators, improved food security, cleaner environment and more products in the market. Most of the lost by-products include blood and internal organs including offals. There is limited information about how different communities utilize various by-products despite the modern processing technological advancement. Blood has been identified as one of the most important by products after slaughter operations of food animals in Kenya. This study was therefore designed to assess how blood is utilized by different communities in the Kenyan pastoral regions with the aim of potential commercialization. The counties of Turkana, Garissa, Kajiado and Marsabit were purposively selected because they are the largest livestock producers among the pastoral Counties. Data was collected using structured questionnaires, key informant interviews and focus group discussions. Visual observations in selected slaughterhouses were also employed. The by-products that were used for food were prepared for consumption in different ways by the different communities. The utilized portions however especially that of blood was too small leading to high losses. For instance about 97% of blood in all the study areas went to waste. The study established that the by-products were not effectively utilized leading to high post slaughter wastage. The inedible by-products such as the hides were sold to tanneries, the horns were used for ornamentals, skins as dry-skin containers and hooves were just cast away.

Blood was shown to be the major by-product lost in most slaughter houses and was therefore utilized in value added product development. Sausages were formulated in which fat was partially substituted with fresh bovine blood as a replacer. Fat was substituted with blood at the rate of 5% from the conventional sausage which contained 20% fat down to 0%. The sausages were evaluated for sensory properties, analysed for proximate composition, texture, cooking loss, water holding capacity, iron contents, fat oxidation as peroxide value and microbial profile and cost benefits. All the analysis for the sausages concluded that the acceptable blood/fat ratio in percentage points is 10:10. The sausages showed significant fat reductions from the full fat ones at $P<0.05$, protein increased on the other hand from 14% to 15.9%. Iron increased significantly $P<0.05$ from 236mg/kg to 576mg/kg with the blood increase such that a 50g sausage at10% (575.6mg/kg) replacement can supply the iron RDA for an adult man. The cooking losses, water holding capacity were not significantly affected by blood use. Use of blood as fat replacer saves the processor up to Kenya shillings 25 per kilo which translates to 9% decrease in cost of production. The sausages were also chemically stable through-out the 14 days at 18.3meq/kg for full fat sausages well below the recommended 20 meq/kg. The microbial count for the 14 day storage period showed levels that are within the KEBS standards. *Salmonella spp, Listeria monocytogens, Campylobacter and Escherichia coli were all absent.* The highest counts for *Staphylococcus aureus, Clostridium Perfringens,* and TVC were 1.16 $\log_{10}$cfu/g, 1.47 $\log_{10}$cfu/g and 5.27 $\log_{10}$cfu/g respectively. These were lower than the KEBS recommended values of below 6.0 $\log_{10}$cfu/g, 2.0 $\log_{10}$cfu/g and 2.0 $\log_{10}$cfu/g for TVC, *staphylococcus aureus and Clostridium perfringens* respectively. There is need to pursue other potential uses of blood and other by-products to ensure no losses occur after slaughter.

Key words: *Bovine Blood, By-products, Loss reduction, Pastoral, Production cost, Slaughterhouse losses, Value addition.*

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background information.

Livestock sector in Kenya is one of the most important sectors in more multifaceted perspective. The livelihoods of small scale livestock producers and pastoral communities are characterized by low incomes and food insecurity. Kenya has an estimated livestock resource of 14.1 million indigenous cattle, 3.4 million exotic cattle, 17.1 million sheep, 27.7 million goats, 3.0 million camels and 1.8 million donkeys (KNBS, 2010).Consequently this contributes 12.5% of the national GDP and 47% of the agricultural based GDP.

The food value Chain comprise all activities required to bring food commodities to the consumer, including agricultural production at the farm level, transport, storage, processing, marketing, distribution and consumption (Gomez *et al.*, 2011). The meat value chain is very important area of focus if the losses are to be reduced or minimized.

In Kenya, about two- thirds of the red meat is produced in the arid and semi-arid lands (ASALs) under pastoral production system. Approximately 96 % of beef cattle in Kenya are kept by subsistence farmers and pastoralists distributed based on rainfall patterns. About 67% of the national meat production goes through formal slaughter process, while the rest uses informal channels. Cattle account for approximately 77 % of Kenya's ruminant off-take for slaughter (Behnke and Muthami, 2011).These facts emphasises on the need to harness and take up the reduction meat post-harvest losses seriously.

By products vary from those that can be utilised elsewhere to the wastes that may not be used otherwise. The specific amounts of generated waste vary for each type of animal (Sielaff, 1996; Grosse, 1984) and the specific waste index ranges from 0.1 (sheep) to 0.87 (calf).If animal by-products are not effectively utilised, of course a valuable source of potential

revenue is lost, and the added and increasing cost of disposal of these products is incurred by the industry. Today the cost of live animal often exceeds the selling price of its carcass; therefore, the value of the by-products must pay for the expense of slaughter and generate the profit for the meat slaughtering operations. According to Bowater and Gustafson, 1988, 15% of slaughterhouse income could come from by-products. In the last several years, the value of animal by- products relative to their value of the live animal has declined (Hedrick *et al*., 1994) because of technological progress in producing competitive products from non-animal sources. Blood has however been applied either as whole or part of in processing food products. Locally in Kenya, *mutura,* a local sausage which is very popular has blood as a major ingredient. Some Italian and Mexican blood sausages are a delicacy in respective countries and consume high amounts of blood. Blood plasma is widely used as a binder across the world according to Ofori and Hsieh (2012).

Non utilisation of animal by-products would create a major aesthetic and catastrophic public health problem. The effective utilization of animal by-products treatment plants undoubtedly has had major influences in upgrading public health in the recent past. This project was tailored to find out the by-product loss loopholes in the slaughterhouses and address them by developing acceptable products using the generally wasted animal edible parts.

1.2 Problem Statement

Post slaughter losses account for huge losses of meat in the ASALs. Generally 48.7 percent of food stuffs are lost between postharvest handling and processing and packaging (FAO, 2011). 29.7 percent of meat specifically goes to waste during the postharvest period (FAO, 2011). Blood is one of the by-products which is generally discarded and has been an environmental menace to the authorities and population at large. Among the most important reasons for high losses along the meat value chain is that meat and meat products are highly perishable and

unless appropriately handled, quality and physical losses are high. The underlying reasons for high losses are lack of appropriate meat processing technologies, storage and packaging facilities (MOLD, 2010; Lewa, 2010).

In small and medium size slaughterhouses and abattoirs, problems exist with regard to lack of technical facilities and respective technical skills for processing, packaging and storage (Abegaz, 2009). In sausage processing, fat plays an important role yet it costs as much as the meat itself. Replacing the fat with blood will enable the processor save during production of the designed sausages. This will present a positive aspect especially because the cost of production has always been a big challenge for Kenyan manufacturers.

Another important dimension is lack of capacity for new product development and diversification as well as lack of information on postharvest losses along the value chain (Pavanello, 2010). The stakeholders who suffer the losses especially in the ASALs region in Kenya will find a reprieve in new ways of utilising their meat by-products after slaughter. Cheap, simple, sustainable and easily adoptable technologies will be transmitted to the stakeholders with the aim of developing new acceptable products which consequently lowers the losses through enhanced by-products intake.

1.3 Justification

Postharvest losses alone contribute about 29.7 percent in meat loss (FAO 2011), along the harvest, storage, transportation, processing and retail. Any endeavor to improve existing technologies or inventing some is always welcome. Technologies that utilize by products or increase their uptake will not only improve farmers' earnings, but will hugely lead to reduced meat postharvest losses.

Reducing postharvest losses may effectively and sustainably increase the volume of available food, without encountering adverse effects on the regional ecological and economic situation (Hensel, 2009).

Innovation plays a key role in economic growth and requires investment to generate new knowledge in a usable format (Henchion and McIntyre, 2010). Innovations in processing and preservation techniques create a potential platform for value addition. That coupled with conventional meat processing techniques will further improve the products for small-scale commercialisation. Successful innovations are those driven by the consumer expectations and perceptions.

Developed meat processing technologies haven't being adequately adopted by stakeholders in the beef value chain and therefore low-cost traditional meat processing and packaging techniques will improve household food security. These techniques have not been successfully adopted; the low adoption rate of such processing and preservation technologies and the poor quality of locally processed products are the main challenges to value addition along meat value chains. This would provide a good income opportunity to households especially in ASAL regions. Investment and revamping meat distribution systems (especially informal channels) will enhance value addition (Abegaz, 2009).

1.4 Study objectives

The main objective of the study was to develop sausages using blood as fat replacer to facilitate utilization of blood as a by-product from slaughterhouses.

Specific objectives:

1. To characterize the slaughter by-products utilization in the pastoral areas.
2. To formulate beef sausages by replacing fat with blood from slaughterhouses.
3. To assess sensory properties of the sausages.
4. To determine the physico-chemical properties and production line of the sausages.
5. To determine the shelf life of the sausages.

1.5 Hypothesis

1. Slaughter by-products can be improved for commercial processing.
2. Blood can be effectively used as fat replacer in sausages processing.
3. Blood can be used to process sensory acceptable sausages.
4. Blood sausages can be physico-chemically stable.
5. Blood sausages can have a stable shelf life.

CHAPTER TWO: LITERATURE REVIEW

2.1 Meat value chain in Kenya

Meat value chain constitutes several different stakeholders who are interdependent. The most commonly consumed meat is the beef. Sheep and goat meat are largely luxurious commodities and are hardly consumed at home except by a very small group of high-income households (Gamba, 2005). According to Farmer and Mbwika 2012, the urban middle class accounts for the large majority of meat consumed. A study by Muthee (2006) puts the national pa capita meat consumption at 10.8 kilograms per person. Mombasa and Nairobi were the highest ranked with 15 kilograms and 18.25 kilograms per person respectively. Meat value chain constitutes several different stakeholders who are interdependent. Top among the stakeholders are the producers who in Kenya are the livestock keepers. Traders to whom farmers sell to are also prominent in the value chain (Muthee, 2006). Many traders act as the middleman between the farmer and the processor at the slaughterhouses in various counties. Slaughterhouses are classified according to the available facilities and the quantity of animal slaughtered. Low class and middle class butcheries sell low grade meat and by-products while high end butcheries sell high grade meat and in some cases value added products (Muthee, 2006). Different markets for different meat qualities can be clearly outlined.

High end market is characterized by high quality meat from well finished animals. It consists of high end butcheries and supermarkets (Gamba, 2005). The animals are mostly from well-maintained ranches and where the meat is cut into prime cuts. High earning consumers are the major buyers of such meat with high propensity to buy value added products like beef sausage and tenderized meat. High-end butcheries charge a premium for choice beef cuts based on the source of supply, which are primarily ranches (Gamba, 2005).

Middle market is mostly patronized by medium income earners. The market offers meat on bone, boneless steaks, liver and tripe. The retail outlets in this market have a deep freezer where meat is stored overnight, but have limited refrigeration (Farmer and Mbwika, 2012). Low end market basically forms the largest share of the meat market in Kenya. They mostly sell meat on bone, liver and tripe. The low-class butcheries offer beef on bone, which is usually openly displayed without refrigeration (Farmer, 2012).

Livestock movement especially in the ASALs follow certain defined routes to various destinations. Nairobi and Mombasa form the core destinations for the livestock.

Other very important stakeholders are the meat industry regulators. Meat inspectors and public health officials ensure the safety and health of the meat consumers. Located at the slaughterhouses and abattoirs, they thoroughly inspect the carcass before every edible part is allowed to go to the market. On the other end, veterinary officers ensure that the animals are in good health even as they trespass the counties. Travel permits and quarantine enforcements where necessary are a jurisdiction of the veterinary department. The veterinary and public health workers all come from the government to ensure impartiality in their operations

The value chains are primarily geared towards the domestic market, which consumes approximately 99 per cent of domestic production. Small volumes of meat are exported by the newly re-operationalized Kenya Meat Commission (KMC) and private meat exporters who use KMC's facilities for a fee. Choice Meats (a subsidiary of Farmers Choice), and other individual ranchers export small volumes of live animals to Mauritius, Burundi (mainly goats), and Uganda (Farmer and Mbwika, 2012).

2.2 Post harvest losses along the meat value chain

Post-harvest losses generally account for 29.7% of meat harvested (FAO, 2011). Losses occur at various stages after harvest and interestingly, 76.3% is lost between production to processing and packaging (FAO, 2011). Any attempt to arrest any type of a loss in the chain there is hugely welcome. Optimization of sinews and blood into important commercial sausage processing components is one of such attempts. With good binding characteristics, sinews can provide a more natural, easily available and accessible, cheap, and more significantly provide an alternative ingredient and reduce a potential meat loss.Lack of monitoring input quality and processing parameters, Poor equipment, skills and lack of capacity for new product development have been cited as the factors which lead to such amount of wastage at processing.

2.3 By-products from slaughterhouses

Slaughter by-products are classified into two; edibles and inedible. They all serve different purposes and function depending on a multifaceted school of thought. Cultural, educational, economic and social status of the people within a locality impact directly the way the products are utilised. The common edible by products are; liver, heart, tongue, tripe brains, oxtail, trimmings, intestines, testicles, blood and spleen. On the other hand the most common inedible by products are; bones, hooves and hides and skins. Different by products have been utilised in many different ways in different parts of the world.

Animal blood has a high level of protein and heme iron, and is an important edible by-product (Wan *et al.,* 2002).In Europe, animal blood has long been used to make blood sausages, blood pudding, biscuits and bread. In Asia, it is used in blood curd, blood cake and blood pudding (Ghost, 2001). It is also used for non-food items such as fertilizer, feedstuffs and binder. Blood is used in food as an emulsifier, a stabilizer, a clarifier, a colour additive,

and as a nutritional component (Silva and Silvestre, 2003).Blood plasma has ability to form a gel, because it contains 60.0% albumin (Silva and Silvestre 2003).Blood plasma has an excellent foaming capacity as well (Del *et al*., 2008).

Glands and organs from the slaughterhouse are utilised in diverse ways. Those used as human foods include the brain, heart, kidneys, liver, lungs and spleen (Jayathilakan *et al*., 2011). Others are the tongue, the bovine pancreas and udder, the rumen, reticulum, omasum and absomasum of sheep and cattle, and the testes and thymus of sheep (Liu, 2002). Liver is the most widely used edible by product. It is used in many processed meats, such as liver sausage and liver paste (Devatkal *et al.,* 2004). In America and Europe, calf and lamb lungs are mainly used to make stuffing and some types of sausages and processed meats (Darine *et al.,* 2010).

The cholesterol in the brain has been used as an emulsifier in cosmetics (Ejike and Emmanuel, 2009). The most important use of the intestines is as sausage casings (Bhaskar *et al*., 2007). However, intestines form a very important delicacy in the menu of most domestic settings in Kenya. In Nairobi County though, they are significantly used as pet food since the residents are of different class with the high class who keep pets viewing them as more of a waste than human food. Fat although attached in carcass and many by-products, is also a very important component. Traditionally, tallow was used for deep frying (Weiss, 1983). Presently due to health concerns, its use for frying has noticeable decreased. Tallow has in some cases been used to process margarines and shortenings (Ghotra *et al.,* 2002).

Hides and skins have found many uses both in the traditional settings and in the modern world manufacturing. The hides represent a remarkable portion of the weight of the live animal, from 4% to as much as 11% e.g. cattle: 5.1–8.5%, average: 7.0%; and sheep: 11.0–11.7% (Jayathilakan *et al*., 2011). Traditional musical instruments like the drums, packaging

equipments, seats and body covers were made from hides and skins. Other examples of finished products from the hides of cattle and from sheep pelts, are leather shoes and bags, rawhide, athletic equipment, reformed sausage casing and cosmetic products, sausage skins, edible gelatine and glue (Benjakul *et al.,* 2009). In more advanced cases, gelatin has been extracted from hides through hydrolysis of collagen to gelatin. Gelatin itself has many applications in several industries. For example gelatin extracted from animal skins and hides can be used for food (Choa *et al*., 2005). Gelatin is added to a wide range of foods, as well as forming a major ingredient in jellies and aspic (Jamilah and Harvinder, 2002).The marrow in the bones which account for 4.0-6.0% the carcass weight (West and Shaw, 1975), is also used as food.

2.4 Sausage production from meat

Sausage production in Kenya is predominantly carried out by formal manufacturers. These are the well-established meat processing plants that process pork, beef and chicken. Some processors utilize more than one type of meat. The most known sausage processors in Kenya are Kenya Meat Commission (KMC), Farmers choice, Quality Meat Products (QMP), alfa fine foods, Kenchick and Meatons among others. Recently, many backyard and cottage industries have come up and have added another dimension in sausage production. The informal processors however bring with them the quality and hygiene challenges.

Sausages processed in Kenya are either fresh or pre-cooked, with the fresh sausage taking the large chunk. Processors across the board employ almost the same technical knowledge in sausage formulation. The seasoning and curing mix is made up of more less the same combination albeit on a varied preferred quantities.

2.4.1 Blood utilization

Blood in Kenya lack proper utilisation, the reason much of it from the slaughterhouses has always been left to go to the drain. Despite having a lot of nutritional value, blood can pose very unpleasant health and environmental hazard. This would constitute a larger harm caused by improper use or disposal of slaughter by-products. Non-utilization of animal by-products in a proper way may create major aesthetic and catastrophic health problems (Jayathilakan *et al.,* 2011). Waste disposal and by-product management in food processing industry pose problems in the areas of environmental protection and sustainability (Russ and Pittroff, 2004).

Blood is usually sterile in a healthy animal. It contains high protein content (17.0), with a significantly good balance of amino acids (Jayathilakan *et al*., 2011).

2.4.2 Blood based products

There are myriads of blood based products all around the world from human food to animal feeds. Locally in Kenya, blood forms a very important component of the African sausage commonly known as *mutura*. The challenge with the local African sausages or blood laced products is lack of proper technological incorporation. Consequently, that threatens the product's safety and keeping quality since no proper quantities have been defined for inclusion.

Morcilla de Burgos is a Spanish blood based sausage made by mixing blood and some other ingredients in presence of select spices (Diez *et al*., 2008). There is a huge potential in blood utilization either as whole or a component of it. It can be has long been used to make blood sausages, blood pudding, biscuits and bread. In Asia for instance, it is used in blood curd, blood cake and blood pudding (Ghost, 2001). It is also used for non-food items such as fertilizer, feedstuffs and binder. Blood is used in food as an emulsifier, a stabilizer, a clarifier, a colour additive, and as a nutritional component (Silva and Silvestre, 2003).Blood plasma

has ability to form a gel, because it contains 60.0% albumin (Silva and Silvestre, 2003).Blood plasma has an excellent foaming capacity too (Del *et al*., 2008).

2.4.3 Sausage binders.

Binders are proteinaceous agents used to improve the bind of meat and fat when making sausage, improving fat and moisture retention. They are principally applied for their functional (binding, extending, emulsifying) attributes rather than for their nutritional fortifications. They are mostly used when mixing sausage to improve bind characteristics. Precisely binders are used to improve sausage flavor, stability, moisture retention or sliding characteristics (Hippisley *et al*., 1996).

The most common sausage binders applied are soy protein concentrate, soy dairy blend, rusk, starch and non-fat dried skimmed milk. Often in commercial cases, the calcium-reduced form of skim milk powder is used as calcium is said to interfere with protein solubility. The soy protein concentrate which is usually available as coarse granules, powder or grits is used in emulsion type sausages.

2.4.4 Soy protein concentrates.

Soy protein concentrate is about 70% soy protein and is basically defatted soy flour without the water-soluble carbohydrates. It is made by simply removing part of the carbohydrates (soluble sugars) from de-hulled and defatted soybeans (Singh *et al*., 2008). Soy protein concentrate retains most of the fiber of the original soybean. It is widely used as functional or nutritional ingredient in a wide variety of food products, mainly in baked foods, breakfast cereals, and in some meat products. In coarsely chopped meats (such as meat patties, sausages, meatballs, chili, Salisbury steaks, pizza topping, and meat sauces) textured soy protein concentrates and soy flours are the preferred ingredients to obtain the desired texture (Jindal and Bawa, 1988). Soy protein concentrate is used in meat and poultry products to

increase water and fat retention and to improve nutritional values (more protein, less fat). Soy protein play a significant role in the modification of functional properties such as emulsification, water and fat binding capacity and textural properties (Gujral *et al*., 2001).

Generally, the areas with the greatest potential are where casein and non-fat dry milk are used, such as emulsion-type meat products, bakery products, nutritional powder drinks, and soup bases (Onayemi and Lorenz, 1978). Baby foods, cereals, dry food mixes, milk replacers, pet foods, and snacks are just a few more examples where powdered soy protein concentrates could also be used.

2.4.5 Non- fat dried skim milk.

This is basically applied in powder form as it is dried. According to the American dairy products institute (ADPI), it is manufactured by removing water from pasteurized skim milk. It contains 5% or less moisture (by weight) and 1.5% or less milk fat (by weight). The medium heat treated non- fat dry milk is the best recommended for use in meat products. The United States Department of Agriculture (USDA) states that such powder should undergo pre-heating at 160-175°F for 20 minutes, should have 1.51-5.99 mg/gm of un-denatured whey protein nitrogen. Milk powder helps cooked sausages retain moisture. It assists in forming irreversible gels upon heating that holds water and fat.

2.4.6 Rusk.

A rusk is hard, dry biscuit ortwice-baked bread. Rusks can be prepared differently and varies from region to region. Some rusks can be flavoured as is the case with the Swedish skorpor. In South Africa for instance, rusks were a form of bread preservation from the 1960's. Locals dunk rusks in tea, coffee or milk before taking them as breakfast snack. Though originally made from stale bread, now called bread-rusk, a yeast-free variety called simply rusk is now more commonly used.

According to Ripon select foods, a British food ingredient manufacturer, rusks are used for various purposes with key among them being a binding agent in sausages and other compound meat products. It also serves as stuffing materials in the sausage. Rusks can absorb as much three times their weight thus making them a very useful component during sausage emulsion making.

2.4.7 Starch.

Starch is basically a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. It is the most common carbohydrate in human diets and is contained in large amounts in such staple foods as potatoes, wheat, maize (corn), rice, and cassava. Starch consists of two molecules; amylose and amylopectin which make up 20-25% and 75-80% respectively by weight (Brown and Poon, 2005). Starch becomes soluble in water when heated. The granules swell and burst, the semi-crystalline structure is lost and the smaller amylose molecules start leaching out of the granule, forming a network that holds water and increasing the mixture's viscosity. This enables the starch in the sausage emulsion to bind the inherent water especially during and after cooking.

2.4.8 Sausage types

Different regions and countries have different ways of naming and coding their sausage types.Various metrics such as types of ingredients, consistency, and preparation are used.

1. **Cooked sausages** are made with fresh meats, and then fully cooked. They are either eaten immediately after cooking or must be refrigerated. Examples include dogs and liver sausage.

2. **Cooked smoked sausages** are cooked and then smoked or smoke-cooked. They are eaten hot or cold, but need to be refrigerated. Examples include kielbasa and

mortadella. Some are slow cooked while smoking, in which case the process takes several days or longer, such as the case for Gyulaikolbász.

3. **Fresh sausages** are made from meats that have not been previously cured. They must be refrigerated and thoroughly cooked before eating. Examples include *Boerewors*, Italian pork sausage, siskonmakkara, and breakfast sausage.

4. **Fresh smoked sausages** are fresh sausages that are smoked and cured. They do not normally require refrigeration and do not require any further cooking before eating. Examples include Mettwurst and Teewurst which are meat preparations packed in sausage casing but squeezed out of it (just like any other spread from a tube).

5. **Dry sausages** are cured sausages that are fermented and dried. Some are smoked as well at the beginning of the drying process. They are generally eaten cold and will keep for a long time.

6. **Bulk sausage**, or sometimes *sausage meat*, refers to raw, ground, spiced meat, usually sold without any casing.

7. **Vegetarian sausage**s are made without meat, for example, based on soya protein or tofu, with herbs and spices. Some vegetarian sausages are not necessarily vegan, and may contain ingredients such as eggs.

2.5 Product development

Product development is the creation of products with new or different characteristics that offer new or additional benefits to the customer. It may involve modification of an existing product or its presentation, or formulation of an entirely new product that satisfies a newly defined customer want or market niche. Product development in this study will be aimed at consuming the beef slaughter by-products in formulation of a stable and acceptable sausage.

Product development and innovation are necessary to offset the growth in the availability of food products competing for disposable income (Resurreccion, 2004).

2.5.1 Models in formulation and process technologies

The selected by-products or any unconventional ingredient must have the capability to be included in the sausage formulation without adversely affecting the aspects of desired qualities. The optimization process allows optimal parameters to be selected from the combination of the various different processing parameters.

Sausage production has several very important process technologies that define the qualities of the final product. Many different manufacturers have formulated sausages by optimizing and varying different aspects. Further, manufacturers due to market demand and economic reasons have shifted to varying and including components in the sausage that have traditionally not been used. The Use of Sweet Potato Starch as Binder in Beef and Pork Frankfurter-Type Sausages has been done. The use of sweet potato starch up to 4% inclusion had no significant effect on cooking loss, meat flavour intensity, flavour liking and overall acceptability of the products (Teye and Teye, 2011). Fortification and substitution is also very common in sausage production. Caceres and others in 2006 designed a meat sausage enriched with extraneous calcium. Osburn and Keeton (2004), utilized konjac gel as fat replacers in sausage produced from desinewed lamb meat.

2.5.2 Gaps in knowledge

There are many knowledge gaps witnessed from the available information regarding by-products usage. The biggest one is the lack of proper technique in utilizing slaughter by-products. Utilization of by-products would certainly improve their uptake, therefore reducing losses after slaughter. Increased usage of by-products has great potential of improving the

income of the slaughter house owners. Lack of knowledge by the people also contributes to the less usage of by-products.

2.6 Thesis layout.

CHAPTER ONE: GENERAL INTRODUCTION.

The chapter gives the background information and the basis around which the research concept was based. It outlines the glaring problems facing pastoralism stakeholders including the poor utilization of by-products and high losses along the meat value chain.

CHAPTER TWO: LITERATURE REVIEW

A review of what has been done elsewhere with regard to by-products is outlined here. Potential utilization ways for several by-products has also been cited. Research objectives and hypothesis are also listed in this chapter which builds a base for the actual research.

CHAPTER THREE: UTILIZATION OF BEEF SLAUGHTER BY-PRODUCTS AMONG THE KENYAN PASTORAL COMMUNITIES.

This chapter comprises of abstract, introduction, study area, methodology, results and discussion and conclusion sections. It characterizes how various pastoral communities in the study area utilize slaughter by-products with the aim of picking up potential ones for use in commercial processing. This is eventually aimed at reducing post-slaughter losses in the slaughter houses.

CHAPTER FOUR: PHYSICO-CHEMICAL, SENSORY, CHEMICAL STABILITY AND COST CHARACTERISTICS OF BEEF SAUSAGES PRODUCED WITH SUBSTITUTION OF FAT WITH BOVINE BLOOD.

This chapter is divided into the following sections; abstract, introduction, methodology, results, discussion, conclusion and recommendations. It outlines the modelling of the sausages with use of blood as fat replacer and laboratory analysis of attributes necessary for a stable sausage.

CHAPTER FIVE: MICROBIOLOGICAL HYGIENE ATTRIBUTES OF SAUSAGES DEVELOPED WITH BLOOD AS FAT REPLACER.

This chapter with introduction, methodology, results, discussions and conclusion sections, explains the hygiene of the sausages. It implies the effect of using blood as an ingredient as well as handling. The results insinuate the sausages hygiene and fitness for consumption.

CHAPTER SIX: GENERAL CONCLUSION AND RECOMMENDATIONS

This chapter outlines the general conclusions against the research objectives. It also states recommendations that can be pursued to heighten further opportunities on the same research line.

CHAPTER THREE: UTILIZATION OF BEEF SLAUGHTER BY-PRODUCTS AMONG THE KENYAN PASTORAL COMMUNITIES

3.1 Abstract

Slaughter of animals (cattle, goats, sheep and camels) is very common in the pastoral areas to satisfy the high local demand for meat. Most of this slaughter is informal. Slaughter is aimed at producing meat but at the same time many by-products are produced. The by-products include blood, kidneys, intestines, lungs, liver, skins and hides among others. However information on the traditional use of slaughter by-products is scanty, in spite of their high potential for commercial utilization. This study therefore was designed to establish how beef by-products are utilized and assess their potential for utilization in commercial processed products. The counties of Turkana, Garissa, Kajiado and Marsabit were purposively selected because they are the largest livestock producers among the pastoral counties. They are inhabited by Turkana (Turkana), Somali (Garissa), Maasai (Kajiado), and Borana, Rendile and Orma (Marsabit) communities. Data was collected using key informant interviews and focus group discussions. Visual observations in selected slaughterhouses were also employed.

Results showed that the by-products could be divided into wastes (hooves and horns), commercial (hides, horns and bones) and food (blood-5%, glands and organs-90% and meat on bones). The by-products that were used for food including kidneys, liver, tongues among others were prepared for consumption in different ways by the different communities. The study established that the by-products were not effectively utilized leading to high post slaughter wastage. The inedible by-products such as the hides were sold to tanneries, the horns were used for ornamentals, skins as dry-skin containers and hooves were just cast away

Keywords: *Beef slaughter, By-products, Pastoral communities, Kenya, Utilization*

This chapter has been accepted as an article for publication in the Journal of Food Science and Quality Management as: Utilization of beef slaughter by-products among the Kenyan pastoral communities. Pius M. Mathi[1*], Catherine N. Kunyanga[1], Josphat N. Gichure[1], Jasper K. Imungi[1]

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3.2 Introduction`

In a meat slaughtering operation, by-products are those parts of the animal that are removed to dress the carcass. There are two categories of by-products .The common edible by products are including liver, heart, tongue, tripe, brains, oxtail, trimmings, intestines, testicles, blood and spleen, and the inedible by-products which include bones, hooves and hides and skins. High losses of by-products are experienced post slaughter because they are not handled with as much care as the main carcass. Their utilization is also not very well organized and due to the high temperature prevailing in the pastoral areas, deterioration can be rapid. According to FAO (2011), 29.7% of meat products are lost post slaughter in East Africa.

Reducing Post harvest losses will effectively and sustainably increase the volume of available food, without encountering the adverse effects on the regional ecological and economic situation (Hensel, 2009). The inedible by-products can be channelled to commercial uses. Hides and skins can be sold to the tanneries, horns used for ornamentals and extraction of glue, bones for animal feed and hooves for production of glue. However, most often, the bones and hooves are thrown away. Today in the pastoral areas the cost of live animal often exceeds the selling price of its carcass. The preparation of saleable part of the carcass can be greatly increased by utilizing the by-products commercially to make the slaughter most cost effective. According to Bowater and Gustafson, 1988, 15% of slaughterhouse income could come from by-products if they are handled properly. However in the last several years, the

economic value of animal by- products has declined (Hedrick *et al*., 1994) because of technological advancement in producing competitive substitutes from non-animal sources.

Improper disposal of animal slaughter by-products may create major aesthetic and health problems (Jayathilakan *et al.,* 2011). By-products disposal and waste management in food processing industry can pose problems in the areas of environmental protection and sustainability (Russ and Pittroff, 2004). The edible slaughter by-products have the potential to be incorporated into the commercial products in the modern processing plants to increase their value without necessarily jeopardising the acceptability of the products. This is an area that needs to be explored because it will boost the economic value of the by-products

This study therefore was designed to identify methods and extent of utilization of by-products in the pastoral areas with the view to evaluating the potential of the by-products for mainstreaming them into the modern commercial meat processing industry.

3.3 Methodology

3.3.1 The study setting

The study was conducted in four pastoral counties namely Turkana, Marsabit, Garrisa and Kajiado. These counties are shown in Figure 3.1. The counties were purposively selected because they are the leading cattle producing areas.

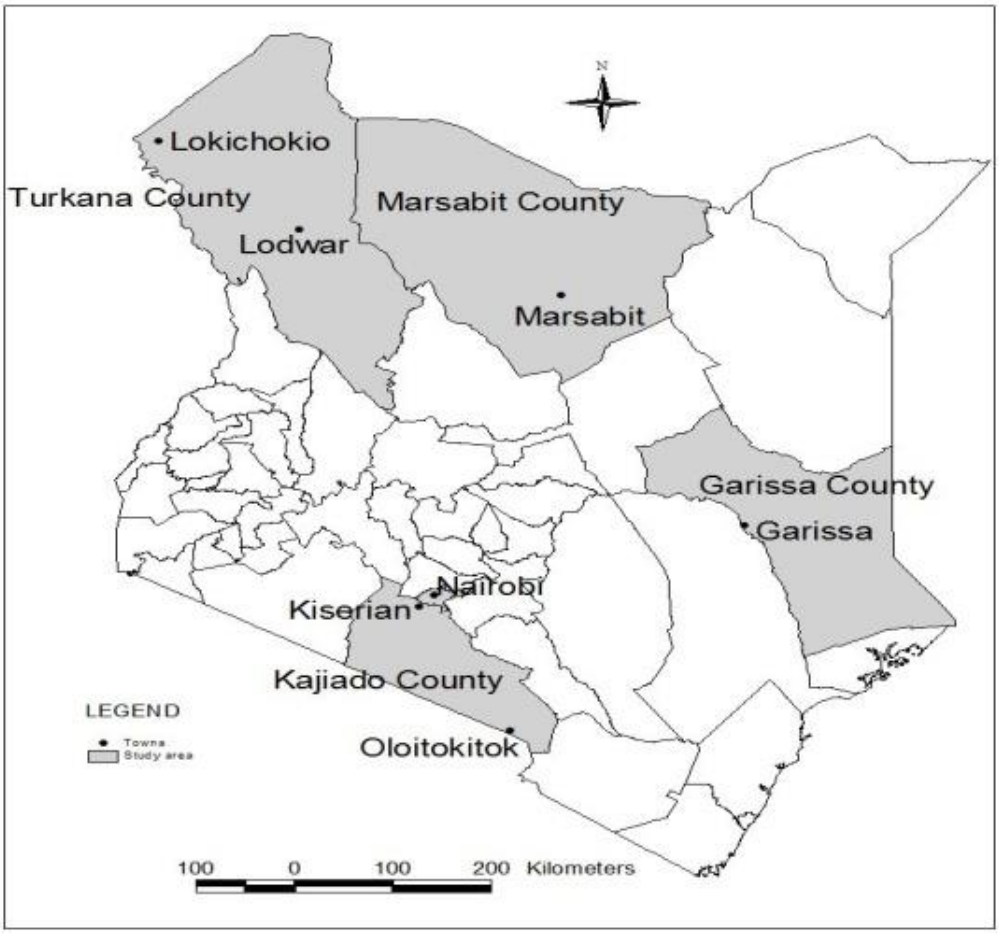

Figure 3.1: The study regions.

Turkana and Marsabit are the largest arid and semi-arid land counties in Kenya therefore constitute an important area of cover; moreover, they fall under the pastoral regions of Kenya. Garissa is one of the largest Counties in the arid and semi-arid land and also the leading livestock market in East Africa moving over 30,000 cattle a week and 500 shoats daily, according to the Department of Veterinary Services. Kajiado County is important as the biggest livestock producer in Kenya.

3.3.2 Study design

The study was cross sectional in design. The residents of the four counties have different socio-cultural backgrounds. A structured questionnaire (Appendix 1) was used to guide the interviews .The persons therefore were therefore assembled, one from each county. The focus groups interviewed were key informants (Checklist shown as appendix 1) who were selected from each county as government veterinary officers, mainly meat inspectors and regularly interact with the local residents at the slaughterhouses and households. Focus groups members were drawn from the local communities within the county with the help of the local government officers. The focus groups consisted of members of the communities who were best knowledgeable on the animal slaughter and products utilization. The two research tools were combined with personal visits and observations of at least two slaughterhouses in each county.

3.3.3 Method of data collection

Key informant interviews were conducted and focus group discussions held to gather information about the current slaughter by-products utilization. Government meat inspectors and knowledgeable people provided the information. A previously pretested semi-structured questionnaire was used as a guide to gather information on various utilization ways. Meat inspectors interact with the slaughter operators at slaughterhouse levels. The knowledgeable people supplied the cultural details and aspects regarding the by-products utilisation by local communities. Further, knowledgeable people from the various communities in each county were involved so that they give the cultural aspect of the by-products utilisation. .

The number of informants varied from county to county depending on the expansiveness as well as the cultural variance of the areas. In total, 22 informants were interviewed. Turkana (6),Garissa (5), Kajiado (7) and Marsabit (4). The focus group discussion membership

constituted six to ten participants with attempts to balance gender of group members. The ages of the group members ranged from 42 to 74 years. These age groups of people were deemed to have the relevant knowledge about the by-products in their respective localities. The languages used were English and Swahili. Where necessary, some terms or questions were interpreted with the help of native field assistants. The interviews of the key informants were conducted individually, to avoid influences from other informants.

3.4 Results and discussion

The four counties reported varied ways of by-product utilization. Ethnic traditions and cultures influenced the way by-products are utilized. Traditions, culture and religion are often important when a meat by-product is being utilized for food (Jayathilakan *et al.,* 2011). So diverse are food preparation methods that the same products are cooked differently for consumption by different communities. These results are summarized in Tables 3.1, 3.2 and 3.3.

3.4.1 Blood utilization

There was a very high amount of blood produced in the slaughterhouses. Most of the blood (about 95%) was left to waste with very little being consumed by humans. That which was consumed was incorporated in the traditional sausage as stuffing or fed to the pets especially dogs. Ethnic Somalis in Garissa County do not consume blood due to cultural and religious convictions. Other communities of the county however, consume in various food formulations. Among all the study areas, Garrisa reported most blood wastage because of the high proportion of the inhabitants of the county being Somalis. Maasais in Kajiado consume blood and it is accepted as food in the homesteads. During grazing especially far away from home, young maasai men (*morans*) pierced the cattle's jugular vein, bled and collected the blood which was mixed with raw milk and consumed. In the commercial slaughterhouses in

Kajiado, most of the blood was left to waste again although some was used in sausage stuffing.

In one of the slaughterhouses the blood was directed together with other ingesta matters into a biogas digester plant. There was very little utilization of blood in Turkana and Marsabit. It was merely left to waste. Similar to other counties, any used blood was mainly for traditional sausage stuffing. The rest of the blood was merely dumped in lagoons. One of the major challenges reported was the maintenance of hygiene in the collection and handling of the edible by-products to maintain safety in consumption. This inhibited utilization of the products for food because the methods of preparation for consumption at the domestic level could not necessarily guarantee safety.

The average percentage of blood that can be recovered from cattle is 3.0–4.0 % (Jayathilakan *et al*., 2011). Blood is usually sterile in a healthy animal. It has high protein content, 17%, with a reasonably good balance of amino acids (Jayathilakan *et al*., 2011) and a high level of heme iron, (Wan *et al.*, 2002). It can therefore be used as protein and iron supplement. There is a huge potential in blood utilization as ingredient in processing of human and animal food, but also as farm inputs. In Europe, animal blood has long been used to make blood sausages, blood pudding, biscuits and bread. In Asia, it is used in blood curd, blood cake and blood pudding (Ghost, 2001). Blood is also used for non-food items such as fertilizer, feedstuffs and binder for sausages. Blood is used in food as emulsifier, stabilizer, clarifier, colour additive, and as nutritional supplement (Silva and Silvestre, 2003). Blood plasma has ability to form a gel, because it contains 60.0% albumin (Silva and Silvestre, 2003) and has excellent foaming capacity (Del *et al*., 2008).

Table 3.1: Edible by-products utilization in the pastoral areas

	County and dominant community			
	Garissa *(Somali)*	**Kajiado** *(Maasai,kikuyu)*	**Marsabit** *(Borana,Rendile,Orma)*	**Turkana** *(Turkana)*
By-product	**Method(s) of utilization**			
Blood	Human food	Human food. Animal food	Human food	Human food
Liver	Human food	Human food	Human food	Human food
Heart	Human food	Human food	Human food	Human food
Kidney	Human food	Human food	Human food	Human food
Tongue	Human food	Human food	Human food	Human food
Brain	Human food	Human food	Human food	Human food
Tripe	Human food	Human food	Human food	Human food
Oxtail	Soup making	Soup making	Soup making	Soup making
Testicles	Human food pet food	Human food	Human food	Human food
Pancreas	Human food pet food	Human food	Human food	Human food
Sinews	Meat on bones Human food	Meat on bones Human food	Meat on bones Human food	Meat on bones Human food
Trotters	For making soup	For making soup	For making soup	For making soup
Bones with meat	For stock making	For stock making	For stock making	For stock making

Table 3.2: Some uses of the inedible by-products in the counties

County	Garissa	Kajiado	Marsabit	Turkana
Product	**Some uses**			
Horns	Wasted	Ornamental fabrication	Musical instruments fabrication	Ornamental Musical instruments
Hides/skin	Sold to tanneries.	Sold to tanneries	Sold to tanneries, packaging	Sold to tanneries, construction of meat package*(enyas)*

3.4.2 Utilization of glands and organs.

Glands and organs form a very important food in many parts of the world. Table 3.3 shows the summary of various preparation methods. Only areas with access to electricity enabled the traders to refrigerate the by-products. The study showed that these products are very important food items in the pastoral regions. They include the brain, heart, kidneys, liver, lungs and spleen. On average, 90% of the brain, heart, kidneys, liver, lungs and spleen were used for human food. Only 2% went to waste while the remaining 8% was used as pet food. Others are the tongue, the pancreas and udder, the rumen, reticulum, omasum, abomasum the testes and thymus (Liu, 2002). Glands and organs were cooked for consumption in almost the same way in all counties. However whereas in other counties the kidneys were roasted, stewed or boiled before consumption, the Maasai morans (warriors) in Kajiado county ate them raw. Other communities within the County cooked kidneys mainly by boiling in water before eating. Liver was cooked and eaten by all communities. Liver is commercially processed into products such as sausage and liver paste (Devatkal *et al.,* 2004). Urban hotels commonly fry liver as part of their daily menu. Lungs were cooked together with other meat trimmings. However, about 3% of lungs and trimming were used as pet food. It has been reported in USA and Europe that calf and lamb lungs are mainly used to make stuffing for some types of sausages and processed meats (Darine *et al.*, 2010).

Brain and tongue were not removed from the head and were therefore sold together and cooked together for consumption, mainly by boiling after scalding the adhering fur on the head. Elsewhere, the cholesterol in the brain has been used as an emulsifier in cosmetics (Ejike and Emmanuel, 2009). The intestines and tripe in general were utilised as local delicacies by all the communities in the study regions. They were either cooked by boiling and /or roasting or used as a local sausage casing. Before cooking they were cleaned of the residual excreta. World over, the intestines constitute an important use as a sausage casing

(Bhaskar *et al*., 2007). Sinews and tendons were not separated from the main carcass and therefore were sold as meat on bones. The fats were also not separated from the main carcass meat. This made the meat fattier in the pastoral areas than in other urban areas where high end butcheries usually trim the fat and hard tendons from the main carcass. Majority of people in the pastoral areas preferred their meat fresh and fatty since they perceived such meat as the most delicious and juicy. They argued that meat which has been stored overnight is not sweet on consumption. 95% of testicles and udders were used as food for human while the remaining 5% was used as pet food. They were mostly boiled or roasted and eaten immediately after slaughter. Similar by- products showed similarities in the way they were prepared in different areas.

Table 3.3: Common methods of storage and preparation of slaughter by-products for consumption in the pastoral areas

By product	State of its preparation	Method of preparation
Blood	Fresh but coagulated	Sausage stuffing, cooked for pet food
Liver	Fresh , refrigerated	Boiled, fried, stewed
Heart	Whole ,fresh ,refrigerated	Fried, boiled, roasted
Kidney	Whole, fresh, refrigerated	Boiled, Roasted, stewed
Tongue	Whole, fresh	Boiled, roasted, stewed
Brain	Whole within the head	Boiled
Tripe	Whole	Boiled, stewed, roasted, sausage
Oxtail	Fresh, frozen ,refrigerated	Boiled, cooked into broth
Testicles	Whole ,sliced, fresh	Roasted, boiled(fur scalded first)
Pancreas	Whole, fresh	Boiled, Fried, sausages stuffing
Sinews	In meat cuts, trotters	Cooked as meat on bones
Trotters	Fresh	Boiled, roasted

3.4.3 Utilization of hides and skins.

Hides and skins were not utilised as food according to the study. The hides and skins were sprayed with salt on the whole surface and partially dried in air, then sold to intermediaries who sold them to tanneries as shown in Table 3.2. Hides and skins from animals slaughtered at homes were usually left to waste since most of the collection centres were far away from households. In a few cases, communities in Kajiado dried them and used them for preparation of meat storage containers, for making traditional ornaments and clothing. Wooden containers were wrapped by a dried softened skin and used to package traditional meat products. These containers were for example the one used to store *Enyas*, a traditional Turkana meat product. The hides represent a remarkable portion of the weight of the live animal, from 4% to as much as 11% e.g. cattle: 5.1–8.5%, average: 7.0% (Jayathilakan *et al*., 2011). Other products from the hides of cattle include leather shoes and bags, rawhide, athletic equipment, reformed sausage casing and cosmetic products, sausage skins, edible gelatine and glue (Benjakul *et al.* 2009). Figure 3.2 shows the Turkana traditional meat product, *enyas* packed in a gourd wrapped in skin.

Figure 3.1: A skin wrapped gourd for storage of enyas

In more advanced cases, the hides have been used for extraction of gelatin through hydrolysis of collagen. Gelatin has many applications including use in food (Choa *et al*., 2005). Gelatin is also the major ingredient in jellies (Jamilah and Harvinder, 2002).

3.4.4 Utilization of hooves and horns.

Hooves and horns were not used for food by the pastoral communities in all the counties studied. Hooves were sold to glue manufacturers. Horns were locally utilised in various ways depending on the cultural practices of the community. Most of the communities used the horns for preparation of music instruments and making ornamentals, by chopping and shaping to desired shapes and sizes. Kajiado County had the most diverse ways of utilizing horns. This probably owed to the fact that people living there were of more diverse cultural backgrounds. Besides being used for ornamental and music instrument, horns were prepared into containers for drinking local brew by the kikuyu community living in Kajiado. It should be realized that only a small proportion of selected horns were used for manufacture of the items indicated. The larger proportion of horns plus the hooves were discarded or sold to the glue manufacturers. If hooves and horns are controllably collected and sold to glue manufacturers, then this can help to increase the profitability of animal slaughter.

3.4.5 Utilization of bones.

The study showed that most of the bones had adhering meat and were sold as meat on bones. Very few slaughterhouses deboned their meat. The few ones who deboned their carcass sold the bones to people who made clear soups/stock in hotels, restaurants and households. Trotters contributed the largest proportion of bones since many people perceived them to be inferior food. Soup from trotters was prepared in most slaughterhouses. To make soup from trotters, they are first flamed to burn off the hairs, and then brushed and washed-off the soot. They are cut into chunks then boiled until soft. Bone soup was reported to be very popular

among people living in the study areas. Since the trotters were the most affordable and available source of bones, majority of soup makers preferred them to the carcass bones. Competition for main carcass bones with pet keepers also contributed to the trotters being more preferred soup making ingredient than main carcass bones. People however seemed to dislike the tendons/sinews and cartilages on the trotters since they were arguably tough to chew. This perception led to some soup makers requiring that the tough tendons be removed before boiling for soup. The sinews were thereafter used for pet food.

The carcass consists of 15% bones with the figures being higher if the meat chips clinging on the bones are included. The marrow in the bones which account for 4.0-6.0% of the carcass weight (West and Shaw, 1975) is also used as food. In some more developed countries in Europe, the bones are used for meat bone meal manufacture. Kenya meat commission (KMC) in Kenya also utilises their bones for bone meal processing and sinews as part of the binders for corned beef.

3.5 Conclusion

By products utilization in the pastoral areas is not well organised and wastage is rampant. However, most of the by-products with the exception of solid bones, hides and skins and hooves are considered as food. Their incorporation into commercially processed products for a wider market will therefore be considered acceptable. There also exist market for inedible by-products like hooves, horns and solid bones. If the marketing of the edible and inedible slaughter by-products is well organized, then this should increase profitability of slaughter.

CHAPTER FOUR: PHYSICO-CHEMICAL, SENSORY AND KEEPING QUALITY OF BEEF SAUSAGES DEVELOPED WITH INCORPORATION OF BOVINE BLOOD AS A FAT REPLACER

4.1 Abstract.

Fat is a fundamental ingredient in sausages formulation and manufacture. However, of late animal fat is increasingly being implicated in ill health world over. This has led to the processors' rethinking and research into low fat formulations. Many fat substitutes have been tested with varied results. Bovine blood is widely accepted as food in Kenya. It is for example used in the preparation of a traditional sausage (*mutura)*, as well as being incorporated in various traditional dishes. In Kenyan abattoirs and slaughterhouses, blood is a waste by-product. If used successfully, blood can be a good and cheap substitute of fat in sausages. The project was therefore designed to produce sausages in which fat was partially substituted with fresh bovine blood. Fat was substituted with blood at the rate of 5% from the conventional sausage which contained 20% fat down to 0%. Fat: blood ratio was as follows; 20:0, 15:5, 10:10, 5:15 and 0:20. The sausages were evaluated for sensory properties, analysed for proximate composition, texture, cooking loss, water holding capacity, iron contents, fat oxidation as peroxide value and microbial profile for the general shelf life and keeping quality determination and cost benefits. Sensory evaluation concluded that it is only possible to include 10% blood as a direct fat substitute in the sausages. The substitution however increased the protein, iron and water contents of the sausages significantly ($P<0.05$) but reduced the water holding capacity while increasing cooking loss. The maximum protein content was 15.93% for 10% fat replacement, iron content increased significantly to 575mg/kg, cook loss increased to 10.7% for lower fat sausages while water holding capacity decreased to 85.12% for the low fat sausages. Chemical stability of the sausages was shown by a lower than the recommended peroxide value of 20 meq/kg at 18.3 meq/kg after 14 days in a 4 degrees Celsius storage conditions. Further, use of blood to replace fat in the process

could save the processor up to 25,000 shillings per tonne of sausage. The study concluded that it is possible to produce a safe, nutritious and acceptable fresh beef sausage by partial substitution of the fat content with bovine blood with lowered production cost.

Key words: *Bovine blood, Fat substitution, Fresh sausages, Production cost, Quality evaluation.*

This chapter has been accepted as an article for publication in the Journal of Animal Sciences Advanced as: Physico-chemical, sensory, chemical stability and cost characteristics of beef sausages produced with substitution of fat with bovine blood. P. M. Mathi[*1], C. N. Kunyanga[*], J. N. Gichure, [*] J. K. Imungi[*]

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4.2 Introduction

Animal blood has been studied in recent years as a low cost source of nutrients, especially protein (18 g/100 g) and iron (30 mg/100 g), with functional and sensory properties that are acceptable for human consumption (Fontes, 2006; Gorbatov, 1988; Pereira, 2000; Santos, 2007). Blood sausage is rich in high biological value proteins, amino acids, essential fatty acids, and iron, (Silva *et al*., 2013). The food industry utilizes animal muscle meat and other sections of the animal for the manufacturing of a variety of marketable products, such as sausages, hams, and bologna (Pearson and Gillett 1996), and these products have a high levels of fat. For instance, frankfurters and bologna may have as much as 30% fat, and fresh pork sausages may contain up to 50% fat (Roth and others 1997; Belloque and others, 2002).

Blood especially in the Kenyan slaughterhouses has not been well utilised either in informal or commercial processing, which provides the opportunity for new product development and slaughter loss reduction. Fat serves a multifaceted purpose in all meat products with nutrition being a major important factor. Nutrition is a very important factor in food selection (Bruhn *et*

al., 1992). In any food, fat serves three basic physiological functions: a source of essential fatty acids, a carrier of fat soluble vitamins and as an energy source (Mela, 1990).

In meat products fat contributes to the flavour, texture, mouth feel, juiciness and overall sensation of lubricity of the cooked products. Therefore, any fat reductions can affect the acceptability of the products (Huffman and Egbert, 1990; Giese, 1996). Applied fat alternatives are guided by different targeted outcomes depending on the consumer needs. The needs could bring negative healthy issues as those dominating the debate on consumption of animal fat or they could be related to aesthetics. Proper formulation is critical in finding a balance between achieving fat reduction and retaining the desired properties in the final products. This is not always easy because; with excessive fat reduction, these products become bland and dry and the texture can be hard, rubbery or mealy (Keeton,1994).

Despite the fat serving such a crucial role in meat products, health organizations all over the world have advocated reduction of its intake particularly fats high in saturated fatty acids and cholesterol, as a mean of preventing obesity and cardiovascular heart disease (AHA, 1986; Department of Health, 1994; NCEP, 1988). According to Lee and others, (1994), and William (2000), there was also an increased prostate cancer risk with increased intake of food rich in animal fat.

4.3 Methodology

4.3.1 Sausage formulation and production

The fat replacement ratios are shown in Table 4.1 as percentages per kilogram. The meat and blood were obtained in a local slaughterhouse (Bahati slaughterhouse, Limuru) which possesses the required authorisation from local authorities about health and hygiene practices. Other ingredients were bought from an authorised supplier in Nairobi city.

Fresh boneless semi membranous beef from the thigh muscle immediately after slaughter was chopped into smaller pieces and minced with a 5mm sieve in a table top mincer. The minced meat was apportioned into five batches of equal quantities each. The batches contained the standard sausage ingredients and fresh blood at different levels of substitution as shown in Table 4.2. Each treatment group was replicated three times. Different additives and seasonings used in the formulations are shown in Table 4.3. Figure 4.1 shows the logical framework of the sausages production.

4.3.2 Experimental design.

The projects experimental design was a complete randomized design because of the three replication effect. The detailed interaction of the blood and fat is shown in Table 4.4.

Table: 4.1 The fat, blood ratios of the sausages

RUN	BLOOD (%)	QUANTITY(g)	FAT (%)	QUANTITY(g)
1	0	0	20	200
2	5	50	15	150
3	10	100	10	100
4	15	150	5	50
5	20	200	0	0

Table: 4.2: Grams of ingredients per kg of the different fat blood level sausages

RUN	BEEF	BLOOD	FAT	ICE	WHEAT FLOUR	RUSK	CORN STARCH	ADDITIVES	SEASONINGS
1	500	0	200	180	60	40	20	19.8	3
2	500	50	150	180	60	40	20	19.8	3
3	500	100	100	180	60	40	20	19.8	3
4	500	150	50	180	60	40	20	19.8	3
5	500	200	0	180	60	40	20	19.8	3
Total	**2500**	**500**	**500**	**900**	**300**	**200**	**200**	**99**	**15**

Table 4.3: Additives and seasonings for a 1 kg sausage formulation

Additives	Quantity (gm.)	Seasonings	Quantity (gm.)
Common salt	16	White pepper	2
Sodium tripolyphosphate(STTP)	3	Nutmeg	0.3
Ascorbic acid	0.3	Mace ground	0.3
Monosodium glutamate(MSG)	0.5	Coriander & Ginger	0.4
Total	**19.8**	**Total**	**3**

Table 4.4: The experimental design of the sausages production

Meat quantity in grams	Replicates	Treatment in %	Run
500	1	Blood 0 + Fat 20	1
500	2	Blood 0 + Fat 20	1
500	3	Blood 0 + Fat 20	1
500	1	Blood 5 + Fat 15	2
500	2	Blood 5 + Fat 15	2
500	3	Blood 5 + Fat 15	2
500	1	Blood 10 + Fat 10	3
500	2	Blood 10 + Fat 10	3
500	3	Blood 10 + Fat 10	3
500	1	Blood 15 + Fat 5	4
500	2	Blood 15 + Fat 5	4
500	3	Blood 15 + Fat 5	4
500	1	Blood 20 + Fat 0	5
500	2	Blood 20 + Fat 0	5
500	3	Blood 20 + Fat 0	5

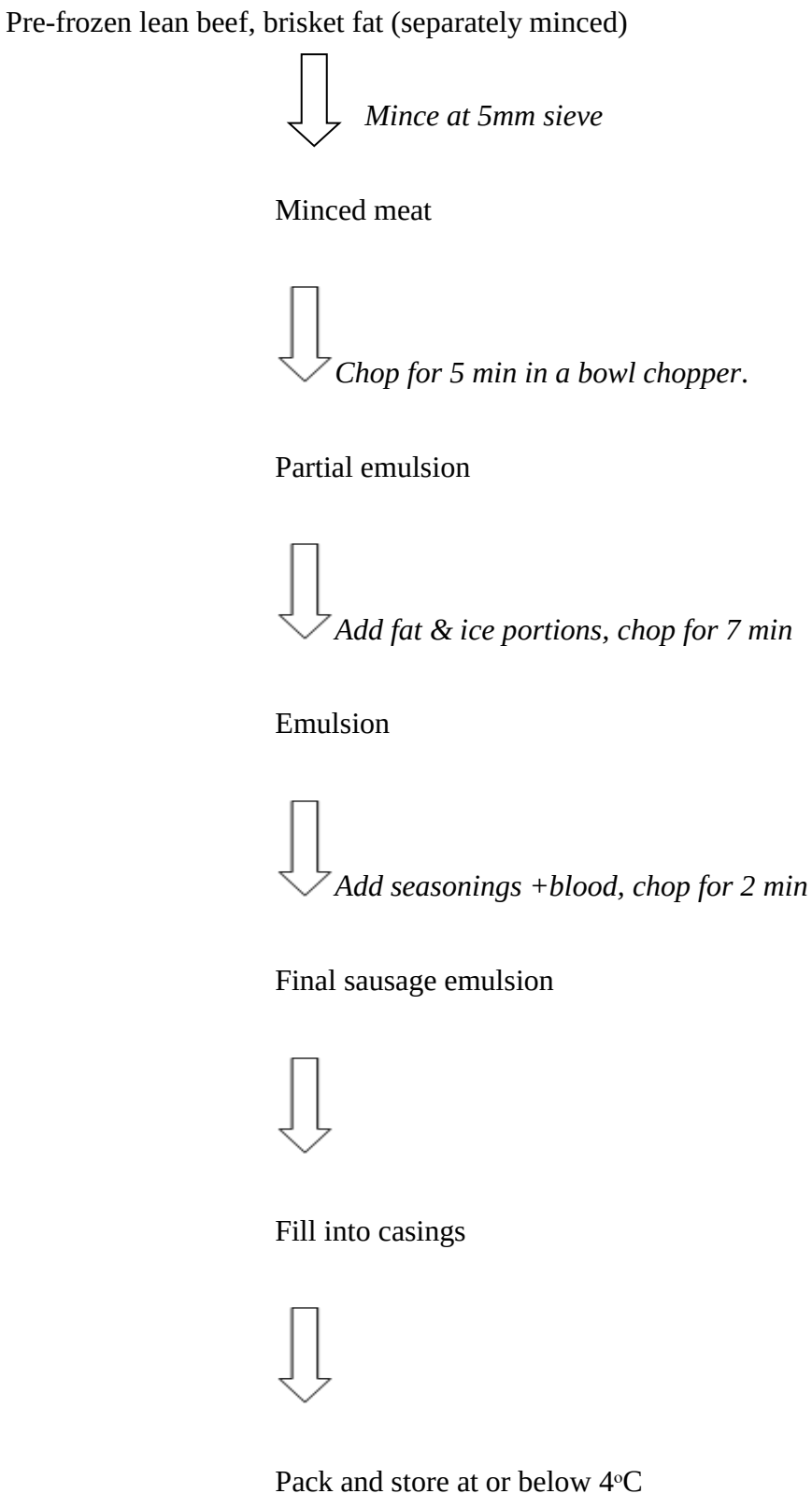

Figure 4.1: The sausages processing logical flow

4.4 Analytical methods

4.4.1 Proximate composition

4.4.1.1 Determination of Moisture content

The moisture content was basically determined using AOAC oven drying method14.0004 (AOAC, 2000). Approximately 5g of the sample was weighed in aluminium made dish which was placed in an oven at 105ºC for approximately 5 hours. Cooling followed and both the dish and the residue were weighed. The difference in weight between the original fresh sample weight and the dried sample gave the moisture content. This was expressed as per cent moisture content.

4.4.1.2 Determination of Fat content

The Sohxlet method according to AOAC Approved method 24.005 (AOAC, 1984) was used to determine the fat content of the sample. Approximately 5g of ground sample was weighed accurately into an extraction thimble and covered with cotton wool. The thimble was then placed into the sohxlet extractor and the fat extracted into a tared flask for 8 hours using petroleum ether (B.P. 40-60ºC). The solvent was evaporated in a rotary evaporator and the residue dried in an air oven at 105ºC for approximately one hour before weighing. The fat content was calculated and expressed as percentage of the sample dry matter content.

4.4.1.3 Determination of Protein content

The approved AOAC (2003) kjeldahl928.08 method was used for crude protein determination. 0.5g of the sample were accurately weighed and placed in a Kjeldahl flask, folded in a nitrogen free filter paper. A catalyst tablet was and sulphuric acid were carefully added to digest the sample in a fume chamber. Phenolphthalein was used as the end point indicator before the Kjeldahl flask was connected to a distillation unit. 40% NaOH solution

was used for back titration against a 0.1N NaOH solution. 6.25 were used as the standard conversion factor for Nitrogen into crude protein content of the sample.

4.4.1.4 Determination iron content

Iron contents of sausages were determined by atomic absorption spectrometry (AAS) according to the methods of AOAC (2003). This was done through wet digestion extraction. Exactly 1g of the powdered sample was taken in digesting glass tube. 12ml of HNO3 was added to the samples and mixture was kept for overnight at room temperature. Then 4.0 ml perchloric acid (HClO4) was added to the mixture and kept in the fumes chamber for digestion. The temperature was increased gradually, starting from 50ºC and increasing up to 250-300 C.

The digestion completed in about 70-85 min as indicated by the appearance of white fumes. The mixture was left to cool down and the contents of the tubes were transferred to 100 ml volumetric flasks and the volumes of the contents were made to 100 ml with distilled water. The wet digested solution was transferred to plastic bottles and taken for mineral determination. The reading was made using the spectrophotometer against a standard of iron prepared before. The conversion from ppm to g/100g was calculated by dividing the reading by 10000.

4.4.2 Determination of cooking loss

A sample of known weight was cooked in hot oil until its centre attained a temperature of 75ºC. Cooking loss was expressed as per cent loss in weight between the initial and the after cooking weights of the sample.

$$\text{Cooking loss} = \frac{\text{weight before cooking- weight after cooking}}{\text{Weight before cooking}} \times 100$$

4.4.3 Determination of water holding capacity (WHC)

Water holding capacity was measured using a modified method of Jauregui *et al.,* 1981. Samples of 1.5 g were placed into pre-weighed thimbles consisting of one piece Whatman No. 3 paper folded around two pieces Whatman No. 50 filter paper. Samples were centrifuged at 1500 G for 10 minutes and the filter paper was re-weighed to determine the total moisture prior to centrifugation. Free moisture was calculated as the weight gained by the filter paper on centrifugation. Moisture was determined as the percentage of the free moisture expressed from the sample.

4.4.4 Hardness measurement

This was determined by letting a rheometer pin penetrate through a sausage slice positioned in a cross sectional alignment. The casing was removed before the tests were done. The penetration speed was set at 100mm/min and the measurements were replicated twice. The hardness of the sausages was recorded as the force used to penetrate through the slice in Newtons.

4.4.5 Colour measurements

Colour was measured using a hand held Hunter lab colour meter in L*a*b* values. Samples were cooked deep fried for 5 minutes at 90^{o} C and sliced into pieces 4mm thick as described by Huda and others, (2000). The colour reading includes lightness (L*), redness (a*) and yellowness (b*). The colorimeter was calibrated throughout the study using a standard white ceramic tile. Two replications were performed for each sample.

4.4.6 Sensory evaluation

Sensory evaluation was conducted on day 1 by lining up a panel of 11 semi-trained members who are familiar with sausages and whose key focus was on colour, odour, taste, texture and overall acceptance. Coding was done randomly using three digit codes to enable subjective,

unbiased and independent exercise among the panellists. They were instructed to finish with all attributes of one sample before proceeding to another to aid objectivity. Rinsing water was duly provided for mouth rinsing between samples tasting. Panellists were subjected to similar operating conditions. A five point hedonic scale was used by the panellist for their judgement, 5 dislike extremely, 4 dislike moderately, 3 neither like nor dislike, 2 like moderately, 1 like extremely as described by Meilgaard *et al.*, (1999).

4.5 Keeping qualityand shelf life analysis

The shelf life was monitored chemically to determine how long it can last without getting spoilt up to standards that are harmful to the consumer. This was done through-out the 14 days of storage at $4^{o}C$, but was divided into three analytical periods at day 0, 7 and 14. Chemical analysis constituted the peroxide value determination.

4.5.1 Determination of peroxide value (PV)

The method Cd 8-53 (AOCS 2003) was used to determine the peroxide value of the samples. It was measured at day 0 just after processing, day seven and day fourteen of storage to determine the quality of the sausages during storage. Approximately 4g of the sample was weighed into a dry 250ml stoppered conical flask. 10ml of chloroform was added and the fat dissolved by careful swirling. 15ml of glacial acetic acid and 1ml of fresh saturated aqueous potassium iodide solution was then added and the flask stoppered. The contents were shaken for 1 minute and the flask placed in darkness for 5 minutes. 75ml of distilled water was then added and mixed well with the flask contents. Titration of the iodine with 0.002M or 0.01M sodium thiosulphate solution using 1% starch solution as an indicator then followed. A reagent blank determination (V_0) was carried out using 0.5ml of 0.01M thiosulphate solution.

4.6 Cost analysis

The cost analysis of the production of the sausages was done by listing down the production facets and comparing their current market costs head to head for the commercially viable sausage formulations. The breakdown costs considered include; cost of raw materials, ingredients, packaging, transport and labour.

4.7 Statistical analysis

The data were analysed using one-way analysis of variance (ANOVA) and the Duncan's multiple range tests for multiple mean comparisons. The data were processed using Genstat software version 15.0 and significance was defined at $P<0.05$. Sensory analysis descriptive data was analysed using SPSS for windows version 20 and the difference in means done using the Duncan's multiple test at $P\leq0.05$.

4.8 Results and discussions

4.8.1 Proximate composition

Results for proximate composition which include moisture content, fat, protein and iron contents are shown in Table 4.5. The moisture content increased with increase in replacement of the fat with blood. The values range from 64.65% for the 10% replacement down to 62.93% for the control sausage. This however did not represent any significant difference for all the three sausages at 0%, 5% and 10% fat replacement at $P<0.05$.

The protein content increased progressively with the increase in fat replacement with blood although that did not impact a significant different among all sausages at $P<0.05$. The highest value was 15.93% for the 10% replacement with the lowest being 14.26% for the control sausage with no blood inclusion. There was an inverse relationship between fat replacement with blood and fat content of the sausages. All the sausages' fat contents were significantly

different at P<0.05. Iron content too increased with the increase with blood increase in the sausages. This was evidenced by the huge leaps from the control all the way up to the 10% fat replacement. At 575.6mg/kg, 283.6mg/kg and 236.3mg/kg for the 10%, 5% and 0% blood inclusion, they all showed significant difference at P<0.05.

The increasing moisture content witnessed in the sausages with increasing blood was as a result of the fact that blood has very high loosely bound water. That water manifested itself on the sausages that consequently led to a higher cooking loss and low water holding capacity as shown in Table 4.7. These results were in agreement with those reported by Olivares *et al*. (2010) and Lorenzo *et al*. (2011) who found higher water content in low fat sausages.

The increasing protein with increasing blood inclusion in the sausages can be explained by the fact that, blood has a high level of protein (Wan *et al.,* 2002). This was also in agreement with earlier studies by Silva *et al.,* (2013), who found blood sausages to be rich in high biological value proteins. The decreasing fat content with the increasing blood was obviously as a result of direct fat removal. Further, the fact that blood does not contain fatty tissues can also explain the decreasing fat content. Blood showed an immense potential as a partial fat replacer in a sausage since all the sausages were significantly different at P<0.05. This is consequently good for health as animal fats are high in cholesterol. Obesity, heart diseases and prostate cancer have all been associated with high cholesterol and animal fat intake, (Lee *et al*., 1994 and Williams, 2000).

Iron is an essential micro nutrient that is very useful for a human body's physiological processes. It plays an integral part in oxygen transport in the body as well as enzyme activities. It is generally recommended that an adult should not take more than 45mg per day. Adult men and women have a recommended daily requirement (RDA) of 8mg and 18mg per day respectively according to the institute of medicine of the national academy of science.

Therefore one average sized sausage weighing 50 grams and containing 5% and10% blood can provide 14.2mg and 28.9mg of iron respectively. This therefore supplies more than both the adult men and women's RDA which is consequently a cheap nutritional advantage. The highest overall acceptable fat-blood substitution sausage at 10% blood provides a significantly higher level of iron compared to the full fat sausage. Blood is very rich in iron, (Fontes, 2006; Gorbatov, 1988; Pereira, 2000; Santos, 2007) therefore leading to an increase in iron with the increasing blood in the sausages.

Table 4.5: Moisture content, crude protein, Fat content and Iron levels of the sausages

FAT LEVEL (%)	20	15	10
BLOOD LEVEL (%)	0	5	10
FAT	25.70 ± 0.6^{a}	23.50 ± 0.62^{b}	22.10 ± 0.3^{c}
CRUDE PROTEIN	14.26 ± 0.76^{a}	15.09 ± 0.22^{ab}	15.93 ± 0.31^{ab}
MOISTURE	62.93 ± 0.28^{a}	62.97 ± 0.86^{a}	64.65 ± 0.71^{ab}
IRON CONTENT(mg/kg)	236.3 ± 2.97^{a}	283.6 ± 1.56^{b}	576.6 ± 1.84^{c}

Superscripts ab Indicates that means along the same row with similar superscripts are not significantly different ($P<0.05$)

4.8.2 Sensory evaluation

Table 4.6 shows the sensory evaluation results for the sausages. Since the sausages were designed for commercial processing, the sensory evaluation showed that only sausages with up to 10% blood inclusion are acceptable and therefore they are the ones that were considered for further quality attributes analysis.

The control sausage that had full fat did not show any significant difference with the 5% blood included sausages at $P<0.05$ in all the evaluated aspects. Increasing the blood further to

10% and reducing fat with the same margin showed a marked difference with the most affected aspect being colour at the mean score of 3.45. The aspects that came closest to the control sausage were taste and texture at 2.82 mean score respectively. This therefore means that with the cut off being 3.00, colour would make the 10% blood inclusion be unacceptable. However, since the mean score 3.45 is not significantly different at $P<0.05$ with mean score 2.82, it makes it overall acceptable to consider blood inclusion at 10% for commercial processing. The more the fat replacement especially after 10%, the less acceptable the sausages became.

Fat plays a very important role in sausages' sensory acceptance including contributing to the flavour, texture, mouth feel, juiciness and overall sensation of lubricity of the cooked products (Huffman and Egbert, 1990). Therefore, any fat reductions can affect the acceptability of the products Giese, (1996). Excessive fat reduction in a sausage as was the outcome with the texture and taste leads to the sausages becoming bland and dry while the texture can be hard, rubbery or mealy according to Keeton (1994).The decreasing color ratings with fat reduction can also be attributed to the fact that increasing the fat content in meat products also influences the overall color appearance (Troutt *et al.,* 1992).

Increased fat reduction agrees with results in earlier studies that when fat is removed from meat products, high cooking loss and low water holding capacity are observed. These issues result in lower palatability and general low acceptability (Lamkey, 1998; Chin et al., 2004a).Dietary fat plays a major role in the texture, juiciness and flavour of comminuted meat products (Crehan *et al*., 2000). Therefore the decreased rating of all the sensory aspects of the sausages with the increased fat reduction is likely to minimize the sensory characteristics of food products (Byers *et al*., 1993).

Table 4.6: Sensory attributes in Color, Odor, Taste, Texture and Overall acceptability of the sausages

SAMPLE		ATTRIBUTES				
FAT (%)	**BLOOD (%)**	COLOR	ODOR	TASTE	TEXTURE	OVERALL ACCEPTABILITY
20	**0**	1.18^{a}	1.27^{a}	1.18^{a}	1.36^{a}	1.18^{a}
15	**5**	1.91^{a}	1.91^{ab}	1.45^{a}	1.73^{a}	1.64^{a}
10	**10**	3.45^{b}	2.82^{bc}	2.82^{b}	2.82^{b}	3.09^{b}
5	**15**	4.18^{b}	2.91^{c}	2.91^{b}	3.09^{b}	3.64^{b}
0	**20**	3.55^{b}	2.36^{c}	2.91^{b}	3.00^{b}	3.64^{b}

Superscripts abc Indicates that means along the same row with similar superscripts are not significantly different ($P<0.05$)

4.8.3 Physical properties of the sausage

Results for cooking loss (CL), water holding capacity (WHC) and hardness of the cooked sausages are presented in Table 4.7. There was an increasing cooking loss with the increasing fat replacement with blood. However, the losses were not big enough to make significance at $P<0.05$ for all the two replacements. The losses ranged from 9.44% to 10.71% for control, 5% and 10% respectively. The cooking loss also increased with the increase in protein contents. A decreasing trend was noted for the WHC from the control to the 10% blood inclusion. This too did not represent a significant difference at $P<0.05$ for all the three sausage samples. There was a general trend of an increasing cooking loss, reducing water holding capacity and increasing moisture content with the increase of blood. The hardness of the sausages decreased with the reducing fat content. The control sausage and the 5% fat replacement had

no significant difference at $P<0.05$. The highest force required to penetrate the sausages was 0.06N for the full fat sausage while the 10% fat replacement required 0.035N.

The high cooking loss and low water holding capacity exhibited by the sausages with the increasing blood can be attributed to the moisture content increase in the sausages. Removing fat that is a good water binder and replacing it with blood that is predominantly water without adding more water binders in the sausage formulation only leads to the availability of more loosely bound water that easily moves off during cooking. The cooking loss increase with the increasing protein content in the sausages can be attributed to the fact that during cooking, high temperatures cause protein denaturation that otherwise binds the water through its network (Lawrie, 1998).

The decreasing water holding capacity with the reducing fat further highlights the importance of fat in a sausage. Fat in meat products can reduce cooking loss and improve water-holding capacity (Carballo *et al*., 1995; Pietrasik and Duda, 2000). Increased fat reduction agrees with results in earlier studies that when fat is removed from meat products, high cooking loss and low water holding capacity are observed (Lamkey, 1998; Chin *et al*., 2004). Reduction of fat level increases the weight losses of sausages (Muguerza *et al*., 2002). The decrease in hardness of the sausages with the increase in blood and moisture content agrees with an earlier study by Serdaroglu (2005) that high moisture content improves the tenderness of a sausage. Huffman and Egbert (1990) further emphasizes on the importance of fat to the textural hardness of the sausages in which excessive low fat leads to excessively tender products.

Table 4.7: Cooking loss, Water holding capacity and Hardness of the sausages at different blood/fat levels (%)

FAT LEVEL (%)	**20**	**15**	**10**
BLOOD LEVEL (%)	**0**	**5**	**10**
Cooking loss (%)	9.44±0.46[a]	9.73±0.61[a]	10.71±0.48[ab]
Water holding capacity (%)	86.52±0.51[a]	86.16±0.5[a]	85.12±0.66[ab]
Hardness(N)	0.06±0.007[b]	0.06±0.00[b]	0.035±0.007[a]

Superscripts ab Indicates that means along the same row with similar superscripts are not significantly different ($P<0.05$). N= newtons.

4.8.4 Color characteristics of the sausages

Colour measurements were classified as lightness (L*), redness (a*) and yellowness (b*) are shown in Table 4.8. The lightness (L*) of the sausages decreased with the reducing fat content whereby all the sausages were significantly different at ($P<0.05$). The values fell between 66.9 and 40.6 for the full fat and 10% fat replacement sausages respectively. The redness (a*) of the sausages exhibited an increase with the increase in blood. They were all significantly different at $P<0.05$. The yellowness exhibited mixed results for the different fat levels although the full fat and the 10% blood inclusion showed no significant differences with a 5% fat reduction.

The color of a product is a fundamental aspect in regard to marketing, branding and acceptability by the consumer. Color is the single most important factor of meat products that influences consumer buying decisions, as it indicates freshness or otherwise of the product (Boles and Pegg, 2010). Color and product appearance are very important criteria that influence consumer patronage (Comfort, 1994).Color represents perceived freshness and is of

vital importance to the meat industry and meat science research (Mancini and Hunt, 2005). The decreasing color ratings with fat reduction can also be attributed to the fact that increasing the fat content in meat products also influences lightness, redness, and yellowness (Troutt *et al.,* 1992; Hughes *et al.,* 1997). Basicallythe reduction of fat level in sausages makes them darker and redder (Muguerza *et al*., 2002).

Table 4.8: Color characteristics of the sausages in Lightness (L*), Redness (a*) and yellowness (b*).

BLOOD LEVEL (%)	FAT LEVEL (%)	COLOR MEASUREMENTS		
		L*	a*	b*
0	**20**	66.9±0.71^{c}	12.7±0.85^{a}	13.9±0.14^{a}
5	**15**	54.30±0.28^{b}	20.1±0.28^{b}	13.0±0.85^{b}
10	**10**	40.6±0.28^{a}	28.7±0.99^{c}	14.3±0.28^{a}

Superscripts abc Indicates that means along the same row with similar superscripts are not significantly different ($P<0.05$).

4.8.5 Cost benefits

The comparative production cost for the control, 5% and 10% blood inclusion showed a cost benefit to the processor (Table 4.9). Since all the other ingredients and materials were constant, only the Fat cost varied from the control. Blood was obtained for free from the slaughterhouse which was the aspect that brought the cost relieve on the fat cost during replacements. The total production cost per kilo for the control was 281 Kenya shillings since it required full fat in its formulation. The 5% and 10% fat replacement cost 268.50 and 256.00 shillings respectively. That accounts for 12.50 and 25.00 shillings cost relief per kilo

respectively which translates to 4.5% and 8.9% cost savings for the blood sausages production.

It is clearly cheaper for the commercial processors to use blood as a sausage production raw material. The highest replacement of fat with blood may however not provide the sausage quality closest to the control one though it is still viable for commercial production. The sausage formulation that comes closest to the control is the one that contains 5% blood that saves 12.50 shillings per kilo. This translates to 12,500 and 25,000 shillings per tonne respectively. The savings can be translated as extra earnings for the processor and the slaughter house operator. This agrees with Bowater and Gustafson (1988) that up to 15% of slaughterhouse income could come from by-products.

Table 4.9: Cost analysis of the developed blood sausages

Item	**Control cost**	**5% blood**	**10% blood**
	Ksh	**Ksh**	**Ksh**
Meat	180	180	180
Fat	50	12.5	25
Blood	-	0	0
Ingredients	30	30	30
Additives/seasonings	3	3	3
Packaging	3	3	3
Transport	10	10	10
Labour	5	5	5
Total	281	268.5	256

*1kg sausage production

4.8.6 Shelf life analysis

4.8.6.1 Peroxide value of the sausages

The fat peroxidation profile for the sausages is shown in Figure 4.2. The highest amount as represented as oxygen milliequivalents (meq) per kilogram was 18.3meq/kg for the full fat sausage at day 14 with the lowest being 8.5 meq/kg for the full fat sausages on day 0. All the sausages showed an increasing trend for the peroxidation through-out the storage period but the quantities reduced with the reduction in fat content of the respective sausages. The 10% fat replacement exhibited the lowest peroxidation with 15.1 meq/kg, 12 meq/kg and 6.4 meq/kg for days 0, 7 and 14 respectively. The lower the fat content the lower the peroxidation for the sausages.

The peroxide value is used to show extent of spoilage through fat oxidation therefore the higher the fat content, the higher the peroxidation and value.Lipid peroxidation in food is of importance, in that it progresses at faster rates in products with higher fat contents (Warriss, 2010). The peroxide value is low during the beginning of a food's shelf life (Abou-Charbia, 2002).The polyunsaturated and unsaturated fatty acids present in the fats, usually react with oxygen to form fatty acid hydro- peroxides.

Hydro-peroxides are unstable, and breakdown into various compounds which consequently produce off-flavours; leading to a stale, rancid flavour in foods (Kerler and Grosch, 1996). Among the sausages however, the peroxide values were much lower than 25millequivalent/kg sausage, which is considered as the limit of acceptability in fatty foods (Evranuz, 1993; Narasimhan*et al*., 1986). According to Bimbo (1998), the acceptable peroxide values was found to be between 3 and 20 meq/Kg. Fat replacement with blood at both 5% and 10% exhibited very good keeping qualities in a sausage since all the sausages were far much below the limits. Sausages with 10% fat replaced with blood had the best storability at less than20

meq/Kg for the 15 days at 4ºC, which is the limit above which deterioration of the organoleptic and nutritional characteristics of foodstuffs occur (Bimbo, 1998).

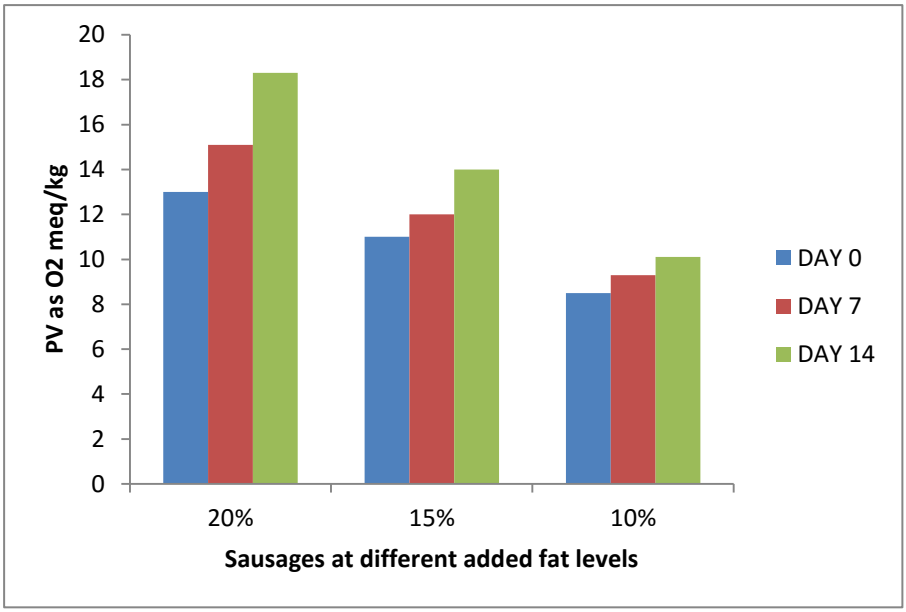

Figure 4.2: Peroxide values of the sausages at different storage times at 4°C

4.9 Conclusions

Low fat acceptable sausages can be processed by using blood up to 10% as a direct fat substitute without significantly affecting the physico-chemical and sensory properties of the sausage. However, the 5% fat replacement produces the sausages closest to the conventional sausages in physico-chemical, nutritional and keeping qualities. Blood sausages are also chemically stable and can be stored for equally the same time as conventional sausages. Blood can be a cheap substitute for the animal fat used in sausage formulation, with its use bringing down the production cost by an impressive 8.9%. Blood inclusion in a sausage brings about many benefits to the consumer including higher proteins, low fat, and high iron content enough to match the RDA for an adult human.

What’s more, the slaughterhouse operator can improve the earnings from the blood sale to the processor that normally would have been released as a waste. It is also a relief to the environment which suffers the wrath of the disposed blood that translates to a health hazard to human and animal lives at large as well.

CHAPTER FIVE: HYGIENE STATUS OF SAUSAGES DEVELOPED WITH BLOOD AS FAT REPLACER.

5.1 Abstract.

Blood is known to favor microbial growth whenever it is incorporated in any food stuff or used as a stand-alone food component. On the other hand, blood has found many uses around the world owing to its diversity in its applicability. While blood can be the major source of microbial contamination in food, personnel hygiene and equipment handling during production is the other potential source of contamination. This project therefore was designed to find out the fitness and suitability for consumption of sausages produced with blood as a direct fat replacer. Sausages were formulated with fresh liquid blood with replacements up to 10% fat replacement. Proportions of 0%, 5% and 10% were the sequences of replacement applied. Standard analytical methods were used for the laboratory analysis in days 0, 7 and 14 for sausages stored at 4 degrees Celsius. The results showed an impressive trend which in turn indicated good handling and hygienic source of blood. The microbial count for the 14 day storage period showed levels that are within the Kenya bureau of Standards (KEBS). *Salmonella spp, Listeria monocytogens, Campylobacter and Escherichia coli were all absent.* The highest counts for *Staphylococcus aureus, Clostridium Perfringens,* and TVC were 1.16 $\log_{10}$ cfu/g, 1.47 $\log_{10}$ cfu/g and 5.27 $\log_{10}$ cfu/g respectively. These were lower than the KEBS recommended values below 6.0 $\log_{10}$ cfu/g, 2.0 $\log_{10}$ cfu/g and 2.0 $\log_{10}$ cfu/g for TVC, *staphylococcus aureus and Clostridium perfringens* respectively. The study therefore clearly indicates that it is possible to use blood as fat replacer in sausage production without adversely affecting the hygiene. Clean and proper handling during processing is paramount to hygienic product.

Key words: *Colony forming units, Hygiene, Microbial contamination, Pathogen*.

This chapter has been submitted for review in the African Journal of Food, Agriculture, Nutrition and Development (AJFAND) as: Hygiene status of sausages developed with blood as fat replacer: P. M. Mathi*[1], C. N. Kunyanga, J. N. Gichure, J. K. Imungi

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5.2 Introduction

Sausages are some of the meat products that possess high potential of microbiological spoilage. Blood is one of the best sources of protein. In fact is has in some cases been referred to as 'liquid protein' (Putman, 1975). All over the world, blood is being produced after slaughter and sadly majority of it goes to waste. This is despite the fact that it has the potential to provide higher and cheaper protein source than eggs. For instance, according to Wang *et al* (2007), it has been estimated that China produces about 1,500,000 tons of blood yearly. This has a protein content equivalent to that of 2,000,000 tons of meat or 2,500,000 tons of eggs (Wang *et al*., 2007).

On the other hand, blood is one of the most potent microbial broths used to proliferate microbial growth in laboratories for research institutions. Inclusion of blood as an ingredient therefore increases the vulnerability of microbial attack on the sausages. It is crucial to ensure that blood used for the sausage or food product processing is fit for human consumption. While blood is not entirely the source of pathogens and harmful microbes, it cannot be ignored as an important source of the same. Improper handling and processing has been blamed for pathogenic invasion of blood related products according to Bachir and Mehio (2001). *Salmonella spp, Listeria monocytogens, clostridium perfrigens* and *campylobacter* are usually of particular interest as they serve as indicators of food safety. They have been found to be dominant in meat products and related to disease outbreaks according to Jacxsens (2009). *Listeria* and *Salmonella* are among the most lethal pathogens and are responsible for

1500 deaths annually in the USA (Hsieh and Ofori, 2011). *Escherichia coli* and *Staphylococci aureus* are important indicators of faecal contamination of the blood and personal hygiene respectively.

There are several ways that are typically employed to protect against that growth of bacteria in blood laced products. Cold treatment remains the most common coupled with HACCP system application in the processing areas (Ramos-Clamont *et al*., 2003). Use of salts like nitrites and nitrates has also been a successful deterrent of bacterial growth in meat products. Bacterial growth inhibitors such as nicin have as well been used to reduce undesirable bacteria growth in meat products (Cutter and Siragusa, 1998). Food hygiene and safety is clearly then ensured through clean portion usage and operational cleanliness.

5.3 Study design and methodology.

The study was carried out at the Department of Food Science, Nutrition and Technology, College of Agriculture and Veterinary Science, University of Nairobi. The Analytical tests were carried out in the food chemistry and food microbiology laboratories.

5.4 Methodology

5.4.1 Sausage production

The fat replacement ratios are shown in Table 5.1 expressed as percentages per kilogram. The meat and blood were obtained in a local slaughterhouse (Bahati slaughterhouse, Limuru) which possesses the required authorisation from local authorities about health and hygiene practices. Other ingredients were bought from authorised suppliers in Nairobi city.

5.4.2 Experimental design

Experimental design is as has been described in chapter 4 section 4.3.2 in table 4.4.

Table 5.1: The fat, blood ratios in both gm and percentages

SAMPLE	BLOOD (%)	QUANTITY(g)	FAT (%)	QUANTITY(g)
1	0	0	20	200
2	5	50	15	150
3	10	100	10	100
4	15	150	5	50
5	20	200	0	0

The blood was carefully collected at the slitting stage. Sharp knives pre-sterilized in boiling water was used to severe the jugular of the cow. The slit cow/animal was swiftly hoisted for bleeding with the head angled in such a way that it did not come into contact with the gushing blood. Collection was done immediately after hoisting in a cooled container; a large mouthed sterile plastic container dipped in ice cubes. It was transported in cool boxes and stored below 4ºC to retain freshness and prevent microbial spoilage.

Fresh boneless semi membranous beef from the thigh muscle immediately after slaughter was chopped into smaller pieces and minced with a 5mm sieve using a table top mincer. The minced meat was divided into five batches of equal quantities. The batches contained the standard sausage ingredients and fresh blood at different levels of substitution as shown in Table 5.2. Each treatment group was replicated three times. Additives and seasonings used in the formulations are shown in Table 5.3. Figure 5.1 shows the sausage production process flow.

Table 5.2: Ingredients in gm/kg for the sausages

SAMPLE	BEEF	BLOOD	FAT	ICE	WHEAT FLOUR	RUSK	CORN STARCH	ADDITIVES	SEASONINGS
1	500	0	200	180	60	40	20	19.8	3
2	500	50	150	180	60	40	20	19.8	3
3	500	100	100	180	60	40	20	19.8	3
Total	**1500**	**150**	**450**	**540**	**180**	**120**	**120**	**59.4**	**9**

Table 5.3: Typical additives and seasonings for a 1 kg sausage formulation

Additives	Quantity (gm.)	Seasonings	Quantity (gm.)
Common salt	16	White pepper	2
Sodium tripolyphosphate(STTP)	3	Nutmeg	0.3
Ascorbic acid	0.3	Mace ground	0.3
Monosodium glutamate(MSG)	0.5	Coriander & Ginger	0.4
Total	**19.8**	**Total**	**3**

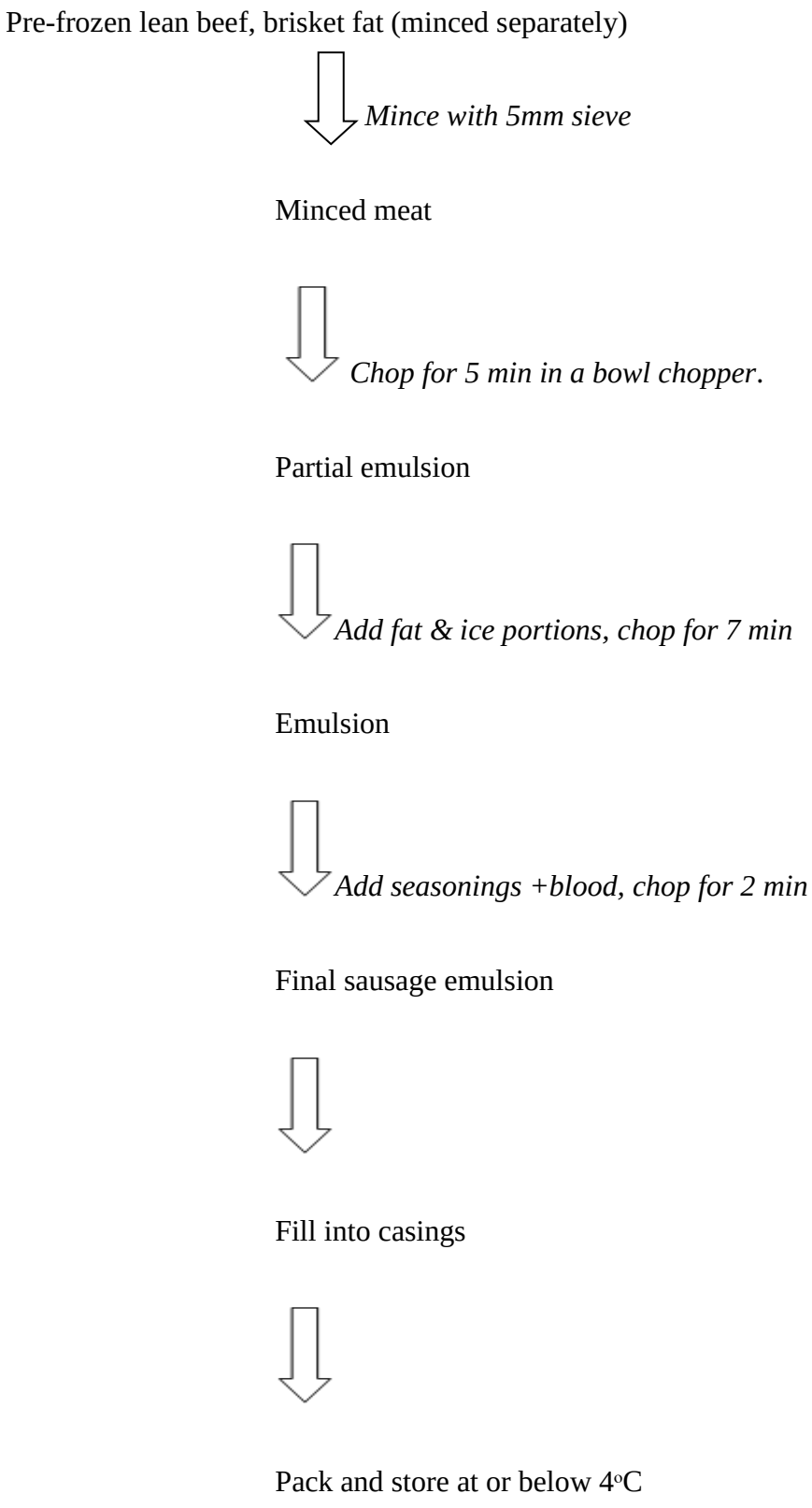

Figure 5.1: Sausage production process flow

5.5 Analytical methods

The sausages were analysed for various microorganisms to determine the microbial profile so as to predict the general hygiene and overall keeping quality. For proper comparison with the sausages in the commercial market, the tests were carried out for a period of 14 days. This was aimed at accurate estimation of microbial shelf life vis-a-vis the conventional sausages.

Tests were carried out in days zero, seven and fourteen on sausages stored in a refrigerator set at a constant 4ºC. Of interest were organisms that are relevant to meat safety problems. *Salmonella spp, Listeria monocytogens, clostridium perfrigens* and *campylobacter* were of particular interest since they serve as indicators of food safety. These have been found to be dominant in meat products and related to disease outbreaks according to Jacxsens (2009). *Escherichia coli* was analysed as hygiene indicator especially for faecal contamination. *Staphylococcus aureus* was used as an indicator of personal hygiene during processing while total viable count (TVC) were analysed as indicator of overall microbial quality of the sausages.

5.5.1 Determination of Total Viable Counts

ISO method 4833:2003 (ISO 2003) was used for the total viable count (TVC) enumeration. These are basically aerobic mesophilic bacteria. Duplicate plates containing plate count agar were used in the process. Incubation was done at 30±1ºC for 72±3 hours after which microbial counts were expressed as numbers of cfu/g of the sample.

5.5.2 Determination of *Escherichia coli*

Based on ISO method 16649-2:2001(ISO 2001), the *E. coli* was accordingly enumerated.10g of sample was homogenized in 90ml peptone water. Decimal serial dilutions of the homogenized solution in sterile peptone water were prepared and plated in duplicate on the selective agar media. Blue green colonies for *E. coli* were counted after 48 hours of

incubation at 44ºC. The number of colony forming units (CFU) of presumptive *E.coli* per gram of sample was calculated.

5.5.3 Determination of *Salmonella*

The ISO method 6579:2002 (ISO 2002) was used to enumerate the *salmonella* species. 25g of sample was blended in buffered peptone water and incubated at 37±1ºC for 18±2 hours. From pre-enrichment broth, the inoculums were transferred to Rappaport- Vassiliadis broth and selenite cysteine broth and then incubated at 41.5±1ºC and 37±1ºC for 24 hours for selective enrichment. A loopful of the selective enrichment was streaked onto two solid selective media: Brilliant green agar (BGA) and xylose lysine desoxycholate agar (XLD). XLD agar was incubated at 37±1ºC and observed after 24±3 hours for typical *salmonella* transparent red halo and a black centre.

5.5.4 Determination of *Staphylococcus aureus*

EN ISO method 6888-1:1999 (ISO 1999) was used for the detection and enumeration of *Staphylococcus aureus.* In a sterile pipette, 0.1ml of the appropriate sample test dilutions were transferred in duplicate onto the Baird Parker agar (BPA). The plates were then incubated at 35-37ºC for 24±2 hours, then re-incubated for further 24±2 hours. Observation ensued for typical colonies appearing black or grey, shining and convex,1-1.5mm in diameter after 24hours and 1.5-2.5mm after 48 hours of incubation, surrounded by a clear zone but partially opaque zone. The coagulase positive staphylococci were then expressed as cfu/g of sample.

5.5.5 Determination of *Listeria monocytogens*

Method 11290-01:2004 (ISO 2004) was used to enumerate the organism *Listeria monocytogen.* 225ml of Fraser broth with reduced concentration of selective agents otherwise known as half Fraser broth as selective enrichment media was blended with 25g of test sample. 0.1ml of the selective enrichment culture was transferred into 10ml of secondary

enrichment media and incubated at 35ºC for 48±3 hours. Plating was done using *Listeria* agar. Agar listeria incubation was done at 37ºC for 24±3 hours. Carbohydrate utilization test was used to confirm the *Listeria monocytogens*. Typical *Listeria* colonies appear blue green with an opaque halo on the *Listeria* agar.

5.5.6 Determination of *Campylobacter*

Enumeration was done using ISO method 10272-2006 (ISO 2006). 225ml of liquid enrichment medium (Bolton broth) was added to 25g of the test sample. The contents were then incubated at 37ºC for 4-6 hours and then 41.5ºC for 44±4 hours after which incubation in a modified charcoal cefoperazonedeoxycholate agar (mCCD) at 41.5ºC for 44±4 hours was done. For confirmation purposes, at least one suspected *campylobacter* colony from each plate of selective media and a further four colonies if the first were negative were taken. Typical colonies are seen as greyish on mCCD agar, often having a metallic sheen and are flat and moist with a tendency to spread. The numbers of *campylobacter* per gram of the sample were calculated from the number of colonies per plate.

5.5.7 Determination of *Clostridium perfringens*

Enumeration was done using the ISO method 7937:2004 (ISO 2004). 1ml of appropriate sample dilutions were transferred and inoculated in a sterile pipette into empty petri dishes. 10ml of the sulphite-cycloserine agar (SC) which maintained at 44-47ºC in the water bath was poured into the petri dishes and mixed well with the inoculum by gently rotating each dish. After the media solidification, a 10ml over layer of the CS was added and allowed to solidify. The plates were then incubated under anaerobic conditions at 37ºC for 20±2 hours. Plates representing successive dilutions and containing less than 150 colonies were selected and counted for the characteristic colonies presumptive *Clostridium perfringens* on each plate after the specified incubation period. Five selective colonies were selected and confirmed

using Lactose sulphite medium test (LS) which has a very specific reaction to *clostridium perfrigens* when incubated at 46^{o}C.

In this particular confirmatory test, each colony selected was inoculated into fluid thioglycolate media and then incubated under anaerobic conditions at 37^{o}C for 24 hours. After incubation, 5 drops of the thioglycolate culture was transferred to the Lactose sulphite media with a sterile pipette. Incubation was then done aerobically at 46 for 24 hours in the water bath. Tubes containing lactose sulphite media were examined for the production of gas and the presence of a black colour (iron sulphate precipitate). Durham tubes with over a quarter full of gas and tubes having the black precipitate were considered positive for the *Clostridium perfringens* which was expressed as cfu/g.

5.6 Data analysis

After enumeration, the counts were represented as colony forming units per gram (cfu/g). Microsoft excel was used to convert the figures into logarithmic version presented as $\log_{10}$.

5.7 Results and discussion

Table 5.4 shows the microbial profile for the sausages at different storage times and fat replacements.

Samonella spp, Listeria monocytogens, Campylobacter and *Escherichia coli* were absent in the sausages. The microbial profile of the sausages as per Kenya bureau of standards (KEBS) legal limits (KS 2455:2013, KS59-2:2013) requires that *Salmonella spp, Listeria monocytogens* and *Campylobacter* be absent. Jacxsens (2009), lists *Listeria Monocytogens, Salmonella spp, Clostrifium perfringens and Campylobacter* as critical organisms that determine food safety status all around the world. What's more, *Listeria Monocytogens,* and

Salmonella spp have been found to cause over 1500 fatalities in the USA through food contamination as reported by Hsieh and Ofori in study done in 2011.

Several ways of microbial infestation control have been discussed in previous research for different meat products. The most effective of them have been to employ a HACCP system during production. However a prerequisite for the development of HACCP programmes is observance of good manufacturing practice (GMP) (Sperber *et al.*, 1998). Cold treatment of meat products have been found to work against most organisms including *Samonella spp*, *Listeria monocytogens*, and *Campylobacter* according to Cutter and Siragusa (1998). *Escherichia coli* have been reported in meat products including sausages and blood laced products. According to Bachir and Mehio (2001), improper or unhygienic food handling increases the chances of contamination by *Escherichia coli.* Therefore, absence of *Escherichia coli* in the sausages demonstrates hygienic handling as well as clean equipment and work area during the sausages processing.

5.7.1 Total viable count (TVC)

The TVC exhibited an increasing trend with the increase in blood content although the general count decreased as days passed by through-out the storage period. The highest value was for the 10% fat blood sausages on day zero at 5.27. On the other hand, there was a declining count throughout the storage period with the full fat sausages having the highest $\log_{10}$ cfu/g while the lowest was 3.97 $\log_{10}$ cfu/g on day 14 for the full fat sausages. The TVC levels are well within the Kenyan safe limits for meat products which should not exceed 6.0 $\log_{10}$ cfu/g.

The high counts trend exhibited in the reducing level content of the sausages contradict with a study done by Reagan and others, (1983) that found increasing microbial numbers with higher fat levels. This could be attributed to the highly nutritious blood in the sausages that make a

good microbial broth as well as the high water activity caused by the increasing blood in the sausages. Lack of nitrite pickling salt in the sausages that act as an anti-oxidant that prevent growth of bacteria could also explain the high number of organisms deep into the storage period according to Marta and others, (2004).

5.7.2 Staphylococcus aureus

The *Staphylococcus aureus* count decreased with the increased fat replacement while also reducing with the prolonged storage period. The highest count for *Staphylococcus aureus* was 1.16 $\log_{10}$ cfu/g for full fat sausage on day zero while the lowest was 1.0 $\log_{10}$ cfu/g for the 10% fat replacement on day 14. The numbers fall within the Kenya Bureau of Standards (kebs), legal limits that set it at 2.0 $\log_{10}$ cfu/g. The decreasing numbers can be explained by the inactivation of the organisms by the low storage temperatures during storage. *Staphylococcus aureus* is usually used to indicate the efficacy of proper human handling of food during processing. As observed by Bachir and Mehio (2001), improper handling has in the past introduced pathogens in food.

5.7.3 Clostridium Perfringens

The highest count for *Clostridium perfringens* was 1.47 $\log_{10}$ cfu/g for the control sausages on day zero while the lowest count was in day 14 for the 10% fat replacement at 1.25 $\log_{10}$ cfu/g. The counts were way below the lethal limit set out by KEBS at 4.0 $\log_{10}$ cfu/g. The decreasing trend for the organism can be accounted for by the low storage temperatures. *Clostridium perfringens* are important food safety indicators that significantly feature in food borne disease outbreaks. The same organisms are actually linked to meat products bans by importing countries (Heinitz *et al*., 2000).

Table 5.4: Microbial profile of the sausages during the storage showing staphylococcus aureus, Clostridium perfringens and TVC in log_{10}cfu/g

	DAY 0			**DAY 7**			**DAY 14**		
Fat:Blood (%)	**20:0**	**15:0**	**10:10**	**20:0**	**15:5**	**10:10**	**20:0**	**15:5**	**10:10**
Staphylococcus aureus	1.16	1.13	1.02	1.09	1.02	1.02	1.06	1.06	1.0
Clostridium perfringens	1.47	1.39	1.42	1.43	1.38	1.34	1.38	1.27	1.25
TVC	5.24	5.26	5.27	4.48	4.59	5.02	3.97	4.39	4.44

5.8 Conclusion

The research proved that it is practically possible to use blood in sausage production without adversely affecting the microbial keeping quality. Proper blood collection and hygienic handling during the sausage production is paramount to ensure an acceptable general hygiene. It is important that other shelf life enhancing ways be established to increase the sausages longevity.

CHAPTER SIX: GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

Utilization of the slaughter by-products found to be very low. The biggest losses occur with blood at the slaughterhouses since other organs are utilized differently by different pastoral communities. The communities showed some ways of blood utilization although lack of technology limits the amount of blood intake and the stability of the local products they made.

Using blood as a partial fat replacer in a fresh sausage is a highly beneficial endeavour that does not only reduce the post slaughter losses but contributes to the nutrition status of the population. While it is possible to replace fat with blood for up to 10%, the sausages with the closest characteristics to the conventional sausages are the ones at 5% replacement. The blood containing sausages exhibited significantly high protein content, lower fat content, high iron and equally desirable eating and keeping qualities. This provides a platform for consumers to experience a product that is healthier and potentially cheaper than the conventional ones in the market without necessarily affecting the attributes they crave for.

The possibility of producing sausages at a cheaper cost for the processors is real. The blood sausage can save the processor up to 25 Kenya shillings per kilo, about 9% less in production cost. The blood uptake has a potential to generate more income to the slaughter house operator through blood sales that will serve as raw material for the processor. It is also possible to produce safe and hygienic sausages using blood as an ingredient as proved by the microbial examination outcome.

Through innovative product development, one is able to address a multifaceted concoction of problems by proper by-products utilization. Blood sausage addresses the food security problem without encountering adverse effects on the regional ecological and economic

situation. The environmental nightmare that is blood in the slaughterhouses has the potential to become gold as has been found out in this study.

6.2 Recommendations

It is important to encourage the population to utilize the by-products using the traditional knowledge they have to reduce the post slaughter losses since it would improve their food security status. Dissemination of information especially new technologies that can help reduce losses should be enhanced since the communities in the study area seemed eager to learn and embrace new ideas. There is need to come up with more technologies in new product development that will uptake more by-products to reduce wastage. This will also enhance income to the slaughter house operators through sales of what will be new raw materials.

Other ways to utilize blood need to be pursued to enhance more utilization. Some of the potential uses include; inclusion in other meat products such as smokie, meat balls, nutritious biscuits for vulnerable groups among others. Non-food uses may include processing the blood for animal feeds and for use as a fertilizer component.

Awareness creation among communities is highly necessary to remove the traditions and beliefs that hinder some by-products intake. Educating the communities that by-products especially blood intake is healthy and nutritious would see a leap in nutritional health and food security assurance. There is need to research further and come up with better, simple and efficient blood collection methods to enhance improved hygiene of the sausages.

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APPENDICES

Appendix 1: Slaughterhouse and focus group discussion interview guide

Surveyor **……**

Project **…….**

Date **…….**

Establishment/slaughter house…….

Qn 1. Which type of animals do you slaughter? Tick appropriately.

a) Cattle
b) Goats
c) Sheep
d) Camel
e) Donkey
f) Others (specify)……

Qn 2. Where do you source your animals? Tick appropriately.

a) Ranches
b) Dairy culls
c) Pastoralist areas/ ASALS
d) Other sources (specify)……

Qn 3. How many animals do you slaughter on a daily basis?

a) 1-10
b) 10-50
c) 50-100
d) 100-200
e) Above 200.

Qn 4. What are the by-products you get after slaughtering your animals? Tick appropriately.

a) Blood
b) Offal
c) Legs
d) Skin and hide
e) Horns and hooves
f) Tendons and sinews
g) Others (specify)……

Qn 5. How do you utilize the above mentioned by products? Briefly describe.

a) Blood……
b) Offal……..
c) Legs……
d) Skin and hides……..
e) Horns and hooves…….
f) Tendons and sinews…….
g) Others…… …… ……..

NB. For legs

a) Sell to soup/stork makers
b) Give away to domestic animals
c) Sell as meat on bone
d) Other ways (specify)……

Qn 6. Which meat products do you make? Tick appropriately.

a) Fresh cuts

b) Canned products

c) Sausages/smokies

d) Others (specify)

Qn 7. Which ingredients do you use for the above products?

a) Fresh cuts……
……
……..

b) Canned products……
……
……
……

c) Sausages/smokies……
……
……
……..

d) Others……
……
……
……

Q 8. In your opinion, do you think there are other meat products that can be made using either fresh meat or by-products? If yes, which product and how would they be made.

……
……
……
……

Appendix 2: Sensory evaluation score sheet.

Score sheet number ……

Date of analysis ……/……/2015

Name/initials of analyst ……....

Sensory evaluation

You are provided with coded samples of fresh beef sausages. Please evaluate each of the samples presented to you for five 5 sensory attributes namely; **odour, colour, texture, taste and overall acceptability**. Each sensory attribute is represented by a hedonic scale ranging from "1=like extremely) and "5=dislike extremely". Score each attribute using the scale provided at the bottom of the page and record the score of your response in the appropriate space on the grid provided. You will be provided with water and crackers in between samples. Please provide a general comment on the sensory properties of the samples at the end of the exercise.

Sample	**Odour**	**Colour**	**Texture**	**Taste**	**Overall acceptability**
XKL					
MPZ					
WTR					
ASN					
DCF					

Scale

Score

1……like extremely
2……like moderately
3……neither like nor dislike
4……dislike moderately
5……dislike extremely
Comments……
……
……
……
……