STUDIES ON THE USE OF HERBS TO PRESERVE MEAT AND MILK AMONG THE PASTORAL COMMUNITIES OF WEST POKOT IN KENYA

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Studies on the Use of Herbs to Preserve Meat and Milk among the Pastoral Communities of West Pokot in Kenya

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DECLARATION

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

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DEDICATION

This thesis is dedicated to my wife Josephine K. Ongeri, my children Wendy Moraa Ongeri, Richard Nyaberi Ongeri Jnr and Winphine Bosibori Ongeri, who gave me a reason to study.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Analytical Chemistry
ASAL	Arid and Semi Arid Land
ATCC	American Type Culture Collection
BSLB	Brine Shrimp Lethality Bioassay
BMPA	British Meat Processors Association
BHT	Butylated hydroxytoluene
BHA	Butylated hydroxyanisole
DMSO	Dimethyl sulphoxide
DPPH	2, 2 diphenyl picrylhydrazyl
EU	European Union
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agricultural Research Institute
KNBS	Kenya National Bureau of Statistics
LAB	Lactic Acid Bacteria
LC	Least Concentration
MIC	Minimum Inhibitory Concentration
MA	Malonaldehyde
MS	Sodium metabisulphite
PDA	Potato dextrose agar
PG	Propyl gallate
PCA	Plate Count Agar
SL	Sodium lactate

SDA	Sodium diacetate
SA	Sodium acetate
SAS	Statistical Analysis System
TBARS	Thiobarbituric acid reactive substances
TVB-N	Total volatile base nitrogen
TBHQ	Tert-butyl hydroquinone
TCA	Trichloroacetic acid
TVC	Total viable count
UV	Ultraviolet
VBN	Volatile Base Nitrogen
VRBGA	Violet-red bile glucose agar
WHO	Word Health Organization
WFP	World Food Program
OTI	*Tamarindus indica* L
AZA	*Ziziphus abyssinica* A. Rich
KOIC	*Ozoroa insignis* Del.
SSDC	*Senna didymobotrya* Fresen Irwin and Barneby

ABSTRACT

The pastoral communities of West Pokot have been using herbs to preserve both meat and milk throughout the ages. These naturally occurring herbs are gaining popularity as the chemical preservatives become less acceptable among consumers due to health concerns. The purpose of this study therefore, was to determine the preservative potential of herbs used by the pastoralists of West Pokot in the preservation of meat, milk and their products.

The study involved collection and identification of samples using a structured questionnaire. Water, methanol and chloroform extracts were obtained, from which, antimicrobial, antioxidant, phytochemical and toxicity tests were performed on each of the herbs. The quality changes during preservation of milk treated with the herb *Ozoroa insignis* Del was analyzed for Titratable acidity, pH, plate and LAB count within 14 Days of storage. The quality changes in meat were assessed by making pork sausages and analyzing for total viable count (TVC), *S. aureus* and *E. coli*, TVB-N and rancidity tests over a period of 14 days. Samples were stored at both 4ºC and 25ºC.

The phytochemicals mostly found in the identified herbs, *Ozoroa insignis* Del, *Senna didymobotrya* Fresen Irwin and Barneby, *Tamarindus indicus* L and *Ziziphus abyssinica* include, reducing compounds, sterols and steroids, alkaloids, saponins, flavonoids and condensed tannins.

Methanol and chloroform extracts of *Ozoroa insignis* Del and *Senna didymobotrya* Fresen Irwin and Barneby had antimicrobial activity against *E. coli* and *P. aeruginosa,* while water and methanol extracts of *Tamarindus indicus* L had antimicrobial activity against all the test microorganisms. *Ziziphus abyssinica* inhibited *E. coli* and *S. aureous* only. *Tamarindus indica* L, *Ozoroa insignis* Del, *Senna didymobotrya* Fresen Irwin and Barneby and *Ziziphus abyssinica* had antioxidant capacity reducing DPPH by 86%, 88%, 92%, and 96% respectively.

Milk incorporated which *Ozoroa insignis* Del had its Percentage acidity stabilized at 2.8% and the pH stabilized at 3.8. All the herbs were unable to preserve sausages beyond the third day at 25ºC, while at 4ºC the sausages incorporated with the aqueous extracts of *Tamarindus indica* preserved for up to fifteen days. Those with the aqueous extracts of *Ziziphus abyssinica* at 4ºC were able to preserve for 10 days. *Tamarindus indica* was a strong antimicrobial agent while *Ziziphus abyssinica* was a strong antioxidant. These results indicated that the herbs had the potential to preserve meat sausages.

Further research on quantitative analysis of the phytochemicals needs to be done. A product incorporating the related identified herbs such as *Ziziphus abyssinica*, *Tamarindus indica* and *Ozoroa insignis* Del, *Senna didymobotrya* Fresen Irwin and Barneby should be developed.

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CHAPTER 1

1.0. INTRODUCTION

1.1. Background

The word herb refers to herbaceous or short-stemmed plants that are valued for their preservative, flavour, fragrance and curative properties. Herbs are and have been an integral part in the life of many indigenous communities for centuries. They provide building materials, fodder, weapons, medicine, flavour enhancers, nutritional additives and appetizers (Uebelherr, 2005; Sidigia et al., 1990). As much as the people of West Pokot use herbs extensively for various purposes including preservation of their food, other communities such as the Maasai, Borana, Sabot and the Kikuyu practice the same (Kareru et al., 2007). Different parts of these herbs are used and include leaves, roots, seeds, stems, stem barks, and even the whole plant. In some cases a mixture of various herbs are used to meet a specific objective. These herbs are prepared and utilized in different forms including, liquid extracts, charcoal, ash, powders and even whole fresh plant parts.

Herbs like many other plants readily synthesize phytochemicals for defense against attack by insects, herbivores and microorganisms (Majorie, 1999). A number of these phytochemicals are known to retard microbial growth and oxidative degradation thereby improving the quality and nutritional value of foods. The most important of these phytochemicals in plants are alkaloids and phenolic compounds such as tannins and flavonoids (Hill, 1952). The presence of these phytochemicals has made herbs be of great interest to the food industry.

Highly complex, value added processed products, have been introduced into the market, these products require longer shelf life and greater assurance of freedom from food borne pathogenic micro organisms. In order to attain longer shelf life, various preservation techniques have been put into use. These include temperature reduction, moisture reduction and direct microbial inhibition. This is achieved by freezing, drying, heat treatment or the use of synthetic preservatives.

Synthetic preservatives such as, *butylated hydroxytoluene* (BHT), *butylated hydroxyanisole* (BHA), *tert-butyl hydroquinone* (TBHQ), *propyl gallate* (PG), sulphites, sorbic, sorbates benzoic, nitrites, sodium metabisulphite and many others, have for a long time been the choice of food industries that produce large amounts of products. These chemical preservatives have all along been used due to their affordability and effectiveness (Daniells, 2006). However, these chemical preservatives are perceived to be harmful by health conscious consumers due to their potential toxicity. This has lead to increased pressure on food manufacturers to either completely remove them from food products or adopt more natural alternatives for food preservation (Steinman, 2006; Murray et al., 1999). Herbs provide a viable natural alternative source of preservatives that are readily acceptable by health conscious consumers. (Voravuthikunchai et al., 2004a).

1.2. Statement of the problem

Synthetic preservatives are being discouraged in the market because of their actual or presumed side effects. Conventional methods of preserving meat and milk products are expensive and inaccessible particularly in developing countries were supporting infrastructure is absent. If this indigenous knowledge of preserving food

using herbs, that the people of West Pokot have was adapted, and the herbs analyzed, better preservatives can be realized, that will be effective and much better accepted by a wide range of consumers. Presently very little research has been done on the herbs that the pastoralists of West Pokot claim to preserve meat and milk. This information is passed on orally from one generation to another. If it is not harnessed, it is likely to be lost with time. Therefore, research in this area was timely to facilitate the preservation of knowledge on herbs used to preserve meat and milk.

1.3. Justification

Modern preservation methods have been unable to solve the problems of preserving meat and milk for the rural and pastoral people in the developing countries. The use of herbs is therefore preferred because they are relatively cheap, of medicinal importance, have no known side effects, are abundantly available and are consumed without fear of toxicity or residues that result from increased concentrations conventional preservatives in attempt to kill resistant strains of microorganisms. Foods preserved with these natural products are more acceptable and perceived as safe and nutritious. While a lot has been documented on the use of herbs as medicine, very little has been documented on their use as food preservative agents. This is because knowledge of herbs used as preservatives among the pastoralists of West Pokot is still transferred to generations by word of mouth. To avoid losing this information, while at the same time giving confidence among the new users of these herbs, it is important therefore, that a comprehensive research is undertaken to understand the constituents of these herbs and their capacity to preserve meat sausages and milk.

1.4. Objectives

The overall objective of this study was to determine the preservative potential of herbs used by the pastoralists of West Pokot in the preservation of meat, milk and their products.

The specific objectives were to: -

a) Collect, taxonomically identify and determine the herbs and parts thereof being used to preserve meat and milk by the communities in West Pokot District.
b) Identify and evaluate possible and potent active phytochemicals.
c) Determine possible effect of the herbs extracts on test organisms.
d) Evaluate the preservative action of the herb extract on milk and meat sausages.

1.5. Hypothesis

The hypothesis tested was: -

a) Herbs used by the pastoral communities of West Pokot to preserve their food do not have the capacity to preserve meat sausages and milk.

1.6. Research Questions

The research questions were: -

a) What type of herb is used?
b) Which parts of the herb are used and how are they administered?
c) Do these herbs have antimicrobial properties?
d) What compounds inherent in the herb contribute to their preservative effect?
e) Do these herbs really preserve food products?

CHAPTER 2

2.0. LITERATURE REVIEW

2.1. Geographical spread of herbs

Herbs are herbaceous or short-stemmed plants that are valued for their preservative, flavour, fragrance, and curative properties. They are used in preservation, perfumery, cosmetics and medicinal purposes. Most herbs grow to a maximum height of 30-90cm. Such are the herbs in this study which include *Ozoroa insignis*, *Senna didymobotrya (Fresen)* Irwin & Barneby and *Ziziphus abyssinica* A. Rich. A few aromatic trees, such as bay, *Tamarindus indicus*, grow to a height of 6-9 meters, yet they are also considered herbs. The valuable parts of many herbs are the leaf, flower, seed, stem, root, fruit pulp or entire plant. (Wikipedia, 2008).

Africa is one continent that is endowed with the richest biodiversity in the world. It has a variety of plants many of which are not only utilized as food but also for preservative and therapeutic purposes. The major reason is that, it consists of a geographical spread, spanning approximately 216,634,000 hectares of closed forest areas. Over 5000 different species of plants have been known to occur in these areas, among which many of them are useful in traditional preservation of food and curative purposes (Iwu, 1993). The use of herbs for preservation has been there for many years dating back as far as 3000BC (Cowan 1999, Maffi 1999). Despite enormous advances in conventional practices, preservation and curative practices using traditional herbs has been encouraged by the Word Health Organization, partly because some conventional drugs have failed to prove effective, and have serious side effects, on the users (Juhee et al., 2004).

2.2. **Preservation using herbs**

Herbs have been used by the pastoralists of West Pokot to preserve their meat and milk for generations. They have upheld their rich culture to date and depend on meat, milk, blood and herbs for their food. Meat and milk are highly perishable commodities because of their high protein and water content which is a good media for the growth of spoilage bacteria. As a result of the harsh climatic conditions, traditional methods of handling food, and their nomadic lifestyle, food spoilage is accelerated. Therefore, all their food is consumed at once to avoid losing it all together.

Traditional preservation techniques that improved flavour and keeping quality were later introduced. Meat was preserved by adding the fruit paste of *Tamarindus indica* L (Oron) or *Ziziphus abyssinica* A. Rich (Angau), while milk gourds were treated with several herbs including *Ozoroa insignis* Del (Kromwo), *Senna didymobotrya* Fresen Irwin and Barneby (Senetwo), *Lippia javanica* (Burm.f.) Spreng (Chepyetwo) or *Leucas calostachys* oliv (*Chepku-surwa*). These herbs were prepared differently depending on the product being preserved.

2.2.1. Preparation of *Kromwo* and *Senetwo* herbs for the preservation of milk

To prepare *Kromwo* and *Senetwo* for use in the preservation of milk, the stems of the herbs were peeled and dried. One end of the stick was burnt till it starts smouldering. The charcoal formed was dusted on the inner wall of the gourd till the wall was completely covered with the charcoal. The excess charcoal dust is removed using a brush made from the stem of palm leaves, and then milk is introduced. After

three days, whey is emptied and more milk introduced with the addition of more charcoal by sprinkling. The formed whey was poured out periodically till the gourd was full of milk. In this condition the pastoralists of West Pokot claim the milk is able to keep for up to a year without getting spoilt. The milk processed in this way is known as *mursik* (Mathara et al., 1995). The preservation process makes milk to store for longer in addition to enhancing its flavour, Milk within the West Pokot community and many other African communities is preferred sour, rather than fresh due to lactose intolerance (Swagerty et al., 2002). Though these methods of preservation have proved to work locally in many homes, they cannot be reproduced en masse and therefore not helpful during the dry spells.

2.2.2. Preparation of *Oron* and *Angau* herbs for the preservation of meat

These herbs are both harvested around the end of December and January. At this time most of the herbs have fruited and the rains have subsided and therefore the concentration of metabolites is at the highest (Freitas and Glories 1999). *Angaun* (AZA) fruit is harvested when ripe and has turned red in colour. It is dried and crushed. The hard particles of the seeds are separated and the remaining powder dissolved in water to form a paste. Meat that has been cut in strips and dried is immersed and left to dry. This herb preserves meat for a long time and the taste of meat remains the same. On the other hand, the fleshy part of *Oron* (OTI) fruit is dissolved in water to form a paste. Meat already cut in strips and dried is immersed in the paste and then put under the sun to further dry. In both these cases the meat remains in good state free from moulds and any other spoilage indicators for as long as a year, though the sour taste of *Oron* (OTI) remains in the meat.

Most of the preservation techniques basically involve the reduction of water activity, alteration of the pH and prevention of rancidity due to oxidation of the lipids in meat. Addition of herbs mainly prevents rancidity and is a deterrent to microbial infestation (Christoffell and William, 1997). Herbs have been in use for a long time in the field of preservation. “The wacky Romans” introduced coriander to Britain, where it become semi naturalized in some areas. It was used along with vinegar and cumin to preserve meat, legions would march carrying it to flavour their bread. In Roman Britain it was seen as a high status food as it was considered fairly exotic” (Jacobs, 2007).

2.3. Properties of herbs

Herbs exhibit various characteristics that include antimicrobial, antioxidant, antifungal, nutritive and anti nutritive properties (Lupina and Cripps, 1987). This has been brought about by the plants synthesizing chemical substances that bring about these effects. The reason why the plants synthesize these chemical substances was provided by Majorie (1999) as reported by Aboaba et al., (2005) that plants readily synthesize substances for defense against attack by insects, herbivores and same microorganisms. They also synthesize some of these products to enable them survive adverse conditions in order to propagate themselves.

2.3.1. Antibacterial properties

Natural antimicrobial systems are set to become an increasingly important component in food preservation methodology. One reason for this is that the consumers are rejecting the use of chemical preservatives but still demand food with

an acceptable shelf life (Gould, 1992). Herbs that have been found to have this kind of activity to name a few include the extract of water soluble arrowroot tea. This was able to inhibit some intestinal pathogens including *E. coli* 0157:H7. Rosemary extracts inhibited *staphylococcus aureus* in culture medium. Crude saponin extract from *S. bicolor* has useful antimicrobial properties (Nkere et al., 2005).

A recent study published by Juhee (2006), gives a boost to the sector of natural products by reporting that grape seed extract (*ActiVin*) and pine bark extract (*Pycnogenol*) performed better than butylated hydroxyanisole (BHA) and butylated hydroxytoluene(BHT) in retarding microbial contamination by *E. coli, Listeria,* and *Salmonella* of freshly ground beef. The same extracts also performed better at reducing oxidation of beef than the synthetic alternatives after nine days. Phenolic compounds are known to have antibacterial and antifungal properties. These are found in the skin of bananas and peels of unripe mangoes. They suppress the growth of *colletotrichum gloesporiodes hypae* till the fruit ripens. Antimicrobial activity of the *V. doniana* extract could also be attributed to the presence of phenolic compounds that have been linked with antimicrobial properties (Kilani, 2006).

Mathabe et al., (2005) found out that methanol, ethanol, acetone and hot water extracts from the bark, leaves, roots and stem rhizome of *Ozoroa insignis* (KOIC) were able to act against *Vibrio cholera*, *Escherichia coli* and *Staphylococcus aureus*, *Shigella* spp., *Salmonella typhi*. Many of these plant extracts owe their potency to the presence of phenolic compounds in the form of gallo tannins (hydrolysable tannins) and ellagi tannins, alkaloids, saponins, terpenoids, and steroids

(Hostettmann and Nakanishi 1979; Leven et al., 1979). These compounds display powerful protein-denaturing activity attributed to astringency nature. This astringent action also contributes to antimicrobial and antifungal activity. These findings are consistent with previous published reports that specific saponins could have antimicrobial properties (Soetan et al., 2006).

Proteins, lipids, salts, pH and temperature are also factors that affect the antimicrobial activity of phenolics (Tassou, 1993). Rico-Munoz and Davidson (1983) found that the antimicrobial effects of *Butylated hydroxyanisole* (BHA) and *Tert-butyl hydroquinone* (TBHQ) were influenced by the presence of different amounts of casein and corn oil. They reported that an increase in protein content of the media influenced their inhibitory effect against *S. aureus, Pseudomonas fluorescens and Saccharomyces cerevisiae*.

2.3.2. Antifungal properties

Herbs have also been found to have antifungal effect. The antifungal activities of four spice decoctions *sage*, *wild thyme*, *oregano* and s*avory* that grows wild in Turkey, were examined against six moulds (*Fusarium oxysporum f.* sp. *phaseoli, Macrophomina phaseoli, Botrytis cinerea, Rhizoctonia solani, Alternaria solani* and *Aspergillus parasiticus*). These herbs were tested for fungi static and fungicidal activity *in vitro*. Decoctions were added at 5% and 10% levels to 250 ml culture medium. The results showed that the decoctions investigated varied in antifungal activity. *Sage* had weak activity, whilst *wild thyme,* o*regano and savory* were active against all moulds tested. All test fungi were completely inhibited by both the

concentrations of *wild thyme* and the 10% level of *oregano* decoctions in all the incubation periods. It was concluded that some decoctions may be useful as mould inhibitors at food additive levels (Paster et al., 1995).

Numerous studies have documented the antifungal properties of plant essential oils (Bouchra et al., 2003; Daferera et al., 2003; Sokmen et al., 2004). These properties are caused by many active phytochemicals, including flavonoids, terpenoids, carotenoids, coumarins and curcumines (Tepe et al., 2005). Because of health and economic considerations, the search for antifungal agents is extensive (Paster et al., 1995). Natural plant extracts may provide an alternative way to protect foods or feeds from fungal contamination (Viuda-Martos et al., 2007).

The activity of plant extracts from Tanzania was tested on six different organisms: two fungi, yeast and three bacteria, namely *Aspergillus fumigatus* Fres, *Fusarium culmorum, Candida albicans*, *Pseudomonas syringae* pv. *Erwinia amylovora* and *Staphylococcus aureus*, all plants showed activity against several test organisms (Hugo and de Boer et al., 2004). The leaves of citrus plants contain fungi toxic methylated flavones, such as tangeretin and nobiletin (5, 6, 7, 8, 3', 4'-hexamethocyflavone).

According to Daferera *et al.* (2003) another serious problem about the use of certain non herbal chemical agents in the protection against fungal action is the development of resistance. The application of higher concentrations of chemicals in

an attempt to overcome this problem increases the risk of high-level toxic residues in the products (Viuda-martos et al., 2007).

2.3.3. Antioxidant properties

The oxidative deterioration of lipids is a great concern in the shelf life of foods. Lipid peroxidation leads to development of undesirable off-flavors and decreases the acceptability of foods (Namki, 1990). In addition, lipid oxidation decreases food safety and nutritional quality by formation of potentially toxic products and secondary oxidation products during cooking (Gould, 1992). To prevent and retard lipid oxidation, synthetic antioxidants such as BHA, BHT and PG to name a few are added to lipid-containing foods (Winata and Lorenz, 1996). However, potential health hazards of synthetic antioxidants in foods, including possible carcinogens, have been reported several times (Ford et al., 1980; Hettiarachchy et al., 1996; Juhee, 2006). This, therefore, results in the need to replace these synthetic products with natural alternatives. Indeed, the synthetic antioxidant market is in decline, while natural antioxidants, such as herb extracts, *tocopherols* (vitamin E) and *ascorbates* (vitamin C) are growing, pushed by easier consumer acceptance and legal requirements for market access. (Singh et al., 2005; Shahidi et al., 1992).

Strawberries are one of the best natural sources of antioxidants. They contain abundant vitamin C and E which are essential antioxidants. However, non-essential phytochemical antioxidants such as phenolic compounds in strawberries have been increasingly found to be even stronger antioxidants. They have also been found to work synergistically with the vitamins in the elimination of free radicals. Some of

the different phenolic compounds found in strawberries are: - anthocyanidins, procyanidins, phenolic acids and flavonoids. However, the concentration and composition of these phytochemicals vary depending on the variety, location of cultivation and growing seasons (Prindle and Wright, 1977). It was found that the average total phenolic content of wild strawberries were much higher than those of the cultivated strawberries. By using three different in vitro antioxidant assays, extracts of strawberries were found to be better antioxidants than the commercial antioxidants, used in food at the recommended concentration. The antioxidant activity of the strawberry is attributed to the phenolic compounds, which were also found to possess high antioxidant activity (Malliard et al., 1996).

Antioxidants from herbs have been found to increase the shelf-life and usage time of cooking oils, by increasing the resistance of the oil to oxidation. Ingestion of these herbs could also bring resistance of polyunsaturated fatty acids of cell membranes and lipoproteins to oxidation within the body. Herbs extracts with antioxidant capacity could also be used directly as stabilizers of meat fat and indirectly as feed additives, in order to improve quality and shelf-life of meat, fat-containing foods and milk (Vichi et al., 2000; Shahidi et al., 1992; Malliard et al., 1996).

2.3.4. Anti nutritive properties

Herbs contain a number of phytochemicals, some which substances are known to bind enzymes, minerals and other nutrients thereby inhibiting their absorption. These phytochemicals are known as anti nutrients. The commonly alleged anti-nutrients are protease inhibitors, amylase inhibitors, phytic acid, and polyphenolic

compounds such as condensed tannins. They are known to function by binding with minerals and vitamins making them unable to get absorbed in the alimentary canal. This makes them unavailable in the body despite the fact that they are consumed with food. Research has shown that, anti-nutritional factors such as enzyme inhibitors and other anti-nutrients are greatly decreased to insignificant levels or to nothing during soaking while normal cooking removes most of the remaining anti-nutrients. This has been seen to happen with mung bean, lentil, chicken pea (*garbanzo bean*) (Peary and Peavy, 1995).

Peary and Peavy (1995), also found out that the second mistake often made in talking about natural toxins or anti-nutrient is to call something toxic that, in the body, is not toxic at all but rather, is beneficial. Such is the case with saponins. Saponins are compounds found in legumes and legume sprouts. They are toxic to red blood cells only *in vitro* but harmless when ingested.

2.3.5. Odours and flavour properties

Plant preservatives have since shown potential in reducing odour in sheep meat as claimed by the pastoralists of West Pokot. Sheep meat has a characteristic odour and flavour which make it very low in preference in the affluent markets. The principle source of this odour is the fat (Priolo et al., 2003 and Voravuthikunchai et al., 2004a, b, Kim and Lindsay, 2006). When meat is dried and preserved in either *Oron* or *Angaun* herbs, the sheep odour tends to disappear and acquire the herbs odour.

2.4. Health benefits of herbs

Herbs contain several phytochemicals including some which are considered to be anti-nutrients. These anti-nutrients are known to bind nutrients in the body thereby denying the body these nutrients. On the other hand these anti-nutrients are actually powerful cancer-protecting phytochemicals. These include protease inhibitors and tannins. The problem in most diets is that, they do not contain these substances. Substantial research shows that protease inhibitors are one of the most powerful anti-carcinogens in existence. They have proven to be particularly protective against cancer of the colon, breast, and prostate (Ramel, 1986; Troll and Kennedy, 1993; Troll and Wiesner, 1983; Wattenberg, 1983; Yauclow et al., 1983). Tannins have been shown to give substantial protection against cancer including cancer of the stomach and lungs when ingested orally (Yauclow et al., 1983). Tannins and other polyphenols may play a role in fighting tooth decay. Evidence shows that some tannin inhibits the growth of bacteria that cause tooth decay (Moles and Waterman., 1985).

Phytates, like tannins, may also interact with digestive processes in a beneficial way. Small amounts in food slow down the absorption of sugars and regulate insulin levels. This is beneficial in the prevention and treatment of diabetes and hyperlipidemia (high blood fats). Small amounts of protease inhibitors, tannins, and phytates are beneficial and can be considered to be a normal part of our nutritional ecology (Kakiuchi, 1986).

The use of natural additives has extra advantages of health. The antioxidant activity of grape seed extracts has been linked to boosting cardiovascular health by limiting oxidation of low density lipoproteins (LDL), bad cholesterol. This extract is also claimed to have beneficial effects on a wide range of medical conditions from diabetes to asthma, from boosting male fertility to improving the memory of mice (Stephen, 2006).

Minerals are nutritionally important elements in the human body. They are inorganic, meaning they do not contain carbon, as do vitamins and other organic compounds. They have many roles in metabolism and body functions and are essential for the proper function of cells, tissues, and organs. They also play a role in the building of muscle and bone tissue. Some of the minerals if found in the herbs would increase their nutritional value are; calcium, selenium, iron, potassium, manganese, magnesium, zinc, phosphates, copper, nitrates, nitrites, chlorides and iodides (Alvarez et al., 2002).

Flavonoids are widely distributed in plants fulfilling many functions including producing yellow or red/blue pigmentation in flowers and protection from attack by microbes and insects. The widespread distribution of flavonoids, their variety and their relatively low toxicity compared to other active plant compounds such as alkaloids, mean that many animals, including humans, ingest significant quantities in their diet. Flavonoids are also referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-

allergic, anti-inflammatory, anti-microbial and anti-cancer activity. Flavonols unlike other phenolic compounds are quickly excreted from the body so they make very good preservatives in that they don't remain in the body to exert negative reactions (Viuda-martos et al., 2007).

2.5. Mode of action of phytochemicals on microorganisms in food

Prindle and Wright (1977) mentioned that the effect of phenolic compounds is concentration dependent. At low concentrations, phenols affect enzyme activity, especially of those enzymes associated with energy production. At higher concentrations, they denature proteins. The effect of phenolic antioxidants on microbial growth and toxin production could be the result of the ability of phenolic compounds to alter microbial cell permeability, permitting the loss of macromolecules from the interior. They could also interact with membrane proteins, causing a deformation in their structure and functionality (Fung et al., 1977).

Lis-Balchin and Deans (1997) reported that strong antimicrobial activity could be correlated with essential oils containing a high percentage of monoterpenes, eugenol, cinnamic aldehyde and thymol. Conner and Beuchat (1984) suggested that the antimicrobial activity of the essential oils of herbs and spices or their constituents such as thymol, carvacrol and eugenol could be the result of damage to enzymatic cell systems, including those associated with energy production and synthesis of structural compounds.

Nychas (1995) indicated that phenolic compounds could denature the enzymes responsible for spore germination or interfere with the amino acids involved in germination. Once the phenolic compounds have crossed the cellular membrane, interactions with membrane enzymes and proteins would cause an opposite flow of protons, affecting cellular activity. Davidson (2001) also reported that the exact cause–effect relation for the mode of action of phenolic compounds, such as thymol, eugenol and carvacrol, has not been determined, although it seems that they may inactivate essential enzymes, react with the cell membrane or disturb genetic material functionality. Several studies have attempted to determine the efficacy of extracts from selected plants as antimicrobial and antifungal agents (Lopez et al., 2000). Some studies have shown that specific essential oils and phenolic compounds can control the growth rate and spore germination time of spoilage fungi (Hope et al., 2003). Many antibacterial agents may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis (Franklin et al., 1987).

2.6. Quality changes in meat and milk

In normal circumstances when animals are healthy, the meat and milk is sterile, therefore the quality changes of the above products solely depend on externally introduced factors. These factors include unhygienic handling, and lipid oxidation.

2.6.1. Microbial spoilage

When meat or milk is not stored in unhygienic conditions, growth of spoilage microorganisms is inevitable. Spoilage of these products is primarily due to bacterial

activity and the large concentration of non protein nitrogen extractives in the meat muscles, Mayer and Ward, (1991) and rancidity due to fat oxidation. Meat and milk are common foods that effectively support microbial growth because they mostly consist of water, protein and fat. Even after cooking, these products retain about 50% moisture. Therefore, from the time of initial exposure, bacteria begin to grow at a rate dependant on, natural acidity (pH), water activity (a_w), handling practices and temperature. According to Lawrie (1985), when surface bacteria reach high limits and the chemical by products of their growth reach concentrations regarded objectionable then the following takes place; off flavors, off texture, putrefaction, off odours and discoloration. The meat or milk is said to be spoiled. This therefore, indicates that the shelf life of these products is limited by bacterial growth (Vichi et al., 2000).

The main pathway for the bacterial utilization of the nitrogenous compounds is through oxidative deamination. This results in accumulation of ammonia, volatile fatty acids and sulphur containing compounds such as, methyl mercaptan, hydrogen sulphide, dimethyl sulphide. These are typical components of spoiling meat and meat products (Gram et al., 1990). Determination of total volatile bases-nitrogen (TVBN) has been used to evaluate the extent of spoilage in meat and meat products. TVBN evolution shows a gradual and stepwise increase during the storage of meat and meat products (Owaga, 2004). A linear correlation between the microbiological quality and the sensory scores has been established with bacterial counts of 10^6cfu/g. These counts are regarded as the limit above which it is indicative of organoleptic spoilage (Ryder et al., 1984). In fish as in meat the TVBN content values of 30 – 40

mg N/100g are commonly considered as the maximum allowable limits for good quality fish (El – Marrakchi et al., 1990).

When meat is not handled hygienically, it is normally contaminated by varied flora depending on environmental factors. However, some genera have been reported as predominant. These include:- *Bacillus spp, Micrococcus spp, Pseudomonas spp, Actinobacter spp (aerobes), Coliforms, Salmonella spp, Staphylococcus spp, Lactobacillus spp, Streptococcus spp (facultative anaerobes), Clostridium sporogenes, Clostridium butilinum, Clostridium perfigens, Clostridium putrefaciens (anaerobes), Yeasts and Moulds. Coli forms, Salmonella spp, and Staphylococcus spp* are commonly associated with food poisoning (Kvenberg, 1991; Ahmed, 1991).

2.6.2. Lipid Oxidation (Rancidity)

Lipid oxidation is the process by which unsaturated fatty acids react with oxygen via a free radical chain mechanism to form fatty acyl hydroperoxides or primary products of oxidation. There are three mechanisms by which lipid oxidation takes place. These are free radical oxidation, photosensitized oxidation (singlet oxygen oxidation) and enzyme catalyzed oxidation (Colby *et al*., 1993; Christoffell and William, 1997).

With increased consumption of prepackaged raw meat and precooked convenience meat items, control of oxidation has become increasingly important. Antioxidants and chelating agents are the most effective inhibitors of lipid oxidation. Phytochemical antioxidants have been proved to retard and even inhibit the progress

of oxidation. (Abdel-alim *et al*., 1999). Herbal extracts have various kinds and amounts of antioxidants, associated with the phytochemicals. Most natural antioxidants often work synergistically with each other, to produce broad spectrum of anti oxidative activities that creates an effective defense system against free radical attack (Lu and Foo 2001). Huang and Frankel (1997); Baratta et al., (1998) and Wang and Wixon (1999) also indicated that phytochemicals such as flavonoids, and biflavones are associated with antioxidant activity.

2.7. Preservation of milk using herbs

Preservation of milk using herbs is an art that has been going on within the pastoral communities of West Pokot for a very long time. Though this kind of preservation has been on going very little is known about it. Other communities that have been known to do something similar are the Maasai, Kalenjin, Luhya and Samburu.

2.8. Total Lactic acid bacteria (LAB)

LAB comprise a group of Gram positive, acid tolerant, non-sporulating, non-respiring rod or cocci associated by their common metabolic and physiological characteristics (Christensen et. Al., 1999). These bacteria are usually found in decomposing plants and lactic products. They produce lactic acid as the major metabolic end product of carbohydrate fermentation. This trait has historically linked LAB with food fermentations as acidification inhibits the growth of spoilage agents. LABS are characterized by an increased tolerance to a lower pH range. This aspect partially enables them to out compete other bacteria in natural fermentation. Laboratory media used to culture LAB typically includes a carbohydrate source as

most species are incapable of respiration. The genera that comprise the LAB are at its core *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus* as well as the more peripheral *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Teragenococcus*, *Vagococcus*, and *Weisella*. These microbes are broadly used in the production of fermented food products, such as yogurt (*Streptococcus* spp. and *Lactobacillus* spp.), cheeses (*Lactococcus* spp.), sauerkraut (*Leuconostoc* spp.) and sausage. This acidification process is one of the most desirable side-effects of their growth. The pH may drop to as low as 4.0, low enough to inhibit the growth of most other microorganisms including the most common human pathogens, thus allowing these foods to keep for longer (Wikipedia, 2008).

2.9. Conventional Preservation techniques

Food preservation is a method of maintaining foods at a desired level of properties, or nature for their maximum benefits. Different preservation techniques were developed, to satisfy current demands of economic preservation and consumer satisfaction, in nutritional and sensory aspects. They include freezing, irradiation, cooking, fermentation, dehydration, use of conventional preservatives. These methods generally involve temperature manipulation, water reduction and the exclusion or elimination of spoilage microorganisms (Fox and Cameron, 1977).

2.9.1. Freezing and vacuum packaging

Freezing is done at -18°C, it works by completely stopping enzyme activity and inhibiting spoilage microorganisms like bacteria, yeast and molds. The only disadvantage is that freezing does not kill all microorganisms, others like spores

only become dormant and resurface once the product is thawed. Low storage temperatures also suppress the residual activity of the oxygen utilizing enzymes. Vacuum packaging enhances the keeping quality during freezing (Rozbeh *et al*., 1992).

2.9.2. Radiation

This is a relatively new process to make food safe. It works by exposing meat to radiant energy, which destroys most, but not all microorganisms. Foods that are approved for irradiation by the USA centre of Disease Control against pathogenic contamination and increased life include meat, poultry, spices, spinach lettuce and mollusk shellfish such as oyster mussels and clams. Irradiation reduces spoilage while leaving meat still as nutritious. (Rogachev, 1969, Ragheb, 2009).

2.9.3. Cooking

Cooking targets the microorganisms by killing them at high temperatures. There are two ways of cooking, Pasteurization and Sterilization. Pasteurization is cooking done between 72°C for 15 seconds though not all microorganisms are killed, immediate freezing is recommended. This part elimination of microorganisms is geared towards elimination of thermopiles, mesophiles and psychrophiles. Sterilization, is divided into dry heat and moist heat sterilization. The products are cooked under pressure at 121°C for 15 minute or 134°C for 3 minutes this eliminates all microorganisms. All the above methods have a danger of re infestation with the same microorganisms (Montville and Matthews 2005; Al-Baali 2006).

2.9.4. Chemical preservatives

Preservatives are chemical agents that prevent growth of microorganisms in food products. They render them safe to use and increase their shelf life. Preservatives are typically used in liquid products that do not have extreme pH values or high concentration of surfactants. For example products with a pH between 3 and 10 generally require preservatives. As a group, preservatives consist of many structurally different substances (Madsen *et al.,* 2001). They are used to inhibit microorganism growth, add flavour, improve product shelf life and develop a pink cured-meat color. Some of chemicals used include brine, sodium nitrite and sodium lactate. All chemicals applied should be approved by the relevant government bodies since some have unfavorable effects (USDA-FSIS 2000).

2.9.5. Fermentation

Fermentation is breakdown of carbohydrates under limited supply of oxygen or under anaerobic conditions to result in the production of alcohol and carbon dioxide. Other reactions would result in the production of acetic acid. This acid and alcohol inhibits the growth of selected microorganisms. Fermentation can preserve foods, but it also makes food more nutritious since micro organisms responsible for fermentations can produce vitamins as they ferment. This produces a more nutritious preserved end product from the ingredients. (Elizabeth and Ryan 2001; Nummer, 2002).

2.9.6. Dehydration

Dehydration is the oldest form of preserving meat, it removes water from the product through sun drying, heating and salting. The main concept is of reducing the water activity of the product. Reducing water activity can attain microorganism control. This is achieved by drying meat to a very low water activity level. Low water activity prevents microbial growth thus increasing shelf life. Large changes in textural characteristics occur at about $a_w = 0.65$ and reduces the rate of chemical reactions for example increasing hydrophobic reactions but reducing hydrophilic aqueous-diffusion-limited reactions. Growth of most bacteria is inhibited below about $a_w = 0.91$, similarly most yeasts cease growing below $a_w = 0.87$ and most molds cease growing below $a_w = 0.80$. The absolute limit of microbial growth is about $a_w = 0.6$ (Martin, 2006).

CHAPTER 3

3.0. MATERIALS AND METHODS

3.1. Study Design

The study was divided into three parts consisting of (i) collection of information and samples, (ii) chemical and bacteriological analysis of the herbs and (iii) determination of the preservative potential of the herbs on milk and pork sausages.

The first and the second part of the study were set in a completely randomized design where the herbs were randomly collected for identification with the help of key informants and then analysed to asses their efficacy in relation to antimicrobial, phytochemical, antioxidation and toxicity levels. The third part of the study was divided into two main blocks. (i) The first block was set to test the length of time the sausages could store under various conditions. (a) Sausages with herb extract of OTI, (b) sausages with herb extract of AZA, (c) sausages with preservative sodium metabisulphite and (d) a control were no preservative was added. Each of the above was split into the two storage temperatures of 4℃ and 25℃. The analysis was set in randomized complete split plot design. (ii) The second block was set to test the length of time the herb extract KOIC could preserve milk compared to the control. The daily analysis was set in randomized completely block design. In all cases n was equal to 3).

3.2. Study Site

This study was carried out in Chepareria division of West Pokot District, (Figure 1). According to the ASAL report of the Government of Kenya of 1998, West Pokot is one of the 20 districts of the Rift Valley province which covers an area of 9100km^2. The district is surrounded by the Turkana people in the north the Karamajong cluster of Uganda in the west, Transzoia in the south, Baringo and Marakwet in the east.

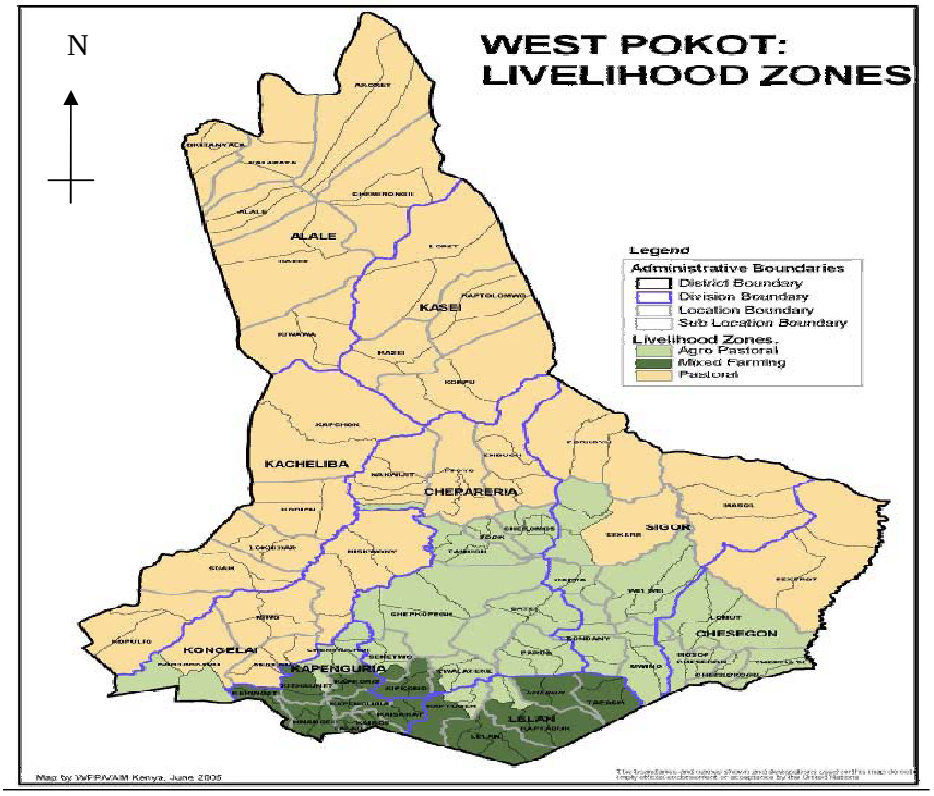


Figure 1: Map of West Pokot District showing the study site which includes Chepareria and Kongelai divisions (United Nations World Food Program (WFP), 2005)

In 1993 the population was estimated by the Kenya National Bureau of Statistics as 320,000, with an annual growth rate of 4.2%. The community is predominantly Christian. The district has three different farming activities, mixed farming in the south, agro pastoral in the central and pastoralism in the north. The central area, covered partly by forest vegetation is the major source of herbs. It constitutes of 44% rangeland, 28% marginal land, 19% land not suitable for agriculture and only 3% and 6% in the southern of the district are considered high potential and medium potential respectively.

3.3. Materials

3.3.1. Collection of information and samples

During the collection of samples, introduction to the community was done with the assistance of the office of the District Agriculture Officer, Kapenguria. The information on the type of herbs used and the way preservation of both meat and milk was undertaken, was obtained by personal observation and focus for discussions guided by a questionnaire (Appendix 1). Three groups of respondents were involved, elders, women and youth groups. From each group fifteen respondents were interviewed. All the respondents were from Chepararia Divisions of West Pokot District.

Four different types of samples were collected in triplicates. The first batch consisted of six herbs, whose leaves, stems, fruits and flowers were collected for taxonomic identification at the East African Herbarium of the National Museums of Kenya (Table 1). The second consisted of the parts of the six herb samples to be

used for analysis in the Department of Food Science and Technology of Jomo Kenyatta University of Agriculture and Technology (JKUAT). The last two samples consisted of milk in two gourds, one containing milk treated with herbs and the other, milk not treated with the herbs. Immediately after collection, the samples were stored in cool boxes containing ice packs, these ice packs maintained a temperature of approximately 4°C while on transit to JKUAT Food Science Laboratory.

Table 1: List of herbs collected for analysis from West Pokot District

Local name (Code)	Scientific Name	Class	Plant Part used	Product preserved
Oron (OTI)	*Tamarindus indica* L	Caesalpinoideae	Fruit paste	Meat
Angau (AZA)	*Ziziphus abyssinica* A. Rich	Rhamnaceae	Fruit paste	Meat
Kromwo (KOIC)	*Ozoroa insignis* Del.	Anacardinaceae	Charcoal of peeled stem	Milk
Senetwo (SSDC)	*Senna didymobotrya* Fresen Irwin and Barneby	Caesalpinoideae	Charcoal of peeled stem	Milk
Chepuyetwo	*Lippia javanica* (Burm.f., Spreng)	Verbenaceae	Leaves	Milk
Chepku-surwa/ chepku	*Leucas calostachys* oliv.	Labiatae	Leaves	Milk

- Identified at the National museums of Kenya 2007

3.3.2. Test microorganisms

Non resistant clinical isolates of selected microorganisms were obtained from Kenya Agricultural Research Institute (KARI) (Table 2). These included, gram positive *Staphylococcus aureus* (ATCC 22923) and *Bacillus subtillis*, gram negative *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922), and a fungus *Candida albicans* (ATCC 90028). The cultures of bacteria were maintained on nutrient agar slants at 4 ± 2°C.

Table 2: Microorganisms used to test the inhibition capability of the herbs extracts

Microorganisms	Agar	Incubation time and temperature
Bacillus subtillis (locally isolated in the KARI laboratory Nairobi)	Nutrient agar	37°C for 24 hours
Pseudomonas aeruginosa (27853 ATCC)	Pseudomonas Agar	37°C for 24 hours
Candida albicans (90028 ATCC)	Potato Dextrose Agar with 10% tartaric acid	25°C for 5days
Escherichia coli (25922 ATCC)	Violet-Red Bile Glucose Agar	37°C for 24 hours
Staphylococcus aureus (25923 ATCC)	Baird Parker with egg yolk Tellurite	37°C for 24 hours

3.4. METHODS

3.4.1. Preparation of samples and extraction

Herbs samples were prepared as shown in Figure 2. A portion of each of the fresh herbs OTI, AZA, SSDC and KOIC weighing 10kg was gently cleaned using running tap water to remove soil. The samples were dried at ambient temperatures of 25 ± 2°C in a room for six days. Herb samples were ground into moderately coarse powder using an electric grinder (model M10R Japan) and stored until needed for use (Onoruvwe and Olorunfemi, 1998). A 500g portion of each of the dried herb sample were taken and cold extracted with two solvents; chloroform and methanol, using the method of Regnier and Macheix, (1996), with modifications. In water extraction, distilled water alone was used (Bautista-Banos et al., 2003). In each of the solvents, each herb sample was completely immersed and the container shaken for 30 minutes to ensure sufficient contact with the solvent using a Kika Labortechnik Shaker, (Model KS 250 Basic, Staufen, Germany). The mixture was left to stand for four days in an enclosure at 25 ± 2°C. The mixture was then centrifuged at 4,000rpm for 10 minutes at a temperature of 4°C using a Kokusan Centrifuge from Kokusan Corporation (Model 2000C, Tokyo Japan). The supernatant was later filtered using No. 1 Whatman paper filter. The solvent was evaporated to dryness under vacuum at 80°C using a rotary evaporator (Model RE 100, Staffordshire, England). The extract obtained was put in a glass light proof container and store at 4°C till a time when it would be used.

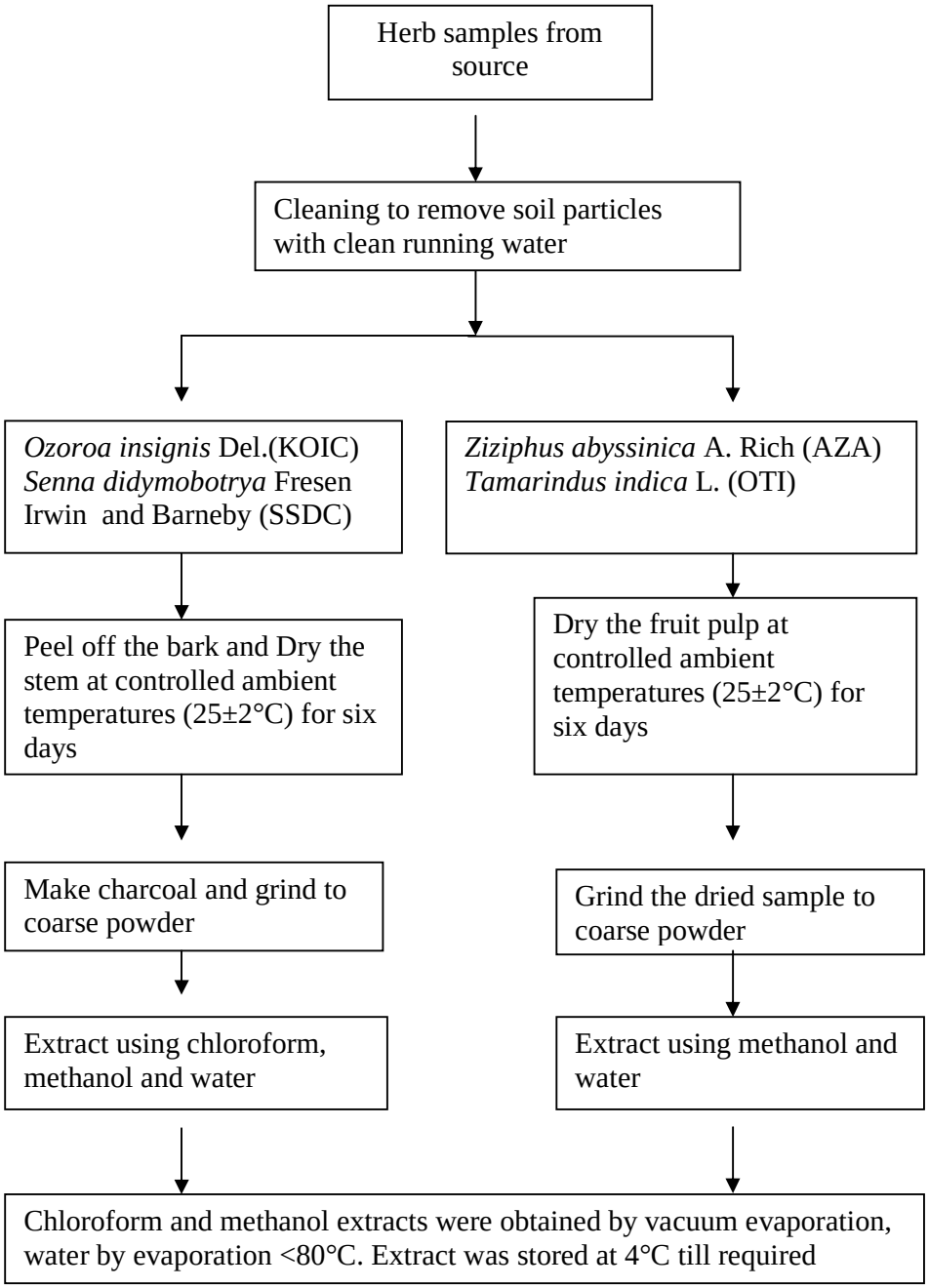


Figure 2: Preparation process of herbs for analysis

For samples extracted using water, 500gm of the freshly ground sample was placed in a round-bottomed flask. Deionized water was added till the sample was completely immersed and shaken gently for 30 minutes to ensure adequate mixing. The mixture was boiled for one hour, cooled and filtered, then centrifuged at 4,000rpm for 10min at 4°C. The supernatant was filtered using a No. 1 Whatman filter paper and evaporated at below 80°C to dryness. (Mekbib et al., 2007). From each of the three extracts, concentrations of 0.1, 0.2 and 0.3g/ml were prepared. The extracts were dissolved in distilled water and also in 50% dimethyl sulphoxide (DMSO) for those extracts insoluble in water.

3.4.2. Phytochemical screening of selected herbs extracts

The crude herb extracts of chloroform, methanol and distilled water, were subjected to qualitative chemical screening for the identification of various classes of phytochemicals using standard procedures of plant constituent's identification as described by Trease and Evans (1987), El-Olemyl et al., (1994) and Wall et al., (1954). The phytochemicals tested for included alkaloids, saponins, tannins, steroids, flavonoids/polyphenolics, cardinolids and reducing compounds.

3.4.2.1. Determination of alkaloids

Mayers test

A sample of extract weighing 0.1g was dissolved in 2ml of ethanol, and then 3drops of 5% HCl was added followed by 6drops of Mayers reagent. Alkaloids were indicated by the production of a creamy colour (Trease and Evans, 1987).

To confirm the presence of alkaloids 0.1g of sample was taken and dissolved in 2ml of ethanol and then 3drops of 5% HCl was added followed by 6drops of Dragendroffs reagent. Alkaloids were confirmed by the indication of a reddish brown color (Trease and Evans, 1987).

3.4.2.2. Tests for Saponins

A 1g sample of the extract was mixed with 5ml of distilled water. The mixture was shaken and heated to boiling point. The presence of saponins was indicated by frothing (Trease and Evans, 1987).

A confirmatory test of saponins was done by adding 5ml of distilled water to 1g of sample. The mixture was shaken and filtered with a No. 1 Whatman filter paper. To the filtrate, 3ml of olive oil was added and the resulting mixture thoroughly shaken for a stable emulsion to form, the emulsion was allowed to stand for 5min and the presence of saponins was indicated by the stability of the emulsion (Trease and Evans, 1987).

3.4.2.3. Test for Tannins

A 0.1g sample of extract was mixed with 1ml ethanol. To the mixture several drops of 5% ferric chloride solution were added. The presence of gallo tannins or egalli tannins (hydrolysable tannins) was indicated by the development of a dark blue or purplish color. The presence of condensed tannins was indicated by a Greenish black or brown precipitate. A confirmatory test for hydrolysable tannins was done by mixing 0.1g of sample with 1ml ethanol and 5 drops of potassium iodide added. The

presence of gallo tannins was indicated by a pink color and free gallic acid was confirmed by an orange color. Confirmatory test for condensed tannins, 0.1g of sample was mixed with 1ml ethanol. To the mixture 2 drops of vanillin-HCl was added. The presence of condensed tannins was indicated by a red color. (Trease and Evans, 1987).

3.4.2.4. Test for Flavonoids

A portion of extract weighing 0.1g was mixed with 4 drops of conc. HCl and 4 pieces magnesium turnings in a test tube. The presence of flavonoids was indicated by the development of a pink or magenta red color (Trease and Evans, 1987).

3.4.2.5. Test for Sterols and steroids

A portion of extract weighing 1g was mixed in a test tube with 0.5ml each of conc. sulfuric acid, acetic anhydride and chloroform in equal amounts. The presence of sterols was indicated by the development of red color, steroids green color (El-Olemyl et al., 1994).

3.4.2.6. Test for reducing compounds

A 2ml portion of the extract was mixed in a test tube with 5ml mixture of equal volumes of Fehling's solution A and B. The mixture was boiled in a water bath for 2 minutes. The presence of reducing compounds was indicated by the development of a brick red precipitate (Trease and Evans, 1987).

3.4.3. Antioxidant activity

The antioxidant activity of herb extracts was measured as hydrogen donating radical scavenging ability using the stable radical 2, 2 diphenyl picrylhydrazyl (DPPH) (Brand et al., 1995). A 0.1 ml methanolic sample solution of various concentrations (0.1-0.5mg/ml) was placed in a cuvette and 4ml of 6×10^{-5} mol/ml methanol solution of DPPH solution added. The mixture was then shaken vigorously and absorbance measurement done immediately. The decrease in absorbance at 515nm was determined and data taken after 1 minute for ten consecutive minutes. Methanol was used as the blank and zero readings were made from the absorbance of the DPPH solution before mixing it with the extract. Antioxidant capacity is expressed as a percentage and calculated as shown below.

$$[(Ao - A_f)/ Ao] \times 100$$

Where: Ao is the Initial absorbance of DPPH,

A_f is the Final absorbance of the sample after reduction by the DPPH.

3.4.4. Evaluation of antibacterial activity of herb extracts

Pure cultures of the test microorganisms were inoculated into nutrient broth (Oxoid, England), incubated for 24 h at 37°C, diluted with sterile nutrient broth to a density of 9×10^{8} cfu/ml by serial dilution. Sterile disposable plates were used and appropriate media (Table 2) was prepared and poured into sterile disposable plates according to AOAC method 966.23 (AOAC, 1995). Inoculation of the prepared plates with the organism was done using a sterilized pipette to transfer 0.1ml of the

suspensions into the plates followed by spreading with a Canards rod to achieve uniform spread on the plate.

Sensitivity of all the organisms to the various extracts was done using the cork and bore diffusion method of (Bauer et al. 1966; Barry et al. 1985; Rojas et al. 2003) with some modifications. Using a sterile cork-borer of 6 mm diameter, three holes were made into the set agar in Petri-dishes containing the bacterial culture. Then 0.1ml of each of the concentrations 0.1, 0.2 and 0.3g/ml of the extracts was poured in to the wells in triplicates. Standard preservative (sodium metabisulphite 0.1 and 0.2g/ml) was used as reference or positive control. A control was set up for all the organisms, in which, 50% DMSO in water was used instead of plant extract to ascertain that the 50% DMSO did not inhibit growth of microorganisms. A second control was set up to check the viability of the microorganisms. The microorganisms were inoculated in the corresponding agar media and placed in the incubator at 37°C overnight with pure sterile water in the holes. The controls without plant extract were examined for growth and those with the plant extracts were examined for zones of inhibition of growth. This was estimated by measuring the linear diameter of the inhibition zone. Antibacterial activity was recorded if the zone of inhibition was greater than 9mm (Hassan et al., 2006).

3.4.5. Minimum inhibitory concentration (MIC) of sample extracts

The MIC of the water extracts from the herbs used to preserve meat was determined using the standard method of Wariso and Ebong (1996) with modifications. Plates that showed significant inhibitory activity (more than 9mm inhibitory diameter) on

the test microorganisms were considered for this test. Nutrient broth was prepared and sterilized using an autoclave at 121ºC for 15min. a sample of 1ml of the prepared broth was dispensed in to the test tubes numbered 2-12 using a sterile pipette and 1ml plant extract containing 0.1-0.2g/ml extract in water was dispensed into each of the tubes numbered 1 and 2. Subsequently from tube 2, serial dilution was carried out and 1ml from tube 2 was transferred up to tube number 10 and 1ml from tube number 10 was discarded. Tube 11 was control for sterility of the medium and tube 12 for viability of the organisms.

Pure cultures of the organisms were inoculated into nutrient broth (Oxoid, England), incubated for 24 h at 37°C, diluted with sterile nutrient broth to a density of 9×10^8 cfu/ml by serial dilution. From this dilution a 0.1ml of inoculums were transferred into each of the tubes 2- 12 with the exception of tube 11, to which another sterile broth was added. The final concentration of the plant extract in each of the test tubes numbered 1-10 after dilution were 100,000; 50,000; 25,000; 12500; 6250; 3125; 1562.5; 781.25; 390.625; 195.3125 µg/ml, respectively. They were incubated at 37ºC for 24-48hrs and examined for growth. The last tube in which growth failed to occur was the MIC tube.

3.4.6. Brine shrimp lethality test

The lethality test was carried out using brine shrimp larvae (*Artemia salina*, Aqua farm, USA) as the test organism. Clean Seawater (500ml) from the Indian Ocean was filled into an incubation tank divided into two unequal compartments. These compartments were separated by a perforated polystyrene wall. Brine shrimp eggs

(0.5g) were sprinkled into the larger compartment, which was darkened by covering with hard paper while the smaller compartment was illuminated with an electrical bulb. The phototropic nauplii were collected from the illuminated compartment by using a micropipette 24 hours of hatching (Meyer et al., 1982a).

Aqueous solutions of 100,000; 50,000; 25,000; 12500; 6250; 3125; 1562.5; 781.25; 390.625; 195.3125 µg/ml were prepared by serial dilution for the AZA and OTI plant extracts in different vials, each with a volume of 10ml. Each solution was made to 10ml by adding clean seawater and then 10 brine shrimps were transferred into each of the four vials. The experimental vials were maintained under illumination conditions. Controls were placed in artificial seawater. The nauplii were counted microscopically in the stem of the pipette against a lighted background. Each assay was repeated three times and the number of survived larvae after 24 hours was recorded. A lethality concentration (LC50 Values) for each assay was obtained statistically by probity analysis using the SAS statistical package at 95% confidence (Alkofahi and Rupprecht 1989).

3.4.7. Potential of herbs to preserve milk

3.4.7.1. Milk pretreatment

To ascertain that the herbs actually have the capacity to preserve milk, several tests were carried out on milk treated with KOIC herb (MS1) and a control consisting of milk that had not been treated with either of the herbs (MS2). All the tests commenced on the third day having taken two days to transport the samples from West Pokot to the Food Science laboratory of JKUAT. The samples were

transported in a cool box that with ice packs which maintained the temperature of melting ice, at approximately 4±3°C. The process of collection and preparation of milk samples is shown in Figure 3. The milk collection was carried out three times and each time the tests were replicated three times.

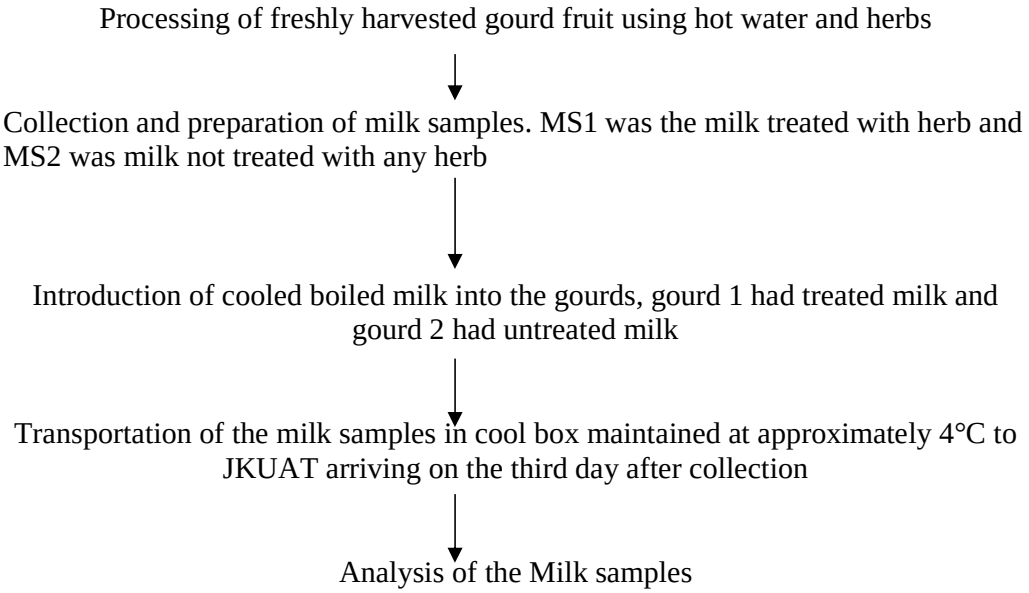


Figure 3: Collection and preparation of milk samples

The samples were both stored at approximately 20ºC throughout the period of research, which are the average temperatures in Chepararia Division of West Pokot. The gourds used to collect the milk samples are shown in Plate 1. The samples were tested daily for titratable acidity, changes in pH, accumulation of microorganisms and total lactic acid bacteria. The evaluation of phytochemical constituents, antioxidant activity and microbial activity of SSDC and KOIC herbs are described in section 3.5.2.-3.5.5. The tests were replicated three times.

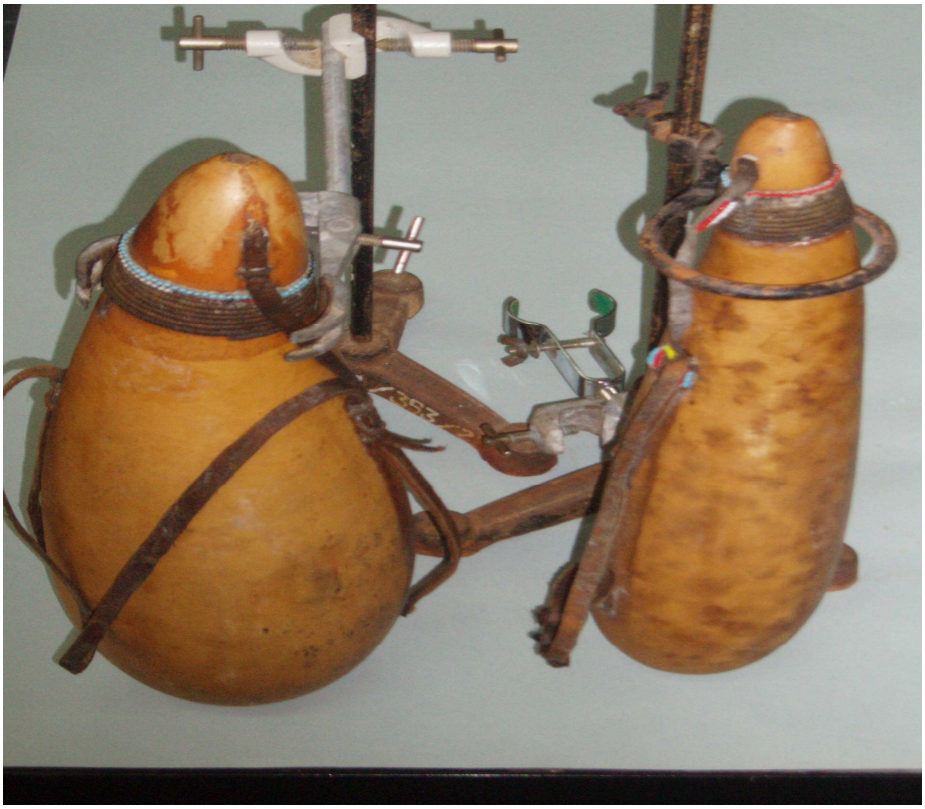


Plate 1: Milk gourds used by the pastoralists of West Pokot to collect and preserve milk (Photo by Nyaberi M. O. 2007).

3.4.7.2. pH of milk sample

A 10ml sample was taken into a 50ml beaker and the pH was determined using a calibrated electrode probe pH meter (model 20, Denver Inc, Denver, USA).

3.4.7.3. Total acidity of milk sample

A 10ml portion of milk sample was pipetted into a conical flask and 3 drops of phenolphthalein indicator were added and the mixture swirled to mix. It was then titrated using 0.1N NaOH until a permanent pink color appeared for about 1 minute

and the titter was recorded. Since the lactic acid is predominant in milk, to calculate percentage lactic acid the following formula is used. (Conversion Factor=0.009)

$$\% \text{ Lactic Acid} = \frac{\text{Volume of 0.1N NaoH (titre) x C.F x 100}}{\text{Volume of sample used}}$$

3.4.7.4. Total viable count

Total viable counts were determined according to the AOAC method 966.23 (AOAC, 1995), with modifications. 1ml of milk sample was homogenized in 9ml of sterile triptone (Oxoid, England) water containing 0.1% triptone water (Oxoid, England) for 30 seconds. Serial dilutions were made to 10^9. 0.1ml Aliquots of the dilution 10^5 to 10^9 were inoculated on plate count agar and aerobically incubated at 37°C for 24 hours. Plates containing 20 – 200 colonies were counted and the number was multiplied by the dilution and then expressed in log cfu/ml.

3.4.7.5. Total Lactic acid bacteria count

Total viable counts of LAB were prepared according to section 3.4.7.4. using PCA instead of nutrient agar. The PCA was mixed with 0.015g per liter bromo-cresol purple (Oxoid, England) during preparation (Amoa-Awua et al., 2006). Bromo-cresol purple is a pH indicating dye which is purple when in the free acid form at pH 6.8 and turns yellow in the protonated form at pH 5.2. Colonies producing lactic acid change the pH of the agar and cause it to turn from purple to yellow. The colonies with a yellow ring were considered positive for lactic acid production and were therefore counted.

3.4.8. Potential of herbs to preserve sausages

A 10kg leg part was obtained from freshly slaughtered chilled pork from the Pork Center Butchery in Thika (Kenya). It was washed, deboned and stored at 4°C. The meat was divided into four batches of 1.8 kg each. Sausages were made from each batch using ingredients shown in Table 3. Batch 1 was preserved with sodium metabisulphate; Batch 2 with OTI; Batch 3 with AZA and Batch 4 had no preservatives and were the control. Sausages were stored at 4±2°C and 25±2°C respectively and analyzed for rancidity using the thioberbituric acid reacting substances test (TBARS), total volatile base nitrogen (TVB-N), total viable count (TVC), *E. coli*, *S. aureus* and sensory properties.

Table 3: Ingredients used in preparation of sausages

No	Ingredients	Batch 1 (g)	Batch 2 (g)	Batch 3 (g)	Batch 4 (g)
1	Lean meat	1,800	1,800	1,800	1,800
2	Fat	600	600	600	600
3	Water	540	540	540	540
4	Salt	33	33	33	33
5	Sugar	30	30	30	30
6	Protein binder	120	120	120	120
7	Non protein binder	450	450	450	450
8	Colour	0.015	0.015	0.015	0.015
9	Phosphate	6	6	6	6
10	MSG	4.5	4.5	4.5	4.5
11	Mixed spices	8.1	8.1	8.1	8.1
	Preservatives				
1	Sodium metabisulphite	4.5	0	0	0
2	AZA	0	5.6	0	0
3	OTI	0	0	5.6	0

(Njoroge et al., 2008).
MSG Monosodium gluamate, AZA- Ziziphas abssynica and OTI- Tamarindus indica.

3.4.8.1. Evaluation of rancidity in meat

Rancidity development was measured using the thiobarbituric acid reactive substances (TBARS) test using the method of Tarladgis et al., (1960) as modified by Izumimoto et al., (1990). A sample of 10g of sausage was homogenized with 50ml

distilled water and allowed to stand for 30 minutes at room temperature before adding 20ml of 20% trichloroacetic acid (TCA) solution (BDH Ltd. England). The sample was mixed thoroughly before filtering, active charcoal was used to eliminate interferences caused by the colour pigmentation in the extract. Five ml of the filtrate (5ml) was mixed with 5ml of 0.02M thiobarbituric acid (TBA) reagent (BDH Ltd England) in test tubes then heated in a water bath at 90°C for 35minutes. Absorbance of the reddish/pink colour formed was measured at 532nm using a Shimadzu UV- VIS spectrophotometer mini 1240, and recorded as D value. A blank was prepared in a similar way excluding the sample. The TBARS was calculated as Malonaldehyde (mg/kg) according to the following equation:

E532 x 12.9

Where: E is the extinction value at 532nm, 12.9 is a conversion factor. The evaluation was done in triplicates.

3.4.8.2. Total volatile base nitrogen (TVB-N)

The process of development of putrification was determined by the production of volatile base nitrogen (VBN) using the method suggested by the European Union (EU) Directive No. 95/149/EEC (1995) over a period of 15 days at two days interval.

A 10g of sample of meat, to which 90ml of 6% perchloric acid (BDH Ltd. England) was added, was homogenized at high speed with a stomacher blender (400 Circulator. Seward stomacher. England). The homogenate was filtered to obtain a 50ml extract. This was transferred to a distillation apparatus and ultimately made alkaline by 6.5 ml of 20% NaOH solution. The extract was then steam distilled and

the volatile bases received in 3% boric acid solution containing mixed indicator. A blank test was done, instead of the extract; 50ml of 6% perchloric acid solution was used. The amounts of the bases were determined by titration using 0.01M HCL solution. The samples were done in triplicates.

TVB-N (expressed in mg N/100g sample) was calculated as show:

$$\text{TVB-N} = \frac{V_1 - V_2 \text{ X } 2 \text{ X } 0.14 \text{ X } 100}{M}$$

Where V_1 = Volume of 0.01 HCl in ml for sample.

V_2 = Volume of 0.01 HCl in ml for blank

M = Weight of sample in g.

3.4.8.3. Total viable count (TVC), *E. coli* and *S. aureus,* yeast and molds

Total viable counts were determined according to the AOAC method 966.23 (AOAC, 1995) with modifications. Approximately 10g sausage sample was homogenized in a stomacher blender (400 Circulator. Seward stomacher. England) using 90ml of sterile diluents (0.1% Tryptone water) and then serial decimal dilutions were made from $10^1 - 10^9$. Triplicate plates of each dilution were done using the spread plate technique onto dry sterile plates of plate count agar(Hi Media Ltd, Bombay), Violet-red bile glucose agar (VRBGA; Hi Media Ltd, Bombay) and Baird Parker medium (Hi Media Ltd, Bombay) with egg york tellurite (Hi Media Ltd, Bombay) for TVC of *E. coli* and *S. aureus*, respectively. The plates were inverted and incubated at 37ºC for 24 – 48hrs. *E. coli* colonies were identified as those surrounded by a purple zone of growth. *S. aureus* colonies were circular,

smooth, convex, moist 2-3mm in diameter, gray-black to jet black. The plates with colonies were counted and results expressed in log cfu/ml.

Yeast and molds were enumerated by the surface plate method using potato dextrose agar (PDA) (Hi media Ltd, Mumbai) supplemented with 75ppm chloramphenical antibiotic using a sterile glass rod. (Harrigan, 1998). Aliquots of 0.1ml of sample dilutions were spread onto the pre dried agar. All the plates were incubated at 25℃ for 5days.

3.4.8.4. Sensory analysis

The sensory analysis of the sausage in which the herbs extracts OTI and AZA had been incorporated was done by a team of fifteen untrained panelists from the Department of Food Science and Technology. Each of the panelists was presented with four deep fried sausage samples. One was the control while the other three contained AZA, OTI or sodium metabisulphate. The samples were coded using random numbers and placed on plastic plates and presented to the panelists. Each panelist was asked to evaluate the samples for colour/appearance, flavour, texture, hardness of casing and general acceptability. Rinsing the mouth with water was done between testing samples. The assessment was carried out under natural light at a room temperature of 26 ± 2°C. Evaluation was done using a 9-point hedonic scale (Ihekoronye and Ngoddy, 1985). The parameters evaluated were all scored between 9 (like extremely) and 1 (Dislike extremely) using the questionnaire shown in Appendix II.

A further sensory analysis was undertaken to find out if the samples with the herbs could be differentiated from the ones without the herb. This was done using the triangular test as described by Jellinek (1985) with some modifications. Three samples were presented to fifteen panelists, two were the same and one was different (Appendix III). The results were analyzed using SAS© statistical program version 9.1.

3.4.9. Statistical analysis

All the data was analyzed for variance (ANOVA) using SAS computer program version 9.1. The comparison of the means, standard error and standard deviations at 5% level of significance were done using Duncan's multiple range tests (Steel and Torrie, 1980).

CHAPTER 4

4.0. RESULTS AND DISCUSSION

4.1. Herbs

From the Six herbs collected (Table 1) four were selected for further tests as they were commonly used for food preservation by the pastoralists of West Pokot (Plate 2). These herbs included OTI, AZA, SSDC and KOIC.

(a)

(b)

(c)

(d)

Plate 2: Herbs collected from West Pokot for analysis (a) *Kromwo* (KOIC); (b) *Senetwo* (SSDC); (c) *Oron* (OTI) and (d) *Angaun* (AZA). (Photos by Nyaberi M. O. 2007)

4.2. Phytochemical analysis

4.2.1. Herbs extract of KOIC and SSDC used in milk preservation

The results of the phytochemical screening of charcoal from the stem of KOIC and SSDC species investigated are presented in Table 4. The qualitative phytochemical results of both KOIC and SSDC show that methanol solvent produced wider range of various phytochemicals from the extract followed by chloroform and the least was water. The range of various phytochemicals of extracts obtained from the methanol and chloroform solvents compared to water suggest that there were higher proportions of water-insoluble plant components in the extracts of KOIC and SSDC (Nkere and Iroegbu 2005). Both the herbs contained hydrolysable tannins, flavonoids, saponins, alkaloids, sterol and triterpenes, in addition to the above compounds SSDC had condensed tannins. The presence of condensed tannins may be the reason why the pastoralists of West Pokot preferred to use KOIC to SSDC in the preservation of milk. This is because condensed tannins that are known to have an unpleasant taste.

Alkaloids were not detected in the aqueous extract an indication that either no water soluble alkaloids were present or the method for extraction encouraged their decomposition. Alkaloids are known to readily decompose if the drying of the herbs proceeds for a long period before extraction (Aboaba et al., 2001).

Table 4: Phytochemicals present in the aqueous and organic extracts of KOIC and SSDC

Phytochemicals	Water extract		Chloroform extract		Methanol Extract	
	KOIC	SSDC	KOIC	SSDC	KOIC	SSDC
Condensed Tannins	-	-	-	+	-	+
Hydrolysable Tannins	-	-	-	+	+	+
Flavonoids	+	-	+	-	+	+
Saponins	+++	+	+	+	++	+
Sterol and steroids	+	+	+	+	+	+
Reducing compounds	-	-	-	-	-	-
Alkaloids	-	-	+	-	+	+

- absent, + present, ++ present in higher proportion, SSDC - *Senna didymobotrya (Fresen) Irwin & Barneby*; KOIC - *Ozoroa insignis.*

4.2.2. Herb extract of OTI and AZA used to preserve meat

Results of the phytochemical screening of the fruit paste of OTI and AZA species investigated, based on the qualitative techniques adopted, indicated that both methanol and water were capable of extracting different phytochemicals (Table 5). Both extracts of OTI and AZA had saponins, alkaloids, flavonoids and polyphenolics, flavonone aglyones, sterols, triterpenes and reducing compounds. AZA had condensed tannins but absent in OTI. This explains why water is successfully used by the pastoralists of West Pokot during extraction of these herbs for preservation. The preference for OTI to AZA among the pastoralists of West Pokot can be attributed to the absence of condensed tannins in OTI (Lin, et al., 2004).

Table 5: Phytochemicals present in the aqueous and organic extracts of OTI and AZA

Phytochemicals	Methanol extract		Water extract	
	OTI	AZA	OTI	AZA
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Flavonoids and polyphenolics	+	+	+	+
Hydrolysable tannins	-	-	-	-
Condensed tannins	-	+	-	+
Reducing compounds	+	+	+	+
Sterols and steroids	+	+	+	+

- absent, + present, AZA - *Ziziphus abyssinica A.Rich*, OTI (Mkwaju) - *Tamarindus Indica* L

The phytochemical compounds detected such as saponin, tannin, flavonoids, terpenoid and alkaloids, have been reported to have antimicrobial activity (Leven et al. 1979). Prindle and Wright (1977) explained that the effect of phenolic compounds is concentration dependent. At low concentrations, phenols affect enzyme activity, especially those enzymes associated with energy production. At greater concentrations, they denature proteins and alter microbial cell permeability thus permitting the loss of macromolecules from the interior. They could also interact with membrane proteins, causing a deformation in their structure and functionality (Fung et al., 1977).

4.3. Antimicrobial analysis

4.3.1. Herbs extract of KOIC and SSDC used to preserve milk

The chloroform extract of SSDC, did not produce a clear zone of inhibition against all test organisms except *B. subtillis.* On the other hand the chloroform extract of KOIC inhibited the growth of *E. coli* and *P. aeroginosa.* The methanol extract of SSDC produced a zone of inhibition with all test microorganisms except *B. subtillis*. *E. coli* was the most inhibited with a zone of inhibition of 15.3 ± 0.6mm followed by *P. aeruginosa* and lastly *C. albicans*. On the other hand the methanol extract of KOIC produced a smaller zone of inhibition with *E. coli* and *P. aeruginosa* of 9.6±0.5 and 12.3±0.6 respectively. Therefore, the methanol extract of SSDC had a higher antimicrobial activity than the methanol extract of KOIC, while the chloroform extract of KOIC showed a higher antimicrobial activity than the chloroform extract of SSDC. This difference can be attributed to the fact that chloroform may be able to extract more phytochemicals from KOIC while methanol was able to extract more phytochemicals from SSDC. The antimicrobial results are shown in Table 6.

Other studies that have been done on the antimicrobial activity of the plant parts of KOIC include the extracts from leaves, roots, bark and stem rhizome. They all indicated that methanol, ethanol, acetone and hot water extracts, were active against test microorganisms *Vibrio cholera*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella* spp. and *Salmonella typhi* (Mathabe *et al*. 2006 and Moshi et al., 2004). Another study done by Njoroge and Bussman (2006) indicated that fresh extract

Table 6: Average inhibition zone diameter IZD (mm) of extracts of SSDC and KOIC against test organisms.

	Chloroform				Methanol		
	SSDC		KOIC		SSDC		KOIC
	0.2g/ml	0.1g/ml	0.2g/ml	0.1g/ml	0.2g/ml	0.1g/ml	0.2g/ml
E. coli	-	-	15.4±0.6[a]	12.9±0.6[b]	15.3±0.6[a]	13±0.5[b]	12.3±0.6[c]
C. albicans	-	-	-	-	10.3±0.6	-	-
P. aeruginosa	-	-	15.4±0.9[a]	13.0±0.9[b]	13.6±0.5[b]	10.0±0.6[c]	9.6±0.5[c]
*B. subtillis**	10.5±1.5[c]	-	-	-	-	-	-

- indicates that the diameter of the zone of inhibition is below 9mm. Values followed by the same small letter within the same raw are not significantly different (P>0.05). All the figures are in millimeters (mm). * gram positive, values are mean ± SD of n = 3, SSDC stands for *Senna didymobotrya* (Fresen) Irwin & Barneby, KOIC stands for *Ozoroa insignis*.

of SSDC are used as an antiseptic against animal wounds which shows that it also contains ingredients that are able to act against microorganisms.

4.3.2. Herbs extract of OTI and AZA used to preserve meat

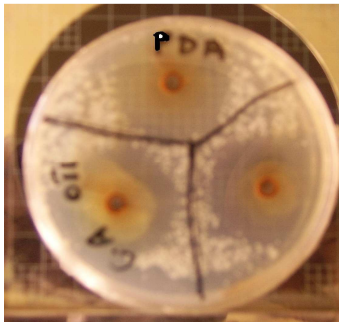
Table 7 indicates that the methanol and water extracts of OTI were strong antimicrobial agents. The extracts inhibited the growth of all the test microorganisms. This was unlike the water and methanol extracts of AZA which could not inhibit the growth of *B. subtillis*, *P. aeruginosa* and *C. albicans*. This shows that the methanol and water extracts of OTI were much better at inhibiting the test microorganisms than those of AZA. There was no significant difference ($P>0.05$) in inhibition between the water and methanol solvents of OTI. Therefore, both the water and methanol extracts of OTI are able to be used against all the test microorganisms while the water and methanol extracts of AZA have activity against *E. coli* and *S. aureus*. Sodium metabisulphite was a better antimicrobial agent than both the herbs extracts. Since water is a common medium of extraction and is easily available to communities of West Pokot, it was selected for further tests (Shale *et al*., 1999). The manner in which inhibited microorganisms appeared can be viewed in Plate 3.

Table 7: Average inhibition zone diameter IZD (mm) of extracts of OTI, AZA and sodium metabisulphite (SM) against test organisms

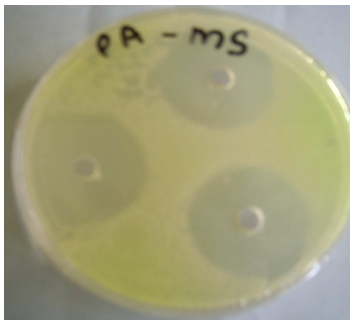
Solvent	Methanol				Water					
Extracts	OTI		AZA		OTI		AZA		MS	
Dilutions	0.2g/ml	0.1g/ml	0.2g/ml	0.1g/ml	0.2g/ml	0.1g/ml	0.2g/ml	0.1g/ml	0.2g/ml	0.1g/ml
B. subtilis	17.5±2.5^{b}	-	-	-	14.5±0.5^{c}	10.8±0.9^{d}	-	-	29.7±0.3^{a}	23.7±0.9^{b}
*S. aureus**	24.0±1.0^{b}	10.2±1.0^{d}	15.6±0.7^{c}	9.0±0.8^{d}	17.7±0.7^{c}	16.5±0.4^{c}	14.7±0.3^{c}	-	52.7±1.5^{a}	50.0±1.2^{a}
P. aeruginosa	19.5±0.5^{c}	13.3±0.5^{d}	11.3±0.3^{d}	-	25.0±0.6^{b}	11.3±0.9^{d}	-	-	33.7±1.9^{a}	26.7±0.5^{b}
C. albicans	12.5±0.5^{b}	10.0±0.8^{c}	-	-	14.0±0.5^{b}	10.0±0.8^{c}	-	-	62.3±1.5^{a}	60.0±1.5^{a}
E. coli	19.8±0.3^{b}	9.5±0.4^{d}	12.7±0.3^{c}	10.7±0.5^{d}	21.7±3.3^{b}	18.3±0.5^{c}	13.0±0.6^{c}	9.7±0.5^{d}	42.0±1.2^{a}	33.0±1.4^{a}

- indicates that the diameter of the zone of inhibition is below 9mm. Values followed by the same small letter within the same line are not significantly different (P>0.05). All the figures are in millimeters, values on mean ± SD of n = 3, * gram positive microorganisms, AZA stands for *Ziziphus abyssinica* A.Rich, OTI (Mkwaju) stands for *Tamarindus Indica* L, MS stands for Sodium metabisulphite,

(a)



(b)



(c)

Plate 3: Area of inhibition of (a) *E. coli*, (b) *C. albicans*, using the water extract of OTI and (c) *P. aeruginosa* using Sodium metabisulphate at a concentration of 0.1mg/ml (Photo by Nyaberi M. O. 2007)

4.4. Minimum inhibitory concentration (MIC) of aqueous extracts of OTI and AZA

The MIC of the aqueous extract of the fruit pulps of OTI and AZA, were determined because these extracts showed significant ($P<0.05$) antibacterial activity compared to others in Table 8. The MIC values of OTI, ranged between 0.39mg/ml and 3.13mg/ml, while for AZA it ranged between 3.13mg/ml to 50mg/ml. That of sodium metabisulphite was between 0.195mg/ml and 0.39mg/. MIC with respect to *C. albicans* was not done since the antibacterial activity measured by the zone of inhibition was below 9mm.

Table 8: Minimum inhibitory concentration in mg/ml of aqueous extracts of OTI and AZA against test microorganisms

Test microorganism	OTI (mg/ml)	AZA (mg/ml)	Sodium Metabisulphite (mg/ml)
Bacillus subtillis	1.56	25	0.20
Staphylococcus aureus	0.39	3.13	0.20
Pseudomonas aeruginosa	3.13	50	0.39
Candida albicans	3.13	nd	0.39
Escherichia coli	0.78	25	0.20

AZA - Ziziphus abyssinica A.Rich, OTI (Mkwaju) - Tamarindus Indica L, nd – Not determined

The values in mg/ml show the minimum concentrations of the aqueous extracts able to inhibit the growth of the test microorganisms as shown in Table 8. These values were useful in estimating the amount of herbs extract to be used in sausages for the

purpose of preservation. This is because it gave an indication of the minimum amount of extract utilizable for effective inhibition of growth of microorganisms.

4.5. Antioxidant activities of herbs KOIC, SSDC, AZA and OTI

4.5.1. Antioxidant activity of KOIC and SSDC

The results in Figure. 4 show that absorbance decreased in the first minute due to scavenging effect of the herbs extracts. All the herbs significantly ($P<0.05$) reduced DPPH, between KOIC and SSDC, there was no significant difference in their scavenging capacities ($P>0.05$).

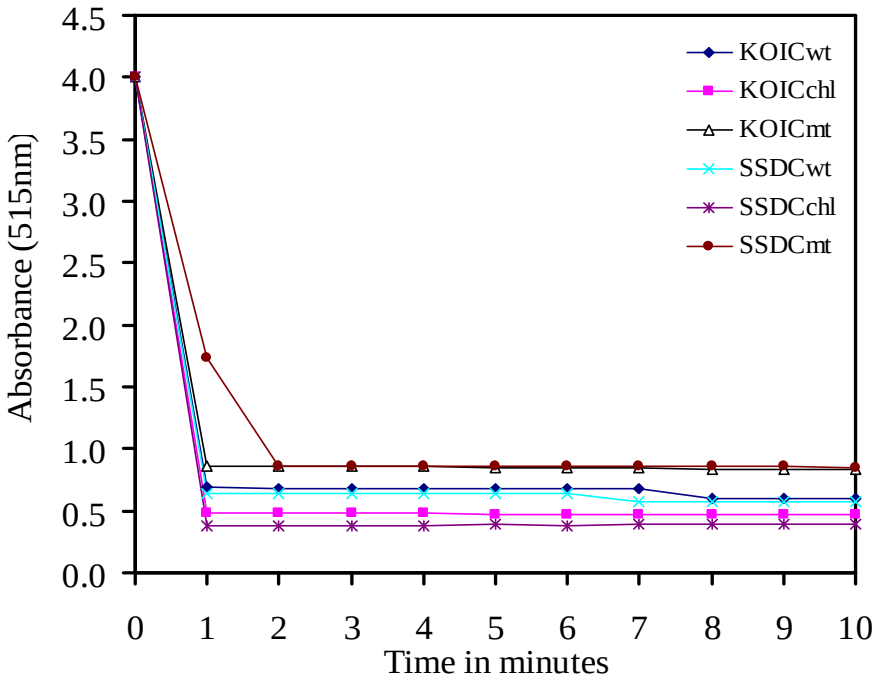


Figure 4: Antioxidant activity of the water (wt), chloroform (chl) and methanol (mt) extracts of KOIC and SSDC at 515nm.

Table 9 shows that, there were no significant differences (P>0.05) in antioxidant activity between KOIC and SSDC herbs, when the concentration was varied between 0.1 – 0.5mg/ml. Therefore, either of the herbs could be used for preservation and addition of flavour to sour milk.

Table 9: Percent antioxidant activities at different concentrations of the water, chloroform and methanol extracts of KOIC and SSDC

	Concentration of extract in mg/ml				
	0.1	0.2	0.3	0.4	0.5
KOIC (wt)	84.9±1.4^{ab}	85.0±0.06^{a}	85.0±0.08^{a}	85.0±0.03^{a}	85.0±0.05^{a}
KOIC (CHL)	87.9±0.5^{a}	87.9±0.05^{a}	88.5±0.03^{a}	88.5±0.3^{a}	88.5±0.5^{a}
KOIC (MT)	83.4±0.3^{ab}	80.2±0.08^{a}	74.1±3.2^{b}	79.2±2.6^{ab}	88.3±0.04^{a}
SSDC (wt)	85.9±0.04^{a}	85.9±0.03^{a}	85.6±1.4^{a}	85.8±0.5^{a}	85.6±1.4^{a}
SSDC (CHL)	87.9±0.3^{a}	89.5±0.3^{a}	90.1±0.2^{a}	92.3±1.3^{a}	91.1±3.2^{a}
SSDC (MT)	78.0±0.2^{b}	80.8±0.6^{a}	77.6±2.4^{ab}	78.8±2.9^{ab}	79.4±0.9^{ab}

Values followed by the same small letter within the same row are not significantly different (P>0.05) values are means ± SE and n=3, wt – water extract, CHL – chloroform extract and MT – methanol extract

4.5.2. Antioxidant activity of OTI, AZA and Sodium metabisulphate

Both water and methanol extracts of AZA reduced DPPH in the first one minute to a minimum low while both water and methanol extracts of OTI reduced DPPH gradually with time. In both AZA and OTI there was no significant difference in activity between their respective water methanol extracts. The maximum activity of sodium metabisulphite was reached within the first minute but it did not completely

reduce DPPH showing that it never had the same reducing potential as both AZA and OTI (Figure 5).

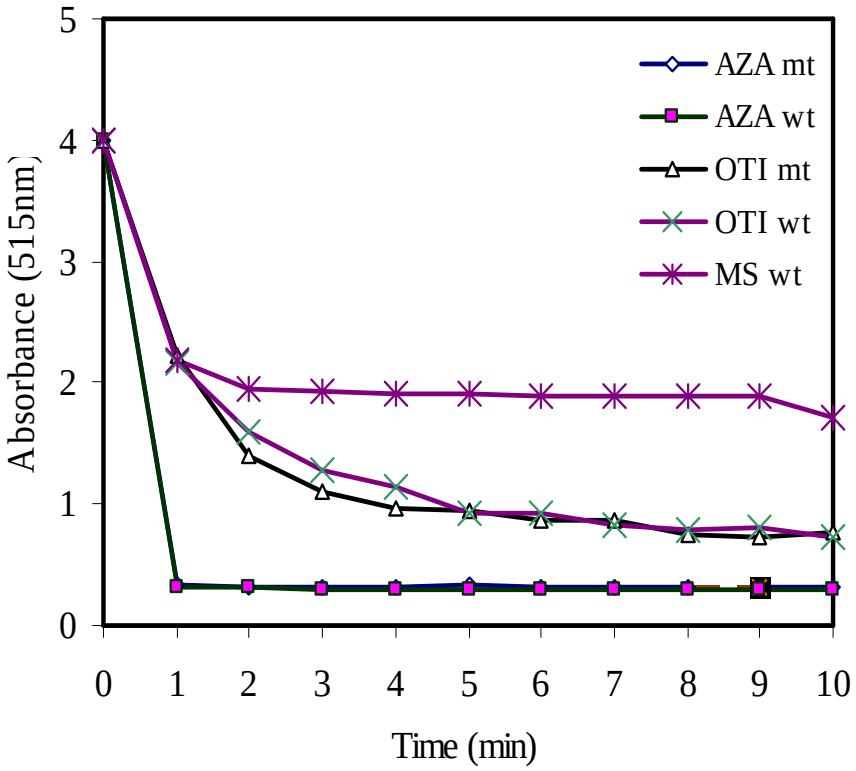


Figure 5: Antioxidant activity of methanol (mt) and water (wt) extracts of OTI, AZA and sodium metabisulphite (MS).

When the concentrations of the herbs were varied between 0.1 – 0.5mg/ml the methanol and water extract of AZA and the control sodium metabisulphite did not show significant differences in their antioxidation capacities ($P>0.05$). However, water and methanol extracts of OTI increased in antioxidation capacity with the increase in concentration indicating that the antioxidation capacity of the water and methanol extracts of OTI are concentration dependant. Sodium metabisulphite

reduced DPPH rapidly but to a lesser extent compared to the herbs extracts indicating low antioxidation capacity (Table 10).

Table 10: Percent antioxidant capacity of sodium metabisulphite (MS) and the water and methanol extracts of OTI and AZA.

	Concentrations of extracts in mg/ml				
	0.1	0.2	0.3	0.4	0.5
OTI wt	44.2±1.6^{c}	49.2±4.9^{bc}	55.8±1.2^{b}	76.3±5.9^{ab}	86.2±0.9^{a}
OTI (mt)	74.8±0.1^{b}	85.6±2.2^{a}	87.3±0.3^{a}	84.3±0.9^{ba}	86.9±1.3^{a}
AZA (wt)	96.2±0.1^{a}	95.7±0.1^{a}	95.3±0.1^{a}	92.7±0.1^{a}	89.6±0.4^{a}
AZA (mt)	93.4±0.1^{a}	92.9±0.5^{a}	92.3±0.1^{a}	91.5±0.2^{a}	90.7±0.1^{a}
MS (wt)	51.3±2.4^{b}	59.5±4.6^{ab}	57.8±1.7^{b}	64.1±1.9^{a}	69.4±1.3^{a}

Values followed by the same small letter within the same row are not significantly different (P>0.05) values are means ± SE and n=3.

4.6. Toxicity test on AZA and OTI herbs extracts

The herbs extracts exhibited moderate toxicity on the brine shrimps, giving an LC_{50} value greater than 100µg/ml. The LC_{50} value of the aqueous extract of AZA was 270µg/ml while that of OTI was 650µg/ml. According to Meyer et al. (1982) and Parra et al. (2001), LC_{50} values lower than 100µg/ml are considered very highly toxic while those between 100 - 1000µg/ml are considered moderately toxic. Above 1000µg/ml are mildly toxic (Kamrin, 1997). Therefore AZA is more toxic to brine shrimps than OTI. Although OTI was more active against bacteria and fungi than AZA it exhibited lower toxicity on brine shrimps compared to AZA (Table 11). This

may suggest inherent selectivity of the extract, thus making the OTI herb more suitable for preservation purposes than AZA (Mbwambo et al., 2007).

Table 11: Toxicity levels of the aqueous extracts of OTI and AZA

Concentration (µg/ml)	AZA (%)Mean Survival of brine shrimps	OTI (%)Mean Survival of brine shrimps
781.3	0	35.7±7.5
390.6	22.3±4.0	79.2±8.8
195.3	68.7±4.5	100.0
97.7	100.0	100.0
LC_{50} (µg/ml)	270	650

AZA - *Ziziphus abyssinica A.Rich,* OTI (Mkwaju) - *Tamarindus Indica L.* values are means ± SD n=3.

4.7. Preservative potential of herb KOIC and SSDC on Milk

4.7.1. Total viable counts of milk samples

The total viable count of both MS1 and MS2 increased between day three and day six and growth of microorganisms was not inhibited. On day six the maximum growth was attained with 11.5±2 log cfu/g for MS1 and 11.8±0.6 log cfu/g for MS2. The competition for food and accumulation of acidity by the lactic acid forming bacteria increased stress on the microorganisms and their counts started to drop. The numbers stabilized at about 9.0 log cfu/g as shown in Figure 6.

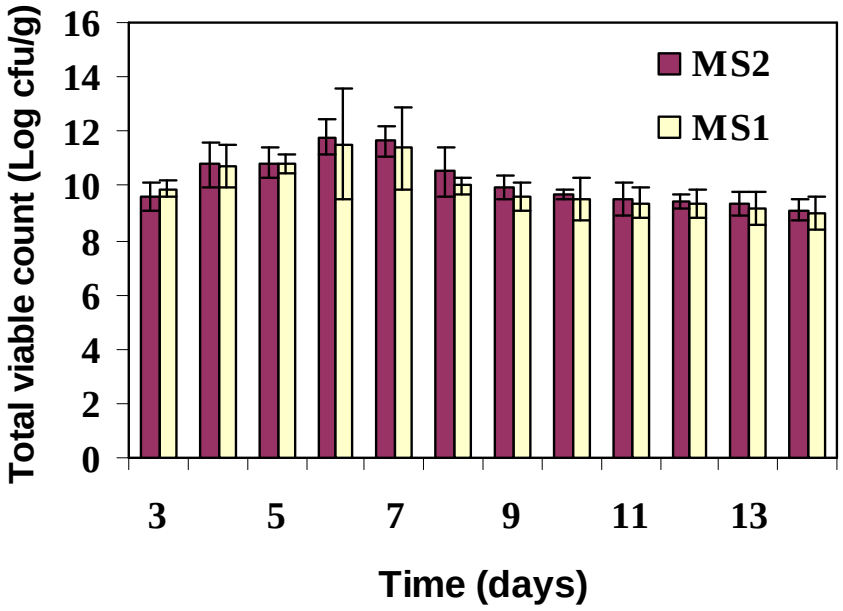


Figure 6: Total viable counts of milk treated with KOIC herbs charcoal during 14 days of storage.

The microbial counts of up to 11.5±2 log cfu/g for MS1 and 11.8±0.6 log cfu/g for MS2 were generally very high. This was attributed to the high temperatures that supported the proliferation of microorganisms, handling practices of the milk and the gourds, health condition of the cow's adder during milking and the hygiene condition of the one milking. All these contributed to the high bacterial load observed (Desmasures *et al*., 1997).

From the fourth to the fourteenth day the total counts of MS1 were consistently lower for milk treated with herbs compared to the control (MS2). This implies that the herbs charcoal had inhibitory effect on the growth of microorganisms. The

inhibitory effect may have reduced accumulation of LAB as a result lowering the accumulation of acidity in the milk. Therefore, milk continued to be palatable even when held over a long period of time as is customary with pastoralist communities.

4.7.2. Total lactic acid bacterial counts of milk samples

From day three to day six the LAB increased constantly to a maximum 10.7 and 11.6 log cfu/gm for MS1 and MS2 respectively. As from day six there was a constant reduction in count of LAB to a minimum of 6.0 log cfu/gm for both MS1 and MS2. The LAB counts for MS1 were consistently significantly lower ($P<0.05$) than those of MS2 from day 3 to day11 (Figure 7). The difference may have resulted in selective inhibition of some of the LAB.

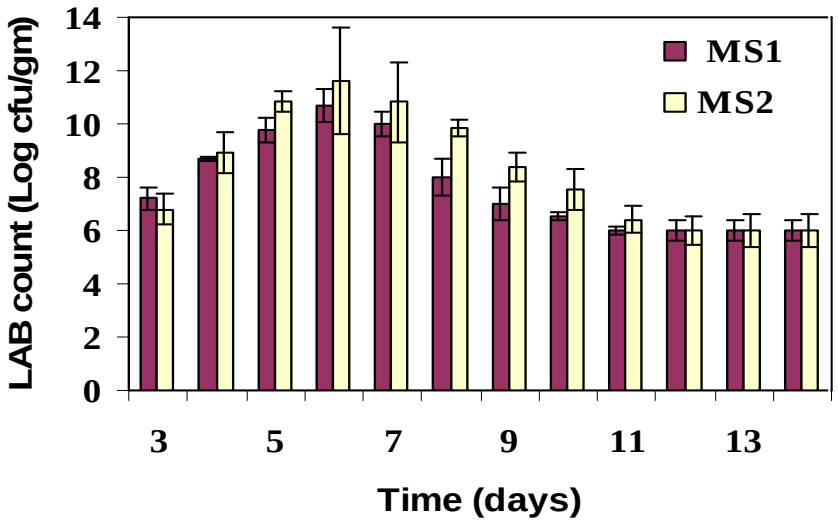


Figure 7: LAB counts of milk treated with KOIC herbs charcoal during 14 days of storage.

4.7.3. Changes in pH and total titratable acidity of milk samples

While fresh milk is known to have a titratable acidity of between 0.14 and 0.18% (Wanjala, 2007), both MS1 and MS2 samples had a titratable acidity of 0.6% lactic acid on the third day after collection indicating that fermentation had set in. From the third to the eleventh day, the titratable acidity of both MS1 and MS2 increased without any significant difference ($P<0.5$) between them. Between day 11 and 14 there was significant difference ($P<0.5$) between the two. The acidity of MS2 increased from 2.1% on the day 11 of storage to 3.5% in day 14. On the other hand the treated milk increased slightly from 2.0% on the day 11 to 2.8% on day 14 (Figure 8A).

At 3.5% and 2.8% MS2 and MS1 stabilized respectively. This indicated that MS2 accumulated more acid faster as it was not protected by the presence of herbs. This shows that the herb had a capacity to control the increase in acidity by either inhibiting the growth of the microorganisms such as the lactic acid producing bacteria responsible to the increase in acidity or acting as a buffer.

The pH of both samples MS1 and MS2 on day three was 6.4. Fresh milk has a pH of 6.7. The milk samples had started fermenting (Amoa-Awua et al. 2006). The pH of both MS1 and MS2 decreased constantly till the 9^{th} day with no significant difference between them. From the 10^{th} to the 14^{th} day the pH of MS1 reduced very gradually to stabilize at a pH of 4.8, while the pH of MS2 continued to decline to a pH of 3.8. At a pH of 3.8 milk is already too bitter to consume, while the pH of 4.8 that MS1 had attained in day14, milk was palatable (Figure 8B). It can be observed

that in all days between the 5th and the 14th day MS2 had a lower pH than that of MS1. This shows that the herb was able to control acidity in MS1. Since acidity is as a result of accumulation of acid producing microorganisms, then the herb extract of KOIC is able to control the growth of selected microorganisms (Shelef 1994; Mbandi and Shelef 2002).

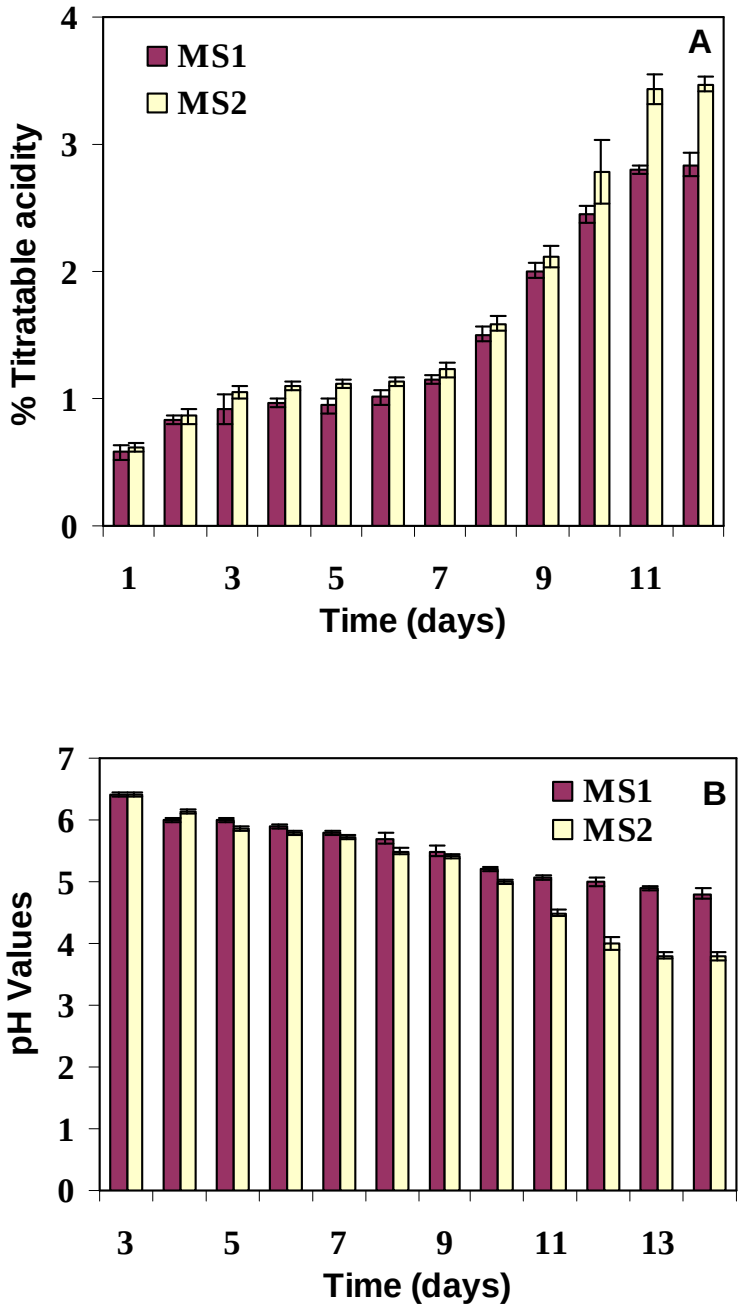


Figure 8: Percentage titratable acidity (A) and pH (B) of MS1 and MS2 during 14 days of storage.

4.8. Preservative potential of the herbs OTI and AZA on pork sausages

4.8.1. Microbial activity of sausages treated with herb extracts

4.8.1.1. Total viable count (TVC)

The total viable counts in sausages stored at 4 and 25ºC ± 2 increased significantly ($P<0.05$) in all the samples (Figure 9 A+B). At 4ºC the initial viable plate counts of sausages treated with OTI, AZA, sodium metabisulphite and control were <1.3, <1.3, 1.8 and 1.4 log cfu/g respectively. The British Meat Processors Association (BMPA, 2006) recommends a maximum upper limit of 6 log cfu/g (Brewer et al., 1992). While OTI and sodium metabisulphite therefore qualify to preserve sausages for 15 days, AZA preserves sausages for 10 days at 4ºC. (Figure 9A). On the other hand AZA can also be used but for a shorter storage duration.

At 25 ± 2°C, the total viable counts increased significantly ($P<0.05$) in all the samples (Figure 9B). Initially the sausages treated with sodium metabisulphite and OTI had very low counts that was unlike those treated with AZA and the control which had 1.8 and 1.4 log cfu/g. This showed that the antimicrobial effect of OTI and sodium metabisulphite possibly took effect immediately resulting in the initial low counts. On the second day the counts were 8.5, 8.3, 7.2 and 7.6 log cfu/g for OTI, AZA, sodium metabisulphite and the control respectively. The counts were high and considered objectionable (El – Marrakchi et al. 1990; Ryder et al. 1984). There was no significant difference in the growth of microorganism among the sample sausages and therefore the preservatives OTI, AZA and sodium metabisulphite had minimal preservative capacity when stored at room temperature.

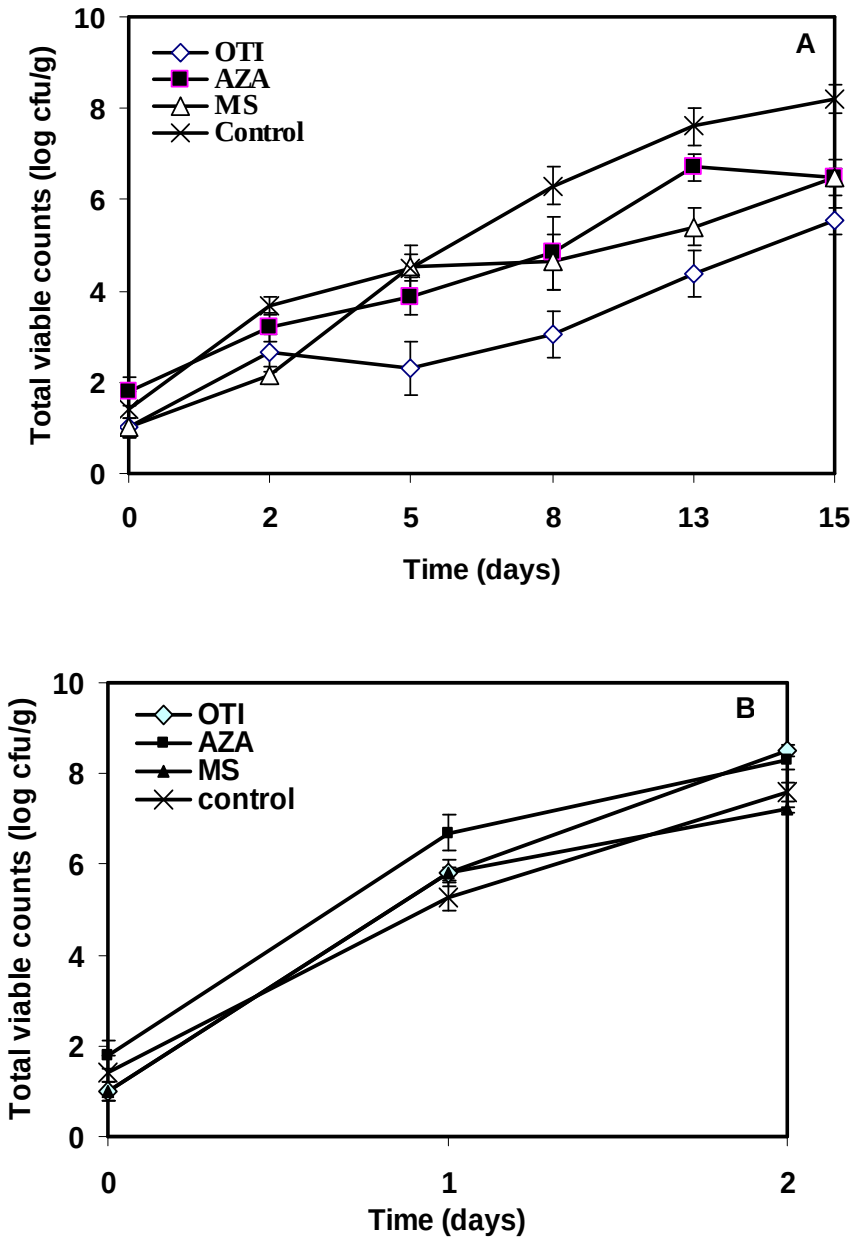


Figure 9: Total viable counts of sausages treated with herb extracts, sodium metabisulphite and a control at (A); 4°C and (B); 25°C.

4.8.1.2. *E. coli* and *S. aureus* counts

During storage at 4 ± 2°C, growth of *E. coli* and *S. aureus* was detected and increased constantly until the thirteenth day when the counts started to decrease. There was no significant difference in growth of *E. coli* in OTI, AZA or sodium metabisulphite. This was attributed to temperature, competition for food and the preservatives. According to the BMPA the microorganisms were within the acceptable limit of below 4.0 log cfu/g (Domańska and Różańska, 2002). The presence of OTI and AZA delayed initial growth of *E. coli*. The herb OTI had the highest antimicrobial effect on both *E. coli* and *S. aureus*. This was because it exhibited the highest inhibitory effect with subsequent low counts (Figure 10 A and B).

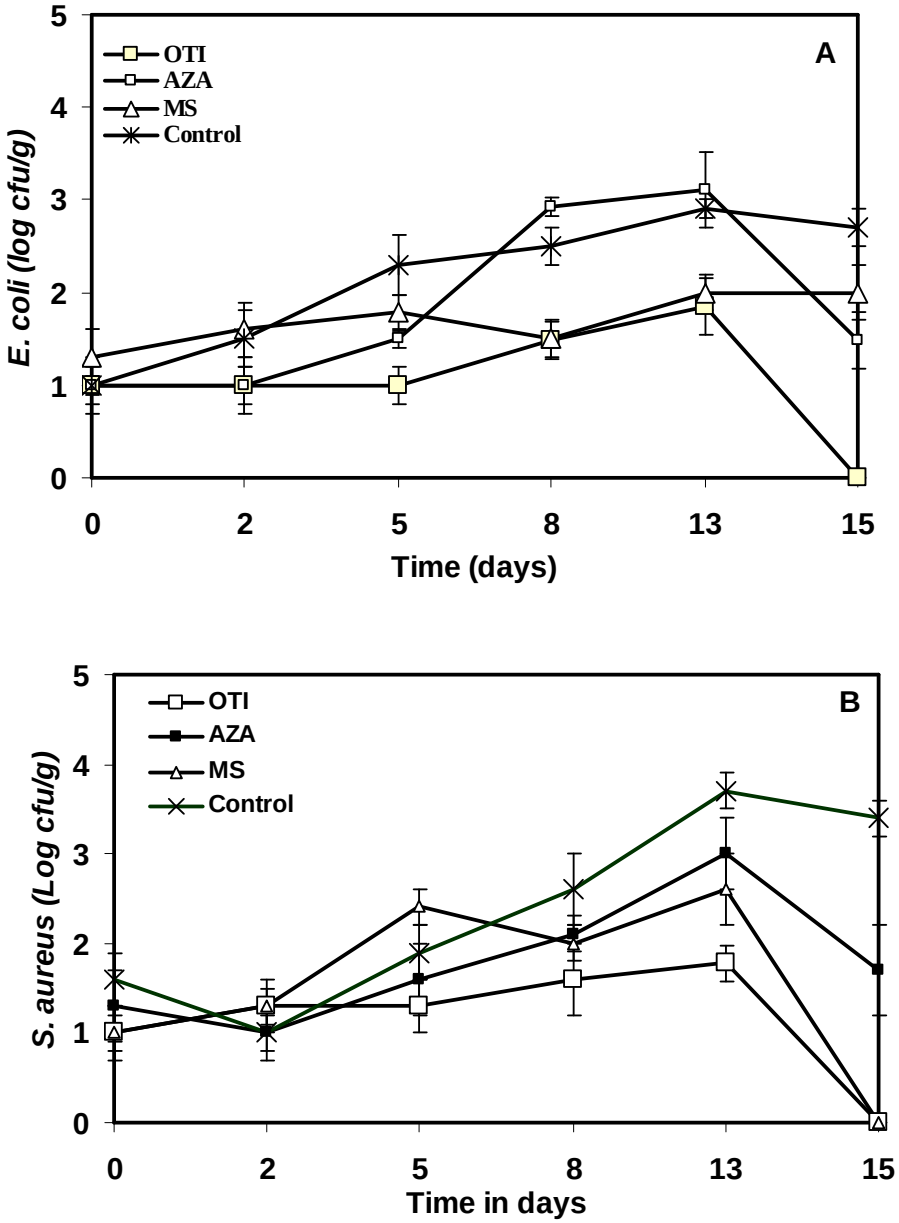


Figure 10: Growth of *E. coli* (A) and *S. aureus* (B) in sausages treated with herb extracts, sodium metabisulphite and a control at 4°C.

In hygienically processed sausages, and stored at room temperature of 25 ± 2°C the *E. coli* and *S. aureus* counts increased significantly in all samples (Figure 11 A and

B). The sausages treated with OTI herbs extract and sodium metabisulphate had significantly lower ($P<0.05$) counts compared to those with AZA herbs extract and the control. These microorganisms *E. coli* and *S. aureus* are indicators of the presence of pathogens. Therefore the presence of *E. coli* and *S. aureus* is attributed to the poor processing, handling and storage of meat during and after slaughter.

On day 0, *E. coli* and *S. aureus* detected was in very low counts. The sausages treated with AZA and the control at <1.3 and 1.5 log cfu/g respectively. On day 2, the sausages treated with OTI and sodium metabisulphite had lower counts of *E. coli* at 3.3 and 2.8 log cfu/g respectively. The sausages treated with AZA and the control had 5.3 log cfu/g. This shows that OTI and sodium metabisulphite have higher inhibitory effects on *E. coli* than AZA. Thus concurs with observations that OTI and sodium metabisulphite had a significantly higher antimicrobial capacity than AZA based on MIC. Thus samples treated with AZA and the control had significantly higher counts of *S. aureus* and *E. coli* than those treated with OTI and MS (Figure 11A and B).

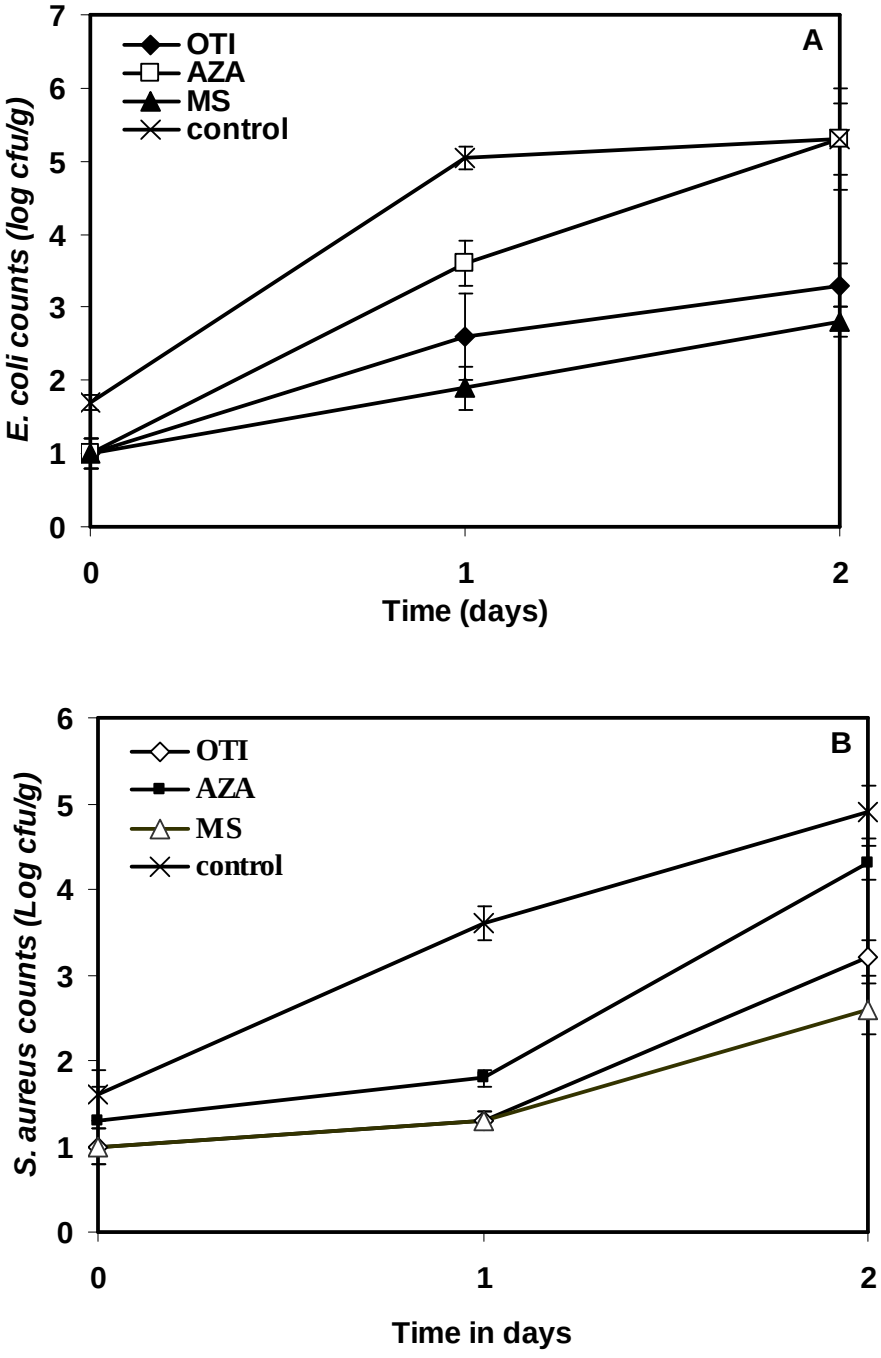


Figure 11: Growth of *E. coli* (A) and *S. aureus* (B) in sausages treated with herb extracts, sodium metabisulphite and a control at 25°C.

4.8.1.3. Yeast and moulds count

The initial yeast counts in the sausages treated with herbs extracts of OTI, AZA and sodium metabisulphite were 3.8, 2.7, 2.6 and 3 log cfu/g respectively. The presence of yeast and moulds was attributed to the ingredients used to prepare sausages. When the sausages were stored at 4 ± 2°C, those preserved with OTI and sodium metabisulphite had significant ($P<0.05$) inhibitory effect against yeast and moulds compared to AZA and the control (Figure 12A).

When the sausages were stored at 25±2°C the yeast and moulds counts rose steadily, there were no significant differences ($P>0.05$) in the action of all the herbs on the yeast and moulds. This implies that none of the herbs including sodium metabisulphite was able to control the proliferation of yeast and moulds at 25 ± 2°C (Figure 12B).

Temperature alone cannot control the growth of yeast and moulds; there is a need to introduce a preservative to control the growth of yeasts and moulds. OTI and sodium metabisulphite may be a better option to control the yeast and moulds.

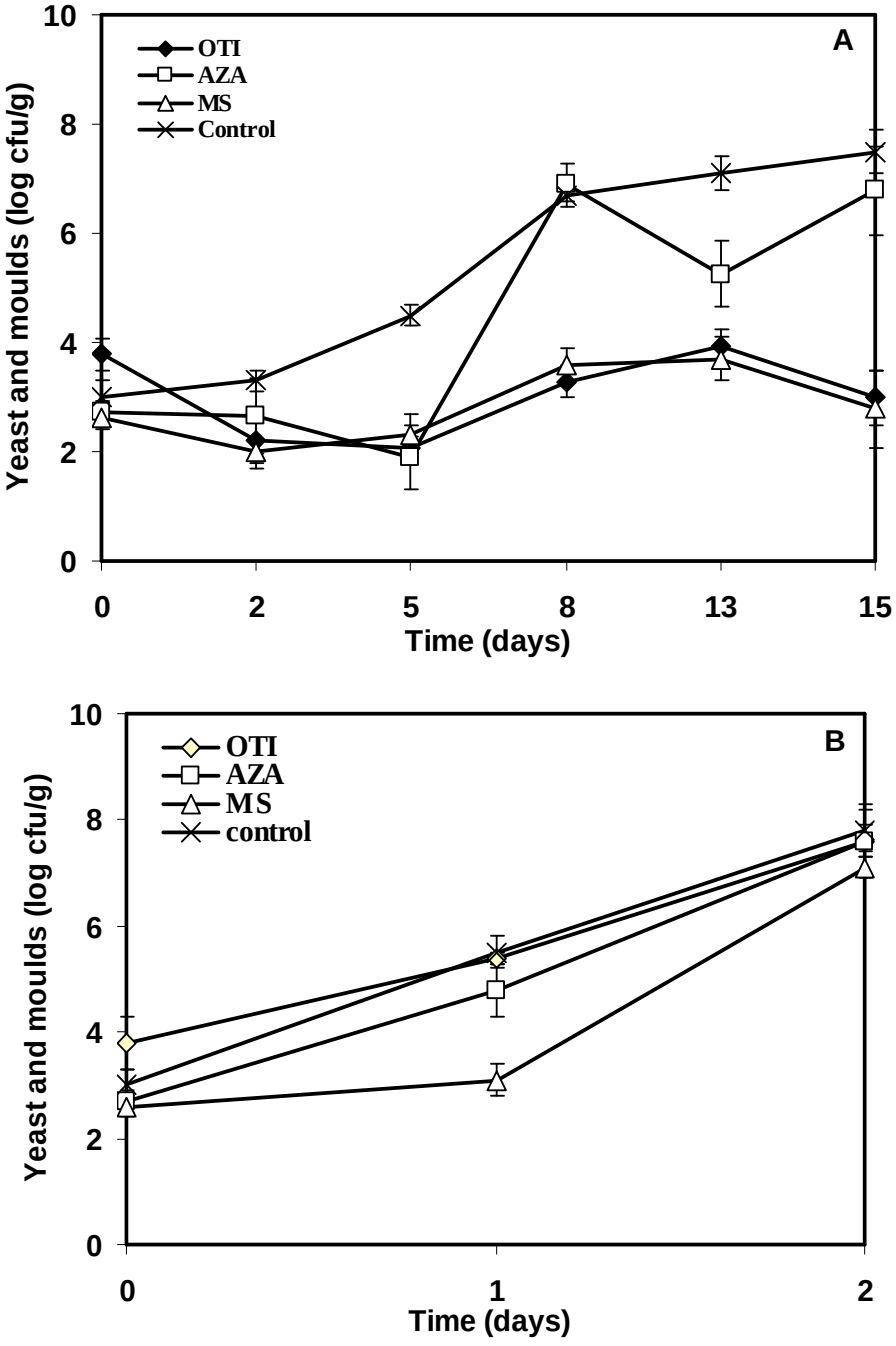


Figure 12: Growth of yeast and moulds in sausages treated with herb extracts and sodium metabisulphite at 4°C (A) and 25°C (B).

4.8.2. Evaluation of rancidity (TBARS) in sausages treated with herbs

Freshly prepared sausages had a thiobarbituric acid reactive substance (TBARS) value of 0.1mg MA/kg (MA) which was within the limits recommended by Lee and Decker (1997) of 0.3mg MA/kg. Sausages stored at 4 ± 2°C showed a significant ($P<0.05$) increase in TBARS values across treatments. Samples treated with OTI showed an increase up to a maximum of 2.4mg MA/kg while samples treated with sodium metabisulphate attained 1.53 mg MA/kg on the fifteenth day. Samples treated with AZA experienced the least increase in rancidity. This is because AZA is a strong antioxidant which prevents rancidity (Figure 13A).

Storage of sausages at 25 ± 2°C resulted in a constant increase in TBARS values. The sausages treated with the herb OTI had the highest increase while those treated with AZA had the least. The difference were significant at ($P<0.05$). All the TBARS values were within the acceptable levels in the sixth day of storage at approximately 3 mg MA/kg, whereby OTI, AZA and sodium metabisulphite had 0.9, 0.6 and 0.7 mg MA/kg respectively. Physically the sausages were already decomposed. Therefore this method is not reliable to measure acceptability of a food product for consumption. Nevertheless, AZA was the most effective herb in the prevention of rancidity due to the low TBARS values, than the control (Figure 13B).

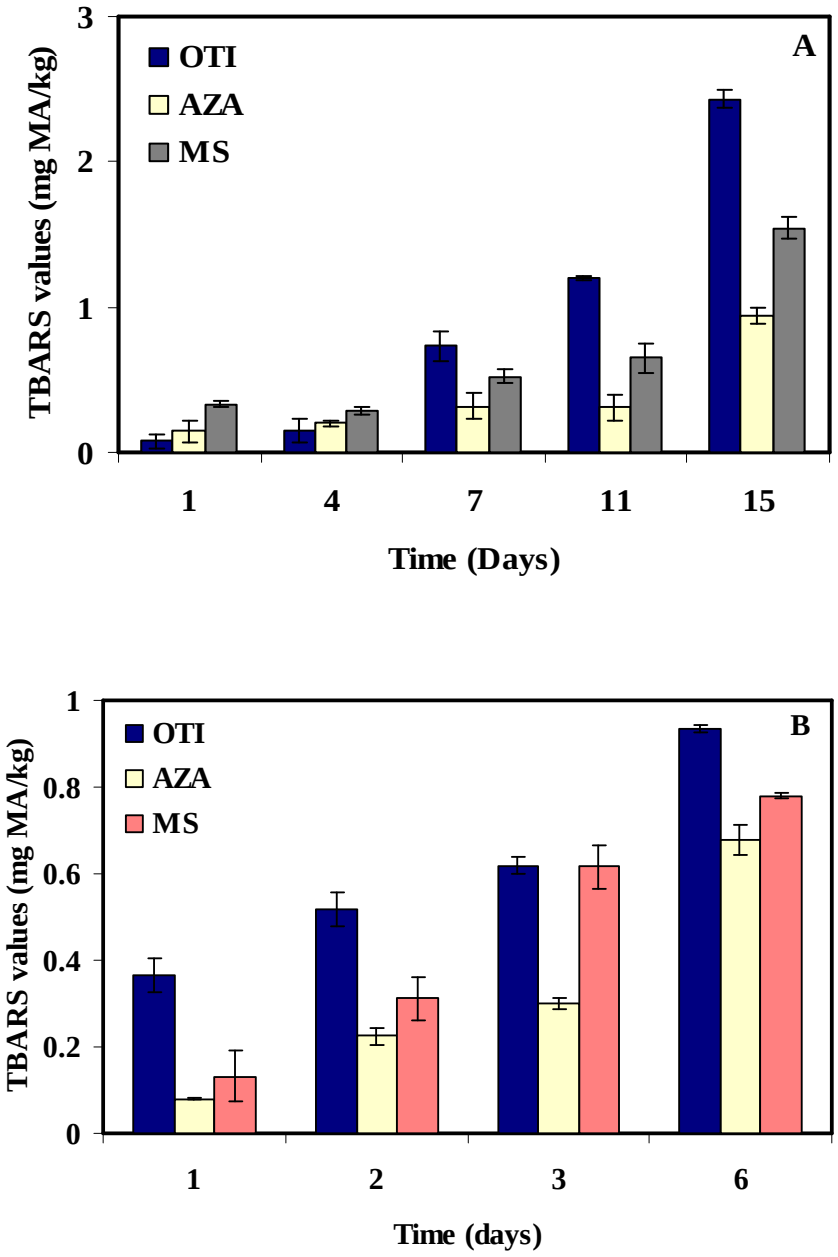


Figure 13: TBARS values at 4°C (A) and 25°C (B) for sausages preserved with OTI and AZA herb extracts and sodium metabisulphite.

Sausages treated with commercial preservatives BHA/BHT had TBARS value of 1.19 mg MA/kg on the 14th day which was higher than that of AZA in the 15th day at 0.9 mg MA/kg (Kawole et al 1996; Lee and Decker 1997). This implies that, AZA is a better antioxidant than the commercial products BHA/BHT.

4.8.3. Total volatile base nitrogen (TVB-N)

The recommended upper limit for total volatile base nitrogen (TVBN) in pork sausages is >25 mg/100g (Lannelongue et al. 1982). On the eleventh day at 4 ± 2°C the total volatile base nitrogen values of sausages treated with OTI, AZA herbs and sodium metabisulphite were 11.5, 16.1 and 17.6 mg/100g respectively. This was within the recommended upper limit. It indicated that the sausages were of good quality till the eleventh day. Total volatile base nitrogen is produced as a result of microbial activity and the low values obtained concurred with earlier findings of low total counts (>5.5 log cfu/g) for all treated sausages (Figure 14A). On the fifteenth day all the sausages treated with herbs extracts and sodium metabisulphate were slightly above the recommended upper limit. AZA had higher TVBN value than OTI and the control of between 0.3 and 33.3 mg/100g on the fifteenth day. This too is consistent with the TVC of 6.5log cfu/g just above the recommended upper limit, were microbial spoilage was observed to be highest. Therefore, taking TVBN into consideration the sausages would remain edible till the eleventh day. The TVBN values can be used for rapid assessment of spoilage.

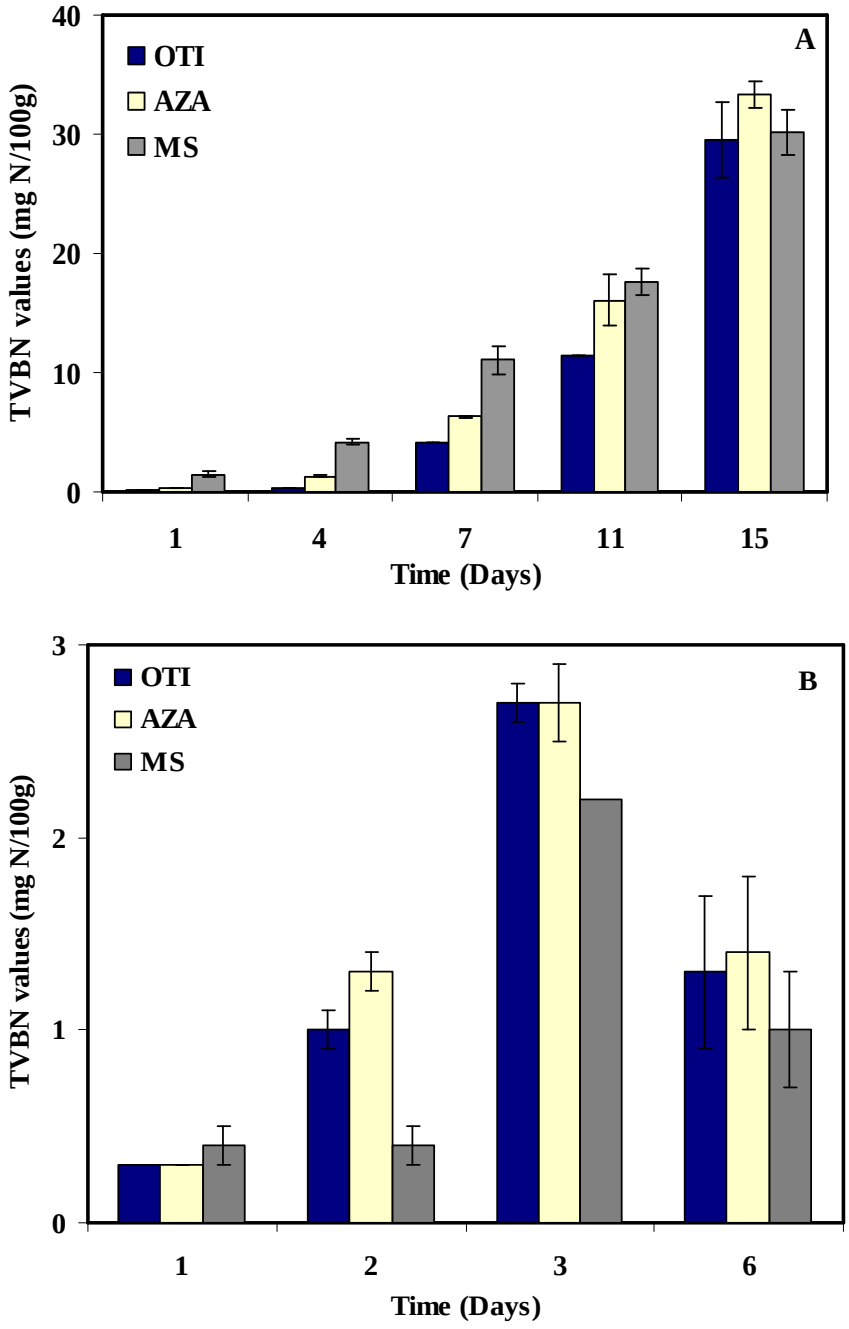


Figure 14: TVBN at 4°C (A) and 25°C (B) for sausages preserved with herbs extract OTI and AZA and Sodium metabisulphite.

Sausages stored at 25 ± 2°C deteriorated significantly in quality by the sixth day (Fig 14B). The TVBN values remained below 3 mg/100g indicating that the sausages were of good quality. Therefore, TVBN is not a good indicator test for spoilage during short storage. From the results OTI is a good antimicrobial agent while the AZA is a good antioxidant.

4.8.4. Sensory analysis

Sausages treated with the herbs, were not significantly different ($P>0.05$) from the controls in the sensory evaluation ratings. However, those treated with OTI were rated lower at 6 or "slightly good" compared to those treated with AZA and sodium metabisulphite which were rated 6.8 and 7.2 respectively, indicating they were "moderately good". This implies that OTI has properties such as acidity that adversely affect the texture of the casing making it slightly less desirable than those treated with AZA and sodium metabisulphite sausages (Krithika and Radhai 2007). The sausages treated with OTI were rated considerably higher for flavour at 7 or moderately good (Figure 15). All sausages were found to be of moderately good overall acceptability. These herbs used together can therefore be considered favorably for protection of sausages against microbial and oxidative spoilage.

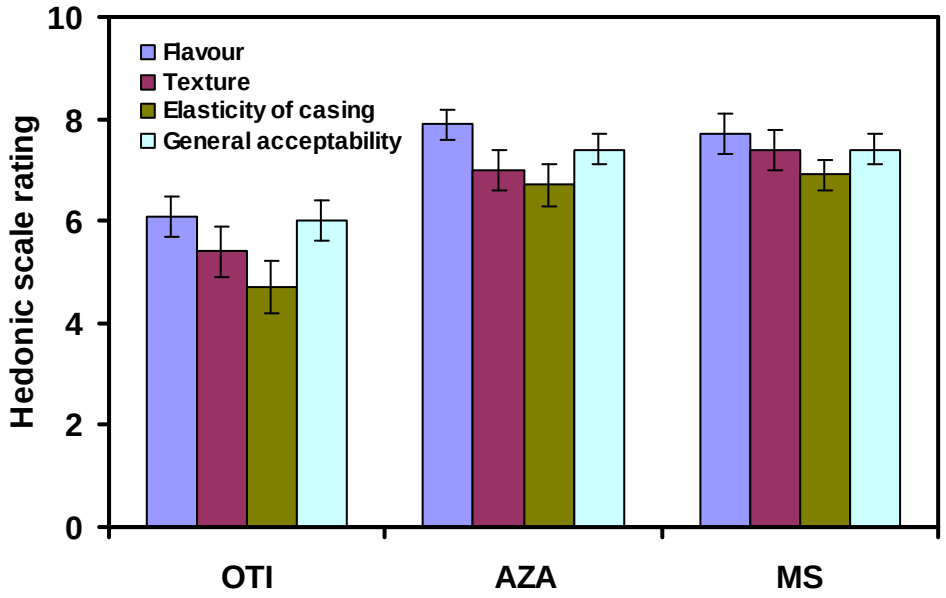


Figure 15: Mean sensory hedonic scores for sausages with herb extracts, sodium metabisulphite (MS) and a control

Treatment with OTI caused sausages to develop a distinct flavour that was distinguishable by 60% of the panelists. The control was distinguishable by 10% of the panelists while 30% of the panelists were able to distinguish sausages that had AZA from the sausages treated with OTI and the control (Figure 16).

Since more than a half of the panelists could distinguish the taste of OTI then the preservative OTI influences the taste of the sausages. The panelists were not able to distinguish the taste of AZA or the control meaning that the herb AZA did not impart any taste on the sausages. This concurred with survey findings where the pastoralists of West Pokot claimed that the herb AZA did not impart any taste to the

meat unlike OTI. Though OTI imparted some flavour to the sausages the taste was found to be appealing by the majority of the panellists. This implies that the herbs did not only impart protection against spoilage but also enhanced the taste of products. These herbs can therefore find use as preservatives (Jellinek 1985).

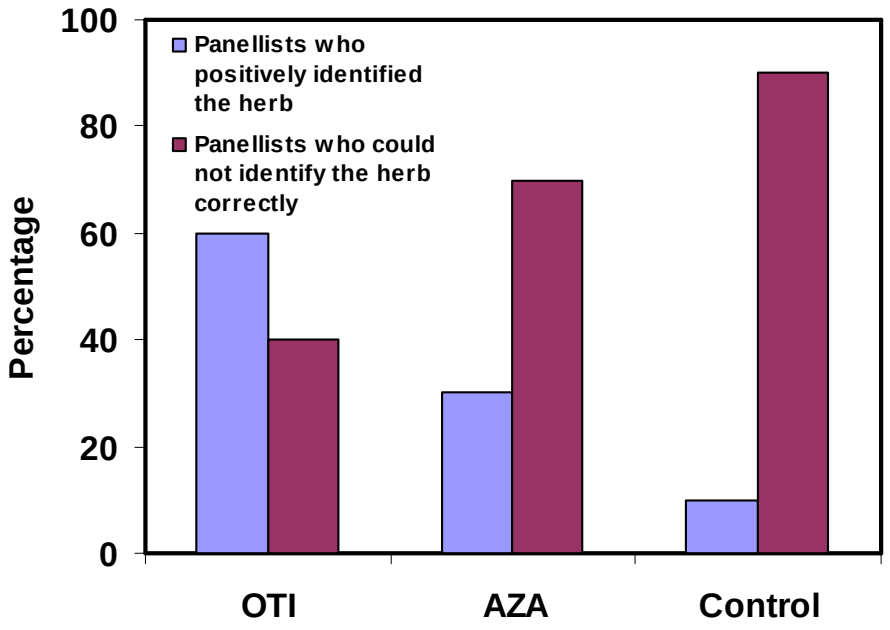


Figure 16: Ability to distinguish sausages treated with the herbs extracts.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The study established that several herbs are used by pastoral communities of West Pokot to preserve both meat and milk. KOIC and SSDC are mainly used to preserve milk while OTI and AZA are commonly used to preserve meat. The four herbs that are preferably used were found to be rich in phytochemicals. The main ones are reducing compounds, sterols and steroids, alkaloids, saponins, flavonoids, polyphenolics and condensed tannins. AZA and SSDC both had condensed tannins and this may be why they are less preferred.

The herbs had antimicrobial activity against test microorganisms that included *B. subtillis, P. aeruginosa, S. aureus, E. coli, C. albicans*. The herbs extract of OTI was the most active; it inhibited the growth of all the test microorganisms while AZA inhibited the growth of *S. aureus* and *E. coli* only. The herbs extracts of KOIC inhibited *E. coli* and *P. aeruginos* while SSDC inhibited all the test microorganisms. The herbs extract AZA, SSDC and KOIC were good antioxidants, OTI exhibited the least antioxidant activity compared to AZA, SSDC, and KOIC.

The herb KOIC, used to preserve milk, was able to control acidity and the growth of both total plate and lactic acid producing bacteria. Therefore, the herbs not only added flavour to the milk as claimed by the pastoralists of West Pokot but also preserved the milk by controlling the growth of microorganisms and enhanced palatability by controlling acidity development. The herbs extract of OTI is able to

preserve pork sausages for at least 15 days at 4 ± 2°C. This compares well with the sausages in the market that have a shelf life of 12 – 14 days. The herbs extract of AZA was able to preserve sausages for at least 10 days. Since OTI is a strong antibacterial agent and AZA is a strong antioxidant, blending them would result in a commodity that can reduce microbial growth and rancidity in meat products. In the absence of any preservatives sausages keep for 7 days at 4ºC.

Sensory evaluation showed no difference in preference between sausages with herb extracts and those found in the market. Unlike AZA, OTI flavour was preferred, the casing was adversely affected. In conclusion, the herbs used by the pastoralists of West Pokot have properties that are effective against the spoilage of milk and pork sausages. Therefore their use as antimicrobials and antioxidants is possible and should be up scaled for possible commercialization.

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Areas for further research

The following are recommended as areas for further research.

1) Other pathogenic microorganisms such as *Salmonella spp. L. monocytogenes, Y. enterocolitica, C. jejuni/coli* were not considered yet they can too have a hand in the spoilage of pork sausages. Therefore further research can be considered to cover them and see how the herbs OTI and AZA directly affect them.
2) Several microorganisms are classified as LAB, further research is required to ascertain the particular lactic acid bacteria present inhibited, and how they are affected by the herbs KOIC and SSDC in milk preservation.

3) The active principle compound of the herbs AZA and OTI should be identified. This will now confirm the actual compound that is exerting the inhibitory effect to the test microorganisms used and also find the effective ratio for antimicrobial and antioxidant action.
4) A refined herbal product should be produced and tested for commercialization.

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APPENDIX I

QUESTIONNAIRE ON USE OF INDIGENOUS PLANTS AND PLANT PRODUCTS IN PRESERVATION OF MILK AND MEAT

Respondent No. ----------

Location ----------

Interviewer's name ----------

Duration of interview ----------

DEMOGRAPHIC DATA

1. Name----------Male---------- Female----------

2. Age 15---------- 15-20yrs---------- 20-30----------

   30-40yrs-------40-50yrs----------->50yrs----------

3. Head of household----------Marital status----------

4. Number of people in the household----------

5. Occupation of members of the household ----------

---

6. Level of education: Primary----------Secondary----------

   Tertiary----------Others----------

7. Farm size----------

USE OF HERBS IN FOOD PROCESSING AND PRESERVATION

1. Do you use herbs in preservation of livestock products?

   □ Yes

   □ No

2. What are the commonly used herbs in preservation of livestock products in the community?

Local name of plant		Plant part/parts used in processing and preservation						
		Roots	Barks	Leaves	Seeds (Dry)	Seeds (Fresh)	Stem	Whole
1.								
2.								
3.								
4.								
5.								
6.								
7.								

3. Which of the plants are used in the fermented milk/meat products?

Fresh/Fermented Milk	Meat products	Fish Products

4. From where do you get the herbs you use?

……
……..

5. What method do you use in the collection and processing of the herbs?
   - Drying
   - Smoking
   - Sprinkling with ashes
   - Using herbs charcoal
   - Any other comments

6. During which season of the year do you collect the plant and what time of the day do you collect them?

……

……

……

7. If they are stored how are they preserved (include temperature, method of storage and duration of storage)?

……

……

……

8. Once preserved do the foods require any special treatments?

……

……For how long can the food be preserved using the preservative named above?

- □ 1 week
- □ 2 weeks
- □ 1 month
- □ 3 months
- □ 6 months
- □ Any other

9. Before consuming the milk/meat products are there any post treatments required?
   - □ Yes
   - □ No

If your answer to the above is yes, explain?

……

……Does the preservation effect imparted by your treatment affect the quality of the food in any way?

- □ Yes
- □ No

If your answer to the above is yes, explain?

……

……Any suggestions for improving the preservative affects described in the questionnaire?

……

……

Appendix II

SENSORY EVALUATION OF POCK SAUSAGES TREATED WITH DIFFERENT CONCENTRATIONS OF NATURAL PRESERVATIVES AND A CONTROL.

Please bite and taste samples of fried pock sausages. Make a judgment of each sample by checking and ticking the appropriate phrase under each of the quality parameters provided. The samples are randomly coded to avoid biased judgment. Rinse between samples.

Name……. Code No.……

1	Extremely bad	6	Slightly good
2	Very bad	7	Moderately good
3	Moderately bad	8	Very good
4	Slightly bad	9	Extremely good
5	Neither good nor bad		

Flavour	
Texture	
Hardness of casing	
General acceptability	

Appendix III

SENSORY EVALUATION OF POCK SAUSAGES TREATED WITH DIFFERENT CONCENTRATIONS OF NATURAL PRESERVATIVES AND A CONTROL.

Please bite and taste samples of fried pock sausages. Make a judgment of each sample by checking and ticking the appropriate phrase under each of the quality parameters provided. The samples are randomly coded to avoid biased judgment. Rinse your mouth between samples.

Panelist number ……

1. Five point deference scale for plate 1, 2 and 3

5	**4**	**3**	**2**	**1**
Extremely different	Very different	Moderately different	Slightly different	Neither different nor similar (almost similar)

Plate No						
	S	D	S	D	S	D
Indicate which of the 3 samples are similar and which one is different?						
How different is the different one? Rate it using the 5 point deference scale						

S – Similar, D - Different