UNIVERSITY FOR DEVELOPMENT STUDIES

THE EFFECTS OF SWEET BASIL *(Ocimum basilicum)* LEAVES AND DAWADAWA *(Parkia biglobosa)* SEED POWDERS ON THE SENSORY, NUTRITIONAL AND MICROBIAL QUALITIES OF BEEF AND FRANKFURTER SAUSAGES

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BY

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2015

DECLARATION

I hereby declare that this work is the results of my own work for the award of Master of Philosophy in Meat Science and Technology. This work has not been submitted in whole or part in University for Development Studies and any other University. Literature referred has been duly acknowledged in the references.

Candidate: Abu Alexander …………………

Signature: ……………………. Date

Supervisors' declaration

We hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

Principal Supervisor: Prof. Gabriel A. Teye ………………..

Signature: ……………………………….. Date

Co-supervisor: Dr. Frederick Adzitey ……………….

Signature: ……………………….. Date

ABSTRACT

The excessive use of artificial additives and preservatives in meat products is receiving a lot of mixed feelings from consumers for health reasons. This study was conducted with the aim of finding out the effects of basil leaf and fermented dawadawa seed powders on the sensory, nutritional and microbial qualities of beef and frankfurter sausages. The experiment involved the use of dawadawa and basil leave powders at 4, 6 and 8g/kg meat in place of adobo® a commercial spice in both beef and frankfurter sausages. Sensory characteristics such as colour, aroma, flavour intensity, flavour liking and texture were evaluated on the 1st, 7th and 14th day after product formulation. The control and 4g of both test materials in beef and frankfurter sausages had acceptable sensory attributes as compared to the higher levels (6 and 8g/kg). Both dawadawa and basil products were significantly (P<0.001) higher in minerals such as iron, calcium, copper, manganese, selenium and zinc. All the frankfurter sausages had peroxide values below the acceptable limit of 25 meq/kg of product. However, the basil frankfurter sausages had significantly (P<0.001) higher peroxide values as compared to the control. The microbial load for the products ranged between 3 – 6 CFU/g of sausage. The values were below 7CFU/g of sausage in which most foods spoilage begins, therefore the storability of the products prepared with both basil and dawadawa were not negatively affected.

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DEDICATION

I dedicate this work to my family for their prayers and support throughout my studies.

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CHAPTER ONE

1.0 INTRODUCTION

World food programme (WFP) estimated that 1.2 million people of the population of Ghana were food insecure (WFP, 2009), out of which about 34% are from Upper West, 15% are from Upper East and Northern Region with 10%, amounting to approximately 453,000 people (WFP, 2009). This calls for the use of technology to provide sufficient quantity of food and meat for the ever increasing population in the country.

Meat processing can serve as a basic technology to solve the problem of food insecurity. Processing of raw meat into products adds value, increases the yield, extends the shelf life of meat and also serves as a source of employment (Smith and Hui, 2004). To reduce spoilage, meat is processed by the addition of ingredients and/ or mechanical action to convert it into specific products which may include sausages, burgers and many more, to meet the desires of consumers (Teye, 2007).

Meat products such as sausages and burgers require the use of spices. Spices are esoteric food adjuncts that are used as flavouring agents and as preservatives in meat products (Srinivisan, 2005). Spices and herbs are valued for their distinctive flavours, colours, and aromas and are among the most versatile and widely used ingredients in food preparation hence the meat industry cannot be imagined without the utilization of spices (Skrinjar *et al.,* 2012). More than 400 spices have been used in the world (Ceylan and Fung, 2004). Herbs and spices have tremendous importance in the lives of humans as ingredients in food, alcoholic beverages, medicine and cosmetics (Ravindran *et al.*, 2002). The excessive use of artificial additives and preservatives is receiving lot of mixed feelings from consumers for health reasons such as cancer. Hypertension and obesity are perceived to be associated with these additives (Lawrie and Ledward, 2006). One way of solving this problem is by the use of natural spices which are believed to have beneficial health effects.

Dawadawa is the fermented seed of *Parkia biglobosa*. It is a black strong flavouring substance, high in protein, lipids and vitamin B2 (Ohenhen *et al.*, 2008). It is used in traditional soups and stews which are usually eaten with millet-based, maize-based or sorghum-based dishes particularly in Northern Ghana (Owolarafe *et al.*, 2011).

Another popular local spice is sweet basil (*Ocimum basilicum*) which is commonly cultivated throughout the Mediterranean region (Ganasoundari *et al.*, 1997). It is locally known as “Akokobesa’’ in “Akan’’ in Ghana. It is an aromatic bushy herb and a dicotyledonous plant from the family of *Lamiaceae* (Dokosi, 1998).

A preliminary study on fermented dawadawa seed powder and basil leaf paste in sausages and burgers respectively showed a promising results in terms of nutritional qualities especially crude protein content when included up to 4g/kg (Taame, 2012; Teye *et al.*, 2013). As is the case with many other agricultural products, spices and herbs may be exposed to a wide range of microbial contamination pre and post-harvest (Hashem and Alamri, 2010). These microorganisms can cause spoilage of the product by their metabolic activity, consequently resulting in significant economic losses. Also some spices and herbs have antimicrobial properties which inhibit the growth of microbes when incorporated in meat products. The maximum inclusion levels of these spices, mineral composition, storability and their antioxidants properties.

The aim of the experiment was to determine the effect of dawadawa seed and basil leaf powders on the sensory, nutritional and microbial qualities of beef and frankfurter sausages.

The specific objectives were to determine:

- The sensory characteristics of sausages prepared with dawadawa seed and basil leaf powder.
- The nutritional qualities (mineral, fat, moisture and protein content) and pH of the sausages.
- The peroxide value of the sausages.
- Total microbial count.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The composition of meat and its nutritive value

Meat has long been known for its nutritive value which could explain why it is being consumed by many people worldwide. The protein profile of meat consists of amino acids that have been described as excellent due to the presence of all essential ones required by the body (FAO, 2010). A large proportion of the world's population relies on meat as a source of food. It has also been proven that some protein and vitamins (especially A and B12) in meat could not be substituted for by plant protein sources, further justifying the nutritive importance of meat (FAO, 2010).

The composition of meat, after rigor mortis but before post-mortem degradative changes, can be approximated to 75% water, 19% protein, 3.5% soluble, non-protein substances and 2.5% fat (Lawrie and Ledward, 2006). The proteins in muscle can generally divided into those which are soluble in water or dilute salt solutions (the sarcoplasmic proteins), those which are soluble in concentrated salt solutions (the myofibrillar proteins), and those which are insoluble in salt solution (Lawrie and Ledward, 2006). The sarcoplasmic proteins are a mixture of several hundred molecular species. Several of the sarcoplasmic proteins are enzymes of the glycolytic pathway and may be present in more than one form of isozymes (Lawrie and Ledward, 2006). Proteins of beef consist of essential amino acids such as leucine, isoleucine, lysine, methionine, cystine, phenylalanine, threonine, tryptophan, valine, arginine and histidine; of these, the last two are considered essential for infants. Amino acids are important for maintenance and repair of body tissues in human (Lawrie and Ledward, 2006).

Meat is also a very good source of various micronutrients: low-fat pork contains 1.8 mg iron, 2.6 mg zinc; and pigs' liver contains 360 mg magnesium, 20 mg iron and 60 µg selenium per 100 µg (Biesalski, 2005). A daily intake of 100 g of meat and liver can supply up to 50% of the recommended daily allowance for iron, zinc, selenium, vitamins B1, B2, B6, B12 and 100% of vitamin A (Biesalski and Nohr, 2009). The importance of meat as an essential source of some micronutrients is due to the fact that it is either their only source, or they have a higher bioavailability. Vitamins A and B12 occur exclusively in meat and can hardly be compensated for by plant-derived pro-vitamins (Biesalski, 2005). Iron has a higher bioavailability from meat than from plants, as has folic acid which is nearly 10-fold more, especially from liver or eggs, compared to vegetables (Biesalski and Nohr, 2009). Deficiency and repeated respiratory infections can be treated therapeutically with moderate vitamin A supplementation (Biesalski, 2005). Vitamin A is also responsible for lung development and maturation and for the development of other tissues, and control of these processes seems to be dependent on the expression of Retinoic Acid (RA receptors) (Biesalski and Nohr, 2009). Although liver is the best available source of vitamin A, it has a 'bad reputation' due to other potentially harmful constituents such as heavy metals, hormones or xenobiotics (Biesalski, 2005).

In order to obtain the recommended 1 mg retinol per day from vegetables, 500 mg of mixed and β-carotene-rich vegetables have to be eaten daily, whilst 100 g of liver twice a month is sufficient and is neither toxic nor teratogenic (Biesalski, 2005; Nohr and Biesalski, 2007). In addition, elderly people are particularly at risk of vitamin B12 deficiency, mainly due to suboptimal intestinal absorption (Biesalski and Nohr, 2009).

There are several groups of people especially elderly people that could be at risk of deficiencies of one or more micronutrients: A, D, E, folate, iron and calcium, mostly because of diseases and an age-adapted lifestyle, less because of physiological problems (with the exception of iron and vitamin B12 uptake due to gastric mucosal atrophy) (Biesalski and Nohr, 2009)

2.2 Meat processing by comminution

Communition is the process of reducing whole muscle to small particles (FAO, 1991). The degree of comminution differs among various processed products. It ranges from coarsely, finely comminuted to form an emulsion (FAO, 2010). Meat trimmings, meat pieces and fatty tissues that could otherwise not be utilized are comminuted and spiced to form high quality meat products (FAO, 2010). Typical examples of comminuted meat products include; sausages, meat loaves, burgers and liver patties.

2.2.1 Sausages

Sausages are defined as comminuted seasoned meats stuffed into casings. They may be cured, dried or fermented. They are made from any edible part of animals and some non- meat ingredients (FAO, 2010).Meat processing provides farmers with a ready market for their animals thereby providing necessary encouragement for improved and increased production (FAO, 1991; Teye, 2010).

Based on the method of processing, there are six types of sausages. These include; fresh, cooked, smoked and cooked and uncooked smoked, semi- dried, dried and specialty sausage (FAO, 1992).

2.2.2 Ingredients, additive, and casings

An ingredient is a component of a recipe that is added in a specific quantity to the products. Most ingredients may be purchased at local supermarkets or meat markets unless it comes from the livestock farm or game. Homegrown or custom meat should come from healthy and disease-free livestock. Certain cuts of meat, generally of lower economic value, are suggested for sausage making (FAO, 1991).

Non-meat ingredients are used to impart flavour, slow bacterial growth and increase the yield of the sausage. These include water, salt, sugar, nonfat dry milk, extenders and binders, and spices (FAO, 2007).

2.2.3 Water and ice

Water and ice are added to provide moisture and keep the sausage cold. Cold temperature delays microbial growth and also ensures a better final product texture. Ice and water can also be added to increase the yield of sausage, but there are upper limits for wholesale or retail marketing. Water also aids in dissolving salt to facilitate its distribution within the meat. Texture and tenderness of the finished sausages are markedly affected by added water content (Pearson and Gillet, 1996; FAO, 2007).

2.2.4 Salt

Salt is an ingredient that is always used in sausage products. Technically, it is the only non-meat substance required for a product to be considered a sausage (FAO, 2007). Salt serves three functions in the meat. It lowers the amount of available water (which allows for preservation or shelf-life extension), extracts the meat myofibrillar proteins needed to make the product bind and to emulsify fat, and for flavour enhancement (FAO, 2007). In general, salt is added at a concentration of 1% to 2% (w/w) of the total sausage batter weight.

2.2.5 Sugar

Sugar is used for flavour and to counter the slight bitter taste of salt. It is also added as a medium for the microbial fermentation process used to reduce the pH of dry and semi-dry sausages (for example pepperoni). The lactic acid produced by fermentation of the sugar (usually dextrose) reduces the meat pH and gives these sausages their characteristic tangy flavour (Meat Board, 1991).Additives can be included in sausage products but under strict conditions and legal limits. They are used to impact the colour, minimize rancidity or to inhibit microbial growth. Examples of these are sodium nitrite, phosphates, sodium ascorbate and sodium erythorbates (Feiner, 2006).

2.2.6 Sodium nitrite

Sodium nitrite is used for curing meat. It inhibits the growth of a number of pathogenic and spoilage microorganisms, most importantly *Clostridium botulinum* (Tronsky *et al.*, 2004). It is also used to retard the development of rancidity, stabilize colour of lean meat and to contribute to the flavour of cured meat. It is usually manufactured as a pink colored salt (to distinguish it from normal sodium chloride salt) that can be purchased from ingredient suppliers as "Rapid Cure." It is highly undesirable to add too little or too much nitrite to sausage (Tronsky *et al.*, 2004).

2.2.7Ascorbates and erythorbates

Ascorbates and erythorbates are chemicals used interchangeably in cured sausages to which nitrite has been added. They are active reducing agents that react with nitrite to accelerate the curing process. Ascorbate is derived from ascorbic acid (that is vitamin C) (Heinz and Hautzinger, 2010).

2.2.8 Milk-protein

Milk-protein derived extenders are used widely in processed meat products. These include non-fat dry milk, dried whey, and butter milk solids and are added to improve binding qualities, flavour, cooking yields and slicing characteristics. They also help to stabilize meat emulsion products such as bologna and frankfurters (FAO, 2007).

2.2.9 Casings

After the meat has been chopped or ground, it is formed into patties or placed into a container. The containers, such as pans for loaves and casings for links, will hold their shape during cooking (Bradley, 2002). Traditional sausage casings are made from parts of the alimentary canal of various animals. These natural casings are largely made up of collagen which has the unique characteristic of variable permeability. Moisture and heat make casings more porous and tend to soften them. Natural casings readily permit smoke penetration and do not contribute any undesirable flavors (INSCA, 2003).When stuffed, natural casing sausages have a characteristic curved shape. Natural casings are readily purchased from local meat markets. Natural casings are usually obtained from hogs, beef cattle and sheep (INSCA, 2003).

There are five classifications of hog casings: bungs, middles, small intestine, stomachs and bladders. Bungs and middles are generally used for liver sausage. Middles are used for dry sausage. Small intestine casings are used for fresh sausage, bockwurst, Polish sausage, frankfurters, and chorizos. Head cheese is generally stuffed into stomachs. Bladders are used for minced luncheon meats).

Similar to the hog, almost the entire beef gastrointestinal tract can beused. Beef rounds are the most common of all beef casings. Rounds are used for ring bologna, holsteiner, and mettwurst. Commercial sausage makers often use “sewed-casings.” Sewed casings are obtained from two natural casings that are slit, matched up, and stitched together. This increases the uniformity and strength of the casings (INSCA, 2003).

Each type of casing can be stored for a reasonable length of time if salted in a controlled, refrigerated environment. All natural casings need to be prepared before use. The casings are rinsed thoroughly in lukewarm water to remove salt before using. Dried middles, bladders and similar casings are softened by soaking in warm water (INSCA, 2003).

The alternatives to natural casings are synthetic casings made from edible or inedible materials. The three most common types of synthetic casings are collagen, cellulose, and artificial casings (All About Sausage, 2004).Collagen casings are made from the gelatinous substance found in the connective tissue, bones and cartilage of all mammals. The substance is harvested from the animals and reconstructed in the form of a paper-like edible casing (All About Sausage, 2004).

Cellulose casings are made from solubilized cotton linters, the short fibers that adhere to cottonseed (All About Sausage, 2004). The interior surface of the cellulose casings can contain a water soluble dye which colours the sausage surface during heat processing (FAO, 1985). Briefly submerging cellulose casings (for example 30 minutes) in room temperature water can facilitate the stuffing process. They are uniform, very strong, and generally used for slicing-sausages such as salami. Skinless hotdogs are made with this form of inedible casing; the casing is removed after smoke processing and before consumption (All About Sausage, 2004).

Artificial, inedible casings are made from plastics and do not require refrigeration. Artificial casings are used by commercial producers and can be made in different colors. For example some manufacturers use red casings for bologna, clear casings for some salami and white casings for liverwurst. Artificial casings' strength and uniformity are similar to cellulose (All About Sausage, 2004). Synthetic casings are more consistent in diameter throughout their length, have a higher tensile strength than natural casings, and are cost effective for large manufacturers (All About Sausage, 2004).

2.3 Lipid oxidation

Lipid oxidation occurring in food products is one of the major concerns in food technology. It is responsible for rancid odours and flavours of the products, with a consequent decrease in nutritional quality and safety caused by the formation of secondary, potentially toxic compounds (Wood *et al.*, 2003). The problem of ensuring a high quality of lipids and lipid-containing products and prolonging their storage time is directly associated with their optimum stabilization by addition of suitable antioxidants. This is very important to human health protection and also economically important (Wood *et al.*, 2003).

Muscle proteins are susceptible to oxidative reactions initiated by oxidizing lipids, metal ions and other pro-oxidants generated during processing (Estevez *et al.*, 2005). The oxidation of muscle protein involves the loss of amino acids and decreases protein digestibility, and hence has negative effects on the nutritional value of meat (Xiong, 2000).

2.4 Spices

A spice is a dried seed, fruit, root, bark or vegetative substance used in nutritionally insignificant quantities as a food additive for the purpose of flavouring and sometimes as a preservative by killing or preventing the growth of harmful bacteria (Adamson, 2004). Though the term spice can be used to incorporate herbs, the distinction between herbs and spices can be described as follows, in the kitchen, spices are distinguished from herbs, which are leafy, green plant parts used for flavouring purposes (Bender and Bender, 2005). Herbs, such as basil or oregano, may be used fresh, and are commonly chopped into smaller pieces. Spices, however, are dried and often ground or grated into a powder.

According to Hirasa and Takemasu (1998) and Sivaraman and Peter (1999) spice can be defined as the dry part of a plant such as roots, leaves and seeds, which impart to food a certain flavour and pungent stimuli. By clubbing spices and condiments into one group the International Organization for Standardization (ISO) explained that the term spice or condiment refers to natural plant or vegetable products or mixtures or thereof, that are used in a whole or ground form, for imparting flavour, aroma and piquancy to and for seasoning food (Manay and Shadaksharaswami, 1997).

Many of these substances are also used for other purposes, such as medicine, religious rituals, cosmetics, perfumery or eaten as vegetables. For example, turmeric is also used as a preservative; licorice as a medicine and garlic as a vegetable (Dalby, 2001).Spices are used as diet components often to improve colour, aroma, palatability and acceptability of food. Spices are among the most versatile and widely used ingredients in food processing. Apart from their traditional role in food flavouring and colouring, they are increasingly used as natural preservatives in active packaging (Seyidim and Sarikus, 2006; Mubeen *et al.*, 2009).

Spices have played an important role in the history of civilization, exploration and commerce as these had a universal acceptance as condiments and flavours in human diet as well as in treatment of ailments. There are evidences of plant derived aromatic compounds especially spices being used by almost all ancient civilizations - the Indian, the Egyptian, the Babylonian, the Persian, the Jews, the Chinese, the Greek, and the Roman (Bakhru, 1992). A notable use of spices and herbs in very early times were in medicine in the making of holy oils unguents and aphrodisiacs (Hemphill, 2000).

Moreover, in the last three decades, mainly because of their medicinal values, the use of spices and other herbs have increased markedly in most regions of the world, including Europe and North America. For instance, during this period, herbal medication in the USA has grown into an industry worth an average of $1.5 billion per year, with projected annual growth of 15% (Abebe, 2006).

2.5 The roles and microbial quality of spices

2.5.1 Influence on salivary and gastric secretions

Spices are well recognized to stimulate gastric function. They are believed to intensify salivary flow, gastric juice secretion and to help indigestion. Salivary and gastric secretions are increased when the nerve centers are stimulated by the sense of smell and by the presence of pungent principles in the foodstuff (Conte *et al.*, 2002). Glatzel (1968), whilst studying the effect of spices on the secretion and composition of saliva in humans, observed that red pepper, ginger, capsicum, black pepper and mustard enhanced the secretion of saliva and the activity of salivary amylase. Furthermore the saliva stimulating capacity was greatest for red pepper and mustard among these spices. Pathak and Pai (1956), in their study with

Indian foods reported a slightly increased acid secretion after food rich in spices were given along with pulses. Orally administered capsaicin was found to increase gastric acid secretion and mucosal blood flow in the rat possibly through the release of endogenous gastric secretagogues (Limlomwongse *et al.*, 1979).

The role of spices in digestion is not limited to a single effect but is a combination of their influences on salivary, gastric, biliary and pancreatic secretions and the terminal digestive enzymes present on the mucosa of small intestine (Platel and Srinivisan, 2000).There has been a renewed interest on their role in aiding digestion through a stimulatory influence on bile secretion and activities of enzymes responsible for digestion, the two prime factors contributing to the process of digestion (Conte *et al.*, 2002).

2.5.2 Stimulation of pancreatic enzymes

A report on the effect of spices on digestive enzymes indicated that capsaicin, piperine, ginger, coriander and curcumin stimulate pancreatic enzymes such as lipase, amylase, trypsin and chemotrypsin (Platel and Srinivisan, 2000).There was an enhanced pancreatic amylase activity by as afoetida in vitro but with no significant effect on intestinal esterases, phosphatases and peptidases (Patwardhan and Sastry, 1957).

The influence of dietary intake and single dose administration of commonly used spices or their active ingredients on the pancreatic digestive enzymes and the terminal digestive enzymes of the small intestinal mucosa has been reported (Platel and Srinivasan, 2001). The dietary intake of spice principles-curcumin, capsaicin and piperine and the spices fenugreek, ginger, asafoetida, and ajowan significantly increase lipase activity. Curcumin stimulated lipase activity by 80% while capsaicin and piperine and whole spices increased the activity of this enzyme by 26-43 percent of the control (Platel and Srinivasan, 2001).

2.5.3 Influence of dietary spices on food transit time

The evidence that the beneficial digestive stimulant action of spices is mediated through an appropriate stimulation of the activities of pancreatic digestive enzymes and digestive enzymes of small intestinal mucosa, and a stimulation of the liver to produce and secrete bile enriched in bile acids has led to a study on the influence of spices on food transit time (Platel, 2001).

The food transit time in the gastrointestinal tract was examined in experimental rats by feeding test spices at levels similar to those proven to produce digestive stimulant action (Platel, 2001). Generally, all spices except fenugreek and mustard shortened the food transit time. This reduction was more prominent for ginger, ajowan, cumin, piperine, coriander and as afoetida, which produced a decrease in food transit time by 24-31 percent. Capsaicin, mint, onion, curcumin and fennel also decreased the food transit time, to a lesser extent (12-19%) (Platel, 2001).

2.5.4 Antimicrobial properties of spices

Spices are used to enhance food flavor, colour and palatability. However, the reasons not address the ultimate (evolutionary) questions of why cuisines that contain pungent plant products appeal to people and why some phytochemicals are tastier than others is not certain (Kennedy and Wightman, 2011). These chemicals evolved in plants to protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites (Kennedy and Wightman, 2011; Hemsmeier *et al.*, 2001).

Most spices contain dozens of secondary compounds. These are the plants' recipes for survival legacies of their coevolutionary races against biotic enemies. Throughout recorded history, foodborne bacteria (especially species of *Clostridium*, *Escherichia*, *Listeria*, *Salmonella*, *Shigella*, and *Vibrio*) or their toxins have been serious health concerns, and they still are (CDC, 2010; WHO, 1999). Microbiologists and food-product developers have conducted laboratory experiments that involve challenging numerous foodborne bacteria, fungi, and yeasts with phytochemicals extracted from spice plants.

Multiple techniques have been used to investigate inhibition, and the primary data vary considerably in quality and in quantity for different spices. Nevertheless, it is now clear why many spices have potent antimicrobial properties (Dormans and Deans, 2000; Ceylan, 2003; Takikawa *et al.*, 2002; Sagdic *et al*., 2006). Unrefrigerated meats spoil faster than vegetables and are more often associated with food borne disease outbreaks suggesting that vegetables (plant tissues) contain more antimicrobial compounds than meat tissue (Sockett, 1995).Uncooked meats and meat dishes that are prepared in advance and stored at room temperatures for more than a few hours typically build up massive bacterial populations, especially in tropical climates (Ceylan and Fung, 2004). Therefore the use of spices should be of the greatest benefit in hot climates, where unrefrigerated foods easily spoil.

2.5.5 Antioxidant activity of spices

Spices have achieved some commercial importance as antioxidants. Beneficial influence of certain ground herbs and spices in fat stability has been known (Lai, 2004). Phenolic compounds are the primary antioxidants present in spices and there is a linear relationship between the total phenolic content and antioxidant properties (Pokomey *et al*., 2001).Several food models have been tested under various conditions including lard active oxygen method (AOM), emulsion: Warburg apparatus; ground pork: frozen storage; mayonnaise: storage at room temperature; pie crust: storage at 63 °C and the peroxide values determined.

The antioxidant efficacy was expressed as a protection factor indicating the ratio of carbon dioxide absorption in food model without spice. This is a measure of stability. The effectiveness of spices and herbs depend not only on variety and quality but also on substrate and storage conditions (Lai, 2004). Rosemary, carnosic acid has been described as the most active antioxidant constituents by (Karre *et al*., 2013).

Other phenolic compounds have been investigated, for example, rosemaric acid, which has in model system in activity comparable to that of caffeic acid (Shylaja and Peter, 2004). Depending on their content of active substances, it is recommended they should be used at levels between 200 and 1000mg/kg of finished product to be stabilized (Bin-Shan *et al*., 2009). Bin-Shan *et al*. (2009) observed that clove was the most effective for retarding lipid oxidation and presented the highest antioxidant activity in meat.

2.5.6 Spice synergism

Pepper, lemon and lime juice are among the most frequently used spices but they are unusual in that the frequency with which they are used does not change much across the temperature gradient. Pepper and citric acid play special roles, that is, as synergists. Citric acid potentiates the antibacterial effects of other spices because low pH disrupts bacterial cell membranes (Booth and Kroll, 1989). Foods to which lemon or lime juice are added require less heating to cause the same levels of bacterial mortality that take place in foods cooked at higher pH and temperature for a longer time.

Black pepper, an exclusively tropical plant has several useful properties and contains piperine that inhibits the deadly bacterium *Clostridium botulinum* (Nakatani, 1994). Black pepper is also a "bioavailability enhancer," meaning that it acts synergistically to increase the rate at which cells, including microorganisms, absorb phytotoxins (Holley and Platel, 2005).Many other spices exhibit greater antibacterial potency when they are mixed than when used alone (Agioni *et al.*, 2004).

Some are combined so frequently that the blends have acquired special names. Sausages are rich medium for bacterial growth and have frequently been implicated as the source of botulinum toxin. Other blends, such as curry powder (which contains 22 different spices), pickling spice (15 spices), and chili powder (10 spices), are broad spectrum antimicrobial melanges. In addition to their uses in cooking, individual spices and blends are employed as colouring agents (Thomas, 2007). Among traditional societies, many spice plants also have ethno pharmacological uses, often as topical or ingested antibacterial and vermicides (Chevallier, 1996; Cichewicz and Thorpe, 1996). A few spices, particularly garlic, ginger, cinnamon and chilis, have for centuries been used to counteract a broad spectrum of ailments, including dysentery, kidney stones, arthritis, and high blood pressure (Duke, 1994; Surh, 2002).

2.5.7 Microbial quality of spices

The presence of pathogenic and spoiling microorganisms in spices could act as vehicles for microorganisms to enter into foods. Frequently, spices are grown and harvested in warm and humid areas where the growth of wide variety of microorganisms is readily supported (Mousuymi and Sarkat, 2003). As many other agricultural commodities, spices are exposed to a wide range of environmental microbial contamination during harvest, processing, and in retail markets by dust, waste water, and animal and even human excreta (Freire and Offord, 2003).

2.6 Macro and micro minerals in Spices

The presence of essential metals like iron, copper, nickel and zinc are very useful for the healthy growth of the body though, very high levels are intolerable.

In medical usage, heavy metals are loosely defined and include all toxic metals irrespective of their atomic weight: Heavy metal poisoning can possibly include excessive amounts of iron, manganese, aluminium, or beryllium or such as semimetal as arsenicas (Duffus, 2002). Some of these metals are naturally found in the body and are essential to human health. Zinc is an important cofactor in over 100 enzyme reactions it is particularly necessary in cellular replication and in the development of the immune response (Salgueiro *et al.*, 2002). However its high levels of zinc in human body can be toxic due to its interference with copper metabolism (Uruj –Adams and Keen, 2005). Therefore, dietary zinc intake should be appropriate. High levels of zinc can result in a deficiency of copper, another metal required by the body. Similarly, iron plays an essential role in many metabolic processes including oxygen transport, oxidative metabolism, and cellular growth (Salgueiro *et al.*, 2002).

In human beings, it is absorbed primarily in the duodenum, transported through the blood stream and extracellular fluid bound to transferring, and stored intracellularly predominantly in the form of ferritin (Lynch, 1997).

Metals like mercury, lead and cadmium are toxic at very low concentrations. Their presence in spices and/ or condiments is a major concern. The addition of spices that may be contaminated with trace and heavy metals to food as a habit may result in accumulation of these metals in human body organs and lead to different health troubles (Al-Ed *et al.*, 1997). There is often little information available about the safety of those herbal plants and their products in respect to heavy metal contamination. Due to the significant amount of spices consumed, it is important to know the toxic metal contents in these spices (Choudhury and Garg, 2007).

2.7 Dawadawa and sweet basil as spices

2.7.1 Dawadawa as a spice

Locust bean (*Parkia biglobosa*) is a tree of the genus *Parkia* in the family *Fabaceae*. In West Africa, its fruits are fermented to a condiment called dawadawa (Bonkoungou, 1987).The striking red spherical inflorescences, which appear in the dry season, are often used by children for games (Burkill, 1995). The yellowish powder inside the seed pods is sweet and can be eaten without preparation or also be made into drinks. The pods are boiled to make a black liquid used for sealing floors (Hall *et al.*, 1996).

Its seeds are fermented to make dawadawa, a black, strong scented, tasty food high in protein (Steinkraus, 1996). In the tropics, especially in Nigeria, locust bean is processed locally into consumable dish and is a part of traditional dishes in most parts of the country.

The most popular form of consumption of African locust beans is in its traditional fermented tasty food condiment called dawadawa which is used as a flavor intensifier for soups and stews and also adds protein to a protein-poor diet (Campbell-Platt 1980; Dike and Odunfa, 2003; Ikenebomeh and Kok, 1984; Odunfa, 1986). Nutritionally, African locust bean has an outstanding protein quality and its protein and amino acid composition has been reported by several researchers (Cook *et al.*, 2000; Lockeett *et al.* 2000; Alabi *et al.*, 2003; Elemo *et al.*, 2011) and is reported to have crude protein values between 23.5-33.4 percent depending on the days of fermentation (Odunfa, 1985).

Barks are distinctly longitudinally fissured, often with more or less regular scales between the fissures, thick, ash-grey to greyish-brown, slash fibrous and reddish brown, exuding an amber gum; crown dense, wide spreading and umbrella-shaped, consisting of heavy branches Leaves are alternate and dark green (Burkill, 1995).

2.7.2 Geographical distribution

P. biglobosa has a wide distribution across the Sudan and Guinea savanna ecological zones. The range extends from the western coast of Africa in Senegal across to Sudan. *P. biglobosa* is found in 19 African countries: Senegal, The Gambia, Guinea Bissau, Guinea, Sierra Leone, Mali, Cote d'Ivoire, Burkina Faso, Ghana, Togo, Benin, Niger, Nigeria, Cameroon, Chad, Central African Republic, Zaire, Sudan, and Uganda (Hall *et al.*,1997).

2.7.3 Traditional uses

P. biglobosa is a non-timber forest product (NTFP), which include wood energy (fuel wood and charcoal) and all other tangible products other than timber (Chandrasekharan, 1993).Non-timber forest products derived from *P. biglobosa* are food, medicine, animal fodder, soil amendments, charcoal, and firewood are the most significant product from *P. biglobosa* is food. The food products collected from *P. biglobosa* are especially important due to the seasonality of fruit maturation and food availability. The seeds are used in preparation of dawadawa, a protein and fat rich food. The yellow starchy pulp that surrounds the seed is an important food supplement rich in Vitamin C and carbohydrates (Abbiw, 1990).

The dried powder is often mixed with water to produce a drink called dozim (Abbiw, 1990). Dawadawa is not only used as flavouring agent but also adds protein to a protein-poor diet which could contribute to protein content in diet of human.

2.7.4 Process of producing dawadawa

The greatest economic value derived from *Parkia biglobosa* is the fermented product of dawadawa made from the seeds (Shao, 2002). The seeds can be obtained from trees that are present on family owned farmland. From April and May, the fruits are fully matured and women with long sticks harvest the pods with a crook at the end to pull down the pods. Small, agile children also climb the trees to harvest the pods. The outer shells or husks of the pods are brown, when dried and each pod containing 10-18 seeds encased in a yellow, sweet, farinaceous endocarp. In Kandiga this is known as “dobulong” or “dozim”. To separate the seeds from the sticky endocarp, the husks are removed and the seeds and pulp are deposited into a wooden mortar. The pulp is pounded with a large pestle until the seeds are separated from the endocarp. The endocarp is set aside and eaten as it is, or used as flour to make porridge. The seeds are laid out to dry in the sun for a day. They can be used immediately or stored for future use. The seeds are then selected for use in dawadawa preparation. The first step requires sorting the seeds and removing small stones or small unusable seeds. The seeds are then placed in a large aluminum pot with sufficient water for boiling for about twelve to fourteen hours. Exclusively, “Dawadawa” is produced by women.

The seeds are boiled, adding water as necessary to keep the seeds covered. This lengthy boiling is necessary to soften and separate the hard seed coat or testa from the cotyledons. After the initial boiling, the seeds are drained and the water is poured off. The seeds are transferred into a large mortar with wood ash. The wood ash acts as an abrasive to help remove the testa from the cotyledons. After several minutes of pounding with a pestle, the seeds are collected and laid out in a single layer to dry in the sun. After drying, the seeds are poured from one calabash to another at arm's length to winnow away the testa.

The boiled cotyledons are brought to a water source and washed several times. This is an extremely tedious task using copious amounts of water. The washings remove the wood ash and testa leaving the clean cotyledons. The exposed cotyledons are now boiled for an additional one to two hours to further soften the cotyledons. The seeds are drained again then packed into a nylon or jute bag. The cotyledons are packed tightly in the bag, placed in a basket, and weighted down with large stones to press out any excess water. The bacterium, *Bacillus subtilis*, is a naturally occurring microbe that attaches to the softened cotyledons and begins the fermentation process (Odunfa, 1985). The cotyledons are left for three full days, until the dawadawa has fully fermented (Odunfa, 1985). The temperature and pH of the dawadawa increased over the three days. The jute bag is opened and the dawadawa is deemed ready by taste and its pungent sweetly rancid aroma (Shao, 2002).

2.7.5 Microbiology and fermentation of dawadawa

Microorganisms associated with the fermentation of dawadawahave been identified in several published studies (Antai and Ibrahim, 1986; Ikenebomeh *et al.*, 1986; Konlani *et al.*, 1999; Odunfa, 1985). In the household or cottage industry preparation of dawadawa, the techniques employed are simple and non-sterile materials are used. Fermentation relies on natural inocula under uncontrolled fermentation conditions. This uncontrolled fermentation can lead to inconsistent products and shorter shelf life (Latunde-Dada, 1997).

In industrial manufacturing, the process is more closely monitored and controlled with direct inoculation with isolated and purified microorganisms. Longer shelf life is an advantage of the commercial product. Studies by Antai (1986) and Odunfa (1985) found several microorganisms associated with dawadawa but the most abundant and the major agent of fermentation after 72 hours of fermentation was *Bacillus subtilis*. These bacteria have also been identified as the agent for the fermentation of soybean into Japanese "natto" (Shao, 2002).

Other microorganisms present after fermentation were *Leuconostocmes enteroide s*and *Staphylococcus spp*. Antai and Ibrahim (1986) and Konlani *et al.* (1999) found that the fermentation process to produce dawadawa is exothermic, that is heat is produced during fermentation, and pH also increases during the fermentation. The result of fermentation increases the digestibility and nutritional value of *P. biglobosa* (Shao, 2002).

2.8 Sweet basil as a spice

Basil (*Ocimum* spp.) belonging to the *Lamiaceae* family, is a pleasant smelling perennial shrub which grows in several regions all over the world (Akgul, 1993; Bari-Aux *et al.*, 1992). Basil is one of the spices used for commercial seasoning. It is commonly known that the presence of essential oils and their composition determine the specific aroma of plants and the flavour of the condiments. Many species of aromatic plants belonging to the *Lamiaceae* family grow wild in the Mediterranean basin (Akgul, 1989; Martins *et al.*, 1999; Marotti *et al.*, 1996; Sanda *et al.*, 1998).

There are usually considerable variations in the contents of the major components within this species. In a study of essential oils of different geographical origins, Lawrence (1988) found that the main constituents of the essential oil of basil are produced by two different biochemical pathways, the phenylpropanoids (methyl chavicol, eugenol, methyl eugenol and methyl cinnamate) by the shikimic acid pathway, and the terpenes (linalool and geraniol) by the mevalonic acid pathway.

Sweet basil is a popular culinary herb and a source of essential oils extracted by steam distillation from the leaves and the flowering tops which are used to flavour foods, indental and oral products, and in fragrances (Akgul, 1989; Guenther, 1952; Heath, 1981; La-Chowicz *et.al.*, 1996; Machale *et al.*, 1997; Simon *et al.*, 1990). There aremany types, some are large and some are small, with a range of leaf colours from green to purple up to variated. Basil is native to India but is grown commercially all over the Mediterranean region and in California (Heath, 1981).

Basil is a condimental plant cultivated in some parts of Turkey, and used frequently in soups, desserts, pickles, pizza, spagetti sauce, egg, cheese dishes, tomatoes juice, dressings, confectionery, salads and meat products as a flavouring agent. Also, basil is well known as a plant of a folk medicinal value and as such is accepted officially in a number of countries (Heath, 1981; Lawrence, 1985). The leaves of basil are also used in folk medicine as tonic and vermifuge. Also, basil tea taken hot is good for treating nausea, flatulence and dysentery (Bay-Top, 1984).

Basil is used in pharmacy for diuretic and stimulating properties, in perfumes and cosmetics for its smell; in fact, it is a part of many fragrance compositions (Bariaux *et al.*, 1992; Khatri *et al.*, 1995). Its oil has been found to be beneficial for the alleviation of mental fatigue, colds, spasms, rhinitis, and as a first aid treatment for wasp stings and snake.

2.8.1 Morphology of basil

Morphologically, basil is an erect much branched bushy aromatic herb. The stem is glabrous or sub-glabrous, up to about 60cm high. The leaves peteolates, ovate-lanceolate, cuneate base, acute tip of about 8cm long, 40cm broad and coarsely toothed. The flowers are white or tinged with purple in loose racemes, corolla is 7mm across, twice as calyx lip to 8mm long, the anthers is brown and fruit has 4 achene-like nutlets (Dokosi, 1998).

2.8.2 Chemical composition and aroma profile of basil

Basil is composed of citronellol, linlool, myrcene, pinene, ocimene, terpineol, linalylacetate, fenchyl acetate, trans-ocimene, 1, 8- cineol, camphor octane, mythyleugenol, eugenol and beta- caryophyllene (Klimankova *et al.*, 2008). The aromatic nature of basil is as a result of the presence of compounds such as 1, 8-cineol, Bergamotene, linlool, methyleugenol, phenyl propanoids and trans-b-ocimene (klimankova *et al.*, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

This work consists of two experiments. Experiment 1 involved using sweet basil leaf and dawadawa seed powders as spices in beef sausages. Experiment 2 involved using sweet basil leaf and dawadawa seed powders as spices in frankfurter sausages. All experiments were in triplicates.

3.1 Study site

The study was conducted at the University for Development Studies (UDS), Tamale. The products formulations took place at the Meat processing unit of UDS, whilst microbiological and chemical analyses were carried out at laboratories of University for Development Studies, Nyankpala Campus and Ghana Atomic Energy, Accra respectively.

3.2 Experimental design

Completely randomized design was used in all the trials. The spices were randomly assigned to the minced meat and each treatment was replicated three times. Treatment means of the various levels of ingredients were compared against their respective controls.

Treatment 1(T1) contained no basil or dawadawa to serve as the control, Treatment 2 (T2) contained 4 g of either basil or dawadawa, Treatment 3 (T3) contained 6 g of either basil or dawadawa and Treatment 4 (T4) contained 8 g of either basil or dawadawa.

3.3 Processing of the dawadawa seed, and basil leaf powder

Fermented Dawadawa seeds were obtained from Tamale, sun dried for two days and ground into powder. Basil leaves were obtained from the environs of Aseseeso - Akuapem in the Eastern region sundried for two days and ground into powder.

3.4 Sausage and burgers formulations

Muscles from the hindquarters of mature bull and castrate (hog); comprising of *Longissimusdorsi*, *Semitendinosus*, *Semimembanosus* and *Quadriceps femoris* were obtained from the UDS Meat Processing Unit, thawed overnight at a temperature of 1°C, cut into smaller sizes and minced using a 5mm-sieve table top mincer (Taller Ramon, Spain). A total of 32 kg of both beef and pork was used for the experiment. A total of 32 kg of both beef and pork was used for the experiment

3.4.1 Spices

Ingredients were added as in the stated amounts (g/kg) to the various sausage formulations.

Table 1: Spices used in the formulation of products

Ingredient (spices)	quantities added (g/kg meat)
Curing salt	15.0
Red chillies	0.5
White pepper	1.0
Black pepper	1.0
"Adobo"	2.0
Polyphosphate	5.0

3.4.2 Comminution of meat

Basil and dawadawa powders were included at 4, 6 and 8 g/kg to the various sausage formulations. The minced meat was comminuted in a 3-knife, 30 litre-capacity bowl chopper (Talleres Ramon, Spain) until a meat butter temperature of 16°C was attained. Crushed ice was added to each set of products during comminution to obtain the desired consistency and temperature of meat butter and to minimise the risk of fat separation from the muscles. The meat butter was immediately stuffed into natural casings, using a hydraulic stuffer (Talleres Rammon, Spain) and manually linked into equal length of about 10 cm. Frankfurter sausages were prepared by a similar method as the beef sausages but the sausage formulation contained 50% beef and 50% pork. The sausages were hung on smoking racks and smoked for 45 minutes after which they were scalded to a core temperature of 70°C. They were then cooled in cold water and hung on racks for adhering water to drain before packaging.

3.4.3 Packaging of products

The products were bagged in transparent polythene bags, vacuum sealed using electronic vacuum sealer (Busch, Rammon, Spain), labeled and stored in a refrigerator at 2°C for sensory, chemical and microbial analyses.

3.5 Sensory evaluation of the products

A total of 15 panelists were selected from the students and staff of UDS Nyankpala Campus and trained according to the British Standard Institution guidelines (BSI, 1993) for panel selection and training to form the sensory panel for the evaluation of the products. The panelists were made up of 5 females and 10 males.

3.5.1 Sensory analyses

Sensory analysis according to the British Standard Institution (BSI, 1993) was conducted by panelist to determine the colour, aroma, flavor, juiciness and overall acceptability of the sausages. A five point category scale was used to evaluate the products.

3.5.2 Panel selection and training

Prospective candidates were screened and trained for their tasting acuity. The selection was done by presenting the potential panelist with three products wrapped in coded aluminium foils; two of which were the same products and the third was a different product. Each individual was asked to pick the odd out of these products. Those who were unable to pick the odd product were disqualified. The selected panelists were trained for 14 day period to detect differences in aroma, flavour, colour and texture.

3.5.3 Preparation of products for sensory analyses

The stored products were removed from the refrigerator and allowed to thaw for three hours under normal room temperature. They were then grilled in an electric oven (Turbonfan, Blue seal, UK), sliced into 2cm thickness and wrapped with coded aluminium foil.

3.5.4 Sensory evaluation of products

Sensory evaluation was carried out on the day 1, 7 and 14 of storage to determine the effect of storage period on the sensory characteristics of the products. The grilled products were presented to the panelists under conditions of controlled lightening. Panelists were provided with water and pieces of bread to serve as neutralizers in between products. The panelists were provided with content validated questionnaires to indicate their impression of the products offered to them.

Five point category scale was used to rate the sensory characteristics of the products:

Colour: 1=very pale red; 2= pale red; 3= intermediate; 4= dark red; very dark red

Aroma: 1= very offensive; 2= offensive; 3= intermediate; 4= pleasant; 5= very pleasant

Flavour intensity: 1= very weak; 2= weak; 3= intermediate; 4= strong; 5= very strong

Flavour liking: 1= dislike very much; 2= dislike; 3= intermediate; 4= like; 5= like very much

Texture: 1= very smooth; 2= smooth; 3= intermediate; 4= rough; 5= very rough

Overall liking: 1= dislike very much; 2= dislike; 3= intermediate; 4= like; 5= like very much

3.6 Laboratory analyses

The crude protein, crude fat, moisture, pH, and peroxide values of the products were determined according to AOAC (1999) methods. Microbial quality of the products was examined by following the procedures in the Bacteriological Analytical Manual of the FDA-USA.

3.6.1 Determination of moisture content of the products

The sample (5g each) was weighed into an electronic moisture analyzer for determination of the moisture content.

3.6.2 Determination of the crude protein content of the product

The crude protein content of the product was determined according to the AOAC method (1999). A piece of sausage (1g) was weighed on filter paper and placed on

Kjeldhal digestion tube, 15ml concentrated H_2SO_4 and three tablets of Kjeldhal tabs were added. The flask was placed in Kjeldhal digestion blocks and heated at 420°C for 30 minutes. The setup was allowed to cool and 75ml of distilled water was added and distilled with an automated distillation unit. The distillate was collected and titrated against 0.2N hydrochloric acid. The same procedure was followed without sausage sample to obtain the blank titre. The titre value was used to calculate the nitrogen and crude protein percentage using the formular:

$$\%Nitrogen = \frac{(T-B) \times N \times 14.007)}{\text{Weight of sample (mg)}} \times 100\%$$

% protein = % Nitrogen × 6.25

Where: T-Titre value, B-Blank value, N-Normality of hydrochloric acid (0.2N).

3.6.3 Determination of fat content of the products

Empty thimbles were weighed together with their holders. The samples (3g) each were weighed with an analytical scale into each of the empty thimbles and plugged with cotton wools. Extraction cups were weighed and weight recorded, 50ml of petroleum ether was measured into each of the extraction cups. With the help of thimble holders thimbles were inserted into the condenser of the soxtec apparatus. The extraction cups were then placed into the soxtec apparatus. It was allowed to boil for 45minutes at 150°C. The air button on the unit was pressed to open the evaporation valve on the soxtec apparatus to evaporate traces of solvent from the extraction cups in 20 minutes. The oil was then allowed to cool for about

30 minutes in the service unit after which the extraction cups with the oil were weighed. Percentage fat and oil was calculated using the formula:

$$\text{Percentage (\%) fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100\%$$

Weight of fat = W2-W1, where

W1-Weight of the extraction cup

W2-Weight of extraction cup + oil

3.6.4 Determination of the peroxide value of the products

Samples of each beef sausage and frankfurter (3 g) were weighed into 250 ml Erlenmeyer flasks and heated in water bath at 60°C for 3mins to melt the fat, then thoroughly agitated for 3mins with 30 ml acetic acid – chloroform solution to dissolve the fat. The samples were then filtered using a filter paper to remove meat particles. Saturated potassium iodide solution (0.5 ml) was added to the filtrates in each of the flask. They were then titrated against standard solution of sodium thiosulphate (25 g/l) with 1% starch solution as indicator.

Peroxide value was calculated as:

$$POV = \frac{T \times N}{W}$$

Where:

T-Titre value

N-Normality of sodium thiosulphate solution

W-The sample weight (kg)

3.6.5 Determination of pH of the products

Samples of 10g each were ground with laboratory mortar and pestle and homogenized with 50 ml distilled water. The pH value of the products was measured with digital pH meter (CRISON, Basic 20, Spain).

3.7 Mineral analysis

3.7.1 Glassware and apparatus

The following apparatus were used during the digestion; 50ml of measuring cylinder, 100ml class a beaker, test tube, fume chamber, clean film, hot plate, a 3 ml dropper and wash bottle.

3.7.2 Acid digestion

This technique was accomplished by exposing a sausage sample to a strong acid at moderate temperature which led to a thermal decomposition of the sample and the solubility of heavy metals in solution, This made it possible to quantify the sample through elemental techniques. Thirty percent of concentrated Hydrogen Peroxide (H_2O_2) and 65-67 percent of concentrate Nitric Acid (HNO_3) were used as reagents.

3.7.3 Hot plate acid digestion of sausage sample

Procedure:

A total of 2 g of each sausage sample was put into a 100 ml borosilicate beaker and 6 ml of concentrated nitric (HNO_3) acid and 1 ml of hydrogen peroxide (H_2O_2) were added to it in a fume chamber. The beaker was covered with a cling film and placed on a hot plate and digested for 3 hours at a temperature of 45°C. After the acid digestion, the sample was transferred into a 100ml measuring cylinder and was topped with50ml of distilled water. After this, the whole content was transferred into test tube for AAS analysis.

The digest was then assayed for Iron (Fe), Manganese (Mn), Copper (Cu), Cobalt (Co), Zinc (Zn), Selenium (Se), Chromium (Cr), Potassium (K),Sodium (Na) and Calcium (Ca) using VARIAN AA 240FS Atomic Absorption Spectrometer in an acetylene- air flame. Reference standards (Sigma-Aldrich Chemie GmbH) was used for the elements of interest, blanks and duplicates of samples were digested under the same conditions as the samples. These served as internal positive controls.

The following Quality Control and Quality Assurance techniques were used during the analysis:

Blanks: They were to check contamination during sample preparation.

Duplicates: To check the reproducibility of the method used.

Standards: To check the efficiency of the equipment being used.

The Recommended instrument parameters during atomic absorption are summarized in table 2.

Table 2: Atomic absorption

Element	Wavelength (nm)	Lamp current (nA)	Slit width (H nm)	Fuel	Support
Fe	248.3	5	0.2	Acetylene	Air
Mn	279.5	5	0.2	Acetylene	Air
Cu	324.7	4	0.5	Acetylene	Air
Zn	213.9	5	1	Acetylene	Air
Na	240.7	7	0.2	Acetylene	Nitrous oxide
K	257.9	7	0.2	Acetylene	Nitrous oxide
Se	196.0	10	1	Acetylene	Nitrous oxide
Ca	422.7	10	0.5	Acetylene	Nitrous oxide

Ref: VARIAN. Publication No 85- 100009-00 Revised March 1989.

Table 3: Mineral composition of dawadawa and basil leaves powder (in mg/kg)

Minerals	Sweet basil	Dawadawa
Calcium	12800	1898
Cobalt	<0.005	<0.005
Copper	0.11	0.115
Iron	12.446	10.339
Potassium	90600	9600
Manganese	0.118	0.16
Sodium	72800	77600
Lead	<0.001	<0.001
Selenium	0.011	0.005
Zinc	0.169	0.166
Chromium	<0.001	<0.001

3.8 Aerobic plate count of microbes in products

The products were analysed on days 1, 7 and 14 of storage to determine their microbial qualities in storage.

3.8.1 Preparation of media

Plate count agar was used to determine the microbial load of the products. The Petri dishes were wrapped with aluminium foil and kept in an oven at a temperature of 250^{o}C for three hours to sterilize them. Seventeen grams of the plate count agar was weighed into 1 litre Erlenmeyer flask and 1 litre of distilled water was added. It was then kept in an autoclave at temperature of 131^{o}C at 1 bar to sterilize the media. The media was then allowed to cool and 20 ml of the media was then poured onto each Petri dish and allowed to solidify.

3.8.2 Sample preparation

The frozen sausages were thawed and 10g taken and placed into stomacher bag and mashed with 90ml of peptone buffered water and 1ml of the diluents was drawn and emptied into a serial bottle to serve as a stock. Serial dilution was prepared from the stock. 1 ml of each level of dilution was drawn and poured onto media contained in each of the Petri dishes. They were then allowed to dry and incubated at 37^{o}C for 24hours. Colony counting was done using the colony counter to determine the microbial load on each sausage.

3.9 Statistical analysis

The data obtained were analysed using General Linear Model (GLM) of Analysis of Variance (ANOVA) of GenStat 3rd edition. Where significant differences were found, the means were separated using Tukey Pair Wise comparison at 5% significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Sensory characteristics of the sausages

The dawadawa leaves powder had a significant effect (P<0.01) on the colour of the beef sausages throughout the storage period as compared to the control products.

Table 4: Sensory characteristics beef sausages spiced with dawadawa

Storage periods (days)	Parameters	T1	T2	T3	T4	S.e.d	Sig
1	Colour	2.337^b	2.652^b	3.000^a	3.251^a	0.229	**
	Aroma	3.500	3.420	3.330	3.420	0.377	ns
	Flavour intensity	2.750	2.833	2.833	2.667	0.215	ns
	Flavour liking	3.670	4.250	3.500	3.670	0.362	ns
	Texture	2.500	2.670	2.420	2.420	0.414	ns
	Overall liking	4.333^a	3.583^b	3.667^b	3.667^b	0.268	**
7	Colour	2.385^b	2.615^b	3.154^a	3.200^a	0.211	***
	Aroma	3.769^a	3.538^a	2.846^b	3.077^b	0.536	***
	Flavour intensity	2.692^a	2.846^a	3.385^b	3.385^b	0.231	***
	Flavour liking	3.920	3.540	3.460	3.380	0.302	ns
	Texture	2.690	2.850	2.850	2.850	0.288	ns
	Overall liking	3.920^a	3.850^a	3.310^b	3.230^b	0.287	*
14	Colour	2.188^b	2.500^b	2.750^a	2.812^a	0.232	*
	Aroma	3.880^a	3.620^a	3.120^b	2.940^b	0.579	***
	Flavour intensity	2.500^b	2.875^b	3.312^a	3.438^a	0.219	***
	Flavour liking	3.750	3.620	3.250	3.440	0.295	ns
	Texture	3.560	3.690	3.000	3.250	0.366	ns
	Overall liking	3.810^a	3.810^a	3.120^b	3.33^b	0.243	*

ns = no significant difference Sed = standard error of difference of means. Means on the same row with the same superscript are not significantly different, *=P<0.05, **=P<0.01, ***=P<0.001.

There was no significant difference in aroma of the products on the first day of product preparation. However the aroma of the products differed significantly (P<0.001) as the storage period increases with the control and T2 being significantly pleasant than T3 and T4. The flavour intensity of the product was not affected (P>0.05) during the first day of product tasting but significant differences (P<0.001) were observed as the storage days increases with T3 and T4 being significantly stronger (P<0.05) than T1 and T2.There were no significant differences (P<0.05) among the treatment in terms of the flavor liking throughout the storage period.

The dawadawa seed powder did not have a significant effect (P<0.05) on the texture of the beef sausages throughout the storage period. Overall likeness was significant differences (P<0.05) among treatment means of the products throughout the storage period. The control was much liked on the first day of product storage as compared to the other treatments. However, on days 7 and 14 the control and T2 were both liked as much compared to T3 and T4.

Table 5 shows the sensory characteristics of sausages prepared with basil leaves powder. The addition of basil leaves powder had significant (P<0.001) effect on the colour of the sausages throughout the storage period as compared to the control products. There were no significant (P>0.05) effect of aroma, flavour intensity, flavour liking, texture and overall liking of the products throughout the storage period.

Table 5: Sensory characteristics of beef sausages spiced with basil leaf powder

Storage period (days)	Parameter	T1	T2	T3	T4	S.e.d	Sig
1	Colour	2.911[b]	3.310[a]	3.310[a]	3.620[a]	0.297	***
	Aroma	3.154	3.000	3.154	2.932	0.255	ns
	Flavour intensity	2.692	2.846	3.308	3.000	0.261	ns
	Flavour liking	3.540	3.380	3.150	2.850	0.329	ns
	Texture	2.690	2.770	2.620	3.310	0.591	ns
	Overall liking	3.770	3.540	3.230	3.080	0.293	ns
7	Colour	2.920[b]	3.321[a]	3.410[a]	3.533[a]	0.277	***
	Aroma	3.161	3.020	3.120	2.814	0.264	ns
	Flavour intensity	2.574	2.744	3.316	3.200	0.273	ns
	Flavour liking	3.520	3.461	3.212	2.821	0.337	ns
	Texture	2.686	2.770	2.633	3.332	0.593	ns
	Overall liking	3.778	3.561	3.332	3.051	0.296	ns
14	Colour	2.940[b]	3.330[a]	3.320[a]	3.630[a]	0.296	***
	Aroma	3.230	3.110	3.134	2.941	0.241	ns
	Flavour intensity	2.673	2.853	3. 361	3.110	0.273	ns
	Flavour liking	3.430	3.360	3.356	2.970	0.339	ns
	Texture	2.691	2.768	3.003	3.340	0.583	ns
	Overall liking	3.761	3.640	3.242	3.180	0.295	ns

ns = no significant difference Sed = standard error of difference of means. Means on the same row with the same superscript are not significantly different, ***=P<0.001.

Table 6 shows the sensory characteristics of dawadawa frankfurter sausages. The dawadawa seed powder had a significant effect ($P>0.05$) on the colour of the sausages throughout the storage period.T3 and T4 were significantly dark red ($P<0.05$) as compared to the control and T2. There were no significant differences ($P>0.05$) in terms of the aroma flavour intensity, flavour liking and texture of the products throughout the storage period with the exception of flavour liking which was affected significantly ($P<0.01$) during the subsequent period of storage. There were significant differences ($P<0.001$) in the overall liking of the products on day 7 and 14 where the control product was more preferred but not day 1.

Table 6: Sensory characteristics of frankfurter sausages spiced with dawadawa

Storage period (days)	Parameter	T1	T2	T3	T4	S.e.d	Sig
1	Colour	1.501^{b}	1.920^{b}	2.330^{a}	2.500^{a}	0.323	**
	Aroma	3.670	3.420	3.670	3.250	0.292	ns
	Flavour intensity	2.667	2.833	2.917	2.917	0.196	ns
	Flavour liking	4.000	3.670	3.500	3.500	0.318	ns
	Texture	2.583	2.917	2.833	2.917	0.2169	ns
	Overall liking	4.170	3.920	3.750	3.670	0.323	ns
7	Colour	1.600^{b}	2.000^{b}	2.500	2.700	0.3700	**
	Aroma	4.000	3.700	3.700	3.500	0.2656	ns
	Flavour intensity	2.300^{c}	3.300^{ab}	3.700^{a}	4.000^{a}	0.352	***
	Flavour liking	4.500^{a}	3.700^{b}	3.600^{b}	3.500^{b}	0.293	**
	Texture	2.500	3.000	3.000	3.000	0.231	ns
	Overall liking	4.600^{a}	3.900^{b}	3.600^{bc}	3.300^{c}	0.314	***
14	Colour	1.600^{b}	2.020^{b}	2.531^{a}	2.611^{a}	0.3600	**
	Aroma	4.010	3.600	3.600	3.410	0.2754	ns
	Flavour intensity	2.320^{c}	3.300^{b}	3.810^{a}	4.120^{a}	0.353	***
	Flavour liking	4.502^{a}	3.700^{b}	3.630^{b}	3.502^{b}	0.296	***
	Texture	2.530	3.002	3.100	3.012	0.233	ns
	Overall liking	4.670^{a}	3.820^{b}	3.602^{b}	3.310^{bc}	0.314	***

ns = no significant difference Sed = standard error of difference of means. Means on the same row with the same superscript are not significantly different, **=P<0.01, ***=P<0.001

Table 7: Sensory characteristics of basil frankfurter sausages

Storage period (days)	Parameter	T1	T2	T3	T4	S.e.d	Sig
1	Colour	1.870^{c}	2.770^{b}	3.460^{a}	3.79^{a}	0.372	***
	Aroma	3.429	3.643	3.429	3.357	0.2740	ns
	Flavour intensity	2.420^{c}	3.460^{b}	3.620^{b}	4.240^{a}	0.394	***
	Flavour liking	4.431^{a}	3.690^{b}	3.570^{b}	3.350^{b}	0.319	**
	Texture	2.571	2.640	2.910	2.500	0.343	ns
	Overall liking	4.562^{a}	3.790^{b}	3.560^{b}	3.451^{b}	0.3670	**
7	Colour	1.670^{a}	2.674^{b}	3.440^{c}	3.780^{c}	0.371	***
	Aroma	4.000	3.440	3.560	3.220	0.312	ns
	Flavour intensity	2.440^{c}	3.560^{b}	3.670^{b}	4.220^{c}	0.391	***
	Flavour liking	4.440^{a}	3.670^{b}	3.560^{b}	3.330^{b}	0.317	**
	Texture	2.560	3.220	3.000	3.000	0.389	ns
	Overall liking	4.560^{a}	3.781^{b}	3.560^{b}	3.440^{b}	0.3660	**
14	Colour	2.360^{b}	2.451^{ab}	2.820^{a}	3.180^{a}	0.290	**
	Aroma	3.550	3.450	3.270	3.270	0.315	ns
	Flavour intensity	2.820^{b}	3.000^{ab}	3.550^{a}	3.450^{a}	0.295	*
	Flavour liking	4.340^{a}	3.700^{b}	3.640^{b}	3.513^{b}	0.294	**
	Overall liking	4.600^{a}	3.920^{b}	3.622^{bc}	3.311^{c}	0.315	**

ns= no significant difference S.e.d = standard error of difference of means. Means on the same row with the same superscript are not significantly different, *=P<0.05, **=P<0.01, ***=P<0.001

Table 7 shows the sensory characteristics of the basil frankfurter sausages. The addition of basil leaves powder resulted in a significant (P<0.001) effect on the colour of the frankfurter sausages throughout the storage period. The addition of basil leaf powder did not affect the aroma of the products throughout the storage period.

The flavour intensity of the product was highly affected (P>0.001) by the basil leaves powder throughout the storage period with the basil products having significantly stronger flavour. There were significant differences (P<0.01) among the treatment in terms of flavour liking and overall liking of the products throughout the storage period with the control products being significantly preferred (P<0.05).

4.2 Proximate composition of the sausages

Table 8 shows the proximate composition of the basil beef sausages. All the parameters measured were not significantly affected (P>0.05) by the basil leaves powder.

Table 8: Proximate composition beef sausages spiced with basil leaf powder

Parameter	T1	T2	T3	T4	S.e.d	Sig
Moisture	74.470	76.330	76.250	77.930	1.447	ns
Protein	18.280	19.890	18.840	18.630	0.469	ns
Fat	8.510	7.750	7.890	7.850	0.421	ns

S.e.d = standard error of difference of means.

Table 9 shows the proximate composition of the dawadawa beef sausages. The dawadawa seed powder did not affect (P>0.05) on all the parameters measured.

Table 9: Proximate composition of beef sausages spiced with dawadawa

Parameter	T1	T2	T3	T4	S.e.d	Sig
Moisture	74.07	74.27	75.12	75.59	1.04	ns
Protein	19.10	16.48	16.34	17.46	1.37	ns
Fat	10.48	10.63	10.57	10.54	0.56	ns

S.e.d = standard error of difference of means.

Table 10 shows the proximate composition of the dawadawa frankfurter sausages. There were no significant (P>0.05) differences in the moisture, protein and fat contents of the frankfurter sausages.

Table 10: Proximate composition of frankfurter sausages spiced with dawadawa

Parameter	T1	T2	T3	T4	S.e.d	Sig
Moisture	68.65	70.14	69.98	70.04	1.05	ns
Protein	19.37	18.48	18.68	18.71	3.72	ns
Fat	14.60	12.44	13.43	13.44	2.27	ns

Table 11: Proximate composition of basil frankfurter sausages

Parameter	T1	T2	T3	T4	S.e.d	Sig
Moisture	74.30	74.27	75.12	75.59	1.04	ns
Protein	21.70	21.90	20.30	19.30	4.74	ns
Fat	17.74^{a}	15.15^{b}	14.93^{bc}	13.66^{c}	1.13	*

ns = no significant difference S.e.d = standard error of difference of means, Means on the same row with the same superscript are not significantly different, *=P<0.05.

Table 11 shows the proximate composition of the basil frankfurter sausages. The moisture and protein content of frankfurter sausages were not affected (P>0.05). There was significant difference (P<0.05) in the fat content with the control being significantly higher in fat than the basil products.

4.3 pH of the sausages

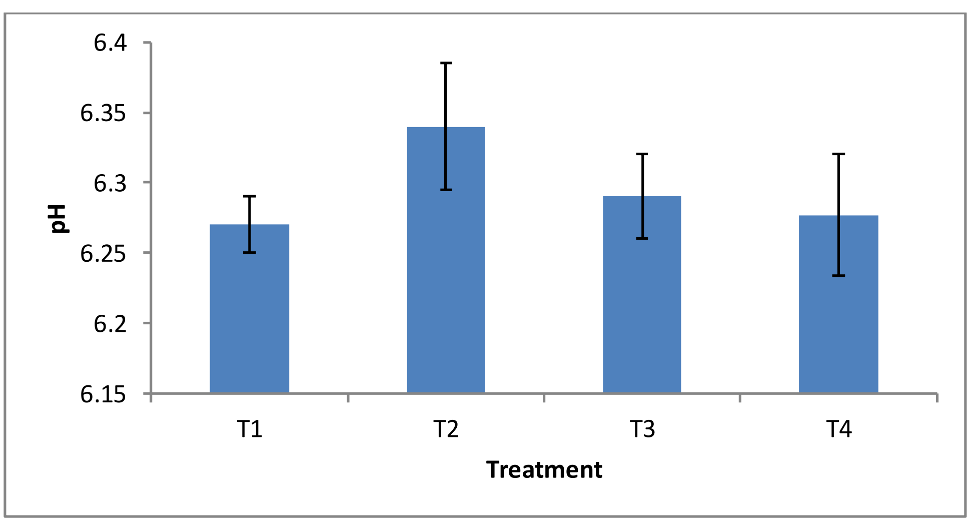

Fig. 1 pH of basil frankfurter sausages

Figure 1 shows the pH of frankfurter sausages prepared with and without basil. The pH values of the products ranges from 6.27 to 6.37. The pH was not different (P>0.05) among the frankfurter sausages.

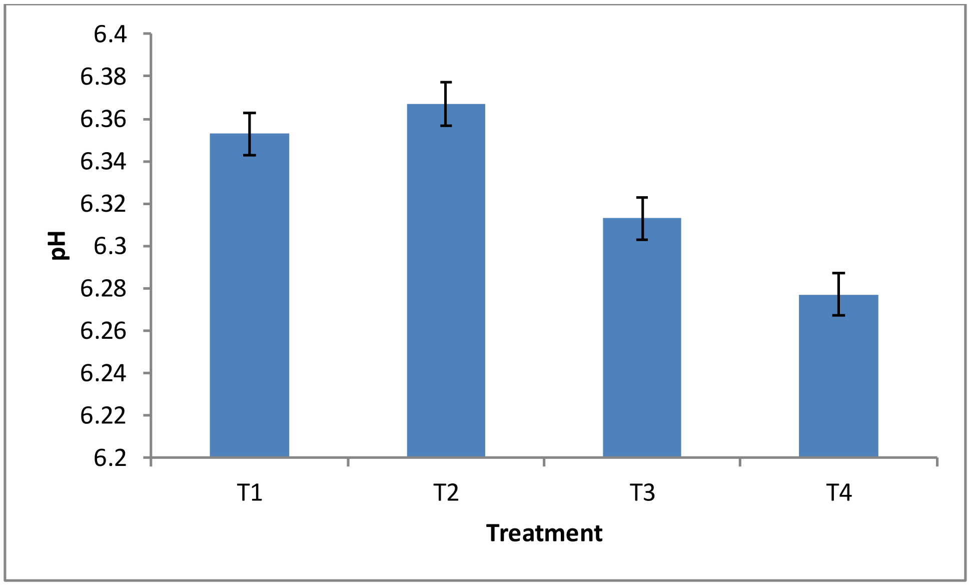

Fig. 2 pH of basil beef sausages

Figure 2 shows the pH of the basil beef sausages. The pH values ranges between 6.277 and 6.367. The pH differed (P<0.001) treatment

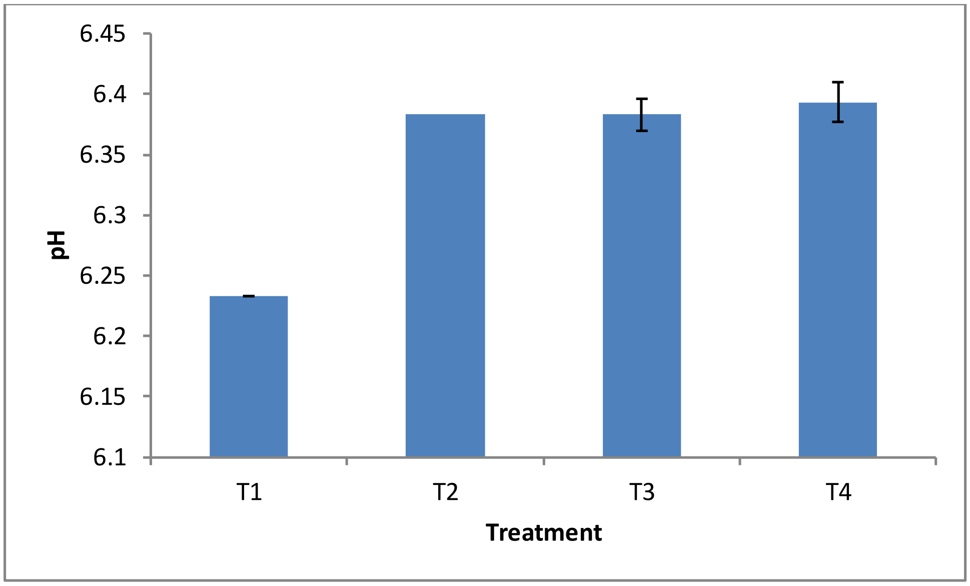

Fig. 3 pH of dawadawa beef sausages

Figure 3 shows the pH of dawadawa beef sausages. The pH values ranges between 6.233 and 6.393. There was significant difference (P<0.05) among treatment means with respect to pH.

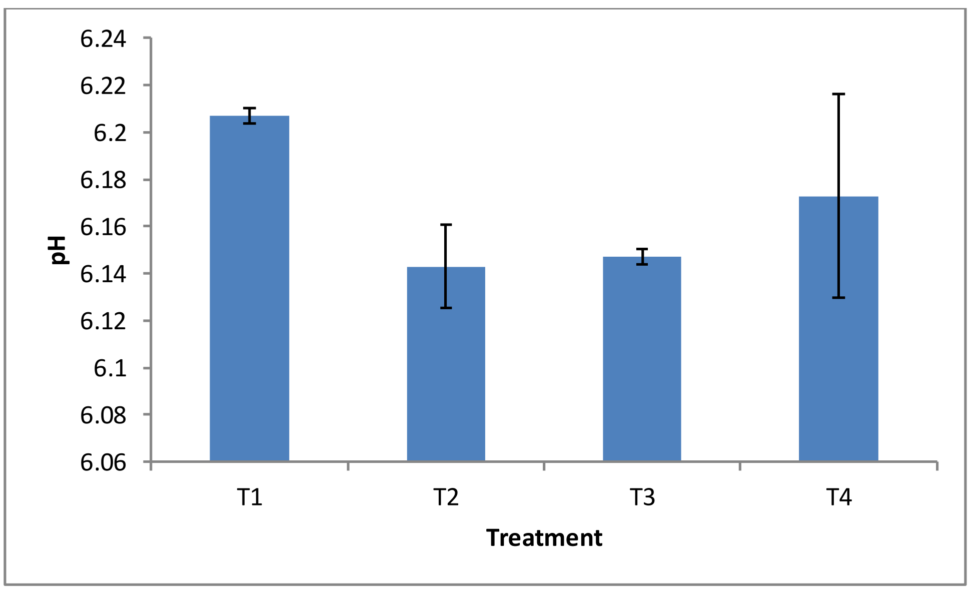

Fig.4 pH of dawadawa frankfurter sausages

The pH values ranges between 6.143 and 6.207. Addition of dawadawa did not affect (P>0.05) the pH of the frankfurter sausages.

Figure 5 shows the peroxide values of the frankfurters prepared with and without dawadawa. The peroxide value ranges between 2.670 and 3.633 milliequivalents per kilogram of product throughout the storage period. There were no significant differences (P>0.05) with respect to treatment-storage period interaction (Fig.5).

4.4 Peroxide values of the frankfurter sausages

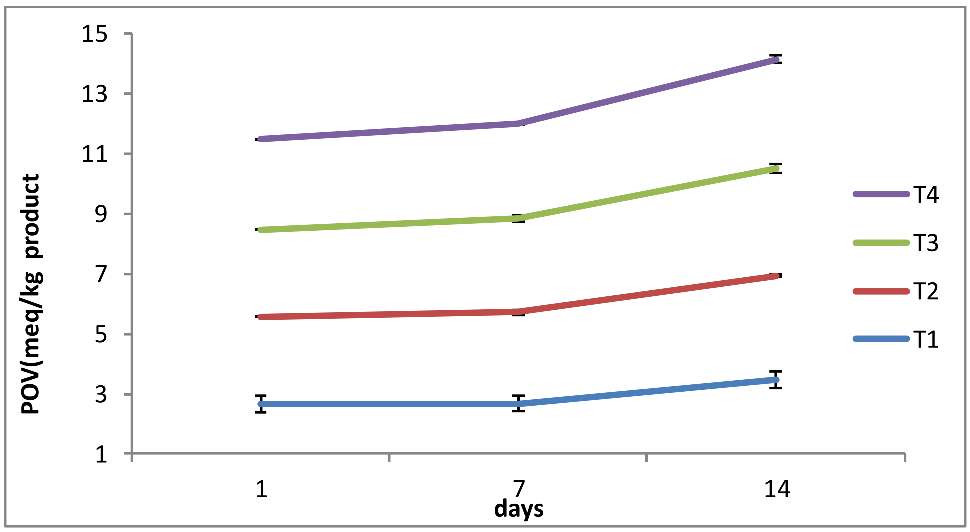

Fig.5 peroxide value of dawadawa frankfurter sausages

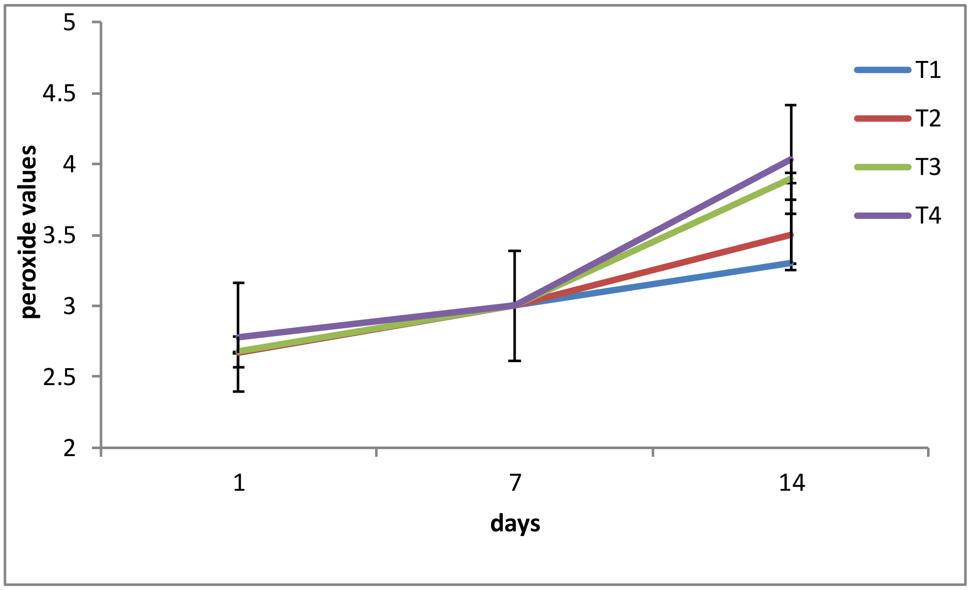

Fig.6 peroxide value of basil frankfurter sausages

Figure 6 shows the trend of lipid peroxidation of frankfurters prepared with basil. The peroxide value of the products ranged between 2.70 and 4.033 milliequivalents per kilogram of product throughout the storage period. There were significant differences (P<0.001) among the treatment means. The basil products had higher peroxide values than the control. The difference was only observed on day 14 of storage. No difference was observed during the first week of product storage.

4.5 Mineral composition of the sausages

There were significant differences (P<0.001) in the concentration of calcium among frankfurter sausages (Table 12). The control had a higher (P<0.001) calcium content than the basil sausages. The chloride content of the basil products were also significantly higher (P<0.001) than their control. The chloride content increases as the basil inclusion increases (Table 12). The iron content of the basil product was significantly higher (P<0.001) than the control product with T3 and T4 being the highest. There were significant differences in terms of the fluoride content of the products (P<0.001). T3 and T4 were significantly higher than T1and T2. The potassium content of the basil products was significantly higher (P<0.001) than the control with T4 being the highest. There were significant differences (P<0.001) in the concentration of manganese among the treatment with the basil products having higher concentration. Sodium content of the basil products was significantly higher (P<0.001) than the control products. The selenium content of all the products did not differ from each (P>0.05).

Table 12: Mineral composition of basil frankfurter sausages

Minerals	T1	T2	T3	T4	S.e.d	Sig.
Calcium	797.50^{a}	792.50^{a}	369.00^{c}	633.00^{b}	2.398	***
Copper	0.0140^{b}	0.01530^{b}	0.0341^{a}	0.0361^{a}	0.000687	***
Iron	1.60050^{c}	1.63750^{c}	1.80400^{b}	4.20850^{a}	0.000612	***
Potassium	31100.00^{c}	35006^{b}	34500^{b}	39100^{a}	308.3	***
Manganese	0.12550^{b}	0.13000^{b}	0.43000^{a}	0.44000^{a}	0.0016	***
Sodium	80200^{d}	83700^{c}	85200^{b}	94200^{a}	291.6	***
Selenium	0.0190	0.0215	0.0225	0.0260	0.00328	ns
Zinc	0.4955^{c}	0.5590^{b}	0.5915^{b}	0.6415^{a}	0.00352	***

ns = no significant difference S.e.d = standard error of difference. Means on the same row with the same superscript are not significantly different, ***=P<0.001.

Table 13 shows the mineral composition of beef sausages prepared with basil leaf powder. The concentration of copper also differed (P>0.001) among treatments with T3 and T4 being than T1 and T2.

Table 13: Mineral composition of basil beef sausages

Minerals	T1	T2	T3	T4	S.e.d	sig
Calcium	777.50^{a}	792.50^{a}	369.00^{c}	633.00^{b}	2.398	***
Copper	0.0150^{b}	0.0160^{b}	0.0360^{a}	0.0370^{a}	0.001	***
Iron	1.00^{a}	0.76700^{c}	0.81150^{b}	0.87350^{b}	0.005	***
Potassium	17400	17400	24800	28300	4174	ns
Manganese	0.1195^{a}	0.1150^{b}	0.1075^{c}	0.1155^{b}	0.003	***
Sodium	63100^{c}	70400^{b}	77500^{a}	78200^{a}	1706	***
Selenium	0.00950^{c}	0.02150^{b}	0.01300^{b}	0.01650^{a}	0.002	**
Zinc	0.2395^{c}	0.2640^{bc}	0.2955^{b}	0.3155^{a}	0.004	***

ns = no significant difference Sed = standard error of difference of means. Means on the same row with the same superscript are not significantly different, **=P<0.01, ***=P<0.001.

The copper content of T3 and T4 was significantly (P<0.001) than T1 and T2. It can also be seen that the iron content of the control product was significantly higher (P<0.001) than the basil products (Table 13). The potassium content of the products did not differ (P>0.05) from each other. The manganese content of the control products was significantly higher (P<0.001) than the basil products.

Sodium content was significantly higher (P<0.001) in the basil products than the control products. The basil products had significant higher (P<0.001) in selenium content than the control products. The zinc concentration differed significantly among the various treatment with T4 being higher than the rest. The basil products had a higher (P<0.001) selenium and zinc concentration than the control products.

Table 14 shows the mineral composition of dawadawa beef sausages. The calcium content of the dawadawa beef sausages was significantly higher (P<0.001) than the control. The copper content of the dawadawa beef sausages was significantly higher (P<0.001) than the control with T4 being more significant. The dawadawa beef sausages were significantly (P<0.001) higher in iron as compared to the control. T4 was higher in iron content than the other treatments.

Potassium content of the products did not differ from each other. Manganese content was significantly lower (P<0.001) in T3 and T4 compared to T1 and T2. Sodium content of the products did not differ significantly (P>0.05) from each other. The dawadawa beef sausages were significantly higher (P<0.001) in selenium compared to the control. There was significant difference (P<0.001) among the treatment means in terms of the zinc content of the products with T3 and T4 being higher than T1 andT2.

Table 14: Mineral composition of dawadawa beef sausages

Minerals	T1	T2	T3	T4	S.e.d	Sig
Calcium	23.00^{c}	24.3600^{b}	26.400^{b}	30.040^{a}	0.01061	***
Iron	1.540^{c}	1.630^{b}	1.770^{b}	2.0100^{a}	0.000354	***
Potassium	24700	22800	20600	24900	1609.300	ns
Manganese	0.12350^{a}	0.13900^{a}	0.06200^{b}	0.0700^{b}	0.002669	***
Sodium	75200	62500	44300	25850	14609.60	ns
Selenium	0.0080^{d}	0.01250^{c}	0.01750^{b}	0.02100^{a}	0.001323	***
Zinc	0.5170^{c}	0.5295^{c}	0.5695^{b}	0.5725^{a}	0.003790	***

ns = no significant difference Sed = standard error of difference. Means on the same row with the same superscript are not significantly different, ***=P<0.001.

Table 15 shows the mineral composition of dawadawa frankfurter sausages. The calcium content of the dawadawa frankfurter sausages differed (P<0.001) significantly from the control with T4 being the highest.

The zinc concentration was higher (P<0.001) in T1 and T2 than T3 and T4.

There were significant differences (P<0.001) in the concentration of copper among the dawadawa frankfurter sausages.

Table 15: Mineral composition of dawadawa frankfurter sausages

Minerals	T1	T2	T3	T4	S.e.d	sig
Calcium	23.100[c]	24.5400[b]	26.8200[b]	31.060[a]	0.02140	***
Copper	0.06250[a]	0.03500[b]	0.03150[b]	0.04900[a]	0.000866	***
Iron	0.77800[c]	0.85350[c]	1.54150[b]	2.41850[a]	0.000935	***
Potassium	24700[a]	10400[c]	14400[b]	14100[b]	408.200	***
Manganese	0.12350[a]	0.045[c]	0.0490[b]	0.0590[b]	0.00350	***
Sodium	75200[a]	34500[c]	49700[b]	45600[b]	1301.3	***
Selenium	0.0080[c]	0.01300[b]	0.01600[a]	0.01900[a]	0.001000	***
Zinc	0.5170[a]	0. 2950[c]	0. 2845[c]	0.3965[b]	0.00555	***

ns = no significant difference S.e.d = standard error of difference. Means on the same row with the same superscript are not significantly different, ***=P<0.001.

There was significant difference (P<0.001) in the concentration of iron among the dawadawa frankfurter sausages. T1 and T2 were significantly lower in iron compared to T3 and T4.The fluoride content of the dawadawa products was significantly lower than the control(P<0.001). Potassium content of the dawadawa products was significantly (P<0.001) lower than the control. The control products were significantly higher (P<0.001) in manganese compared to the dawadawa products. Sodium content was significantly higher (P<0.001) in the control products than dawadawa products. The selenium content of the dawadawa frankfurter sausages were significantly (P<0.001) higher than the control. The control products were significantly higher in zinc compared to the dawadawa products.

Table 16: Aerobic plate counts of basil beef sausages (CFU/g of sausage)

Storage period(days)	T1	T2	T3	T4
1	3.00	4.51	4.53	4.00
7	3.72	5.42	5.88	5.42
14	3.26	4.00	4.18	4.55

Table 16 shows the aerobic plate counts of beef sausages prepared with basil. The counts range between 3.000 and 5.875 CFU/g of sausage throughout the storage period.

Table 17: Aerobic plate counts of dawadawa beef sausages (CFU/g of sausage)

Storage period (days)	T1	T2	T3	T4
1	3.20	3.78	4.54	4.32
7	3.20	3.23	3.04	3.26
14	4.26	4.11	4.23	4.30

Table 17 shows the aerobic plate counts of beef sausages prepared with dawadawa. The counts range between 3.204 and 4.544 CFU/g of sausage throughout the storage period.

Table 18: Aerobic plate counts of dawadawa frankfurter sausages (CFU/g of sausage)

Storage period (days)	T1	T2	T3	T4
1	4.52	4.63	4.65	4.65
7	4.69	4.58	4.76	4.70
14	4.67	4.69	4.73	4.80

Table 18 shows the aerobic plate counts of frankfurter sausages prepared with dawadawa. The counts range between 4.519 and 4.797 CFU/g of sausage throughout the storage period.

Table 19: Aerobic plate counts of basil frankfurter sausages (CFU/g of sausage)

Storage period (days)	T1	T2	T3	T4
1	4.56	4.38	4.63	4.70
7	4.68	4.56	4.74	4.69
14	4.67	4.61	4.76	4.76

Table 19 shows the aerobic plate counts of frankfurter sausages prepared with basil. The counts range between 4.380 and 4.763 CFU/g of sausage throughout the storage period.

CHAPTER FIVE

5.0 DISCUSSION

5.1.1 Sensory characteristics of dawadawa beef sausages

There were significant variations among the treatment of the dawadawa beef sausages in terms of colour. The colour of the product refers to what is perceived by the consumer and it is an important factor in determining meat quality. Alteration of the colour of meat and their products as per consumers' previous knowledge may affect acceptability. The natural black colour of dawadawa was imparted to the products with increasing levels of the dawadawa. The result agrees with that of Taame, (2012) who found significant differences in the colour when fermented dawadawa seed powder was incorporated in smoked pork sausages. Consumers, if made aware of the beneficial properties of dawadawa may patronize these products irrespective of colour difference. However, its inclusion up to 4g/kg will not have any effect on the colour of beef sausages.

Consumers did not detect differences in aroma on the first day of product storage this agrees with Taame (2012) who found no significant difference when dawadawa was incorporated in smoked pork sausage on the first day of product storage. However, as the period of storage increases there was great difference in the aroma of the product this could be attributed to certain biochemical changes that takes place during product storage such as lipid peroxidation. The differences in flavour intensity of the products may be due to the impartation of the strong flavour of fermented dawadawa seed onto the products. Dawadawa is reported to have high levels of glutamic acid which enhances the flavouring effect in food

(Shao, 2002). This result however disagrees with Teye *et al.* (2013) when fermented dawadawa seed powder was incorporated into smoked pork sausages and hamburgers respectively up to 4g/kg meat. The significant differences among the treatment could be due to the higher inclusion levels of dawadawa seed powder in the current study.

The dawadawa sausages were more liked in terms of flavour as the control. Although there was significant differences with respect to the flavour intensity of the products, its liking was not affected. This finding also agrees with Teye *et al.* (2013) when fermented dawadawa seed powder was incorporated into smoked pork sausages and hamburgers respectively up to 4g/kg meat. The texture of the products was not affected by dawadawa seed powder. According to Lawrie and Ledward (2006) texture is one of the sensory attributes currently rated most important by the average consumer. The similar effect indicates that dawadawa seed powder would not have any negative influence on the texture of products.

There were significant effects of treatment on the general acceptability of the products. The control and T2 were more acceptable than the other treatments. This means that dawadawa seed powder can be included up to 4g/kg in beef sausages for without affecting it acceptability.

5.1.2 Sensory characteristics of the dawadawa frankfurter sausages

There was significant effect of dawadawa on the colour of the frankfurter sausages. The natural black colour of dawadawa imparted the products with T4 being more obvious. This result agrees with that of Taame, (2012) who found significant differences in terms of colour when fermented dawadawa seed powder was incorporated in smoked pork sausages. This result however disagrees with the findings of Teye *et al.*, (2013) who found no significant differences in colour when dawadawa seed powder was incorporated in both beef and hamburgers at 4g/kg. Although the colour became darker in T3 and T4, consumers who are aware of the colour of dawadawa may not be discouraged in the patronage of the product.

Consumers did not detect differences in aroma throughout the storage period. This agrees with Taame (2012), who found no significant difference when dawadawa was incorporated in smoked pork sausage. It was expected the pungent odour of fermented dawadawa seed would impart the products. The flavour intensity and flavour liking of the products were not affected during the first day of product tasting but were affected as the storage increases with the flavour of the dawadawa products being more intense. Biochemical processes such as protein and lipid oxidation could have contributed to this.

The texture of the products was not affected by the treatment. The similar differences indicate that dawadawa seed powder will not have any negative influence on the texture of frankfurter sausages when included up to 8g/kg. As the storage period increases the general acceptability also became more significant with the control and T2 being more acceptable than the rest. This implies that frankfurter sausages can be prepared with dawadawa seed powder up to 4g/kg without affecting its acceptability by consumers.

5.1.3 Sensory characteristics of the basil frankfurter sausages

The dark brown colour of the basil leaves powder was imparted the sausages. This result however disagrees with Teye *et al.*, (2013) when basil leaf paste was incorporated up to 6g/kg meat in beef burgers. The original pale red colour of the frankfurters might have contributed to the dark brown colour of the basil leaves being obvious in the product. Moreover the higher inclusion levels might have contributed to the dark coloration of the test products.

It was expected that the aroma of sweet basil would have been imparted onto the products but that was not the case. Drying of the leaves of basil causes it to lose its characteristic aroma and therefore the insignificant effect on the aroma of the products. Similar observation was detected when basil leaf paste was included in beef burgers by Teye *et al*., (2013).The flavour intensity and flavour liking of the basil frankfurters differed from that of the control suggesting that basil flavour was imparted on to the products. Consumers who are aware of basil may patronise these products. The texture of the basil frankfurters did not differ from the control throughout the storage period. This indicates that basil leaves powder did not have any negative effect on the texture of frankfurter sausages. In terms of general acceptability, the control products and the T2 were accepted more than the rest of the products. This indicates that basil can be included up to 4g/kg without affecting its acceptability.

5.2 Proximate composition of the sausages

5.2.1 Proximate composition of dawadawa beef sausages

High moisture content in meat products makes them juicy and susceptible to microbial attack. The similar differences in moisture content indicate that products prepared with dawadawa would equally be juicy as the control.

It was expected that the protein in the dawadawa would have caused significant increase in the crude protein content of the beef sausages but that was not the case. This result also disagrees with the findings of Teye *et al.,* (2013) when dawadawa seed powder was incorporated in smoked pork sausages. Proteins are required in higher levels in growing children and also for reproductive functions such as pregnancy and lactation (Pond *et al.*, 1995). It is also the criterion for the quality and value of finished meat products (FAO, 1991). The protein content of meat and meat products also indicates their biological value (Lawrie and Ledward, 2006).Even though the crude protein content was not improved with the incorporation of dawadawa, it did not have any negative impact on the crude protein content of the beef sausages.

5.2.2 Proximate composition of basil beef sausages

The moisture content of the beef sausages was not affected by the inclusion of basil leaves powder. The moisture content of meat products has an effect on their storability. High moisture content enhances microbial growth whereas low moisture content reduces microbial growth (Lawrie, and Ledward, 2006). The similar variation implies that basil products can equally be stored as the control products. Similar observation was made when basil leaves paste was included in beef burgers Teye *et al*., (2013).

Basil leaves powder did not affect the protein content of the beef sausages. It was expected that crude protein content would have been increased in consistence with that of Teye *et al.* (2013) who reported increase crude protein content of beef burgers when basil leaves paste was included.

The crude fat content of the product was not affected by the treatment. High dietary fat especially those high in saturated fatty acids are reported to have been implicated in coronary heart diseases (Desmond, 2006). Consumers would not be worried about the consumption of basil products since it did not have any negative effect on the fat content of the products.

5.2.3 Proximate composition of dawadawa frankfurter sausages

Fermented dawadawa contains an appreciable amount of fat which was expected to have an impact on the frankfurter sausages but that was not the case. Both the control and dawadawa sausages had similar crude fat content. Similar result was observed by Teye *et al.* (2012) when dawadawa seed powder was included at 6g/kg in frankfurter type sausages. Fat is the most variable component of processed meat and is extremely important because it directly affects flavour, texture, shelf life and profit (FAO, 2007).

The protein content was not affected negatively with the inclusion of dawadawa in the frankfurters. Consumers will still obtain similar amount of protein as the control products. This result is in contrary to that of Teye *et al.* (2012) when dawadawa seed powder was included at 6g/kg in frankfurter type sausages. The moisture content of the beef sausages was not affected by the inclusion of dawadawa seed powder. Similar results were observed by Teye *et al.* (2012) when dawadawa seed powder was included in frankfurter type sausages.

5.2.4 Proximate composition of basil frankfurter sausages

The protein and the moisture contents of the frankfurter sausages prepared with basil were not different from the control. This means basil did not have a negative effect on the crude protein content of the product. However, the inclusion of basil reduced the crude fat content. Although less fat in meat makes it less palatable, this would be of health benefit to consumers who are cautious about the consumption of fatty meat and meat products.

5.3 pH of the sausages

5.3.1 pH of the basil beef and frankfurter sausages

The use of basil leaf powder did not have any negative influence on the pH of the products. pH is important for the storage of meat. The lower the pH the less favourable conditions for the growth of harmful bacteria (FAO, 2007). Lower pH is as a result of accumulation of lactic acids due to some microbial activities in the meat products (Lawrie and Ledward 2006). The lactic acid creates an acidic condition resulting in a low pH of the products which creates unfavorable environment for microbial survival (Young *et al.,* 2003).

The current study reveals that products prepared with basil can equally be stored as their control. Moreover, the results of the microbial count (Table 19) indicated that the storability of the products was not affected. This strongly gives an indication that basil leaf powder would have positive influence on shelf life of sausages.

5.3.2 pH of the dawadawa beef sausages

The pH of the dawadawa beef differed from that of the control. It was expected that the pH of the dawadawa beef sausages would have been lower than the control but that was not the case. This result agrees with that of Teye *et al.*, (2013) who reported lower pH of the dawadawa products lower than the control when dawadawa was used. There could have been a variation with respect to the extent of fermentation of the dawadawa used in the current study and that of Teye *et al.* (2013). As the rate of fermentation increases more lactic acid is produced by the lactic acid bacteria making the test material more acidic.

The pH of the dawadawa frankfurter sausages was similar to their control. This gives an indication that frankfurters prepared with dawadawa would have almost the same shelf life as the control.

5.4 Lipid peroxidation of the sausages

5.4.1 Lipid peroxidation of the dawadawa frankfurter sausages

Throughout the storage period the peroxide values of both the control and the dawadawa products were not affected. Peroxide values obtained were below the maximum permissible limit of 25mill-equivalent of active oxygen/kg product (Evranus, 1993). The unsaturated and polyunsaturated fatty acids components of meat react with oxygen to form fatty acid hydro-peroxide (Warris, 2010).

Hydro-peroxides are unstable and break down into various compounds which can produce off-odours and flavours, leading to a stale, rancid flavour in foods (Kerler and Grosch, 1996). Even though the acceptability of the products was affected as the inclusion level of the test material increased it might not be as a result of lipid peroxidation but rather other inherent characteristics of the test material such as the strong flavour of dawadawa. The peroxide values obtained shows that the health of consumers would not be at risk and storability of the products would not also be affected negatively.

5.4.2 Lipid peroxidation of basil frankfurter sausages

The basil products had higher peroxide values than their control. The values obtained throughout the storage period were however, far below the maximum permissible limit of 25milli-equivalent of active oxygen/kg of product. Basil contains some essential oils which have antioxidant properties and this might have contributed to the lower peroxide values obtained during the product storage. The peroxide values obtained still inform consumers that their health is not at risk and the storability of the product would be prolonged.

5.5 Mineral composition of the sausages

5.5.1 Mineral composition of the basil frankfurter sausages

The calcium content of the control was significantly lower than basil products. This is a good finding to the health and nutrition of human. The calcium content of the test material was over 1800mg/kg. This might have contributed to the high calcium content of the basil frankfurter sausages. Calcium salts provide rigidity to the skeleton and calcium ions play roles in many, if not most, metabolic processes (FAO and WHO, 1962). Adult females from age 19 years to menopause need 1000mg of calcium per day. Females postmenopause need 1300 mg and males between the ages of 19–65 years need 1000mg and males from 65+years need 1300mg per day (FAO and WHO, 1962). This means that the daily intake of basil frankfurter sausages can provide up to about 70% of the daily requirement of calcium for this entire category of people. People who are mineral deficient in calcium can utilize these products to correct the situation.

The iron content of the basil products (T3 and T4) was found to be significantly higher than the control. The basil leaves powder was found to contain 12.446 mg/kg of iron. This might have contributed to the higher content of iron in the basil products than the control. Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues (Brock *et al.*, 1994).

Iron deficiency also negatively influences the normal defense systems against infections. In animal studies, the cell-mediated immunologic response by the action of T-lymphocytes is impaired as a result of a reduced formation of these cells. A recent estimate based on WHO criteria indicated that around600–700 million people worldwide have marked iron deficiency anaemia (DeMaeyer *et al.*, 1985), and the bulk of these people live in developing countries. The finding of this study is good for people who are anemic.

The zinc content of the basil products was significantly higher than that of the control. The basil leaves contained an appreciable amount of zinc (0.169 mg/kg) and this might have been imparted the products to increase the zinc content. Zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients. Zinc stabilizes the molecular structure of cellular components and membranes and in this way contributes to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression (FAO and WHO, 2004). Fundamental activities probably accounts for the essentiality of zinc for all life forms. It recommended that an individual must take in 3.6mg of zinc per day (FAO and WHO, 2004). It implies that basil frankfurter sausages would be able to provide up to about 17.8% of the daily requirement of zinc by humans. The values obtained shows that basil is not harmful to health of humans when included in these products since the zinc content was not above the acceptable limit of human body requirement.

Both control and basil had appreciable amount of selenium. Selenium has been linked to the protection of body tissues against oxidative stress, maintenance of defenses against infection, and modulation of growth and development (FAO and WHO, 2004). A daily intake of 26 -34 µg of selenium is good for the health of humans (FAO and WHO, 2004). All the treatments had above the daily requirement and hence humans will need small amount of these products to supply the amount of selenium needed daily by the body.

The basil products had significantly higher copper content than the control product. The basil leaves contained 0.11mg of copper and this might have contributed to the significant increase in the copper content of the basil products. Copper plays an important role in metabolism largely because it allows critical enzymes to function properly (Harris, 2001). It is essential for maintaining the strength of the skin, blood vessels, epithelial and connective tissues throughout the body. It is also for the production of haemoglobin (Harris, 2001).An adult human needs an average of 1mg of copper daily. Since all the values were not above the acceptable limit per day products prepared with basil at this level of inclusion would not have negative health implication on humans.

The sodium content of the basil frankfurter sausages was significantly higher than that of the control. The sodium content of the basil leaves is supports this finding. The result (Table 12) show that basil leaves are high in sodium and this might have contributed to the high sodium content of the basil products. Common salt was also added during the sausages formulation which already contains sodium. Sodium is an important electrolyte that helps in maintaining water balance within the cells and is involved in proper functioning of both nerve impulses and muscles within the cells. It also plays a role in blood pressure regulation (Advanced Nutrition, 2009).The human body needs only about 200mg of sodium per day (American Heart Association, 2012). All the products had higher sodium content than the acceptable limit.

The manganese content of the basil products was higher as compared to the control. Basil leaves from this study contains some amount of manganese (0.118mg). This value might have had an effect on the manganese content of the basil products. Manganese serves as part of many enzymes and is involved in fat and carbohydrate synthesis. It is needed for normal tendon, bone structure, and pancreas development. An adult human needs 2.3mg of manganese per day (Nutrition Reference Guide, 2013). The results from Table 12 show that basil frankfurter sausages alone can provide up to about 19% of the daily requirement of manganese (Nutrition Reference Guide, 2013).

The potassium content of the basil frankfurter sausages was significantly higher than the control. From the results of the study basil leaves contained 90,600 mg of potassium. This is good enough to have a significant effect on the potassium content of the basil products. Potassium helps to regulate body fluids and mineral balance in and out of body cells. It is involved in maintaining blood pressure, transmitting nerve impulses and helping muscles and heart to contract. A daily requirement of 3.2g is essential (Nutrition Reference Guide, 2013). From Table12 it can be seen that all the treatments were able to supply more than 100% of the daily requirement of potassium. This means that consumers need only small amount of these sausages to obtain their recommended daily intake of potassium.

The chloride content of the basil frankfurter sausages was significantly higher than the control. From the results of the study basil leaves contained 10,996.60 mg of chloride. This value might have contributed to the higher chloride content of the basil products. Chloride helps to regulate fluids in and out of the body cells. It is part of hydrochloric acid, a stomach acid important for digestion of food and absorption of nutrients. It helps to transmit nerve impulse. A daily intake of about 3.8g for both adult males and females is recommended (Nutrition Reference Guide, 2013). From the results it can be seen that all the treatments were able to supply more than 100% of the daily requirement of chloride. It implies that the products are a good source of chloride for proper functioning of the body.

The fluoride content of the basil frankfurter sausages was significantly higher (T3 and T4) than the control. Fluoride hardens tooth enamel and results in decrease in tooth decay. It also helps to retain calcium in the bones of older adults, therefore strengthening the bones. A daily recommended intake of 4mg of fluoride per day is necessary (Nutrition Reference Guide, 2013).The significant increase in fluoride content of the basil frankfurter sausages would be good for people with teeth problems.

5.5.2 Mineral composition of the basil beef sausages

The calcium content of the basil products (T3 and T4) was higher than the control. Calcium is good for the development of strong bones. Even though the acceptability of the products reduces as the inclusion of basil goes high, it inclusion even at higher levels would be beneficial to the health of humans.

The copper content of the basil products was significantly higher than the control. The inclusion of basil was able to provide up to about 3% of the daily requirement of copper needed by humans. The control products had higher iron content than the basil products. Even though the basil leaves had an appreciable amount of iron

(10.388 mg/kg) this did not impart the beef products. The basil beef sausages had higher zinc content (T3 and T4) as compared to the control. It means that basil products were better source of zinc compared to their control. This would be an advantage to the health of humans when basil beef sausages are consumed. The selenium content of the basil products was highly improved than the control. Selenium works with vitamin E to protect cells from damage that may lead to cancer, heart related disease and other health problems (Nutrition Reference Guide, 2013). The inclusion of basil increased the selenium content of the beef sausages which is good for consumers. The manganese content of control was significantly higher than the basil products. It was expected that this would have followed a consistent trend but that was not the case which is difficult to explain. The basil products were high in sodium than the control. The high sodium content in the basil leaves contributed to a higher sodium content of the beef sausages. All the products were able to supply in abundance of the recommended daily intake of sodium. This means that just small amount of the beef sausage consumed can supply enough sodium for the body.

The fluoride and potassium content of the products did not differ from each other. It was expected that the basil products would have been higher in both fluoride and potassium but that was not the case. The irregularities cannot be explained.

5.5.3 Mineral composition of the dawadawa beef sausages

The calcium content of the dawadawa products was significantly higher than their control. The calcium content of the test material was over 12,800 mg/kg. This might have contributed to the high calcium content of the dawadawa frankfurter sausages. Calcium salts provide rigidity to the skeleton and calcium ions play a role in many, if not most, metabolic processes. This means that daily intake of dawadawa frankfurter sausages can provide up to about 70% of the daily requirement of calcium by humans. People who are deficient in calcium can utilize these products to correct the situation.

The dawadawa products had significantly higher copper content than the control product. The dawadawa seed contained close to 0.115 mg/kg of copper and this might have contributed to the significant increase in the copper content of the dawadawa products. Dawadawa inclusion in beef sausages would be good for human health since it was able to produce this appreciable quantity of copper. The iron content of the dawadawa products was found to be significantly higher than the control. The fermented dawadawa seed powder was found to contain about 10.339 mg/kg. This might have contributed to the higher content of iron in the dawadawa products than the control.

The selenium content of the dawadawa products was highly improved than the control. Selenium works with vitamin E to protect cells from damage that may lead to cancer, heart related disease and other health problems (Nutrition Reference Guide, 2013). This finding is good for the health and nutrition of humans.

The dawadawa beef sausages (T3 and T4) had significantly higher zinc content as compared to the control. The inclusion of dawadawa was able to supply an appreciable amount of zinc. This implies that dawadawa products would be a better source of zinc compared to their control. This would be a positive advantage to the health of humans when dawadawa beef sausages are consumed. It was expected that the inclusion of dawadawa at higher levels would have improved the manganese content of the products but that was not the case. It was expected that the dawadawa products would have been higher in potassium but that was not the case.

5.5.4 Mineral composition of the dawadawa frankfurter sausages

The calcium content of the dawadawa products was higher than the control. Calcium is good for the development of strong bones. The results show that the inclusion of dawadawa did not have any negative effect as far as calcium deficiency is concern.

The iron content of the products was significantly improved with the inclusion of dawadawa. Iron serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues. The improvement of the iron content of the products by dawadawa inclusion may help curb anaemic situation in humans. The copper was significantly higher in T1 and T4 than T2 and T3. The results did not show a particular trend.

The manganese content was significantly reduced in the frankfurters when dawadawa was included. An increase in this mineral in the dawadawa products was expected but that was not the case. Similarly the sodium and zinc content of the dawadawa products were significantly reduced in this study. The chloride and fluoride content of the dawadawa was not imparted onto the products.

The selenium content of the dawadawa products were highly improved than the control. This indicates that the selenium in the fermented dawadawa imparted the products. Selenium works with vitamin E to protect cells from damage that may lead to cancer, heart related disease and other health problems (Nutrition Reference Guide, 2013). This finding is good for the health and nutrition of humans.

5.6 Aerobic plate counts of the sausage

5.6.1 Aerobic plate counts of the basil beef sausage

The counts obtained from both the control and the basil beef sausages were all within the acceptable limit of $7\log_{10}$ CFU/g of product. The basil had no effect on the aerobic plate counts of the products.

5.6.2 Aerobic plate counts of the dawadawa beef sausage

The fermented dawadawa had no effect on the aerobic plate counts of the products. The counts obtained from both the control and dawadawa beef sausages were all within the acceptable limit of $7\log_{10}$ CFU/g of product. This indicates that dawadawa had no effect on the storability of the beef sausages.

5.6.3 Aerobic plate counts of the dawadawa frankfurter sausage

The fermented dawadawa had no effect on the aerobic plate counts of the products. The counts obtained from the study were all within the acceptable limit of $7\log_{10}$ CFU/g of product. This implies that dawadawa had no effect on the storability of the frankfurter sausages.

5.6.4 Aerobic plate counts of the basil frankfurter sausage

The counts obtained from the study were all within the acceptable limit of $7\log_{10}$ CFU/g of product. Basil leaves powder had no effect on the storability of the frankfurter sausages.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The inclusion of dawadawa seed and basil leaves powders did not have adverse effect on the sensory attributes of both beef and frankfurter sausages with the exception of colour. Both basil and dawadawa improved most of the mineral composition of the sausages. The crude protein, crude fat and moisture content of the sausages were not negatively affected by both basil and dawadwa. Basil and dawadawa were found to be rich sources of most major and micro minerals. The storability of the sausages was not negatively affected by the inclusion of basil and dawadawa.

6.2 Recommendations

- Meat processors can produce sausages with dawadawa seed and basil leaves.
- Dawadwa and basil leaves powders can be used up to 8g/kg of sausage without any adverse effect.
- Proper packaging of dawadawa seed and sweet basil leaves powders should be introduced on our local markets.

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