EFFECT OF BANANA PEEL ASH ON MICROBIAL, COMPOSITIONAL AND SENSORY PROPERTIES OF DEHYDRATED BEEF

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A Thesis Submitted to the Graduate School in partial fulfillment for the requirements of the Master of Science Degree in Food Science of Egerton University.

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DECLARATION AND RECOMMENDATION

This thesis is my original work and has not, wholly or in part, been presented for the award of a degree in any other university.

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This thesis is the candidate's original work and has been prepared with our guidance and assistance; it has been submitted with our approval as the official university supervisors.

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DEDICATION

This work is dedicated to my parents, Mr and Mrs Moses Nato and my children, Gladys and Karel. May God bless you abundantly.

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Mr. Samwel Muyoma Nato.
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ABSTRACT

The association between excessive sodium intake and hypertension has prompted public health and regulatory authorities to recommend reduction of dietary intake of sodium. The study investigated the antimicrobial effect of 1%, 2% and 3% of both common salt (CS) and water soluble ash (WSA) from green banana peels in dehydrated beef. The proximate properties, mineral composition and sensory properties of beef treated with the three levels of CS and WSA were also evaluated. Portions of beef weighing 500 g were each treated with one of the three levels of CS or WSA while the control was treated with distilled water. The samples were then cut into thin strips and dried at 60°C for 15 hours and packed in polythene pouches. Microbial analysis was done immediately after drying; and after every 5 days for 30 days of storage at 22°C. Proximate, mineral, and sensory analysis was carried out immediately after drying and after 30 days of storage. The experiment was done in three replicates. Data analysis was done using the General Linear Model procedure of the Statistical Analysis System to identify any significant differences in mean microbial CFU/g, proximate values, mineral levels and sensory scores. Mean separation was done by Duncan's Multiple Range Test, at $p<0.05$. On the 30^{th} day of analysis, the results showed that both CS and WSA inhibited mesophilic and thermophilic microbial growth compared to the control. For mesophiles, the control had 8.2 $\log_{10}$ CFU/g compared to samples treated with 3% WSA which had 5.6 $\log_{10}$ CFU/g, while the samples treated with 3% CS recorded the least count of 3.8 log10 CFU/g. For thermophiles, the control recorded 4.6 $\log_{10}$ CFU/g which was significantly higher than the sample treated with 2% WSA which recorded a count of 4.1 $\log_{10}$ CFU/g, while the sample treated with 3% CS recorded 3.5 $\log_{10}$ CFU/g. For Enterobacteriaceae, samples treated with 3% WSA recorded 3.2 $\log_{10}$ CFU/g while that treated with 3% CS recorded the least at 2.4 $\log_{10}$ CFU/g. The sample treated with 3% WSA retained 30% moisture which was significantly higher than 17% retained by the sample treated with 3% CS. Further, the 3% WSA sample recorded significantly less sodium at 900 ppm compared to that treated with 3% CS which recorded 2300 ppm of sodium. Sensory evaluation indicated that there was no significant difference in preference between beef treated with 1% WSA with 1% CS and 2% CS. There is therefore a potential for 1% WSA to be used in dehydrated beef to enhance sensory properties with the specific intention of reducing dietary sodium intake which is associated with high prevalence of hypertension.

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ABBREVIATIONS/DEFINITIONS

WSA: Water soluble ash: It is a residue obtained by evaporation of aqueous filtrate from ash.

CS: Common salt fortified with potassium iodate.

DWB: Dry weight basis

WWB: Wet weight basis

CFU: Colony forming units.

Day0: First day of analysis.

Day5: Fifth day of analysis.

Day10: Tenth day of analysis.

Day15: Fifteenth day of analysis.

Day20: Twentieth day of analysis.

Day25: Twenty fifth day of analysis.

Day30: Thirtieth day of analysis.

CHAPTER ONE

INTRODUCTION

1.1 Background to study

Current studies report various possible strategies for developing healthier meat and meat products. One of the most important of these is to design meat products that have reduced amounts of sodium (Kim and others 2010). Dietary intake of sodium, from all sources, influences blood pressure levels in populations and should be limited in order to reduce the risk of coronary heart disease and stroke. Increased sodium intake is associated with increased blood pressure, whereas increased potassium and calcium intake may slightly decrease blood pressure. Moreover, magnesium intake has been inversely associated with blood pressure (Alino and others 2010). Accordingly, due to the role of sodium in the development of hypertension in sodium-sensitive individuals, public health and regulatory authorities have recommended a reduced dietary intake of sodium chloride (Dimitrakopoulou and others 2005). The World Health Organization (WHO) recommends no more than 5 g/day/person (Zanardi and others 2010). Thus, sodium chloride reduction in meat products is an important component in the goal of an overall decrease in dietary sodium (Dimitrakopoulou and others 2005).

Sodium chloride is used in the production of meat products because of its effects on texture, flavour and shelf life. Salt reduction in meat products has adverse effects on water and fat binding, impairing overall texture and increasing cooking loss, and also on sensory quality especially taste (Ruusunen and others 2005). Sodium reduction requires partially or totally substituting the sodium chloride added to meat derivatives by other compounds that have similar effects on sensory, technological and microbiological properties (Wirth 1991; Alino and others 2010). There is however no panacea in terms of a single ingredient that can be used to replace sodium chloride in meat products, therefore a range of functional ingredient combinations must be developed and/or optimized (Desmond 2006). Gelabert and others (2003) studied the effect of substitution of sodium chloride with potassium chloride, potassium lactate, or glycine (0–40% substitution) on some sensory, microbiological and physicochemical characteristics of fermented sausages. They concluded that this had little effect on microbiological stability though differences in sensory properties were detected. Indeed, Munoz and others (2009) found that potassium lactate could reduce the excessive hardening at the surface of salted meat products. Hoffman and others (2008) too found that phosphates and lactates could be applied successfully to enhance the sensory attributes of beef.

Ruusunen and Puolanne (2005) suggested that the use of mineral salt mixtures is a good way to reduce the sodium content in meat products; while studies done by Kim and others (2010) on the effect of bamboo salt on the physicochemical properties of meat emulsion systems found that bamboo salt (37.4% Na, 0.29% K, 0.46% Ca, 0.92% Mg) effectively improved the physicochemical properties of the meat batter. There is however no published study on the effect of water soluble ash (WSA) from banana peels on microbial, compositional and sensory properties in dehydrated beef in spite of its use to season meat among some Kenyan communities. This was the basis for the study.

1.2 Statement of the problem

Research has indicated a positive relationship between dietary sodium intake and blood pressure levels in human populations. The high sodium intake is partly attributed to high sodium chloride levels in processed meat products. There is therefore a need to explore alternatives to sodium chloride in meat products. The alternatives should serve the traditional role of sodium chloride in processed meat which include preservation and flavour enhancement.

1.3 General objective

The purpose of this study was to investigate the effect of banana peel ash on microbial, compositional and sensory properties in dehydrated beef as an alternative for common salt.

1.4 Specific objectives

1. To determine the antimicrobial effect of common salt and WSA from green banana peels in beef.
2. To determine the proximate and mineral composition of beef treated with common salt and WSA from green banana peels.
3. To compare sensory characteristics of beef treated with common salt and WSA from green banana peels.

1.5 Hypotheses

1. There is no significant difference in antimicrobial effect in beef treated with different levels of WSA from green banana peels and common salt.
2. There is no significant difference in proximate and mineral composition of beef treated with different levels of WSA from green banana peels and common salt.
3. There is no significant difference in sensory characteristics of beef treated with different levels of WSA from green banana peels and common salt.

1.6 Justification

Hypertension is an important public health challenge worldwide (Addo and others 2007). Many studies indicate a high prevalence of hypertension (blood pressure ≥140/90 mmHg) among populations. Tesfaye and others (2009) reports that 25% of the world population was hypertensive in the year 2000 and forecasts that this is likely to increase to 29% by the year 2029. In Ghana, the prevalence is 28.3% (Cappuccio and others 2004) while Sacks and others (2001) reports that hypertension affects about 50 million Americans. Bovet and others (2008) reports 40% hypertension prevalence in Seychelles and states that this situation may not be unique in Sub-Saharan Africa because of a high prevalence of cardiovascular risk factors; one of which is a high sodium intake (Gelabert and others 2003; Desmond and others 2006; O'Shaughnessy and Karet 2006). This association between excessive sodium intake and the development of hypertension has prompted public health and regulatory authorities to recommend reducing dietary intake of sodium chloride and increased consumption of foods rich in potassium (Kuller 2007). In addition, the consumer demands for healthier meat and meat products with reduced level of fat, cholesterol, decreased contents of sodium chloride and nitrite, improved composition of fatty acid profile and incorporated health enhancing ingredients are rapidly increasing worldwide (Zhang and others 2010). Consequently, a new class of foods, the so-called "functional foods" are being developed that either contain components that have beneficial physiological effects or that are void of components that depending on intake amounts may negatively impact consumers health (Weiss and others 2010).

It is therefore desirable in meat processing that a safe, as well as nutritious, product is supplied to the consumer (Arnau and others 2007). The use of WSA from green banana peels in meat preservation and sensory improvement may be an option to reduction of sodium chloride while increasing the potassium level in meat. Studies done by Emaga and others (2007) have indeed, indicated that green banana peels have low sodium level of 0.5 g/Kg dry matter compared to a potassium level of 49g/Kg dry matter. Calcium, phosphorous and magnesium levels are 1.9 g/Kg, 3.5 g/Kg and 1.8 g/Kg respectively.

CHAPTER TWO

LITERATURE REVIEW

2.1 Banana distribution and uses

Banana (*Musa* spp.) is one of the most important food and cash crops in Kenya and Over a million tonnes are produced per year (Njuguna and others 2008). Banana occupies 30% of the cultivated land of the East African highlands (Kenya, Uganda, Tanzania, Rwanda, Burundi and the Democratic Republic of Congo). About 20 million people in East and Central Africa depend on banana for food and income. The main banana cultivars grown are the East African Highland Bananas, the brewing types, the cooking types, and the desert types like the 'Gros Michel' and 'Kampala' (Mbaka and others 2008). Bananas are usually processed into chips, flours, wines, spirits, and jam. Before use, the bananas are usually peeled. Significant quantities of banana peels, equivalent to 40% of the total weight of fresh banana, are for these reasons generated. It is thus significant and even essential to find applications for these peels. Moreover, these peels cause a real environmental problem (Emaga and others 2007).

2.2 Meat and human health

Meat and meat products are important sources of protein, vitamins and minerals and other bioactive compounds (Jimenez-Colmenero and others 2001; Arihara 2006), but they also contain fat, saturated fatty acids, cholesterol, salt, etc. In order to produce ''healthier'' meat products we need to fully understand their positive and negative effects on health. Only then shall we be able to devise suitable strategies to effectively control and adjust their characteristics to suit our needs (Jimenez-Colmenero and others 2001). Among the additives that impart negatively on human health include sodium chloride and nitrites/nitrates. It has recently been recommended that salt intake be reduced in light of the relationship between high sodium levels and arterial hypertension. A large percentage of the population possesses a hereditary predisposition to arterial hypertension, the incidence of which is further affected by excess weight and high sodium intake. Sodium comes from a wide variety of foods, among them meat and meat derivatives. Meat as such is relatively poor in sodium, containing only 50–90 mg of sodium per 100 g (Jimenez-Colmenero and others 2001; Strasburg and others 2008). However, the sodium in meat derivatives is much higher because of the salt content, which can be as much as 2% in heat-treated products (e.g. sausages) and as much as 6% in uncooked cured products, in which drying (loss of moisture) increases the proportion even further. Estimation taking eating habits into account suggests that approximately 20–30% of common salt intake comes from meat and meat derivatives (Jimenez -Colmenero and others 2001).

Meat and its derivatives may be considered functional foods to the extent that they contain numerous compounds thought to be functional (Jimenez-Colmenero and others 2001). However, only a limited number of studies on the possible health benefits of functional meat and meat products in human have been reported. Most conclusions are drawn from the fact that a functional ingredient itself may be beneficial to human (Zhang and others 2010) and thus the idea of using food for health purposes rather than for nutrition opens up a whole new field for the meat industry (Jimenez-Colmenero and others 2001). And as the economy develops, meat and meat products are not only utilized to provide necessary nutrients but also expected to have additional functions to reduce risk of disease occurrence and improve wellbeing of consumers (Zhang and others 2010). In addition to traditional presentations, the meat industry can explore various possibilities, including the control of the composition of raw and processed materials to produce design foods reformulated to have specific properties. For example, fatty acid profiles, the inclusion of antioxidants, dietary fibre and probiotics. In many such products it may be necessary to use new ingredients and/or methods which affect the technological, microbiological and sensory properties (Jimenez-Colmenero and others 2001).

2.3 Microbiological contamination of meat

Meat from healthy animals is sterile. However, it may be contaminated by skin, hooves, hair, intestinal contents, knives, cutting tools, personnel, polluted water, air, faulty slaughtering procedure, post slaughter handling, and storage (Kadim and Mahgoub 2007). The high water activity and abundant nutrients make meat an excellent medium to support microbial growth, since changes that occur during spoilage take place in the aqueous phase of the meat. These substrates are catabolized by almost all bacteria in muscle food microbiota (Argyri 2010) Among the more common species of microorganisms occurring on fresh meats are *pseudomonas, staphylococci, micrococci, enterococci, coliforms,* yeasts and moulds (Pelczar and others 1993; Kadim and Mahgoub, 2007; Ercolini and others 2009; Adeyinka and others 2011). *Listeria monocytogenes, Salmonella* and *Staphylococcus aureus* are important food-borne pathogens and outbreaks associated with these pathogens have been reported in meat products. *L. monocytogenes* is particularly problematic for the food industry, because it is widespread in the environment and because of its ability to grow in a wide range of temperatures. The incidence of *L. monocytogenes* in cooked meat products varies from 3.5 to 85% (Bersot and others 2000) and several hypotheses can be formulated to explain this contamination. These are survival of *L. monocytogenes* to the initial cooking process, post processing contamination and recuperation and growth of the injured micro-organisms during storage. It is also documented that *L. monocytogenes* may become established within the processing environment surviving cleaning and disinfection routines (Borch and Arinder 2002). Listeriosis is of public health concern because of its high case-fatality (20–30%). *S. aureus* also has the ability to grow at a wide range of temperatures and pH and staphylococcal food poisoning, together with salmonellosis, are two of the main food-borne diseases (Ananou and others 2005; Jofre and others 2008). *S. aureus* is often found closely associated with the human body. It may also be found in many parts of our environment, including dust, water, air and feces and on clothing or utensils (Bremer and others 2004). Attachment to the meat surface may be considered a first step in the microbial spoilage of meat and, for organisms that attach to meat surfaces in low numbers; their continued presence will depend on their ability to remain attached to the meat surface (strength of attachment). Research reported by Benito and others (1996) indicated that *Staphylococcus aureus, Clostridium perfringens* and *Yersinia enterocolitica* attach to meat surfaces very strongly. Human infections caused by microorganisms from the Enterobacteriaceae family such as *E. coli* O157:H7 have also been reported and cattle appear to be the chief source of infection; moreover, many outbreaks of the disease have also been linked, in particular, to the consumption of beef (Cadirci and others 2010).

Research indicates that spoilage of meat products is in general attributed to bacteria. However, new processing and storage techniques inhibiting the growth of bacteria may provide opportunities for yeasts to become the dominating micro flora causing the spoilage of meat products. The yeast flora isolated from salted, dry and semi-dry products, such as fermented sausages, consists mainly of the salt tolerant *Debaryomyces hansenii*, while *Yarrowia lipolytica* and members of the *Candida*, *Trichosporon*, *Cryptococcus* and *Rhodotorula species,* including *Candida zeylanoides*, *Trichosporon ovoides* and *T. beigelii*, *Cryptococcus albidus* and *Rhodotorula mucilaginosa* are also present (Nielsen and others 2008).

2.4 Effect of dehydration on microbial levels in meat

Dehydration has been used for centuries for meat preservation (Pelczar and others 1993; Rahman and Perera 2007). Meat is an ideal medium for bacterial growth because of high moisture and protein content. Examples of dry meat products are bitong in South Africa, Pemmican and Jerky in North America and Charqui in South America. In the case of bitong and Charqui, the meat may be salt-dried (Waris 2000). The preservative effect of dehydration is due to microbistasis; the microorganisms are not necessarily killed. Growth of all microorganisms is prevented by reducing the moisture content below a critical level. The critical level is determined by the characteristic of the particular organism and the capacity of the food item to bind water so that it is not available as free moisture (Pelczar and others 1993).

2.5 Effect of sodium chloride content on microbial population and chemical activity in meat

Sodium chloride is added to muscle foods for a variety of purposes including inhibition of microbial growth (Puolanne and others 2001; Dimitrakopoulou and others 2005). The preservation and shelf life of processed meats is therefore of vital importance when reducing sodium chloride levels. Reduction of sodium chloride content below those typically used without any other preservative measure has been shown to reduce product shelf life. Reduction of sodium chloride content by 60% to 1.5% results in a more rapid growth in natural flora of frankfurters. Similarly, a reduction by 50% to 1.25% in ground pork results in slight increases in the growth of Lactobacillus species (Desmond 2006). Curing of meat with sodium chloride is a process used globally to enhance various meat quality attributes such as preservation and taste enhancement but it can also influence binding of water to the meat matrix (Hansen and others 2008). Sodium chloride added to meat products improves their binding and water-holding properties. Sodium chloride induces solubilization of myofibrillar proteins and it has been long known that this exudate serves as binding agent between the meat pieces (Dimitrakopoulou and others 2005). Chloride ions tend to penetrate into the myofilaments causing them to swell (Puolanne and others 2001; Devatkal and Naveena 2010) while sodium ions form an ion "cloud" around the filaments which leads to an increased osmotic pressure within the myofibrils and also causes the filament lattice to swell. Simultaneously, the enlargement of negative net charge within the myosin filaments loosens the filaments and finally causes the disintegration of the filament. However, sodium chloride has been demonstrated to accelerate lipid oxidation in various meat products (Devatkal and Naveena 2010).

2.6 Effect of sodium chloride content on proximate composition of meat

The proximate composition of separable lean tissue of beef is somewhat variable, but in general, water accounts for about 70% of the weight of the fresh, lean muscle. The water is largely trapped within or between the muscle cells, with lesser amounts bound in varying degrees to proteins. Variation in water content is generally offset by changes in lipid composition (about 6%), while protein composition ranges from 18% to 23% and ash or mineral content is approximately 1-1.2%. This includes K (356 mg/g), P (199 mg/g), Na (63 mg/g), Mg (22 mg/g), and Ca (6 mg/g) (Strasburg and others 2008). Dimitrakopoulou and others (2005) reported that sodium chloride level affects the moisture, protein, fat and ash content of restructured cooked pork shoulder and; the higher the sodium chloride level the higher the moisture content. Protein and fat content were inversely proportional to the moisture content of the product. The higher the salt level, the higher the ash level, probably due to the increased sodium content of the product.

2.7 Effect of sodium chloride content on sensory properties of meat

Sodium chloride added to the meat products affects flavor (Puolanne and others 2001; Dimitrakopoulou and others 2005), texture characteristics and consumer acceptability. Traditionally, sodium chloride has been used in restructured meat products to bind meat pieces along with heat treatment (Dimitrakopoulou and others 2005). In the modern meat industry, sodium chloride is used as a flavoring or flavor enhancer and is also responsible for the desired textural properties of processed meats. Sodium chloride imparts a number of functional properties in meat products and this includes activation of proteins to increase their hydration and water-binding capacity which results in an improved texture of the meat (Desmond 2006).

2.8 Sodium chloride partial replacement in meat processing

Alternatives for sodium chloride in meat processing have been investigated for decades. Such interventions are delicate because of the universal role of sodium chloride in texture as well as in flavor development and safety of processed meats (Desmond 2006). Sodium reduction requires partially substituting the sodium chloride added to meat derivatives by other compounds that have similar effects on sensory, technological and microbiological properties. The extent to which sodium chloride levels can be limited depends on the product type (Wirth 1991). A number of compounds have been used for this purpose, among them chlorides other than sodium chloride, such as potassium and magnesium salts. Although total substitution of sodium chloride does not seem possible because of sensory reasons, a combination of sodium, potassium and magnesium salts may produce satisfactory results (Jimenez-Colmenero and others 2001). Bawa and Jayathilakan (2010) reported that calcium and magnesium ions activate Calcium-activated-neutral-proteinase enzymes which contribute to tenderization of meats by causing breakdown of specific myofibrillar proteins.

Research has also demonstrated that phosphates can be very useful in lowering the sodium chloride content in meat products. Desmond (2006) investigated the use of phosphates in reduced sodium cooked meat products. He found that it is possible to produce reduced-salt (1.0–1.4% sodium chloride) bolognas and cooked hams provided that phosphates are added. Further reduction of sodium content in reduced-salt meat products is possible by replacing sodium phosphate with potassium phosphate. It has also been shown that addition of phosphates to post-rigor meat improves its water binding and that there is a synergistic interaction between sodium chloride and phosphates. Phosphate enhances the effect of sodium chloride; and with its addition, good water binding can be reached at a rather low sodium chloride content of about 1.5% (Dimitrakopoulou and others 2005). One way that phosphates increase water binding ability is by increasing meat pH and solubilizing proteins, allowing for myofibrillar swelling (Boles and Swan 1997). Phosphate is often used as a basic ingredient in the tumbling pickle to increase cooking yield and it also chelates ions that acts as catalysts for oxidation. The functions of alkaline phosphates are to increase the pH and ionic strength, and tie up some pro-oxidants (Cheng and Ockerman 2003). The pH is elevated from around 5.5-5.6 (which is close to the isoelectric point of actomyosin) to 5.8-6.0 where myosin and most other muscle proteins will bind water more strongly due to increased net charges. A pH elevation would also allow inter-fillamental spaces to further expand via electrostatic repulsions for additional water to be immobilized (Strasburg and others 2008). This has a direct effect on tenderness which is one of the most important quality attributes of beef and depends on many physical, chemical and biochemical factors. Beef tenderness is very important especially that its inconsistency is one of the major factors affecting consumer satisfaction and has been identified as one of the major problems facing the beef industry (Christensen and others 2011). Another important reason for using phosphates is their ability to increase meat pH and to slow discoloration by stabilizing vitamin C (Dusek and others 2003). However, the presence of excessive amounts of phosphates in the diet may influence the calcium, iron and magnesium balance in the human body, and can increase the risk of bone diseases (Dimitrakopoulou and others 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Source of beef and green banana peels for preparation of the WSA

Lean beef (15 kg) was obtained for each of the three replicates, from a retail outlet in Egerton 24 hour's post-mortem. Green bananas (Uganda green) were sourced from a farmer in Webuye (0^o 37' 0" North, 34^o 46' 0" East; approximate altitude, 1350m above sea level; annual rainfall, 1200-1500mm per annum; approximate average annual temperature, 23 ^oC). The bananas were washed and peeled, and the peels dried in the sun for four days then transported to Egerton University.

3.2 Preparation of WSA

The dry green banana peels were incinerated in a furnace till there was no visible organic matter. Solvent extraction using distilled water was done to obtain a filtrate. The filtrate was then evaporated in an open pan to obtain the WSA.

3.3 Meat treatment and experimental layout

The levels of CS and WSA applied were 1%, 2% and 3 %. Treatment of beef portions was done as described by Mapesa and others (2010) with modifications. Each level was made into a solution of 50 ml per 500 g meat using distilled water and injected evenly (using a single-needle hand-held injector) into the meat. The control samples were injected with 50 ml distilled water per 500 g meat. The injected cuts were then immersed in 1L of their respective solutions to equilibrate for 1 hour. The treated samples were then cut into strips of measuring 5 cm by 2 cm by 2 cm and dehydrated at 60 ^oC for 15 hours, and continually turned after every 2 hours for uniform drying. The samples were then packed in polythene pouches and kept on the shelf at ambient temperature. The experiment was conducted in three replicates and laid out in a completely randomized design (CRD) for microbial and sensory analysis and a randomized complete block design (RCBD) for compositional analysis.

3.4 pH and Mineral analysis

3.4.1 pH analysis of CS, WSA and beef

The pH of CS and WSA was determined using a pH meter (PHS-3B, Nanjing T-Bota Scietech Instruments & Equipments co. Ltd, China). The pH meter was calibrated with pH buffers at pH 4.0 and 7.0 for CS and pH 7.0 and 9.0 for WSA (Nielsen 2003). Sample solutions with 1%, 2% and 3% (w/v) concentration were prepared by dissolving 1 g, 2 g and 3 g respectively of CS or WSA in distilled water to make 100 ml of solution. The pH of the meat was determined on 2 g of meat homogenized in distilled water (10/1 water/sample, w/w) as described by Zanardi and others (2010).

3.4.2 Determination of minerals in CS, WSA and beef

Sodium, potassium, magnesium and calcium were determined as described by AOAC (1995). A sample weight of 1 g was wet ashed using 10 ml concentrated nitric acid and 5 ml concentrated sulphuric acid in a digester (2012 digester Foss Tecator, Burlandegen Germany). The samples were carefully heated at 250^{o}C until vigorous reaction stopped. Heating continued to 450^{o}C for 3hours. The digested samples were cooled then filtered (Whatmans filter paper no. 4) into a 100 ml flat-bottomed flask and contents topped to the mark with distilled water. Lanthanum chloride (10% w/v) measuring 1 ml was added to each sample and an Atomic Absorption Spectrophotometer (AA-6300 Shimadzu Corporation, Tokyo) was used for the analysis.

3.4.3 Determination of Phosphorous and Chloride in CS, WSA and beef

Phosphorous and chloride were analyzed as described by Nielsen (2003). A sample measuring 2 g was ashed at 600 ^{o}C for 4 hours for determination of phosphorous. To dissolve the ash completely, 6 M hydrochloric acid and several drops of nitric acid were added. The sample was cooled, put into 100 ml volumetric flask and topped to the mark with distilled water. An aliquote containing about 0.5-1.5 mg (10 ml) of phosphorous was pipetted into a 100 ml volumetric flask and 20 ml of molybdovanadate reagent added, and brought to the mark using distilled water. The colour was allowed to develop in 10 minutes and the absorbance read at 400 nm using a UV/Visible spectrophotometer (UV-1700 Shimadzu Corporation, Tokyo). A phosphorous standard curve was used for calibration. Chloride level was determined by Volhard titration using 0.1M silver nitrate (excess). The titrant was filtered using a retentive filter paper and the residue washed thoroughly. Concentrated nitric acid measuring 3 ml was added and excess silver titrated with 0.1M potassium thiocyanate using ferric ion as an indicator. The net volume of silver nitrate used was determined and amount of chloride in the sample in mg/Kg calculated. All the reagents used were of analytical grade. Glass ware was acid washed and thoroughly rinsed with distilled water.

3.5 Determination of water activity of dehydrated beef treated with CS and WSA

This was done as described by Mapesa (2004). A durotherm (Aw Messer-Germany) was calibrated using a saturated solution of Barium chloride and left to stand for 3 hours until the water activity reading was at 0.900. The sample was prepared by finely chopping 10 g of each treatment into small pieces and placing them into the durotherm. Water activity levels were recorded after 3 hours.

3.6 Determination of antimicrobial effect of CS and WSA in beef

Each sample weighing 10 g was blended in 90 ml of peptone diluent for 2 minutes (Waring commercial blender, Waring Corporation, USA). Serial dilutions of up to 10^{-3} (this was increased with time while eliminating lower dilutions with very high counts) were prepared in sterile peptone water in universal bottles. This was plated onto nutrient agar and stored at 30 °C for mesophiles and 37 °C for thermophiles. Enterobacteriaceae was enumerated using pour plates with an overlay of violet red bile agar and stored at 35 °C (Chabela and others 1999). This was done in duplicates and enumeration was done after 48 hours. All the media was supplied by Sigma-Aldrich Corp., St. Louis, Mo, USA.

3.7 Determination of proximate composition of beef treated with CS and WSA

3.7.1 Moisture content

Moisture content was done according to AOAC, 1995. It was determined by oven drying 2.00 g of the sample at 105 °C for 8 hours to constant weight in a cabinet drier (Electrolax, Sweden), cooled in a dessicator and weighed. The results were recorded as percentage moisture loss.

% moisture = (weight moisture in sample x 100)/ weight of sample.

3.7.2 Ash content

Ash content was done according to AOAC, 1995. It was determined by ignition (2.00 g) of dried sample at 550 °C for 4 hours in a muffle furnace (Bie & Bernstein, Denmark), cooled in a dessicator and weighed. The results were recorded as percentage mass retained.

% ash, wet weight basis = (weight of ash x 100)/ weight of dry sample.

The respective moisture values recorded earlier were used to transform the data to wet weight basis (WWB) as follows;

WWB score = DWB score x Dry matter co-efficient.

*Dry matter co-efficient = % solids/100

3.7.3 Fat content

Crude fat content was done according to AOAC, 1995. Fat content was determined on dry matter (2.00 g) by the Soxhlet method (M-tops extraction mantle, Indonesia) using petroleum ether as a solvent, for 16 hours. The formula used was;

% crude fat, dry weight basis = {(Initial weight of sample + thimble + pan) – (Final weight of sample + thimble + pan)}/ Initial weight of sample.

The respective moisture values recorded earlier used to transform the data to wet weight basis (WWB) as described for ash determination.

3.7.4 Crude protein content

Crude protein content was done according to AOAC, 1995. It was determined by micro-Kjeldahl method using 0.2 g of dry sample. Concentrated sulphuric acid was used for digestion (DK Heating digester-VELP Scientifica, Italy) at 430 °C for 1 hour with selenium as a catalyst. After distillation using a UDK 127 distillation unit (VELP Scientifica, Italy), the gas was received in 20 ml of 0.1 M hydrochloric acid with mixed indicator (0.1% methyl red, 0.2% bromocresol green, 0.5% phenolphthalein). The resultant distillate was titrated against 0.1M sodium hydroxide and the following formula applied.

$\%N$ = Normality of HCl* x (Corrected acid volume**/ g of sample) x (14 g N/mole) x 100

*Normality is in mol/1000ml

**Corrected acid volume = [Standard acid (ml) for sample] – [Standard acid (ml) for blank].

Nitrogen to protein conversion factor of 6.25 was used, thus;

% Protein = % N x 6.25

The respective moisture values recorded earlier used to transform the data to wet weight basis (WWB) as described for ash determination;

3.7.5 Total Carbohydrates

Total carbohydrate content was done according to AOAC, 1995. It was obtained by subtracting moisture content, protein content, fat content, and ash content from 100%.

% Total carbohydrates = 100% - (% moisture content + % protein content + % crude fat content + % ash content).

3.8 Sensory evaluation of dehydrated beef treated with CS and WSA

Sensory evaluation was carried from the sensory evaluation room with booths for individual assessors. This was done immediately after dehydration and after 30 days of storage of the meat at ambient temperature. Each treated sample weighing 250 g was immersed in 1 litre of distilled water at ambient temperatures to imbibe water for 1 hour (Mapesa and others 2010). The pieces were then boiled at 100 °C till all the water evaporated. A five member trained sensory panel aged between 20 and 40 years was then used to evaluate the cooked meat for colour, tenderness, juiciness, saltiness and flavour as described by Dimitrakopoulos and others (2005) and Gacula and others (1984) with modifications. A preparatory session was held to discuss and clarify each attribute to be evaluated. Testing was initiated after the panelists agreed on the specifications and each session took 10 minutes to accomplish. Attribute intensities were rated on graphical intensity scales, which were anchored from both their ends as shown in the table 1. One piece of cooked meat from each treatment was randomly chosen. The pieces with

Table 1: Graphical intensity scales

Property	0	10
Color	Red	Brown
Tenderness	Tough	Tender
Juiciness	Dry	Juicy
Saltiness	Not at all	Extremely salty
Flavor (odor and taste)	Weak	Strong

three-digit codes were presented to the panelists in random order and a questionnaire (appendix I) used to record results. Warm water was provided for rinsing the mouth between samples.

An untrained panel (consumer panel) of 16 members aged between 20 and 40 years then evaluated the cooked meat pieces for overall acceptability (preference) on a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) (Gacula and others 1984). Panel members were selected from students and staff of the Department of Dairy, Food Science and Technology. The panelists were instructed to express their evaluation for overall acceptability of cooked meat after considering the colour, tenderness, juiciness, saltiness and flavour of the product. Samples were prepared and offered randomly to the panelists as described for trained panel evaluation. Each session took 5 minutes to accomplish and the results recorded in a questionnaire (appendix II).

3.9 Data analysis

The data on proximate composition, mineral composition, microbial quality and sensory evaluation of the meat was subjected to analysis of variance (ANOVA) followed by multiple comparisons between means by Duncan's Multiple Range Test (DMRT) to identify any significant differences in mean proximate values, mineral composition, microbial CFU/g and sensory properties at $p<0.05$. The model used for microbial quality and sensory evaluation was; $Y_{ij}=\mu+\tau_i+\varepsilon_{ij}$. Y_{ij} is the j^{th} observation of the i^{th} treatment (0, 1.0%, 2.0% and 3.0% levels of CS and WSA), μ is overall mean, τ_i is the effect of each treatment and ε_{ij} is random error effect with mean zero and variance σ^2; and data for microbial analysis was transformed to $\log_{10}$ before statistical analysis. For proximate and mineral composition, the model used was; $Y_{ij}=\mu+\tau_i+\beta_j+\varepsilon_{ij}$. Y_{ij} is the j^{th} observation of the i^{th} treatment (0, 1.0%, 2.0% and 3.0% levels of CS and WSA), μ is overall mean, τ_i is effect of each treatment, β_j is the effect of the j^{th} block (Day0 and Day30) and

ε_{ij} is random error effect with mean zero and variance σ^2. Partial correlations were determined to find the relationship between moisture retention and mineral composition of treatments; and sensory scores of the trained panel and the consumer panel. The general linear model (GLM) of SAS (Statistical Analysis System) version 9.1 was used for all the computations.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Composition of CS and WSA solutions

4.1.1 Mineral composition of CS and WSA solutions

The analysis of mineral composition of CS and WSA was carried out and the results recorded in Table 2. Sodium and Chloride content was significantly higher in CS compared to

Table 2: Mineral composition of CS and WSA solutions.

Additive	Mean mineral composition (gKg^{-1}) ± SD^{α}					
	Na	K	Ca	Mg	P	Cl
CS solution	256.3 ± 4.4^{a}	55.5 ± 4.9^{b}	14.5 ± 1.1^{b}	16.9 ± 2.2^{a}	$0.02\pm.01^{b}$	347.1 ± 5^{a}
WSA solution	5.7 ± 0.3^{b}	51.4 ± 0.4^{a}	$63.2\pm.03^{a}$	10.8 ± 0.1^{a}	21.1 ± 0.7^{a}	11.4 ± 0.2^{b}

For every mineral component for CS and WSA solutions, mean values with different superscripts in the same column are significantly different ($p<0.05$).

WSA. WSA on the other hand had significantly higher levels of potassium, Calcium and Phosphorous. The level of Magnesium was not significantly different in CS and WSA solutions.

4.1.2 pH of CS and WSA solutions

The pH of solutions of CS and WSA at different concentrations was determined and the results are shown in Table 3.

Table 3: pH of CS and WSA solutions at different concentrations

Additive	CS solution			WSA solution		
Concentration	1%	2%	3%	1%	2%	3%
pH	7.00 ± 0.0^{d}	6.59 ± 0.0^{e}	6.27 ± 0.0^{f}	10.80 ± 0.0^{b}	10.81 ± 0.0^{a}	10.79 ± 0.0^{c}

For every additive, mean values (pH) with different superscripts in the same row are significantly different ($p<0.05$).

It was found that the pH levels of CS and WSA at different concentrations were significantly different. The pH of CS solution was found to reduce with increasing concentration while the pH of WSA was highly alkaline. The reduction of CS solution pH is due to cationic hydrolysis of contaminant salts in the common salt which results in lowering of the pH of the solution (Bahl and others 1997). The high and fairly constant pH of WSA is due to presence of phosphates and other soluble alkaline compounds.

4.2 Water activity and microbial growth

4.2.1 Water activity of dehydrated beef treated with CS and WSA.

Water activity was determined for all the treatments at Day0 and Day30. The results are shown in Table 4.

Table 4: Water activity of dehydrated beef treated with CS and WSA of different concentrations for Day0 and Day30

Day of	Treatment						
Analysis	DW	1S	2S	3S	1W	2W	3W
Day0	0.7925^{b}	0.7700^{c}	0.7500^{e}	0.7425^{f}	0.7600^{d}	0.7625^{d}	0.8100^{a}
Day30	0.8250^{a}	0.7725^{b}	0.7525^{b}	0.7525^{b}	0.7475^{b}	0.7700^{b}	0.8325^{a}

Key.

DW: Control.

1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

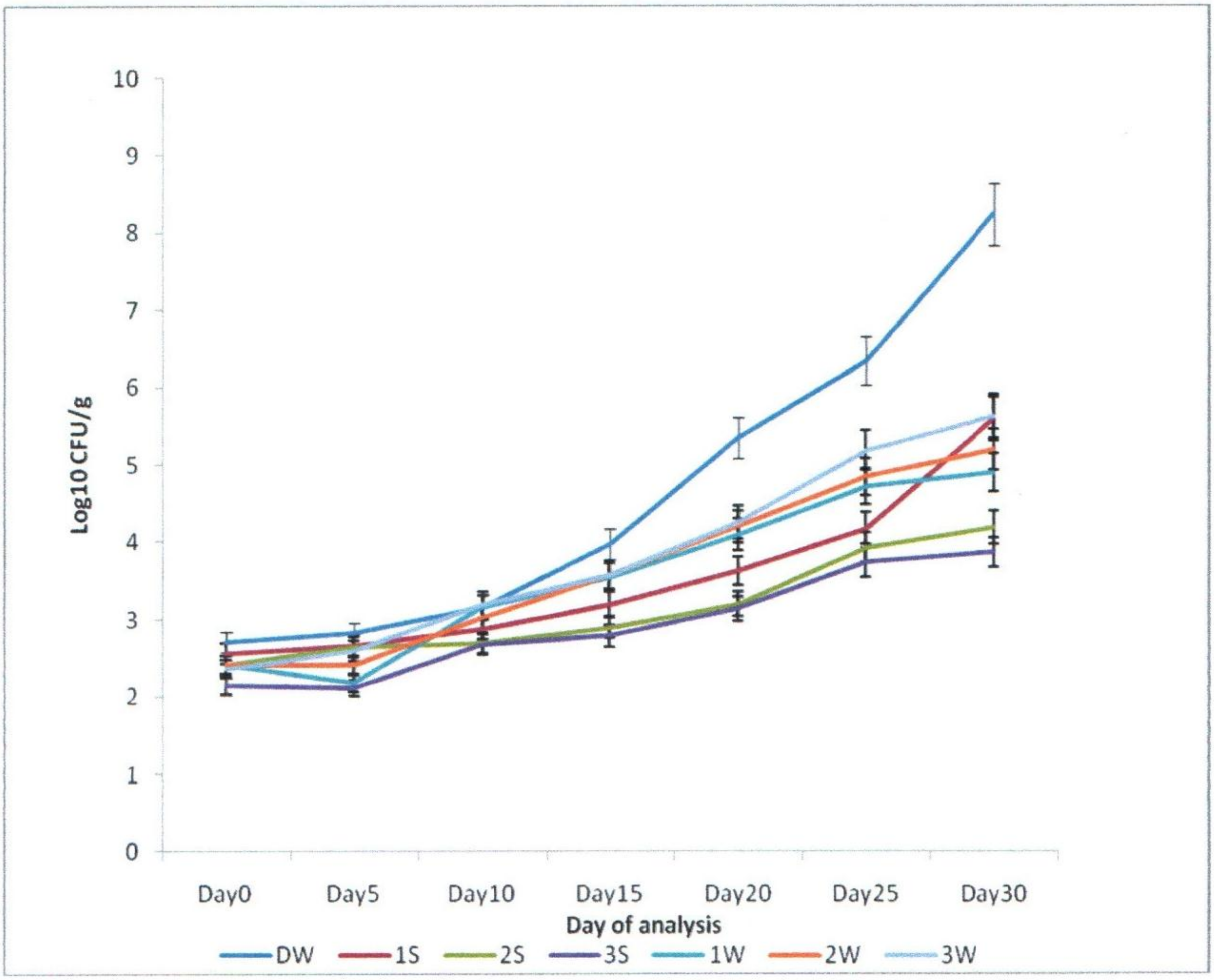
1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

Water activity values with different superscripts in the same row are significantly different ($p<0.05$).

The results indicate that for Day0, water activities were significantly different for all the treatments. The treatment which recorded the highest water activity was 3W while 3S recorded the least. At Day30, water activity of DW and 3W were significantly higher than the rest of the treatments, which were not significantly different from each other. The high water activity in 3W was probably due to high phosphates which enhance water holding capacity in meat (Boles and Swan 1997; Young and others 2005). The least water activity for Day0 was recorded in 3S due to a higher osmotic pressure exerted by sodium chloride (Strasburg and others 2008). It has also been suggested that some salts may increase water activity while others such as calcium and magnesium ions may increase water activity (Jay and others 2005). The variation in water activity may therefore be to the contribution of other salts not covered under this study.

4.2.2 Growth profile of mesophiles for 30 days in dehydrated beef treated with CS and WSA

Microbial analysis for mesophiles was carried out after every 5 days for 30 days (fig.1). The results showed a generally higher count for DW and a low count for 3S throughout the period of analysis. DW had a significantly higher count at Day0 than the other treatments



DW: Control.

1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

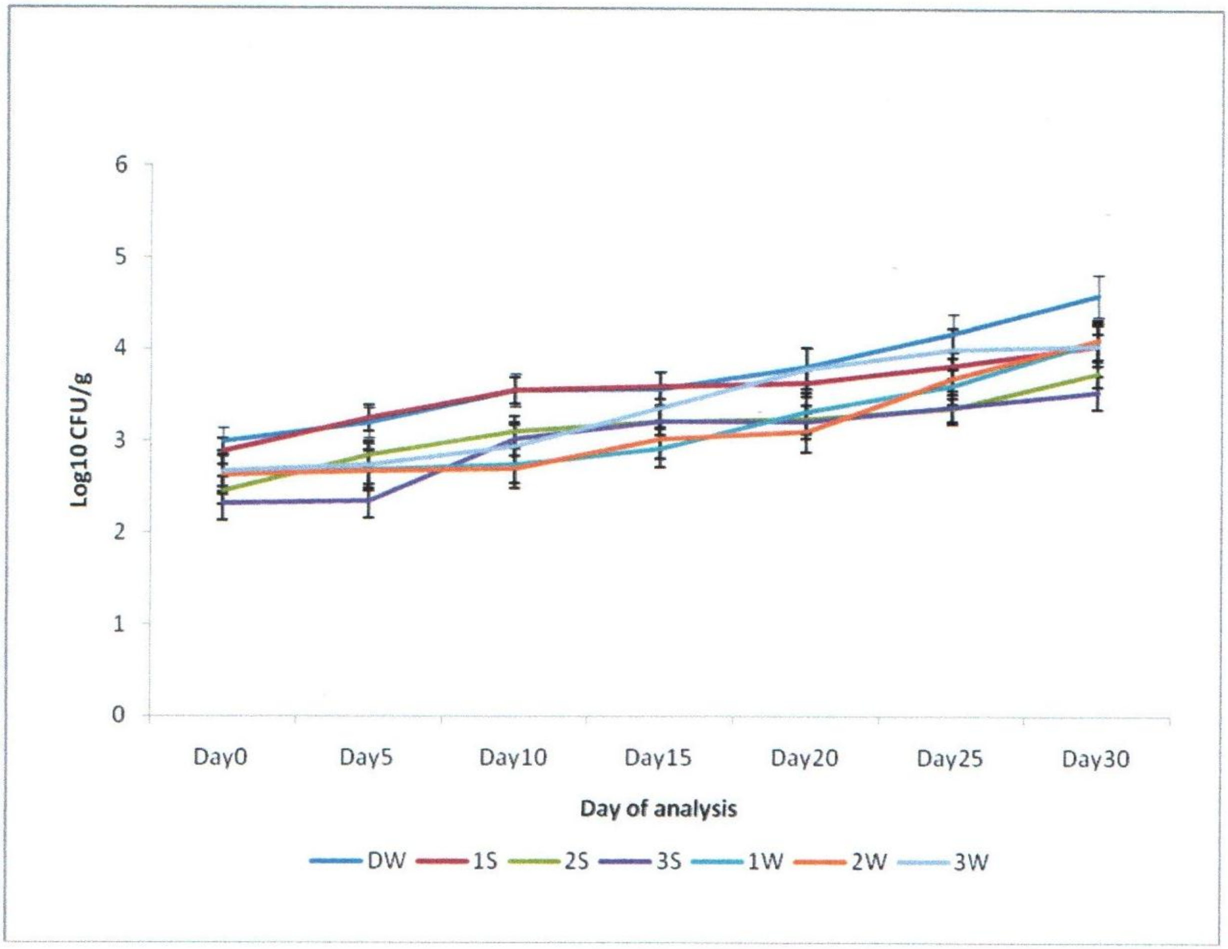
1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

Figure 1: Growth profile of mesophiles in dehydrated beef treated with CS and WSA

followed by 1S. The treatment with the lowest count was 3S. Microbial counts for 1W, 2W and 3W were not significantly different. At Day30, growth of mesophiles in DW was significantly higher than the rest of the treatments. DW had higher counts than 3W despite 3W recording a higher water activity. This indicates some antibacterial effect probably due a higher pH. DW had a significantly higher count on Day0 than the other treatments followed by 1S. A low count was recorded in 3S probably due to a lower water activity and pH (Pelczar and others 1993). Microbial counts for 1W, 2W and 3W were not significantly different despite the fact that water activity for 3W was higher than 1W and 2W. This could be attributed to a higher pH of the treatments. For Day30, microbial count for DW was beyond threshold for meat spoilage which is 10^7 CFU/g (Mapesa 2004). This was significantly higher than the rest of the treatments followed by 1S and 3W, 2W, 1W, 2S then 3S.

4.2.3 Growth profile of thermophiles for 30 days in dehydrated beef treated with CS and WSA

Microbial analysis was carried out for thermophiles, after every 5 days, over a period of 30 days and the results shown in figure 2. The trend of thermophiles was similar to that of mesophiles. DW had the highest count throughout the period of study. The microbial counts were however not significantly different from 1S between Day5 and Day20. Unlike in mesophiles where the microbial level in DW rose to 10^8 CFU/g, in thermophiles, the level was only 10^4 CFU/g. For Day30, microbial counts for thermophiles for 1W, 2W and 3W were not significantly different despite 3W having a higher moisture content, for Day0, of 29.3% compared to 23.2% of 1W and 26.3% of 2W; and a water activity of 0.81 compared to 0.76 of both 1W and 2W. This could therefore be attributed to 3W having a high pH of 8.2 compared to 6.7 and 7.2 of 1W and 2W respectively. The high pH contributes to its antimicrobial activity (Pelczar and others 1993).



DW: Control.

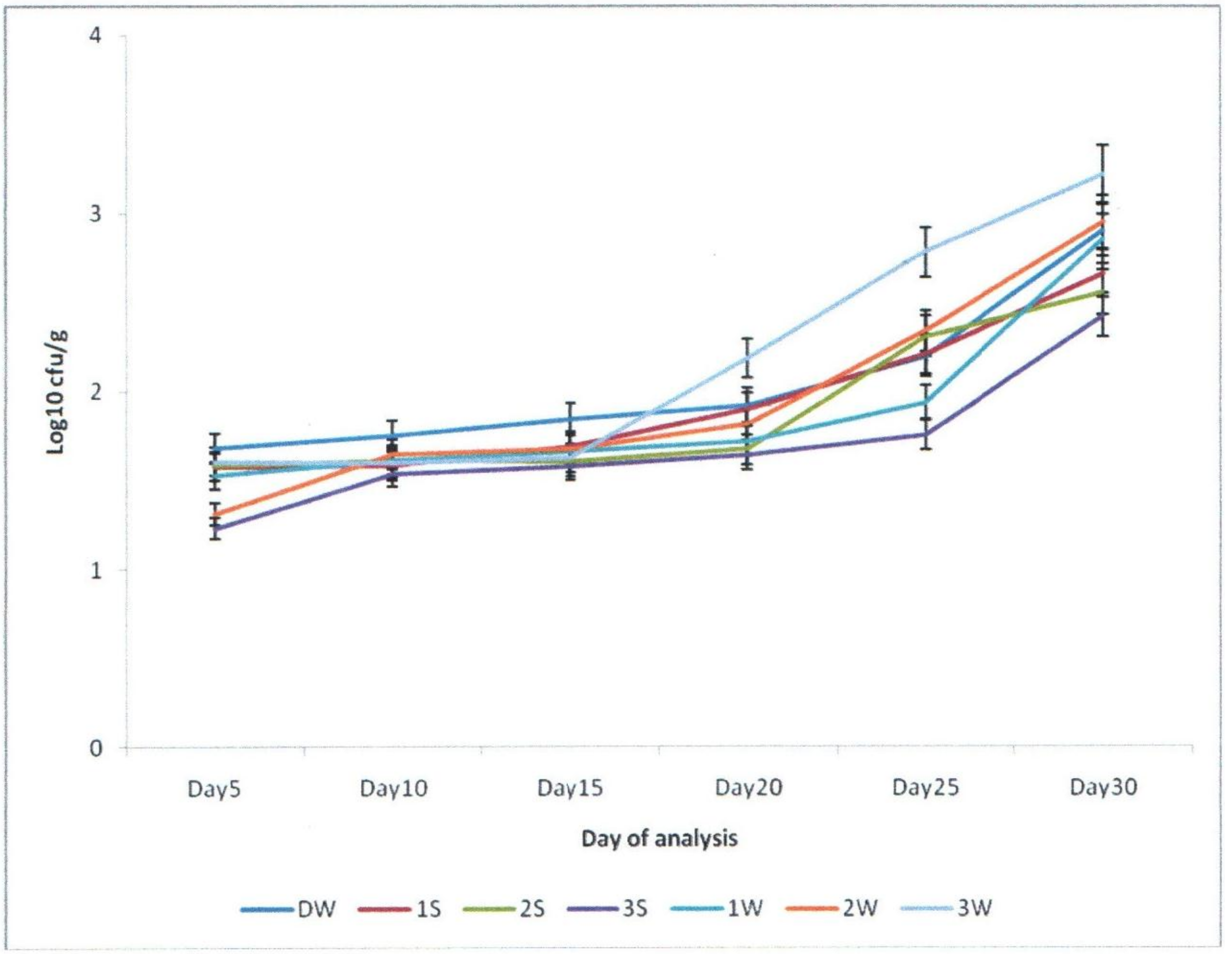
1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

Figure 2: Growth profile of thermophiles in dehydrated beef treated with CS and WSA.

4.2.4 Growth profile of Enterobacteriaceae for 30 days in dehydrated beef treated with CS and WSA

The trend in microbial counts for DW is similar for both mesophiles and thermophiles (figure 3). It was however noted that on Day20 up to Day30 microbial counts for 3W exceeded DW. Similarly counts for 3W for Day20 up to Day30 were significantly higher than 1W and 2W. Significantly lower counts were recorded 1S, 2S and 3S compared to the rest of the treatments. These levels were found to correspond to those found by Crowley and others (2005), despite use of different ingredients, of 0.52 to 6.98 $\log_{10}$ CFU/g in minced beef and beef burgers sold across the republic of Ireland. The levels were however high and are of great concern to the consumer



DW: Control.

1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

Figure 3: Growth profile of Enterobacteriaceae in dehydrated beef treated with CS and WSA.

due to the association of *Enterobacteriaceae, especially Salmonella, E.coli* and *Shigella,* with food borne disease outbreaks (Brenner and Farmer 2005). The high levels were indicative of poor hygienic conditions in the abattoir and possible fecal contamination of the carcass (Styles and Lai-King 1981; Chabela and others 1999). Such pathogens are likely to have been transferred from the gut and feces to the carcass during slaughter and dressing operations (Troy and Kerry 2010). Besides, the microorganisms might have been driven into the interior of the meat during the treatment process when a needle injector was used to introduce the additives. This procedure had the disadvantage of disrupting the muscle structure by cutting through muscle tissues, fibers and connective tissue. At the same time, bacteria were translocated to the meat interior where they grew to dangerous levels ultimately posing a threat to the consumer in the event that proper cooking methods are not used (Wicklund and others 2007).

4.3 Proximate and mineral composition of dehydrated beef treated with CS and WSA for Day0

4.3.1 Proximate composition for Day0.

Proximate analysis was carried out on all the treatments at Day0 and the results recorded in Table 5. Moisture content was significantly different for all the treatments.

Table 5: Proximate composition of dehydrated beef treated with CS and WSA for Day0

Treatment	Mean Proximate Property (percent composition) ± SD^{α}				
	Moisture content	Protein	Crude fat	Ash	CHO
DW	21.0 ± 0.71^{d}	65.0 ± 0.54^{a}	5.4 ± 0.15^{b}	3.9 ± 0.46^{e}	4.8 ± 1.86^{a}
1S	18.6 ± 0.32^{e}	65.5 ± 0.46^{a}	5.3 ± 0.15^{b}	5.5 ± 0.35^{d}	5.2 ± 0.36^{a}
2S	17.3 ± 0.30^{e}	66.4 ± 0.49^{a}	5.4 ± 0.09^{b}	6.4 ± 0.32^{bc}	4.5 ± 0.59^{a}
3S	14.3 ± 0.57^{f}	66.5 ± 0.23^{a}	5.8 ± 0.20^{a}	7.5 ± 0.15^{a}	5.9 ± 0.45^{a}
1W	23.2 ± 0.29^{c}	62.2 ± 0.24^{b}	5.2 ± 0.27^{b}	6.0 ± 0.21^{cd}	3.3 ± 0.98^{a}
2W	26.3 ± 0.89^{b}	59.3 ± 0.30^{c}	4.3 ± 0.16^{c}	6.3 ± 0.14^{bcd}	3.9 ± 1.83^{a}
3W	29.3 ± 0.59^{a}	55.4 ± 0.81^{d}	3.9 ± 0.06^{c}	7.0 ± 0.42^{ab}	4.4 ± 1.77^{a}

CHO: Carbohydrate

DW: Control.

1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

For each proximate property, mean values with different superscripts in the same column are significantly different ($p<0.05$).

Moisture content was highest in 3W followed by 2W and 1W. Moisture content reduced significantly from 18.57% to 14.34% with increase in CS concentration from 1% to 3% respectively. The protein content of 3W was significantly lower, followed by 2W then 1W. The protein contents of the rest of the treatments were not significantly different. This trend was similar to the crude fat content. DW had the lowest ash content while the ash content in 3W was not significantly different from 3S. Though the level of total carbohydrates was higher in 3S than the rest of the treatments, it was not significantly different.

The high moisture retention in 3W, 2W and 1W is attributed to high level of phosphates which enhance water retention (Young and others 2005). Compared to the rest of the treatments, 3W had a puffy appearance. Moisture content reduced significantly with increase in the concentration of common salt. This was due to immersion of the meat in salt solution that exerted a high osmotic pressure unlike in meat batters where salt increases water holding capacity (Weiss 2010). There was apparent lower protein content in 2W and 3W but this is only due to the high level of moisture content. This also explains the lower crude fat content. The ash level in 3W however is not significantly different from 3S due to the minerals in both treatments. Though the level of total carbohydrates is higher in 3S than the rest of the treatments, it is not significantly different.

4.3.2 Proximate composition for Day30.

Proximate analysis was carried out for all treatments at Day30 and the results recorded in Table 6. The moisture content of 3W was significantly higher than the rest of the treatments while 3S has the least due to high phosphate content which enhances moisture retention (Young and others 2005). The protein contents of DW, 1S, 2S and 3S were not significantly different from one another. The lowest protein was recorded in 3W while the protein content of 1W and 2W were not significantly different. The crude fat content of 2W and 3W were not significantly different. They were however lower than the rest of the treatments, which were not different from one another. The treatment that recorded the highest ash content was 3S but the level was not significantly different from 2S, 2W and 3W while DW had the lowest ash content. The carbohydrate contents of all the treatments were not significantly different from each other. All the differences in the proximate composition were brought about by the differences in moisture content which relatively affects the rest of the properties.

Table 6: Proximate composition of dehydrated beef treated with CS and WSA for Day30

Treatment	Mean proximate property (percent composition) ± SD^{α}				
	Moisture content	Protein	Crude fat	Ash	CHO
DW	21.9 ± 0.18^{cd}	64.7 ± 0.74^{a}	5.1 ± 0.12^{a}	3.6 ± 0.67^{d}	4.7 ± 1.41^{a}
1S	19.7 ± 0.50^{de}	64.9 ± 0.71^{a}	5.0 ± 0.14^{a}	5.5 ± 0.53^{c}	4.9 ± 0.60^{a}
2S	19.0 ± 1.94^{e}	65.9 ± 0.84^{a}	5.3 ± 0.13^{a}	6.4 ± 0.25^{abc}	3.4 ± 1.48^{a}
3S	17.7 ± 0.43^{e}	66.3 ± 1.46^{a}	5.2 ± 0.45^{a}	7.3 ± 0.25^{a}	3.6 ± 1.28^{a}
1W	22.8 ± 1.22^{cb}	61.6 ± 1.72^{b}	5.5 ± 0.13^{a}	6.2 ± 0.35^{bc}	3.9 ± 0.73^{a}
2W	25.2 ± 1.07^{b}	59.4 ± 0.84^{b}	4.3 ± 0.23^{b}	6.5 ± 0.15^{abc}	4.8 ± 2.29^{a}
3W	30.4 ± 0.51^{a}	53.8 ± 0.00^{c}	3.9 ± 0.26^{b}	7.2 ± 0.35^{ab}	4.7 ± 1.12^{a}

CHO: Carbohydrate

DW: Control.

1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

For each proximate property, mean values with different superscripts in the same column are significantly different ($p<0.05$).

4.3.3 Comparison between proximate compositions for dehydrated beef treated with CS and WSA for Day0 and Day30.

Statistical analysis for blocking effect was done to determine if there was significant difference in proximate composition of dehydrated beef at Day0 and Day30 and the results recorded in Table 7. It was found that only moisture content in Day30 was significantly higher than Day0. Protein, fat, ash and carbohydrates for Day30 were lower than Day0 but the differences were not significant. Statistical comparison of proximate data for Day0 and Day30 indicated that only moisture content was significantly higher in Day30 compared to Day0. Protein, fat, ash and total carbohydrates for Day30 were lower than Day0 but the differences were not significant. This increase in moisture content could be attributed to solubilization of myofibrillar proteins which releases water from muscle fibre. This biochemical change is catalysed by salts which include sodium chloride (Dimitrakopoulou and others 2005).

Table 7: Comparison between proximate compositions for dehydrated beef treated with CS and WSA for Day0 and Day30

Day	Proximate composition				
	Moisture content	Protein	Crude fat	Ash	Carbohydrates
Day0	21.4^{b}	62.9^{a}	5.1^{a}	6.1^{a}	4.5^{a}
Day30	22.4^{a}	62.4^{a}	4.9^{a}	6.1^{a}	4.2^{a}

For each proximate component, mean values with different superscripts in the same column are significantly different ($p<0.05$).

4.3.4 Mineral composition and pH of dehydrated beef treated with CS and WSA for Day0.

Mineral analysis was carried out on all treatments for Day0 and the results recorded in Table 8. Sodium and chloride levels were significantly high in 3S, 2S and 1S respectively compared to the rest of treatments which, for sodium, were not significantly different when compared amongst themselves. For chloride, DW, 1W and 2W were not significantly different from one another. Potassium levels were significantly different in all the treatments while the phosphorous level was highest in 3W but not significantly different in DW, 1S, 2S and 3S. Magnesium was highest in 3W but not significantly different in the rest of the treatments.

Sodium and Chloride levels were significantly high in 3S, 2S and 1S and this was attributed to the added sodium chloride. The sodium content in the rest of the treatments did not differ significantly from each other due to low content of sodium in WSA. Potassium levels were significantly different in all the treatments because of the high potassium level in both WSA and common salt which is commercially fortified with potassium iodate. The pH of treatment 1S, 2S and 3S were found to be inconsistent with level of sodium chloride added while that of 1W, 2W and 3W were found to increase with the level of WSA added. This means the pH of the additives did not have a consistent effect on the pH of the treatments which may be attributed to buffering capacity of meat (Weiss and others 2010).

Table 8: Mineral composition and pH of dehydrated beef treated with CS and WSA for Day0

Treatment	Mean mineral composition (ppm) /pH ± SD$^{\alpha}$						
	Na	K	P	Ca	Mg	Cl	pH
DW	775±93^{d}	5505±.220^{g}	3524±118^{d}	113±3^{c}	440±29^{b}	508±24^{e}	5.7±0.0^{de}
1S	1295±49^{c}	6293±131^{f}	3572±127^{d}	125±3^{c}	436±17^{b}	1508±50^{c}	5.8±0.3^{de}
2S	1780±69^{b}	7068±83^{e}	3582±54^{d}	132±2^{bc}	445±44^{b}	2417±96^{b}	5.4±0.1^{e}
3S	2361±171^{a}	7569±72^{d}	3535±82^{d}	136±5^{bc}	463±32^{b}	2980±50^{a}	5.9±0.0^{d}
1W	790±90^{d}	9374±86^{c}	4298±97^{c}	142±28^{bc}	471±28^{b}	561±0.0^{de}	6.8±0.2^{c}
2W	881±13^{d}	12847±159^{b}	4538±80^{b}	170±15^{ab}	513±67^{ab}	596±49de	7.2±0.0^{b}
3W	903±6^{d}	15566±219^{a}	4858±26^{a}	204±22^{a}	590±10^{a}	647±25^{d}	8.2±0.0^{a}

DW: Control.

1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

Mean mineral composition (ppm) ± SD$^{\alpha}$

For each mineral component or pH, mean values with different superscripts in the same column are significantly different ($p<0.05$).

Table 9: Mineral composition and pH of dehydrated beef treated with CS and WSA for Day30

Treatment	Mean mineral composition (ppm) /pH ± SD						
	Na	K	P	Ca	Mg	Cl	pH
DW	738 ± 28^{f}	5515 ± 32^{g}	3418 ± 96^{c}	112 ± 5^{d}	444 ± 9^{c}	543 ± 25^{d}	5.5 ± 0.1^{de}
1S	1308 ± 13^{c}	6312 ± 132^{f}	3445 ± 174^{c}	125 ± 1^{cd}	432 ± 1^{c}	1473 ± 99^{c}	5.4 ± 0.1^{e}
2S	1804 ± 64^{b}	6858 ± 188^{e}	3489 ± 54^{c}	136 ± 5^{c}	467 ± 20^{bc}	2484 ± 0^{b}	5.3 ± 0.1^{e}
3S	2319 ± 27^{a}	7252 ± 43^{d}	3425 ± 46^{c}	144 ± 2^{bc}	476 ± 37^{bc}	2945 ± 93^{a}	5.8 ± 0.1^{d}
1W	794 ± 13^{ef}	9358 ± 2^{c}	4102 ± 195^{b}	145 ± 13^{bc}	483 ± 13^{bc}	578 ± 25^{d}	6.6 ± 0.1^{c}
2W	$874\pm24d^{e}$	12898 ± 89^{b}	4361 ± 141^{b}	166 ± 10^{b}	511 ± 26^{b}	614 ± 29^{d}	7.3 ± 0.0^{b}
3W	919 ± 54^{d}	15606 ± 216^{a}	4711 ± 101^{a}	196 ± 14^{a}	589 ± 17^{a}	649 ± 33^{d}	7.9 ± 0.1^{a}

DW: Control.

1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

Mean mineral composition (ppm) ± SD^{α}

For each mineral component or pH, mean values with different superscripts in the same column are significantly different ($p<0.05$).

4.3.5 Mineral composition and pH of dehydrated beef treated with CS and WSA for Day30.

Mineral composition for Day30 had a similar trend as Day0 (Table 9). The sodium level of 3W was however higher than that in 2W and 1W. The level in 1W was not significantly different from that in DW. Potassium level was highest in 3W and lowest in DW and so was the level of calcium. Phosphorous level was also highest in 3W but the levels in DW, 1S, 2S and 3S were not significantly different from each other. Similarly, magnesium level was highest in 3W but not significantly different in DW, 1S, 2S, 3S and 1W. The pH of 3W was highest while that of 1S and 2S was the lowest. Phosphorous level was also highest in 3W but the levels in DW, 1S, 2S and 3S were not significantly different from each other. Similarly, magnesium level was highest in 3W but not significantly different in DW, 1S, 2S, 3S and 1W. The pH of 3W was highest due the high concentration of WSA added, while the pH of 1S and 2S were the lowest. However, since the pH of CS reduced with concentration, it was expected that the pH of 3S would be the lowest but this result was inconsistent. This could also be attributed to the buffering capacity of the meat (Weiss and others 2010).

4.3.6 Comparison of mineral compositions and pH of dehydrated beef treated with CS and WSA between Day0 and Day30.

Analysis for blocking effect was done to determine if there was significant difference in mineral composition at Day0 and Day30 and the results are shown in Table 10. It was found out

Table 10: Comparison of mineral compositions and pH of dehydrated beef treated with CS and WSA between Day0 and Day30

Day	Mineral composition (ppm) and pH						
	Na	K	P	Ca	Mg	Cl	pH
Day0	1254.98a	9174.31a	3986.69a	146.431a	486.046a	1326.53a	6.42a
Day30	1250.96a	9114.10a	3850.08b	146.024a	479.594a	1316.88a	6.25b

For each mineral component/pH, mean values with different superscripts in the same column are significantly different ($p<0.05$).

that only phosphorous composition was significantly different between Day0 and Day30; with Day30 registering a lower level. The pH level was also significantly lower for Day30 compared to Day0. The reduction in phosphorous could be explained by the fact that residual phosphatase hydrolyzed phosphate esters in the beef over time to produce inorganic phosphorous. The method used to assay phosphorous concentration was based on conversion of polyphosphates to orthophosphates. Since some of the polyphosphates had probably been reduced, less was available for conversion to orthophosphate contributing to perceived reduced levels on Day30 (Anna and others 2010). The pH is also significantly lower for Day30 compared to Day0. Reduced pH may be attributed to hydrolysis of fats releasing fatty acids and oxidation of carbohydrates to organic acids which increase acidity (Pelczar and others 1993).

4.3.7 Partial correlation of mineral levels/pH with moisture retention of dehydrated beef treated with CS and WSA for Day0.

Partial correlation was carried out to determine the influence of mineral level/pH on moisture retention. The results of correlation analysis are shown in Table 11.

Table 11: Correlation between mineral and pH levels with moisture retention for dehydrated beef treated with CS and WSA for Day0

Component	Pearson correlation Coefficient	Probability of getting a larger absolute value
P	0.89351	<0.0001
Mg	-0.59875	0.0237
Na	-0.35949	0.02068
Ca	-0.53744	0.0475
K	0.90964	<0.001
Cl	-0.91913	<0.001
pH	-0.6378	0.0141

This statistical analysis was done to establish which component among those analyzed had a stronger relationship with moisture retention in the treatments. It was found that potassium and phosphorous strongly correlated positively with moisture retention. This confirms the role of phosphates in binding water in processed meat (Young and others 2005). The role of potassium is however not clear. It may as well be a coincidence that high levels of phosphorous coincided with high levels of potassium. The relationship between pH and moisture retention however contradicts the fact that high pH in meat enhances water binding (Boles and Swan 1997; Strasburg and others 2008). Sodium too has a negative correlation with the amount of moisture bound in the dehydrated beef though it is well known that sodium chloride enhances water binding in meat (Weiss and others 2010).

4.4 Sensory evaluation

4.4.1 Sensory scores for dehydrated beef treated with CS and WSA for Day0.

Five trained panelists were used to evaluate the sensory attributes of the treatments. The evaluation was based on color, tenderness, juiciness, saltiness and flavor of the cooked samples and the scores recorded in Table 12. Scores for color captured the transition from red meat color of fresh beef to the brown color of cooked beef. Red meat color was retained more by 3W, followed by 2W then 1W (see appendix III). The reason for the color retention in 1W, 2W and 3W were not known. This may be attributed to the presence of phosphates which increase meat pH and slow discoloration by stabilizing vitamin C (Dusek and others 2003) and also sequester metals responsible for oxidation of lipids (Anna and others 2010). The rest of the treatments changed color significantly to the cooked meat brown color. The tenderest of all the treatments were 3S and 3W. This could be ascribed to high sodium chloride level and high phosphate level respectively which are known to improve tenderness (Rhee and Ziprin 2001; Young and others 2005). The treatment found to be least tender was 2S though not significantly different from DW, 1S and 1W. The juiciest treatment was 3W while 1S, 2S and 3S the least. Assessment of saltiness indicated that 3S tasted significantly more salty than the rest of the treatments. This was followed by 2S while the remaining treatments were not significantly different from one another. Flavor (odor and taste) profiling was not specific on meat flavor but the strength of the flavor. The treatment with the strongest flavor was 3W while DW recorded the least strength in flavor.

Table 12: Sensory scores of dehydrated beef treated with CS and WSA for trained and consumer panel for Day0

Treatment	Sensory property					
	Color	Tenderness	Juiciness	Saltiness	Flavor	Preference
DW	0.72 ± 0.07^{a}	0.66 ± 0.08^{bc}	0.54 ± 0.07^{cd}	0.19 ± 0.12^{c}	0.45 ± 0.05^{e}	5.4 ± 1.1^{c}
1S	0.72 ± 0.05^{a}	0.68 ± 0.07^{abc}	0.51 ± 0.06^{d}	0.29 ± 0.16^{c}	0.52 ± 0.05^{de}	6.2 ± 1.0^{abc}
2S	0.74 ± 0.06^{a}	0.65 ± 0.06^{c}	0.50 ± 0.06^{d}	0.50 ± 0.13^{b}	0.65 ± 0.09^{bc}	6.3 ± 1.0^{ab}
3S	0.74 ± 0.06^{a}	0.76 ± 0.07^{a}	0.49 ± 0.06^{d}	0.69 ± 0.78^{a}	0.71 ± 0.09^{b}	6.8 ± 1.4^{a}
1W	0.60 ± 0.05^{b}	0.74 ± 0.05^{abc}	0.61 ± 0.07^{c}	0.18 ± 0.06^{c}	0.60 ± 0.07^{cd}	5.6 ± 1.1^{bc}
2W	0.45 ± 0.06^{c}	0.75 ± 0.06^{ab}	0.73 ± 0.06^{b}	0.24 ± 0.11^{c}	0.73 ± 0.06^{ab}	3.9 ± 1.0^{d}
3W	0.35 ± 0.07^{d}	0.77 ± 0.08^{a}	0.89 ± 0.06^{a}	0.26 ± 0.11^{c}	0.82 ± 0.08^{a}	2.8 ± 1.3^{e}

DW: Control.

1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

Mean sensory score± SD^{α}

For each sensory property, mean values with different superscripts in the same column are significantly different ($p<0.05$).

A sixteen member consumer panel was used to rank the meat pieces for preference on a graphical intensity scale. The results of the evaluation are similarly recorded in Table 13. The panel ranked 3S as the most preferred treatment. It was however not significantly different from 1S and 2S. This was followed by 1W which was not scored significantly different from 1S. The least preferred treatment was 3W. The result confirmed the role of sodium chloride in enhancing typical meat flavor in processed meat (Weiss and others 2010). Most of the consumer panel members had similar comments to those of the trained panel that 3W had a soapy taste and therefore undesirable. The soapy flavor could be due to saponification of free fatty acids by cations of potassium in the WSA (Jain and others 2006). It should however be noted that functional products are considered as a different class of products and improvement in functionality is more important than taste (Zhang and others 2010).

4.4.2 Partial correlation between sensory scores of trained and consumer panel for dehydrated beef treated with CS and WSA for Day0.

Partial correlation analysis was carried out to find out which attribute from the trained panel scores contributed more to the preference recorded by the consumer panel. The results of the correlation analysis are shown in Table 13.

Table 13: Correlation between scores of trained and consumer panel for dehydrated beef treated with CS and WSA for Day0

Attribute	Pearson correlation coefficient	Probability of getting a larger absolute value
Color	0.95725	0.0007
Tenderness	-0.49908	0.2542
Juiciness	-0.97008	0.0003
Saltiness	0.53605	0.2149
Flavor	-0.56253	0.1886

The results of partial correlation analysis indicated that meat color and saltiness were positively correlated to preference while tenderness, juiciness and flavor were negatively correlated. It is however widely acknowledged that beef tenderness is the most important factor in consumer perception of beef palatability or quality (Strydom and others 2000; Li and others

2001). However, it was noted that tenderness and juiciness may not be key to consumer's preference when the flavor is undesirable. This indeed is in agreement with Troy and Kerry (2010) that tenderness, juiciness, flavor and overall palatability remain the most sought after attributes by consumers. It follows, therefore, that tenderness and juiciness and flavor, in tandem, have a negative correlation in this study.

4.4.3 Sensory scores (trained panel) for dehydrated beef treated with CS and WSA for Day30.

Sensory evaluation using a trained panel of five members was repeated on Day30. However, only two attributes which do not involve tasting were considered because of the high levels of Enterobacteriaceae in the treatments. The results are results are shown in Table 14, and they indicate that 1W, 2W and 3W significantly retained red meat color compared to 1S, and 3S just like in the evaluation for Day0. However, 2S was not significantly different from the rest of the treatments. This was inconsistent with results for Day0. Compared to 1S, 2S and 3S, 1W, 2W and 3W had significantly stronger odor. The panel evaluated only color and odor because tasting the beef samples could expose them due to diarrhea and intestinal infections associated with high levels of Enterobacteriaceae (Brenner and Farmer 2005). Red meat color was significantly retained in 1W, 2W and 3W compared to 1S, 2S and 3S just like in the evaluation for Day0. Similarly, 1W, 2W and 3W had stronger odor than 1S, 2S and 3S. This may be attributed to higher microbial activities in addition to the various constituents in WSA with unknown interactions with the meat constituents.

Table 14: Sensory scores for trained panel for dehydrated beef treated with CS and WSA for Day30

Treatment	Sensory property	
	Color	Odor
1S	0.742±0.112^{a}	0.444±0.093^{b}
2S	0.728±0.094^{ab}	0.460±0.117^{b}
3S	0.750±0.086^{a}	0.504±0.127^{b}
1W	0.638±0.078^{b}	0.672±0.099^{a}
2W	0.642±0.060^{b}	0.766±0.091^{a}
3W	0.526±0.089^{c}	0.768±0.094^{a}

DW: Control.

1S, 2S, 3S, are dehydrated beef treated with 1%, 2% and 3% common salt respectively.

1W, 2W, and 3W are dehydrated beef treated with 1%, 2% and 3% water soluble ash respectively.

Mean sensory score± SD$^{\alpha}$

For each sensory property, mean values with different superscripts in the same row are significantly different (p<0.05).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The results showed that both CS and WSA inhibited mesophilic and thermophilic microbial growth compared to the control. Microbial analysis for mesophiles on the 30th day recorded 8.2 $\log_{10}$ CFU/g for the control compared to samples treated with 3% WSA which had 5.6 $\log_{10}$ CFU/g, while the samples treated with 3% CS recorded the least count of 3.8 log10 CFU/g. For thermophiles, the control recorded 4.6 $\log_{10}$ CFU/g which was significantly higher than the sample treated with 2% WSA which recorded a count of 4.1 $\log_{10}$ CFU/g, while the sample treated with 3% CS recorded 3.5 $\log_{10}$ CFU/g. For Enterobacteriaceae, samples treated with 3% WSA recorded 3.2 log10 CFU/g while that treated with 3% CS recorded the least at 2.4 log10 CFU/g. This demonstrated the inhibitory effect of WSA against both mesophillic and thermophilic microorganisms in dehydrated beef.

Sample treated with 3% WSA retained 30% moisture which was significantly higher than 17% retained by the sample treated with 3% CS. This was due to the high level of phosphate recorded in 3W at 3500 ppm compared to 3S at 4800 ppm. Further, the 3% WSA sample recorded significantly less sodium at 900 ppm compared to that treated with 3% CS which recorded 2300 ppm of sodium. Dehydrated beef treated with WSA would therefore contribute less sodium to the diet.

Sensory scores for the consumer panel indicated that there was no significant difference in preference between dehydrated beef treated with 1% WSA and that treated with 1% CS and 2% CS. There is therefore a potential for 1% WSA to be used in dehydrated beef to enhance sensory properties with the specific intention of reducing dietary sodium intake. It is however important consider the fact that most consumers are accustomed to higher concentration of common salt in beef products and will need to adjust to the new product if good health is given priority.

5.2 RECOMMENDATIONS

1. There is need to carry out a cost-benefit-analysis on the use of WSA in beef for the purpose of commercialization. This is in view of the high cost of health care associated with hypertension and related conditions.
2. There is need to need to find out if ash from other plant materials such as bean trash and potato peels can be equally beneficial. This will not only reduce the health burden due to hypertension, but also solve disposal challenges related to such wastes.

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APPENDIX I

QUESTIONNAIRE

Date……………………..

Sample code……………

You are provided with seven samples of food to evaluate. The samples are coded as XXX, XXY, XXZ, XYX, XZX, ZXX and YXX.

Please evaluate the sample by placing a short vertical line across the scale where in your opinion the sample should be classified for that characteristic. An honest expression of your personal feeling will help us decide.

A. COLOUR

Look at the coded samples and score the colour intensity on the line below as shown by the descriptors.

____

Brown (Normal) Dark brown

B. TENDERNESS

Express the force needed by teeth to cut the sample on the line below.

____

Tough Tender

C. JUICINESS

Taste the coded samples and mark on the line below the level of juiciness

____

Dry Juicy

D. SALTINESS

Taste the coded samples and mark on the line below the level of saltiness

____

Not at all. Extremely salty.

E. ODOUR AND TASTE

Taste each coded samples and score for odour and taste (flavour) on the line below

____

Weak Strong

APPENDIX II

QUESTIONNAIRE

Date.........................

You are provided with seven samples of food to evaluate. The samples are coded as XXX, XXY, XXZ, XYX, XZX, ZXX and YXX.

Please evaluate the acceptability of the sample and give a brief comment. An honest expression of your personal feeling will help us decide.

OVERALL ACCEPTABILITY

Taste each sample and indicate how much you like or dislike it.

Descriptor/Sample	**XXX**	**XXY**	**XXZ**	**XYX**	**XZX**	**ZXX**	**YXX**
9.Like extremely							
8.Like very much							
7.Like moderately							
6.Like slightly							
5. Neither like nor dislike							
4.Dislike slightly							
3.Dislike moderately							
2.Dislike very much							
1.Dislike extremely							

COMMENT

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APPENDIX III

Appearance of beef after treatment with CS and WSA.

Key.

1st column: Control.

2nd column from top: beef treated with 1%, 2% and 3% common salt respectively.

3rd column from top: beef treated with 1%, 2% and 3% water soluble ash respectively.