

**CHEMICAL COMPOSITION OF SELECTED SEAWEEDS
AND THEIR UTILIZATION IN CHICKEN SAUSAGES.**

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**Chemical composition of selected seaweeds and their utilization in
chicken sausages.**

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DECLARATION

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

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DEDICATION

Dedicated to my wife, son and daughters and to each member of the larger family

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LIST OF ABBREVIATIONS / ACRONYMS

AACC	American Association of Cereal Chemistry
AFDO	Association of food and drugs officials
AMF	Alkali modified flour
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ARC	Alternatively refined carrageenan
CFU	Colony forming units
DW	Dried weight
DHA	Docosahexanoic acid
EA	Emulsifying activity
EEC	European union regulator
EPA	Eicosapentanoic acid
EPG	Esterified propoxylated glycerol esters
GC	Gas chromatography
HPLC	High performance liquid chromatography
KMFRI	Kenya Marine Fisheries Research Institute

MUFA	Mono unsaturated fatty acids
NA	Nutrient agar
PDA	Photodiode array
PUFA	Poly unsaturated fatty acids
RPM	Revolutions per minute
SFA	Saturated fatty acids
TACTA	Triakolytri- carboxylate
TSI	Triple sugar iron agar
WHC	Water holding capacity
WIO	West Indian Ocean
VBRA	Violet red bile agar
XLD	Xylose lysine desoxycholate agar

ABSTRACT

Seaweeds have the potential of being a source of nutrients and additives in the food industry. The aim of this work was to identify a seaweed from the five harvested species with the best potential of being utilized in formulation of chicken sausages. The chemical composition and functional properties of five seaweed species namely; *Hypnea musciformis*, *Euclima denticulatum*, *Laurencia intermedia*, *Sargassum oligocostum*, and *Ulva fasciata* from the Kenya coast were studied. The standard association of official analytical chemists (AOAC) methods were used for proximate analysis of seaweeds and chicken sausages while mineral composition was determined using atomic absorption spectrophotometry. The fatty acid profile was determined through gas chromatography (GC) and cholesterol by High Performance Liquid Chromatography (HPLC). Chicken sausages containing 0, 1, 3 and 4% seaweed powder with the best functional properties were formulated and analyzed. The physical properties (colour) and chemical properties (pH, water holding capacity, and cooking loss and emulsion capacity) of the formulated chicken sausage were determined. The shelf life and sensory properties were also determined. The analyzed seaweeds showed considerable amounts of saturated fatty acids with a range of 53.03 to 71.05% with *Euclima* recording the least and low amounts of polyunsaturated fatty acids (PUFA) with a range of 2.75 to 10.13%. Sodium and calcium were the dominant minerals in the seaweeds with a range of $(9.88 \pm 1.25$ to $21.88 \pm 0.71\text{mg}/100\text{g})$ and $(7.03 \pm 1.97$ to $56.38 \pm 4.55\text{mg}/100\text{g})$, respectively. The seaweeds had considerably high emulsion capacity with a range of 59.19 ± 0.97 to $75.69 \pm 3.62\%$ with *Euclima* and *Ulva* recording the highest while the water holding capacity of the seaweeds was within 8.42 ± 0.84 to $13.75 \pm 0.87\text{ml}/\text{g}$. Based on proximate analysis, fatty acid profile and emulsion activity of the five seaweed species, *Euclima* with the optimum emulsifying activity, ash and crude fat content was selected for sausage formulation. The chicken sausage with 0% seaweed powder (control) recorded the highest amount of cholesterol (10.73 %) while the 4% recorded the least (2.95 %) ($P < 0.05$). The pH of the chicken sausages (5.52 to 5.75) was within the normal

range of sausage products. Sodium was the dominant mineral followed by potassium while zinc was the least in the seaweed based sausage. In the chicken sausages with varying amounts of seaweeds, saturated fatty acids (SFA) were the highest (27.74 to 37.45%), while monounsaturated fatty acids were the least (22.06 to 23.77%). The lightness (L^*) of the chicken sausages with varying amounts of seaweeds was 68.50 to 73.37, redness (a^*) was 5.43 to 8.80, yellowness (b^*) was 14.63 to 15.53 and the firmness was 11.67 to 13.33 N. The chicken sausage with 0% seaweed recorded the highest emulsion stability while those with 3% seaweed the least. The chicken sausages showed significance difference in water holding capacity ($P= 0.007$) with 4% seaweed recording the lowest water holding capacity (55.10%). There was an increase in total plate count of *Staphylococcus aureus*, yeast and molds in the chicken sausages containing varying amounts of seaweeds with increase in storage time (0,7, 30 days). Based on taste, firmness, aftertaste and overall acceptability, the chicken sausage with 0% and 1% seaweed were more liked by consumers compared to 3% and 4% seaweed chicken sausage. The findings of this study indicate the potential of seaweeds in improving the chemical and functional characteristics of processed meat products with reduced cholesterol levels.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

The consumption of marine products has been gaining attention as people are becoming more aware of the relationship between diet and health. This has resulted in such products being sold as “functional foods” due to their enhanced health benefits and the potential to decrease the risk of diseases (Jimenez, 2000), and the need for reducing consumption of high energy foods to our sedentary lifestyles (Jimenez, 1996). As a result, demand for healthier foods such as low-fat meat products has increased in both developed and developing countries (Mohammadi & Oghabi, 2012). The changes in consumer demand and growing market competition have also prompted the need to improve the quality and image of processed meat products, not only to prevent loss of market share resulting from negative perception of processed meat products but also create new market niches, as in the case of products with healthier –beneficial properties (Fernández-Martín et al., 2009).

Among the marine organisms, seaweeds or marine macroalgae are one of the richest sources of bioactive compounds. Seaweeds are rich in dietary fiber, protein, minerals, vitamins, essential unsaturated fatty acids particularly long chain n-3 polyunsaturated fatty acids (PUFA), polyphenols, carotenoids and tocopherols with potential health benefits (Cofrades et al., 2011 a). Seaweeds polysaccharides are a potential source of soluble and insoluble fibres. These compounds exhibit higher water holding capacity than cellulosic (insoluble) fibers. The soluble dietary fibers demonstrate the ability to increase viscosity, form gels and /or act as emulsifiers. This has opened new possibilities for development of functional foods (Elleuch et al., 2011). Technologically, seaweeds have successfully been incorporated into meat products formulations including beef sausages, patties, meat balls and frankfurters to reduce the proportion of fat in the final product as well as improve on water retention, gelling, and mouth feel (Fernández-Ginés et al., 2005).

The addition of red or brown seaweed powder to meat products has shown a high potential to enhance reduction of saturated lipids levels, reduce postprandial absorption rates of glucose and lipids (Moreira et al., 2013). A number of researchers have shown the potential of seaweed in protection against new and emerging lifestyle diseases and complications due to reduced cholesterol and calorie intake (Choi et al., 2012; L'opez-L'opez et al., 2009). High levels of fat in food products have been associated with increased risk of obesity, coronary heart diseases, and certain types of cancer, e.g. breast, colon and prostate cancers (Lambert, 2001; Viuda-Martos et al., 2010). Immense sensitization in this area has led to consumers across all divides to develop a growing interest towards wholesome and natural foods. This trend has led to a rapid growth in the market for health and wellness products as well as intensified research relating to the association between diet and dietary constituents to health benefits (Cengiz & Gokoglu, 2005).

Sausage making is generally a highly accepted art of mixing ground meat with spices due to their delicious, nutritious and portable features (Feng et al., 2013). They contain up to 30% fat, which is important in the processing, textural and sensory characteristics of the products. Fat contributes key emulsifying properties in sausages, heat transfer, pigment carrier, moisture absorption, and improvement of aroma, flavor and acceptability of food (Mohammadi & Oghabi, 2012). Most importantly, fat contributes to superior textural and palatability properties, presentation of combined prediction of mouth feel, and taste (Cengiz & Gokoglu, 2005). In comminuted meat products, fat exerts considerable influence on the binding, rheological and structural properties of the products, which affects the formation of meat emulsion (Liu et al., 1991). According to Cofrades et al. (2011) seaweed application in the meat industry is attributable to their ability to improve gelling, stabilizing and thickening properties in reduced fat products. The authors documented improved texture and water-binding properties of low-salt; low-fat fresh restructured poultry steak. Consequently, it appears that fat replacements can help to cut down fat and calorie levels in food products. In other studies modified starches are being used as fat replacements in low-fat meat products such as meat emulsions, bologna, pork nuggets, ham, patties, and frankfurters (Feng et al., 2013; Mohammadi

& Oghabi, 2012). Chicken sausages are becoming a popular diet in middle and high class population in Kenya due to their distinctive sensory properties and the demand for healthier white meat (Hanrahan, 2010). These categories of consumers are increasingly becoming aware of what they consume hence the sustained calls on meat processors to become innovative and provide healthy and affordable products. This condition is prompting the emergence of new “healthier” meat products. Most physiologically active substances come from plants, and when combined with other foods such as meat, they provide a food with “functional” effects (Jimenez-Colmenero, 2007). To develop acceptable reduced fat products, considerations have to be made for evaluation of consumer perceptions, the most important quality aspects being that they taste good, appear wholesome, and have nutritional value. The product must also be safe, healthy, and tasty (Mohammadi & Oghabi, 2012)

There are several seaweeds in Kenya and some of them have high nutritive value (Muraguri et al., 2016). Seaweeds are known to have the potential to decrease the risk of diseases and to possess enhanced healthy benefits (Fleurence et al., 1994). This study aimed to evaluate the physico-chemical and sensory properties of a formulated seaweed-based chicken sausages produced using a suitable Kenyan seaweed.



Plate 1.1: Fresh seaweed, dry seaweed and seaweed powder

1.2 Statement of the problem

Currently available chicken sausages contain about 20% -30% animal fats which are mostly saturated fat with high levels of high density lipoproteins and no fibre (Mohammadi & Oghabi, 2012). Negative perceptions have emerged based on the link between consumption of those meat constituents (e.g. fat, saturated fatty acids – Free fatty acids, cholesterol) and the risk of developing some of the modern society's most common chronic diseases. Sausages high in saturated fat (approximately 20–30%) cause an increasing health concern associated with dyslipidemia, cardiovascular disease and cancer (Viuda-Martos et al., 2010). The increasing consumer demand for both vegetarian products and meat products such as sausages with reduced fat has led to a lot of research in food processing. Due to the increased consumers' desire to lose weight, demand for meat products with lower fat contents or healthier fatty acid compositions has increased, this has been driven due in part by the increased knowledge sharing recommending reduced saturated fat intake (Jimenez-Colmenero, 2007). Because of their importance, lipids are among the bioactive components (functional ingredients) that have received most attention, particularly (in quantitative and qualitative terms) with respect to the development of healthier meat products

In Kenya there has been little attention given to utilization of seaweeds despite its commercial cultivation which started in 2010 in south coast. Currently, there are seven villages practicing commercial seaweed farming for the Tanzanian market. Kenya has still not found ways of utilizing the seaweeds which is a potential gap that needs exploitation. Furthermore in Tanzania the seaweed is only utilized in processing of soaps and animal feeds. In addition there is potential of high losses due to limited market (Msuya et al., 2013). Seaweed in Kenya has a market potential of 40 million that needs to be exploited. Kibuyuni, Mkwiro and Nyumba sita are the major villages that have substantial production for commercial uses. Efforts to increase seaweed production upscaling is currently being spearheaded by the Kenya Coast Development Project in the Kenyan coast.

1.3 Justification of the study

Seaweed has been used as human food since ancient times, more commonly in Asian countries, and to a lesser extent in Europe and America. Like other marine organisms, seaweed is an important source of food and bioactive ingredients that can be applied to many aspects of food production. Seaweed utilization and value addition in West Indian Ocean (WIO) has really focused in making fertilizers, animal feeds, soaps, body creams, massage oils and foods such as juice, jam and pickles as well as consumption as salads (Msuya et al., 2013). Technologically, seaweed has successfully been incorporated into meat products formulations including beef sausages, breakfast sausages, patties, meat balls and frankfurters to reduce the proportion of fat in the final product as well as improve on water retention, gelling, and mouth feel. (Fernández-Ginés et al., 2005). Therefore the utilization of seaweed in food processing will help diversify the utilization of seaweed in Kenya and as a result open up market avenues for farmers. Seaweeds have low fat content ranging from 0.92%- 5.2% on dry matter basis. Their lipids are rich in Eicosapentanoic acid (EPA) and Docosahexanoic acid (DHA) which have shown to have beneficial effects like reducing the risk associated with cardiovascular disease and hypertension (Cofrades et al., 2011). The use of algae as an ingredient in meat products will open up new horizons for the meat industry affecting nutritional and technological benefits based on the use of an abundant natural resource currently little used in the western societies. Therefore this study will explore the effect of replacing upto 4% of chicken fat in the chicken sausage using selected seaweeds.

1.4 Objectives

1.4.1 General objective

To evaluate the chemical composition of selected seaweeds and the effects of seaweed powder incorporation on physical, chemical, shelf life and sensory characteristics of chicken sausage.

1.4.2 Specific objectives

1. To determine chemical composition of selected seaweeds along the Kenya coast.
2. To determine the chemical and, physical properties of a formulated seaweed-based chicken sausage.
3. To establish the shelf life and consumer acceptability of a formulated seaweed-based chicken sausages

1.5 Hypothesis

There is no difference between the physico-chemical and sensory properties of a seaweed containing chicken sausage and the conventional sausage (control).

CHAPTER TWO

LITERATURE REVIEW

2.1 Meat industry and consumer perception

Until recently, it was not evident to most consumers that most comminuted products made from meat and meat analogues such as sausages, frankfurters, and bologna contained up to 30% fat (Mohammadi & Oghabi, 2012). This is because fat is not visible to the naked eye; it is the dispersed phase (fat droplets) in a complex continuous phase composed of water, solubilized proteins, cellular components, and miscellaneous spices and seasonings (Gunter, (2007). Recent years have seen increased interest on the part of consumers, researchers, and the food industry into how food products can help maintain the health of an individual, these efforts include trials to reduce the dietary total fat intake to no more than 30% of total calories (Liu et al., 1991).

Besides fulfilling nutrition needs, diet modulates various functions in the body and may exhibit detrimental or beneficial roles in some diseases (Sarkar, 2007). There is increased consumer demand for high-quality food products, which has led to growth in the use of new technologies and ingredients. Factors that influence changes in consumer demand include health concerns such as obesity, cholesterol, cancer, changes in demographic characteristics such as ethnicity, aging population ; changes in distribution systems and price; and the need for convenience (Viuda-Martos et al., 2010).

Over the past decades, meat products have been under investigations due to reports of their associations between their consumption and the risk of different diseases (e.g. Ischemic heart disease, cancer, hypertension and obesity). Therefore, preparation of meat-based functional foods is being seen as an opportunity to improve the perception of meat, address consumer needs and update the nutritional and dietary goals (Jimenez-Colmenero, 2007) Generally, meat is low in dietary fiber; thus adding ingredients containing high fiber content such as seaweeds to processed meat

products would be beneficial (Roohinejad et al., 2016). Use of fat replacers or substitutes is a common method for fat reduction in meat products. Extracts rich in dietary fiber obtained from plants could be used as functional ingredients because they provide numerous health benefits that go far beyond supporting bowel regularity (Cengiz & Gokoglu, 2005; Mohammadi & Oghabi, 2012). The increasing demand for low-fat diets has led the food industry to undertake extensive research to develop low fat meat products (Choi et al., 2012; Cofrades et al., 2011). Meat processors are constantly trying to reformulate sausages from the existing products. Product reformulations with fat replacers have not been successful due to the resulting inferior qualities and storage instability which result in products with sensory limitations, cooking loss, emulsion instability, texture and color parameters effects (Fernández-Martín et al., 2009). However, making low-fat products similar to the full fat ones in terms of flavor, appearance and texture has continued to be a challenging task to manufacturers (Limberger et al., 2011). Sedef and Sebnem (2012) documented a number of successful application of fat-replacers in commercial products including; LITA (zein), simplese (egg protein, milk protein), trailblazer (whiter egg protein, serum protein with xanthan gum), simplese 100 (whey protein), n-flate (nonfat milk, gums, emulsifier, and modified starch), olestra (sucrose polyester), sorbestrin (hexa-fatty acid ester of sorbitol), esterified propoxylated glycerol esters(EPG), and (trialkoxxytri-carballylate (TACTA) (Sedef & Sebnem, 2012).

2.2 Utilization of seaweeds in meat products

Seaweeds are marine macroalgae that mainly forms the diet for the Asian countries such as China, Japan, Korea and Sri-lanka (Aliet al., 2000). They are classified as a high nutrition value food due to their valuable composition. They are rich in minerals such as Ca, K, P, and Na, and also approximately 54 trace elements that are required for human physiological processes (Dawczynskiet al., 2007). It has also been reported that seaweeds are rich in dietary fiber (non-starch polysaccharides) as well as proteins and vitamins (Roohinejad et al., 2016).

The use of seaweeds as partial replacers of animal fat has been reported (Cofrades et al., 2011; Fernández-Martín et al., 2009). Fernández-Martín et al. (2009) demonstrated the effectiveness of brown seaweed - sea spaghetti (*Himanthalia elongata*) as a substitute of pork fat at reinforcing water/oil retention capacity, hardness and elastic modulus in pork meat batter gelation. Agar from red seaweed (*Gelidium cartilagineum*) has been used successfully as a fat replacer in sausages due to its water binding properties (Sartal et al., 2011). Other documented applications of seaweeds in the meat industry include its incorporation in breakfast sausages (Barbut & Mittal, 1992), frankfurters, cooked beef rolls, burgers and meat balls (Hsu & Chung, 2001). Incorporation of whole dehydrated processed seaweed powder has also been reported. Kim et al., (2010) reported the use of *Laminaria japonica* (sea tangle) powder in sausages *Undaria pinnatifida* (Wakame) and *Porphyra umbilicalis* (Nori) *Himanthalia elongata* (sea spaghetti), in frankfurter with a lot of success achieved in developing acceptable products. *L. japonica* used in formulation of beef sausages at a level of 1-4% produced acceptable texture, cooking loss, emulsion stability, color and high acceptability (Kim et al., 2010)

Seaweed are used since they possess important chemical constituents such as polyunsaturated fatty acids (PUFAs), glycolipids, and polyphenols among others. Polyunsaturated fatty acids and glycolipids have been reported to exhibit health-improving activity (Biesalski et al., 2009). Polyphenolic substances express strong antioxidants and antimicrobial activity (Chojnacka et al., 2012). Seaweeds, such as the brown seaweed, *Undaria pinnatifida*, has been reported to contain fucoxanthin and fucoxanthinol (Mohamed et al., 2014). These compounds may reduce plasma and hepatic triglyceride concentrations and the activities of hepatic fatty acid and triglyceride synthesis, adipocytic fatty acid synthesis, and cholesterol regulating enzymes (Fleurence et al., 1994).

Seaweed polysaccharides have the ability to undergo gelation and thus possess a very important property required in the development sausage. This property enables production of rigid, firm, soft, sliceable and rubbery products(Barbucci, 2002; Nishinari & Zhang, 2004). Another important property of the seaweed polysaccharides that makes them an ideal additive in sausage making is their ability to form oil-in-water emulsions, (a fine dispersion oil droplets in water) (Menon, 2011).

Several studies have been conducted on the corresponding saturated fat substitutes by using a variety of oligosaccharides and polysaccharides. For instance 2–12% oligo-fructose was added into sausage, achieving a reduction of the fat content to 40%(Garcia et al., 2007).In addition, other documented seaweed polysaccharides as fat –replacers in sausage include β -glucan, maltodextrin, carboxyl methyl cellulose(Morin et al., 2004), amorphous cellulose gel, konjac gel, dietary fiber prepared from wheat, oat, peach, apple and orange and the mixture of orange fiber and soybean concentrate protein (Feng et al., 2013; Garcia et al., 2007). Various forms of seaweed preparation are practiced including blanched and salted seaweed prepared from wakame (*Undaria pinnatifida*) (Menon, 2011). Other forms of usage include addition as a component in development of salads and wrap. Seaweed has also found wide application as a constituent of composite foods such as addition into pasta (Wakame – *Undaria pinnatafida*- up to 10% w/w) and baking into bread (*Ascophyllum nodosum* up to 5% w/w) (Brownlee et al., 2012).

2.2.1 Physical properties of seaweed based meat products

The physical properties of sausages can be influenced by addition of seaweeds. In a study by Méndez et al. (2015) the addition of dietary fibre was seen to have an effect on water holding capacity and colour. The water holding capacity improved with increased dietary fibre and the colour was affected especially the lightness (L*) values due to reduced fat and dietary fibre incorporated. Kim et al. (2010) observed similar results where lightness values decreased with increasing sea tangle seaweed powder. Cofrades et al. (2008) who studied additions of diverse types of edible

seaweeds such as Sea spaghetti (*Himanthalia elongata*), Wakame (*Undaria pinnatifidia*) and nori (*Porphyra umbilicalis*) obtained similar results. The pH decreased with increased sea tangle seaweed and the texture increased in terms of hardness.

2.2.2 Chemical properties of seaweed based sausages

Chemical composition of seaweeds has been reported to be dependent on the source (temperate/tropic climate) and the time of harvesting (Chojnacka et al., 2012). For example, the protein content of *Palmaria palmata* (.dulse) varies from between 9-25% depending on the season of collection and harvesting. The highest percentage protein per gram of dried whole seaweed is normally found in *Palmaria palmata* collected during the winter season (October - January). Valuable amino acids such as leucine, valine and methionine are well represented in *Palmaria palmata* (El Gamal, 2010).

The use of seaweeds for their interesting functional properties has been reported (Menon, 2011). Polysaccharides from seaweeds are able to bind large amounts of water, because of the presence of various functional groups in their molecules (Barbucci, 2002), therefore are ideal for application in sausage to improve on their functionality. For instance a group of seaweed products known as alginates are referred to as 'hydrocolloids' due to their ability to bind water several times their weight, this occurs due to the presence of carboxylic groups; carrageenans, sulfonic groups and for chitosan, amino groups (Roohinejad et al., 2016). With the aid of these properties, these compounds are able to modify the texture, control syneresis (a phenomenon of separation of water during freeze–thawing of foods) and stabilize the food matrix. The water retention and gel formation property of seaweed has found its application in low fat meat products, which has been made possible due-in-part by extracted algal components such as alginates and carrageenan (polysaccharides from red seaweed) and furcellaran from *Furcellaria fastigiata*) (Sartal et al., 2011).

These products (seaweed extracts) have been tested in breakfast sausages, frankfurters, cooked beef rolls and meat balls. In addition to their technological characteristics, these compounds also provide numerous potential physiological benefits, hence considered as functional ingredients (Bocanegra et al., 2009; Cofrades et al., 2011)

The effects of sea tangle (*Laminaria japonica*) powder on quality characteristics of breakfast sausages have been analyzed by Kim et al. (2010). In this study by Kim the moisture content of all the samples ranged from 59.51-60.13%, other results included protein (7.8%), fat (1.2%) and ash (31.3%).

2.2.3 Sensory evaluation of seaweed based sausages

The effect of adding sea spaghetti (10-40 % w/w) as a source of dietary fibre on sensory traits of cooked beef patties was investigated (Cox & Abu-Ghannam, 2013). The sensory analysis showed that aroma, appearance, texture and taste of the seaweed patties were acceptable by consumers. Patties prepared with 40% seaweed had the highest overall acceptability due to texture and mouthfeel improvement. Therefore it was concluded that adding seaweed in the formulation of beef patties could enhance nutritional and technological quality along with an acceptable sensory quality. The breakfast sausages enriched with *Lamina japonica* revealed a direct relationship to the percentage of sea tangle powder added in terms of colour scores, flavor, juiciness, tenderness and overall acceptability (Kim et al., 2010).

2.2.4 Antimicrobial properties of seaweeds

Seaweed and seaweed extracts have evoked interest as sources of natural products with antimicrobial activities. For instance, the antibacterial properties of 1,8-dihydroxy-anthraquinone, isolated from the red algae (*Porphyra haitanesis*), against *Staphylococcus aureus* has been reported (Wei et al., 2015).

The results showed that the isolated molecule strongly inhibited the cell growth in the logarithmic phase, and this antimicrobial activity is related to its interaction with the cell wall and cell membrane. In fact, an increase of the permeability of the cell envelope was reported, which leads to the leakage of cytoplasm and to cell deconstruction.

The effect of adding sea spaghetti (10-40%) as a source of antioxidants and dietary fibers on microbial traits of cooked beef patties was investigated throughout chilled storage (Cox & Abu- Ghannam, 2013). Microbiological counts and lipid oxidation were found to be lower in patties containing seaweed. Moreover, after 30 days storage, no bacteria growth was observed in the samples treated with more than 20% seaweed.

2.3 Seaweed resources utilization in Kenya and in other countries

The seaweeds of Kenya are fairly well-studied floristically, relative to other Indian Ocean countries (Bolton et al., 2003). According to Bolton et al. (2007) there are 386 species of seaweeds in Kenya ,214 red algae, 116 green algae and 56 brown algae. Kenya's first ever seaweed farm was established in Kibuyuni village in Shimoni, Kwale County. The farm has gained technical support from Kenya Marine Fisheries Research Institute (KMFRI). Additional sites include Mkwiro, Funzi and Gazi near Ukunda trading center. The farmers usually wait for the Tanzanians to buy their produce. <https://www.standardmedia.co.ke/business/article>.

Among the seaweeds collected for this study it included *Eucheuma denticulatum* which is a rhodophyta. These are spiny bushy plants of about 50 cm high, which grow on reefs and in shallow lagoons around the Philippines and Indonesia and other island coasts (Bolton et al., 2007). *E. denticulatum* contains iota carrageenan Carrageenans represent between 30% and 80% of the cell wall constituents of algae. These concentrations are influenced by season, species, and growth conditions of algae (Matanjun et al.,2009). *Ulva fasciata* has Individual blades that can grow to be more than 400 mm (16 in) in size, but this only occurs when the plants are growing in sheltered areas. It's a macroscopic alga light to dark

green in colour and it is attached by disc holdfast. It's used as a salad (Bolton et al., 2007). *Hypnea musciformis* is a red algal, and a well-known carrageenophyte plant producing polysaccharide carrageenan. *Laurencia intermedia* is a genus of red algae that mostly occurs in the sea near islands (MacArtain et al., 2007). *Sargassum oligocystum* is a genus of brown seaweed. Numerous species are distributed throughout the temperate and tropical oceans of the world, where they generally inhabit shallow water and coral reefs, and the genus is widely known for its planktonic (free-floating) species (El Gamal, 2010).

In Western Indian Ocean seaweed utilization and value addition has really focused in making fertilizers, animal feeds, soaps, body creams, massage oils and foods such as juice, jam and pickles as well as consumption as salads. Some of the species available for consumption by coastal people particularly East Asian countries include; *Ulva* spp., *Cladophora* spp., *Chaetomorpha* spp., which are also consumed by the Japanese (Brownlee et al., 2012). Common species cultivated under mariculture in Japan include nori (*Porphyra* spp), kombu (*Laminaria* spp.) and wakame (*Undaria* spp.) (Bolton et al., 2007). Some of the products derived from seaweeds include processed *Eucheuma* seaweed (PES) also known as Philippines Natural Grade (PNG), semi refined carrageenan (SRC), alternatively refined carrageenan (ARC) or alkali modified flour (AMF) prepared from *E. cottonii* and *E. spinosum* (Chang-Suk & Kim, 2012).

2.4 Physical properties of sausages

The pH of chicken meat declines with time due to conversion of glucono-delta lactone into gluconic acid. The pH of meat is influenced by other factors like water activity, salt, temperature, oxide- reduction potential and preservatives to inhibit growth of pathogenic microorganisms. (Fellows, 2000).

The water holding capacity is the ability of meat to retain its inherent moisture even though external pressures (like gravity, heating, centrifugation, pressing) are applied to it. This characteristic can be measured by drip loss. Much of the water in the meat muscle is entrapped in the structures of the cell, including the intra and extra

myofibrillar spaces (AFDO, 1990).The characteristic is an important property of fresh meat since it affects the yield and quality of the end product. The mechanism by which drip is lost from the meat is influenced by pH and amount of spaces in the muscle cell and particularly the myofibril that exists for water to reside. Cooking yield determines how well juices are retained in the cooked product. The entire system of live animal production and handling through initial chilling and finally storage and handling of the meat all play significant roles in influencing the amount of moisture that is lost from the product. (Kristensen, 2001).

The muscle color of chicken meat, at point of purchase is an indicator of freshness and anticipated palatability for consumers. Myoglobin is the principle protein responsible for meat color, although other heme proteins such as haemoglobin and cytochrome may also play a role in beef, pork and poultry color. Colour is influenced by age of the animal, species, sex, diet and even the exercise it gets. The colour of raw poultry meat has less fat under the skin, which can cause the bluish coat and the yellow skin could be as a result of marigolds in the feed. Colour is important when poultry meat are purchased, stored and cooked. Often an attractive, bright colour is a consideration for the purchase of chicken sausages (Kelly, 2012).

Tenderness is considered by many consumers as the most important quantitative characteristic of meat and meat products. Protein oxidation can affect the quality of meat and meat products especially loss of enzymatic activity, solubility and formation of protein complexes that are linked to meat tenderness (Kelly, 2012). Hardness and firmness or tenderness is the most significant feature to satisfy preferences of consumer's eating satisfaction. Tenderness or hardness is the force required to attain a given deformation or a penetration in a product (Morin, 2004).

2.5 Ingredients used in sausage formulation

The finished sausage is only as good as the ingredients it contains. The product should have the proper lean to fat ratio and have good binding properties. The spices should be selected well to complement each other so as to create a satisfying product (Toldrá, 2011).

The meat used in processing of meat products must be clean, sound and wholesome. Lean meat from chicken is meat with no visible fat and skin (AFDO, 1990). Lean chicken is skinless meat with no skin and fat. The ratio of lean to fat has a decided bearing on the quality of the product, particularly in controlling the shrinkage that occurs during cooking. Leaner formulae shows less shrinkage than formulae with more fat.

Fat gives sausages its texture and flavor. The percentage of fat in sausages ranges between 25 and 30 % on average. Fat is an essential component of meat products as it provides several features related to their sensory and technological quality, contributing to the appearance, flavor, texture, mouthfeel, juiciness and overall sensation of lubricity of meat (Jimenez, 1996).

Non meat ingredients are a wide range of substitutes of non-meat origin that are used as ingredients in processed meat products. Some of them are absolutely necessary, such as salt and spices. Most of these ingredients are functional which describes their ability to introduce or improve certain quality characteristics e.g. texture, appearance, water binding, counteracting fat separation and preservation. Others are used to add volume of the meat products e.g. extenders and fillers (Deda et al., 2007).

Extenders such as dry milk powder, cereal flours, and soy protein are used to lower production cost, improve binding qualities and slicing characteristics. The binders and extenders are added up to 3-5% in sausages (Kelly, 2012).

Water is a naturally occurring component of meat. It is added in the formulation in specific amounts (up to 3% of the total formulation according to FSIS regulators). Water helps in improving the consistency of the mixture (AFDO ,1990). In commercial production, sausages meat is chopped in a bowl chopper which creates a lot of friction. In order to minimize bacterial growth crushed ice water is added during sausage production. In cooking or during storage some water is lost and to make up for this factor water is added during sausage production.

Salt is basic to all meat curing mixtures and is the primary ingredient necessary for curing. It acts by dehydration and alters osmotic pressure, inhibiting bacterial growth and subsequent spoilage. Processed products like sausages comprise one of the major sources of sodium in the diet in the form of sodium chloride (Toldrá, 2011). Salt is added between 1.5-3% in the sausage formulation (Deda et al., 2007).

Color is an important attribute of processed meat products because it is one of the main factors that influence consumer's choice and preferences. The colour must be right and consistent (Boles et al., 1998)

Spices are aromatic vegetable substances that are intended to function as contributing flavorings in food instead of contributing to the nutritional substance of food and seasoning are also substances that impart flavor. Spices, seasonings and flavorings are used to add flavor to the sausage and also affect the consistency of the ground mixture (Kelly, 2012)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection and preparation of seaweed powder

Five seaweed species *Hypnea musciformis*, *Eucheuma denticulatum*, *Laurencia intermedia*, *Sargassum oligocystum*, and *Ulva fasciata* were randomly collected at the intertidal zone near Mombasa along the Kenya coast (Latitude 4° 3'0''S and Longitude 39°40'0''E). The five seaweeds were selected because of the availability throughout the year and are in abundance in the study area. The seaweed samples were handpicked and immediately washed using seawater to remove any foreign particles. The clean samples were kept in buckets and transported to Kenya Marine and Fisheries Research Institute (KMFRI) laboratories in Mombasa for identification and drying. Thereafter, a second washing was carried out on the samples using deionized water to remove surface adhering salts. The seaweeds were then sun dried, ground into a powder using a blender (MBLR-4731, Mika Dubai, United Arab Emirates) for one minute then passed through a 20 mesh sieve. The powder was vacuum packed and refrigerated at -20⁰C until further analysis. The collected seaweed voucher specimens are preserved at the Department of Botany in Jomo Kenyatta University of Agriculture and Technology (JKUAT).

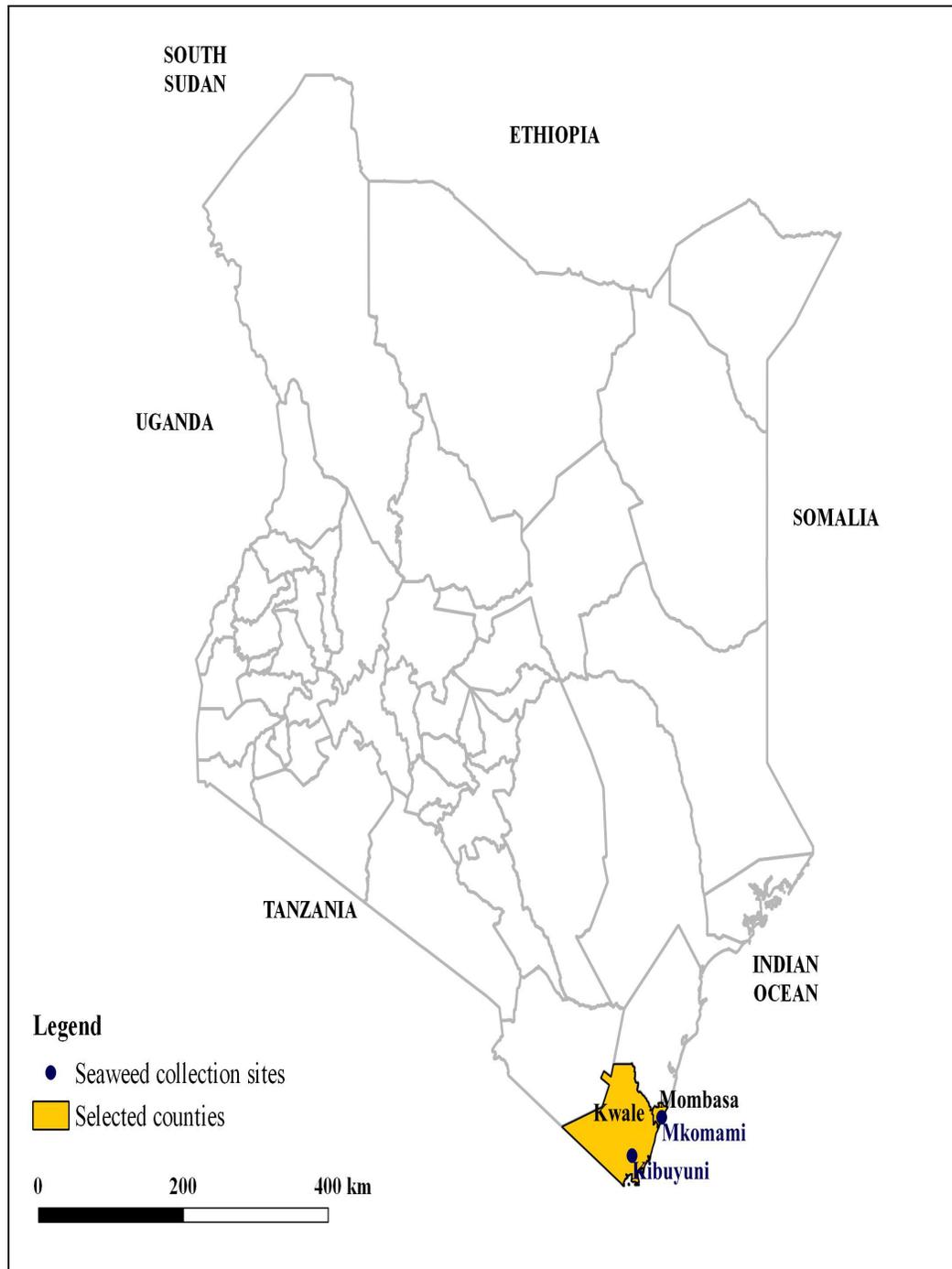


Figure 3.1: Map of Kenya showing the location of Mombasa town in relation to Indian Ocean (seaweeds sites)

3.2 Chemical analysis of seaweed samples

3.2.1 Determination of proximate composition

The moisture, crude ash, crude fat, crude protein and crude fiber of the seaweed samples were determined by using AOAC methods (AOAC, 2005). The moisture content was determined by oven drying method at 105°C where two grams of the sample were used. The ash content was analyzed by incineration where five grams of the sample were ashed in an electric muffle furnace (Shimadzu KL- 420, Japan) at 550 °C for 16 h to a constant weight. The crude fat content was extracted from five grams of algal sample using the Soxhlet apparatus with petroleum ether as the solvent and determined gravimetrically after oven-drying at 70°C for 1 hour. The crude protein content of seaweeds was determined by Kjeldahl method and a conversion factor of 6.25 was used to calculate the crude protein content from the nitrogen content. The crude fibre was determined by sequential digestion of seaweed samples with 1.25% H₂SO₄ and 1.25% NaOH using the fibre glass as a container. For drying and ashing, the crucible with the sample was dried in an oven for five hours at 105°C and ashed in the muffle furnace at 550°C for 16 hours. The weight of crucible with sample after drying and ashing was recorded and the crude fibre content was calculated. The carbohydrate was calculated based on weight difference using crude protein, crude fat, crude fibre, and crude ash and calculation was done as follows : Carbohydrates (%) = 100 - (crude fibre + crude protein + crude ash + crude fat) .

3.2.2 Determination of fatty acids profile

The fatty acid profile of the seaweed samples was determined by gas chromatography (GC) after fat extraction using a modified method by Bligh and Dyer (1959). One gram of the algal sample was put in a 50 mL glass stoppered centrifuge tube and methanol and chloroform were then added in a ratio of 2:1. The contents were centrifuged at 30,000 rpm for 10 minutes. The supernatant was transferred to a conical flask and 15mL of chloroform was added to the remnant. The contents were centrifuged at 30,000 rpm for 10 minutes and the first and second

supernatants were combined and then passed through a defatted cotton wool. The contents were then put in a rotary flask and evaporated to dryness. Five millilitres of 95% methanol and 5% hydrochloric acid were added followed by refluxing at 100°C for 1 hour. The contents were then cooled in running water before transferring to a separating funnel where 10 mL of hexane was added followed by shaking vigorously. The contents were then left to settle where the upper layer of hexane was collected in a conical flask and the lower layer was further re extracted using hexane and the two hexane layers were mixed. The hexane layers were put in a separating flask and washed with water. The contents were then passed through a plugged funnel with cotton wool and anhydrous sodium sulphate. The contents were evaporated in a rotary evaporator to 0.5-1 microlitre and put in vials. 1 µl of the sample was drawn and injected into a gas chromatography (Shimadzu GC-9A, No.41991A, Tokyo, Japan). Known concentrations of fatty acids standards were fed into the GC and identification and quantification done by comparing the retention time and the reference spectrum.

3.2.3 Determination of minerals composition

The mineral content of seaweeds was determined by dry ash method (AOAC, 2005). Two grams of algal samples were ashed at 550°C for 16 hours then diluted using 1N HCl and filtered using Whatman filter paper No. 4. The absorbance of the standards followed by the samples was determined using an atomic absorbance spectrophotometer (Shimadzu, AA-6200, Tokyo, Japan).

3.2.4 Determination of emulsifying activity and water holding capacity

The emulsifying activity of seaweed samples was analyzed using the method of Naczka et al. (1986) with some modifications. Two grams of seaweed powder were dissolved in 20 mL of distilled water then the suspension was vortexed for 10 min using vortex mixer (model TM- 151 Tokyo, Japan). At the fifth minute, 20 mL of corn oil was added continuously with stirring. The emulsion was then centrifuged at 2100 rpm for 10 min at 25° C (Beckman CS-6 centrifuge). The volume of the

emulsified layer was then recorded and the emulsifying activity (EA) was calculated according as follows:

$$\text{EA (\%)} = (\text{volume of emulsified layer/ volume of the suspension}) * 100.$$

The water holding capacity (WHC) was determined according to the methods of the American Association of Cereal Chemistry (AACC 1983) as the maximum amount of water retained by one gram of sample under low-speed centrifugation (2060 rpm for 10 min; Beckman CS-6 centrifuge) at 25°C. Sixty (60) mL of distilled water was added to one gram of dry algal sample powder in a 100 mL centrifuge tube. The sample was stirred and left at room temperature for 1 hr. After centrifugation, the supernatant was discarded and the residue was weighed and water holding capacity expressed as gram of water/gram of dry sample.

3.3 Formulation and processing of chicken sausages

3.3.1 Selection criteria of seaweed for sausage formulation

The selection of the seaweed was based on proximate analysis, fatty acid profile, water holding activity and emulsion activity .The seaweed with the best profile was selected for the sausage formulation.

3.3.2 Formulation of chicken sausages

Fresh chicken meat for formulation of sausages was sourced from Kenchic Ltd (Nairobi, Kenya) accompanied by certificate of meat inspection. The meat was selected from broiler chicken breast boneless and thigh boneless that had no skin and visible fat. The meat products were stored at 4 °C. Food grade spices (nitrites, polyphosphates (E452), sodium ascorbate (E300), sausage condiment and preservatives (E221)) and fillers such as rusk and corn starch were selected carefully to avoid contamination. Portable water was used to make ice for the processing as per Kenya Bureau of Standards for portable water (KEBS, 2014).

The chicken sausage was formulated according to Deda et al. (2007) and Kim et al. (2010) with some modifications by reducing fat and incorporating seaweed. The

formulations are given in Table 1. The quantity of chicken fat was controlled as per the seaweed powder levels. Three batches (each 4.155 kgs) were prepared for each treatment. Each batch of chicken sausage meat, fat, ice water and other ingredients were mixed in a reverse action mixer (Mainca model RM-90, Saint Louis, USA,).

Table 3.1: Formulation of the chicken sausages

Ingredients/ seaweed level	Formulation (grams per batch)			
	0% (control)	1%	3%	4%
Chicken meat	1250	1250	1250	1250
Chicken fat	1000	958	874	832
Ice water	1000	1000	1000	1000
Rusk	325	325	325	325
Corn starch	325	325	325	325
Food colour-F139	5	5	5	5
Spice (E 221,E451and E300)	250	250	250	250
Seaweed powder	0	42	126	168
Total	4155	4155	4155	4155

The temperature of the sausage mix was recorded after mixing the emulsion. The chicken sausages were vacuum stuffed (Handtmann stuffer Model # VF 80, Biberach, Germany) into oxygen impermeable casings (Devro, Johannesburg South Africa). The chicken sausage samples for each treatment were identified by securing a tag to the packaging. The sausages were then packed in oxygen impermeable polythene bags under vacuum and then stored at 4⁰C for experimental analysis.

3.4 Chemical analysis of chicken sausages

3.4.1 Proximate analysis

The proximate composition (moisture, crude ash, crude fat, crude protein and crude fiber) of chicken sausages were determined as outlined in section 3.2.1.

3.4.2 Determination of pH

The pH value was determined using a pH meter (Model HI 8519N, Hanna, Amorim, Portugal) as described by Tan et al. (2007). Raw fresh chicken sausage samples were randomly collected just after stuffing and five (5) gram samples were weighed into a 50 mL conical tube with 20 mL of deionized water. Samples were homogenized for 30 seconds with a Labortechnik homogenizer (Model KS250, Labortechnik Staufen Instruments Inc., Funkentstort, Germany) and pH was determined using an automatic temperature compensation, epoxy-body probe attached to a bench meter (Model HI 8519N, Hanna Instruments, Portugal) calibrated using three buffers (pH 4,7 and 10). The pH values of the sausages were measured in triplicate.

3.4.3 Determination of total cholesterol

Direct saponification method was used for cholesterol determination in chicken sausages as described by Stajić et al. (2011). To 100 mg of each homogenized sausage sample was added 2 mL of 0.5 M KOH in methanol in a tube and the mixture was vortexed for 30 seconds. The mixture was directly saponified at 80°C for an hour and after cooling, 2 mL of distilled water, saturated with sodium chloride, were added. The tubes were vortexed for 30 s, followed by the addition of three mL diethyl ether/hexane (1:1, v/v) and then centrifuged for 10 min at 300 rpm. The upper phase was transferred to a clean tube and the diethyl ether/hexane extraction step was repeated twice. All the three extracts were combined and evaporated to dryness under a stream of nitrogen. The dry extracts were dissolved in 1000 µl of mobile phase used for high performance liquid chromatography (HPLC) analysis and after filtration of the dissolved contents, 10 µl was immediately injected into HPLC. Cholesterol determination was performed using HPLC/PDA (photodiode array) system (Waters 2695 Separation module/Waters photodiode array detector, USA), on an ODS (octadecyl silica) C18(2) reverse/phase column, 150 mm x 3.0 mm, 5-µm particle size, with C18 analytical guard column, 4.0 x 2.0 mm, at room temperature. Quantification of cholesterol was done by external standardization in a linear concentration range from 25 mg/100g to 125 mg/100g.

3.4.4 Determination of fatty acid profile

The fatty acid profile of the chicken sausage samples was determined using gas chromatography (GC) after fat extraction using a modified method of Bligh and Dyer (1959) as outlined in section 3.2.2.

3.4.5 Determination of mineral composition

The mineral content of the formulated chicken sausages was determined by the dry ash method (AOAC, 2005), as outlined in section 3.2.3.

3.5 Physical analysis of chicken sausages

3.5.1 Instrumental colour analysis

The color of meat batter for fresh chicken sausages was determined according to the procedure of Lin and Huang (2003). A cross-section of each piece of sausage was determined for lightness (L^*), redness (a^*) and yellowness (b^*) using a colorimeter (Minolta spectrophotometer CM 3500d, Minolta, Japan). The instrument was calibrated on the International Commission on Illumination (CIE LAB) color space system using a white calibrated plate (calibration plate CR- A43, Minolta Cameras). The hue angle was calculated by $\tan^{-1} (b^*/a^*)$. Colour analysis was performed in triplicate for each sample.

3.5.2 Determination of sausage firmness

Firmness in sausages was determined using a rheometer universal testing instrument, model CR-100D (Scientific Ltd, Tokyo Japan) equipped with a conical penetrometer of 7 mm upper width and 10 mm height (Andersson & Hansson, 1979). Measurements were taken perpendicular to the cut surface of samples which were 3 cm long with casing on. The penetration speed was 2.5 cm/min. Firmness was calculated as the average maximum force needed to penetrate the cross-section of the sausages.

3.5.3 Water holding capacity (WHC) determination

The WHC of chicken sausages was determined using the compression method as described by Tsai and Ockerman (1981) and Dzudie et al. (2005) with some modifications. Approximately 0.3 grams of sausage meat batter was placed between two what man filter papers No 1 and then placed between two plexi glass plates, and a force of 4.0 kg applied for 20 min. Due to the force exerted on the sample, the released liquids were impregnated in the paper, and they were considered as meat – free water. The WHC was calculated as follows:

$$\% \text{ of free water} = (I_w - F_w) \times 100$$

$$\text{WHC} = 100 - \% \text{ of free water}$$

Where I_w is the initial weight of the sample (0.3g) and F_w is the final weight.

3.5.4 Determination of emulsion stability

The meat batters were analyzed for emulsion stability using the method of Jiménez-Colmenero, et.al, 2010) with some modifications. Fifty grams of meat batter was placed in tubes, which were then centrifuged (5 min, 2632g, at 2°C) and heated (40°C/15 min followed by 20 min /70°C).The tubes were left to stand upside down (for 40 min to cool to 4°C) to release the exudate. The fat released was the weight on heating the exudate for 16 h at 103 °C to separate water and fat. Fat released was expressed as a percent of the sample

3.5.5 Determination of cooking loss

The cooking loss of the sausages was determined as described by Choi et al. (2012). The weighed chicken sausages were cooked on a preheated electric grill (CG20, Hobart, OH, USA) at a grill surface temperature of 150°C for 3 min on one side and for 3 min on the opposite side until they attained targeted core temperature of 75°C. After cooling for 30 min, cooked sausages were weighed and a percentage of cooking loss calculated from the weights as follows:

Cooking loss (%) = (Weight of sausage before cooking – Weight of sausage after cooking) ×100/ Weight of sausage before cooking

3.6 Determination of consumer sensory evaluations

An effective sensory test of attributes was conducted by measuring the level of satisfaction of 25 trained consumers using a questionnaire (see Appendix 1). The consumers evaluated color, taste, aroma, hardness, overall acceptability and preference to buy, using a nine point hedonic scale: 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike, 5=neither like nor dislike, 6=like, 7=like moderately, 8=like very much and 9 = like extremely as described by Lawless and Heyman (2010).The sausages were cooked for five minutes until the internal temperature reached 75°C. The cooked chicken sausages were then cooled to 45°C and cut into four pieces and placed on plastic plates coded as S01, S02, S03 and S04 for the levels (0%, 1%, 3%, and 4%) seaweed powder levels, respectively. Distilled water was provided for rinsing the palate. The trained consumers were instructed to indicate their degree of liking or disliking of the sausage samples.

3.7 Determination of shelf life

Determination of shelf life was carried out according to the classical method of Association of Official Analytical Chemists, AOAC Official Method 966.23 (AOAC, 2011). Raw fresh sausage samples were evaluated for *Staphylococcus aureus*, yeast and moulds, coliforms, total plate count, *Salmonella* spp, and *Shigella* spp, during the trials. Three samples were randomly obtained from each sausage formulation stored at 4°C and at - 20°C for shelf life determination at day zero, and subsequent counts at day seven and thirty in order to monitor the microbial dynamic changes during storage of the fresh sausages, frozen sausages and their hygienic quality. In particular, 25 g of each sample were transferred into a sterile stomacher bag and 225 mL of saline/peptone water (8 g/l NaCl, 1 g/L bacteriological peptone, Oxoid) were added and mixed for 1.5 min in a Stomacher machine (PBI, Milan, Italy). Further decimal dilutions were made and the following analyses were carried out in triplicate agar plates:

(a) *Staphylococcus aureus* on Baird-Parker medium (Oxoid) with added egg yolk tellurite emulsion (Oxoid) incubated at 37°C or 24– 48 hr.

(b) Yeasts and moulds on Potato on dextrose agar: (CMO 139 Oxoid Hampshire, England) incubated at 25°C for 48 – 72 hr.

(c) *Escherichia coli* on violet red bile agar (VRBA) plates incubated at 37°C for 24 hours. The plates were examined for colonies appearing on the medium, which were then counted while on coliforms lactose broth with Durham tubes k2 were used for gas detection. Incubation was done at 37°C for 48 h.

(d) Total plate count on Nutrient agar (CM3: Oxoid Hampshire UK).

(e) *Salmonella* and *Shigella* isolation

Salmonella and *Shigella* species were determined through the following procedures:

i. Pre-enrichment

Minced meat from fresh chicken sausages (25 g) were homogenized in 225mL of lactose broth (CM137: Oxoid, Basingstoke, UK) and incubated at 37°C for 24 hr to allow recovery of injured cells.

ii. Selective enrichment: After pre-enrichment for 24 hr, 1mL of culture from lactose broth (CM137: Oxoid, Basingstoke) was transferred to 10 mL selenite broth (CM699: Oxoid, Basingstoke, UK). This was further incubated at 37°C for 24 hr. Loopfuls of growth from the enrichment broth were then streaked onto selective/differential media: Salmonella Shigella agar (SS agar) (CM 533: Oxoid, Basingstoke, UK), xylose lysine desoxycholate agar (XLD) (CM469: Oxoid, Basingstoke, UK). Presumptive Salmonella colonies (black centred colonies on XLD and SS agar, were purified by streaking on nutrient agar plates (NA) (CM003: Oxoid, Basingstoke, UK).

iii. Identification: Presumptive Salmonella colonies were identified through biochemical tests. The biochemical tests performed were on triple sugar iron

agar (TSI) for identification. Acid production on the slants and butts and gas for H₂S production were observed in triple sugar iron (TSI) agar slopes (CM277: Oxoid, UK).

3.8 Statistical analysis

All data were expressed as mean \pm standard error. Data concerning physical, chemical, and microbial properties for seaweed species and or chicken sausages were subjected to Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) using statistical package for social scientists (SPSS®) Version 20.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Chemical compositions of seaweeds

4.1.1 Proximate compositions

The proximate compositions of the seaweeds species are shown in Table 4.1. The results indicated significant difference ($P < 0.0001$) among the seaweed species in carbohydrates with *Hypnea musciformis* and *Sargassum oligocystum* recording the highest levels of carbohydrates (Table 4.1), respectively. The carbohydrates values for *Hypnea musciformis* were considerably higher than those reported from the Martin's island in Bangladesh by Siddique (2013) and Mwalugha et al. (2015) in Kenya, who obtained 20.60% and 43.76%, respectively. In addition, the carbohydrates values for *Sargassum oligocystum* was higher than 53.21% reported by Azad and Xiang (2012) from the seaweeds collected from Banggi island in East Malaysia and 30.30% reported by Kumar et al.(2011) from Saurashtra coast of India. These variation could be attributed to differences in species, habitat and seasonality (Marinho-Soriano et al., 2006). The least proximate component was crude fat (0.87%) as shown in Table 2, where *Eucheuma denticulatum* had the highest crude fat among the five seaweed species, which was within the range of 1-6%(Fleurence et al., 1994) and close to 1.5% reported by Kumar et al. (2011) in *Kappaphycus alvarezii* (*Eucheuma cottonii*) from the Indian coast. In addition, the value was close to 1.10% reported by Matanjun et al. (2009) for *Eucheuma cottonii* from Malaysia.

Eucheuma denticulatum had the highest ash content (27.13%) and crude fat content of 1.78% while *Sargassum oligocystum* had the highest crude fibre (9.40%) and carbohydrates (71.42%) ($P < 0.05$). The highest values of crude protein (10.06%) were obtained from *Ulva fasciata*.

There was a significant difference in crude ash ($p < 0.001$) among the studied species with *Eucheuma denticulatum* and *Laurencia intermedia* recording the highest ash content. These ash values differed with 22.82 % and 24.17 % obtained by Mwalugha

et al. (2015) respectively. Similarly, the ash content of *Hypnea musciformis* differed with the ash value of 21.57% reported by Siddique (2013) and 20.77% reported by Mwalugha et al. (2015). In this study, the ash content of *Sargassum oligocystum* was considerably lower than the ash value of 24.88% reported by Azad and Xiang (2012) and 42.40% in *Sargassum polycystum* obtained by Matanjun et al. (2009). The variation in ash content could be related to habitat, or temperature and pH which could have an influence on mineralization as reported by Mendis & Kim, (2011) and Polat & Ozogul, (2009).

Table 4.1: Proximate composition (dry weight basis) of seaweed species from the Kenya coast

Seaweed species	Ash (%)	Crude fiber (%)	Crude fat (%)	Crude protein (%)	Carbohydrates (%)
<i>Eucheuma denticulatum</i>	27.13 ± 1.46 ^c	5.22 ± 1.67 ^a	1.78 ± 0.67 ^b	5.06 ± 0.36 ^a	60.81 ± 1.95 ^{ab}
<i>Hypnea musciformis</i>	12.79 ± 2.58 ^a	6.49 ± 1.21 ^{ab}	0.77 ± 0.17 ^a	6.88 ± 1.22 ^b	73.07 ± 0.22 ^c
<i>Laurencia intermedia</i>	25.62 ± 1.36 ^c	7.50 ± 0.79 ^b	0.47 ± 0.17 ^a	8.48 ± 0.41 ^c	57.93 ± 0.20 ^a
<i>Sargassum oligocystum</i>	13.08 ± 2.74 ^a	9.40 ± 1.39 ^c	0.46 ± 0.07 ^a	5.64 ± 0.19 ^{ab}	71.42 ± 2.13 ^c
<i>Ulva fasciata</i>	19.92 ± 3.42 ^b	7.10 ± 0.32 ^b	0.86 ± 0.11 ^a	10.06 ± 0.90 ^d	62.06 ± 3.10 ^b
P value	<0.0001	0.003	0.003	<0.0001	<0.0001

All values are means ± SE, n=3.

Values in the same column bearing different superscript letters are significantly different

The crude fiber accounts for the indigestible components of the seaweed (Dawczynski et al., 2007). A significant difference was noted in crude fibre (p<

0.003) with *Sargassum oligocystum* recording the highest amount which varied slightly from the 7.74% obtained by Marinho-Soriano et al. (2006) and 7.58% reported by Azad and Xiang (2012). *Eucheuma denticulatum* and *Hypnea musciformis* recorded the lowest amount of crude fiber. The crude fiber content of *Eucheuma denticulatum* was similar to that found in *Eucheuma cottonii* from north Borneo Malaysia obtained by Matanjun et al. (2009). The variation in crude fiber content could be attributed to differences in photosynthetic activity, growth stage and seasonality which is as a result of changes in the environment that influences photosynthesis and nutrient absorption (Siddique, 2013; Wong & Cheung, 2000).

The crude protein of *Ulva fasciata* 10.06% obtained in this study was slightly higher than the 7.31% obtained from *Ulva rigida* in Tunisia (Frikha et al., 2011). The crude protein of *Eucheuma denticulatum* was slightly lower than the 9.76% obtained by Matanjun et al. (2009). The protein content of *Sargassum oligocystum* was in agreement with 5.63% reported by Mwalugha et al. (2015). The variation in protein content in seaweeds could be attributed to differences in seasonality and growth conditions in the environment (Dawczynski et al., 2007).

4.1.2 Fatty acid composition

The fatty acids profile of seaweeds are shown in Table 4.2. The total saturated fatty acids were not significantly different ($P = 0.075$) among the seaweed species, however *Hypnea musciformis*, *Ulva fasciata* and *Laurencia intermedia* had the highest values of capric (36.09%), lauric (2.09%) and myristic (6.09%) respectively. *Sargassum oligocystum* had the highest levels of mono unsaturated fatty acids (MUFA) (17.71%) and poly unsaturated fatty acids (PUFA) (10.13%) ($P < 0.05$). *Eucheuma denticulatum* had the lowest amount of total MUFA (4.83%) ($P < 0.001$). The total MUFA in *Sargassum oligocystum* was lower than the 23.8% and 27.5 % from *Ulva lactuca* and *Sargassum ilicifolium* respectively reported from Persian Gulf seaweeds by Rohani-Ghadikolaei et al. (2012). The total MUFA in *Eucheuma denticulatum* was within the range of 1.8-12.5% obtained in *Eucheuma Cottonii* from Malaysia (Khotimchenko et al., 2002) ,however the total MUFA in

Eucheuma denticulatum was lower than the range 6.6% to 10.5% reported in seaweeds by Gressler et al., (2010).

In this study, the total saturated fatty acids in *Sargassum oligocystum* was higher (61.63%) than the 49.6% reported by Kumari et al. (2013). However the total saturated fatty acids of *Ulva fasciata* (68.73%) obtained in this study was close to 68.97% reported for *Ulva lactuca* (Yaich et al., 2011), but the value was higher than the 38.50% reported for *Ulva* species (Durmaz et al., 2008). The palmitic acid was the most dominant saturated fatty acid among the seaweeds, though *Hypnea musciformis* and *Ulva fasciata* recorded lower amounts of palmitic acid. The results of this study were in agreement with that of Gressler et al. (2010) who reported for palmitic acid as the dominant saturated fatty acids in *Laurencia filiformis* (49.4%) and *Laurencia intricata* (39.7%) from the Brazilian seaweeds. Khotimchenko et al. (2002) attributed the dominance to the influence of environmental factors and /or characteristic features of the individual genera.

Table 4.2: Fatty acid profile of seaweed species oil from the Kenya coast.

Fatty acids (%)	<i>Eucheuma denticulatum</i>	<i>Hypnea musciformis</i>	<i>Ulva fasciata</i>	<i>Laurencia intermedia</i>	<i>Sargassum oligocystum</i>	P value
Caprylic (C8:0)	0.31 ± 0.54 ^a	0.07 ± 0.13 ^b	0.26 ± 0.45 ^a	nd	0.33 ± 0.50 ^a	0.778
Capric (C10:0)	2.62 ± 4.54 ^a	36.09 ± 24.14 ^b	23.40 ± 1.75 ^{ab}	13.37 ± 1.05 ^a	11.40 ± 10.06 ^a	0.50
Lauric (C12:0)	0.34 ± 0.31 ^{ab}	0.22 ± 0.37 ^{ab}	2.09 ± 1.81 ^c	1.81 ± 0.21 ^{bc}	0.02 ± 0.04 ^a	0.035
Myristic (C14:0)	4.24 ± 0.39 ^c	2.08 ± 0.87 ^b	0.72 ± 0.63 ^a	6.09 ± 0.06 ^d	4.50 ± 0.23 ^c	<0.0001
Palmitic (C16:0)	43.22 ± 17.76 ^b	21.06 ± 3.88 ^a	34.74 ± 3.85 ^{ab}	46.88 ± 0.37 ^b	41.56 ± 3.27 ^b	0.027
Stearic (C18:0)	2.30 ± 1.06 ^a	4.60 ± 0.58 ^a	4.28 ± 1.85 ^a	2.90 ± 0.07 ^a	2.86 ± 0.32 ^a	0.153
Arachidic (C20:0)	nd	nd	3.24 ± 0.45 ^c	nd	0.95 ± 0.58 ^b	<0.0001
SFA¹	53.72 ± 11.55^a	64.12 ± 20.82^a	68.73 ± 0.41^a	71.04 ± 0.49^a	61.63 ± 6.17^a	0.324
Oleic (C18:1)	4.83 ± 3.43 ^a	8.41 ± 1.64 ^b	11.77 ± 1.55 ^b	11.22 ± 0.13 ^b	17.71 ± 1.12 ^c	<0.0001
MUFA²	4.83 ± 3.43^a	8.41 ± 1.64^b	11.77 ± 1.55^b	11.22 ± 0.13^b	17.71 ± 1.12^c	<0.0001
Linoleic (C18:2)	2.27 ± 1.50 ^a	3.27 ± 2.43 ^a	3.81 ± 0.94 ^a	2.70 ± 0.19 ^a	6.33 ± 0.23 ^b	0.03
Linolenic (C18:3)	0.49 ± 0.18 ^a	1.48 ± 1.41 ^a	5.64 ± 0.77 ^c	0.37 ± 0.15 ^a	3.80 ± 0.56 ^b	<0.001
PUFA³	2.75 ± 2.10^a	4.75 ± 3.84^a	9.45 ± 1.69^b	3.07 ± 0.06^a	10.13 ± 0.79^b	0.0487

nd = not detected

¹Total saturated fatty acids (SFA) = the sum of C8:0 to C20:0

²Total mono unsaturated fatty acids (MUFA) = the amount of C18:1

³Total polyunsaturated fatty acids (PUFA) = the sum of C18:2 and C18:3

All values are means ± SE, *n*=3. Values in the same row bearing superscript different letters are significantly different

The total polyunsaturated fatty acids (PUFA) included Linoleic acid and Linolenic acid. *Sargassum oligocystum* and *Ulva fasciata* recorded the highest amount of PUFA i.e. 10.13% and 9.45% respectively which are lower than the 32% from *Ulva Lactuca* reported by Dawczynski et al. (2007) from the North Atlantic. The PUFAs in *Ulva fasciata* 9.45% was considerably lower than the 33.76% reported by Durmaz et al. (2008). Similarly, the value in this study was lower than the 56.9% obtained in *Ulva lactuca* (Khotimchenko et al., 2002). *Euclima denticulatum*, *Hypnea musciformis* and *Laurencia intermedia* recorded the lowest amount of PUFAs (Table 4) ($P < 0.003$), the total PUFA in *Laurencia intermedia* was lower than 70.5% reported in *Laurencia okamuria* (Li et al., 2002) and 16.7% obtained in *Laurencia filiformis* (Gressler et al., 2010). The total PUFA in *Euclima denticulatum* was lower than 26.97% reported in *Kappaphycus alvarezii* (*Euclima cottonii*) (Kumar et al., 2011). Arachidic acid was only found in *Sargassum oligocystum* and *Ulva fasciata*. The difference in the fatty acid among the seaweed is related to difference in species and habitat (Khotimchenko et al., 2002). Additionally, the difference in PUFAs could be attributed to environmental temperature since algae normally accumulates PUFAs when there is a decrease in environmental temperature (Kayama et al., 1985).

4.1.3 Mineral and heavy metal composition of seaweeds

The mineral content of five seaweed species are shown in Table 4.3. Among the seaweeds, the highest values of calcium (1536.93), and zinc (4.25) were obtained from *Euclima* while *Laurencia* had the highest magnesium content (445.09). Calcium and magnesium were found to be the major minerals in brown and red seaweeds analyzed in Spain (Rupérez, 2002).

In this study, the highest amount of calcium was obtained in *Euclima denticulatum* while the lowest values were in *Hypnea musciformis* ($p < 0.001$). The calcium content reported here for *Euclima denticulatum* was considerably higher than the 422 mg/100g obtained in *Euclima* spp from South China (Krishnaiah et al., 2008). The calcium content in all the seaweed species in this study was

substantially higher compared to common vegetables such as lettuce (35mg/100g), cabbage (40mg/100g) and spinach (99mg/100g) (USDA, 2016). From these findings, seaweeds showed a potential of being utilized as a supplement to vegetables in the human diet. Seaweeds have been found to possess diverse mineral content due to their habitat in the marine environment. As a result ,minerals such as calcium are able to accumulate in much higher content compared to terrestrial plants (MacArtain et al., 2007).

Table 4.3: Mineral composition and heavy metal content of seaweed species from the Kenya coast.

Seaweed species	<i>Hypnea musciformis</i>	<i>Sargassum oligocystum</i>	<i>Ulva fasciata</i>	<i>Eucheuma denticulatum</i>	<i>Laurencia intermedia</i>	P value
Minerals (mg/100g)						
Sodium	20.50 ± 5.61 ^b	18.79 ± 1.82 ^b	17.42 ± 2.43 ^b	9.88 ± 1.25 ^a	21.88 ± 0.71 ^b	0.004
Potassium	2.55 ± 0.18 ^c	0.50 ± 0.03 ^a	2.56 ± 0.10 ^c	1.36 ± 0.25 ^b	0.75 ± 0.06 ^a	<0.0001
Calcium	868.75 ± 14.10 ^a	949.20 ± 44.75 ^b	1210.88 ± 50.89 ^c	1536.93 ± 22.06 ^c	1360.70 ± 28.80 ^d	<0.0001
Phosphorus	2.21 ± 0.53 ^a	3.35 ± 0.24 ^a	2.36 ± 0.49 ^a	2.43 ± 1.35 ^a	2.96 ± 1.03 ^a	0.458
Magnesium	411.56 ± 7.66 ^b	397.12 ± 2.19 ^a	429.88 ± 2.47 ^c	436.97 ± 1.06 ^c	445.09 ± 2.68 ^d	<0.0001
Zinc	1.29 ± 0.23 ^a	1.51 ± 0.22 ^a	4.12 ± 0.30 ^b	4.25 ± 1.85 ^b	0.99 ± 0.11 ^a	0.001
Iron	7.34 ± 1.71 ^a	25.82 ± 1.34 ^b	51.39 ± 2.58 ^c	48.80 ± 1.0 ^d	30.71 ± 1.27 ^c	<0.0001
Heavy metals (mg/100g)						
Cadmium	0.17 ± 0.01 ^a	0.18 ± 0.05 ^{ab}	0.24 ± 0.05 ^{ab}	0.30 ± 0.10 ^b	0.21 ± 0.05 ^{ab}	0.163
Lead	nd	nd	nd	nd	nd	

nd=Not detected

All values are means ± SE, *n*=3.

Values in the same row bearing different superscript letters are significantly different

The highest levels of magnesium was recorded in *Laurencia intermedia*, while the lowest in *Sargassum oligocystum* (Table 4). The content of magnesium in *Laurencia intermedia* obtained in the current study was lower than the 640 mg/100g reported by Kumar et al. (2011) in *Laurencia cruciata* harvested in Indian coast. In addition, the level of magnesium in *Sargassum oligocystum* was lower than the 487.81 mg/100g reported by Matanjun et al. (2009) in *Sargassum polycystum* harvested in Malaysia, 700 mg/100g obtained in Indian *Sargassum swartzii* (Kumar et al., 2011) and 1160 mg/100g reported in *Sargassum echinocarpum* (McDermid et al., 2007) from Hawaiian Islands.

Both *Ulva fasciata* and *Hypnea musciformis* had the highest levels of potassium while *Laurencia intermedia* and *Sargassum oligocystum* had the lowest (Table 4). The potassium content of *Ulva fasciata* was lower than 520mg/100g reported by Rohani-Ghadikolaei et al., (2012) in *Ulva Lactuca*, 2450mg/100g reported by MacArtain et al. (2007) and 4340mg/100g obtained by Kumar et al. (2011) in *Ulva* species. The potassium content of *Laurencia intermedia* was considerably lower than (6040mg/100g) reported by Kumar et al. (2011) in *Laurencia cruciate*.

High zinc content was obtained in *Eucheuma denticulatum*. The Zinc content of *Eucheuma denticulatum* was slightly higher than 0.545mg/100g obtained in *Eucheuma isiforme* (Robledo & Freile Pelegrin, 1997) but lower than 6.63mg/100g in *Eucheuma denticulatum* from Malaysia (Krishnaiah et al., 2008). The variation in mineral composition could be attributed to the difference in geographical location and species (Rao et al., 2007).

There was no significant difference in the cadmium content among the seaweed species ($p > 0.05$). The cadmium content in *Ulva fasciata* was higher than 0.05 mg/100g reported by Topçuoğlu et al. (2001) in *Ulva lactuca* and a range of 0.0031-0.0033mg/100g reported by Besada et al. (2009). The cadmium content of *Sargassum oligocystum* obtained in this study was lower than 0.70 mg/100g in *Sargassum wightii* stem and higher than 0.14mg/100g in *Sargassum polycystum* (Jothinayagi & Anbazhagan, 2009). The presence of cadmium in seaweeds around

Mombasa could be attributed to water contamination by sewage, effluent from metal product manufacturers, fertilizers and by- products from oil refining (Wong et al., 1982).However, cadmium concentration in seaweeds is above the European Union regulation of 0.5mg/kg d.w (EEC 889/2008).

4.1.4 Emulsion activity

The emulsion activity of the seaweeds is presented in Figure 4.1.The emulsifying activity of the seaweed species ranged from 59.19% to 75.69% with *Eucheuma denticulatum* and *Ulva fasciata* recording the highest values of 75.68% and 75.66% respectively while *Sargassum oligocystum* (59.19%) recorded significantly lower values ($P<0.001$)(Figure2).

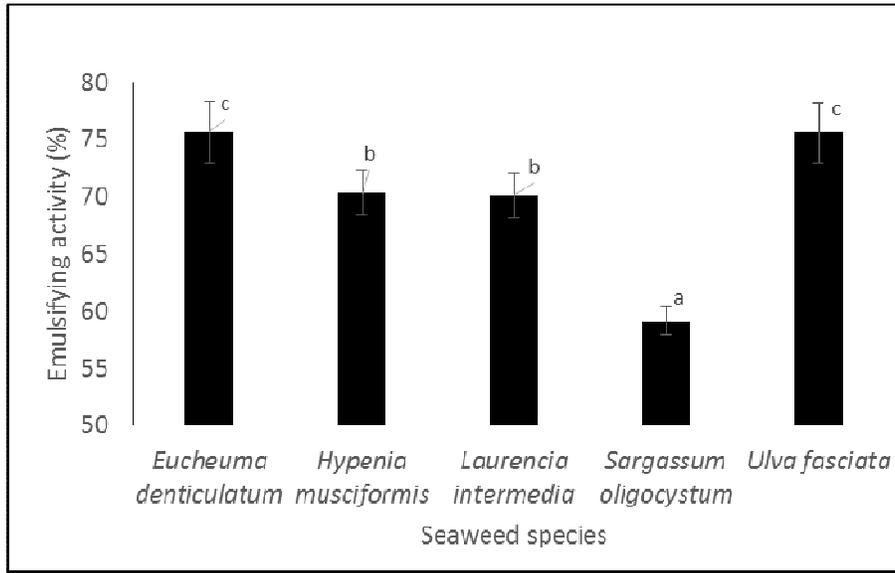


Figure 4.1: Emulsion activity (%) of five seaweed species from the Kenya coast, means \pm SE, n=3

The emulsion activity of *Eucheuma denticulatum* was similar to 75.68% in *Kappaphycus alvarezii* (*Eucheuma cottonii*) obtained by Kumar et al., (2014). In this study the emulsion capacities of the seaweed species compares well with lupin seed (60%) and soya bean (70%) whose extract are used as emulsifiers in the food industry (Tömösközi et al., 2001). The good emulsifying ability of seaweeds is attributed to phycocolloids which are widely used in meat processing as a result of their good thickening, emulsifying, and stabilizing properties (Cofrades et al., 2011).

4.1.5 Water holding capacity of seaweeds

The water holding capacity of seaweeds is presented in Figure 4.2. The seaweed *Sargassum oligocystum* had the highest water holding capacity(13.75 ml/g) while the lowest values were obtained in *Ulva fasciata* (9.16 mL/g) and *Eucheuma denticulatum* (8.42 mL/g) $P < 0.001$ (Figure 4.2).

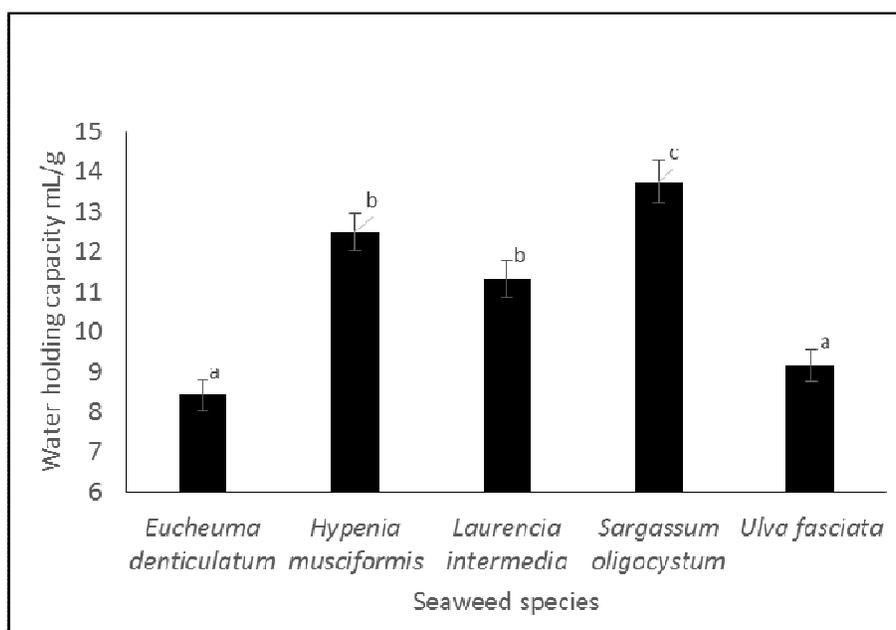


Figure 4.2: Water holding capacity of seaweed species from the Kenya coast, means \pm SE, n=3

The water holding capacity of *Ulva fasciata* reported in this study was higher than 6.66-7.00 mL/g reported in *Ulva lactuca* (Yaich et al., 2011) from Tunisia .The water holding capacity of *Eucheuma denticulatum* was lower than 17.7 mL/g in Indian *Eucheuma* powder (Senthil et al., 2005). The water holding capacity of seaweed species in this study was slightly higher than 6.60-9.00 mL/g reported for commercial dietary fibre supplements like cereals and fruit fibres (Goñi & Martín-Carrón, 1998). The variation in water holding capacity could be as a result of varying fibre and protein content of the seaweeds that have an influence on the functional properties (Fleurence, 1999; Yaich et al., 2011). In this study, *Sargassum oligocystum* had the highest crude fiber and considerably high amount of crude protein while *Eucheuma denticulatum* had the lowest crude fiber and crude protein a pattern that was mirrored by water holding capacity. Seaweeds have been shown to improve the water holding capacity of emulsions, thus having an influence on firmness, hardness and chewiness of cooked meat products (Cofrades et al., 2008).

4.2 Selection of seaweed for sausage production

Eucheuma seaweed was selected for formulation in chicken sausages. The selection was based on the proximate output e.g. highest ash and crude fat contents (Table 4.2), highest calcium, zinc, and iron contents (Table 4.4) and highest emulsion activity (Figure 2) among the five seaweeds. The seaweed is also being farmed in large quantities by the coastal communities in south coast (Msuya et al., 2014).

4.3 Chemical properties of seaweed based chicken sausages

4.3.1 Proximate composition of chicken sausages

The proximate compositions of the chicken sausages containing *Eucheuma denticulatum* powder are presented in Table 4.4. The highest proximate component of chicken sausages was moisture while crude fiber was the lowest (Table 4.4). There was no significant difference in moisture among the chicken sausages ($P= 0.066$).

The moisture contents of all the chicken sausages ranged from 49.94-52.64%. Choi et al.(2010 a) obtained similar results of 59.94% in chicken emulsions sausages. A

higher moisture content of 70.9% was obtained in turkey sausage (Pereira et al., 2000). Kim et al. (2010) also obtained similar results in breakfast sausages with added *Laminaria japonica* a brown seaweed. No significant difference were observed in protein and crude fat contents among the batches of chicken sausages. Similar observations were reported by other authors (Cofrades et al., 2008; Kim et al., 2010). The crude ash and crude fibre contents increased with increasing amount of *Eucheuma* powder ($P < 0.05$). The observed result was probably due to the high ash content of *Eucheuma denticulatum* (27.13%) (Table 4.1), including potassium, calcium, magnesium, zinc, and iron (Table 4.3). López-López, et al. (2009) reported similar results in frankfurters. The results shows that addition of seaweed had a significant impact of increase in ash and fiber content in the chicken sausages. Seaweeds have been shown to contain considerable amounts of ash (Polat & Ozogul, 2009, Muraguri et al., 2016, Mwalugha et al., 2014)

In the present study cholesterol contents decreased with increasing amount of *Eucheuma denticulatum* powder ($P < 0.05$) (Table 5). Cengiz and Gokoglu, (2005) reported a cholesterol range of 179-371 mg/100g in frankfurters which was considerably higher than the values obtained in this study.

Table 4.4: Proximate compositions% (wet weight basis), cholesterol and pH of chicken sausage containing *Eucheuma denticulatum* seaweed powder

Seaweed Level (%)	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Crude fiber (%)	Carbohydrate (%)	Cholesterol (mg/100g)	pH
0	49.95 ± 1.04 ^a	8.70 ± 1.88 ^a	10.80 ± 1.73 ^a	2.10 ± 0.02 ^a	0.50 ± 0.02 ^b	27.95 ± 2.36 ^{ab}	10.73 ± 0.17 ^d	73 ± 0.04 ^b
1	51.89 ± 1.38 ^{ab}	8.34 ± 0.39 ^a	8.33 ± 1.37 ^a	2.35 ± 0.12 ^b	0.42 ± 0.04 ^a	28.67 ± 2.54 ^b	5.87 ± 0.06 ^c	5.52 ± 0.03 ^a
3	52.64 ± 0.92 ^b	8.62 ± 0.31 ^a	10.91 ± 0.21 ^a	2.78 ± 0.13 ^c	0.53 ± 0.05 ^{bc}	24.52 ± 1.08 ^a	4.16 ± 0.04 ^b	5.55 ± 0.08 ^a
4	50.62 ± 1.05 ^{ab}	9.40 ± 1.09 ^a	9.56 ± 1.32 ^a	3.11 ± 0.03 ^d	0.59 ± 0.05 ^c	26.7 ± 0.24 ^{ab}	2.95 ± 0.05 ^a	5.70 ± 0.09 ^b
<i>P</i> value	0.066	0.695	0.120	<0.001	0.007	0.096	0.029	0.008

All values are means ± SE, *n*=3.

Values in the same column bearing different superscript letters are significantly different

The cholesterol content of the chicken sausages was lower than 300 mg per day which is the maximum recommended daily intake for an adult (American Heart Association, 2004). The reduction in cholesterol could probably be linked to ash and dietary fibre that have been reported to have the capacity of absorbing organic components such as cholesterol and glucose (Bocanegra et al., 2009; Candogan & Kolsarici, 2003).

The pH values of the sausages ranged from 5.52 to 5.73. The highest pH were obtained in the sausages containing 0 and 4% seaweed powder (Table 5). Other studies have recorded a pH range of 5.4 – 6.24 in precooked chicken sausages (Yilmaz, 2002; Zaritzky et al. 2006), which is close to values obtained in this study (Table 5). The pH values of the chicken sausages was within the limit set for meat products (5.4-5.8) (Avendaño-Reyes et al., 2006). The pH values of breakfast sausages decreased with increasing seaweed powder as reported by Kim et al. (2010).

4.3.2 Fatty acid composition

The fatty acid compositions of the *Eucheuma* seaweed powder added chicken are shown in Table 4.5. The fatty acid compositions in the chicken sausages showed significant differences in the levels of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ($P < 0.005$) (Table 4.5). The saturated fatty acids (SFA) levels decreased with increasing amounts of *Eucheuma* seaweed powder and this probably was due to the partial replacement of chicken fat. The chicken sausage with 0% seaweed recorded the highest SFA content while those with 4% recorded the least. The dominant SFA was palmitic acid which was also dominant in the seaweeds.

study was similar to the 35.6% and 33% obtained by Pereira et al., (2000); Baggio & Bragagnolo, (2006), respectively, in raw chicken sausages. Yilmaz (2002) reported 50.3% SFA in chicken sausages which was higher than the values obtained in this study.

The chicken sausage with 0% seaweed recorded the highest MUFA content while the 4% recorded the least. The seaweed seemed to have contributed to the reduction of the MUFA content. The MUFA content of the chicken sausages was lower than 42% reported in chicken sausages by Baggio and Bragagnolo, (2006) and 39.19 - 40.03% reported in chicken frankfurters processed from chicken fed with supplemented fish oil by Jeun-horng et al. (2002). The low MUFA content in this study could be because other monounsaturated fatty acids were not detected.

Table 4.5: Fatty acid profile (%) of chicken sausage containing *Eucheuma denticulatum* powder

Fatty acids	Chicken sausage samples					P value
	0%	1%	3%	4%		
Caprylic (C8:0)	0.29 0.01 ^a	± 1.55 0.04 ^c	± 0.84 ± 0.04 ^b	± 0.85 ± 0.04 ^b		<0.0001
Capric (C10:0)	0.29 0.01 ^b	± 0.36 0.03 ^c	± 0.64 ± 0.03 ^d	± 0.15 ± 0.04 ^a		<0.0001
Lauric (C12:0)	0.39 0.01 ^a	± 0.59 0.01 ^{ab}	± 0.46 ± 0.04 ^{ab}	± 0.35 ± 0.04 ^a		0.441
Myristic (C14:0)	1.55 0.04 ^c	± 1.76 0.04 ^d	± 1.36 ± 0.03 ^b	± 1.16 ± 0.03 ^a		<0.0001
Palmitic (C:16:0)	22.78 0.03 ^d	± 18.16 0.05 ^c	± 17.56 0.04 ^b	± 16.85 0.13 ^a	±	<0.0001
Stearic (C18:0)	12.14 0.06 ^c	± 12.09 0.02 ^c	± 10.46 0.04 ^b	± 8.37 ± 0.04 ^a		<0.0001
SFA¹	37.45 0.012^a	± 34.50 0.12^a	± 31.31 0.18^a	± 27.74 0.29^a	±	0.440
Oleic (C18:0)	23.77 0.04 ^c	± 23.71 0.01 ^c	± 22.06 0.04 ^a	± 22.68 0.04 ^b	±	<0.0001
MUFA²	23.77 0.04^c	± 23.71 0.01^c	± 22.06 0.04^a	± 22.68 0.04^b	±	<0.0001
Linoleic (C 18:2)	24.88 0.03 ^b	± 24.28 0.02 ^a	± 26.15 0.04 ^c	± 26.36 0.04 ^d	±	<0.0001
Linolenic (C18:3)	1.28 0.02 ^a	± 1.28 0.04 ^a	± 1.37 ± 0.05 ^b	± 1.46 ± 0.04 ^c		0.001
PUFA³	26.16 0.02^b	± 25.55 0.04^a	± 27.52 0.06^c	± 27.82 0.09^d	±	<0.0001

All values are means ± SE, $n=3$.

Values in the same row bearing different superscript letters are significantly different

¹ Saturated fatty acids (SFA) = the sum of C8:0 to C 18:0

² Monounsaturated fatty acids (MUFA) = the amount of C18:1

³ Polyunsaturated fatty acids (PUFA) = the sum of C18:2 and C18:3

There was significance difference in the PUFA content of the chicken sausages. The chicken sausages in this study showed higher PUFA content than 14.2% in chicken sausages and 17.9% in turkey sausages reported by Pereira et al., (2000). Similarly, Hoz and Cambero (2004) reported PUFA contents of 15.52 – 22.91% in Spanish meat sausages which was slightly lower compared to values obtained in this study. The variation in PUFA content could be attributed to the formulation used where seaweed was used in processing and it is reported to have substantial PUFAs (Yilmaz, 2002) and the type of chicken fat used which is less in saturated fat compared to pork back fat (Jeun-horng et al., 2002).

4.3.3 Mineral composition of chicken sausages

The mineral content of the *Eucheuma* seaweed powder added chicken sausages are shown in Table 4.6. Sodium (743.18 ± 97.02 %) was the dominant mineral followed by potassium while zinc (0.61 ± 0.19 %) was the least. The chicken sausages containing 4% seaweed recorded the least sodium content (Table 4.6). The sodium content obtained in this study were within the range (580-1036 mg/100g) reported by Huda et al. (2010) in chicken sausages from Malaysia. However, the sodium content of the chicken sausages was twice higher the 320 mg/100g reported in lean poultry meat (Mariam et al., 2004). There was no significant difference ($P = 0.437$) in potassium content among the chicken sausage samples. The potassium content of the sausage with 0% seaweed was lower than 259mg/100g in lean poultry meat (Mariam et al., 2004). Similarly, González-Tenorio et al., (2012) obtained 440mg/100g in Mexican sausages which was higher than values reported in this study due to incorporation of paprika. Potassium is very essential in maintaining the integrity of the cellular membrane (Mariam et al., 2004).

Table 4.6: Mineral composition of chicken sausages containing *Eucheuma denticulatum* powder (mg/100g

Chicken sausages					
	0%	1%	3%	4%	P value
Sodium	655.72 ± 47.66 ^b	743.18 ± 77.02 ^b	644.68 ± 28.23 ^b	461.51 ± 17.57 ^a	0.005
Potassium	145.86 ± 25.31 ^a	165.45 ± 16.99 ^a	176.76 ± 16.79 ^a	150.61 ± 34 ^a	0.437
Calcium	31.97 ± 1.39 ^a	22.71 ± 3.52 ^a	27.53 ± 4.34 ^a	30.83 ± 4.17 ^a	0.376
Magnesium	7.61 ± 0.36 ^{ab}	7.94 ± 0.34 ^{ab}	8.41 ± 0.27 ^b	7.03 ± 0.89 ^a	0.066
Iron	3.31 ± 0.35 ^a	2.35 ± 0.74 ^a	2.85 ± 0.70 ^a	3.20 ± 0.85 ^a	0.376
Zinc	0.82 ± 0.21 ^b	0.59 ± 0.23 ^{ab}	0.61 ± 0.18 ^{ab}	0.43 ± 0.12 ^a	0.161

All values are means ± SE, $n=3$.

Values in the same row bearing different superscript letters are significantly different

Addition of seaweed had no significant effect on the calcium content of the chicken sausages ($P = 0.376$). The calcium content of the chicken sausages was similar to 27-116mg/100g obtained in different chicken sausage formulations (Huda et al., 2010). Rahman et al., (1997) reported 666- 2228mg/100 g of calcium in chicken sausages which was higher than the values obtained in this study. The variation in the calcium content could be attributed to the type of meat and ingredients used in processing. It has been demonstrated that when deboned chicken meat was used in chicken sausage processing, high values of calcium are observed due to presence of bone extracts in the meat(Babji et al., 1995).

Zinc was not significantly different in the chicken sausage samples ($P=0.161$). The zinc content of the chicken sausages was close to 1.11mg/100g observed in chicken sausages from Turkey (Uluozlu et al., 2009). The zinc content of the seaweed based

chicken sausage was less than the human recommended daily intake (RDI) of zinc among male (11mg) and female (8mg) adults over 19 years (USNIH, 2016). Zinc is involved in key metabolic pathways in the human body and its deficiency could result into loss of appetite, retardation in growth and malfunctioning of the immune system (Uluozlu et al., 2009).

There was no significant difference in magnesium content of the seaweed based chicken sausages (P= 0.066). Values obtained in this study were lower than 43mg/100g reported in Mexican sausages and 22mg/100g obtained in chicken meat (González-Tenorio et al., 2012; Tunj et al., 2008).

The iron content of the chicken sausages was similar to 3.82mg/100g reported in chicken sausages from Turkey (Uluozlu et al., 2009) and lower than 9.27mg/100g obtained in Chicken meat from Southern Nigeria (Iwegbue et al., 2008). Seaweed had no significant effect on the iron content of the chicken sausages (P= 0.376).

4.4 Physical properties of sausages containing *Eucheuma* seaweed powder

4.4.1 Color and texture of chicken sausages

The colour and texture of the chicken sausages containing *Eucheuma* seaweed powder are shown in Table 4.7. The lightness (L*) of the chicken sausages was similar in all the samples except those sausages containing 4% seaweed powder. Several studies have reported decreased lightness of sausage with increasing seaweed powder (Hsu et al., 1999; Kim et al., 2010). This significant reduction in lightness in sausages containing 4 % seaweed powder could be due to the lower lightness of the added seaweed as reported by kim et al. (2010).

Table 4.7: Colour, texture and cooking loss values of chicken sausage containing *Eucheuma denticulatum* powder

Seaweed Level	Lightness (L*)	Redness (a*)	Yellowness (b*)	Firmness (N/mm ²)	Cooking loss
0%	73.13 ± 1.27 ^b	5.43 ± 0.46 ^a	15.53 ± 0.51 ^{ab}	11.67 ± 0.58 ^a	16.22 ± 1.48 ^a
1%	73.37 ± 0.78 ^b	6.27 ± 1.27 ^a	15.03 ± 0.06 ^b	12.67 ± 0.58 ^b	14.87 ± 0.10 ^a
3%	71.20 ± 1.06 ^b	6.10 ± 0.30 ^a	15.43 ± 0.21 ^b	13.00 ± 0.00 ^b	14.39 ± 0.59 ^a
4%	68.50 ± 1.85 ^a	8.80 ± 0.61 ^b	14.63 ± 0.49 ^a	13.33 ± 0.58 ^b	13.72 ± 2.19 ^a
P value	0.006	0.003	0.063	0.017	0.221

All values are means ± SE, $n=3$.

Values in the same column bearing different superscript letters are significantly different

Yilmaz et al. (2002) reported a range of 38.11- 63.60 for lightness (L*) in sausages which was slightly lower than the values obtained in this study. To the contrary ,Zaritzky et al. (2006) reported a range of 80.5- 86.2 for lightness in chicken sausage which was slightly higher than the values in (Table 9). According to Resurreccion (2004) high lightness value implies that a product is lighter and therefore desirable by consumers. The lightness of sausages can also be attributed to reduced fat and dietary fibre incorporation (Trespacios & Pla, 2007). Heating has been shown to cause oxidation of heme pigments and as a result discolouration occurs (García-Segovia et al., 2007).

The chicken sausage with 4% seaweed levels showed the highest redness (a*) while the rest of the chicken sausage samples recorded no significant difference ($p < 0.003$). The significant redness at 4% could be attributed to *Eucheuma denticulatum* a red seaweed that was used in this study in chicken sausage formulation. The redness (a*) of the chicken sausages was higher than 0.9-2.3 reported by Zaritzky et al. (2006) and lower than 14.82 obtained in chicken sausages and 20.84 in beef sausages by Yilmaz (2002). The chicken sausage samples showed no significance difference in the yellowness (b*). The yellowness of the chicken sausage samples was close to a

value of 13.6 obtained in chicken sausages stored for a period of 6 weeks (Mielnik et al., 2002) and slightly higher than 10.6- 12.3 reported in chicken sausages (Zaritzky et al., 2006). The difference in the colour attributes could be attributed to inherent carotenoids and xanthophyll in the seaweeds(Yilmaz, 2002).

The firmness values of the chicken sausages containing seaweed powder were significantly higher than that without seaweed ($P = 0.017$) The chicken sausages showed slightly higher firmness values than 3.81-10.24 reported by (Zaritzky et al., 2006). Meat emulsions with the highest concentration of *Undaria pinnatifida* seaweed showed the highest values 34.53 for firmness(Cofrades et al., 2008). Similar results of increasing firmness by addition of sea tangle powder and sea spaghetti seaweed were obtained by. Cofrades et al.(2008)and Fernandez -Martin et al.(2009), this could be probably due to the fibre content from seaweeds. Dietary fiber has normally been added to foods to improve the texture and especially insoluble fibers due to their good water holding and swelling capabilities (Thebaudin et al., 1997).

4.4.2 Cooking loss of chicken sausages samples

The cooking loss of the seaweed powder containing chicken sausages is shown in Table 4.7. There was no significant difference ($p > 0.221$) in cooking loss among the chicken sausages samples including the control. However, sea tangle powder was observed to reduce cooking loss in breakfast sausages (Kim et al., 2010). In various foods such as bread and cookies, the addition of seaweed powder increases hardness (Choi et al., 2010). The addition of dietary fibre to meat products increased hardness (Choi et al., 2010; Kim et al., 2010). The cooking loss values obtained in this study were close to 10% reported in beef batters (Sikes et al., 2009). However, Choi et al. (2010 a) reported values that were slightly lower than these obtained in this study. The variation in cooking loss of sausages is highly attributed to cooking time and temperature, ingredients used in processing and natural sources of dietary fiber and technology used in processing (Choi et al., 2010 c; Sikes et al., 2009). Compounds with high dietary fibre has been incorporated in meat emulsions to improve physical and functional properties such as reducing cooking loss, improving water holding capacity and emulsion stability (Turhan et al., 2005).

4.4.3 Emulsion stability of chicken sausage batter

Figure 4.3 shows the emulsion stability of chicken sausages containing *Eucheuma* seaweed powder. Seaweed concentration in the chicken sausages had a significant effect on emulsion stability $P < 0.05$. Chicken sausage with 3% seaweed recorded the highest emulsion stability (2.37%) while those with 0% seaweed recorded the least (0.81%) (Figure 4.3.).

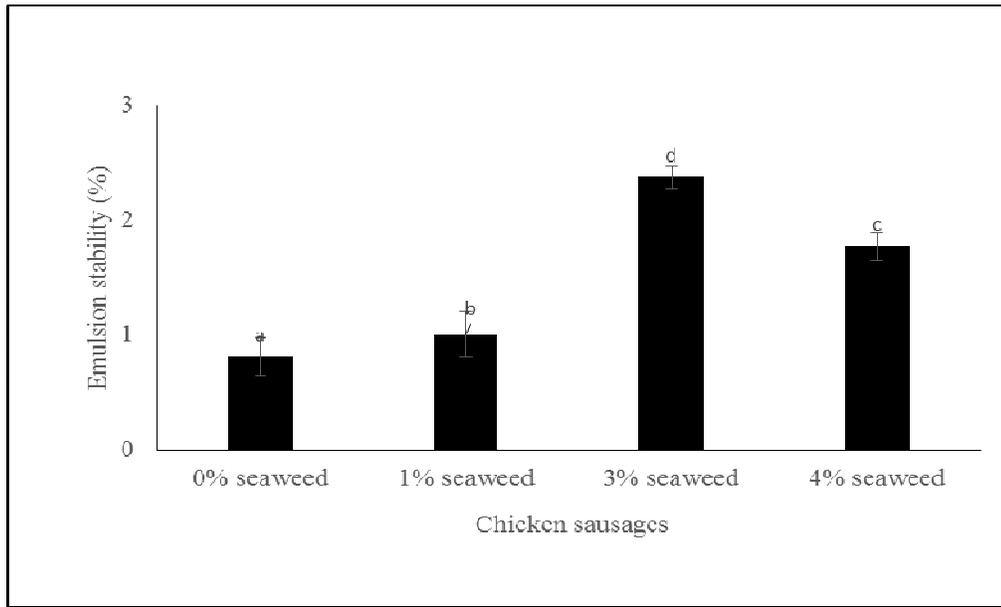


Figure 4.3: The emulsion stability of chicken sausages containing *Eucheuma denticulatum* powder, means \pm SE, n=3

The chicken sausage with 0% and 1% seaweed recorded an emulsion stability close to 0.85% reported in chicken emulsion sausages (Choi et al., 2010 b). A good emulsion is characterized by low or absolutely no loss of water or fat. Good emulsion therefore have good water holding capacity. This could therefore explain the trend in emulsion stability of the chicken sausages since 0 and 1% had higher water holding capacities reported. Based on this study, addition of seaweed to chicken sausage upto 3% showed the highest emulsion stability. Therefore the amount of seaweed and other ingredients aimed at improving functional properties should be highly considered in improving emulsion stability of sausages (Yang et al., 2007).

4.4.4 Water holding capacity of chicken sausages

Figure 4.4 shows the water holding capacity (WHC) of chicken sausage samples. The chicken sausages containing 4% *Eucheuma* seaweed powder had the lowest water holding capacity values compared to the other samples ($P = 0.007$)

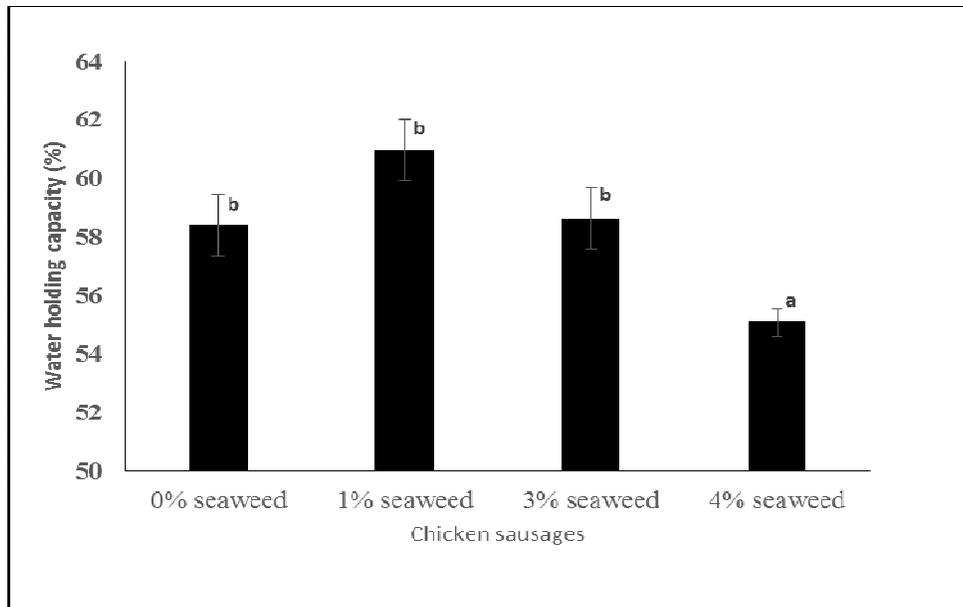


Figure 4.4: Water holding capacity of chicken sausages containing *Eucheuma denticulatum* powder, means \pm SE, n=3

The water holding capacity values obtained in this study were close to the 56.19% obtained in chicken thigh muscles (Kim et al., 2010). However, Ayadi et al. (2009) obtained a water holding capacity of 98.1- 98.7% in turkey sausage incorporated with carrageenan extracted from seaweed which was higher than values obtained in this study and this could have been due to the type of meat used and seaweed. Similarly, the water holding capacity of the chicken sausages was higher than (87.25-95.19%) recorded in pork sausages with added tofu and oatmeal by Yang et al. (2007). The variation in water holding capacity could be attributed to recipe difference such as incorporation of *Eucheuma* which contributed to fibre and protein content (Ayadi et al., 2009). Sausage with 4% seaweed had also the highest fibre content (see Table 4.5).

4.5 Determination of microbial load

Table 4.8 shows the microbial load of the chicken sausages. The total plate count of the chicken sausages at day 0 were within the Kenya Bureau of standards (KEBS) limit of 6 log₁₀ colony forming units (cfu) /g. By the seventh day there was an increase in the total plate count with 1%, 3% and 4% seaweed based chicken sausages having higher values than the Kenya Bureau of Standard on sausages. This could be attributed to too much handling and lack of automation during sausage processing.

Table 4.8: Microbial load (log₁₀cfu/g) of the chicken sausages at varying storage temperature (+4⁰ C and - 20 ° C) and time (0, 7, 30 days)

Micro organisms	Total microbial count log ₁₀ (cfu/g)						KEBS limits
	Storage period (days)	0% seaweed		3% seaweed		4% seaweed	
		0%	1%	3%	4%		
Total plate count	0	4.69 ± 0.11 ^a	4.56 ± 0.02 ^a	5.24 ± 0.04 ^b	5.91 ± 0.14 ^c	6.00	
	7	5.91 ± 0.09 ^a	6.09 ± 0.05 ^b	6.09 ± 0.06 ^b	6.34 ± 0.04 ^c		
	30	6.33 ± 0.07 ^b	6.18 ± 0.03 ^a	6.23 ± 0.02 ^a	6.85 ± 0.04 ^c		
<i>Staphylococcus aureus</i>	0	4.64 ± 0.03 ^b	4.24 ± 0.03 ^a	5.20 ± 0.03 ^c	5.99 ± 0.02 ^d	3.00	
	7	5.43 ± 0.04 ^b	5.35 ± 0.04 ^a	6.47 ± 0.02 ^c	6.58 ± 0.03 ^d		
	30	5.83 ± 0.02 ^b	5.75 ± 0.04 ^a	7.25 ± 0.02 ^c	7.85 ± 0.04 ^d		
Coliforms	0	2.30 ± 0.02 ^a	2.96 ± 0.03 ^b	3.54 ± 0.04 ^c	4.15 ± 0.04 ^d	3.00	
	7	4.47 ± 0.05 ^a	5.45 ± 0.04 ^c	5.05 ± 0.04 ^b	5.72 ± 0.21 ^d		
	30	4.66 ± 0.03 ^a	5.56 ± 0.04 ^b	5.64 ± 0.03 ^c	5.94 ± 0.04 ^d		
Yeast and moulds	0	5.18 ± 0.02 ^b	4.75 ± 0.04 ^a	5.74 ± 0.03 ^c	6.54 ± 0.04 ^d	n/a	
	7	6.32 ± 0.03 ^b	5.35 ± 0.03 ^a	6.35 ± 0.03 ^b	7.44 ± 0.03 ^d		
	30	6.75 ± 0.03 ^b	5.64 ± 0.03 ^a	7.16 ± 0.03 ^c	7.86 ± 0.04 ^d		
<i>Salmonella</i> spp	0	0	0	0	0	0.00	
	7	0	0	0	0		
	30	0	0	0	0		
<i>Shigella</i> spp	0	0	0	0	0	0.00	
	7	0	0	0	0		
	30	0	0	0	0		

All values are means ± SE, n=3.

Values in the same row bearing different superscript letters are significantly different

The microbial values obtained in this study were lower than 5.41 log₁₀cfu/g and 6.47 log₁₀cfu/g recorded at day 0 and day 5 in Greece for pork sausages (Georgantelis et al., 2007). Similarly, the values in this study were lower than 7log₁₀cfu/g point at which spoilage in terms off odors and sliminess of meat based products begin (Verma & Sahoo, 2000).

There was an increase in *Staphylococcus aureus* numbers in chicken sausages with increase in storage time. The *Staphylococcus aureus* concentration in the chicken sausages was higher than the KEBS limit (3 log₁₀cfu/g). Only 0%and 1% of the seaweed based chicken sausages at day 0 had a coliform concentration within the KEBS limit. Increase in storage duration resulted to increase in the number of coliforms. Coliforms are a symbol of faecal contamination of the food product and this could have originated from the chicken meat (Gurtler et al., 2005). However coliforms are heat sensitive and are destroyed during frying or cooking of sausages (Andrés et al., 2006). Similarly, yeast and moulds in the chicken sausages increased with increase in storage period. Georgantelis et al. (2007) recorded 4.90 log₁₀cfu/g and 6.3 log₁₀cfu/g at 0 and 5 at 4°C, respectively which were close to values obtained in this study. The presence of yeast and moulds is influenced by high water activity, therefore semi drying of sausages would be a suitable technique of increasing shelf life by lowering yeasts and moulds count (Ahmad & Srivastava, 2007).

Salmonella and *Shigella* species were not detected in the seaweed sausages. The *shigella* species have the potential to cause large outbreaks at low infection doses of about 10 cells (Cetinkaya et al., 2008). Shigellosis or bacillary dysentery characterized by diarrhea is one of the infections associated with *Shigella* species (Mead et al., 1999). *Salmonella* is also one of the foodborne pathogenic bacteria that is of public health concern in many parts of the world (Erdem et al., 2005). The absence of *Shigella* and *Salmonella* indicate that the chicken sausages were considerably safe for consumption. However, the production process of sausages should be done aseptically and the processors should ensure that they observe good hygiene and good manufacturing practices to avoid cross contamination.

4.6 Consumer sensory evaluation

The sensory evaluations of the chicken sausages containing *Eucheuma* powder are shown in Table 4.9. The chicken sausage samples showed no significant difference in scoring color ($P = 0.151$) and aroma ($P = 0.291$). The addition of *Eucheuma* seaweed powder did not affect the colour scores of the sausages. Contrary to several studies, the addition of brown algae to meat and cookies have received lower scores than control (Choi et al., 2010, Hsu et al., 1999, Kim et al., 2010). However, regardless of the addition of large amounts of *Lamina japonica* (brown seaweed, Choi et al., (2012) reported high colour scores in sulgidduk. Similarly the colour scoring in this study could be attributed to the seaweed being red in colour. There was significance difference in taste ($P= 0.001$), hardness ($P= 0.008$), aftertaste ($P= 0.01$), and overall acceptability ($P= 0.01$) scores (Table 4.9).

Table 4.9: Comparisons on sensory properties of the chicken sausages containing *Eucheuma denticulatum* powder

Attributes	Mean hedonic ratings				P value
	0% seaweed	1% seaweed	3% seaweed	4% seaweed	
Color	6.5 ± 1.5 ^a	7.1 ± 1.2 ^a	6.3 ± 1.4 ^a	6.4 ± 1.4 ^a	0.151
Taste	6.8 ± 1.4 ^c	6.0 ± 1.4 ^{bc}	4.8 ± 1.9 ^a	5.6 ± 2.0 ^{ab}	0.001
Aroma	6.7 ± 1.4 ^a	6.2 ± 1.6 ^a	6.0 ± 1.4 ^a	6 ± 1.8 ^a	0.291
Hardness/texture	6.8 ± 1.8 ^c	6.4 ± 1.6 ^{bc}	5.3 ± 1.7 ^a	5.7 ± 1.7 ^{ab}	0.008
After taste	6.5 ± 1.2 ^c	6.2 ± 1.6 ^{bc}	5.1 ± 1.8 ^a	5.4 ± 2.0 ^{ab}	0.01
Overall acceptability	6.8 ± 1.6 ^b	6.3 ± 1.6 ^{ab}	5.4 ± 2.0 ^a	5.4 ± 1.9 ^a	0.01

All values are means ± SE, n=3.

Values in the same row bearing different superscript letters are significantly different

In terms texture, the panelists sensed a soft texture in sausages containing 3 and 4 % seaweed powder. The soft texture is probably due to the dietary fiber in seaweed which disintegrates upon heating during sausage cooking.

4.6.1. Preference to buy chicken sausages

Figure 4.5 shows how the panelist rated the chicken sausages in terms of preference to buy

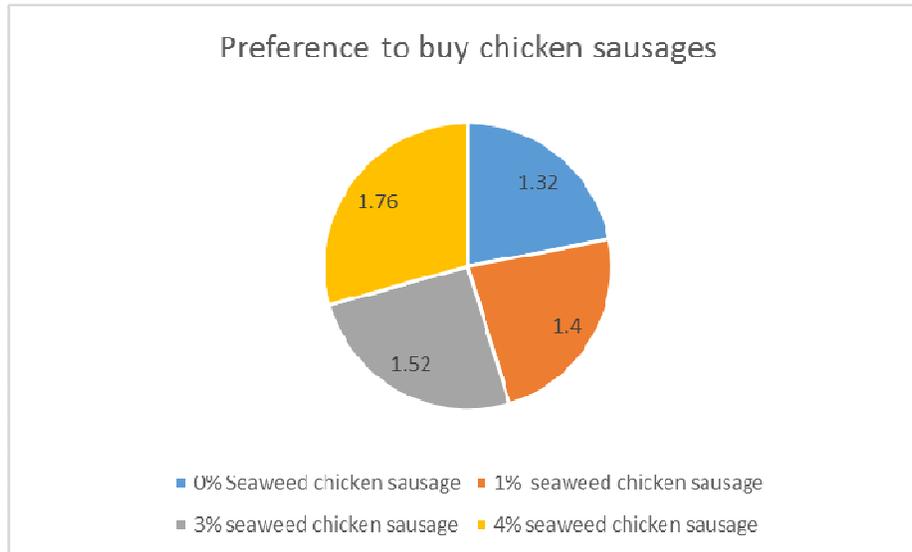


Figure 4.5: Preference to buy chicken sausages

Based on taste, hardness, aftertaste and overall acceptability the 0% and 1% seaweed were more liked compared to 3% and 4% seaweed chicken sausage. However, more consumers preferred to buy the 3% and 4% seaweed chicken sausage (Figure 4.4) and this could have been attributed to the redness of the chicken sausages which influenced their perception. Therefore, incorporation of a well thought and determined proportion of seaweed is critical in influencing the acceptability and suitability of the chicken sausages.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

This study revealed that the chemical composition of the selected seaweeds could be a good source of dietary fibre, proteins and carbohydrates. The seaweeds have potentially good mineral content especially calcium and magnesium compared to conventional vegetables. For this reason, seaweeds could be consumed as food supplement to aid in achieving the recommended daily intake of macro and trace minerals. Lead was not detected in the seaweed species however cadmium was detected and this could be due to sea contamination. In the seaweed species, the SFA were the highest followed by MUFA and finally PUFA. The emulsion activity of the seaweeds were similar to that of soybean and even higher than that of lupin seeds. The water holding capacities of the seaweeds were relatively higher than that of commercial fibre products. These characteristics show that seaweeds can be incorporated in food products to provide nutrients as well as improve the functional properties, specifically *Eucheuma denticulatum* due to its high ash content, crude fat content, calcium, zinc and iron and high emulsion activity.

The incorporation of seaweed in chicken sausage formulation was shown to improve the chemical, physical and sensory characteristics of these samples. Specifically; there was increase in PUFA, ash content, potassium and Iron. The addition of seaweed was also shown to significantly decrease the cholesterol content in the chicken sausages. The redness of the chicken sausages was also high probably due to the red colour of *Eucheuma denticulatum*. Higher concentrations of seaweed in chicken sausages seemed to negatively influence the sensory attributes such as taste, hardness and aftertaste since the chicken sausage with 0% and 1% seaweed were more liked. The obtained results especially in cholesterol reduction may provide practical applications in chicken sausage and become a positive impact on consumers' health therefore seaweed has shown the potential of being utilized in formulation of meat based products.

In general, the hypothesis that guided the study is not supported by the results and *Eucheuma denticulatum* seemed to have an impact on the physico- chemical and sensory properties of the chicken sausages.

5.2 Recommendations

Based on the results of this study, the following recommendations are advanced:

1. There is need for heat treatment of seaweed before incorporation in food products to avoid microbial cross contamination and grow seaweed in safe environment that mimics the ocean environment to avoid heavy metal contamination
2. There is need to conduct more chemical analysis of seaweeds in Kenya e.g. amino acids profile and extraction of protein for human protection in order to increase seaweed utilization
3. The growing of seaweeds needs upscaling in Kibuyuni, Mkwiro and Nyumba sita in order to have consistent production for commercial uses and thereafter food processors can have access to utilize in their food processing.
4. Further research should be conducted to investigate functional properties and other parameters such as phytochemical and antioxidant properties of other seaweeds along the Kenyan coast.

REFERENCES

- Ahmad, S., & Srivastava, P. K. (2007). Quality and shelf life evaluation of fermented sausages of buffalo meat with different levels of heart and fat. *Meat Science*, 75(4), 603-609.
- American Heart Association, (2004). Heart and Stroke Encyclopedia. Dietary Guidelines for Healthy American Adults. Cholesterol, Retrieved from: <http://www.americanheart.org>.
- Andrés, S. C., Garcia, M. E., Zaritzky, N. E., & Califano, A. N. (2006). Storage stability of low-fat chicken sausages. *Journal of Food Engineering*, 72(4), 311-319.
- Andersson, L. & Hansson, K. E. (1979). Use of a penetrometer to determine firmness in emulsion-type sausages. 25th European Meeting of Meat Research Workers, Budapest, 333-35.
- AOAC (2011). Official methods of analysis of the Association of Official Analytical Chemists, Retrieved from: <http://www.eoma.aoac.org/Methods/>.
- AOAC (2005). Official methods of analysis of the Association of Official Analytical Chemists .International Retrieved from;<http://www.eoma.aoac.org/Methods/>
- AACC, (1983). *Approved methods of the American Association of Cereal Chemistry* (8th Ed.). (Method 88-04). St. Paul, MN: American Association of Cereal Chemistry.
- AFDO, (1990). Association of food and drugs Officials. Safe practices for sausage production manual.
- Avendaño-Reyes, L., Torres-Rodríguez, V., Meraz-Murillo, F. J., Pérez-Linares, C., Figueroa-Saavedra, F., & Robinson, P. H. (2006). Effects of two β -adrenergic agonists on finishing performance, carcass characteristics, and

meat quality of feedlot steers. *Journal of Animal Science*, 84(12), 3259-3265.

Ayadi, M. A., Kechaou, A., Makni, I., & Attia, H. (2009). Influence of carrageenan addition on turkey meat sausages properties. *Journal of Food Engineering*, 93(3), 278–283. <http://doi.org/10.1016/j.jfoodeng.2009.01.033>

Azad, S. A., & Xiang, T. Z. (2012). Suitability of Seaweed Meal Incorporated with *Rhodovulum* sp. Bacterium as Feed Supplement for Finfish Larvae. *Borneo Science*, 30, 57-61.

Babji, A. S., & Yusof, S. C. M. (1995). The nutritional value of some processed meat products in Malaysia. *Malysian Journal of Nutrititon*, 1, 83-94.

Baggio, S. R., & Bragagnolo, N. (2006). The effect of heat treatment on the cholesterol oxides, cholesterol, total lipid and fatty acid contents of processed meat products. *Food Chemistry*, 95(4), 611-619.

Barbucci, R. (2002). Polysaccharides based hydrogels for biological applications. *Macromolecular Symposia*, 204, 37-58.

Barbut, S., & Mittal, G. S. (1992). Use of carrageenans and xanthan gum in reduced fat breakfast sausages. *Food Science and Technology*, 25, 509-513.

Besada, V., Andrade, J. M., Schultze, F., & González, J. J. (2009). Heavy metals in edible seaweeds commercialised for human consumption. *Journal of Marine Systems*, 75(1), 305-313.

Biesalski, H. K., Dragsted, L. O., Elmadfa, I., Grossklaus, R., Müller, M., Schrenk, D. & Weber, P. (2009). Bioactive compounds: Safety and efficacy. *Nutrition*, 25(11), 1206-1211.

Bligh, E.G. & Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. *Canadian Journal Biochemistry Physiology* 37, 911-917

- Bocanegra, A., Bastida, S., Benedi, J., Rodenas, S., & Sánchez-Muniz, F. J. (2009). Characteristics and nutritional and cardiovascular-health properties of seaweeds. *Journal of Medicinal Food*, *12*(2), 236-258.
- Boles, J. A., Mikkelsen, V. L., & Swan, J. E. (1998). Effects of chopping time, meat source and storage temperature on the colour of New Zealand type fresh beef sausages. *Meat Science*, *49*(1), 79-88.
- Bolton, J. J., Oyieke, H. A., & Gwada, P. (2007). The seaweeds of Kenya: Checklist, history of seaweed study, coastal environment, and analysis of seaweed diversity and biogeography. *South African Journal of Botany*, *73*(1), 76–88. <http://doi.org/10.1016/j.sajb.2006.08.006>.
- Bolton, J.J., De Clerck, O., & John, D.M. (2003). Seaweed diversity patterns in sub-Saharan Africa. In Proceedings of the Marine Biodiversity in Sub-Saharan Africa: The Known and the Unknown. *Cape Town, South Africa*, 23. 229-241.
- Brownlee, I., Andrew, F., Anna, H., & Jenny, P. (2012). *The potential health benefits of seaweed and seaweed extracts*. Hauppauge, New York: Nova Science Publishers.
- Candogan, K., & Kolsarici, N. (2003). The effects of carrageenan and pectin on some quality characteristics of low-fat beef frankfurters. *Meat Science*, *64*(2), 199-206.
- Cengiz, E., & Gokoglu, N. (2005). Changes in energy and cholesterol contents of frankfurter sausages with fat reduction and fat replacer addition. *Food Chemistry*, *91*(3), 443-447.
- Cetinkaya, F., Cibik, R., Soyutemiz, G. E., Ozakin, C., Kayali, R., & Levent, B. (2008). Shigella and Salmonella contamination in various foodstuffs in Turkey. *Food Control*, *19*(11), 1059-1063.

- Choi, Y. S., Choi, J. H., Han, D. J., Kim, H. Y., Lee, M. A., Kim, H. W., & Kim, C. J. (2010). Optimization of replacing pork back fat with grape seed oil and rice bran fiber for reduced-fat meat emulsion systems. *Meat Science*, *84*(1), 212-218.
- Choi, Y.S., Park, K.S., Choi, J. H., Kim, H.W., Song, D.H., Kim, J.M., & Kim, C.J. (2010). Physico-chemical properties of chicken meat emulsion systems with dietary fiber extracted from makgeolli lees. *Korean Journal for Food Science of Animal Resources*, *30*(6), 910-917.
- Choi, Y.-S., Choi, J.-H., Han, D.-J., Kim, H.-Y., Kim, H.-W., Lee, M.-A., Chung, H.-J., & Kim, C.-J. (2012). Effects of *Laminaria japonica* on the physico-chemical and sensory characteristics of reduced-fat pork patties. *Meat Science*, *91*, 1-7.
- Chojnacka, K., Agnieszka, S., Zuzanna, W., & Łukasz, T. (2012). Biologically active compounds in seaweed extracts - the prospects for the application. *The Open Conference Proceedings Journal*, *3*, 20-28.
- Cofrades, S. (2009). Low-fat frankfurters enriched with n À 3 PUFA and edible seaweed : Effects of olive oil and chilled storage on physico chemical , sensory and microbial characteristics. *Meat Science*, *83*(1), 148–154. <http://doi.org/10.1016/j.meatsci.2009.04.014>
- Cofrades, S., López-López, I., & Jiménez-Colmenero, F. (2011). *Applications of Seaweed in Meat-Based Functional Foods Handbook of Marine Macroalgae* (pp. 491-499). New York: John Wiley and Sons, Ltd.
- Cofrades, S., López-López, I., Ruiz-Capillas, C., Triki, M., & Jiménez-Colmenero, F. (2011). Quality characteristics of low-salt restructured poultry with microbial transglutaminase and seaweed. *Meat Science*, *87*(4), 373-380.
- Cofrades, S., López-López, I., Solas, M. T., Bravo, L., & Jiménez-Colmenero, F. (2008). Influence of different types and proportions of added edible

seaweeds on characteristics of low-salt gel/emulsion meat systems. *Meat Science*, 79(4), 767-776.

Cox, S., & Abu-Ghannam, N. (2013). Enhancement of the phytochemical and fibre content of beef patties with *Himanthalia elongata* seaweed. *International Journal of Food Science and Technology*, 48(11), 2239-2249.

Dawczynski, C., Schubert, R., & Jahreis, G. (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry*, 103(3), 891–899. <http://doi.org/10.1016/j.foodchem.2006.09.041>

Deda, M. S., Bloukas, J. G., & Fista, G. A. (2007). Effect of tomato paste and nitrite level on processing and quality characteristics of frankfurters. *Meat Science*, 76, 501–508.

Durmaz, Y., Duyar, H. A., Gökpinar, S., Taskaya, L., Öğretmen, Y. O., Bandarra, N. M., & Nunes, M. L. (2008). Fatty Acids, α -tocopherol and total pigment contents of *Cystoseira* spp., *Ulva* spp. and *Zosteraspp.* from Sinop Bay (Turkey). *International Journal of Natural and Engineering Sciences*, 2(3), 111-114.

Dzudie, T., Scher, J., Tchiégang, C., & Hardy, J. (2005). Effect of fat sources on the physico-chemical nutritional and textural properties of beef sausages. *Journal of Food Technology*, 3(2), 220-225.

El Gamal, A.A. (2010). Biological importance of marine algae. *Saudi Pharmaceutical Journal*, 18(1), 1-25.

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., & Attia, H. (2011). Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. *Food chemistry*, 124(2), 411-421.

Erdem, B., Ercis, S., Hascelik, G., Gur, D., & Aysev, A.D. (2005). Antimicrobial resistance of *Salmonella enterica* group C strains isolated from humans in

Turkey, 2000–2002. *International Journal of Antimicrobial Agents*, 26(1), 33-37.

Fellows, P. J. (2000). Properties of foods and processing theory, p. 26– 43. In P. J. Fellows (ed.), *Food processing technology: principles and practice*, (vol. 1.) Boca Raton, FL: CRC Press.

Feng, T., Ye, R., Zhuang, H., Rong, Z., Fang, Z., Wang, Y., Gu, Z., & Jin, Z. (2013). Physicochemical properties and sensory evaluation of Mesona Blumes gum/rice starch mixed gels as fat-substitutes in Chinese Cantonese-style sausage. *Food Research International*, 50(1), 85-93.

Fernández-Ginés, J. M., Fernández-López, J., Sayas-Barberá, E., & Pérez-Alvarez, J. A. (2005). Meat Products as Functional Foods. *Journal of Food Science*, 70(2), 37-43. <http://dx.doi.org/10.1111/j.1365-2621.2005.tb07110.x>

Fernández-Martín, F., López-López, I., Cofrades, S., & Colmenero, F. J. (2009). Influence of adding Sea Spaghetti seaweed and replacing the animal fat with olive oil or a konjac gel on pork meat batter gelation. Potential protein/alginate association. *Meat Science*, 83, 209-217.

Fleurence, J. (1999). Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends in Food Science and Technology*, 10(1), 25–28. [http://doi.org/10.1016/S0924-2244\(99\)00015-1](http://doi.org/10.1016/S0924-2244(99)00015-1)

Fleurence, J., Gutbier, G., Mabeau, S., & Leray, C. (1994). Fatty acids from 11 marine macroalgae of the French Brittany coast. *Journal of Applied Phycology*, 6(5-6), 527–532. <http://doi.org/10.1007/BF02182406>

Frikha, F., Kammoun, M., Hammami, N., Mchirgui, R. A., Belbahri, L., Gargouri, Y., & Ben-Rebah, F. (2011). Chemical composition and some biological activities of marine algae collected in Tunisia *Ciencias Marinas*, 37(2), 113-124.

- García, M. L., Cáceres, E., & Selgas, M. D. (2007). Utilisation of fruit fibres in conventional and reduced-fat cooked-meat sausages. *Journal of the Science of Food and Agriculture*, 87(4), 624-631.
- García-Segovia, P., Andrés-Bello, A., & Martínez-Monzó, J. (2007). Effect of cooking method on mechanical properties, color and structure of beef muscle (Pectoralis major). *Journal of Food Engineering*, 80(3), 813-821.
- Georgantelis, D., Ambrosiadis, I., Katikou, P., Blekas, G., & Georgakis, S. A. (2007). Effect of rosemary extract, chitosan and α -tocopherol on microbiological parameters and lipid oxidation of fresh pork sausages stored at 4 °C. *Meat science*, 76(1), 172-181.
- Goñi, I., & Martín-Carrón, N. (1998). In vitro fermentation and hydration properties of commercial dietary fiber-rich supplements. *Nutrition Research*, 18(6), 1077-1089.
- González-Tenorio, R., Fernández-Diez, A., Caro, I., & Mateo, J. (2012). *Comparative Assessment of the Mineral Content of a Latin American Raw Sausage Made by Traditional or Non-Traditional Processes*. Shanghai, China: Intechopen.
- Gressler, V., Yokoya, N. S., Fujii, M.T., Colepicolo, P., Filho, J.M., Torres, R. P., & Pinto, E. (2010). Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. *Food Chemistry*, 120(2), 585–590. <http://doi.org/10.1016/j.foodchem.2009.10.028>
- Gunter, H. (2007). *Meat processing technology for small- to medium-scale producers*. Bangkok: Food and Agriculture Organizations of the United Nations, Regional Office for Asia and the Pacific

- Gurtler, J.B., Kornacki, J.L., & Beuchat, L.R. (2005). Enterobacter sakazakii: a coliform of increased concern to infant health. *International Journal of Food Microbiology*, 104(1), 1-34.
- Hanrahan, W. (2010). *Understanding and developing qualitative research methods in Kenya*. Unpublished PhD dissertation, United Kingdom: University of Warwick.
- Hoz, L., D'arrigo, M., Cambero, I., & Ordóñez, J. A. (2004). Development of an n-3 fatty acid and α -tocopherol enriched dry fermented sausage. *Meat Science*, 67(3), 485-495.
- Huda, N., Wei, L. H., Jean, A. T., & Ismail, I. (2010). Physicochemical properties of Malaysian commercial chicken sausages. *International Journal of Poultry Science*, 9(10), 954-958.
- Hsu, S. Y., & Chung, H. Y. (2001). Effects of κ -carrageenan, salt, phosphates and fat on qualities of low fat emulsified meatballs. *Journal of Food Engineering*, 47, 115-121.
- Hsu, S. Y., & Chung, H.-Y. (1999). Comparison of 13 edible gum-hydrate fat substitutes for low fat Kung-wan (an emulsified meatball). *Journal of Food Engineering*, 40, 279-285
- Iwegbue, C. M. A., Nwajei, G. E., & Iyoha, E. H. (2008). Heavy metal residues of chicken meat and gizzard and turkey meat consumed in southern Nigeria. *Bulgarian Journal of Veterinary Medicine*, 11(4), 275-280.
- Jeun-Horng, L., Yuan-Hui, L., & Chun-Chin, K. (2002). Effect of dietary fish oil on fatty acid composition, lipid oxidation and sensory property of chicken frankfurters during storage. *Meat Science*, 60(2), 161-167.
- Jimenez, C.F. (2000). Relevant factors in strategies for fat reduction in meat products. *Trends in Food Science and Technology*, 11(2), 56-66.

- Jiménez-colmenero, F., Cofrades, S., López-lópez, I., Ruiz-capillas, C., Pintado, T., & Solas, M. T. (2010). Technological and sensory characteristics of reduced / low-fat, low-salt frankfurters as affected by the addition of konjac and seaweed. *Meat Science*, 84(3), 356–363. <http://doi.org/10.1016/j.meatsci.2009.09.002>.
- Jiménez-Colmenero, F. (2007). Healthier lipid formulation approaches in meat-based functional foods. Technological options for replacement of meat fats by non-meat fats. *Trends in Food Science and Technology*, 18(11), 567-578.
- Jimenez, C.F. (1996). Technologies for developing low-fat meat products. Trends in *Food Science and Technology*, 7(41), 41-48.
- Jothinayagi, N., & Anbazhagan, C. (2009). Heavy metal monitoring of Rameswaram coast by some Sargassum species. *American-Eurasian Journal of Scientific Research*, 4(2), 73-80.
- Kayama, M., Iijima, N., Kuwahara, M., Sado, T., Araki, S., & Sakurai, T. (1985). Effect of water temperature on the fatty acid composition of *Porphyra* spp. *Nippon Suisan Gakkaishi*, 51(4), 680 - 687.
- Kenya Bureau of Standards, (2009). *Specifications for sausages: Raw or cooked poultry sausages*. Nairobi: KEBS.
- Kelly, J. P. (2012). *Advanced in meat, poultry and seafood packaging*. Sawston, Cambridge: Woodhead.
- Khotimchenko, S. V., Vaskovsky, V. E., & Titlyanova, T. V. (2002). Fatty acids of marine algae from the Pacific coast of North California. *Botanica Marina*, 45(1), 17-22.
- Kim, H. W., Choi, J. H., Choi, Y. S., & Han, D. J. (2010). Effects of sea tangle (*Laminaria japonica*) powder on quality characteristics of breakfast sausages. *Korean Journal of Food Science and Animal Resources*, 30, 55-61.

- Krishnaiah, D., Sarbatly, R., Prasad, D. M. R., & Bono, A. (2008). Mineral content of some sea weeds from Sabah's South China Sea. *Asian Journal of Scientific Research*, 1(2), 166-170.
- Kristensen, L., & Purslow, P. P. (2001). The effect of ageing on the water-holding capacity of pork: role of cytoskeletal proteins. *Meat science*, 58(1), 17-23.
- Kumar, K.S., Ganesan, K., Selvaraj, K., & Rao, P.S. (2014). Studies on the functional properties of protein concentrate of *Kappaphycusalvarezii* (Doty) Doty—An edible seaweed. *Food Chemistry*, 153, 353-360.
- Kumar, M., Kumari, P., Trivedi, N., Shukla, M. K., Gupta, V., Reddy, C. R. K., & Jha, B. (2011). Minerals, PUFAs and antioxidant properties of some tropical seaweeds from Saurashtra coast of India. *Journal of Applied Phycology*, 23(5), 797-810.
- Kumari, P., Bijo, A. J., Mantri, V. A., Reddy, C. R. K., & Jha, B. (2013). Fatty acid profiling of tropical marine macroalgae: An analysis from chemotaxonomic and nutritional perspectives. *Phytochemistry*, 86, 44–56. <http://doi.org/10.1016/j.phytochem.2012.10.015>
- López-lópez, I., Cofrades, S., & Jiménez-Colmenero, F. (2009). Low-fat frankfurters enriched with n-3 PUFA and edible seaweed: Effects of olive oil and chilled storage on physicochemical, sensory and microbial characteristics. *Meat Science*, 83, 148-154.
- Lambert, N. (2001). *Diet, phytochemicals and cancer*. In: *Food choice in Europe* (pp. 131-151). Munich, Germany: Springer Verlag.
- Lawless, H.T., & Heymann, H. (2010). *Sensory evaluation of food: principles and practices*. Washington, DC: Springer Science and Business Media.

- Limberger, V.M., Brum, F.B., Patias, L.D., Daniel, A.P., Comarela, C.G., Emanuelli, T., & Silva, L.P.D. (2011). Modified broken rice starch as fat substitute in sausages. *Food Science and Technology*, 31(3), 789-792.
- Lin, K.W., & Huang, H.Y. (2003). Konjac/gellan gum mixed gels improve the quality of reduced-fat frankfurters. *Meat Science*, 65(2), 749-755.
- Liu, M. N., Huffman, D. L., & Egbert, W. R. (1991). Replacement of beef fat with partially hydrogenated plant oil in lean ground beef patties. *Journal of Food Science*, 56(3), 861-862.
- López-López, I., Bastida, S., Ruiz-Capillas, C., Bravo, L., Larrea, M.T., Sánchez-Muniz, F., & Jiménez-Colmenero, F. (2009). Composition and antioxidant capacity of low-salt meat emulsion model systems containing edible seaweeds. *Meat Science*, 83(3), 492-498.
- MacArtain, P., Gill, C.I., Brooks, M., Campbell, R., & Rowland, I.R. (2007). Nutritional value of edible seaweeds. *Nutrition Reviews*, 65(12), 535-543.
- Mariam, I., Iqbal, S., & Nagra, S.A. (2004). Distribution of some trace and macrominerals in beef, mutton and poultry. *International Journal of Agriculture and Biology*, 6(5), 816-820.
- Marinho-Soriano, E., Fonseca, P. C., Carneiro, M. A. A., & Moreira, W. S. C. (2006). Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresource Technology*, 97(18), 2402-2406.
- Matanjun, P., Mohamed, S., Mustapha, N.M., & Muhammad, K. (2009). Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. *Journal of Applied Phycology*, 21(1), 75–80. <http://doi.org/10.1007/s10811-008-9326-4>

- McDermid, K. J., Stuercke, B., & Balazs, G. H. (2007). Nutritional composition of marine plants in the diet of the green sea turtle (*Cheloniemydas*) in the Hawaiian Islands. *Bulletin of Marine Science*, 81(1), 55-71.
- Mead, P.S., Slutsker, L., Dietz, V., Mccaig, L.F., Bresee, J.S., & Shapiro, C., (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, 5(5), 607–625.
- Méndez-Zamora, G., García-Macías, J. A., Santellano-Estrada, E., Chávez-Martínez, A., Durán-Meléndez, L. A., Silva-Vázquez, R., & Quintero-Ramos, A. (2015). Fat reduction in the formulation of frankfurter sausages using inulin and pectin. *Food Science and Technology (Campinas)*, 35(1), 25-31.
- Mendis, E., & Kim, S. K. (2011). Present and future prospects of seaweeds in developing functional foods. *Advances in Food and Nutrition Research*, 64, 1-15.
- Menon, V.V. (2011). *Seaweed Polysaccharides–Food Applications*. In: *Handbook of Marine Macroalgae* (pp. 541-555). London: John Wiley and Sons, Ltd.
- Mielnik, M. B., Aaby, K., Rolfsen, K., Ellekjær, M. R., & Nilsson, A. (2002). Quality of comminuted sausages formulated from mechanically deboned poultry meat. *Meat Science*, 61(1), 73-84.
- Mittal, G.S., & Barbut, S. (1992). Effects of fat reduction on frankfurters' physical and sensory characteristics. *Food Research International*, 27, 425-431.
- Mohamed, G.A., Ibrahim, S. R. M., Elkhayat, E.S., & El Dine, R.S. (2014). Natural anti-obesity agents. *Bulletin of Faculty of Pharmacy, Cairo University*, 52(2), 269-284.
- Mohammadi, M., & Oghabi, F. (2012). Development of low-fat and low-calorie beef sausage using modified starch as fat replacement agent. *Journal of the Science of Food and Agriculture*, 92(6), 1291-1296.

- Moreira, A.R.S., García-Fernández, R.A., Bocanegra, A., Méndez, M.T., Bastida, S., Benedí, J., & Sánchez-Muniz, F.J. (2013). Effects of seaweed-restructured pork diets enriched or not with cholesterol on rat cholesterolaemia and liver damage. *Food and Chemical Toxicology*, *56*, 223-230.
- Morin, L.A., Temelli, F., & McMullen, L. (2004). Interactions between meat proteins and barley (*Hordeum*spp.) β -glucan within a reduced-fat breakfast sausage system. *Meat Science*, *68*, 419-430.
- Muhammad, S.A., Muhammad, J., Muhammad, S., Muhammad, K.P., Shaista, H., & Viqar, U. A. (2000). Metabolites of marine algae collected from Karachi-coasts of Arabian Sea. *Natural Product Sciences*, *6*(2), 61-65.
- Muraguri, E.N., Wakibia, J.G., & Kinyuru, J.N. (2016). Chemical Composition and Functional Properties of Selected Seaweeds from the Kenya Coast. *Journal of Food Research*, *5*(6), 114 -125.
- Msuya, F.E., Buriyo, A., Omar, I., Pascal, B., Narrain, K., Ravina, J., & Wakibia, J.G. (2014). Cultivation and utilisation of red seaweeds in the Western Indian Ocean (WIO) Region. *Journal of Applied Phycology*, *26*(2), 699-705.
- Msuya, F. E. (2013). Social and economic dimensions of carrageenan seaweed farming in the United Republic of Tanzania. Social and economic dimensions of carrageenan seaweed farming, Fish Aqua Tech Paper, 580, 115-146.
- Mwalugha, H.M., Wakibia, J.G., Kenji, G.M., & Mwasaru, M.A. (2015). Chemical composition of common Seaweeds from the Kenya Coast. *Journal of Food Research*, *4*(6), 28 - 35. <http://doi.org/10.5539/jfr.v4n6p28>

- Naczek, M., Rubin, L. J., & Shahidi, F. (1986). Functional properties and phytate content of pea protein preparations. *Journal of Food Science*, *51*(5), 1245-1247.
- Nishinari, K., & Zhang, H. (2004). Recent advances in the understanding of heat set gelling polysaccharides. *Trends in Food Science and Technology*, *15*, 305-312.
- Pereira, N. R., Tarley, C. R., Matsushita, M., & de Souza, N. E. (2000). Proximate composition and fatty acid profile in Brazilian poultry sausages. *Journal of Food Composition and Analysis*, *13*(6), 915-920.
- Polat, S., & Ozogul, Y. (2009). Fatty acid, mineral and proximate composition of some seaweeds from the northeastern Mediterranean coast. *Italian Journal of Food Science*, *21*(3), 317-324.
- Rahman, S.A., Shamsoul Bahrein, M., Tew, W.A., Abdullah, A., & Babji, A. S. (1997). Komposisi proksimat dan mineral dalam sepi. *Sains Malaysiana*, *26*(3&4), 31-37.
- Rao, P. V. S., Mantri, V. A., & Ganesan, K. (2007). Mineral composition of edible seaweed *Porphyra vietnamensis*. *Food Chemistry*, *102*(1), 215–218. <http://doi.org/10.1016/j.foodchem.2006.05.009>
- Resurreccion, A.V.A. (2004). Sensory aspects of consumer choices for meat and meat products. *Meat Science*, *66*(1), 11-20.
- Robledo, D., & Freile-Pelegrin, Y. (1997). Chemical and mineral composition of six potentially edible seaweed species of Yucatan. *Botanica Marina*, *40*(1-6), 301-306.
- Rohani-Ghadikolaei, K., Abdulalian, E., & Ng, W.K. (2012). Evaluation of the proximate, fatty acid and mineral composition of representative green, brown and red seaweeds from the Persian Gulf of Iran as potential food

and feed resources. *Journal of Food Science and Technology*, 49(6), 774-780.

Roohinejad, S., Koubaa, M., Barba, F.J., Saljoughian, S., Amid, M., & Greiner, R. (2016). Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. *Food Research International*, 1-42.

Rupérez, P. (2002). Mineral content of edible marine seaweeds. *Food Chemistry*, 79(1), 23-26.

Sarkar, S. (2007). Functional foods as self-care and complementary medicine. *Nutritional Food Science*, 37(3), 160-167.

Sartal C. G., Alonso, M. C. B., & Bermejo Barrera, P. (2011). Application of seaweeds in the food industry. *Handbook of Marine Macroalgae: Biotechnology and Applied Phycology*, 522-531.

Sedef, N.E.I., & Sebnem, S. (2012). Food technological applications for optimal nutrition: An overview of opportunities for the food industry. *Comprehensive Reviews in Food Science and Food Safety*, 11(1), 2-12.

Senthil, A., Mamatha, B.S., & Mahadevaswamy, M. (2005). Effect of using seaweed (*Euclima*) powder on the quality of fish cutlet. *International Journal of Food Sciences and Nutrition*, 56(5), 327-335.

Siddique, M.A.M. (2013). Proximate chemical composition and amino acid profile of two red seaweeds (*Hypnea pannosa* and *Hypnea musciformis*) collected from St. Martin's Island, Bangladesh. *Journal of Fisheries Sciences*, 7(2), 178-186. <http://doi.org/10.3153/jfsc.com.2013018>

Sikes, A.L., Tobin, A.B., & Tume, R.K. (2009). Use of high pressure to reduce cook loss and improve texture of low-salt beef sausage batters. *Innovative Food Science and Emerging Technologies*, 10(4), 405-412.

- Stajić, S., Živkovića, D., Perunovića, M., Šobajićb, S., & Vranićc, D. (2011). Cholesterol content and atherogenicity of fermented sausages made of pork meat from various breeds. *Procedia Food Science*, 1, 568-575.
- Tan, Y.W., Zhang, Y., Bolotin, K., Zhao, Y., Adam, S., Hwang, E.H., & Kim, P. (2007). Measurement of scattering rate and minimum conductivity in graphene. *Physical Review Letters*, 99(24), 246803.
- Thebaudin, J.Y., Lefebvre, A.C., Harrington, M., & Bourgeois, C.M. (1997). Dietary fibres: nutritional and technological interest. *Trends in Food Science and Technology*, 8(2), 41-48.
- Toldrá, F., & Reig, M. (2011). Innovations for healthier processed meats. *Trends in Food Science and Technology*, 22(9), 517-522.
- Tömösközi, S., Lásztity, R., Haraszi, R., & Baticz, O. (2001). Isolation and study of the functional properties of pea proteins. *Journal of Molecular Nutrition and Food Research*, 45(6), 399–401.
- Topçuoğlu, S., Güven, K.C., Kırbaşoğlu, Ç., Güngör, N., Ünlü, S., & Yılmaz, Y.Z. (2001). Heavy metals in marine algae from Şile in the Black Sea, 1994–1997. *Bulletin of Environmental Contamination and Toxicology*, 67(2), 288-294.
- Trespalcios, P., & Pla, R. (2007). Simultaneous application of transglutaminase and high pressure to improve functional properties of chicken meat gels. *Food Chemistry*, 100(1), 264-272.
- Tsai, T.C., & Ockerman, H.W. (1981). Water binding measurement of meat. *Journal of Food Science*, 46(3), 97-701. <http://dx.doi.org/10.1111/j.1365-2621.1981.tb15328.x>
- Tuni, O.M., Carbajal, Á., Forneiro, L.C., & Vives, C.C. (2008). *Tablas de composición de alimentos*, Spain: Ediciones Pirámide.

- Turhan, S., Sagir, I., & Ustun, N. S. (2005). Utilization of hazelnut pellicle in low-fat beef burgers. *Meat Science*, *71*(2), 312-316.
- Uluozlu, O. D., Tuzen, M., Mendil, D., & Soylak, M. (2009). Assessment of trace element contents of chicken products from Turkey. *Journal of Hazardous Materials*, *163*(2), 982-987.
- United States Department of Agriculture (USDA), (2016). National Nutrient Database for Standard Reference Release 28. Retrieved from: <https://ndb.nal.usda.gov/ndb/search/list>.
- USNIH, (2016). *United States National Institute of Health. Strengthening knowledge and understanding of dietary supplements*. USA: USNIH.
- Verma, S.P., & Sahoo, J. (2000). Improvement in the quality of ground chevon during refrigerated storage by tocopherol acetate preblending. *Meat Science*, *56*(4), 403-413.
- Viuda-Martos, M., López-Marcos, M.C., Fernández-López, J., Sendra, E., López-Vargas, J.H., & Pérez-Álvarez, J.A. (2010). Role of Fiber in Cardiovascular Diseases: A Review. *Comprehensive Reviews in Food Science and Food Safety*, *9*(2), 240-258.
- Wei, Y., Liu, Q., Yu, J., Feng, Q., Zhao, L., Song, H., & Wang, W. (2015). Antibacterial mode of action of 1, 8-dihydroxy-anthraquinone from *Porphyrahaitanensis* against *Staphylococcus aureus*. *Natural Product Research*, *29*(10), 976-979.
- Wong, K. H., & Cheung, P.C. (2000). Nutritional evaluation of some subtropical red and green seaweeds: Proximate composition, amino acid profiles and some physico-chemical properties. *Food Chemistry*, *71*(4), 475-482.
- Wong, M.H., Kwok, T.T., & Ho, K.C. (1982). Heavy metals in *Ulva lactuca* collected within Tolo Harbour, an almost landlocked sea. *Hydrobiological Bulletin*, *16*(2-3), 223-230.

- Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C., & Attia, H. (2011). Chemical composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia. *Food Chemistry*, *128*(4), 895–901.
- Yang, H.S., Choi, S.G., Jeon, J.T., Park, G.B., & Joo, S.T. (2007). Textural and sensory properties of low fat pork sausages with added hydrated oatmeal and tofu as texture-modifying agents. *Meat Science*, *75*(2), 283-289.
- Yılmaz, I., Şimşek, O., & Işıklı, M. (2002). Fatty acid composition and quality characteristics of low-fat cooked sausages made with beef and chicken meat, tomato juice and sunflower oil. *Meat Science*, *62*(2), 253-258.
- Zaritzky, N., Andrès, S., & Califano, A. (2006). The effect of whey protein concentrates and hydrocolloids on the texture and colour characteristics of chicken sausages. *International Journal of Food Science and Technology*, *41*(8), 954-961.

APPENDICES

Appendix 1: Sensory evaluation questionnaire

CONSENT FORM

Date: __ __

Panellist Code: _____

SENSORY EVALUATION OF COOKED CHICKEN SAUSAGE

You are invited to participate in a research study of perception of cooked chicken sausage. We ask that you read this form and ask any questions that you may have before agreeing to be in the study.

This is a voluntary exercise to determine acceptability of a newly developed chicken sausage.

Please do not participate in the study if you have any allergic reaction or intolerance to **Seafood and Gluten**.

The results of your performance as a panellist will be kept strictly confidential.

Kindly fill in your details in the section below

SEX

Male Female

AGE: (yrs.)

Less or equal to 20 21-25; 26-30; 31-35;
36-40; 41 and above

How often do you consume chicken sausage?

Daily; Weekly; Fortnightly; Monthly; Never

STATEMENT OF CONSENT

I have read the information about the conditions of this sensory evaluation and all my concerns about the study have been addressed. I hereby give my voluntary consent for participation in this study.

Name: _____

Signature: _____

SENSORY EVALUATION QUESTIONNAIRE

Date: __ __

Instructions

You have been provided with three coded samples of sausages.

Please take a sip of water to clean your palate before and after tasting the sample.

Taste the sausage and hold in the mouth while chewing for 5 seconds.

Record your perception in the scale below by ticking in the box against each statement. Please look and taste each of the (4) coded sausage samples. Indicate how much you like or dislike each sample by checking the appropriate sample attribute and indicate your reference (1-9) in the column against each attribute. Put the appropriate number against each attribute

9 – Like extremely

8 – Like very much

7- Like moderately

6- Like

5- Neither like nor dislike

4- Dislike

3- Dislike moderately

2- Dislike very much

1- Dislike extremely

Attributes	Sample Codes			
	S01	S02	S03	S04
Color				
Sensory taste				
Aroma				
Hardness(texture)				
After taste				
Overall acceptability				
Would you prefer to buy a product?	Yes/ No	Yes/ No	Yes/ No	Yes/ No

Additional comments:-

Thank you for participating in the study.