EFFECTS OF RIGOR STATE, CURING TEMPERATURE AND SPICE EXTRACTS ON THE QUALITY AND SHELF-LIFE OF BROILER MEAT PRODUCTS

By

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NIGERIA.

JULY, 2014.

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JULY, 2014

DECLARATION

I declare that the work in this thesis "Effects of rigor state, curing temperature and spice extracts on the quality and shelf-life of broiler meat products" was carried out by me in the Department of Animal Science, Ahmadu Bello University. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other university.

……. Date ……

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CERTIFICATION

This thesis entitled EFFECTS OF RIGOR STATE, CURING TEMPERATURE AND SPICE EXTRACTS ON THE QUALITY AND SHELF-LIFE OF BROILER MEAT PRODUCTS by Maryam Hajia HARUNA meets the regulations governing the award of the degree of Masters of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to Allah the most beneficent and most merciful without whom I will not be where I am today; His grace and light has brought me this far and saw me through in my times of struggle.

To my late dad and beloved mum Mr and Mrs Haruna Abdul; for your relentless and sufficient prayers, encouragement and support all the way, I will always love you. May jannatul-firdaus be your home, amin.

To Aisha Haruna Kolo, Jamila Haruna, Imran Haruna and Arome A. Musa, you are a manifestation of Allah's favour and guidance upon me, may He also guide and protect you at all times.

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ABSTRACT

Two studies were conducted using 20 broiler chickens with the aim of improving the yield, quality and shelf-life of meat products. In the first study, eight broiler chickens were used to investigate the effects of rigor state and curing temperature on yield and quality of smoke-cooked meat using a 2 x 2 factorial arrangement. The results showed that the yield was not significantly ($P>0.05$) affected by rigor states but was affected ($P<0.05$) by curing temperatures. The yield of broiler meat was higher in cold cure (14.60%) than in hot cure (6.31%). Furthermore, the interaction between curing temperature and rigor state was also significant ($P<0.05$) with post rigor and cold cure interacting best with a yield of 15.88%. Effect of rigor on sensory properties of the product showed no significant difference ($P>0.05$) except for flavour which was higher (2.17) in the pre-rigor carcasses, curing temperature showed no significant effect ($P>0.05$) while their interaction effect was significant ($P<0.05$) for the parameters and ranged between 1.75 and 1.92, 2.00 and 2.06, 2.03 and 2.20, 1.56 and 1.74, 2.03 and 2.20,2.44 and 2.64 for texture, juiciness, colour, saltiness, smoky flavour and general acceptability respectively were pre-rigor hot cure meat was most preferred. In the second study, five kilogrammes of manually deboned and comminuted broiler meat was used to determine the antioxidant and antimicrobial activities of cloves, ginger, pepper, black pepper and African nutmeg extracts on the quality and shelf-life of nuggets using a completely randomised design. The results for the antioxidant effect of spice extracts revealed that lipid oxidation of chicken nuggets was improved by the inclusion of spice extracts with cloves, ginger, pepper and their combinations performing better ($P<0.05$) than the other spices. Total plate count, coliform count and fungal count were significantly ($P<0.05$) reduced with the addition of extracts during storage. It is

concluded that optimum yield can be achieved through cold curing in post- rigor state. More so, addition of spice (cloves, ginger, and pepper) extracts led to retarded oxidative rancidity and microbial growth during refrigerated storage, thereby improving the quality and shelf-life of chicken nuggets.

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CHAPTER ONE

1.0 INTRODUCTION

The demand for livestock products, including poultry is increasing in West Africa as a result of population growth and urbanisation, but the sector faces constraints. The constraints include safety concerns due to inadequate sanitary controls, processing technology and marketing, adversely affecting the supply of meat, an important source of animal protein in the region (Dupaigre *et al.*, 2004).

Meat is the flesh of animals used as food (Lawrie and Ledward, 2006), and is one of the most perishable commercial foods (Aymerich *et al*., 2008). The nourishing components of meat are susceptible to degradable modification by microbiological and physico–chemical agents. In fact, meat represents an ideal substratum for the growth of potential pathogens such as *Escherichia coli, Salmonella spp., Listeria monocytogenes, Staphylococcus aureus and Bacillus cereus* (Kotula and Kotula, 2000). It is, therefore, essential that adequate preservation technologies are applied to maintain its safety and quality. Food safety and shelf-life are both important microbial concerns in relation to broiler meat production. The shelf-life of meat is generally determined by appearance, texture, flavour, colour, microbial activity and nutritive value (McMillin, 2008). The production of high-quality meat with good appeal to consumers is expected to translate into increased revenue for meat producers and processors; consequently, boosting the entire meat industry. The quality of meat and its overall acceptability are determined by its juiciness, flavour, tenderness and colour. Reduced product quality results in decreased consumer acceptance (Kotula and Pandya, 1995). Therefore, improving

customer satisfaction and maintaining the consistency of meat products are a major concern and challenge for the meat industry (Behrends *et al.*, 2005).

Meat processing enables the processor to convert low-priced meat cut into high-priced processed product (FAO, 1995). Processing of meat to products also facilitates the packaging, handling, distribution and marketing of the products (Omojola *et al.*, 2004). Meat products include chicken nuggets, frankfurters, sausages, bologna, and local meat products such as *kilishi, suya* and *dambun nama*. Chicken nugget is a comminuted meat product of chicken, which offers an alternative avenue for better utilization of the meat especially from spent chicken. These pre-processed products are ready-to-fry and serve, and are gaining importance in the consumer market. Thermal processing, curing and smoking may be considered as the three most commonly encountered operations in meat preservation and processing (Kordylas, 1990). Current meat-curing practice, based on the ancient art of preserving meat with salt, employs the addition of nitrite along with salt, sugar, reducing agents and phosphates to meat (Rubin *et al.*, 1992). There are two methods of curing namely: wet and dry curing which can be done at the pre-rigor or post-rigor state. In the wet cure method, the curing solution can be either hot or cold. Some researchers (Lauritzsen *et al.*, 1993; Ogunsola and Okubanjo, 2001) have reported that curing temperature and state of rigor prior to curing affects the quality and yield of the processed product. Lauritzsen *et al.* (1993) report on fish showed that pre-rigor salting resulted in a greater reduction in weight, higher water loss and a lower sodium chloride uptake than the post-rigor salted. Pre-rigor curing treatment with hot brine at 45^0C has also been shown to accelerate curing process of rabbit carcass (Ogunsola and Okubanjo, 2001).

Over the past 10 years there has been an on-going development of curing processes with natural ingredients designed to meet consumer demands and safety requirements for natural and organic processed meats (Sebranek *et al*., 2012). Bio-preservation has gained increasing attention as a means of naturally-controlling the shelf-life and safety of meat products (Ogunbanwo and Okanlawon, 2006). The utilization of spices possessing antimicrobial and antioxidant properties in various forms like powder and extract or essential oils have been variously reported (Meena and Sethi, 1997; Subbulakshmi and Naik, 2002; Rajkumar and Berwal, 2003). Examples of such spices are clove, rosemary, cassia bark, liquorice, aniseed, pepper, round cardamom, nutmeg and angelica. Their addition also affects the taste, flavour, texture, water activity, pH and colour of some meat products (Zhang *et al*., 2009; Ibrahima *et al.,* 2011).

1.1 Justification

Over 10 per cent of animal producers in Nigeria are involved in poultry production on small-scale or medium-scale farms. However, availability of quality animal proteins to the populace, especially in the developing countries is still a mirage (Ogunbanwo and Okanlawon, 2006) due to marketing inefficiency at the end of the production season, unstable power supply for preservation of dressed carcass and inadequate knowledge of an alternative processing/preservation procedure. There is also paucity of literature on the interactive effect of rigor state and curing temperature; and organic spices on meat quality and shelf-life of poultry meat in Nigeria.

1.2 Objectives of the study

The objectives of this study were:

1. To compare the effect of rigor state and curing temperature on the yield and meat quality of processed broiler chickens.
2. To determine the antioxidant activities of ginger, African nutmeg, pepper, black pepper and cloves on the quality and shelf-life of chicken nugget, stored at 4^0C for 12 days.
3. To determine the antimicrobial activities of Ginger, African nutmeg, Pepper, Black pepper and Cloves on the quality and shelf-life of chicken nugget, stored at 4^0C for 12 days.

1.3 Hypotheses

The present work was, therefore, designed to evaluate the following alternate hypotheses:

H_A: Pre-rigor and post-rigor hot and cold cure have effect on the quality of smoked-cooked broiler meat.

H_A: The antioxidant activities induced following the incorporation of spice extracts in chicken nugget have effect on the quality and shelf-life of the product.

H_A: The antimicrobial activities induced following the incorporation of spice extracts in chicken nugget have effect on the quality and shelf-life of the product.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Meat industry in Nigeria: An overview of poultry production

Throughout the world, consumption of poultry meat continues to rise in both developed and developing countries. In 1999, global production of broiler chickens reached 40 billion for the first time and, by 2020, poultry is predicted to become the overall meat of choice (Bilgili, 2002). World production of poultry amounted to 72 000 000 tonnes in 2002, which was five times greater than in 1970 (FAO, 2003). In Nigeria, the poultry industry has also been rapidly expanding, increasing from 185,300 MT in 2001 to 268,000 MT in 2011 and projected to reach 400,000 MT by 2021(FAOSTAT, 2013).

The trend is due to the fact that the product is relatively inexpensive and widely available. A major advantage of poultry meat as human food is that there are no major taboos on their consumption. In particular the absence of religious obstacles has facilitated its geographical expansion, popularity and acceptance. In addition, a chicken provides a meal for the average family without the need for a refrigerator to store left-overs. The breakthrough of the poultry industry globally can also be attributed to the ever increasing demand for varieties of new poultry products by consumers and prompt response to these demands from the stand point of the processors to widen the range of poultry based foods on offer. This has also driven the poultry products available in the market from whole and raw products to ready-to-cook (RTC) and ready-to-eat (RTE) products like marinated, spiced preparation, emulsified and formed products. Consumers have also become better informed on a variety of issues, including food safety and quality, and their expectations have risen accordingly (Mead, 2004).

The introduction of chicken nuggets in the early 1980s opened a whole new market for the meat industry. Today, the chicken nugget and patty are some of the most popular convenience poultry items available, being sold at virtually every fast food restaurant and grocery store in the U.S. (Owens, 2001).

2.2 Characteristics and nutritional value of poultry meat

Poultry meats are highly nutritious. It is not only tasteful, easy to prepare and appropriate for different food combinations, but also a natural source of vitamins, minerals, proteins and healthy fats when compared to red meat **(**Leskanich and Noble,1997). It is devoid of fiber with percentage water, protein, lipid, ash and carbohydrate/energy (Cal /100g) contents of fresh chickens of 75.0, 22.80, 0.90, 1.2 and 105 respectively (Heinz and Hautzinger, 2007); 74.4, 22.6, 1.47,1.15 and 406.7 ME (kJ/100g) respectively (Nkukwana *et al.,*2014) . The percentage proximate composition of smoked chicken as reported by Ogunmola *et al.,* (2013) were moisture (0.50), ash (4.00), fat (3.70), protein (68.97), and carbohydrate (22.83). Studies conducted by Yogesh *et al.* (2013) on the proximate analysis of raw chicken nuggets revealed a percentage moisture, protein, lipid, ash and carbohydrate of 62.4, 18.5, 10.7, 1.2, and 7.3 respectively. The studies of Kumar and Tanwar (2011) compared the proximate composition of optimised (clove powder) chicken nuggets and control but observed that the values obtained were not statistically significant.

2.3 Poultry meat products

Several studies have been conducted in many countries of the world on how to increase the per capita consumption of poultry meat, and the most important growth area of recent times has been the development of value-added, further-processed products. Birds grown for meat are sold as whole or processed as cut-up parts, such as drumsticks,

breast meat, thighs, half chicken or quarter chickens. They are either cooked or used in further processing; about 25-30% of broiler meat goes into further processed products. In the USA, for example, less than 10% of all broilers are sold as whole carcasses (Thornton and O'Keefe, 2002). The majority of carcasses are cut up, deboned or further processed as portioned, sliced, ground, flavored, marinated or cooked products. There is also a large production of breaded and coated items.

Suya, tsire, kilishi, balangu, dambu and cured-smoked chicken meat are poultry products which are commonly available in Nigeria, West Africa and other parts of Africa. *Suya*/ *tsire*, is a spicy shish kebab like skewered meat which is a popular food item in various parts of Nigeria and West Africa (Eke *et al*., 2013), but *tsire* may not necessarily be skewered. The thinly sliced meat is marinated in various spices which include groundnut cake, salt, vegetable oil, and other flavourings, and then barbecued (Egbebi, 2014). A dried version of *suya* is *kilishi*, except that the slices are thinner (Agence France-Presse, 2012), while *dambu* is a dry, shredded and spiced meat product which can also be made from poultry meat.

Innovation in product development has resulted in a wide variety of combinations of curing, smoking, and cooking. Cured-smoked meat products possess a unique, delicate flavour and pink colour with increased refrigerator storage life (Ogunsola and Okubanjo, 2001). Other chicken products that are readily available and have gained world-wide acceptance includes chicken frankfurter, chicken bologna, chicken sandwiches and chicken hot dogs, chicken sticks or fingers (muscle strips) or chicken schnitzel (breast muscle slices), chicken soups (Darre, 2014). In addition to chicken sausages, the chicken meat industry also developed new value added products such as chicken nuggets, which contributed significantly to meeting the global increase in

demand for poultry and has been identified as the best way to increase poultry meat consumption. These pre-processed products are ready-to-fry and serve, and are gaining importance in the consumer market. Improvement of such product quality widens the marketability of chicken products. The characteristic of such products is the coating of meat surfaces with flour, fat/flour mixes and/or breadcrumbs (Yogesh *et al.*, 2013). It can be prepared from either whole muscle trimmings or specific muscle parts depending on the locality. In the U.S., the breast meat is usually chosen because of the uniformity and colour. The dark part of the meat can be incorporated into the formulation in the ratio 70:30 to improve flavour (due to fat content) and reduce cost. Dark meat increases the susceptibility of the product to oxidative rancidity due to the fat and iron content. It also makes the nugget darker and softer (Owens, 2001).

2.4 Meat quality and safety

Meat quality is a generic term used to describe properties and perceptions of meat. It includes attributes such as carcass composition and conformation, the eating quality of the meat, health issues associated with meat such as microbial contamination, diseases and production-related issues including animal welfare and environmental impact. These factors combined to give an overall assessment of meat quality by the ultimate arbiter, the consumer (Boleman *et al.,* 1997).

Many factors have been reported to affect meat quality before and after slaughter and these factors play important part in deterioration of meat quality (Adzitey, 2011). The pre-slaughter factors affecting meat quality includes stress (Miller, 2002), genotype (Baeza *et al*., 2002), sex (Zanusso, 2002), age (Sonaiya *et al*., 1990), feeding (NRC, 1994), rearing condition and production practices (Debut *et al*., 2003) while the post slaughter factors affecting meat quality includes stunning, electrical stimulation, hot

processing and boning, hanging methods, chilling rate, ageing time, packaging and cooking (Cerveny *et al*., 2009).

There are three main mechanisms of spoilage of meat and meat products after slaughtering and during processing and storage; microbial spoilage, lipid oxidation and autolytic enzymatic spoilage (Dave and Ghaly, 2011).

2.4.1 **Microbial spoilage**

Meat and meat products provide excellent growth media for a variety of microflora (bacteria, yeasts and molds) some of which are pathogens (Kotula and Kotula, 2000; Jay *et al*., 2005). Russell *et al*. (1996) stated that a favorable pH for the growth of spoilage bacteria for meat is in the range of 5.5-7.0. Slime formation, structural components degradation, off odors and appearance change were found in meat as a result of microbial growth within this pH range. Storage temperature of meat also determines microbial spoilage. Sources of microbial contamination of meat include air of slaughter house, slaughter slabs and utensils, skin and feathers, gastro intestinal tract of the animal, wash water and poor hygienic practices (Conner *et al.*, 2001).

2.4.1.1 ***Causes of spoilage defects***

Multiplication of psychrotrophic bacteria on poultry surface and the action of their extracellular enzymes causes the buildup of metabolic byproducts. Some of these byproducts become detectable as off-odors and slime as bacteria exploit nutrients on the surface of meats. The metabolic byproducts from spoilage bacteria vary depending on the energy source available to them. When populations of bacteria are low, their cells utilize glucose as their primary source of energy which is not usually odorous and do not significantly contribute to spoilage defects. However, as bacterial populations

increase and glucose availability begins to decrease, these bacteria begin utilizing other substrates, such as protein, which yields much more odorous end products. Proteolysis of the skin and muscle tissue begins with low concentrations of glucose and/or gluconate or when they have been exhausted. Initial off-odors result from direct microbial utilization of low molecular weight nitrogenous compounds such as amino acid which are present on the skin and muscle rather than protein in the skin and muscle (Lampropoulou *et al.*, 1996).

Venugopal (1990) reported that bacteria growing on the surface of muscle (meat or fish) secrete a variety of extracellular enzymes that degrade the muscle tissue, causing extensive damage. These enzymes were able to degrade myofibrillar proteins broadly into heavy meromyosin, light meromyosin, and meromysin. Schmitt and Schmidt-Lorenz (1992) demonstrated that there was an increase of low molecular weight peptides (less than 50,000 Da) and free amino acids on chicken carcasses stored at 4°C.

Nychas and Tassou (1997) monitored the progression of spoilage as it relates to depletion of substrates in the muscle tissue. The concentration of glucose decreased progressively and more rapidly toward the end of the storage period and occurred to a greater degree for samples held at higher temperatures. Similar observations were made for concentrations of L-lactate. Concentrations of free amino acids increased as proteolysis occurred throughout the storage period.

2.4.1.2 *Bacteria involved in spoilage of poultry*

The three genera of bacteria that were most commonly isolated and identified from spoiled chicken were *Pseudomonas, Acinetobacter,* and *P. putrefaciens* (Russell, 2001). Pseudomonas fragi and Pseudomonas fluorescens cause quality deterioration of meat

products due to the production of extracellular proteases and lipases at low temperatures (Lebert *et al*., 1998). These bacteria produced off-odours which resembled "sulfur," "dishrag," "ammonia," "wet dog," "skunk," "dirty socks," "rancid fish," "unspecified bad odour," or a sweet smell resembling "canned corn." However, odours produced by the spoilage bacteria were varied. Odours most associated with spoiled poultry, such as "dishraggy" or "sulfurous" odours, were produced by the bacteria that were most consistently isolated, such as *Salmonella putrefaciens* and the pseudomonads (Russell, 2001).

Lactic acid bacteria (LAB) were also identified as the major spoilage population of vacuum packaged emulsion-type sausages and other processed meats stored at refrigerator temperatures (Sakala *et al.*, 2002).

2.4.1.3 *Number of bacteria needed for spoilage*

High numbers (10^5cfu/cm^2) of psychrotrophic spoilage bacteria are required on poultry surfaces before off-flavors, off-odours, and appearance defects can be detected organoleptically. In some studies, off-odours caused by bacteria on uneviscerated broiler carcasses were not detected until bacterial concentrations reached $2.5x10^6$ to $1x10^8$ cfu/cm^2. Dainty and Mackey (1992) determined that proteolysis and slime production under aerobic conditions begins when bacterial numbers reach $10^7$to 10^8 cfu/g.

Other researchers observed that odor and slime were not present until bacteria exceeded $1x10^8$cfu/cm^2 (Rusell, 2001).

2.4.1.4 *Pathogenic microorganisms on processed poultry: Pathogens of concern*

Raw and processed poultry meat naturally harbors bacteria that are responsible for the spoilage of poultry meat. A number of food-borne pathogens have been isolated from processed poultry, of these are, *Salmonella* serotypes, *Campylobacter, L. monocytogenes, C. perfringens,* and *Staphylococcus aureus* are of major concern (Waldroup, 1996; Bryan and Doyle, 1995).

In the *Campylobacter sp*., *C. jejuni and C. lari* comprise the thermotolerant group of the family Campylobacteriaceae (Benefield, 1997). *L. monocytogenes* is a psychrotrophic, gram positive bacillus, facultative anaerobe, that is catalase-positive, oxidase-negative, and a non-spore forming foodborne pathogen (Adams and Moss, 1995) which is sometimes associated with poultry products. *L. monocytogenes* tends to be an environmental contaminant found in damp areas of a poultry processing plant, and can withstand extreme temperatures (as low as −0.4 °C and as high as 45 °C),low pH and high salt (Jay, 2000; Barker and Park, 2001). *L. monocytogene*s could be introduced to cooked meat during slicing and packaging (Enan *et al*., 1996). *C. perfringens* are most prevalent in meat products that provide an anaerobic environment and are held at improper temperatures, this pathogen can enter a growth cycle in which the numbers of spores will double every 15 minutes, and to prevent the germination and outgrowth of *C. perfringens,* cooked meat products must be cooled rapidly to stabilize the product (Conner *et al.*, 2001). Staphylococcal intoxications involving poultry products are mostly related to recontamination of cooked product by food handlers, followed by improper holding temperatures (NACMCF, 1997). *Escherichia coli* O157:H7 is another potential health risk and poultry are highly susceptible to the infection. It is highly pathogenic causing hemorrhagic enteritis in humans (Hargis *et al.,* 2001).

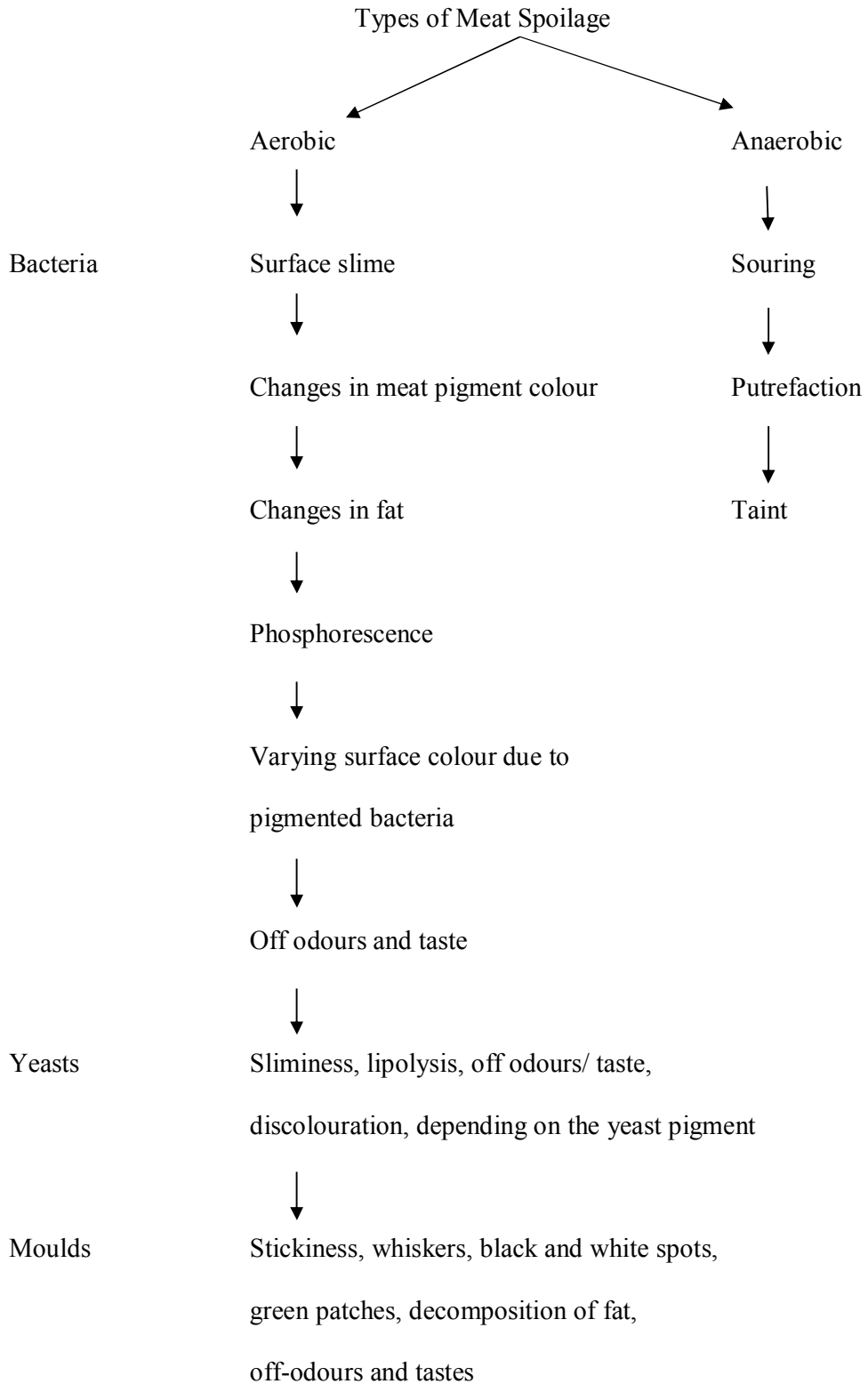


Figure 1: A summary of microbial spoilage of meat

Source: Frazier, (1977).

2.4.2 **Lipid oxidation**

Autoxidation of lipids and the production of free radicals are natural processes which affect fatty acids and lead to oxidative deterioration of meat and off-flavours development (Simitzis and Deligeorgis, 2010). After slaughtering of animals, the fatty acids in tissues undergo oxidation when the blood circulation stops and metabolic processes are blocked (Linares *et al*., 2007). Lipid oxidation is the reaction of oxygen with double bonds of fatty acids (Hultin, 1994). It involves three stages of free radical mechanisms: initiation, propagation and termination (Fernindez *et al*., 1997). Their breakage causes secondary reaction products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (MDA) as well as other oxygenated compounds such as aldehydes, acids and ketones (Fernindez *et al*., 1997). These secondary products can cause loss of colour and nutritive value due to sever effects on lipids, pigments, proteins, carbohydrates and vitamins (Simitzis and Deligeorgis, 2010) and are directly related to carcinogenic and mutagenic processes (Liu *et al*., 1995).

2.4.3 **Enzymatic actions**

These are natural processes in the muscle cells of the animals after they have been slaughtered and are the leading cause of meat deterioration. The enzymes have the ability to combine chemically with other organic compounds and work as catalysts for chemical reactions that finally end up in meat self-deterioration (Tauro *et al*., 1986). In the autolysis process, the complex compounds (carbohydrates, fats and protein) of the tissues are broken down into simpler ones resulting in softening and greenish discoloration of the meat. These autolysis changes include proteolysis and fat hydrolysis which are prerequisite for microbial decomposition. Excessive autolysis is termed "souring" (Dave and Ghaly, 2011).

2.5 Meat processing

Poultry processing is a complex combination of biology, chemistry, engineering, marketing, and economics. The primary aim of meat processing is to convert fresh low priced meat to high priced product, to improve the culinary attribute and nutritive value of the meat, to inhibit meat spoilage and extend shelf life (FAO, 1995). The processing steps to which birds are subjected are designed to produce wholesome and safe final products. It also involves the incorporation of preservative agents which improves the organoleptic properties of the meat.

There are several methods of meat preservation which may be conventionally grouped into three broad categories based on control by temperature, by moisture and, more directly, by inhibitory processes (bactericidal and bacteriostatic), although a particular method of preservation may involve several antimicrobial principles (Zhou *et al.,* 2010)

2.5.1 **Curing/ marination**

Marination is the soaking of meat in vinegar, oils, or both, in combination with spices for improved sensory properties (flavour, colour, moisture and texture), improved functionality of product (water-holding capacity, stability and cooked yield), improved yield for the processor and extended shelf-life. The marinade produces a tenderer product, with more flavour, lower cooking losses and increased juiciness. Marinades were mainly a mixture of salt, organic acids, nitrates and spices in a solution in which the meat was soaked, or with which it was injected prior to smoking and/or curing. Market forms of marinated poultry include whole birds, cut-up parts, and boneless meat. The mechanism of curing through marination in improving meat quality and yield is by chemically binding water within the muscle tissue termed as Water Holding Capacity, which can be water that is either already in the meat or that is added to it. Higher water-

holding capacity usually equates to juicier and more palatable sensory perception and an overall improvement in meat quality. From the processor's perspective increased water-holding capacity equals increased yield, or the sale of water at meat prices. Other benefits include flavor enhancement, both by the addition of spices and flavorings, and through reduction in rancidity that develops during storage. Marinade also improves physical appearance/colour imparted by the inclusion of spice or seasoning particles in the marinade, tenderness has been improved through the inclusion of salt and phosphate, plus calcium chloride and papain enzymes (Young and Lyon 1997).

2.5.2 Smoking and drying

Smoking, drying of meat over fire or under the sun has been used for centuries to preserve meat. The principle behind these preservation methods is based on reducing the water activity to a level low enough to inhibit microbial growth. Also the phenols, ketones, aldehydes and organic acids found in various kinds of wood smoke have antioxidant, bacteriostatic and bactericidal effects and thus can inhibit or kill microorganisms.

Curing and smoking impart a unique, delicate flavour and pink colour to poultry meat and increase the refrigerator storage life. Moisture migration and evaporation at the surface result in product "shrink" or weight loss. Although the yield is reduced, the drying effect during the latter stages of cooking is usually beneficial for enhancing the product's appearance. U.S. Federal regulations specify an internal temperature of 71°C for uncured and 68°C for cured poultry (Code of Federal Regulations, 1999).

2.6 Effect of rigor and temperature on meat quality

The post-mortem temperature of the muscle strongly interacts with the rate of rigor mortis development in determining the quality of poultry meat (Sosnicki *et aI.*, 1998).

Exposure to low temperatures when ATP is still present in the muscle cell, such as prior to rigor mortis development, has been found to toughen the meat through a process termed "cold shortening" (Hamm, 1982). At low temperatures, the sarcoplasmic reticular membranes become less efficient at sequestering Ca^{2+} and allow it to "leak" into the myofibrillar space. If ATP is present, the Ca^{2+} initiates the contraction cycle and causes the sarcomere to shorten. Fast chilling help to reduce microbial growth on carcass surface, evaporative loss, manifestation of PSE meat, improve lean colour and water holding capacity, all of which would have had negative effect on meat quality (Warriss, 2000; Adzitey and Nurul, 2011). Different cooling rates affects pH drop through lactic acid production, the disappearance of adenosine triphosphate (ATP) and creatine phosphate, hasten up the occurrence of rigor mortis (Warriss, 2000). Chilling can also result in weight loss (Stanisz *et al*., 2009). When muscles are cooled below 10°C before the onset of rigor, cold shortening occurs which makes the meat tough upon cooking, and slow freezing may produce cold shortening before freezing whilst rapid freezing may results in thaw rigor (Warriss, 2000). Thaw rigor meat losses large amount of drip or water during thawing and are tough upon cooking. Cooking and packaging function to protect meat from contamination and inhibit microbial growth, increase tenderness, reduce or eliminate evaporative weight loss and surface drying, and enhance the colour of the product (Warris, 2000). Cooking especially (grilling, roasting, smoking) melts fats and some volatiles fatty acids from the meat that are lost by evaporation; also some lipids can be lost in the cooking container or energy source (McCance and Widdowson, 1997).

pH and Water holding capacity (WHC) are physico-chemical parameters used to determine meat quality. Drop in pH is closely associated with the development of rigor

mortis in postmortem muscle. The intracellular osmotic pressure (i.e., ionic strength) increases nearly twofold and has a close relationship with pH during the time course of *rigor mortis* (Ouali, 1991). WHC describes the ability of the meat products to maintain water within its fibres. Mendes (2003) stated that WHC can be measured by the difference in weight of the pectoralis muscle before and after cooking. Pre-rigor meats have high moisture content and water-holding capacity as a result of high pH and ATP (Heinz and Hautzinger, 2007). A high WHC of pre-rigor meat depicts higher yield and better sensory properties (Cia and Marsh, 1976; Ogunsola and Okubanjo, 2001; Okubanjo *et al*., 2010). The state of rigor prior to curing has been shown to affect yield. Hot processing or boning (the removal or cutting of carcasses into parts or meat while it is still hot about 37- 39 °C) can increase yield, promote more uniform colour and better water holding capacity but reduces tenderness, promote abnormal shape of joints and difficulty in handling such meats (Warriss, 2000; Fletcher, 2002). Lauritzsen *et al.* (1993) reported that pre-rigor salting leads to a larger reduction in weight, a higher water loss and a lower uptake of salt in fish. Ogusola and Okubanjo (2001) also reported lower yield in pre-rigor cured rabbit meat.

Curing temperature has also been reported to affect yield and quality of meat. Cold cured carcass has been reported to give higher yield than hot cured carcass, due to the longitudinal shrinkage of the muscle fibre and connective tissue network, with increased temperature which leads to higher water loss (Offer, 1984), but increasing the temperature of curing has been shown to accelerate curing time and also bring about uniformity in cured products. The studies conducted by Ogunsola and Okubanjo (2001) and Owen *et al.* (1986) on rabbit meat revealed a significantly higher yield observed in

the meat cured at 2^0C (conventional curing temperature) than the hot cured carcass (45^0C).

2.7 Bio-preservation: Spices

The aims of preservation methods are to inhibit microbial spoilage and to minimize the oxidation and enzymatic spoilage. Traditional methods of meat preservation such as drying, smoking, brining, fermentation, refrigeration and canning have been replaced by new preservation techniques such as chemical, bio-preservative and non-thermal techniques. Current meat preservation methods are broadly categorized into three methods controlling temperature, controlling water activity and use of chemical or biopreservatives (Zhou *et al*., 2010). A combination of these preservation techniques can be used to diminish the process of spoilage (Bagamboula *et al*., 2004).

Spices are aromatic substances taken from various plant parts (Kochlar, 1986) or herbs. Cloves, nutmeg, pepper, mace, cardamon, coriander and mustard, cinnamon, sage, thyme and marjoram, onion, garlic and ginger are derived from flower bud, fruit, aril (fleshy covering of the seed), aromatic seeds, bark, dried leaves, vegetable bulbs and rhizome respectively. Plant derived spices are generally used in foods for flavouring and medicinal purposes (Zhang *et al.*, 2009). Utilization of spices in various forms like powder, extract or essential oils has been well documented for inhibiting the growth of many spoilage bacteria and fungi in foods (Meena and Sethi, 1997; Subbulakshmi and Naik, 2002; Rajkumar and Berwal, 2003). Spices also contribute to flavour and colour of food (Ekeanyanwu and Etienajirhevwe, 2012)

The aromatic properties of spices are found in the oleoresins and essential (volatile) oils. Piperine, for example, is the oleoresin from pepper (5 to 12%). Essential oils are the

volatile aromatic fractions of spice derived by steam distillation. These compounds contribute to the odour and flavour value and include hydrocarbons, terpenes and sequiterpenes (unstable) while alcohols, esters, aldehydes, and ketones are the main aroma carriers (Flamini *et al.,* 2007). Oussalah *et al*. (2007) evaluated 28 essential oils for their antibacterial properties against four pathogenic bacteria (*E. coli* O157:H7, *L. monocytogenes*, *Salmonella typhimurium* and *S. aureus*) and showed that most of these essential oils possess antibacterial activity while Zhang *et al.*(2009) evaluated 14 spice extracts for their antibacterial properties against four common meat spoilage and pathogenic bacteria (*Listeria monocytogenes, Escherichia coli, Pseudomonas fluorescens and Lactobacillus sake*) in cultured media which revealed their antimicrobial properties . These include eugenol in cloves and allyl isothiocyanate in mustard seed. Other compounds include non-volatile residues such as waxes and paraffins. Oleoresins are thick resinous materials extracted with solvents (acetone, ethanol, isopropyl alcohol, ethylene dichloride, hexane, and petroleum ether) and contain both volatile and non-volatile fractions. These are more complete than essential oils, contain natural antioxidants, are free of enzymes and molds, and can be standardized for flavour and strength. Oleoresins can be made in liquid form when combined with emulsifiers (Polysorbate 80) and monoglycerides to make the oleoresin water soluble. Dry dispersed spices may be placed on soluble carriers like salt, dextrose, flour, or yeast or the oleoresin may be encapsulated with modified food starch, gum Arabic, maltodextrins, gelatin, solid fat, or oil. The use of spices with other food ingredients, such as sodium chloride, sugar and organic acid might provide a synergistic effect in controlling microbial growth (Giese, 1994).

2.7.1 **Ginger (*Zingiber officinale*)**

Ginger is a member of the family zingiberaceae; a small family with less than 45 genera and 800 species. Its scientific name is *Zingiber officinale* (Foster, 2011). It is an erect perennial plant growing from one to three feet in height; its stem is surrounded by the sheathing bases of two ranked leaves. A club-like spike of yellowish, purple lipped flowers has greenish yellow bracks which rarely flowers in cultivation (Tyler, 2002). Ginger is the washed and dried cleaned rhizome of the root stock of *Zingiber officinale* with a greyish skin and a pale yellow fibrous interior. The root of ginger has been widely cultivated for over 3000 years. It is found throughout the tropics especially far eastern countries. Ginger has been reported to have a wide range of medicinal activities against nausea, asthma, cold and headaches. Ginger contains gingerol and shogaol (Wilkinson, 2003). The extracts of ginger could have an anti-fungal, anti-histamine and antibacterial effect (Schulick, 1993). The report of Kikuzaki *et al.* (1994) revealed that most of the isolated compounds from ginger exhibited stronger antioxidant effect by inhibiting lipid oxidation and scavenges super-oxide anions (Lee *et al.*, 1986; Reddy and Lokesh, 1992; Krishnakantha and Lokesh, 1993). Lee *et al.,* 1986 also reported that ginger contains enzyme Zingibain which improves meat tenderness.

2.7.2 **Cloves (*Eugenia caryophyllus*)**

Clove is a naturally occurring spice, which has been shown to possess antioxidant and antimicrobial activity in food products. Eugenol, the essential oil of clove, is a 2-methoxy phenolic derivative, which has been reported to possess 90% of the antioxidant activity of butylated hydroxy anisole (Suresh *et al.*, 1992; Gulcin *et al.*, 2004). Clove has been reported to possess the highest antioxidant index followed by rose petals, cinnamon, nutmeg and other spices in pastourma sausages and also the strongest

antioxidant in an oil/water emulsion (Al-Jalay *et al.*, 1987).The antimicrobial properties of cloves have been documented by various authors; antibacterial (Suresh *et al.*, 1992), antimycotic (Illicim *et al.*, 1998) and yeast inhibitory (Farag *et al.*, 1989) activity. Its inhibitory action against various gram positive and gram negative bacteria in meat and meat products is also recorded (Mannie, 1999). Eugenol also strongly inhibits the growth of *Listeria monocytogenes*, *Salmonella enteritidis*, *Escherichia coli* and *Staphylococcus aureus* in various agar mediums (Cressy *et al*., 2003; Mytle *et al*., 2006). The terpenoids in clove are thought to exert inhibitory action against microorganisms by membrane disruption (Lambert *et al*., 2001). However, to have an inhibitory effect on microorganisms, higher levels of cloves are required in the formulation (Mannie, 1999). Crude extracts of many spices and herbs at higher level impart an objectionable color, odor and taste to food (Draughon, 2004). Kumar and Tanwar (2011) reported that clove powder used at 0.1% level (w/w) of meat emulsion has beneficial effect on physico-chemical and sensory qualities of chicken nuggets stored at refrigeration temperature (4±1°C) for 15 days in Low Density Poly Ethylene bags with good acceptability.

2.7.3 **African nutmeg (*Monodora myristica*)**

Monodora myristica commonly called African nutmeg is a perennial edible of the Annanacea family. It is a berry that grows wild in evergreen West Africa forests (Burubai *et al.*, 2009). Its seeds usually embedded in a white sweet smelling pulp, was reported to possess valuable economic and medicinal value (Okafor, 1987). In Nigeria and other African countries, the kernel obtained from the seeds is a popular spicing agent to improve flavour of food resembling that of nutmeg (*Myristica fragrans*) as well as an aromatic stimulating addition to medicine and snuff (Ekeanyanwu *et al.,* 2010).

Also, the seeds when ground into powder and taken acts as stimulant, relieving constipation and can as well be sprinkled on sores especially those caused by guinea worm (Burkill, 1985).

Monodora myristica is rich in glycosides, flavonoids, saponins and steroids but with very little cyanogenic glycosides, tannins, oxalates and phytates. Flavonoids, saponins and tannins are known to have antimicrobial activity, as well as other physiological activities (Ekeanyanwu *et al.*, 2010). In fact, flavonoids have a wide range of biochemical and pharmacological activities in mammalian and other biological systems. They possess anti-inflammatory, anti-oxidant, anti-allergic, hepatoprotective, anti-thrombic, antiviral and anticarcinogenic activities (Middleton *et al.*, 2000)

2.7.4 **Pepper (*Capsicum spp*.)**

These include a wide variety of spices, among which are hot red pepper, cayenne pepper, paprika and chilli pepper. The small fruited varieties are hotter or more pungent while the large fruited are sweeter or milder. The pungency of the spice is due to capsaicin present in the specie as the active ingredient which is said to increase as the plant matures (Purseglove, 1981). Pepper is a good source of antioxidant, contains vitamin c and beta carotene (Coon, 2003).

2.7.5 **Black pepper (*Piper guineense*)**

This is the unripe fruit of *Capsicum spp*. picked when it begins to turn red and dried. It is the drying process that turns it black and makes it to shrivel. It is used in the meat industry for curing and preservation of meat. Black pepper is mostly used for its characteristic delicate penetrating aroma and pungent biting taste (Yildirim *et al.*, 2000; Kong *et al.,* 2007)

2.8 Effect of spices on TBARS, microbial load and sensory properties of meat

A number of interrelated factors influence the shelf life and keeping quality of meat, specifically holding temperature, atmospheric oxygen (O_2), endogenous enzymes, moisture (dehydration), light and, most importantly, micro-organisms. All of these factors, either alone or in combination, can result in detrimental changes in the physicochemical and culinary properties of the meat (Faustmann and Cassens, 1990). Although deterioration of meat can occur in the absence of micro-organisms through proteolysis, lipolysis and oxidation, microbial growth is by far the most important factor in relation to the shelf life of meat (Lambert *et al*., 1991). Other reports have shown microbial growth and oxidative rancidity as the major problems causing shelf life quality deterioration in stored meat and meat products (Eburne and Prentice, 1996). Also processed products, which are minced, mixed and/or heated, are also reported susceptible to lipid oxidation and off flavor development. In post mortem muscle, during aging of whole muscle products, oxidation of myofibrillar and sarcoplasmic proteins increases (Rowe *et al*. 2004a; 2004b). This results in the conversion of some amino acid residues, including histidine, to carbonyl derivatives and can cause the formation of intra - and/or inter - protein disulfide cross - links (Martinaud *et al*., 1997). In general, both these changes reduce the functionality of proteins in post-mortem muscle (Xiong and Decker 1995). In post mortem muscle, these redox systems may also play a role in influencing meat quality. Oxidation does occur early in postmortem meat, and it does influence proteolysis (Harris *et al*., 2001; Rowe *et al.,* 2004b). Also increase in pH value during storage period causes break-down of meat protein of the product (Yadav and Sanyal, 1999; Kumar and Tanwar, 2011).

Synthetic preservatives have been used in foods for decades; however, an increasing perception by consumers that synthetic compounds may lead to negative health consequences has led to a reduced acceptance for their use in foods (Namiki, 1990). Natural compounds, such as essential oils, oleoresins, chitosan, nisin and lysozyme, have been investigated to replace synthetic preservatives and to obtain 'green label' products. Organically processed meat products are gaining increasing acceptance by consumers (Sloan, 2011) due to the detrimental effects poised by preservative chemicals such as nitrate and nitrite when consumed by humans over time. This is because nitrite can under certain circumstances such as low pH and high temperature react with the amines in the meat to produce nitrosamines which is carcinogenic (Pegg and Shahidi, 2000). This has resulted in an increase in research on using natural or alternative additives, such as plant and animal derivatives (Ennajar *et al.*, 2009). Bio preservatives can be useful in extending shelf life of foods, by reducing or eliminating survival of pathogenic bacteria and increasing overall quality of food products through inhibition of oxidative rancidity.

A number of studies have demonstrated that compounds existing in many spices also possess antimicrobial activity. Examples of such spices are ginger, nutmeg, pepper, black pepper, cassia, clove, garlic, sage, oregano, pimento, thyme, rosemary, scutellaria and forsythia suspense (Yildirim *et al.*, 2000; Kong *et al.,* 2007) and as such naturally extending the shelf-life and safety of meat products (Ogunbanwo and Okanlawon, 2006). Many herbs, spices, and their extracts are known to possess antioxidant effects (Zheng and Wang, 2001; Shan *et al.*, 2005), especially clove (Myrtaceae), cinnamon (*Cinnamomum zeylanicum*), blume (Lauraceae), and oregano (Lamiaceae) (Shan *et al.*, 2005). The antioxidant activity of these plants have been attributed to their phenolic

compound content, resulting in a positive linear correlation between them (Zheng and Wang, 2001; Shan *et al.*, 2005). Species with a strong antioxidant capacity have high phenolic content. One example is clove that had the highest antioxidant capacity as measured by three methods and was related to it having the highest phenolic content (Kumudavally *et al*., 2011).

Shan *et al.* (2005) reported phenolic volatile compounds called essential oils to be the main active ingredients in most spices. The main components of essential oils are terpenoids, specifically monoterpenes (C10) and sesquiterpenes (C15), as well as a variety of low molecular weight compounds (Flamini *et al.*, 2007).

The antioxidant activity of some plants can be affected by number of samples, small differences between the highest and lowest values, method used to extract antioxidant compounds, and method used to measure antioxidant capacity (Su *et al.*, 2007; Tabart *et al.*, 2009). The advantages of natural antioxidants in foods are high consumer acceptance and safety. Other positive effects of natural antioxidants on meat characteristics are retarding lipid oxidation (Fasseas *et al.*, 2007; Camo *et al.*, 2008), color loss and microbial growth (Cam o *et al.*, 2008; Zinoviadou *et al.*, 2009). Some authors have reported that natural antioxidants have no effect on sensory characteristics of meat. Chaves *et al.* (2008) did not detect any effect of essential oil compounds (carvacrol and cinnamaldehyde), added to the diet of growing lambs, on the sensory characteristics of sirloins. The same was observed in pork where different essential oils were included in the diet of pigs (Janz *et al.*, 2007). The only evidence of the effect of natural antioxidants on inhibiting off-odour formation and discoloration of meat is active packaging (Camo *et al.*, 2008). The disadvantages are their higher cost and lower effectiveness (Fasseas *et al.*, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study location

The study was conducted at Ahmadu Bello University, Samaru-Zaria, Kaduna State, located within the Northern Guinea Savannah zone of Nigeria (latitude $11^{o}12^{'}$N and Longitude $7^{o}33^{'}$E) at an altitude of 610 m above sea level (Akpa *et al.*, 2002). The climate is relatively dry, with mean annual rainfall ranging from 700 to 1400 mm and occurring between the months of April and September. The dry season begins around the middle of October, with cold weather that ends in February. This is followed by relatively hot, dry weather from March to sometime in April, when the rains begin. The mean minimum and maximum daily temperatures are 14^{0}C and 24^{0}C, respectively during cool season and 19^{0}C and 36^{0}C, respectively during the hot season. Relative humidity varies between 19 and 35% in the dry season, and between 63 and 80% in the rainy season (Alphonsus *et al.,* 2012).

3.2 Study 1: Effect of rigor state and curing temperature on quality and broiler carcass yield

3.2.1 Slaughter and processing treatments

Eight (8) broiler chickens of about 3.0 kg average live weight and about 12 weeks of age were used in this study. The birds were fasted overnight and weighed prior to slaughter. They were placed in the slaughter cones, the jugular vein and carotid artery were then severed using a sharp knife. The birds were allowed to bleed for about two minutes, weighed to take the bled weight and then scalded using hot water at a temperature of 56^{0}C for two minutes and feathers were removed using a picking machine. The eviscerated weights were taken after evisceration and washing of the

carcasses. Thereafter, the carcasses were split longitudinally into two equal halves. The half carcasses were weighed and immersed in ice for 30 minutes before allotting randomly into four (4) curing treatments: (1) pre-rigor hot cure (2) pre-rigor cold cure (3) post-rigor hot cure (4) post-rigor cold cure.

3.2.2 **Curing**

The half carcasses used for pre-rigor study were cured immediately after chilling before rigor mortis sets in. The post-rigor carcasses were placed in a zip loc bag and transferred to the refrigerator for 24 hours to allow rigor mortis to fully set in before the carcasses were cured. The curing brine was prepared according to the method described by Ogunsola and Okubanjo (2001) with slight modification. The brine composed of 10 litres water, 1020.58g salt, 100g sodium nitrite, 170.10g sugar. Heating of the curing brine for the hot cure treatment was done in a stainless steel medium with the aid of a water bath to a temperature of 35^0C. The carcasses were immersed in the curing brine after injection with the brine solution of the same strength to 110% of the green weight for three (3) hours. The cold cure brine was chilled in a refrigerator at 2^0C. Thereafter, carcasses were immersed in the cold brine after injection with cold brine of the same strength to 110% of the green weight, followed by curing at that temperature for twelve (12) hours.

After the appropriate time schedule for each treatment, the carcasses were rinsed with cold water, drained, weighed and then placed in ziploc bags for equilibration in the refrigerator. This was done to prevent shrinkage until the samples for the longest treatment had gone through its normal schedule (Ogunsola and Okubanjo, 2001).

3.2.3 **Primal cuts and smoking**

The cured carcasses were cut into primal cuts; breast, thigh, drumsticks, back and wing and then weighed prior to smoking. A locally designed smokehouse consisting of two layers was used for smoking the meat. The lower layer was where charcoal was burnt; while the upper was covered with thick wire mesh, where the cut up parts were laid during smoking. The meat was smoked to impart smoky flavour for approximately two hours to an internal temperature of about 69 ± 1^{0}C with the use of a digital thermometer.

The smoked meat was briefly cooled, weighed and packed for further analysis.

3.2.4 **Processing yield**

Yield was determined as described by Naveena *et al.* (2006) as follows:

$$\text{Percentage (\%) cured yield} = \frac{\text{Half carcass weight after curing}}{\text{Half carcass weight before curing}} \times 100$$

3.2.5 **Proximate, nitrite and salt analysis**

Fresh and smoked meat samples were taken to the laboratory for analysis of dry matter, ash, moisture and crude protein, using the procedure outlined by AOAC (2000).

3.2.5.1 ***Dry matter***

5g of the meat sample was oven dried at 105^{0}C overnight to remove all moisture and then allowed to cool in a dessicator for about 20 minutes.

The dry matter was determined by:

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

3.2.5.2 *Ash*

5g of meat sample was ashed in a muffle furnace at a temperature of 500^0C for about 24 hours to obtain the inorganic matter (ash) and the cooled ash was weighed. The percentage ash was determined by:

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

3.2.5.3 *Lipid*

1g of ground meat sample was folded in filter paper, placed in a thimble and extracted in a Soxhlet extractor using 250 ml of 40 to 60% (v/v) solvent (petroleum ether) for approximately 6 hours. The sample was removed and dried in an oven at a temperature of 50^0C and then cooled.

The percentage lipid was determined by:

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

3.2.5.4 *Crude protein*

A 0.53 g ground meat sample was placed into a macro Kjedahl flask and one tablet of $CuSO_4$ was added to raise the reaction (catalyst)**.** A volume of 40 ml of concentrated H_2SO_4 was also added and then digested. The tube was allowed to cool and then 50 ml of water was added. The solution was transferred to a 100-ml volumetric flask and then made up to the mark with distilled water. Distillation was carried out by measuring 10 ml of boric acid in a pipette and poured into a clean empty flat bottom pyrex flask. Then, 2 drops of mixed indicator (100 parts methyl blue 0.1 % in 95 % alcohol and 20 parts methyl red 0.1% in 95 % alcohol) was used**.** 10 ml of 40% sodium hydroxide

(NaOH) was also added and distilled. After collecting about 40 ml of distillate into the green boric acid/indicator solution, the distillate was titrated with 0.1 normal sulphuric acid until the intensity of the colour was same as that of the boric acid/indicator solution, diluted to the same extent as the distillate being trapped. A reference solution of diluted boric acid/indicator was used as blank.

Percentage nitrogen was determined by:

$$\frac{\text{Normality of acid x NH3 x volume of dilution x 100 x titre value}}{\text{Sample weight x volume of digest (aliquot)}}$$

Percentage crude protein was determined by: % crude protein = % nitrogen x 6.25

3.2.5.5 ***Nitrite***

5g of meat sample and about 40 ml of water was thoroughly mixed in a beaker and heated to 80^0C, then transferred to a volumetric flask and hot water was added to a volume of 300 ml and heated for 2 hours with occasional shaking. The mixture was allowed to cool, diluted with water to 500 ml and filtered. After filtration the solution was centrifuged until a clear solution was obtained. Sulphanilamide (2.5 ml) was added to aliquot 10 ml of sample in a 50-ml beaker. The mixture was allowed to stand for 5 minutes, after which 2.5 ml of NED reagent was added and allowed to stand until colour was developed in 15 minutes. The absorbance of the solution was read at 540 nm against a blank, containing 45 ml water, 2.5 ml sulphanilamide and 2.5 ml NED reagent. The nitrite present in the solution was determined by comparison with standard curve, prepared by adding 10, 20, 30, 40 ml of nitrite working solution, with 2.5 ml sulphanilamide reagent; and after 5 minutes 2.5 ml of NED was added and absorbance was read.

3.2.5.6 *Salt*

2 g of meat sample and 50 ml distilled water were mixed in a beaker and heated on a water bath. The solution was filtered and residue was thoroughly washed. Dilute nitric acid (20 ml) was added to the solution and standard silver nitrate was added until precipitation occurred. Ferric alum (1 ml) indicator was added and titrated with standard potassium thiocyanate until light brown colour appeared.

Sodium chloride was then calculated using the formula:

$$\text{Sodium chloride (on dry matter basis) m/m} = \frac{5.85 \text{ x } (V_1N_1 - V_2N_2)}{M}$$

Where,

V1 = volume of standard solution of silver nitrate

N1 = Normality of standard silver nitrate solution

V2 = Volume of standard potassium thiocyanate solution

N2 = Normality of standard potassium thiocyanate solution

M = Mass of dried meat sample

3.2.6 **pH**

The pH of the meat samples at each step of the processing chain was determined according to the method described by Alakali (2010). For fresh meat samples, the pH reading was taken directly by inserting the electrode of the pH meter into the breast muscle; while for processed samples, the pH was taken by homogenising 5g of meat

sample to 50 ml of distilled water. The pH reading was then taken by inserting the glass electrode of the pH meter into the homogenate.

3.2.7 **Sensory analysis**

Cubes of meat 1 x 1 x 1 cm^3 cooled to room temperature were obtained from the breast muscle enough for a-20 member panel. The panellists used for the sensory analysis were semi- trained. The meat pieces were obtained from the four treatments and presented to the panellists for evaluation using a standard grading form (Appendix 1).

Evaluation for tenderness, juiciness, flavour, saltiness, smokiness and overall acceptability was carried out and scored on the 5-point hedonic scale form. The samples were scored as 5 being the highest value, 1 being the lowest value and 3 as intermediate (Ogunsola and Okubanjo, 2001).

3.2.8 **Statistical analysis**

Data obtained were subjected to general linear model (GLM) procedure of SAS (SAS, 2002) and significant means were separated using the least significance difference method. The following model was used:

$$Y_{ijk} = \mu + R_i + C_j + RC_{ij} + e_{ijk}$$

Where;

Y_{ijk} = observation on the carcass of the j^{th} curing temperature in the i^{th} rigor state;

μ = overall mean; R_i = effect of the i^{th} rigor state; C_j = effect of the j^{th} curing temperature; RC_{ij}= effect of interaction between the i^{th} rigor state and j^{th} curing temperature; e_{ijk} = random error.

3.3 Study 2: Effects of spice extracts on the quality and shelf-life of chicken nuggets

3.3.1 Preparation of extracts

Clove (*Eugenia caryophyllata*), pepper (*Capsicum frutescens*), African nutmeg (*Monodora myristica*), ginger (*Zingiber officinale*) and black pepper (*Piper guineense*) were purchased from Kurmi market in Kano State. The spices were washed with water, air dried overnight and ground into powder using a hammer mill. The spice extracts were prepared as follows: Aliquots (50 g each) of pulverized and dried spices were mixed into 400 ml of 95% (v/v) ethanol for 12 h in enclosed flasks with constant shaking (100 rpm) using a flask (Stuart: SF1). After filtration with a Whatman No. 2 filter paper, the residue was re-extracted with an additional 200 ml of 95% ethanol for an additional 12h and then filtered. The combined filtrates were concentrated on a rotary evaporator (RE- 100, Bibby Sterilin Co., Ltd., U.K.) at 50^0C with a vacuum pump. The extracts were further evaporated on water bath using Petri dishes. Dried extracts were placed in sealed bottles and stored at 4^0C before use (Zhang *et al*., 2009).

3.3.2 Bacterial culture preparation

Listeria monocytogenes (NICPBP 54002), *Escherichia coli* (strain 0150), *Bacillus* sp. and *Staphylococcus aureus* stocks were obtained from Nigerian Institute of Medical Research, Lagos, Nigeria. The bacteria were subcultured on Mueller-Hinton agar (Oxoid U.K.) which was prepared according to manufacturer's instructions. The media were sterilized by autoclaving at 121^0C for 15 minutes. The agar was then transferred into petri dishes after cooling, and was allowed to dry in a dryer. The bacteria strains were then streaked on the plates, containing Mueller-Hinton agar, and incubated at 37^0C for 24 hours.

McFaland standard solution was prepared using normal saline, and was used as a guide to prepare the solution of bacteria inocula for the sensitivity test.

3.3.3 **Antimicrobial activity testing of extracts in agar media**

The antimicrobial activity of spice extracts was examined in duplicate using the well diffusion test (Kim *et al*., 1995) to detect growth inhibition of *L. monocytogenes*, *E. coli, Bacillus and Staphylococcus* spp. The spice extracts were used singly or in combinations. Tests for individual antimicrobials were conducted at the following diluted spice extract concentrations in 95% ethanol: 80, 40, 20, 10 and 5 mg/ml. A 95% ethanol solution was used as control.

Tests for combined antimicrobials were conducted using mixtures of two of the following spice extracts that exhibited strong inhibitory activity against the four microorganisms when tested individually: clove, ginger and pepper. Each of the combinations was an equal-volume mixture of two spice extracts (1:1 v/v), at their respective pre-mixing concentrations (20 mg/ml).The inhibitory effect was assessed by measuring the disk diameter of the inhibition zone (clear zone) around the extract-containing steel cylinder by means of a vernier caliper (Kong *et al*., 2007). Sterile microsteel cylinders (7.8 X 10 mm) were vertically set on the agar in the plates and 0.1 ml of herb/spice extracts at each dilution was then aseptically transferred into the steel cylinders. The control was 0.1 ml of 95% ethanol only. With the lid on, the plates were incubated at 37^0C for 24 hours.

3.3.4 **Chicken nugget preparation**

Meat was deboned manually and cut into small pieces and minced in a meat mincer (Breville, UK) with a 3 mm plate. Emulsion of each formulation was prepared using

bowl chopper (Breville®, UK). All the nugget formulations consisted of broiler meat, 60%; soy bean oil, 10%; ice flakes, 10%; refined wheat flour, 2.5%; skim milk powder, 2%; whole egg liquid, 5%; table salt, 2%; sugar, 1%; sodium tri-polyphosphate, 0.2%; condiments/spice mix, 2.5%; and spice extract,4.8%. The formulation obtained was carefully cut in nugget size pieces (4×1.5×1.5 cm) and baked at 160^0C for 15 minutes. The cooked product was cooled and weighed. The nuggets were packed in sterilized aluminum foil plates and stored at refrigerated temperature (4±1°C) for analysis (Kumar and Tanwar, 2011).

3.3.5 **Optimization of spice extracts**

The addition of extract was in accordance with the different treatments. Treatment 1 (Control), Treatment 2 (Cloves), Treatment 3 (Ginger), Treatment 4 (African nutmeg), Treatment 5 (Pepper), Treatment 6 (Black pepper), Treatment 7 (Cloves + Ginger), Treatment 8 (Cloves + Pepper), Treatment 9 (Ginger + Pepper), Treatment 10 (Nitrite). All treatments contained the above formulation for nugget, except for treatment 10 which contained 150ppm nitrite level and control which neither contained extract nor nitrite.

3.3.6 **pH**

The pH of the nugget was determined according to the method described by Alakali (2010). 5g of sample was homogenized with 50 ml of distilled water. The pH reading was then taken by inserting the glass electrode of the pH meter into the homogenate.

3.3.7 **Thiobarbituric acid**

Thiobarbituric acid (TBA) was determined according to the method proposed by Kirk and Sawyer (1991). Ten grams of nugget were macerated with 50 ml of water for 2

minutes and transferred to a distillation flask with 47 ml of water. Thereafter, 2.5 ml of 4 M hydrochloric acid were added to adjust pH to 1.5. After the addition of a few glass beads, the flask was heated so as to collect 50 ml of distillate in 10 min from the time boiling commenced. 5ml of the distillate were pipetted into a beaker, and 5 ml TBA reagent were added. The tube was stoppered, shaken and heated on a boiling water bath for 35 min. A blank was prepared in a similar way using 5 ml of water with 5 ml reagent. The beaker were cooled in water for 10 minutes and the absorbance (D) was measured with the aid of a spectrophotometer (Spectro 20D, Pec medicals U.S.A) against the blank at the wavelength of 538 nm using 1 cm cells. The TBA was expressed as mg of malondialdehyde (MDA)/kg sample =7.8 D.

3.3.8 **Antimicrobial activity testing**

3.3.8.1 Collection of samples

Nugget samples from each treatment were collected into sterilised plastic containers and transferred to the laboratory with the aid of ice bags, and analysed within an hour after collection (Nwachukwu *et al*., 2008).

3.3.8.2 ***Media preparation***

The media included plate count agar, McConkey agar, potato dextrose agar, peptone water and nutrient agar which were prepared according to manufacturer's instructions (Oxoid, U.K.). The media were sterilized by autoclaving at 121^0C for 15 minutes.

3.3.8.3 ***Enumeration of Bacteria and fungi***

A sterile knife was used to cut 25 g of each nugget sample, which was weighed using a digital weighing balance. The sample was homogenized in 225 ml peptone water (0.1 peptone water) to form the stock solution. 1ml from each stock solution was transferred

aseptically into 9 ml of peptone water and this was serially diluted to 10^3 dilution. Thereafter, 0.1 ml of 10^3 dilution was transferred aseptically into plates containing plate count agar, Mc Conkey agar and potato dextrose agar. A sterile bent glass rod was used to spread the inocula on the surface of the culture media. The inoculated media were incubated inversely at 37^0C for 24 hours (Cheesebrough, 2004) for total plate count and total coliform count while the inoculated media for total fungal count was incubated at room temperature (28-32^0C) for 7 days. The number of bacterial and fungal colonies were counted, recorded and expressed as colony-forming unit per gram (cfu/g).

$$Cfu/g = \frac{\text{Total Number of Colonies Counted} \times \text{Dilution factor}}{\text{Volume of inocula}}$$

3.3.9 **Sensory analysis**

Cubes of nugget 1 x 1 x 1 cm^3 cooled to room temperature were obtained from the samples enough for a-11 member panel. The panellists used for the sensory analysis were semi- trained. The nugget pieces were obtained from the ten treatments and presented to the panellists for evaluation using a standard grading form.

Evaluation for colour, texture, odour and overall acceptability was carried out and scored on the 5-point hedonic scale form (Appendix 2). The samples were scored as 5 being the highest value, 1 being the lowest value and 3 as intermediate (Ogunsola and Okubanjo, 2001).

3.3.10 **Statistical analysis**

Data obtained were subjected to analysis of variance procedure of SAS (SAS, 2002) and significant means were separated using Duncan Multiple Range Test (Duncan, 1995). The following model was used:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where;

Y_{ij} = dependent variable; μ = overall mean; T_i = effect of the i^{th} treatment; e_{ijk} = random error.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of rigor state, curing temperature and their interaction on the physico- chemical properties of cured and smoked-cooked broiler meats

Tables 1 to 3 show the effect of rigor state, curing temperature and their interaction on the percentage yield, dry matter, moisture, ash, crude protein, lipid, nitrite and salt content of broiler carcasses. The yield of half carcasses as affected by state of rigor showed no significant difference ($P>0.05$) after curing, however the post-rigor carcasses gave a higher value of 11.43%. The effect of state of rigor on broiler meat samples significantly differed ($P>0.05$) for dry matter, moisture, lipid and nitrite. On the contrary, salt, ash and crude protein were not significantly affected ($P<0.05$) by the state of rigor. The percentage dry matter, moisture, lipid and nitrite in the pre rigor and post rigor states were 69.48 and 79.77, 30.52 and 20.23, 12.98 and 10.37, 120.52 and 131.06, respectively.

The hot cured carcasses (Table 2) recorded significantly lower values ($P<0.05$) in the yield of half cured carcass as affected by curing temperature, a yield of 6.31% was obtained for hot cured carcasses, while cold cured carcasses had a higher yield of 14.60%. Curing temperature was observed to have significantly affected ($P>0.05$) the dry matter, moisture, nitrite and salt levels in the broiler meat while on the other hand, ash, crude protein and lipid were not shown to significantly differ ($P<0.05$). The percentage dry matter, moisture, nitrite and salt on the hot cured and cold cured treatments were 81.94 and 67.32, 18.06 and 32.68, 137.66 and 113.92, 2.33 and 1.87, respectively.

In the interaction between rigor state and curing temperature (Table 3), the post-rigor cold cured treatments gave significantly higher yield ($P < 0.05$) of 15.88%; with the least values observed in the pre-rigor and post-rigor hot cured meat. There was no significant difference in crude protein values obtained for all the treatments. The moisture content of the pre-rigor cold cured meat (40.41%) was significantly higher than all other treatments but was not statistically different in the value obtained in the post-rigor cold cure. The ash content was also higher in the pre-rigor cold cure. The mean values obtained in the lipid content showed no statistical difference in the pre-rigor hot cured and pre-rigor cold cured meat and were significantly ($P > 0.05$) of higher values (13.27 and 12.68 respectively). Higher nitrite (143.19) and salt contents were observed in the post-rigor hot cured but the salt content value was not significantly different ($P> 0.05$) from the pre-rigor hot cured treatment.

Table 1: Effect of rigor state on physico-chemical properties of smoked cooked broiler meat

Parameters (%)	Pre-rigor	Post-Rigor	LOS
Cured Yield	9.49± 1.61	11.43± 1.89	NS
Dry Matter	69.48±4.19[b]	79.77± 3.41[a]	*
Moisture	30.52±4.19[a]	20.23±3.41[b]	*
Ash	5.10±1.07	4.48±0.69	NS
Crude protein	51.14±3.50	52.88±2.55	NS
Lipid	12.98±0.27[a]	10.37±0.89[b]	*
Nitrite	120.52±4.43[b]	131.06±4.62[a]	*
Salt	2.06±0.11	2.13±0.10	NS

Values represent means and standard error of inhibition zones; [a,b] Means in each row with different superscript letters differ significantly ($P < 0.05$); NS = Non-significant, LOS = Level of Significance,* = Statistically significant

Table 2: Effect of curing temperature on the physico-chemical properties of smoked cooked broiler meat

Parameters (%)	Hot Cured	Cold Cured	LOS
Cured Yield	6.31 ± 0.60^{b}	14.60 ± 1.08^{a}	*
Dry Matter	81.94 ± 1.57^{a}	67.32 ± 4.36^{b}	*
Moisture	18.06 ± 1.57^{b}	32.68 ± 4.36^{a}	*
Ash	4.41±0.87	5.17±0.92	NS
Crude Protein	50.19±3.07	53.82±2.93	NS
Lipid	11.83±0.90	11.51±0.72	NS
Nitrite	137.66 ± 2.17^{a}	113.92 ± 2.01^{b}	*
Salt	2.33 ± 0.04^{a}	1.87 ± 0.08^{b}	*

Values represent means and standard error of inhibition zones; ^{a,b} Means in each row with different superscript letters differ significantly ($P < 0.05$); NS = Non-significant, LOS = Level of Significance,* = Statistically significant

Table 3: Effect of interaction between rigor state and curing temperatures on the physico-chemical properties of smoked cooked broiler meats

	Pre-rigor		Post-rigor		
Parameters (%)	Hot Cured	Cold Cured	Hot Cured	Cold cured	LOS
Cured Yield	5.66 ± 0.75^{c}	13.33±1.34^{b}	6.97 ±0.91^{c}	15.88±1.59^{a}	*
Dry Matter	79.37±1.57^{a}	59.59±3.76^{b}	84.51±2.13^{a}	75.04±5.89^{ab}	*
Moisture	20.63±1.57^{b}	40.41±3.76^{a}	15.49±2.13^{b}	24.96±5.89^{ab}	*
Ash	4.58±1.60^{b}	5.62±1.61^{a}	4.25±0.99^{b}	4.72±1.11^{b}	*
Crude Protein	43.26±3.39	59.02±2.09	57.13±0.70	48.63±4.22	NS
Lipid	13.27±0.06^{a}	12.68±0.52^{a}	10.39±1.56^{b}	10.35±1.12^{b}	*
Nitrite	132.13±0.36^{b}	108.90±1.35^{d}	143.19±1.18^{a}	118.94±0.43^{c}	*
Salt	2.27±0.06^{a}	1.86±0.16^{b}	2.38±0.04^{a}	1.88±0.06^{b}	*

Values represent means and standard error of inhibition zones; ^{a} ^{,b,c} Means in each row with different superscript letters differ significantly ($P < 0.05$); NS = Non-significant, LOS = Level of Significance,* = Statistically significant

4.2 Effect of rigor state, curing temperature and their interaction on the sensory properties of smoked-cooked broiler meat

Tables 4, 5 and 6 show the effect of rigor state, curing temperature and their interaction on the sensory properties of smoked-cooked broiler meats respectively. The effect of rigor state on the sensory attributes of smoked broiler meats was not significant ($P>0.05$) for all the parameters; except on smoky flavour, where it was significant ($P<0.05$). Results obtained on texture, juiciness, colour, saltiness, smoky flavour and general acceptability for pre-rigor and post rigor meat were 1.88 and 1.80, 2.04 and 2.03, 2.19 and 2.05, 1.69 and 1.59, 2.17 and 2.06, 2.62 and 2.48, respectively, with the pre-rigor meats having higher scores in all the parameters.

The effect of curing temperatures on the sensory attributes of smoked broiler meat were not significant ($P>0.05$) for all the parameters (Table 5). Results obtained on texture, juiciness, colour, saltiness, smoky flavour and general acceptability for hot and cold cured treatments were 1.88 and 1.79, 2.06 and 2.01, 2.13 and 2.10, 1.68 and 1.60, 2.15 and 2.09, 2.58 and 2.52, respectively, with the hot cured meats having higher scores in all the parameters.

There was significant difference ($P<0.05$) in their interaction (Table 6). The ranges for texture, juiciness, colour, saltiness, smoky flavour and general acceptability were between 1.75 and 1.92, 2.00 and 2.06, 2.03 and 2.20, 1.56 and 1.74, 2.03 and 2.20, 2.44 and 2.64, respectively; where post-rigor cold cure had the lowest values. The pre-rigor hot cured carcasses gave the highest values in all the parameters evaluated; except in juiciness where post-rigor hot cured carcasses/ meat had similar value with pre-rigor hot cured carcasses/ meat.

Table 4: Effect of rigor state on the sensory properties of smoked-cooked broiler meats

Parameters (%)	Pre-rigor	Post-rigor	LOS
Texture	1.88±0.08	1.80±0.08	NS
Juiciness	2.04±0.14	2.03±0.15	NS
Colour	2.19±0.17	2.05±0.18	NS
Saltiness	1.69±0.11	1.59±0.10	NS
Smoky flavour	2.17±0.03[a]	2.06±0.04[b]	*
General acceptability	2.62±0.11	2.48±0.10	NS

Values represent means and standard error of inhibition zones; [a, b] Means in each row with different superscript letters differ significantly ($P < 0.05$); NS = Non-significant, LOS = Level of Significance,* = Statistically significant

Table 5: Effect of curing temperatures on the sensory properties of smoked-cooked broiler meats

Parameters (%)	Hot Cure	Cold cure	LOS
Texture	1.88±0.07	1.79±0.09	NS
Juiciness	2.06±0.14	2.01±0.15	NS
Colour	2.13±0.17	2.10±0.18	NS
Saltiness	1.68±0.11	1.60±0.11	NS
Smoky flavour	2.15±0.04	2.09±0.04	NS
General acceptability	2.58±0.11	2.52±0.11	NS

Values represent means and standard error of inhibition zones; NS = Non-significant, LOS = Level of Significance

Table 6: Effect of rigor state, curing temperature and their interaction on the sensory properties of smoked-cooked broiler meats

	Pre-rigor		Post-rigor		
Parameters	Hot Cured	Cold Cured	Hot Cured	Cold cured	LOS
Texture	1.92±0.11^{a}	1.83±0.13^{b}	1.84±0.11^{b}	1.75±0.14^{c}	*
Juiciness	2.06±0.20^{ab}	2.02±0.22^{bc}	2.06±0.22^{a}	2.00±0.24^{c}	*
Colour	2.20±0.25^{a}	2.17±0.27^{b}	2.07±0.27^{c}	2.03±0.28^{d}	*
Saltiness	1.74±0.16^{a}	1.65±0.18^{b}	1.62±0.17^{b}	1.56±0.16^{c}	*
Smoky flavour	2.20±0.04^{a}	2.15±0.04^{b}	2.09±0.06^{c}	2.03±0.05^{d}	*
General acceptability	2.64±0.18^{a}	2.60±0.17^{a}	2.52±0.15^{b}	2.44±0.16^{c}	*

Values represent means and standard error of inhibition zones; $^{a\text{-}d}$ Means in each row with different superscript letters differ significantly ($P < 0.05$); NS = Non-significant, LOS = Level of Significance,* = Statistically significant

4.3 Antimicrobial activities of individual and combined spice extracts at various concentrations of agar media

Tables 7 and 8 show the antimicrobial effect of spice extracts using different concentrations of 0, 5, 10, 20, 40 and 80 (mg/ml) and in combination (20mg/ml) respectively, on *L. monocytogens, E. coli, Bacillus and Staphylococcus spp*. Results obtained on clove extracts showed significant effect ($P<0.05$) on the different microbes. The zones of inhibition obtained on *L. monocytogens, E. coli, Bacillus and staphylococcus spp.* ranged between 9.80 to 21.50, 10.28 to 18.90, 10.50 to 21.15, and 10.35 to 17.20 respectively. The concentration of 80 mg/ml gave the highest and 0 mg/ml showed the lowest zone of inhibition; except for *L. monocytogens,* where 40 mg/ml concentration gave the highest zone of inhibition.

The zones of inhibition on *L. monocytogens, E. coli, Bacillus and Staphylococcus spp.* in agar medium as affected by ginger extracts of 0, 5, 10, 20, 40 and 80 (mg/ml) were significantly different ($P < 0.05$). The ranges obtained were 10.70 to 15.55, 9.45 to 12.55, 9.40 to 18.63 and 10.30 to 15.60 mm. It was observed that the 80 mg/ml concentration had the highest zone of inhibition, while 0 mg/ml concentration of ginger extract had the lowest zone of inhibition.

The zones of inhibition on *L. monocytogens, and E. coli i*n agar medium as affected by extracts of African nutmeg at 0, 5, 10, 20, 40 and 80 (mg/ml) were not significantly different ($P > 0.05$). But the zones inhibited on *Bacillus sp. and Staphylococcus sp.* in agar medium as affected by African nutmeg extracts of 0, 5, 10, 20, 40 and 80 (mg/ml) were significantly different and ranged between 11.95 to 16.10 and 9.90 to 14.18mm, respectively, but the lowest and highest zones occurred at 0 mg/ml and 80 mg/ml, respectively.

The values observed in the effect of black pepper on the zone of inhibition of *L. monocytogens, Bacillus sp. and staphylococcus sp.* showed significant difference ($P < 0.05$), but no significant difference ($P > 0.05$) was observed in the values obtained for the zone of inhibition of *E. coli*. The range of values observed for 0, 5, 10, 20, 40 and 80 (mg/ml) on zone of inhibition of *L. monocytogens, E. coli, Bacillus sp. and Staphylococcus sp.* were 10.00 to 14.40, 9.54 to 11.55, 9.00 to 15.15 and 10.83 to 14.30 mm, respectively.

The zones of inhibition on *L. monocytogens, E. coli, Bacillus and Staphylococcus* in agar medium as affected by pepper extracts of 0, 5, 10, 20, 40 and 80 (mg/ml) concentrations were significantly ($P < 0.05$) different. The ranges obtained were 11.50 to 16.48, 10.43 to 12.95, 12.00 to 15.23 and 9.58 to 15.50 mm. It was observed that the 80 mg/ml concentration had the highest zone of inhibition, while the lowest zone of inhibition was recorded at the 0 mg/ml concentration of pepper extract.

The ranges obtained for single effect of spice extracts at 20mg/ml and in combinations were 13.20 to 18.65, 11.25 to 15.70, 12.48 to 19.55 and 12.85 to 14.65. M1 combination was best in the inhibition of *L. monocytogens, E. coli and Bacillus sp.* except in *Staphylococcus sp.* where M2 combination was best. There was significant difference ($P<0.05$) in the values obtained from *L. monocytogens, E. coli* and *Bacillus sp.*; while there was no significant difference ($P > 0.05$) observed in *Staphylococcus sp.*

Table 7: Antimicrobial activity of individual spice extracts at various concentrations in agar media

		Diameter of inhibition zone (mm)			
Spice	Concentration (mg/ml)	*Listeria monocytogens*	*Escherichia coli*	*Bacillus sp.*	*Staphylococcus sp.*
Cloves	0	9.80±0.80^{e}	10.28±0.78^{d}	10.50±0.60^{d}	10.35±0.75^{b}
	5	13.65±0.65^{d}	11.20±0.60^{d}	10.95±0.35^{d}	12.33±1.68^{b}
	10	13.90±0.70^{cd}	11.70±0.30^{cd}	13.40±0.60^{c}	12.75±0.35^{b}
	20	17.70±0.70^{b}	15.00±1.20^{bc}	18.05±0.95^{b}	13.13±0.13^{b}
	40	21.50±0.90^{a}	15.83±1.43^{ab}	19.85±0.45^{ab}	14.13±1.03^{ab}
	80	16.15±0.05^{bc}	18.90±1.10^{a}	21.15±0.85^{a}	17.20±1.80^{a}
Ginger	0	10.70±1.30^{b}	9.45±0.05^{c}	9.40±1.10^{c}	10.30±0.70
	5	12.30±0.20^{ab}	11.28±0.38^{b}	12.45±0.85^{bc}	12.60±2.60
	10	12.60±0.00^{ab}	11.13±0.13^{b}	16.58±0.58^{ab}	12.65±2.65
	20	14.10±0.90^{ab}	11.25±0.25^{b}	17.58±1.48^{a}	12.85±2.15
	40	15.45±1.65^{a}	12.43±0.23^{a}	18.10±1.90^{a}	14.63±2.53
	80	15.55±0.95^{a}	12.55±0.35^{a}	18.63±2.38^{a}	15.60±2.60
African nutmeg	0	12.35±1.25	10.75±1.15	11.95±0.95^{b}	9.90±1.10^{c}
	5	13.55±1.25	10.10±0.10	13.15±0.15^{ab}	11.65±0.85^{abc}
	10	13.88±1.38	10.75±0.75	13.55±0.45^{ab}	10.28±1.08^{bc}
	20	13.90±1.60	10.50±0.50	14.45±0.55^{ab}	12.45±0.25^{abc}
	40	12.20±0.10	10.50±0.50	15.65±1.55^{ab}	13.40±1.10^{ab}
	80	12.85±1.55	10.25±0.25	16.10±1.90^{a}	14.18±1.08^{a}
Black Pepper	0	10.00±0.00^{b}	9.54±0.83	9.00±0.00^{b}	10.83±0.18^{bc}
	5	11.50±1.00^{ab}	9.60±0.40	11.50±1.70^{ab}	14.15±2.15^{a}
	10	12.25±0.85^{ab}	10.58±0.58	11.50±1.50^{ab}	14.30±1.70^{a}
	20	13.60±0.80^{a}	10.65±0.55	12.60±2.40^{ab}	14.05±1.25^{a}
	40	14.03±1.23^{a}	10.75±0.25	15.15±2.15^{a}	14.05±1.15^{a}
	80	14.40±1.60^{a}	11.55±0.95	11.75±1.75^{ab}	11.98±0.98^{b}
Pepper	0	11.50±0.50^{b}	10.43±0.13^{c}	12.00±1.00^{b}	9.58±1.03^{c}
	5	12.98±0.88^{ab}	11.05±0.05^{bc}	12.35±0.65^{b}	10.90±1.10^{bc}
	10	12.88±0.83^{ab}	11.55±0.45^{abc}	12.35±0.65^{b}	12.70±0.70^{abc}
	20	13.20±1.10^{ab}	11.55±0.25^{abc}	12.48±0.68^{ab}	13.70±1.30^{ab}
	40	16.25±2.05^{a}	12.33±0.83^{ab}	13.55±0.85^{ab}	14.50±1.50^{ab}
	80	16.48±0.33^{a}	12.95±0.85^{a}	15.23±0.93^{a}	15.50±1.20^{a}

Values represent means and standard error of inhibition zones; $^{a–d}$ Within a column, for the same spice, means with different letters differ significantly ($P < 0.05$).

Table 8: Antimicrobial activity of combined spice extracts in agar media

Spice (mg/ml)	*Listeria monocytogens*	*Escherichia coli*	*Bacillus sp.*	*Staphylococcus sp.*
A	$17.70\pm0.70a^{b}$	15.00 ± 1.20^{a}	18.05 ± 0.95^{a}	13.13 ± 0.13^{NS}
B	14.10 ± 0.90^{cd}	11.25 ± 0.25^{b}	17.58 ± 1.48^{a}	12.85 ± 2.15^{NS}
C	13.20 ± 1.10^{d}	11.55 ± 0.25^{b}	12.48 ± 1.68^{b}	13.70 ± 1.30^{NS}
M1	18.65 ± 0.55^{a}	15.70 ± 0.10^{a}	19.55 ± 1.35^{a}	13.40 ± 0.00^{NS}
M2	16.00 ± 1.00^{bc}	14.50 ± 0.00^{a}	18.30 ± 0.60^{a}	14.65 ± 0.35^{NS}
M3	14.45 ± 0.25^{cd}	12.20 ± 0.10^{b}	18.80 ± 0.60^{a}	14.00 ± 0.50^{NS}

Values represent means and standard error of inhibition zones; $^{a-d}$ Means in each column with different superscript letters differ significantly ($P < 0.05$) different, A= cloves, B= ginger, C= pepper, M1= clove + ginger, M2= clove + pepper, M3= ginger + pepper, NS= Non-significant.

4.4 Effect of spice extracts and storage time on pH and thiobarbituric acid reactive substances (mg of malondialdehyde/kg of chickens) of chicken nugget stored at 4°C for 12 days

Effects of spice extracts and storage time on pH and thiobarbituric acid of reactive substances (mg of malondialdehyde/kg of nugget) of chicken nugget stored at 4°C for 12 days are presented in Tables 9 and 10 respectively. The results showed significant difference ($P<0.05$) in pH values across the treatments and as the storage days increased. The ranges obtained across the different treatments on days 1, 3, 6, 9 and 12 were 5.38 to 5.99, 5.68 to 6.01, 5.88 to 6.25, 5.91 to 6.27 and 5.98 to 6.45, respectively; The lowest values were observed in treatments 3, 10, 8 (and 9), 8 and 9, respectively, while the highest values were observed in treatments 4, 4 (and 1), 7, 1 and 6, respectively.

The results on TBARS (Table 10) showed significant differences ($P < 0.05$) in the values across the treatments and as the storage days increased. The range obtained across the different treatments on day 1, 3, 6, 9 and 12 were 0.13 to 0.44, 0.12 to 0.46, 0.21 to 0.66, 0.22 to 0.79 and 0.24 to 1.43, respectively. Treatment 10 had the lowest TBA value on days 1, 3 and 6; while on days 9 and 12, treatments 9 had the lowest TBA values as storage days increased. Treatment 1 showed the highest TBA value. All the spices reduced TBA values as measured on each storage days.

Table 9: Effect of spice extracts and storage time on pH changes of chicken nugget stored at 4°C for 12 days

	Storage days				
Extract	1	3	6	9	12
Treatment 1	5.93±0.11^{a}	6.01±0.02	6.13±0.07^{ab}	6.27±0.02^{a}	6.39±0.03^{ab}
Treatment 2	5.38±0.11^{b}	5.94±0.04	5.99±0.01^{ab}	5.99±0.01^{cd}	6.17±0.12^{abc}
Treatment 3	5.48±0.20^{b}	5.95±0.03	5.99±0.01^{ab}	5.99±0.01^{cd}	6.10±0.06^{bc}
Treatment 4	5.99±0.01^{a}	6.01±0.01	6.25±0.16^{a}	6.18±0.02^{ab}	6.41±0.06^{ab}
Treatment 5	5.84±0.17^{a}	5.85±0.17	6.12±0.13^{ab}	6.08±0.03^{bc}	6.39±0.16^{ab}
Treatment 6	5.90±0.03^{a}	5.96±0.43	6.06±0.10^{ab}	6.16±0.07^{ab}	6.45±0.18^{a}
Treatment 7	5.89±0.00^{a}	5.94±0.11	5.96±0.01^{b}	5.99±0.03^{cd}	6.19±0.09^{abc}
Treatment 8	5.85±0.14^{a}	5.87±0.17	5.88±0.01^{b}	5.91±0.09^{d}	6.05±0.07^{c}
Treatment 9	5.89±0.01^{a}	5.85±0.36	5.88±0.15^{b}	5.95±0.12^{cd}	5.98±0.13^{c}
Treatment10	5.81±0.02^{a}	5.68±0.07^{xy}	5.94±0.11^{b}	5.95±0.03^{cd}	6.21±0.10^{abc}

Values represent means and standard error of pH; $^{a-d}$ Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

Table 10: Effect of spice extracts and storage time on thiobarbituric acid reactive substances (mg of malondialdehyde/kg of chicken) of chicken nugget stored at 4°C 12 days

	Storage days				
Extract	1	3	6	9	12
Treatment 1	0.44±0.03^{a}	0.46±0.04^{a}	0.66±0.05^{a}	0.79±0.11^{a}	1.43±0.22^{a}
Treatment 2	0.17±0.04^{ab}	0.30±0.09^{abc}	0.32±0.02^{b}	0.46±0.15^{bcd}	0.46±0.06^{bc}
Treatment 3	0.21±0.02^{ab}	0.28±0.05^{abc}	0.42±0.07^{ab}	0.43±0.08^{cd}	0.46±0.05^{bc}
Treatment 4	0.38±0.05^{ab}	0.38±0.08^{ab}	0.50±0.24^{ab}	0.69±0.10^{ab}	0.65±0.23^{b}
Treatment 5	0.39±0.18^{ab}	0.33±0.10^{ab}	0.38±0.07^{ab}	0.38±0.05 ^{cd}	0.45±0.12^{bc}
Treatment 6	0.35±0.16^{ab}	0.37±0.08^{ab}	0.49±0.13^{ab}	0.49±0.06^{bc}	0.66±0.13^{b}
Treatment 7	0.33±0.05^{ab}	0.26±0.03^{bc}	0.27±0.05^{ab}	0.27±0.05 ^{cd}	0.28±0.03^{c}
Treatment 8	0.20±0.10^{ab}	0.27±0.03^{bc}	0.29±0.02^{b}	0.29±0.04 ^{cd}	0.33±0.05^{bc}
Treatment 9	0.19±0.10^{ab}	0.24±0.03^{bc}	0.22±0.06^{b}	0.22±0.02^{d}	0.24±0.07^{c}
Treatment 10	0.13±0.05^{b}	0.12±0.03^{c}	0.21±0.06^{b}	0.38±0.07^{cd}	0.42±0.06^{bc}

Values represent means and standard error of thiobarbituric acid reactive substances (mg of malondialdehyde/Kg); $^{a–d}$ Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

4.5 Microbial count (Log cfu/g) of chicken nuggets stored at 4ºC for 12 days

The effect of spice extracts on the total plate count, total coliform count and total fungal counts of chicken nuggets stored at 4ºC for 12 days are presented in Tables 11, 12 and 13 respectively. The Total Plate Count (Log cfu/g) of chicken nuggets stored at 4ºC for 12 days are presented in Table 11 which showed significant difference ($P < 0.05$) across treatments. The ranges obtained for the treatments on days 1, 3, 6, 9 and 12 were between 0.00 to 4.16, 0.00 to 4.69, 0.00 to 4.84, 1.33 to 4.99 and 2.67 to 5.08 in which treatment 7 had the lowest total plate count and treatment 1 had the highest total plate count. All the spices and spice mixtures greatly reduced the Total Plate Count.

Significant difference ($P < 0.05$) was also observed in the treated samples for total coliform count. On the first day, treatment 1 had the highest coliform count, while treatments 2, 3, 5, 7, 8, 9 and 10 had the lowest coliform counts. On day 3 of storage, treatment 2, 3, 5, 7, 8 and 9 had no count, while treatment 1 had the highest count. On day 6 of storage, treatments 2, 3, 7, 8 and 9 had no count, but treatment 1 had the highest count. Treatments 2 and 7 still had no count on the 9^{th} day of storage, while treatment 1 maintained the highest count. Day 12 of storage showed treatment 7 to have the lowest count, but the highest count was obtained in treatment 1.

Significant difference ($P < 0.05$) across the treatments as the storage days increased was observed in total fungal count. There was no count on day 1 across treatments, except treatment 1 which was 2.00 log cfu/g. Day 3 showed a count of 1.00 and 2.00 log cfu/g on treatments 4 and 1, respectively, day 6 showed no counts on treatments 2, 5, 7 and 8, while treatment 1 had the highest count of 3.10 log cfu/g, days 9 and 12 showed a range between 0.00 to 3.46 log cfu/g and 2.00 to 3.53 log cfu/g respectively with the lowest count observed on treatment 7 and highest on the control.

Table 11: Total Plate Count (Log cfu/g) of chicken nuggets stored at 4^0C for 12 days

	Storage period				
Extracts	1	3	6	9	12
Treatment 1	4.16±0.16^{a}	4.69±0.05^{a}	4.84±0.03^{a}	4.99±0.05^{a}	5.08±0.00^{a}
Treatment 2	1.33±1.33^{ab}	1.33±1.33^{ab}	1.43±1.43^{ab}	2.87±1.43^{ab}	4.67±0.03^{a}
Treatment 3	1.33±1.33^{ab}	2.67±1.33^{ab}	2.77±1.39^{ab}	2.77±1.39^{ab}	4.69±0.05^{a}
Treatment 4	2.77±1.39^{ab}	2.87±1.44^{ab}	2.87±1.44^{ab}	4.43±0.13^{a}	4.83±0.02^{a}
Treatment 5	1.33±1.33^{ab}	2.67±1.33^{ab}	2.83±1.42^{ab}	4.00±0.00^{ab}	4.75±0.05^{a}
Treatment 6	2.77±1.39^{ab}	3.03±1.51^{ab}	2.67±1.33^{ab}	4.00±0.00^{ab}	4.84±0.03^{a}
Treatment 7	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	1.33±1.33^{b}	2.67±1.33^{b}
Treatment 8	1.33±1.33^{ab}	1.43±1.43^{b}	1.43±1.43^{ab}	2.67±1.33^{ab}	4.72±0.06^{a}
Treatment 9	1.33±1.33^{ab}	1.49±1.49^{ab}	1.53±1.53^{ab}	2.67±1.33^{ab}	4.78±0.04^{a}
Treatment 10	1.33±1.33^{ab}	2.77±1.39^{ab}	2.97±1.49^{ab}	4.49±0.25^{a}	4.82±0.06^{a}

Values represent means and standard error of total plate counts; ^{a,b} Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

Table 12: Total Coliform Count (Log cfu/g) of chicken nuggets stored at 4^0C for 12 days

	Storage period				
Extracts	1	3	6	9	12
Treatment 1	2.00±1.00^{a}	3.10±0.10^{a}	3.16±0.16^{a}	3.53±0.12^{a}	3.67±0.03^{a}
Treatment 2	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	2.10±1.05^{ab}
Treatment 3	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}	1.00±1.00b^{c}	3.00±0.00^{a}
Treatment 4	1.00±1.00^{ab}	2.00±1.00^{ab}	2.00±1.00^{ab}	2.10±1.05^{abc}	3.53±0.12^{a}
Treatment 5	0.00±0.00^{b}	0.00±0.00^{c}	1.00±1.00^{bc}	2.10±1.05^{abc}	3.00±0.00^{a}
Treatment 6	1.00±1.00^{ab}	2.10±1.05^{ab}	2.10±1.05^{ab}	3.10±0.10^{ab}	3.36±0.06^{a}
Treatment 7	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	1.00±1.00^{b}
Treatment 8	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}	1.00±1.00^{bc}	2.00±1.00^{ab}
Treatment 9	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}	1.00±1.00^{bc}	2.10±1.05^{ab}
Treatment 10	0.00±0.00^{b}	1.00±1.00^{bc}	2.10±1.05^{ab}	2.20±1.11^{abc}	3.30±0.17^{a}

Values represent means and standard error of total coliform counts; ^{a,b} Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

Table 13: Total Fungal Count (Log cfu/g) of chicken nuggets stored at 4^0C for 12 days

Extracts	Storage period				
	1	3	6	9	12
Treatment 1	2.00±1.00^{a}	2.00±1.00^{a}	3.10±0.10^{a}	3.46±0.09^{a}	3.53±0.12^{a}
Treatment 2	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{b}	1.00±1.00^{bc}	3.00±0.00^{a}
Treatment 3	0.00±0.00^{b}	0.00±0.00^{a}	1.00±1.00^{b}	2.10±1.05^{ab}	3.10±0.10^{a}
Treatment 4	0.00±0.00^{b}	1.00±1.00^{ab}	2.00±1.00^{ab}	3.26±0.14^{a}	3.36±0.06^{a}
Treatment 5	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{b}	3.00±0.00^{a}	3.10±0.10^{a}
Treatment 6	0.00±0.00^{b}	0.00±0.00^{a}	1.00±1.00^{b}	3.00±0.00^{a}	3.26±0.14^{a}
Treatment 7	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.47^{b}	0.00±0.00^{c}	2.00±1.00^{b}
Treatment 8	0.00±0.00^{b}	0.00±0.00^{a}	0.00±0.00^{b}	2.00±1.00^{ab}	3.00±0.00^{a}
Treatment 9	0.00±0.00^{b}	0.00±0.00^{a}	1.00±1.00^{b}	2.00±1.00^{ab}	3.00±0.00^{a}
Treatment 10	0.00±0.00^{b}	0.00±0.00^{a}	2.00±1.00^{ab}	3.26±0.14^{a}	3.16±0.16^{a}

Values represent means and standard error of total fungal counts; ^{a,b} Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

4.6 Effect of spice extract inclusion on the sensory properties of chicken nuggets stored at 4ºC for 12 days

Tables 14-18 shows the effect of spice extract inclusion on the sensory properties (colour, texture, odour and general acceptability) and proximate composition of chicken nuggets stored at 4^0C for 12 days.

Results on the colour of the different treatments as affected by spice extracts (Table 14) showed significant difference ($P < 0.05$) as the storage days increased. The ranges obtained on days 1, 3, 6, 9 and 12 were 1.70 to 4.80, 1.70 to 4.60, 1.60 to 4.40, 1.60 to 4.40 and 1.60 to 4.20, respectively. The lowest scores for colour were observed in treatments 6 and 8 on day 1, 2 and 8 on day 3, 8 on days 6, 9 and 12, while the highest colour scores were observed in treatments 1, 1, 10, 10, 10 on days 1,3,6,9 and 12 respectively.

Results on the texture of the different treatments as affected by spice extracts (Table 15) showed significant difference ($P < 0.05$) as the storage days increased. All The ranges obtained on day 1, 3, 6, 9 and 12 were 4.70 to 5.00, 4.30 to 5.00, 3.80 to 5.00, 3.00 to 5.00, 2.60 to 5.00, respectively. The lowest scores were observed in treatment 1, while the highest scores were observed in treatments 2 to 10 on days 1; 2,3,5,6,7,8,9,10 on day 3; 2,3,5,6,7,8,9 on day 6; 2,3,5,6,7,8,9 on day 9 and 2,5,7,8,9 on day 12.

Results on the odour of the different treatments as affected by spice extracts (Table 16) showed significant difference ($P < 0.05$) as the storage days increased. The ranges obtained on day 1, 3, 6, 9 and 12 were 4.00 to 5.00, 3.80 to 5.00, 3.20 to 5.00, 2.60 to 5.00 and 1.90 to 5.00, respectively. The lowest scores were observed in treatment 6 on

day 1 and 1 on the rest of the storage period, while the highest scores were observed in treatments 7 and 8 throughout the storage period.

Scores on the general acceptability of different treatments as affected by spice extracts showed significant difference ($P < 0.05$) as the storage days increased. The ranges obtained on day 1, 3, 6, 9 and 12 were 3.30 to 5.00, 3.30 to 5.00, 2.90 to 5.00, 2.40 to 5.00 and 1.40 to 5.00, respectively. The lowest scores were observed in treatment 10 on day 1 and 1 on the rest of the storage period, while the highest scores were obtained in treatments 3, 7, 8 and 9 on storage days 1 and 3, treatment 2, 3, 7, 8 and 9 on storage days 6, 9 and 12 of the storage period.

Results on the proximate analysis of chicken nuggets shows significant difference ($P < 0.05$) across treatments, and the range obtained for dry matter, crude protein, oil and ash were 85.05 to 88.57, 20.12 to 22.19, 7.28 to 11.14 and 8.69 to 6.46, respectively. The lowest percentage dry matter, crude protein, oil and ash were obtained in treatments 1, 6, 9 and 1, respectively, while the lowest percentage dry matter, crude protein, oil and ash were obtained in treatments 3, 7, 4 and 9, respectively.

Table 14: Effect of spice extract inclusion on colour of chicken nuggets stored at 4^0C for 12 days.

	Storage days				
Extracts	1	3	6	9	12
Treatment 1	4.80 ± 0.2^{a}	4.60 ± 0.22^{a}	4.20 ± 0.25^{a}	4.20 ± 0.25^{a}	3.50 ± 0.22^{cb}
Treatment 2	1.90 ± 0.23^{ef}	1.70 ± 0.15^{e}	2.00 ± 0.00^{fe}	2.00 ± 0.00^{ef}	2.00 ± 0.00^{fg}
Treatment 3	4.10 ± 0.18^{b}	3.90 ± 0.1^{b}	3.60 ± 0.16^{b}	3.60 ± 0.16^{b}	3.60 ± 0.16^{b}
Treatment 4	3.30 ± 0.40^{c}	3.10 ± 0.31^{c}	3.00 ± 0.30^{c}	3.00 ± 0.30^{c}	3.00 ± 0.30^{cd}
Treatment 5	2.40 ± 0.16^{de}	2.40 ± 0.16^{d}	2.40 ± 0.16^{de}	$2.40\pm0.16d^{e}$	2.40 ± 0.16^{ef}
Treatment 6	1.70 ± 0.26^{f}	1.80 ± 0.24^{e}	1.90 ± 0.23^{ef}	2.00 ± 0.21^{ef}	2.00 ± 0.21^{fg}
Treatment 7	2.60 ± 0.16^{d}	2.60 ± 0.16^{cd}	2.60 ± 0.16^{dc}	2.60 ± 0.16^{cd}	2.60 ± 0.16^{de}
Treatment 8	1.70 ± 0.15^{f}	1.70 ± 0.15^{e}	1.60 ± 0.16^{f}	1.60 ± 0.16^{f}	1.60 ± 0.16^{g}
Treatment 9	3.00 ± 0.00^{cd}	3.00 ± 0.00^{c}	3.00 ± 0.00^{c}	3.00 ± 0.00^{c}	3.00 ± 0.00^{cd}
Treatment 10	4.50 ± 0.22^{ab}	4.50 ± 0.22^{a}	4.40 ± 0.27^{a}	4.40 ± 0.27^{a}	4.20 ± 0.25^{a}

Values represent means and standard error of colour; $^{a-g}$ Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

Table 15: Effect of spice extract inclusion on texture of chicken nuggets stored at 4^0C for 12 days

	Storage days				
Extracts	1	3	6	9	12
Treatment 1	4.70 ± 0.15^{b}	4.30 ± 0.15^{c}	3.80 ± 0.13^{c}	3.00 ± 0.00^{c}	2.60 ± 0.16^{c}
Treatment 2	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}
Treatment 3	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	4.80 ± 0.13^{a}
Treatment 4	5.00 ± 0.00^{a}	4.80 ± 0.13^{b}	4.60 ± 0.16^{b}	4.40 ± 0.16^{b}	4.00 ± 0.21^{b}
Treatment 5	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}
Treatment 6	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	4.80 ± 0.13^{a}
Treatment 7	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}
Treatment 8	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}
Treatment 9	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}
Treatment 10	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	4.80 ± 0.13^{ab}	4.60 ± 0.16^{b}	4.20 ± 0.13^{b}

Values represent means and standard error of texture; $^{a-c}$ Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

Table 16: Effect of spice extract inclusion on odour of chicken nuggets stored at 4^0C for 12 days

	Storage days				
Extracts	1	3	6	9	12
Treatment 1	4.20 ± 0.32^{cd}	3.80 ± 0.25^{e}	3.20 ± 0.25^{d}	2.60 ± 0.16^{d}	1.90 ± 0.28^{e}
Treatment 2	4.80 ± 0.13^{ab}	4.80 ± 0.13^{ab}	4.60 ± 0.16^{ab}	4.60 ± 0.16^{ab}	4.60 ± 0.16^{ab}
Treatment 3	4.80 ± 0.13^{ab}	4.80 ± 0.13^{ab}	4.60 ± 0.16^{ab}	4.60 ± 0.16^{ab}	4.60 ± 0.16^{ab}
Treatment 4	4.20 ± 0.25^{cd}	4.20 ± 0.25^{cde}	4.00 ± 0.21^{c}	4.00 ± 0.21^{c}	3.60 ± 0.16^{d}
Treatment 5	4.40 ± 0.27^{bcd}	4.40 ± 0.27^{bcd}	4.20 ± 0.25^{bc}	4.20 ± 0.25^{bc}	4.20 ± 0.25^{bc}
Treatment 6	4.00 ± 0.21^{d}	4.00 ± 0.21^{de}	4.00 ± 0.21^{c}	4.00 ± 0.21^{c}	3.80 ± 0.25^{cd}
Treatment 7	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}
Treatment 8	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}
Treatment 9	4.60 ± 0.16^{abc}	4.60 ± 0.16^{abc}	4.60 ± 0.16^{ab}	4.60 ± 0.16^{ab}	4.60 ± 0.16^{ab}
Treatment 10	4.60 ± 0.16^{abc}	4.40 ± 0.16^{bcd}	4.20 ± 0.13^{bc}	4.00 ± 0.00^{c}	4.00 ± 0.00^{cd}

Values represent means and standard error of odour; $^{a-e}$ Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

Table 17: Effect of spice extract inclusion on the general acceptability of chicken nuggets stored at 4^0C for 12 days

	Storage days				
Extracts	1	3	6	9	12
Treatment 1	3.60±0.22^{b}	3.40±0.22^{c}	2.90±0.18^{d}	2.40±0.16^{e}	1.40±0.16^{e}
Treatment 2	4.90±0.10^{a}	4.90±0.1^{a}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}
Treatment 3	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}
Treatment 4	3.70±0.30^{b}	3.70±0.30^{bc}	4.00±0.21^{b}	3.80±0.13^{c}	3.80±0.13^{c}
Treatment 5	4.90±0.10^{a}	4.90±0.10^{a}	4.80±0.13^{a}	4.80±0.13^{a}	4.80±0.13^{a}
Treatment 6	3.70±0.15^{b}	4.00±0.21^{b}	4.10±0.18^{b}	4.20±0.13^{b}	4.20±0.13^{b}
Treatment 7	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}
Treatment 8	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}
Treatment 9	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}
Treatment 10	3.30±0.30^{b}	3.30±0.30^{c}	3.40±0.27^{c}	3.40±0.26^{d}	3.00±0.21^{d}

Values represent means and standard error of general acceptability of chicken nuggets
$^{a-e}$ Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

Table 18: Effect of spice extracts on the proximate analysis of chicken nuggets

Extracts	Dry matter	Crude protein	Oil	Ash
Treatment 1	85.05±0.80^{c}	20.34±1.23^{b}	10.71±0.33^{ab}	6.46±0.61^{d}
Treatment 2	87.39±0.17^{ab}	20.84±0.52^{ab}	10.60±0.48^{abc}	7.28±0.2b^{cd}
Treatment 3	88.57±0.95 ^{a}	21.26±0.14^{ab}	10.32±0.84^{abcd}	8.09±0.11^{ab}
Treatment 4	86.69±0.03^{abc}	20.83±0.31^{ab}	11.14±0.08^{a}	8.69±0.12^{a}
Treatment 5	86.66±1.27^{abc}	21.29±0.81^{ab}	8.97±0.56^{bcde}	7.06±0.23^{cd}
Treatment 6	86.87±1.00^{abc}	20.12±0.06^{b}	9.06±1.16^{abcde}	6.72±0.04^{d}
Treatment 7	86.68±0.29^{abc}	22.19±0.76^{a}	8.50±0.5^{cde}	7.10±0.38^{bcd}
Treatment 8	87.46±0.23^{ab}	21.45±0.39^{ab}	9.30±0.91^{abcde}	8.01±0.45^{abc}
Treatment 9	87.13±0.97^{abc}	20.40±0.16^{b}	7.28±0.72^{e}	8.69±0.48^{a}
Treatment 10	85.60±0.35^{bc}	20.31±0.02^{b}	8.18±0.98^{de}	8.09±0.30^{ab}

Values represent means and standard error of proximate analysis
$^{a-e}$ Means in each column with different superscript letters differ significantly ($P < 0.05$); Treatment 1= Control, Treatment 2= Clove, Treatment 3 = Ginger, Treatment 4= African nutmeg, Treatment 5= Pepper, Treatment 6= Black pepper, Treatment 7= Clove + Ginger, Treatment 8= Clove + Pepper, Treatment 9= Ginger + Pepper, Treatment 10= Nitrite.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of rigor state, curing temperature and their interaction on the physico- chemical properties of cured and smoked-cooked broiler meat

The higher moisture content observed in the pre-rigor meat could be due to high pH of pre-rigor meat which enhances its water holding capacity. The higher lipid content observed in the pre-rigor meat could be due to the absence of rigor mortis at this state. Rigor mortis causes shrinkage of myofibrils, reduced water holding ability and increase exudate drip loss along with oil. A higher residual nitrite level was observed in the post-rigor carcass but were both within the limits recommended by U.S.D.A. (2010) which is 200ppm for brined and injected meat. The result is in accordance with the reports of Ogunsola and Okubanjo (2001), who observed that curing pre-rigor rabbit meat led to reduction in weight of the meat.

The yield and moisture of cold cured carcass which was observed to be significantly higher than the hot cured carcass, could be due to transverse shrinkage of the fibre axis caused by the high temperature of the brine. The longer time the cold cured meat was retained in the brine, may have also led to its high yield, which may increase the time for uptake of water and salts. The result agrees with the reports of Ogunsola and Okubanjo (2001). They observed that there was lower yield obtained from rabbit meat cured at 45^0C when compared with the conventional method. The nitrite and salt levels which were higher in the hot cured treatment could be due to the fact that in the hot cured treatment, there may be some ease in the binding of salt and nitrite to the protein, hence a higher value.

The interaction between rigor state and curing temperature gave a higher yield in the post-rigor cold cured treatment. This could be due to the synergic effect of longer curing time and higher retention of nutrient absorbed as a result of closed network of myofibrils. The high moisture and ash content observed in the pre-rigor cold cured may also be as a result of the longer curing time in brine, high ATP, high pH of pre-rigor muscle, which promote higher intake of water due to the loose network of actin and myosin filaments (Pisula and Tyburcy, 1996; Claus and Sorheim, 2006), hence, the high ash content as brine is composed of dissolved solutes. The lipid content which was higher in the pre-rigor hot cured and pre-rigor cold cured meat could be due to the absence of rigor which reduces shrinkage loss. The trend observed in the nitrite and salt values could be due to the accelerated distribution of dissolved solutes as a result of high temperature of hot brine. However, Lauritzsen *et al*. (1993) observed a lower salt uptake in pre-rigor salted fish. The results obtained correspond to the findings of Ogunsola and Okubanjo (2001).

5.2 Effect of rigor state on the sensory properties of smoked cooked broiler meat

The effect of rigor state on sensory properties of the product showed that the pre-rigor meat was tender, juicier, flavoured and more acceptable, when compared to the post-rigor meat. The observed result could be due to the high water holding capacity of pre-rigor meat which makes it possible for such meat to maintain desirable culinary properties. Saltiness was also observed to be higher in the pre-rigor meat. The result observed in this study is in accordance with the report of Cia and Marsh (1976), Ogunsola and Okubanjo (2001) and Okubanjo *et al*. (2010).

Hot-cured-treated meat was also observed to be better in terms of tenderness, juiciness, saltiness, smoky flavour and acceptability, while the interaction effect between rigor state and curing temperature showed better qualities in the pre-rigor hot cured products. This result was similar to the report of Ogunsola and Okubanjo (2001).

5.3 Antimicrobial activity of individual spice extracts at various concentrations in agar media

All spice extracts tested in agar media exhibited positive activities against the pathogenic microbes (*Listeria monocytogens, Escherichia coli, Bacillus sp. and Staphylococcus sp*.). However, the extracts from clove, ginger and pepper showed a better antimicrobial effect against all the four organisms. The results indicated that extracts of clove, ginger and pepper are promising antimicrobials, possessing significant activity against *L. monocytogenes*. Subsequent experiments to determine whether there was a synergy between individual antimicrobials were carried out using mixtures of these three spice extracts. The highest synergy was observed on the cloves and ginger combination (M1) as it was able to suppress the growth of *Listeria monocytogens, Escherichia coli* and *Bacillus sp.* better than the other combinations. This could be due to the high phenolic contents of the spice extracts. Antibacterial activity of natural antioxidants has been determined through *in vitro* assays against *Escherichia coli* 0157:H7, *Salmonella typhimurium* (Helander *et al*., 1998; Elgayyar *et al*., 2001), *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Elgayyar *et al*., 2001; Lambert *et al*., 2001). The results of this study agreed with the invitro antimicrobial activity of plant essential oils on several Gram positive and Gram negative bacteria (Sreenivasan *et al*. 2006).

5.4 Effect of spice extracts and storage time on pH and thiobarbituric acid reactive substances (mg of malondialdehyde/kg of chicken) of chicken nugget stored at 4°C for 12 days

The increase in pH value during storage period suggests significant break-down of meat protein on storage of the product. The increase in pH value during storage of meat was also reported earlier (Yadav and Sanyal, 1999; Kumar and Tanwar, 2011). The TBA values showed that there was no significant difference among treatments on day 1; but as storage days increased, there was significant difference, and the combined extracts showed better stability against lipid oxidation in the stored products than when used singly. When the extracts were used singly, clove and ginger showed better stability against lipid oxidation. Comparing the different treatments and the control, all the treatments showed traces of better lipid oxidation stability, while the values obtained in the control increased astronomically.

Chicken nuggets containing treatments maintained significantly ($P < 0.05$) lower TBA values throughout the storage periods. Lower TBA values in optimized product were due to the anti-oxidant properties of the extracts. Spices have also proved to act as effective antioxidants, to stimulate Lactic Acid Bacteria (LAB) activity by supplying Mn, which inhibits undesirable organisms (Hagen *et al.*, 2000; Chi and Wu, 2007), their extracts have been shown to exert positive effect on lipid oxidation by reducing 2-thiobarbituric acid (TBA) or malondialdehyde (MDA) formation on different types of meats during refrigeration storage (Su *et al*., 2007). The studies conducted by Tanabe *et al.* (2002) showed that adding methanolic extracts of 22 selected herbs and spices decreased lipid oxidation in pork with sansho (*Zanthoxylum piperitum* L.) extracts but sage and ginger (*Zingiber officinale* Rosc.) exerted the greatest effect. The rhizome of

the popular ginger species (*Zinger officinale*) is well known to have antioxidant activity (Ibrahima *et al.,* 2011). Clove powder on chicken nugget have been reported to be an effective antioxidant in the product and other products (Kumar and Tanwar, 2010).

5.5 Effect of spice extracts and storage time on microbial count of chicken nugget stored at 4°C for 12 days

An increasing trend in microbial counts was observed with the advancement of storage period, but chicken nuggets containing spice extracts maintained a significantly ($P<0.05$) lower microbial counts (TPC, TCC and TFC) throughout the storage periods than the controls. Lower microbial count observed in nuggets, containing spice extracts suggested antimicrobial properties of the spices. Some authors reported that plant extracts with antimicrobial properties can be used to increase refrigerated meat shelf-life (Djenane *et al*., 2002; Chouliara *et al.*, 2007). The antimicrobial properties of essential oils extracted from plants such as spices are used against a wide range of microorganisms (Chao *et al*., 2000). Essential oils are capable of inducing bacterial cell lysis, damaging both cell wall and membrane of the microbes (Rhayour *et al.,* 2003). The hydrophobicity of essential oils enables them to partition the lipids of the cell membrane and mitochondria, rendering them permeable and leading to leakage of cell contents causing cell death (Burt, 2004).

Extracts of ginger and rosemary significantly decreased the TPC count of treated patty during storage at 4°C for seven days (Baker *et al*., 2012). Similarly, Jitoe *et al.* (1992), Zia-ur-Rehman *et al.* (2003) and Ibrahima *et al.* (2011) found that ginger has antioxidant activity and is an effective antimicrobial agent. It has been reported that extract of rosemary and ginger showed an inhibitory activity against coliform bacteria (Malu *et al.,* 2009; Kenar *et al.,* 2010).

Spices like clove and cinnamon generally used in Indian culinary have been reported to exhibit suppressing action on many food-borne pathogens, with clove exerting antimicrobial effect (Yadav, 2005; Mytle *et al*., 2006). Effect of ethanolic extract of clove on the keeping quality of fresh mutton during storage at ambient temperature 25±2°C showed a positive effect (Kumudavally *et al*., 2011). Inhibitory effect of clove on several spoilage and toxigenic molds has been reported (Rajkumar and Berwal, 2003). The inhibitory effect of clove extract on *E. coli, S. aureus, B. cereus* and spoilage organisms has been shown (Yadav and Singh, 2004; Kong *et al.,* 2007) and on certain fungi (Guo and Cheng, 2006; Viuda *et al.,* 2007). Eugenol (2-methoxy-4-2-propenyl phenol), the phenolic component of clove is known to possess antibacterial activity (Burt, 2004), and clove is reported to possess highest total phenolic constituents (141 mg/g) compared to other spices like cumin and black mustard (Kumudavally *et al*., 2011).

The seed extracts of *Monodora myristica* produced a significant anthelmintic activity (Ekeanyanwu and Etienajirhevwe, 2012).

5.6 Effect of spice extracts inclusion on the sensory properties of chicken nuggets store at 4ºC for 12 days

Stability in colour, texture, odour and general acceptability of nuggets treated with spice extracts were observed to be better than the control as storage days increased. Natural antioxidants such as ginger extracts (Baker *et al.,* 2012), clove powder (Kumar and Tanwar, 2010) have been reported to have better overall acceptability, when compared to other groups.

5.7 Effect of spice extracts on the proximate analysis of chicken nuggets

Results obtained on proximate composition of chicken nuggets with spice extract inclusion indicated that incorporation of spice extracts in chicken nugget formulation caused slight increase in dry matter, crude protein and ash with a decrease in oil content. The result obtained is in accordance with that of Kumar and Tanwar (2010), who reported better proximate composition of chicken nuggets treated with clove powder.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The study was undertaken to determine the effect of rigor state and curing temperature on the quality of smoked and cooked broiler meat. It was also aimed at determining the effects of spice extracts on the quality and shelf-life of chicken nuggets stored at 4^0C for 12 days. The results revealed that rigor state had no significant effect ($P>0.05$) on yield and general acceptability of the meat but had significant effect ($P<0.05$) on dry matter, moisture, lipid and nitrite levels of the products. Curing temperature had significant effect ($P<0.05$) on percentage yield, dry matter, moisture, nitrite and salt but not significant ($P>0.05$) with respect to general acceptability. The interaction between rigor and temperature was statistically significant on percentage yield, dry matter, moisture, ash, lipid, nitrite, salt and general acceptability of the product. Spice extracts inclusion in chicken nuggets significantly ($P<0.05$) decreased the TBA, TPC, TCC, TFC values. Extracts of cloves, ginger, pepper and their combinations had exceptionally high activities against lipid oxidation and microbial growth as storage days increase. Spice extracts significantly ($P<0.05$) affected the sensory properties of the nuggets. Scores on general acceptability was higher ($P<0.05$) in treatments with clove, ginger, clove and ginger combination, clove and pepper combination and nitrite as storage days increased. Proximate analysis of the nugget samples containing different spice extracts was significant. The highest percentage dry matter, crude protein, oil and ash were obtained from treatments ginger, clove and ginger combination, African nutmeg and nitrite.

6.2 Conclusion

The yield of pre-rigor and post-rigor cold cured treatments gave better results when compared to pre-rigor and post-rigor hot cured treatments with the post-rigor cold cured being the best treatment. On the other hand pre-rigor carcasses had better sensory profile and acceptability. The inclusion of spice extracts in chicken nuggets increased the shelf life and sensory attributes of the product.

6.3 Recommendation

Broiler meat should be cured pre-rigor or post-rigor at 2^0C to improve yield and as such increase the profit of the producer and pre-rigor curing should be done to improve the sensory profile of the product.

Extracts of cloves, ginger, pepper and their combinations can be included at 80mg/g of meat to improve sensory properties and increase the shelf life of chicken nuggets.

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APPENDICES

Appendix 1: Sensory evaluation sheet for smoked-cooked meat

Please choose the terms that best reflect your rating of the products displayed before you, assess the sample by writing the appropriate number under the product code. Ensure you rinse your mouth after each tasting.

Date:_____ Sex:_____ Age:_____

	1	2	3	4
COLOUR 1= Very dark 2= Moderately dark 3= Intermediate 4= Moderately light 5= Very light				
ODOUR 1= Very off 2= Moderately off 3= Intermediate 4= Moderately sweet 5= Very sweet				
TEXTURE 1= Very slimy 2= Moderately slimy 3= Intermediate 4= Moderately firm 5= Very firm				
JUICINESS 1= Very dry 2= Moderately dry 3= Intermediate 4= Moderately juicy 5= Very juicy				
GENERAL ACCEPTABILITY 1= dislike very much 2= Moderately dislike 3= Neither like nor dislike 4= Like moderately 5= Like very much				

Additional comment (If any)……

Appendix 2: Sensory evaluation sheet for chicken nugget

Please choose the terms that best reflect your rating of the products displayed before you, assess the sample by writing the appropriate number under the product code. Ensure you rinse your mouth after each tasting.

Date:_____ Sex:_____ Age:_____

	1	2	3	4	5	6	7	8	9	10
COLOUR 1= Very dark 2= Moderately dark 3= Intermediate 4= Moderately light 5= Very light										
TEXTURE 1= Very slimy 2= Moderately slimy 3= Intermediate 4= Moderately firm 5= Very firm										
ODOUR 1= Very off 2= Moderately off 3= Intermediate 4= Moderately sweet 5= very sweet										
GENERAL ACCEPTABILITY 1 = dislike very much 2 = Moderately dislike 3 = Neither like nor dislike 4 = Like moderately 5= Like very much										

Additional comment (If any)

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