EFFECT OF GRADED LEVELS OF WATER LEAF (*Talinum triangulare*) EXTRACT ON OXDATIVE STABILITY OF BROILER MEAT

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CERTIFICATION

This is to certify that this project was carried out by ZUBAIR JAMIU IBRAHIM and has been read and approved as meeting the requirement for the award of master of Science (M.Sc.) in Animal Production, Faculty of Agriculture, University of Ilorin, Kwara state, Nigeria.

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DEDICATION

This work is dedicated to the Almighty Allah, the creator of all things who fore know this time.

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All adoration, honour, praise and thanks to the LORD of Lords, Alpha and Omega, the Author and Finisher of our faith, the I am that I am for his divine grace, love and guidance over me endures forever.

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ABSTRACT

Antioxidant effect of graded levels of *Talinum triangulare* (Waterleaf) extract on cooked and raw broiler meat was evaluated and compared with Butylated Hydroxyl Anisole (BHA), a synthetic antioxidant, using the thiobarbituric acid (TBA) assay. The minced broiler meat was weighed into five samples of 700g each. The treatments consist of a control without additive; waterleaf extract was separately added at a rate of 0.1%, 0.2%, and 0.3% of the weight of the minced meat, while BHA was added at a rate of 0.01%. Each sample was divided into 56 parts of 12.5g each. Twenty eight of these were cooked in microwave oven over 1minute, 30 seconds, while the other twenty eight samples were left raw. Both cooked and raw samples were stored in a refrigerator for 14days at a temperature of 4°c.Oxidative stability of the cooked and raw samples was monitored at 2-day intervals. The result shows that no levels of waterleaf extract were able to reduce lipid oxidation in both raw and cooked meat samples. This was shown by their higher TBARS values which were significantly different. BHA was able to reduce lipid oxidation in both cooked and raw meat samples. However, its antioxidant potency was well expressed in raw meat samples. *Talinum triangulare* (waterleaf) extract should not be considered as

the possible source of natural antioxidant in the prevention of broiler meat against lipid oxidation under refrigeration storage.

Key words: Antioxidant, Waterleaf, Butylated Hydroxyl Anisole (BHA), Thiobarbituric Acids (TBA), broiler meat.

CHAPTER ONE

1.0 INTRODUCTION

Poultry meat is an important source of food in many parts of the world. This is because it is a good source of protein, which is indispensable to balance diet and it is valued in many cultural culinary traditions. During storage, the quality attributes of poultry meat undergo lipid oxidation due primarily to its high unsaturated fatty acids. Lipid oxidation is considered one of the major problems in the meat industry, due to the resultant flavor deterioration and loss of nutritional value (Ladikos and Lougovois, 1990; Ahn *et al.,* 1992). Lipid oxidation is a complex process whereby unsaturated fatty acids react with molecular oxygen via free radicals, and form peroxides or other primary products of oxidation (Gray, 1978), Secondary oxidation products, such as aldehydes, ketones and esters, are responsible for the increase deterioration and rancid flavour (Ladikos and Lougovois, 1990) during frozen storage (Igene *et al.,* 1981). The major strategies for preventing lipid oxidation in

mechanical deboned poultry meat are the use of free radical terminators, such as phenolic antioxidants (Jantawat and Dawson, 1980a; Nolan et al., 1989; Ladikos and Lougovois, 1990; Mielnik *et al.,* 2003).

Antioxidants have been known to play protective role in human body against deleterious effects of reactive free radicals .Antioxidants can be define as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1990). They are chemical compounds that can prevent, stop or reduce reactive effect of free radicals. These effects include oxidative damage to membrane and enhanced susceptibility to lipid peroxidation or enzyme inactivation (Farombi and Fakoya, 2005, Sathishsekar and Subramanian, 2005).

Free radicals include hydroxyl (OH^-), superoxide ($O^{\cdot}_2{}^-$), nitric oxide ($NO^{\cdot}$), nitrogen dioxide ($NO_2^{\cdot}$), peroxyl ($ROO^{\cdot}$), and lipid peroxyl (

$LOO^{\cdot}$), Also, hydrogen peroxide (H_2O_2),Ozone (O_3), singlet oxygen ($^1O_2$), hypochlorus acid (HOCL), nitrious acid (HNO_2), lipid perixynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), lipid perioxide LOOH), are not free radicals but generally, they are called oxidants, although, they can easily lead to free radical reactions in living organisms (Genestra, 2007). Biological free radicals are thus highly unstable molecules that have electrons available to react with various organic substrates such as lipids, proteins, DNA and eventually progress to oxidative stress (OS). Thus, Antioxidant containing foods like fruits and vegetables could have strong protective effect against the risk of major diseases such as Cancer and Cardiovascular diseases (Kaur and Kapor, 2001, Amic *et al.,* 2003). Vegetables and fruits extracts are often employed in folkloric medicine to treat several ailments (Wang *et a*l., 2007).

Talinum triangulare (portulacaceae) is a herb with fleshy green leave, succulent stem and pink flowers which are rarely white (Keay, 1981). It is a season vegetable, grown mostly in West Africa from seed or by

vegetative propagation (Akobundu and Agyakwa, 1998, Imoh and Julia, 2000). *T.tringulare* leaf is found to be a rich source of chemical substances such as flavonoids, alkanoids,tocopherols, carotenoids, which help for the used in folkloric medicine to treat diuretic and gastrointestinal disorders (Mensah *et a*l.,2008) and Oedema.

Lipid oxidation is major cause of deterioration in the quality of meat and meat products. Undesirable changes in color, flavor and nutritive value occur as meat lipid oxidize and interact with other meat constituents, such as pigment and other protein, carbohydrates and vitamins.

Fresh meat and many processed meat products are susceptible to lipid oxidation. Oxidative deterioration occurs in raw meat and stored at refrigerator temperature and in fresh meat in frozen storage. Stored cooked and cured meat, freeze-dried, and irradiated meat products are all susceptible to lipid oxidation. In prepackaged fresh meat, the brown color and lipid oxidation due to ferric hemes are highly undesirable from the consumers' point of view.

Control of lipid oxidation in meat and meat products has become increasingly important with greater production and consumption of precooked meat items for institutional and home use.

1.1 **JUSTIFICATION**

The high incidence of degenerative diseases such as cancer, diabetes, Arthritis and other related diseases in human linked to the consumption of synthetic preservatives in food calls for immediate attention. Thus, the latest trend of returning to the natural sources for health, medicine and prevention of lipid oxidation in meat or lipid food has created a lot of development in the recovery of natural antioxidants. Natural antioxidants are not only effective in preventing lipid oxidation in meat or lipid food, but also have beneficial effects on human health. This is a critical factor in the antioxidant defense mechanism, populations that consume a diet that offers natural antioxidant molecules from variety of sources tend to have lower risk of cardiovascular disease, arthritis, diabetes, hypertension and

cancers. Hence, antioxidants from fruit, green leafy vegetables would prove to be better for health and prevention of lipid oxidation. This study of antioxidants from waterleaf extract (green leafy vegetable) would prove to be better for preservatives of broiler meat against oxidative rancidity and better health for teeming population. This would also serves as a good resources material for further research in the usage of waterleaf extract in meat or food industry especially with the respect to lipid oxidation.

1.2 OBJECTIVE OF THIS STUDY

The objective of this study is to determine the effect of graded levels of waterleaf extract on oxidative stability of broiler meat under refrigerated storage.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 COMPOSITION OF MEAT LIPID

Lipids found in meat can be classified as depot or intermuscular and as intramuscular or tissue lipids. The depot or intermuscular lipids are generally stored in specialized connective tissues in relatively large deposits, whereas tissue lipids are integrated into and widely distributed throughout muscle tissues. The intracellular lipids exist in close association with proteins and contain a large percentage of the total phospholipids. The phospholipids, contribute about 1% of the tissue weight, the triglyceride fraction is about five times as large.

Though the phospholipids content of meat is relatively small, susceptibility of the phospholipids to oxidation makes them important in determining meat quality. Liability of the phospholipids is a result of their high unsaturated fatty acid content for example 19% of the fatty acids in beef phospholipids have four or more

double bonds, why only 0.1% of triglyceride fatty acids from beef show this degree of unsaturation. Particularly high levels of linoleic and arachidonic acids are found in phospholipids. Phospholipids may also exist in closer contact with tissue catalyst of oxidation than do the triglycerides, thus increasing their tendency to oxidize.

The amount of phospholipids has been shown to be relatively constant in muscles from different animals or carcass location, while the amount of total lipids and neutral lipids are more variable.

The fatty acids composition of phospholipids reportedly shows some variation with carcass location. Luddy *et a*l., (1968) reported that phospholipids fatty acids from muscles classified as light colored had a higher content of monoeres, while polyunsaturated fatty acids predominated in the phospholipids in dark muscles.

Kuchmak and Dugan (1968) noted the variation in the composition of fatty acids from phospholipids from different muscles sources. Pork belly muscles phosphatidylethanolamine was observed to have elevated levels of linoleic and arachidonic acids. This factor may

explain the tendency of pork bellies to undergo oxidative deterioration. It seems possible that compositional differences in fatty acids in the phospholipids fraction may result in varying susceptibilities to oxidative rancidity in cuts from different carcass locations.

2.2 OXIDATION OF MEAT LIPIDS

Early studies of rancidity in meat were concerned with oxidation of the adipose tissue and have been reviewed by watts (1962). The role of tissue lipids in rancidity was postulated by Tims and watts (1962). They noted rapid flavor deterioration in cooked meats during storage and proposed that this change in flavor resulted from the oxidation of highly unsaturated protein-bound phospholipids. Later work showed that less oxidation occurred in the neutral lipids fractions separated from rancid cooked pork than in the total lipids extraction or in the fraction referred to as phosphor- or proteolipids.

Hornstein *et a*l; (1967) also observed that phospholipids fraction and the total lipids from pork and beef became rancid quickly when exposed to air.

The neutral fat fraction developed off flavors less readily, leading to the conclusion that phospholipids contribute to poor flavor, particular in excessively lean meat. Neutral fat may trap volatile decomposition products of polar lipids and thus reduce their effect on flavor.

In addition to the rapid lipids oxidation observed in cooked meat, lipids oxidation occurs in raw meats with adverse changes in flavor and colour resulting. Although frozen raw meat is generally fairly resistant to oxidation, rancidity can develop during freezing and thawing. Wide fluctuations in temperature and inadequate protection from oxygen can accelerate the development of rancidity. Under good conditions, however, lean raw meat is quite stable for periods of several months to a year, depending on the species from which it originated and storage conditions.

Lower levels of lipid oxidation have been observed in cooked, cured meat than in uncured samples. As long as the meat pigment is in the pink, cured, ferrous form, lipid oxidation occurs very slowly. On storage of cured meat, however, the pigment is converted to the brown ferric form, which accelerates oxidation and results in increased TBA values for the stored product. Treatments that retard lipid oxidation are effective in preventing pigment breakdown. Frozen, cured, cooked pork samples may be protected from salt-catalyzed lipid oxidation with sodium tripolyphosphophate and 0.108% sodium ascorbate. Increases in lipid oxidation are related to the loss of ascorbate in the stored samples.

Many researchers have noted adverse changes occurring in freeze-dried foods as a consequence of oxygen uptake. Harper and Tappel *et a*l., (1952) have reviewed earlier work on oxidation deterioration in freeze-dried meat. Oxidation of tissue lipids has been reported to occur in two steps, with the phospholipids becoming oxidized first and the neutral fats later.

Protein denaturation, resulting in a decrease in solubility, may also result from pronounced oxidation of the polyunsaturated fatty acids. Deteriorative reactions occurring in freeze-dried meats may not be the oxidative rancidity reactions typical of fresh and frozen meats. Interactions between proteins and cross linking of auto oxidizing lipids and protein may take place. Tuomy *et a*l; (1969) found highly significant positive correlation and coefficients between oxygen uptake and taste panel results for a variety of meat products.

Presumably oxidation of meat lipids contributed to the undesirable flavor and odor. Certain products were also observed to be less susceptible to oxygen uptake and subsequent deterioration, indicating that some natural antioxidant activity was present. Certainly the correlations between oxygen uptake and flavor and odor deterioration indicate the desirability of low levels of oxygen in the storage atmosphere of freeze-dried meat items.

Lipid oxidation is an important cause of flavor deterioration in cooked meat irradiated at pasteurizing levels and refrigerated. Chang

*et a*l., (1950) showed that cooked, irradiation-sterilized beef did not develop oxidative rancidity when stored in air tight containers and postulated that the pigments were converted to a catalytically inactive form during radiation treatment. Greene and watts later indicated that low TBA values observed for stored, cooked irradiated meat were due to a combination of antioxidant development and further reactions undergone by lipid oxidation products.

2.3 CATALYSTS OF LIPID OXIDATION IN MEAT

Much effort has been devoted to identification of catalysts in animal tissues responsible for the oxidation of unsaturated lipids. The accelerating effect of hemoglobin and other iron porphyrins on the oxidation of lipids is a generally accepted phenomenon, and hemoproteins have been implicated as the major prooxidants in meat and meat products. Early work, reviewed by Watts (1962), demonstrated that heme catalyzed lipid oxidation results in destruction of the pigments, as well as oxidation of the fatty tissue.

Ferric hemochromogen is postulated to be the active catalytic form of the muscle pigments. In cooked meat, the pigment is in the active denatured ferric hemochromogen form, accounting for the rapid initiation of lipid oxidation. The lower level of oxidation in cured, stored meat results from the conversion of the pigments to the catalytically inactive ferrous nitric oxide hemochromogens. In fresh meat the pigments exist in three forms: purple reduced myoglobin, red oxymyoglobin and brown metmyoglobin. Metmyoglobin is undesirable from the standpoint of meat color and also the oxidation of unsaturated lipids. Free radical intermediates from this reaction can decompose hemes, resulting in loss of colour. Thus, pigment and lipids oxidation are interrelated in fresh meat, and of crucial importance from the standpoint of consumer acceptability.

Some of the work on iron porphyrins as biocatalysts has been reviewed by Tappel (1952). A mechanism proposed for the prooxidant activity of hemes is based on their known ability to

decompose lipid peroxide. In this theory, free radicals resulting from the peroxide scission initiate new reaction chains.

Hemes compounds can also act as antioxidants rather than prooxidants. Kendrick and Watts (1969) reported that no oxidation occurred at heme concentrations of three to four times the concentration required for optimum catalytic activity. While studies with model systems are of great value, the implications for complex systems, such as meat and meat products, are of a speculative nature. Treatments, such as cooking and freezing, may alter the influence of cellular hemes on the oxidation of tissue lipids.

Other meat components have been attributed catalytic roles in lipid oxidation. Some metals, especially ferrous iron, occurring in meat in trace amounts, are efficient lipid oxidation catalysts. Iron, in combination with ascorbic acid, has been implicated in lipid oxidation in meat, also as the major catalyst of lipid oxidation in tissue homogenates and fractions. Sodium chloride, a common meat additive, has a puzzling effect on oxidative changes in meat. The role

of NaCl in initiating color and flavor changes in cured meat is well recognized but poorly understood. Although, some evidence indicates that trace metal impurities present in salt account for its effects on lipid oxidation, there is evidence for a direct role of sodium chloride in initiating of the stored triglycerides, and the effect of sodium chloride on fat oxidation depends on the level of free moisture in the system. The effect of NaCl on oxidation has been attributed to the action of the reactive chloride ion on lipids, or to a modification of the hemoprotein catalysts of lipid oxidation.

2.4 CONTROL LIPID OXIDATION IN MEAT

Lipid oxidation and the related deterioration in color and flavor can be inhibited by using antioxidants and chelating agents, especially polyphosphates. In raw meat, propylgallate and butylated hydroxyl anisole (BHA) protect meat pigments and inhibit lipid oxidation. Polyphosphates are ineffective in raw meat, presumably due to hydrolysis of the phosphate group by muscle phosphates.

There is also evidence indicated that natural antioxidants may be produced in meat under certain conditions, such as prolong heating at high temperatures and by sterilizing doses of ionizing radiation.

If sufficient reducing activity is present in meat, wrapping fresh meat in an oxygen impermeable wrap should result in reduction of myoglobin, anaerobiosis and retarded lipid oxidation of canned, refrigerated raw meat by evacuation have been reported to be less successful than treatment with antioxidants.

In view of the importance of meat color and flavor to the consumer, efforts to find acceptable ways of limiting lipid oxidation are of great importance.

2.5 CHEMICAL COMPOSITION OF MEAT

Meat contains majorly muscle, connective tissue, blood, water, fat content, protein and carbohydrate. Meat colour is primarily determined by the varying quantity of myoglobin and hemoglobin. Fat content of meat varies between 5-40% depending on the type of breed, nutritive content of feed and age of animal. These fats are deposited subcutaneously and as a protective layer around the organs of the animal.

A value of about 60 - 80% protein on dry matter basis can be obtained from any meat source. Oke (1967) reported that a value of between 68 - 69% proteins on dry matter basis was obtained from the flesh of the white fulani cattle while 29% (on wet basis) was recorded for the guinea fowl (Ayeni *et a*l; 1983).

Connective tissue of all types are present in meat with adipose tissue (fat); cartilage, bone properly predominating (Forest *et a*l; 1975). Connective tissues holds and connect other tissues, skeleton, lymph, organ and blood vessels. The amount of connective tissues and the sarcomere length of the muscle are correlated with tenderness (Herring et al; 1965; Marsh, 1977; Mckeith *et al*; 1985). Connective tissues contain two proteins collagen and elastin. Collagen on heating in the presence of suitable moisture dissolves and yields gelatin. The higher the collagen content, the firmer or tougher the raw meat (Sato et al., 1986: Hatue *et al*., 1986). Thus it is reasonable to speculate that muscle collagen is partly responsible for the texture or tenderness of raw and cooked meat.

Tougheness of meat generally is dependent on connective tissue content (epimisial and intramuscular and myofibrilliar structure of meat as well as binding between meat pieces. Dranfield (1977), reported that muscle with most intramuscular collagen were the toughest. Muscles with higher rates intrasmuscular and / or epmysial

collagen solubilization were reported to have lower shear force and better sensory scores (Light, *et al*., 19854; Goll *et al*., 1964).

Others reported that total intramuscular collagen was unrelated to Warner – Bratiler measurement or that soluble intramuscular collagen had no major influence on the sensory or instron texture characteristics of muscle (Naewbainj *et al*., 1985; Fole *et al.,* 1982; Goll *et al.,* 1963).

The most important functional properties in meat are protein – water interactions, protein– lipid association and protein – protein aggregation (Kinsells, 1976; Acton *et al.,* 1983). Myofibrillar proteins are most responsible for the functionality of comminuted meat products (Acton *et al*., 1983). Proteins unfold and aggregate when heated to form a three dimensional cross – linked protein network which trap fat and macro particulates within the gel matrix. This matrix is responsible for texture and fat and water binding characteristics of finished comminuted product. (Ziegler and Action 1984, Kijonski and Niewiarowicz, 1978) Many studies have been

carried out on the changes of myofibrillar proteins during post mortem storage, the causes of these changes and the relationship between these changes and meat tenderization (Asghur and Bhatti, 1987; Penny, 1980). The important of myofibrillar proteins for gelatin, textual properties and binding of water and fat in processed meat is well documented (Acton et al., 1983: Schimndt *et al*., 1981).

Proteolysis of muscle appears to be the major contributor to the tenderization process during post mortem aging. Proteolysis changes in collagen during post mortem storage comparable to those of myofibrillar proteins (Tarrant, 1987) have not been observed.

Therefore, the principal mechanism of meat tenderization during postmortem storage may be limited to the hydrolysis of myofibrillar proteins by endogenous muscle proteases (Goll *et a*l., 1883a). The use of proteolytic enzymes and fungal proteases has been widely studies (El-Gbarbavi and Whitaker, 1963; Tsen and Tappel, 1959. Miyade and Tappel (1956) reported that papain, ficin and fungal enzymes readily hydrolyzed the soluble fraction of the proteins of

muscle fibers and that all of them hydrolyzed collagen to some extent. In contrast, Wang *et a*l., (1957) reported that the proteolytic enzymes of plant origin act preferentially against connective tissue. Moreover, they also found that ficin and papain was the only proteolytic enzyme that degraded elastin.

Meat fats are rich in saturated fatty acids and it is likely that it produces certain forms of atherosclerosis. The fatty acid in the lean portion of meat contains greater proportions of unsaturated fatty acids than tissue fats. Marbling in meat refers to fats deposits between muscle fibers. Marbling can be desirable in some meat; the amount of fat present consequently influences overall meat juiciness and flavor. Cholesterol which is a poly unsaturated fatty acid, content in meat is about 75mg per 100g. The lean portion of meat contains a high percentage of phospholipids (0.5-1, 0 percent) and these are located in the membranes of the cell.

Moulton (1973) observed the water contents in the muscle of animals to vary between 50-85 percent depending on the amount of

neutral lipids percent. The neutral lipid holds water in an ionic bonding and usually releases this water upon heating.

During cooking of meat, some water, fat and protein are lost resulting in a reduction in the initial weight after cooking. Thompson *et al*., (1986) observed less cooking weight loss for broiler meat cooked 24hours postmortem. Water holding capacity (WHC) which is an essential subjective measurement of raw and cooked meat can be defined as the ability of meat to retain its water during application of external forces such as heating, cutting, grinding, pressing, etc. tenderness of meat is closely associated with WHC, and therefore it is not surprising that factors that affect WHC also affect tenderness.

Glycogen and micro polysaccharides are the two known forms of which carbohydrates are present in lean muscle of animals. They are energy reserves found in areas of concentrated muscle such as thigh, drumstick and breast portion of birds. Mast et al., (1984) concluded that fasting, anxiety and fear affects the release of glycogen reserves to a significant extent.

Most skeletal muscle is attached directly to ligaments, fascia, cartilage and skin. Muscles can be physically divided successfully into smaller longitudinal units each of which is surrounded by a sheath of connective tissue. The muscle itself is surrounded by the epimysium, myofibrils are organelles within the sarcolemma and are suspended in a fluid phase called sarcoplasm.

The relative amount of protein in the muscle of different animals will vary with their nutrition, malnutrition and the species under study. Meat contains 15-20 percent proteins of outstanding nutritive value.

Lean meat contains 20-22 percent protein of the total nitrogen content of meat, approximately 95 percent is protein and 5 percent is smaller peptides and amino acids.

There is a determined relationship between protein and moisture content as well as protein and fat as the animal grows. The percentage protein tends to reduce as the fat content increases (Oke, 1967). Amino acids derivable from meat protein are the essential

amino acids required for the growth and maintenance of cells and tissues of the body.

2.6 EFFECT OF PROCESSING, STORAGE AND FAT COMPOSITION ON LIPID OXIDATION OF MEAT

Sato and Hegarty (1971) postulated that any process causing disruption of the muscle membrane system, such as grinding, cooking and deboning, results in exposure of the labile lipid components to oxygen, and thus accelerate development of oxidative rancidity. Destruction of the extremely well organized structure of living animal cells will bring together lipids, Oxidation catalysts and enzymes responsible for lipid oxidation (Hall, 1987).

Pearson *et al.,* (1977) suggested that chopping and emulsification are at least as likely to cause WOF as grinding or mincing of samples. MacNeil *et al.,* (1973) and Dawson and Gartner (1983) attributed the high oxidative potential of mechanically deboned poultry to the extreme stress and aeration during the process and the compositional

nature (bone marrow, haem and lipids) of the product; TBA values increase most rapidly with decreasing particle sizes, as the latter are related to greater cell disruption. On the other hand, comminuted beef has a storage life similar to that of intact pork, despite the differences in fatty acid composition (Enser, 1987). The extent of lipid oxidation in cooked meat appears to be related to the intensity of heat treatment. In a survey of the malonaldehyde (MA) content of retail meats and fish (Siu & Draper, 1978), it was reported that 38% of all fresh meat samples tested had MA contents less than 1 pg/g whereas 60% were in the range 1-6 *pg/g.* Highest MA values were found among cooked chicken, cooked pork and cooked beef roasts. Keller and Kinsella (1973) observed increases in TBA values on cooking up to 70°C; further increases were observed when cooked samples were stored for 36 days at -18°C.

Pearson *et al.,* (1977), reported that meat heated at 70°C for 1 h developed rancidity rapidly. However, TBA values decreased when the cooking temperature was raised above 80°C. According to Huang

and Greene (1978), meat subjected to high temperatures and/or long periods of heating developed lower TBA values, than did samples subjected to lower temperature for a shorter period of time. This phenomenon was postulated to have resulted from antioxidant substances produced from browning reactions during the heating of meat. Earlier on, Sato *et al.,* (1973) had demonstrated that reductic acid, maltol and products of the amino-sugar reaction were effective inhibitors of development of WOF in cooked ground beef. This is not irrelevant to the observation that precooked beef roasts prepared by low temperature cookery for food service establishments, are not as stable to oxidative rancidity as roasts cooked in a more conventional manner (Allen & Foegeding, 1981). Similarly, Einerson and Reineccius (1977) reported that retorted turkey meat was found to have significant resistance to development of WOF, as opposed to less severely cooked turkey. The active antioxidant material extracted from retorted turkey exhibited strong reducing properties similar to those of reductones (known intermediates of the browning reaction)

and was thought to act as a primary antioxidant, interrupting the free radical mechanism (Einerson & Reineccius, 1978). Tiros and Watts (1958) noted that flavour deteriorated rapidly in cooked beef after only a few hours of refrigerated storage. Keller and Kinsella (1973) revealed marked increases in TBA numbers of frozen stored hamburgers and suggested that uncured cooked meats should not be stored for prolonged periods. Upon removal of cooked hamburger from frozen storage, discoloration and off-odours were perceptible. Keller and Kinsella (1973) also noticed a progressive increase of TBA values of raw hamburger during frozen storage at -18°C and suggested that this temperature may not be optimum for prevention of lipid oxidation. Siu and Draper (1978) reported that flesh fish samples yielded lower MA levels than the frozen samples. The toughened texture, poor flavour and unappealing odour of poorly stored frozen seafood, has been attributed to the binding of oxidized unsaturated lipids to proteins, a process by which insoluble lipid-protein complexes are formed (Khayat & Schwall, 1983).The normal

resistance of meat to the development of rancidity depends on the balance between the presence of antioxidants in the animal tissues andthe level of unsaturation and the concentration of the fatty acids present (Enser, 1987). The most common antioxidant in animal tissue is vitamin E (tocopherol) which, however, is not all available to block oxidation because of the inhomogeneous nature of the animal tissue (Enser, 1987). Poultry meat is composed of relatively high levels of unsaturated fatty acids and low levels of natural tocopherols and thus poultry products are very susceptible to the development of off-flavours due to oxidative rancidity (Dawson & Gartner, 1983). According to Wilson *et al.,* (1976) turkey meat, containing lower levels of natural tocopherol, is most susceptible to WOF development, followed closely by chicken, then by pork, beef and mutton. The use of mechanically deboned poultry meat enhances the tendency of poultry products to oxidize (Moerck & Ball, 1974). However, the use of mechanically deboned beef in beef meat products did not result in flavour deterioration during storage,

compared to control samples made of hand-boned beef, suggesting that lipid oxidation is not a problem as with chicken and fish; this was attributed to differences in the degree of unsaturation of fatty acids (Allen & Foegeding, 1981). The susceptibility of stored sea food products to autoxidation at low temperature may be related to the highly unsaturated long chain fatty acids present in these products (Siu & Draper, 1978). On the other hand, phospholipids have been shown to be the major contributors to development of rancidity in cooked meat because of their high unsaturated fatty acid content (Love & Pearson, 1971; Igene & Pearson, 1979). Wilson *et al.,* (1976) demonstrated that phospholipids play a major role in development of WOF in all cooked meats except pork, where totals lipid level seems to be the major contributor to WOF.

2.7 ANTIOXIDANTS

According to origin, antioxidants can be classified as synthetic or natural. Synthetic antioxidants have been widely used as food

preservatives, because of their effectiveness and relatively low cost. The most used antioxidants are those derived from phenolic structures, like butylated hydroxyanisole (BHA), tertbutyhydroxiquinone (TBHQ) and dodecyl, propyl and octyl gallate. All of them have an admissible daily ingest (ADI). Ethoxyquin (ETOX) is another synthentic antioxidant with a non-phenolic structure. In contrast to the others, its consumption by humans is not allowed, but it is only in animal diets such in the preservation of aviary foods (Baily *et a*l., 1996).

On the other hand, natural antioxidants are general molecules present in plant parts (e.g. leaves, bark, seeds and/or fruits). Among the most important natural antioxidant are the tocopherols (or vitamin E, liposoluble) and ascorbic acid (vitamin C, hydrosoluble). While the former represents an essential nutrient (it must be consumed in the diet), the latter is biosynthesized by poultry (Pardue and Thaxton, 1986). Other natural molecules with antioxidant

characteristics are carotenes (i.e. carotene, lycopene, luthein, asta-, zea- and cantha-xanthin), flavoids

(i.e, catechins, epigallocatechins, quercetin, rutin and morin among others), and non-flavonic phenols (i.e. rosmanol and rosmaridiphenol; boldine and its analogous). Even though antioxidant can protect susceptible substrates by different mechanisms, their main mode of action consists of the removal of free radial initiators and propagators. Clearly, this is the case for antioxidants such as vitamins E and C, and the phenolic antioxidant used as food preservatives. In their interaction with free radical, these antioxidant transfer an H atom, which stabilizes the free radical, becoming themselves low reactivity free radical, thereby stopping the lipoperoxidative chain.

In order to use an antioxidant in humans and animals, its efficicacy and its innocuoudness needs to be priorly established. An example is the use of rosemary leaf extracts (Rosmarinus officinalis L,), first for pigmentation, and latter as food preservative due to its antioxidant

components such as carnosol, rosmanol, isorosmanol and rosmaridiphenol (Wu *et al*., 1982).

Speisky and Cassels (1994) evaluated the antioxidant potential of several Chilean plants, determining that boldo leaves (Peumus boldus, Mol.) contains high concentrations of antioxidant. There are, however may have different molecules in Peumus boldus acting as antioxidant. Aporphine structures are regarded the major antioxidant agents, boldine being the vmost abundant of these.

In live organisms, reduced glutathione (GSH; hydrosoluble tripeptide synthensised by poultry), along with vitamins C and E are responsible for lowering the oxidative stress. GSH acts by releasing a hydrogen atom (attached to its thiol cysteine) and becoming oxidized glutathione (GSSG). GSH helps to stabilize free radical and acts as a factor of glutathione peroxidase (GSHpx), an enzyme that is responsible for transforming LOOH into easily eliminated lipoalcohols. It is important to stress that this enzyme (GSHpx) contains selenium (Se) as a prosthetic group, which makes it highly

dependent to the availability of this metal. It is widely estabilished that dietary Se deficiency can cause oxidative stress in poultry (Noguchi *et al*., 1973; Van Fleet and Ferrans, 1976; Michiels *et al*., 1994; Avanzo et al., 2001; Bozcaya *et al.,* 2001; Surai, 2002a), but this can be avoided through diet management. In fact, in a review published by Surai (2002b), author makes a series of important and practical recommendations to improve the status of Se in poultry. The regeneration of GSH from GSSG is catalyzed by the enzyme glutathione reductase.

Another important enzyme in the antioxidant defence is SOD, whose presence in the cell allows a fast dismutation of O_2, to O_2 and H_2O_2. Two types of SOD exist in eukaryotic cells: the first one incorporates the metals Cu and Zn in its prosthetic group (CU/Zn-SOD) and occurs mostly in cytosol, while the second one incorporates Mn in its structure (Mn-SOD) and occurs in the mitochondria (Fridovich, 1997). Supplementation of poultry diets with copper leads to an increase in the activity of the Cu/Zn dependent SOD isoform (Ozturk-Urek *et a*l.

2001). A similar result was previously reported by Aydemir et al., 2000, who demonstrated that the supplementation of diets with copper result in a high Cu/Zn-SOD activity in chicken erythrocytes and plasma Cu. On the other hand, Cu deficiency produces a decrease in activity of the Cu/Zn-SOD in erythrocytes of chickens (Bozcaya *et a*l., 2001), mice (Liochev and Fridovich, 1994), and sheep (Andrewartha and Caple, 1980). Finally, the catalase enzyme (CAT) acting in concert with SOD, transforms H_2O_2 into H_2O and O_2 (Michiels *et al*., 1994), and as with other antioxidant enzymes, it is also affected by some components of the diets. For example, Bozcaya *et a*l. (2001) reported that the activity of CAT in chicken erythrocytes increases in birds with Cu and Se deficiency. More research is needed to clearly establish how these nutrients affect CAT activity.

A review published by Surai (2002a) points out that in the last 10 years it has been established that the antioxidant system with which living organisms face oxidative processes is formed by different

enzymes, vitamins and minerals, which are organized in three clearly definite levels:

The first level would fit with a preventive level, in which free radical production would be avoided, thanks to the SOD, GSHpx and CAT enzymes, besides the metal-binding proteins. The second level would be simultaneously preventive and “curative “, since it must prevent the damage from spreading. In this level are all those breakers of chain antioxidant (vitamins A, C and E, carotenoids, GSH, uric acid, etc), which prevent the lipoperoxidative chain from proliferating. The third level, covering several enzymatic systems, is absolutely “ curative “, and is responsible for removing or repairing damaged molecules, so they do not damage the organisms.

2.7.1 Effect of antioxidants on oxidative rancidity of broiler meat

The lipid composition of broiler meat is influence by fatty acids present in their diet. As the diet becomes richer in PUFA, there is an increase in the PUFA/ saturated fatty acid balance in the carcass

(Bartov and Borstein, 1977a; Grau *et al*., 2001), promoting lipoperoxidation susceptibility in broiler meat (Marion and Woodroof, 1966; Bartov *et al.,* 1974; Bartov and Borstein, 1976).

It has been demonstrated that the systemic effect of some antioxidant is not restricted to an in vivo effect, since it can persist in the tissue post mortem, protecting the PUFA present in the meat. Bartov and Borstein, (1977b) studied the relation between the unsaturated level of the diet and the effectiveness of some antioxidant (vitamin E, ETOX and BHT) on the oxidative stability of abdominal fat and oxidative (dark) and glycolitic (white) chicken muscle. They reported that all tested antioxidant had a positive effect on the oxidative stability of the abdominal fat of poultry fed with saturated or unsaturated fatty acids.

In turn, in chickens fed with saturated fatty acids, the addition of antioxidants, vitamin E and ETOX to the diet reduced the basal lipoperoxidation and dark muscle susceptibility to lipoperoxidation (they did not find a significant difference for light muscle). In a later

study, the same authors (Bartov and Bornstein 1981) evaluated the effect of ETOX and dietary the oxidative rancidity of adipose tissue; however, no significant increase in the oxidative rancidity of dark muscle tissue was observed. Also there was a significant increase in vitamin E deposition in the adipose tissue, which the authors attributed to a protective effect of synthetic antioxidant on dietary vitamin E, or to a lower consumption of vitamin E (sparing effect). Finally, they found that the additional of these antioxidant in combination with increased the vitamin deposition in the adipose tissue (compared to the addition of vitamin deposition in the adipose tissue (compared to the addition of vitamin E alone at the same concentration) and that it substantial decreased the parameters in such tissue compared to the addition of BHT, ETOX, or vitamin E alone.

Lin *et al*., (1989) demonstrated that poultry fed with diets enriched with (-tocopherol or a mixture of BHA/BHT, showed a better oxidative stability in cooled (4^0C) and frozen meat (-18^0C), in addition

to significant increase in weight gain compared to chicks fed with the control diet (without antioxidant)

Additionally, it has been demonstrated that natural antioxidant can also exert a stabilizing effect on meat. Different authors have evaluated the effect of poultry diets supplemented with (-tocopherol, showing that this antioxidant confers a greater protection against oxidation to broiler (De Winnie and Drink, 1996; Lopez-Bote *et al*., 1998; Grau et al., 2001) and turkey meat (Sheldon, 1997). Moreover, Lopez-Bote *et a*l., (1998) investigated the effect of adding rosemary and sage extracts and vitamin E to the broiler diet on the lipoperoxidation susceptibility of meat. The authors reported a significant decrease in the lipoperoxidation levels of the white muscle of poultry fed with the natural antioxidant, for different cold storage periods (up to 9 days; 4°C). Although they did not find significant differences for cooled dark meat, in the case of frozen (up to 4 month; -20°C), and cooked meat, the protecting trend similar.

The beneficial effects of vitamin E are not restricted to lipid protection, and it has also been demonstrated that they protect proteins present in turkey meat from oxidation provoked by different oxidation methods (Gatellier et al., 2000; Renerre *et al*., 1999; Mercier *et a*l., 2001). However, there are no studies showing that vitamin E has an antioxidant effect against the proteo-oxidation of cooked broiler meat when pro-oxidant conditions are not given. Thus, more investigation is needed concerning the effect that different antioxidants might have on the oxidative processes that involve the proteins contained in the poultry meat.

The research carried out to date shows that the incorporation of antioxidant as additives to poultry diets, not only protects foods components from oxidative processes, but also promotes in vivo and post mortem affects, possibly after their absorption in the gut and incorporation to the bird's metabolism. Apparently, the antioxidant that is more soluble in fat (Vitamin E, BHA, BHT) would be absorbed more rapidly at intestinal level, so that a certain quantity can be

found deposited in tissues (Lin *et al*., 1989), which allows efficient oxidative control of these tissues and of the meat. In case of the AOX that have a comparatively minor liposolubility (carotenoids, polyphenols), the absorption might be slower at intestinal level, or it's deposited in the fatty tissues less efficient. Up to this moment, it is known that the natural antioxidant such as polyphenols, flavonoids, or extracts of rosemary and sage, have an antioxidant effect in meat, but the mechanisms by which this effect takes place are mainly unknown.

2.8 CHEMICAL COMPOSITION TALINUM TRIANGULARE (Water leaf)

Talinum is a small genus of the purslane family (Portulacacae), which appears to possess a unique nutritional potential. Waterleaf is in the genus *Talinum* and the species *triangulare* and is leafy vegetable found in west Africa, the west indies, South America (Adams, *et al*., 1972) and the warmer parts of the world (Mabberly, (1990). The

leaves and tender stems of waterleaf are consumed as a vegetable or as the constituent of a sauce by the populations of the areas where it is grown. Waterleaf grows spontaneously during the growing season, and it is common in a variety of habitats including roadsides, open fields, and abandoned agricultural lands. Although it is extensively consumed in the diet of populations where it is abundant, much is not known about its nutritive value.

Waterleaf's crude protein content compares favorably with that of cowpea, peanut, millet, and cashew nuts (Egwin, (1979). Akachuku and Fawusi (1995) also reported crude protein content of waterleaf leaves and tender stems as high as 29.4% and 13.4%, respectively. Sridhar and Lakahminarayana (1993) reported high total lipids, essential oils, and alpha – tocopherols and beta – tocopherols in *Talinum triangulare*. A paucity of information exists on the health and nutritional benefits of waterleaf in humans and / or animals. Although pursalane has been shown to possess nutritional qualities for reducing serum cholesterol and serum triglycerides in laboratory

animals , the present invention shows for the first time that waterleaf contains superior nutritional qualities that, when consumed by humans, vastly improves human health. Waterleaf contains a rich source of n – 3 fatty acids and other nutrients that the present invention shows reduces harmful total plasma cholesterol and plasma HDL – cholesterol and blood hematocrit in hypercholesterolemic humans. Waterleaf, compared to purslane, is an easier plant to grow, grows more efficiently and abundantly, has more aggressive growth characteristics, and has no known insect pests, thereby potentially serving a larger portion of the population. Waterleaf has higher levels of nutritionally – important vitamins (such as vitamin C, vitamin E, and Beta – carotene), minerals (such as calcium, potassium, and magnesium), and soluble fiber (pectin) than does purslane, all of which contribute to waterleaf's highly elevate antioxidant values and its total biological effect.

The combination in one, plant species of n – 3 fatty acids, antioxidants, and pectin that consequently has a positive and

beneficial influence in reducing the risk of cardiovascular diseases in humans is a unique attribute of waterleaf that has not been shown to exist until the present invention. The inventors have designed food compositions comprising waterleaf and methods of reducing harmful blood cholesterols and simultaneously increasing beneficial blood cholesterols and vitamin and mineral levels using such food compositions and biomedical utilization and applicability to a large segment of the population.

2.9.0 THIOBARBITURIC ACID TEST

The 2-thiobarbutric acid test is an objective measure of lipid oxidation (Tarlagris *et al.,* 1969). TBA values have been reported with sensory evaluation of meat quality. The reaction of malonaldehyde (MDA) with 2- thiobarbituric (TBA) has been widely used for measuring the extent of lipid in muscles food (Gray, 1978, Melton, 1983). The MDA is a secondary oxidation product of polyunsaturated fatty acids (Dehl *et a*l., 1962, Pryor *et al.,* 1976).The absorption

spectrum generated by adding the TBA reagent to meat distillate or filtrates containing MDA was identified to that of the complex formed between TBA and 1,1,3,3 tertracthary propane, thus the TBA reaction is a valid indicator of MDA in meat (Siu and Draper, 1978).

The TBA test can be performed directly on the food product followed by extraction of the colour complex (Sinnburher and Yu, 1958), on a portion of distillate of the food or on an extract of the food (Wuitte *et a*l., 1970). The MDA can be quantified by its reaction with TBA to form a color complex with a maximum absorbance near 532nm or quantified directly by high performance liquid chromatography (HPLC) as shown by Kakuda *et al.,* (1981) determination of free MDA by the HPLIX procedure after distillation excludes the effect of TBA reactive substance other than MDA which may contribute to the color complex at 525nm. The HPLC method used for determination of free MDA in freezer dried chicken samples was found to have a linear relationship with a correlation of 0.95 with the TBA – MDA coloured complex absorbance at 532nm (Kakuda *et al*., 1981).

Dawson *et a*l., (1975) reported that TBA values in broiler patties could be controlled by antioxidants in unfrozen products hold at 3^{o}c up to 10 days. The TBA values remained the same or decrease as storage time at 18^{o}c increases up to 12 months when no antioxidants were used 1,1,3,3, (TEP) hydrolyses with acid to malonaldehyde which resets quantitatively with TBA to give the typical red color.

Tarladgris *et al.,* (1964) reported that treatment is not necessary for the condensation of TBA with malonaldehyde not for maximum colour development. The reaction between malonaldehyde and TBA in water or 90% glacial acetic acid has been investigated at different temperature.

The result shows that acid heat treatment of the reaction mixture should be avoided since the Em530 of the coloured compare is considerably affected by the acid on the contrary heating without acid accelerates the condensation of TBA with malonaldehyde without affecting the Em530.

When ground meat was held for 7 days at 3^{o}c, all TBA number increased considerably and again TBA values of sample with skin increased more than in samples with meat only, (Dawson *et al*., 1976).

TBA analysis demonstrated that broiler meat is most susceptible to warmed over flavor development, followed closely by chicken, then by pork, beef, and mutton in that order, Although freshly cooked muscle from all species except mutton had higher TBA number than fresh raw samples, the most dramatic change occurred during storage of cooked meat at refrigerated temperature (48hr at 4^{o}c). Red muscle had consistently higher TBA values than white muscles under these storage conditions, indicating that red muscles were more susceptible to oxidative deterioration. Correlation coefficients between TBA numbers and total lipid levels and phospholipids suggest that phospholipids plays a major in development of warmed over flavor in all cooked meats, except for pork, where total lipids

levels seems to be the major contributor to warmed over flavor (Wilson *et al.,* 1976).

The TBA assay is the most popular method for measuring oxidative deterioration of lipids in muscles foods (Metton *et a*l., 1983). The TBA number is generally regarded to highly correlate with taste panel scores for oxidized and warmed over flavor in muscle foods (Wilson *et al*., 1976).

Despite its popularity in food industry, the TBA assay has been criticized for its reliability and use fullness as an indicator of food quality. A major technical criticism of the TBA assay is the extreme variability of assay results, which is generally due to different ways that the TBA assay is formed and due to sample autoxidation during the preparation, extraction or distillation, and the heating steps of these assays. These technical problems make comparisons of TBA data among different studies virtually impossible (Rhee; 1978).

A second criticism of the TBA assay is that TBA assay is that TBA number by itself is difficult to interpret without additional

information about the composition of different kinds of food products (Pearson, *et a*l., 1983). These criticisms are also important for edible products because of large differences in fat content and composition among different anatomical parts from the same bird, and also because of differences among bird due to age, sex, and breed.

Pikul *et a*l., (1985) reported that because of the different fat content of tissue, the TBA number of skin was higher than that of leg, which in turn was higher than that of breast.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 COLLECTION OF MATERIALS

Six eight (8) weeks old broilers weighing 2±0.3kg were purchased from Animal Production pavilion, University of Ilorin, Ilorin, Nigeria. *Tanilum triangulare* (Waterleaf) were collected from a farmland at University of Ilorin, Nigeria.

3.2 EXTRACTION OF PLANT MATERIAL

Fresh leaves of *Talinum triangulare* (Water leaf) were air dried and then ground to fine powder. The pulverized sample (200g) were soaked in 150ml of 100% ethanol for 72hr before decanting. The ethanolic extracts were concentrated by allowing the ethanol to evaporate before use.

3.3 PROCESSING OF SAMPLES

The broilers were slaughtered conventionally by cutting through the jugular vein with a sharp knife. They were scalded by dipping in boiling water for a minute and then defeathered by plucking the feather manually. The carcasses were then deskined, deboned and minced by the use of a food processor (National MK5080M).

3.4 APPLICATION OF TREATMENT

The minced meat was weighed into 700g each, into which waterleaf extract was applied at levels 0%, 0.1%, 0.2%, and 0.3%. Butylated hydroxyl Anisole (BHA) was also applied to a separate 700g of minced meat to serve as a reference control at 0.01% level.

Each 700g of meat was divided into twenty eight (56) parts of 12.5g each, twenty eight (2) of these were cooked for 1 minute, 30 seconds using a microwave oven (National NN-555WF), while the other twenty eight (28) parts were left raw. Both cooked and raw samples were used for thiobarbituric acid (TBA) analysis.

3.5 STORAGE OF SAMPLES

The cooked and the raw meats were packed in different nylon bags with labeling corresponding to different treatments.

The samples were stored in a refrigerator for a period of seven (14) days for the cooked and raw meat, respectively.

3.6 DETERMINATION OF LIPID OXIDATION

Lipid oxidations in cooked and raw meat were assessed by the thiobarbituric acid (TBA) test. Thiobarbituric Reactive Substances (TBARS) values were measured in duplicate 10g samples at each storage period using a distillation method of Tarladgris *et al.*, 1960. A 10g sample was homogenized with 47.5ml of distilled water in a mortar using a pestle and then rinsed with 50ml of distilled water in a round bottom flask.

Thereafter, 2.5ml of hydrochloric acid (HCL) was added and the mixture was distilled through condensing assembly to collect about 15ml of distillate. 5ml of distillate was transferred to a separate test tube and 5ml of thiobarbituric acid (TBA) (0.02M) was added to it.

The mixture was heated in a water bath for 35minutes and then cooled for 10minutes with cold tap water for color development. The deeper the colour of the sample, the more the oxidation development.

The duplicate absorbance reading was measured at 538nm against a blank that contained 5ml of hydrochloric acid (HCL) and 5ml of thiobarbituric acid (TBA) reagent using a spectrophotometer (CECILL-2000). The absorbance values were multiplied by a factor of 7.8 (Tarladgris *et al.*, 1964) to obtain the TBARs values in mg per malonaldehyde per kg of sample. Each treatment was replicated four (4) times.

3.7 STATISTICAL ANALYSIS

The experiment followed a completely randomized design (CRD) in a 5x2x7 factorial design using Genstat 5 release 3.2 software (P/Windows NT). The data obtained were analyzed using analysis of variance (ANOVA) model suitable for the design with the aid of a

Genstat 5 program package (payne, lane and Genstat committee, 1987). Duncan multiple range test was used to separate the means. Significance was defined at $p< 0.05$

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Table 1: Main effects of antioxidant treatment, storage days and state of meat on oxidative stability of broiler meat

Parameters	TBARS mg/MDA/kg						
Antioxidants	0	0.1	0.2	0.3	BHA		
	3.316^{c}	3.004^{b}	3.339^{c}	4.942^{d}	2.017^{a}		
State of Meat	Raw			Cooked			
	2.341^{a}			4.306^{b}			
Storage days	0	2	4	6	8	10	12
	1.525^{a}	6.666^{f}	2.071^{b}	3.654^{e}	3.654^{e}	2.596^{c}	2.994^{d}

a, b, c, d, e, f means having different superscript along the same row are significantly different($p<0.05$).

There was significant difference ($p<0.05$) among the TBARS values observed for all antioxidant treatments (Table 1). The lower TBARS value obtained for BHA is an indicator of its antioxidant potency. There was no significant difference ($p>0.05$) between the control sample and 0.2% waterleaf extract. The 0.1% waterleaf extract performed better than other treatments except the BHA treated samples.

There was significant difference ($p<0.05$) between the TBARS values observed for the cooked and the raw meat samples. The higher TBARS values obtained for the cooked meat sample indicates that cooking influence lipid oxidation of broiler meat. This is in line with the report given by Igene *et al.,* (1979) that cooking caused a release of non-heme iron which lead to higher warmed-over flavor (WOF).

There was a significant difference ($p<0.05$) among the TBARS values observed for all meat samples at all storage days. As storage day progresses, the TBARS values observed followed irregular pattern. The reason for this might be that the active antioxidant compounds

such as flavonoids, alkanoids, tocopherols and carotenoids have been denatured and/or not present in sufficient amount to combat lipid oxidation.

Table 2: Combined effects of antioxidants and state of meat on oxidative stability of broiler meat

Antioxidants	Raw	Cooked
0	$1.647^{a}{}_{x}$	$4.984^{b}{}_{y}$
0.1	$3.501^{b}{}_{z}$	$2.507^{a}{}_{w}$
0.2	$1.990^{a}{}_{y}$	$4.687^{b}{}_{y}$
0.3	$3.670^{a}{}_{z}$	$6.214^{b}{}_{y}$
BHA	$0.897^{a}{}_{w}$	$3.138^{b}{}_{x}$

a, b, means having different superscript along the same row are significantly different. x, w, y, z means having different subscript along the same column are significantly different ($p<0.05$).

In raw meat samples, BHA treated samples had the lowest TBARS value that was significantly different from all other treatments. The control samples had lower TBARS value compared to other levels of waterleaf treated samples. This indicates that waterleaf extract is a poor antioxidant.

In cooked meat samples, the 0.1% waterleaf extract had the lowest TBARS value that was significantly different from other treatments. This was closely followed by BHA. Generally, the TBARS values obtained for the cooked meat samples was observed to be higher than the raw meat samples. This result is in line with the report given by Satos and Hegarty (1971) that postulated that any process causing disruption of muscle membrane system, such as grinding, cooking and deboning, results in exposure of the labile lipid components to oxygen, and thus accelerates the development of oxidative rancidity. Hung and Greene (1978) reported that meat subjected to high temperature and/or long periods of heating developed lower TBARS

values than the sample subjected to lower temperature for a shorter period.

Table 3: Interactive effects of antioxidant treatments and storage days on oxidative stability of broilers meat

Antioxidant Treatment	0	2	4	6	8	10	12
0	2.819^{b}_{z}	5.020^{e}_{w}	3.599^{d}_{y}	1.681^{a}_{w}	4.988^{e}_{y}	3.315^{c}_{y}	1.787^{a}_{v}
0.1	1.224^{a}_{x}	8.206^{f}_{x}	1.100^{a}_{x}	3.546^{e}_{x}	1.599^{b}_{v}	2.901^{d}_{w}	2.433^{c}_{w}
0.2	0.744^{a}_{w}	8.997^{f}_{z}	0.752^{a}_{w}	3.799^{d}_{y}	1.880^{b}_{w}	2.991^{c}_{x}	4.208^{e}_{x}
0.3	2.407^{b}_{y}	8.682^{g}_{y}	0.432^{a}_{v}	8.299^{f}_{z}	7.211^{e}_{z}	2.788^{c}_{w}	4.777^{d}_{y}
BHA(0.01)	0.429^{a}_{v}	2.426^{d}_{v}	4.473^{f}_{z}	0.924^{b}_{v}	3.120^{e}_{x}	0.983^{b}_{v}	1.765^{c}_{v}

a, b, c, d, e, f means having different superscript along the same row are significantly different. v, w, x, y, z means having the same subscript along the same column are significantly difference($p<0.05$).

There were significant differences ($p<0.05$) among the all TBARS values observed at various days for various treatments.

On storage day 0, 2, 6, 10 and 12, the lower TBARS values observed for BHA was significantly different ($p<0.05$) from other treatments. This observation corroborates the report of Branen, 1975, who asserted the antioxidant potency of BHA. The higher TBARS values observed for BHA on storage day 4 and 8 was unexpected and could not be explained.

Table 4: Combined effects of Antioxidant, State of Meat and Storage days.

		Storage day						
Antioxidant Treatment	State	0	2	4	6	8	10	12
0	Raw	$0.538^{a}{}_{s}$	$1.841^{c}{}_{t}$	$1.638^{b}{}_{u}$	$1.138^{b}{}_{t}$	$2.317^{e}{}_{t}$	$2.005^{c}{}_{t}$	$2.054^{e}{}_{t}$
	Cooked	$5.100^{c}{}_{w}$	$8.199^{d}{}_{w}$	$5.560^{c}{}_{v}$	$2.223^{b}{}_{u}$	$7.659^{d}{}_{w}$	$4.625^{c}{}_{v}$	$1.521^{a}{}_{s}$
0.1	Raw	$0.281^{a}{}_{s}$	$14.836^{d}{}_{z}$	$0.351^{a}{}_{s}$	$5.346^{c}{}_{v}$	$0.897^{b}{}_{s}$	$1.178^{b}{}_{s}$	$1.419^{b}{}_{s}$
	Cooked	$2.168^{b}{}_{s}$	$1.376^{a}{}_{t}$	$1.849^{a}{}_{u}$	$1.583^{a}{}_{t}$	$2.301^{b}{}_{t}$	$4.625^{d}{}_{v}$	$3.448^{c}{}_{u}$
0.2	Raw	$0.460^{a}{}_{s}$	$7.355^{c}{}_{v}$	$0.507^{a}{}_{s}$	$0.484^{a}{}_{s}$	$0.718^{a}{}_{s}$	$2.216^{b}{}_{t}$	$2.192^{b}{}_{t}$
	Cooked	$1.029^{a}{}_{t}$	$10.639^{f}{}_{y}$	$0.998^{a}{}_{t}$	$7.114^{e}{}_{w}$	$3.042^{b}{}_{u}$	$3.766^{c}{}_{u}$	$6.224^{d}{}_{v}$
0.3	Raw	$0.351^{a}{}_{s}$	$9.142^{e}{}_{x}$	$0.237^{a}{}_{s}$	$5.819^{c}{}_{v}$	$1.053^{b}{}_{s}$	$1.037^{b}{}_{s}$	$8.033^{d}{}_{w}$
	Cooked	$4.462^{c}{}_{s}$	$8.221^{d}{}_{w}$	$0.068^{a}{}_{s}$	$10.779^{e}{}_{x}$	$13.369^{f}{}_{x}$	$4.539^{c}{}_{v}$	$1.521^{b}{}_{s}$

BHA(0.01)	Raw	$0.437^{a}{}_{s}$	$0.460^{a}{}_{s}$	$0.709^{a}{}_{s}$	$0.718^{a}{}_{s}$	$2.036^{c}{}_{t}$	$0.796^{a}{}_{s}$	$1.120^{b}{}_{s}$
	Cooked	$0.421^{a}{}_{s}$	$4.391^{d}{}_{u}$	$8.237^{e}{}_{w}$	$1.131^{b}{}_{t}$	$4.204^{d}{}_{v}$	$1.170^{b}{}_{s}$	$2.410^{c}{}_{t}$

a, b, c, d, e, f, means having different superscript along the same row are significantly different. s, t, u, v, w, x, y, z means having different subscripts along the same column are significantly different ($p<0.05$).

On day 0, there was no significant difference among the treatments. This reason for this could be that oxidative rancidity has not been fully initiated. On storage day 2, 4, 6, and 8, 0.1% waterleaf extracts had the lowest TBARS value in cooked meat samples. These values were significantly different from other treatments. From storage days 2 to 12, BHA maintained its antioxidant potency in raw meat samples. However, in cooked samples, its antioxidant potency was not manifested. The same trend applies to all waterleaf treated samples.

Generally, the TBARS values of cooked meat samples observed at all storage days were significantly higher than the raw meat samples. The higher TBARS values observed for waterleaf extract could be attributed to the destruction of antioxidant and phenolic compound present in waterleaf extract in the course of boiling the meat samples. Aside this, heat could also denature most of the antioxidants phytochemicals to form pro-oxidant compound. The reason could be that cooking, which is mostly associated with increase in temperature, activate some lypolytic enzymes such as lipase and phospholipase in the meat, which promote lipid oxidation (Krinsky, 1994). This is in line with the report of Olorunsanya *et al.,* 2010; who asserted that increase in lipid oxidation in the cooked samples may also be due to increase in ionic concentration from heat induced release of protein bound iron after cooking and the formation of hyper valent ferry myoglobin (or activate met-myoglobin) during cooking.

CHAPTER FIVE

5.0 Conclusion and Recommendation

5.1 Conclusion

No levels of Waterleaf extract reduced lipid oxidation in both raw and cooked meat samples. This was shown by their higher TBARS values, which was significantly different from BHA treated samples. BHA reduced lipid oxidation in both cooked and raw samples. However, its antioxidant potency was well expressed in raw meat samples.

5.2 Recommendation

Waterleaf extract should not be considered as the possible source of natural antioxidant in the preservation of broiler meat against lipid oxidation. Other trials should be conducted to determine the antioxidant potency of other leafy vegetables.

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APPENDIX

Genstat 5 Release 3.2 (PC/Windows NT) 29 September 2011 11:46:06

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Genastat 5 Second Edition (for Window)

Genastat 5 Procedure Library Release 3(3) (PL9)

Identifier	Values	Missing	Levels
Level	280	0	5

Identifier	Values	Missing	Levels
State	280	0	2

Identifier	Values	Missing	Levels

Storage	280	0	7

Identifier	Minimum	Mean	Maximum	Values	Missing
Tbars	0.210	3.319	14.872	280	0 Skew

Identifier	Minimum	Mean	Maximum	Values	Missing
C1	1.000	3.000	5.000	280	0

Identifiers	Minimum	Mean	Maximum	Values	Missing
C2	1.000	1.500	2.000	280	0

Identifier	Minimum	Mean	Maximum	Values	Missing

C3	0.000	6.000	12.000	280

0

Identifier	Minimum	Mean	Maximum	Values

Missing

C4	0.210	3.324	14.872	280

0 Skew

Identifier	Values	Missing	Levels
state	280	0	5

Identifier	Values	Missing	Level
state	280	0	2

Identifier	Values	Missing	Levels
storage	280	0	7

Identifier	Minimum	Mean	Maximum	Values	Missing
tbars	0.210	3.324	14.872	280	0 skew

181……..

***** Analysis of variance *****

Variate: tbars

Source of variation	d.f	s.s	m.s	v.r	F pr
Levels	4	248.0233	62.0058	393.37	<.001
state	1	270.2677	270.2677	1714.59	<.001

storage	6	676.5278	112.7546	715.32
<.001				
levels.state	4	162.1749	40.5448	257.22
<.001				
level.stoage	24	685.5591	28.5650	181.22
<.001				
state.storage	6	163.4362	27.3494	
172.81	<.001			
level.state.storage	24	875.7012	36.4875	231.28
<.001				
Residual	210	33.1020	0.1576	
Total	279	3114.7966		

***** Tables of means *****

Variate: tbars

Grand mean 3.324

Levels	1.00	2.00	3.00	4.00	5.00
	2.017	3.004	3.339	4.942	3.316

State	1.00	2.00
	2.341	4.306

Storage	0.00	2.00	4.00	6.00	8.00	10.00	12.00
	1.525	6.666	2.071	3.657	3.760	2.596	2.664

Levels state	1.00	2.00
1.00	0.879	3.138
2.00	3.501	2.501
3.00	1.990	4.687
4.00	3.670	6.214
5.00	1.647	4.984

Levels	storage	0.00	2.00	4.00	6.00	8.00	10.00	12.00
1.00		0.429	2.424	4.473	0.924	3.120	0.983	1.765
2.00		1.224	8.206	1.100	3.564	1.599	2.901	2.433
3.00		0.744	8.997	0.752	3.799	1.880	2.991	4.208
4.00		2.407	8.682	0.432	8.299	7.211	2.788	4.777
5.00		2.819	5.020	3.599	1.681	4.988	3.315	1.787

State	storage	0.00	2.00	4.00	6.00	8.00	10.00	12.00
1.00		0.413	6.727	0.692	2.741	1.404	1.446	2.964
2.00		2.636	6.605	3.451	4.566	6.115	3.745	3.205

levels	state	storage	0.00	2.00	4.00	6.00	8.00	10.00	12.00
1.00	1.00		0.437	0.460	0.709	0.718	2.036	0.796	1.120
	2.00		0.421	4.391	8.237	1.131	4.204	1.170	2.410
2.00	1.00		0.281	14.836	0.351	5.546	0.897	1.178	1.419

	2.00	2.168	1.576	1.849	1.583	2.301	4.625	3.448
3.00	1.00	0.460	7.355	0.507	0.484	0.718	2.216	2.192
	2.00	1.029	10.639	0.988	7.144	3.042	3.7666	.224
4.00	1.00	0.351	9.142	0.257	5.819	1.053	1.037	8.033
	2.00	4.462	8.221	0.608	10.779	13.369	4.539	
1.521								
5.00	1.00	0.538	1.841	1.638	1.139	2.317	2.005	2.054
	2.00	5.100	8.199	5.560	2.223	7.659	4.625	1.521

Standard error of means

Table	levels	state	storage State	levels
rep.	56	140	40	28
d.f	210	210	210	210
e.s.e	0.0531	0.0336		0.0628
	0.0750			

Table	level Storage	state storage	level state storage
rep.	8	20	4
d.f	210	210	210
e.s.e	0.1404	0.0888	0.1985

Standard error of differences of means

Table	levels	state	storage State	levels
rep.	56	140	40	28
d.f.	210	210	210	210

e.s.e.	0.0750	0.0475	0.0888	0.1061

Table	levels	state	levels
	Storage	storage	state
rep.	8	20	4
d.f	210	210	210
s.e.d	0.1985	0.1256	0.2807