ANTIMICROBIAL AND ANTIOXIDANT EFFECTS OF RED ONION (*ALLIUM CEPA*) ON UNREFRIGERATED BROILER CHICKEN MEAT

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ABSTRACT

This study was to determine the keeping quality of raw broiler chicken meat laced with red onions (Allium cepa) as a means of preservation in comparism with refrigeration. Eighteen (18) broiler chickens were purchased from a reputable poultry farm in Akure and humanely slaughtered. Thirty three thigh samples were aseptically collected, weighed and assigned to treatments with three replicates per treatment using a completely randomized design. The samples in the control group were refrigerated; another group was laced with 150 g of freshly diced red onions per kg of meat; while the last group was not refrigerated and without onions. Samples in the different treatments were kept over a storage period of 36 hours and analyzed for the keeping quality by determining the microbial status and lipid stability at 12 hour interval basis. The length of storage period and methods of preservation had significant (p<0.05) influence on microbial load and oxidative stability of the meat samples. The bacterial load of unrefrigerated samples without onions was the highest (300.00 ± 20.21 cfu/g, 320.00 ± 22.23 cfu/g and 320.00 ± 24.21 cfu/g) throughout the storage period. The unrefrigerated samples without onions showed least oxidative stability during storage with TBARS values of 3.71 ± 0.09 mgMDA/kg meat at 12 hours, 4.66 ± 0.12 mgMDA/kg meat at 24 hours and 8.29 ± 0.14 mgMDA/kg meat at 36 hours. The study suggests that diced red onion can be used to preserve chicken meat up to 12 hours of storage to retain its quality for human consumption.

Keywords: Bacterial load, Broiler meat, Oxidative stability, Red onion

INTRODUCTION

The consumption of poultry meat is gaining popularity, being an excellent source of high quality protein and broiler chicken accounts for more than 90 % of the total poultry population of the world (Biswas *et al.,* 2010). The nutritional 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).

Besides health concerns to the consumer, lipid peroxidation is also a major cause of meat quality deterioration, affecting colour, flavour, texture and nutritional value (Botsoglou *et al.,* 2010; Giannenas *et al.,* 2010).

It is, therefore, essential that adequate preservation technologies are applied to

maintain the quality and safety of meat. After the development and rapid growth of super markets meat preservation became essential for transportation of meat for long distances without spoiling and lose of texture, colour and nutritional value (Nychas *et al.,* 2008).

The use of natural antioxidants to stabilize meat has gained much attention from consumers because they are considered safer than synthetic antioxidants (Jung *et al.,* 2010). These natural antioxidants include spice extracts (El-Alim *et al.,* 1999), fruit juice (Naveena *et al.,* 2008), tea extracts (Rababah *et al.,* 2004) and seed extracts (Brannan and Mah, 2007). The use of spices for meat preservation was due to their antimicrobial properties (Peter, 2012). Plants, including herbs and spices, have many phytochemicals that are potential sources of natural antioxidants, e.g. phenolic diterpenes, flavonoids, tannins and phenolic acids (Dawidowicz *et al.,* 2006). It has also been reported that these natural antioxidants, especially of plant source, have greater application potential due to consumer's acceptability, palatability, stability and prolong shelf life of meat products (Jung *et al.,* 2010). Thus, the application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf life and preventing economic loss (Yin and Cheng, 2003).

Onion belongs to the genus *Allium* along with garlic, chives, shallots and leeks. Onion has been characterised for its flavonol, quercetin and quercetin derivatives (Roldán-Marín *et al.,* 2009) with red onion containing a higher amount of antioxidant compounds. Traditionally, fresh onion bulbs are sliced on chicken meat before or during cooking to improve its flavour but it has not been established if onions have the ability to extend the shelf life of unrefrigerated chicken meat.

This study was therefore undertaken to provide information on the potential of red onion (*Allium cepa)* in improving the lipid stability and microbial status, thus extending the shelf life and overall quality of broiler chicken meat, especially under storage emergencies for fresh meat where there is no refrigeration.

MATERIALS AND METHODS

Collection and Preparation of Red Onions: Fresh red onions (*Allium cepa)* were purchased from Oja Oba Market in Akure, Nigeria. The fresh red onion bulbs were washed and diced manually for application on the broiler chicken meat.

Chicken Meat Sample Collection and Preparation: Eighteen (18) broiler chickens were purchased from a reputable poultry farm in Akure and slaughtered. After evisceration and dissection, thirty three (33) of the thigh samples were aseptically collected, weighed, assigned to treatments and kept in sterile plastic bags. The samples were immediately transferred to the Microbiology Laboratory of Animal Production and Health Department, FUTA for analysis.

Experimental Layout: The experiment comprised of 4 treatments with three replicates per treatment using a completely randomized design. The treatments were: Treatment 1, Control group (One set of control samples was refrigerated, while the other set was left unrefrigerated); Treatment 2 - comprised unrefrigerated chicken thigh samples not treated with diced red onion bulbs; Treatment 3 - refrigerated chicken thigh samples not treated with diced red onion bulbs and Treatment 4 - unrefrigerated chicken thigh samples treated with diced red onion bulbs. Each set of thigh samples to be preserved with onions was laced with diced red onion at 150 g/kg and placed in transparent plastic bags and all the unrefrigerated chicken samples were then stored at room temperature of 23^{o}C. The meat samples in the various treatment groups were analyzed for microbial status and oxidative stability at <1, 12, 24 and 36 hours storage period.

Microbiological Analysis

Bacterial isolation and determination of total viable counts: A portion of each raw chicken thigh (10 g) was macerated using mortar and pestle. Each macerated sample (1 g) was added into test tubes containing sterile

distilled water (9 ml) and was thoroughly mixed to serve as stock. Four fold serial dilutions ($10^{-1}$to 10^{40}) of the stock were done using 1 ml stock homogenate and 9 ml sterile distilled water in order to obtain discrete colonies (Yusuf *et al.*, 2012). The media (Nutrient Agar) used was prepared from commercially dehydrated products and reconstituted according to the manufacturer's directives, sterilized and allowed to cool. 1 ml each of the serially diluted chicken meat sample was dropped at the centre of a Petri dish followed by pouring of the nutrient agar using the pour plate method as described by Begum *et al.* (1986). It was allowed to solidify for some minutes and then incubated at 37 °C for 24 hours. The colonies that emerged were counted and calculation for the colony forming units was expressed as log cfu/ml using the formula as described by Muhammad *et al*. (2016).

Identification and Characterization of Bacterial Isolates: The bacterial colonies that developed on the nutrient agar plates were sub-cultured by streaking on freshly prepared nutrient agar plates and MacConkey agar plates until pure colonies were obtained according to the conventional procedure as described by Fawole and Oso (2001).

Then isolates were characterized and identified based on their morphological and cultural characteristics including shape, size, pigmentation, elevation and marginal characteristics of the colony and Gram staining. Then a series of biochemical reactions which include oxidase test, catalase test and coagulase test were done. Sugar fermentation assay and indomethyl red tests were also carried out as described by Cheesbrough (2006).

Oxidative Stability Analysis: The chicken meat samples were stored in a refrigerator overnight and thereafter transferred to a deep freezer (-20 and -18^{o}C) and kept for 30 days. The thiobarbituric acid (TBA) assay was carried out according to the method described by Pikul *et al.* (1989). The frozen samples were deboned, de-skinned and chopped into smaller pieces. The chopped meat samples were mixed together thoroughly and 50 g of each was blended with 170 ml of 4 % perchloric acid and 5 ml of 2.02 % butylated hydroxyl toluene for one minute. The blended samples were filtered through Whatman filter paper No. 1 using a vacuum pump. About 50 ml of the filtrate was stored in dispensing bottles and frozen.

The frozen samples in the bottles were allowed to thaw and 5 ml of each sample was transferred into screwed capped test tubes. 5 ml of 0.02 M TBA solution was added to each sample and shaken thoroughly for proper mixing. The test tubes were placed in the test tube racks and the racks containing the test tubes were incubated in boiling water for 30 minutes. The racks were removed and allowed to cool in cold water. A pink colour was observed after boiling and the absorbance of the resulting supernatant solution was determined against the blank solution (5 ml of 0.02 M TBA and 5 ml of distilled water) using Spectrum lab 23A Spectrophotometer at 532 nm. The amounts of TBA were expressed as milligrams of malondialdehyde (MDA) per kilogram of muscle.

Statistical Analysis: All data generated were subjected to analysis of variance using SAS (2008). Duncan Multiple Range Test was used to separate mean among factors where there were significant differences.

RESULTS

The methods of preservation, length of storage period and the interaction between storage period and the methods of preservation had a significant ($p<0.05$) influence on bacteria load of the meat samples (Table 1).

The mean bacterial count (278.44 ± 27.71 cfu/g) at 36 hours was the highest compared to that of <1 hour (7.33 ± 3.06 cfu/g). It was also observed that chicken meat samples preserved with onions had lower mean bacterial count (226.92 ± 39.29 cfu/g) when compared with chicken meat samples without red onions (236.92 ± 39.99 cfu/g), while refrigerated chicken meat samples had the lowest mean bacterial count (82.67 ± 26.98 cfu/g).

Table 1: Total bacterial load of refrigerated, unrefrigerated and onion preserved chicken meat samples over a period of 36 hours

Storage period (hour)	Methods of preservation	Total bacterial load (10^4 CFU)
< 1	Refrigerated samples	7.00 ± 5.51[a]
	Unrefrigerated samples without onion	7.67 ± 4.06[a]
	Unrefrigerated samples laced with onion	7.67 ± 4.06[a]
12	Refrigerated samples	25.00 ± 19.02[b]
	Unrefrigerated samples without onion	300.00 ± 20.21[e]
	Unrefrigerated samples laced with onion	260.00 ± 21.21[d]
24	Refrigerated samples	27.67 ± 21.21[b]
	Unrefrigerated samples without onion	320.00 ± 22.23[e]
	Unrefrigerated samples laced with onion	283.67 ± 21.43[d]
36	Refrigerated samples	195.33 ± 22.21[c]
	Unrefrigerated samples without onion	320.00 ± 24.21[e]
	Unrefrigerated samples laced with onion	**291.33 ± 23.51[e]**

Mean ± Standard error; Means values with different superscripts and for the same parameter differ significantly (p<0.05); CFU = Coliform Forming Unit

At <1 hour period, there was no significant (p>0.05) difference among the treatment groups for the bacterial counts. Subsequently, as the storage period increased, the bacterial load of unrefrigerated samples without onions was seen to be highest at 12 hours (300.00 ± 20.21 cfu/g), 24 hours (320.00 ± 22.23 cfu/g) and 36 hours (320.00 ± 24.21 cfu/g) when compared with the refrigerated samples with significantly lower bacterial counts (25.00 ± 19.02, 27.67 ± 21.21, 195.33 ± 23.21 cfu/g) at the various hourly intervals. The use of onions as a method of preservation on the meat samples decreased bacterial counts since samples without onions had bacterial counts of (300.00 ± 20.21 cfu/g) at 12 hours which was significantly (p<0.05) different from bacterial counts of samples laced with onions (260.00 ± 21.21 cfu/g). At the 24th and 36th hour, there was no significant (p<0.05) difference among the various treatments as influenced by the use of onions.

The occurrence of bacteria isolated from the meat samples in the various treatment groups and biochemical characterizations of these isolates revealed 12 types of bacteria were detected (Table 2). The isolates include pathogenic bacteria like *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Staphylococcus epidermidis, Enterobacter aerogenes, Actinomyces* spp. *and Pseudomonas aureginosa*. The other bacteria isolated are *Micrococcus letus*, *Saacharomyces* spp., *Serratia marcescens*, *Proteus vulgaris* and *Klebsiella* spp.

The percentage prevalence of the isolated bacteria on the chicken meat sample in the different treatment groups over the storage period indicated that the refrigerated meat samples had *Saacharomyces* spp. as the most common bacteria isolated with prevalence rate of 50 % (Table 3). The chicken meat samples without onions had *Escherichia coli* with 38 % occurrence as the most common, while *Salmonella* spp. with 30 % prevalence being the most common isolate in meat samples with onions as preservatives.

The storage period, the methods of preservation and the interaction between storage period and the methods of preservation showed a significant (p<0.05) effect on the oxidative stability (Table 4). The mean oxidative value at 36 hours (5.18 ± 0.85 mg MDA/kg meat) was the highest compared to that of <1 hour (0.98 ± 0.05 mg MDA/kg meat). It was observed from the TBARS values that refrigerated samples (1.74 ± 0.18 mg MDA/kg meat) had better oxidative stability than the unrefrigerated samples laced with onions (3.63 ± 0.33 mg MDA/kg meat), while unrefrigerated samples without onions showed least oxidative stability (4.43 ± 0.78 mgMDA/kg meat).

Table 2: Occurrence of bacterial isolates from refrigerated, unrefrigerated and onion preserved chicken meat samples

Storage period (Hour)	Methods of preservation	Bacteria isolates
< 1	Fresh samples immediately after slaughter	*Salmonella* spp., *Staphylococcus aureus, Micrococcus letus, Saccharomyces* spp.
12	Refrigerated samples	*Salmonella* spp., *Micrococcus letus, Staphylococcus aureus, Saccharomyces* spp. `
	Unrefrigerated samples without onions	*Salmonella spp., Escherichia coli, Staphylococcus aureus, Micrococcus letus, Saccharomyces spp., Enterobacter aerogenes*
	Unrefrigerated samples laced with onions	*Salmonella* spp., *Staphylococcus aureus, Micrococcus letus, Saccharomyces* spp., *Serratia marcescens*
24	Refrigerated samples	*Salmonella spp., Micrococcus letus, Staphylococcus aureus, Saccharomyces* spp., *Staphylococcus epidermidis*
	Unrefrigerated samples without onions	*Escherichia coli, Salmonella* spp., *Staphylococcus aureus, Micrococcus letus, Saccharomyces* spp., *Enterobacter aerogenes, Proteus vulgaris.*
	Unrefrigerated samples laced with onions	*Salmonella* spp., *Escherichia coli, Staphylococcus aureus, Micrococcus letus, Saccharomyces* spp., *Serratia marcescens, Actinomyces* spp.
36	Refrigerated samples	*Salmonella* spp., *Micrococcus letus, Staphylococcus aureus, Saccharomyces* spp., *Proteus vulgaris, Staphylococcus epidermidis.*
	Unrefrigerated samples without onions	*Escherichia coli, Salmonella* spp., *Staphylococcus aureus, Micrococcus letus, Saccharomyces* spp., *Enterobacter aerogenes, Proteus vulgaris, Klebsiella* spp.
	Unrefrigerated samples laced with onions	*Escherichia coli, Salmonella spp., Staphylococcus aureus, Micrococcus letus, Saccharomyces* spp., *Serratia marcescens, Actinomyces* spp., *Pseudomonas aureginosa.*

Table 3: **Percentage prevalence of isolated bacteria on the experimental chicken meat samples throughout the storage period**

Isolates	Treatments		
	Refrigerated chicken meat samples (%)	Non-Refrigerated chicken meat without onions (%)	Non-Refrigerated chicken meat with onions (%)
Escherichia coli	0	38	25
***Salmonella* spp.**	5	20	30
Micrococcus letus	20	8	11
***Saccharomyces* spp.**	50	10	7
Staphylococcus aureus	10	7	12
Enterobacter aerogenes	0	6	0
Serratia marcescens	0	0	10
Staphylococcus epidermidis	15	0	0
Proteus vulgaris	0	9	0
***Actinomyces* spp.**	0	0	3
***Klebsiella* spp.**	0	2	0
Pseudomonas aureginosa	0	0	2

Table 4: Oxidative stability of refrigerated, unrefrigerated and onion preserved chicken meat samples over a period of 36 hours

Storage period (hour)	Methods of preservation	Lipid oxidation (mg MDA/kg)
< 1	Refrigerated samples	0.91 ± 0.05[a]
	Unrefrigerated samples without onion	1.05 ± 0.07[a]
	Unrefrigerated samples laced with onion	0.91 ± 0.05[a]
12	Refrigerated samples	1.65 ± 0.07[b]
	Unrefrigerated samples without onion	3.71 ± 0.09[c]
	Unrefrigerated samples laced with onion	2.53 ± 0.08[c]
24	Refrigerated samples	1.94 ± 0.11[b]
	Unrefrigerated samples without onion	4.66 ± 0.12[d]
	Unrefrigerated samples laced with onion	3.59 ± 0.13[c]
36	Refrigerated samples	2.47 ± 0.11[c]
	Unrefrigerated samples without onion	8.29 ± 0.14[e]
	Unrefrigerated samples laced with onion	4.76 ± 0.15[d.]

Means ± Standard error; Mean values with different superscripts and for the same parameter differ significantly (P<0.05), MDA = Malondialdehyde

The result also showed that refrigerated meat samples had the best oxidative stability as seen from the low TBARS values at <1 hour (0.91 ± 0.05 mg MDA/kg meat), at 12 hours (1.65 ± 0.09 mg MDA/kg meat), 24 hours (1.94 ± 0.11 mg MDA/kg meat) and 36 hours (2.47 ± 0.11 mg MDA/kg meat) respectively.

DISCUSSION

The results of this present study showed that the length of storage period and the use of onion as a preservative significantly influenced bacteria count of the chicken meat samples. It was observed that the use of onion as a preservative led to a decrease in bacterial count and this was similar to the findings of Billing and Sherman (1998) that spices kills microorganisms or inhibit their growth before they could produce toxins. Also, Benkeblia (2004) reported that sulfur compounds present in garlic and onion inhibits gram-positive and gram-negative bacteria. Overtime, plant products, particularly spices and extracts of various plant parts have been used extensively as natural antimicrobials. Shan *et al.* (2007) reported that the partial hydrophobic nature of phenolic compounds found in spices might degrade the cell wall, interact with the composition of and disrupt the cytoplasmic membrane, which may eventually lead to cell death of microbes. The results further showed that bacterial counts were higher in the meat samples preserved with onions when compared to the refrigerated meat samples. This may be explained as reported by Haruna (2014) that processing methods of onions such as peeling, cutting, or slicing, may increase the probability of microbial spoilage and contamination due to the increased surface area and tissue injuries that release nutrients and facilitate the growth of microorganisms. In addition, the presence of cut surfaces of onions may have increased the moisture content, which enhanced water activity and favoured the proliferation of spoilage microorganisms (Thompson, 2009).

The storage period and preservation methods in this present study influenced the type of bacteria isolated from the chicken meat sampled. The fresh raw chicken meats sampled were contaminated with bacteria like *Salmonella* spp.*, Staphylococcus aureus, Micrococcus letus* and *Saccharomyces* spp*.* This may probably be due to the fact poultry carcasses are frequently contaminated with pathogens from the intestinal tract or from feacal material on feet and feathers of birds (Sharma and Chattopadhyay, 2015). This is of public health importance because raw meat remains probably the major source of human food borne infection with pathogenic bacteria. However, the bacterial load on the chicken meat sampled at less than one hour was within satisfactory range according to the International Commission of Microbiological Standards of Foods (Onourah *et al*., 2015).

In the present study, the oxidative stability of raw broiler chicken meat was significantly influenced when red onions (rich in

quercetin) was used as preservative as storage period lengthened. This was the case since chicken meat preserved with red onions recorded lower TBARS values as compared to the meat samples without onions. The antioxidant properties of onions in this study was in agreement with the report of Younathan *et al.* (1980) that onion is much valued for its flavouring components and high flavonoids content such as quercetin (284 – 486 mg/kg) which acted as an antioxidant in cooked ground turkey. Jurdi-Haldeman *et al.* (1987) also reported the antioxidant effects of quercetin present in onion on cooked ground lamb. The antioxidant properties of quercetin have been associated with phenolic compound that break free radical chain reactions by electron donation and chelating metal ion (Bekhit *et al.,* 2003). This was in agreement with Zielinska *et al.* (2008), that phenolic compounds in red onions including anthocyanins, flavonoids, quercetin and volatile sulfuric components have chelating and free radical scavenging activities, resulting in shelf life extension of fat-containing food systems. Similarly, Srinivasan *et al.* (2004) and Joe *et al.* (2009) have also reported that onion bulbs have numerous organic sulphur compounds, flavonoids and phenolic acids with proven antibacterial, antioxidant and hypolipidemic efficacy. The results from this study was also in agreement with the findings of Sarah *et al.* (2010) that the minimum oxidative stability value for sturgeon fillets dipped in higher concentrations of onion juice suggests a positive correlation between phenolic content of onion juice and antioxidant properties of these compounds to prevent or retard the formation of malonaldehydes. Similarly, Park *et al.* (2008) reported the efficacy of onion as an antioxidant in fresh pork belly and loin during refrigerated storage.

Conclusion: In this study, data obtained showed that the use of red onions has antimicrobial and antioxidant effects when used as preservative for chicken meat over a period of time. It was ascertained that unrefrigerated meat samples without onion preservation had the lowest meat quality parameters. It was concluded that storage of chicken meat was best with refrigeration but in case of problems with refrigeration, diced onions could be used to preserve chicken meat up to 12 hours of storage.

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