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Full Length Research Paper

Oxidative stability and bacteriological assessment of meat from broiler chickens fed diets containing *Hibiscus sabdariffa* calyces

Gbenga Emmanuel Onibi* and Innocent Bamidele Osho

Department of Animal Production and Health, School of Agriculture and Agricultural Technology, Federal University of Technology, Akure, Nigeria.

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Thigh muscles were obtained from a batch of 120 broiler chickens fed diets containing 0, 1.5, 3.0, 4.5 and 6.0% dried *Hibiscus sabdariffa* calyces (diets A, B, C, D and E respectively). The thigh muscles were studied as uncooked and steam-cooked, and refrigerated for up to 9 days. Oxidative stability of muscle was measured using the thiobarbituric acid test and bacteria isolates were identified and quantified. Moisture and lipid contents of muscle were not affected (P>0.05) by dietary treatments. Oxidation of refrigerated meat decreased (P<0.01) with increasing levels of dietary *H. sabdariffa* calyces. Uncooked meat contained more bacteria load than cooked meat (P<0.001). With increasing levels of dietary *H. sabdariffa* calyces and length of refrigeration, bacteria load of meat decreased. Six types of bacteria (*Staphylococus aureus*, *Bacillus subtilis*, *Corynebacterium* sp., *Escherichia coli*, *Salomnella* sp. and *Lactobacillus salivarius*) were isolated from the fresh-uncooked meat but only three (*S. aureus*, *B. subtilis* and *Corynebacterium* sp.) from the cooked meat. Bacteria type isolated decreased after 9 days of refrigeration. It was concluded that *H. sabdariffa* calyces contain potential antioxidant and antibacterial agents that need further investigation.

Key words: Oxidative and bacteria deterioration, *Hibiscus sabdariffa* calyces, broiler chicken meat.

INTRODUCTION

Meat is important in human nutrition, being an excellent source of high quality protein, vitamins and minerals (except calcium). Chicken meat is derived from broilers, spent layers and cockerels and is a class of meat that has little or no religious taboos attached to its consumption. Like other meat types, chicken meat is subject to deterioration in quality. Lipid oxidation and microbial growth are major factors causing changes resulting in loss of attractive and fresh meat colour (Fautsman et al., 1989), development of off-flavour (Gray and Pearson, 1987), changes in texture and nutritive value (Pearson et al., 1983) and formation of potentially harmful lipid oxidation products such as malonaldehyde (MDA) and cholesterol oxides (Monahan et al, 1992; Onibi and Atibioke, 2004). Many of the microorganisms in meat are introduced from the skin, digestive tract as a result of rupture, and unhygienic processing condition like use of polluted water and poor preservation (Brown, 1982). The number and types of organisms carried at site of skin and gastrointestinal tract reflect both animals' indigenous microflora and its environment. The poultry carcass is not usually skinned; hence skin-associated organisms may likely be sources of contamination. Adams and Moss (1999) observed that the intestinal tract of poultry contains high number of organisms and poultry evisceration therefore poses microbiological hazards. Refrigeration has been identified as a good method of preservation, but it does not completely inhibit oxidative changes (Whang and Peng, 1987; Onibi, 2000a) and is adversely affected by epileptic electricity supply in Nigeria.

There has been resurgence of interests for "all natural" medicinal plants like herbal feed additives, plant extracts with growth, flavour, colour enhancing, antioxidant and antibacterial activities (Adodo, 2002; Omojasola and Awe, 2004). Rosemary extract (Hopia et al, 1996), thyme constituents (Schwarz et al, 1996) and fruits (Wang et al,

*Corresponding author. E-mail: gbengaonibi@yahoo.com. Phone: (234) 803-3503320.

Table 1. Composition of experimental diets (g/kg).

Ingredients	Diet				
	A	B	C	D	E
White maize	447.50	452.50	457.50	462.50	467.50
Maize offal	140.00	115.00	90.00	65.00	40.00
Soyabean meal	280.00	285.00	290.00	295.00	300.00
Fish meal (72%)	50.00	50.00	50.00	50.00	50.00
Brewers dried grain	40.00	35.00	30.00	45.00	60.00
H. sabdariffa calyces	-	15.00	30.00	45.00	60.00
Palm oil	-	5.00	10.00	15.00	20.00
Others*	42.50	42.50	42.50	42.50	42.50
	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated composition					
Crude protein (g/kg)	206.00	205.80	205.70	205.50	205.40
Crude fibre (g/kg)	53.80	51.40	48.90	46.40	44.00
Metabolisable energy (Kcal/kg)	2864.92	2864.69	2864.23	2864.23	2864.00
Calcium (g/kg)	14.90	14.90	14.80	14.80	14.80
Phosphorus (g/kg)	7.50	7.50	7.50	7.50	7.50
Methionine + cysteine (g/kg)	8.00	8.00	8.00	8.00	8.00
Lysine (g/kg)	11.90	12.00	12.00	12.10	12.10
Vitamin C (mg/kg feed)**	-	427.50	855.00	1,282.50	1,710.00

*Others in g/kg: Bone meal = 25.0, oyster shell = 5.0, vitamin/mineral premix = 5.0, methionine = 2.5 and salt = 5.0.
**As supplied by *H. sabdariffa* calyces only, and calculated on basis of 28,500 mg vitamin C kg^{-1} calyces (Akanya et al, 1997).

1996) have been shown to exhibit antioxidant activity in food lipids. Akanya et al. (1997) reported high vitamin C content and acidic condition of sorrel drink made from *Hibiscus sabdariffa* calyces. *H. sabdariffa* is widely grown in North-eastern and Middle-belt region of Nigeria (Akanya et al, 1997). The calyx is readily available and called Zoborodo in Hausa and Isapa in Yoruba languages. It has not been exploited as a source of natural antioxidants and antimicrobial agent in livestock feed and meat. Pigs and poultry are able to incorporate dietary fatty acids and antioxidants directly into adipose and muscle tissues (Monahan et al, 1992; Onibi and Atibioke, 2004; Onibi, 2006). Hence, this study is aimed at investigating the effect of varying levels of dietary *H sabdariffa* calyces on lipid peroxidation and bacterial isolates of meat of broiler chickens.

MATERIALS AND METHODS

A total of 120 chicks, brooded on deep litter and fed commercial broiler starter diets for a 4-week pre-experimental period, were placed on the 6 experimental diets (Table 1) from 4-8 weeks of age in metabolic cages. There were 4 replicates per treatment and 6 birds (3 males and 3 females) per replicate. The diets were isonitrogeneous and isocaloric and had varying levels of ground *H. sabdariffa* calyces (0, 1.5, 3.0, 4.5 and 6.0% in diets A, B, C, D and E, respectively). At the end of the feeding trial, 40 birds were slaughtered, deplumed and dressed. These represented 8 birds per dietary treatment at 2 birds (a male and a female) per replicate. The thighs of each dressed chicken were removed, the muscle dissected and separately wrapped in polythene bags. The left thigh muscle was steam-cooked for 15 min and the right part was left uncooked. The uncooked and cooked muscle were subdivided into 4, individually re-wrapped in polythene bag, sealed and allocated to each of the 4 storage treatments. The storage treatments were:

1 uncooked and cooked fresh muscle.
3 uncooked and cooked muscle refrigerated for 3 days.
6 uncooked and cooked muscle refrigerated for 6 days.
9 uncooked and cooked muscle refrigerated for 9 days.

Storage treatments 3, 6 and 9 were to simulate the effect of refrigerated storage for up to 9 days. The refrigeration temperature was set at 4°C and monitored with a digital thermometer. The refrigerator was supported with a stand-by electric power generator. Samples for bacteriological study were prepared for culturing immediately after each refrigerated period but those for oxidative study were frozen for 3 weeks prior to analysis.

Moisture and lipid contents of muscle were determined according to AOAC (1995) method. The microbial procedure to identify the presence of bacteria on the fresh and refrigerated samples was carried out by aseptically preparing different aliquots of appropriate dilution in triplicates. The various microbiological examination methods were as well followed as outlined by Odepidan and Ilo (1996) for different types of bacteria organisms via acid-forming bacteria, coliforms and clostridia organism. The plates were incubated between 32 and 44°C for 24 to 48 h as applied to the different bacteria organisms' requirements. Count of the typical colonies and calculation of the number of bacteria per gram of meat product were recorded. Identity of the bacteria isolates was done using the morphological, cultural and biochemical characteristics methods (Lamb, 1981). Extraction and assay procedures for measuring extent of muscle lipid oxidation were based on the aqueous extraction 2-thiobarbituric acid (TBA) method described by Pikul et al. (1989). Results were expressed as mg malonaldehyde (MDA)/kg muscle.

Table 2. Moisture and lipid contents (%) of fresh thigh from broiler chickens fed diets containing varying levels of *H. sabdariffa* calyces.

Diets	*H. sabdariffa calyces* level (%)	Moisture	Lipid
A	0	72.67 ± 0.58	4.21 ± 0.39
B	1.5	72.43 ± 0.93	4.03 ± 0.92
C	3.0	71.68 ± 1.19	4.09 ± 0.67
D	4.5	72.07 ± 1.79	4.29 ± 0.39
E	6.0	72.83 ± 1.80	4.12 ± 0.12
Statistical significance		NS	NS

Mean ± SD; N = 8; NS = not significant (P>0.05).

Table 3. Extent of lipid oxidation of thigh muscle (mg MDA/kg muscle) from broiler chickens fed diets containing varying levels of *H. sabdariffa* calyces.

Storage length at 4°C (days)	Meat condition	Diet					Statistical significance
		A	B	C	D	E	
		H. sabdariffa calyces level (%)					
		0	1.5	3.0	4.5	6.0	
1	Uncooked	1.13 ± 0.15	0.96 ± 0.19	0.95 ± 0.14	1.15 ± 0.13	0.98 ± 0.12	NS
	Cooked	3.03 ± 0.33^{a}	2.57 ± 0.19^{b}	3.27 ± 0.23^{ad}	2.10 ± 0.15^{c}	3.48 ± 0.26^{d}	***
3	Uncooked	1.54 ± 0.14^{ac}	1.44 ± 0.12^{a}	1.48 ± 0.11^{b}	1.47 ± 0.16^{ac}	1.64 ± 0.11^{c}	***
	Cooked	3.23 ± 0.33	3.49 ± 0.23	3.23 ± 0.34	3.47 ± 0.29	3.33 ± 0.34	NS
6	Uncooked	2.40 ± 0.16^{a}	2.18 ± 0.09^{bc}	2.04 ± 0.22^{ab}	2.02 ± 0.07^{c}	2.29 ± 0.13^{ab}	*
	Cooked	4.51 ± 0.24^{a}	4.52 ± 0.18^{a}	5.38 ± 0.29^{b}	4.53 ± 0.12^{a}	4.78 ± 0.14^{a}	***
9	Uncooked	2.80 ± 0.18^{a}	2.60 ± 0.14^{ab}	2.39 ± 0.11^{bc}	2.21 ± 0.17^{c}	2.37 ± 0.06^{c}	***
	Cooked	5.00 ± 0.18^{ab}	4.81 ± 0.27^{a}	4.67 ± 0.29^{bc}	4.64 ± 0.22^{c}	4.34 ± 0.19^{c}	***
Statistical significance							
Storage length		***	***	***	***	***	
Meat condition		***	***	***	***	***	
Storage length x Meat condition		NS	***	***	***	***	

Mean ± SD; N = 8; NS = Not significant (P>0.05)
* = P<0.05; *** = P<0.001.
Means with different superscripts along the same row are significantly different (P<0.05).

The data were subjected to one-way analysis of variance (ANOVA) and 4 x 2 factorial analysis where appropriate, using the Minitab Statistical Package (v. 10.2, Minitab Inc. USA).

RESULTS

Moisture and lipid contents of meat

The moisture and lipid contents of the thigh muscle are presented in Table 2. They showed no significant (P > 0.05) dietary effect. The moisture content ranged between 71.68 ± 1.19% and the lipid content between 4.03 ± 0.92%. No trend was observed in moisture and lipid contents in relation to dietary level of *H. sabdariffa* calyces.

Oxidative stability of meat

Results of oxidation of the thigh muscle are presented in Table 3. Muscle lipid oxidation was significantly (P < 0.001) influenced by storage length, meat condition and interaction of the two factors (except for muscle length by meat condition interaction for diet A). Uncooked meat had lower level of oxidation than cooked irrespective of storage length. The values averaged 1.97 vs. 3.94, 1.80 vs. 3.97, 1.72 vs. 4.14, 1.71 vs. 3.68 and 1.82 vs. 3.98 mg MDA/kg muscle for uncooked and cooked meat from chickens fed diets A, B, C, D and E, respectively. On the 9th day of refrigerated storage, extent of oxidation decreased with increasing levels of *H. sabdariffa* calyces in diets. These averaged 3.90, 3.71, 3.53, 3.43 and 3.36 mg MDA/kg muscle for both uncooked and cooked meat for diets A, B, C, D and E, respectively.

Bacteria load and isolates of meat

The bacteria load of meat samples from the broiler chickens is shown in Table 4. The bacteria load was affected

Table 4. Bacteria load (CFU) of thigh muscle from broiler chickens fed diets containing varying levels of *H. sabdariffa* calyces.

Storage length at 4°C (days)	Meat condition	Diets					Statistical significance
		A	B	C	D	E	
		H. sabdariffa calyces level (%)					
		0	1.5	3.0	4.5	6.0	
1	Uncooked	$2.69^{a} \pm 0.12 \times 10^{8}$	$2.65^{a} \pm 0.23 \times 10^{8}$	$2.38^{ab} \pm 0.18 \times 10^{8}$	$2.14^{bc} \pm 0.08 \times 10^{8}$	$2.01^{c} \pm 0.21 \times 10^{8}$	**
	Cooked	$0.40^{a} \pm 0.06 \times 10^{5}$	$0.40^{a} \pm 0.02 \times 10^{5}$	$0.40^{a} \pm 0.03 \times 10^{5}$	$0.30^{b} \pm 0.02 \times 10^{5}$	$0.20^{c} \pm 0.03 \times 10^{5}$	***
3	Uncooked	$2.42^{a} \pm 0.12 \times 10^{8}$	$2.18^{ab} \pm 0.18 \times 10^{8}$	$1.83^{b} \pm 0.23 \times 10^{8}$	$1.83^{b} \pm 0.29 \times 10^{8}$	$1.69^{b} \pm 0.10 \times 10^{8}$	**
	Cooked	$0.40^{a} \pm 0.04 \times 10^{5}$	$0.40^{a} \pm 0.08 \times 10^{5}$	$0.20^{b} \pm 0.03 \times 10^{5}$	$0.20^{b} \pm 0.03 \times 10^{5}$	$0.20^{b} \pm 0.01 \times 10^{5}$	***
6	Uncooked	$2.19^{a} \pm 0.19 \times 10^{8}$	$1.81^{ab} \pm 0.31 \times 10^{8}$	$1.64^{b} \pm 0.24 \times 10^{8}$	$1.42^{b} \pm 0.22 \times 10^{8}$	$1.37^{b} \pm 0.27 \times 10^{8}$	**
	Cooked	$0.50^{a} \pm 0.10 \times 10^{5}$	$0.30^{b} \pm 0.03 \times 10^{5}$	$0.30^{b} \pm 0.02 \times 10^{5}$	$0.20^{c} \pm 0.02 \times 10^{5}$	$0.10^{d} \pm 0.01 \times 10^{5}$	***
9	Uncooked	$1.85^{a} \pm 0.29 \times 10^{8}$	$1.72^{ab} \pm 0.17 \times 10^{8}$	$1.53^{b} \pm 0.13 \times 10^{8}$	$1.52^{b} \pm 0.11 \times 10^{8}$	$1.15^{c} \pm 0.09 \times 10^{8}$	**
	Cooked	$0.40^{a} \pm 0.05 \times 10^{5}$	$0.30^{b} \pm 0.02 \times 10^{5}$	$0.20^{c} \pm 0.02 \times 10^{5}$	$0.20^{c} \pm 0.03 \times 10^{5}$	$0.10^{d} \pm 0.03 \times 10^{5}$	***
Statistical significance							
Storage length		**	***	***	***	***	
Meat condition		***	***	***	***	***	
Storage length x Meat condition		**	*	*	*	*	

Mean ± SD; N = 3.
* = P<0.05; ** = P<0.01; *** = P<0.001.
Means with different superscripts along the same row are significantly different (P<0.05).

by storage length irrespective of dietary treatments (diets A = P<0.01; B, C, D and E = P < 0.001) and decreased with increasing length of refrigeration (day 1 averaged 2.37 x 10^8 vs. 0.34 x10^5 cfu, and day 9 averaged 1.55 x 10^8 vs. 0.24 x 10^5 cfu for uncooked and cooked meat, respectively). The bacteria load of the cooked meat was significantly lower (P<0.001) than that of the cooked meat. The values respectively averaged 2.29 x 10^8 vs. 0.43 x 10^5 cfu for diet A, 2.09 x 10^8 vs. 0.35 x 10^5 cfu for diet B, 1.83 x 10^8 vs. 0.28 x 10^5 cfu for diet C, 1.62 x 10^8 vs. 0.15 x 10^5 cfu for diet D, and 1.15 x 10^8 vs. 0.15 x 10^5 cfu for diet E. With increasing level of H. sabdariffa calyces in the diets through diets A to E, bacteria load decreased (uncooked meat = P<0.01; cooked meat = P<0.01) for all storage periods. The interaction between storage length and meat condition was significant (Diet A = P<0.01, B, C, D and E = (P < 0.01).

Table 5 shows the occurrence of the bacteria isolates in uncooked and cooked meat during refrigerated storage for up to 9 days, irrespective of dietary treatments. Six types of bacteria (*Staphylococus aureus*, *Bacillus subtilis*, *Corynebacterium sp.*, *Escherichia coli*, *Salomnella sp.* and *Lactobacillus salivarius*) were isolated from the uncooked meat but only 3 (*S. aureus*, *B. subtilis* and *Corynebacterium sp.*) from the cooked meat.

DISCUSSION

The moisture and lipid contents of the muscle fell within the range of 65 and 70%, and 4 and 12%, respectively, which was reported for poultry by Ikeme (1990) and Onibi (2000a). The significant effect of diets on meat oxidation observed for uncooked and/or cooked meat on days 1, 3 and 6 of refrigeration did not show any trend in relation to dietary treatments. However, on day 9 of refrigeration, there was a trend of decreasing meat oxidation (P<0.001) with increasing levels of dietary *H. sabdariffa* calyces (diets C, D and E compared with diets A and B). This may be attributed to increasing levels of vitamin C in the diets (Table 1). Mitsumoto et al. (1991) reported the antioxidant ability of vitamin C in ground beef. The storage length and meat condition significantly influenced (P<0.001) the extent of meat oxidation irrespective of dietary treatments. Oxidation increased with increasing period of refrigeration. This

Table 5. The occurrence of bacteria isolates during refrigerated storage of thigh muscle from broiler chickens fed diets containing varying levels of *H. sabdariffa* calyces.

Storage length at 4°C (days)	Meat condition	
	Uncooked	Cooked
1	Staphylococcus aureus Bacillus subtilis Corynebacterium *sp.* Escherichia coli Salmonella *sp.* *Lactobacillus salivarius*	Staphylococcus aureus Bacillus subtilis Corynebacterium *sp.* - - -
3	Staphylococcus aureus Bacillus subtilis Corynebacterium *sp.* Escherichia coli Salmonella *sp.*	Staphylococcus aureus *Bacillus subtilis* *Corynebacterium* sp. - -
6	Staphylococcus aureus Bacillus subtilis - Escherichia coli Salmonella *sp.*	Staphylococcus aureus *Bacillus subtilis* *Corynebacterium* sp. - -
9	Staphylococcus aureus Bacillus subtilis Escherichia coli Salmonella *sp.*	Staphylococcus aureus *Bacillus subtilis* - -

is similar to the results of Sklan et al. (1983) and Onibi (2000a) that deteriorative changes continue to occur in meat during refrigerated storage. The cooked meat significantly ($P<0.001$) had higher MDA concentrations than the uncooked meat. This is consistent with Monahan et al. (1993) and Onibi (2000b) that heat disrupt muscle membranes and break lipoprotein complexes, thereby increasing exposure of tissue lipids to attack by oxygen and catalysts (especially free iron) and making cooked meat more readily susceptible to oxidation. The significant ($P<0.001$) interaction between storage length and meat condition for diets B to E showed increasingly more oxidation in cooked than uncooked meat with increasing storage length.

The decrease in bacteria load with increasing storage length could be attributed to the fact that the temperature condition of the refrigerator might not be conducive for the multiplication and growth of some of the bacteria organisms. Collins and Lyne (1976) reported that bacteria might be cold shocked during refrigeration. Similarly, Adams and Moss (1999) reported that at chill temperatures, microbial growth is restricted to the psychrotrophs organisms.

The lower bacteria load of the cooked meat than uncooked may be attributed to the fact that some of the bacteria might be thermolabile. Also, the surface cover of uncooked meat is soft for easy penetration of bacteria while cooked meat is a little tougher and drier thus making penetration of bacteria more difficult. Decreasing bacteria load with increasing level of *H sabdariffa* calyces suggests that *H. sabdariffa* calyces had preservative effect. Bacteria load of the uncooked meat ranged between 1.15 to 2.69 x 10^8 cfu and this exceeded 1.0 x10^7 cfu surface bacteria count reported by Nottingham (1971) to cause apparent spoilage in meat. However, since the meat is fresh, it is unlikely that spoilage would have set in. The large number of bacteria could have arisen during defeathering, evisceration and dissection of carcass as reported by Adams and Moss (1999). The significant interaction between storage length and meat condition revealed that with increasing storage length, bacteria load, which was higher in uncooked meat, reduced more than in cooked meat.

Reduced types of bacteria found in the cooked meat compared with uncooked showed that the unisolated bacteria were killed during cooking. The large number of bacteria isolates in uncooked meat could have arisen during processing. Bergdoll (1980) reported that handlers of food products are the most likely source of contamination as they cough, sneeze and talk over food. The use of contaminated water and equipment for processing is a likely source of bacteria too (Adams and Moss, 1999). On the third day of storage, *L. salivarius* was not isolated from the uncooked meat. This may be due to the inability of the organism to withstand cold storage. On day 6 of refrigerated storage, *Corynebacterium sp*. was

not isolated from the uncooked meat and on day 9 of storage, this bacterium was not found in both cooked and uncooked meat. *L. salivarius* is non-pathogenic but the others are (Collins and Lyne, 1976).

Conclusion

The feeding of broiler-chickens with diets containing various levels of *H. sabariffa* calyces did not produce significant changes in moisture and lipid contents of the meat. On the 9th day of refrigeration, there was a trend of decreasing meat oxidation with increasing levels of dietary *H. sabdariffa* calyces. Lipid oxidation in meat increased with increasing period of refrigeration irrespective of meat condition and dietary treatments. Uncooked meat contained more bacteria load than cooked meat. With increase in the period of refrigeration and levels of *H. sabdariffa* calyces in the diets, there was a reduction in bacteria load of both fresh and cooked meat. More bacteria types were isolated in the uncooked than cooked meat but the types reduced during refrigerated storage. Thus, *H. sabdariffa* calyces contain potential antioxidant and antibacterial agents that need further investigation. Proper cooking of fresh and refrigerated meat is advocated.

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