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In vitro antioxidant effect of groundnut leaf and shell extracts on cooked and raw broiler meat

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ABSTRACT: Antioxidant effect of Groundnut Leaf Extract (GLE) and Groundnut Shell Extract (GSE) on broiler meat was evaluated and compared with butylated hydroxyl anisole (BHA), a synthetic antioxidant in a 4x2x7 factorial design. To a separate 350g of minced broiler meat, each extract was applied separately at the rate of 1.5%, a control without additive and 0.01% of BHA, which serve as positive control. Each sample was divided into 28 parts of 12.5g each. Fourteen (14) of these were cooked in microwave oven for 1½ minutes while the other 14 parts were left raw. Both cooked and raw samples were stored in a refrigerator for 12 days at a temperature of 4 ± 1^0C. Oxidative stability of the samples was monitored at 2-day interval using the Thiobarbituric acid (TBA) assay. The result shows that all the additives and BHA were able to reduce lipid oxidation in broiler meat. This was shown by lower TBARS values in treated broiler meat samples compared to the control samples (meat without additives). The results indicate that the antioxidant potency of GLE and GSE is not significantly different ($P>0.05$) from that of BHA. Treated raw meat samples have a lower TBARS values than the cooked meat samples. Addition of GLE and GSE was effective in reducing lipid oxidation in both cooked and raw broiler meat under refrigeration.

Keywords: Groundnut leaf; Groundnut shell; Broiler meat; Butylated Hydroxy Anisole; Thiobarbituric acid.

Introduction

Chicken meat and its product have experienced increasing popularity and wide usage mainly for consumption all over the world (Sallan *et al*., 2002). The processed chicken meat provides a means of functional protein. It is also utilized in a wide range of emulsified and restructured meat products. The presence of lipid and its interactions are important factor affecting the stability of meat during storage. The major problem for processed meat is the onset of oxidative rancidity, which results into off-flavour and off-colour (Mielnik *et al*., 2002). Oxidation of lipid in chicken meat is responsible for the changes in its nutritional quality, loss of vitamins, minerals, essential amino acids, colour, flavour and texture (Aguirrezabal *et al*.,2000) and reduced shelf life (Coronado *et al*., 2002).

The oxidation processes primarily produce hydroperoxides, which is tasteless and odourless. As the oxidation reaction proceeds, the hydroperoxides are broken down into carbonyl and other compounds, which are responsible for rancid flavour in oxidized lipids (Gordon, 1991). Products of lipid oxidation are potentially toxic and may lead to adverse effects such as production of carcinogens, mutagenesis and ageing (Yagi, 1990). In order to prevent lipid oxidation, antioxidants were used (Olorunsanya *et al*., 2009).

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Antioxidants are food additives of either synthetic or natural origin that can scavenge free radicals thereby preventing oxidation of unsaturated fats, the cause of rancidity. Synthetic antioxidants such as Butylated hydroxylanisole (BHA) and Butylated Hydroxylanisole (BHT) are typical example of commonly used antioxidants. However, the health and nutritional safety of consumers have been jeopardized as a result of using these synthetic antioxidants, hence their low acceptability by consumers (Pokorny, 1991). Synthetic antioxidants are carcinogenic (Kahl and Kappus, 1993), scarce and expensive. The need to find lasting solution to the aforementioned problems, necessitate the use of natural antioxidants in foods. Phenolic extracts from herbs and spices (Abdallah and Roozen, 1999), cereals and legumes (Onyeneho & Hiettiarachchy, 1992) have been reported to retard lipid oxidation in oils and fatty foods. Natural antioxidants in addition to being cheap and readily available also have antioxidant effect in the human body. Leguminous crops such as groundnut are common in Nigeria where it is mainly used for consumption while the leaves and shells can be used for feeding livestock. Due to the availability of more nutritious forage crops for feeding livestock, groundnut leaves and shells are considered waste in most cases. This fact coupled with the availability of groundnut prompt its selection as a source of natural antioxidant for this study. The objective of this study was to determine the effect of groundnut shell and leaf extracts on oxidative stability of cooked and raw broiler meat in comparison with Butylated hydroxyl anisole, a synthetic antioxidant.

Materials and Methods

Collection of samples

Ten broiler chickens of eight (8) weeks old were purchased from Animal Production Pavilion, Department of Animal production, Faculty of Agriculture, University of Ilorin. Freshly harvested groundnut leaves and shell were obtained from a local farm at Ilemona in Kwara State.

Processing of broiler chickens

The broiler chickens were slaughtered by cutting through the jugular vein. They were scalded manually by dipping into boiled water for a minute, defeathered, washed, eviscerated and de-skinned. The carcasses were cut into different parts. The breast, thigh and drumsticks were manually deboned using a sharp knife and minced using a food processor (National MK-5080M).

Processing of groundnut leaves and shells

The groundnut leaves were thoroughly washed and air-dried for three days. After the separation of the nut from the shell, the shell was thoroughly washed and air-dried for three days. Both leaves and shell were subjected to soxhlet extraction separately with methanol as the solvent.

Application of treatments

The minced meat was weighed into four portions of 350g each. The first portion serve as the negative control (no additive). 0.05% of butylated hydroxylanisole (BHA) was added to the second portion (positive control). 0.05% of Groundnut Leave Extract (GLE) and Groundnut Shell Extract (GSE) were added to the third and fourth portion respectively. Each of the treated minced meat was divided into twenty eight parts of 12.5g each. Fourteen of these were cooked for 1½ minutes using a microwave oven (National-NN-55WF) while the other parts were left raw. Both cooked and raw samples were wrapped in different foil paper with labels corresponding to the applied treatments. They were stored in a refrigerator (HR-170T) at a temperature of 4 ± 1^{0}C for twelve days. The oxidative stability was monitored at 2-day interval for 12days of storage.

Determination of lipid oxidation

Lipid oxidation in the samples was evaluated using the 2-thiobarbituric acid (TBA) test. The thiobarbituric acid reactive substance (TBARS) values were measured on a duplicate 10g samples at each storage day using the distillation method of Tarladgris *et al*, (1964). 10g of the meat sample was homogenized with 47.5ml of distilled

water in a specimen bottle using glass pestle. The homogenized mixture was rinsed with 50ml of distilled water into a 500ml round bottom flask. Thereafter, 2.5ml of 0.1M Hydrochloric acid solution (1:2 v/v) was added and the mixture was distilled through a condensing assembly to collect about 15ml of the distillate. 5ml of the distillate was mixed with 5ml of TBA (0.02M). The mixture was boiled for 35 minutes in boiled water, and then cooled for ten minutes under a running tap water for color development. The duplicate absorbance readings were measured at 538nm against a blank that contained 5ml of 0.1M hydrochloric acid solution and 5ml of thiobarbituric acid (TBA) reagent using a spectrophotometer (COMSPEC-M105). The absorbance values were multiplied by a factor of 7.8 (Tarladgris *et al.*, 1964) to obtain the TBARS values in milligram per malonaladehyde per kilogram of sample (mg/MDA/kg).Triplicate samples were analyzed in duplicate for each treatment.

Statistical analysis

The experiment follows a 4*2*7 factorial design. The data obtained was analyzed using analysis of the variance (ANOVA) model suitable for the factorial design with the aid of a Genstat 5 program package (Payne, Lane and Genstat Committee, 1987). The difference between means was determined by Duncan multiple range test and significance was defined at $P<0.05$.

Results and Discussion

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

Factor		TBARS (mg/MDA/kg)			
Antioxidant treatment		Control	GLE	GSE	BHA
		4.534^{b}	2.461^{a}	2.378^{a}	2.531^{a}
	SEM			0.234	
State of meat			Raw		Cooked
			2.525^{a}		3.425^{b}
	SEM			0.156	

a,b means with the same superscript along the same row are not significantly different. ($P>0.05$)

In Table 1, the control samples had the highest TBARS value which was significantly different ($P<0.05$) from other treatments. This shows that all additives reduced lipid oxidation more than the control samples. There is no significant difference ($P>0.05$) in the antioxidant activity of Butylated hydroxyl anisole (BHA), Groundnut Leaf Extract (GLE) and Groundnut Shell Extract (GSE). The effectiveness of both GLE and GSE may be due to the presence of phenolic compounds such as p-coumaric acid, tannin, flavonoids and phenol. These compounds are mainly concentrated in seed coats (Preet and Punia, 2000). It was observed that cooking speeds up the rate of lipid oxidation in broiler meat. This was manifested by the higher TBARS value (3.425) of cooked meat samples as compared to the raw ones whose TBARS value was 2.525. This observation agrees with the report of Beltran *et al*, (2003) which states that heating accelerates lipid oxidation and the production of volatiles in meat. Cooking also deactivate antioxidant enzymes and release iron from heme pigments (Kanner, 1994). This observation however differs from that of olorunsanya *et al*, (2009) who reported that raw pork patties undergo lipid oxidation than cooked ones. They further asserted that this observation might be due to formation of Maillard reaction products (MRP) during cooking. MRP have been reported to exhibit antioxidant activity (Sato *et al*., 1973; Bailey, 1998 and Guntensperger *et al*., 1998). The mechanism for antioxidant activity of MRPs is that they reduce hydroxyl-peroxides to products that are unable to form free radicals that were formed during oxidative degradation of unsaturated fatty acids (Wijewickreme *et al*., 1999; Wijewickreme and Kitts, 1997). MRPs have also been reported to scavenge free radicals the cause of lipid oxidation (Yen and Hseich, 1995).

Table 2: Interactive effect of antioxidant treatment and storage days on oxidative stability of broiler meat

	TBARS (mg/MDA/kg)							
Antioxidant	Storage days							
	0	2	4	6	8	10	12	
Control	2.80^{a}	4.60^{b}_{y}	4.60^{b}_{y}	4.10^{b}_{y}	7.20^{c}_{y}	3.20^{a}	5.10^{b}_{y}	
GLE	1.90	2.60_{x}	2.60_{x}	2.40_{x}	3.10_{x}	3.10	2.40_{x}	Ns
GSE	2.00	1.90_{x}	3.00_{x}	3.50_{x}	2.35_{x}	1.00	2.00_{x}	Ns
BHA	2.20	2.80_{x}	2.30_{x}	2.50_{x}	3.00_{x}	2.20	2.10_{x}	Ns
	Ns					Ns		
SEM				0.44				

a, b, c means having different superscript along the same row are significantly different (P<0.05). x, y means having different subscript along the same column are significantly different (P<0.05).

As storage days increases, lipid oxidation increases as shown in Table 2. This may be caused by increase in the formation of pro-oxidant compounds. At day 0 and 10, there was no significant difference (P>0.05) among the treatments. At storage day 2, 4, 6, 8 and 12, the control samples had higher TBARS values than other treatments. However, there was no significant difference (P>0.05) between BHA, GLE and GSE. In all treated samples, lipid oxidation progresses at a slower rate. The activity of both GSE and GLE was typical of natural antioxidants. At storage day 10, there was a general decrease in lipid oxidation for all the samples.

Table 3: Interactive effect of antioxidant treatment and state of meat on oxidative stability of broiler meat

State of meat	TBARS (mg/MDA/kg)			
	Antioxidant treatments			
	Control	GLE	GSE	BHA
Cooked	5.267_{y}^{b}	2.737_{x}^{a}	2.500_{x}^{a}	2.913_{x}^{a}
Raw	3.508_{x}^{b}	2.080_{x}^{a}	2.242_{x}^{a}	2.150_{x}^{a}
		Ns	Ns	Ns
SEM		0.82		

a,b means with the same superscript along the same row are not significantly different (P>0.05). x,y means having the same superscript along the same column are not significantly different (P>0.05).

All treated meat samples performed equally (P>0.05) in the cooked and raw state. This is contrary to the report given by Olorunsanya *et al* (2009) that cooking which is mostly associated with increase in temperature activates some lypolytic enzymes such as lipase and phospholipase in the meat, which promote lipid oxidation. Ashgar *et al*, (1998) reported that cooking disrupts muscle membrane systems leading to loss of structural integrity thus exposing membrane lipids to oxidant catalyst. There is no significant difference (P>0.05) in the antioxidant activity of GLE, GSE and BHA in the cooked and raw samples. However, their antioxidant potency is significantly different from that of the control samples. The potency of GSE and GLE may be due to the presence of inherent phenolic compounds that are antioxidant in nature. Phenolic compounds have been reported to scavenge free radicals through hydrogen or electron donation from their phenolic hydroxyl groups (Murthy *et al*., 1998).

Conclusion

All the additives and BHA were able to reduce lipid oxidation in broiler meat. This was shown by lower TBARS values in treated broiler meat samples compared to the control samples (meat without additives). Antioxidant potency of GLE and GSE is not significantly different (P>0.05) from that of BHA. Treated raw meat samples have a lower TBARS values than the cooked meat samples. Addition of GLE and GSE was effective in reducing lipid oxidation in both cooked and raw broiler meat under refrigeration. Thus, readily available and cheap GLE and GSE could be used as a natural source of antioxidant in the control of lipid oxidation in meat.

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