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Research Article

Post-mortem application of garden egg (*Solanum melongena*) peel powder on oxidative stability of pectoralis major muscle in broiler chickens

Hakeem Ishola,* Rukayat Olowoeyo

Department of Animal Production, Kwara State University, Malete, Nigeria

Abstract: This study evaluated the phenolic compounds in garden egg peel powder (GEPP) and the effect of GEPP on lipid oxidation of raw and cooked pectoralis major muscle in broiler chickens. Graded levels (0, 0.5, 1.0 and 1.5%) of GEPP were applied to minced raw and cooked broiler breast meat and compared with 0.01% Butylated hydroxyl anisole (BHA). Lipid oxidation in the meat samples was monitored at 3-day interval for 9 days using the thiobarbituric acid test. The GEPP contains flavonoid, phenols, terpenoids and tannins. Irrespective of antioxidant treatments, cooked meat samples had higher ($P< 0.05$) thiobarbituric acid reactive substance (TBARS) value than the raw meat samples. The raw and cooked control samples had higher ($P< 0.05$) TBARS than meat samples treated with antioxidants. On day 0 and 3, the TBARS value of BHA samples did not differ ($P> 0.05$) from those treated with GEPP. However, on day 6 and 9, BHA samples had lower TBARS value compared with those treated with GEPP. The current results suggest that post-mortem application of 0.5% GEPP could reduce lipid oxidation in refrigerated broiler meat for up to 3 days.

Keywords: Broiler meat; BHA; garden egg peel; lipid oxidation; pectoralis major

1. Introduction

Meat is a nutritious and protein-rich food which has a short shelf life unless effective preservation methods are applied to it (Olaoye et al., 2010). Oxidative changes in lipids and myofibrillar proteins represent the major causes of quality deterioration and reduction in shelf life of meat and meat products (Adeyemi et al., 2017). Broiler meat has several desirable nutritional characteristics such as high protein, low lipid contents and high polyunsaturated fatty acids (Mothershaw et al., 2009). These make it preferable, health wise, when compared to red meat. Broiler meat is however highly susceptible to lipid oxidation and bacterial contamination during storage (Sahin et al., 2010). Lipid oxidation is reported to have adverse effects on meat quality parameters such as colour, juiciness, tenderness and flavour, thus leading to the reduction of the meat's shelf-life (Min et al., 2008). Synthetic antioxidants are capable of inhibiting lipid oxidation in meat (Olorunsanya et al., 2011; Adeyemi et al., 2013).

Nonetheless, synthetic antioxidants could have detrimental effects on the health of consumers (Cao et al., 1996). This has prompted and favoured the use of plant polyphenols as antioxidants in foods (Olorunsanya et al., 2011). Fruits are an important source of many bioactive compounds, such as phenolic acids and flavanone glycosides (Abeysinghe et al., 2007). Peels are the primary by-products during fruit juice processing and if unused, they constitute wastes and a source of environmental pollution. However, it has been shown that peels of various fruits such as citrus, apples, grapes and berries are major sources of natural antioxidants (de Moraes Barros et al., 2012). Phenolic compounds in peels and fruit by-products can be used in food products as active ingredients or as substitutes for synthetic preservatives (Ignat et al., 2011; Adeyemi et al., 2013).

* Corresponding author:
Email: abdulhakeemadisa5@gmail.com

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Oxidative modifications of proteins can change the physical and chemical properties, including conformation, structure, proteolysis and enzyme activities of the meat. These modifications can be involved in the regulation of fresh meat quality and influence the processing properties of meat products (Liu et al., 2016). Oxidative stress occurs when the formation of oxidants exceeds the ability of antioxidant systems to remove the reactive oxygen species (ROS) in organisms. Increased levels of tissue protein oxidation have been associated with various biological consequences including diseases and aging in humans and other animal species (Friguet, 2002). Reactive oxygen species represent a broad category of molecules which give rise to reactive free radicals such as superoxide, hydrogen peroxide and hydroxyl radicals (Lee et al., 2004). These free radicals participate in oxidative reactions that damage organic substrates, such as lipids, proteins and DNA of living organisms, resulting in a deteriorative biological condition called oxidative stress (Kalam et al., 2012). The biological damage of oxidative stress is deleterious at the cellular level and it has been known to cause several degenerative health problems which affect livestock productivity (Yang et al., 2013; Sharma et al., 2011; Avanzo et al., 2001).

Nonetheless, there is limited investigation on the phenolic contents of garden egg peel and its effect on oxidative stability of broiler meat. The objective of this study is to determine the phenolic contents of garden egg peel powder and its effects on lipid oxidation in refrigerated pectoralis major muscle in broiler chickens.

2. Materials and methods

2.1. Collection of samples

Ripe fresh garden egg fruits were obtained at a local market in Ilorin, Kwara State, Nigeria. The preparation of garden egg fruit peel powder and broiler meat processing was carried out at the Agricultural laboratory, Kwara State University, Malete while the extraction, phytochemical screening of the GEPP and lipid oxidation analysis was carried out at the laboratories of Animal Production and Chemistry Departments, University of Ilorin, Ilorin, Nigeria.

2.2. Preparation of garden egg peel powder

The ripe garden egg fruits were peeled using a sterilised knife and their edible part was separated. The peels were weighed using digital weighing machine, then washed with distilled water and air-dried for 5 days. The peels were ground to form a powder with electric blender and kept in a tight container for further analysis.

2.3. Extraction of polyphenols in garden egg peel powder

Using a solvent extraction method, the garden egg peel powder was extracted with 400 ml methanol and ethyl acetate. Approximately 400 ml of methanol was poured in a round bottom flask containing 106 g of garden egg peel powder, shaken and left for 5 days. The methanol was collected by decanting into another bottle. The collected solvent was left to dry off for 5 days to get the extract. The extract was kept in a tight container and used for phytochemical screening (Prashant et al., 2011).

2.4. Qualitative phytochemical screening using methanol extraction

2.4.1. Test for flavanoids

Approximately 10 ml of ethyl acetate was heated with 0.5 ml of the extract for 3 minutes over a steam bath. The mixture was filtered and 4 ml of the filtrate was added to 1 ml of diluted ammonia solution and shaken thoroughly. A light yellow coloration indicated the presence of flavonoids (Prashant et al., 2011).

2.4.2. Test for phenols

About 0.5g of the extract was dissolved in 5 ml of distilled water and 2 drops of neutral 50% of ferric chloride were added. A dark green coloration showed the presence of phenol (Prashant et al., 2011).

2.4.3. Test for terpenoids

About 0.5g of the extract was added to 2 ml of chloroform and 3 ml of concentrated H_2SO_4 was carefully added to form a layer. Reddish brown colour with interface indicated the presence of terpenoids (Prashant et al., 2011).

2.4.4. Test for tannins

Approximately 0.5 g of the extract was boiled in 10 ml distilled water. About 2 drops of ferric chloride were added and a brownish green coloration was observed which indicated the presence of tannins (Prashant et al., 2011).

3. Meat preparation

Six broiler chickens of 8 weeks old, weighing 2-2.5kg were obtained from Affcon Farms, Ilorin. The broilers were slaughtered by cutting through the jugular vein with a sharp knife. They were de-feathered, de-skinned and eviscerated. The pectoralis major (breast) muscle

of the breasts were excised and minced using a food processor (National MK-50M).

3.1. Application of treatment

About 3200 g of the breast meat harvested from the 8 broiler chickens was minced and divided into 160 parts of 20g each. Graded levels of 0% (negative control), 0.5, 1.0 and 1.5% of eggplant peel powder, and 0.01% Butylated hydroxyl anisole was separately added to 20g of meat samples and mixed thoroughly. Eighty (80) parts of the 20g meat samples were cooked for 15 minutes while the other 80 parts were left raw. Both cooked and raw meat samples were put in petri-dishes with labelling corresponding to the applied treatments and stored in a refrigerator (PVDD 240L) for 9 days at 4°C. The oxidative stability was monitored at 3-days interval for both raw and cooked samples.

3.2. Analysis of lipid oxidation in broiler meat

Lipid oxidation in the broiler meat samples were evaluated using the method described by Tarladagris et al. (1964). Approximately 10g of meat sample was homogenised with 47.5ml of distilled water in a test tube using a glass pestle. The homogenised samples were rinsed with 50ml of distilled water into a round bottom flask and 2.5ml of 0.02M hydrochloric acid (HCl) was added. The mixture was distilled through a distillate apparatus (Buchi-distillation unit K- 350 apparatus) to collect about 10ml of the distillate. Approximately, 5ml of the distillate was mixed with 5ml of thiobarbutric acid (TBA), boiled for 30 minutes in a water bath, and cooled for 10 minutes under a running tap water. The duplicate absorbance readings were measured at a wavelength of 538nm against a blank containing 5ml of TBA and 5ml HCl using spectrophotometer (Cecill-2000). The absorbance values were multiplied by a factor of 7.8 (Tarladagris et al., 1964) to obtain the TBARS values in mg/MDA/kg.

3.3. Statistical analysis

The data were analysed as a 5*2*4 factorial arrangement in a completely randomised design with antioxidant treatments (0, 0.5, 1.0, 1.5% GEPP and 0.01% BHA), state of the meat (raw or cooked) and storage days (0, 3, 6, 9).The data obtained were analysed using the MIXED procedure of SAS (SAS, 2003). Means were separated with Tukey HSD test at $P < 0.05$ level of significance.

4. Results and discussion

The results of the phytochemical screening of the garden egg peel powder indicated the presence of various secondary metabolites such as flavonoids, tannins, phenol, and terpenoids (Table 1). These phytochemicals exhibit various pharmacological and biochemical actions (Evans, 2008). The phenolics are responsible for the characteristic door, pungencies and colour of plant, while others give the particular plant its culinary and medicinal properties (Evans, 2008). Flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer properties (Pourmorad et al., 2006). They induce mechanisms that affect cancer cells and inhibit tumour invasion (Rafat et al., 2008). Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues (Okwu & Emineke, 2006). These findings suggest that GEPP is a potential source of natural antioxidants that could serve great importance as therapeutic, anti-inflammatory, anti-analgesic and anti-hyper lipidemic agent.

Table 1: Phytochemical contents of garden egg peel powder

Phytochemical compounds	Methanol extracts
Flavonoids	+
Phenols	+
Tannins	+
Terpenoids	+

All the GEPP and BHA treated meat samples had lower TBARS value ($p<0.05$) than the control samples (Table 2). The low TBARS value obtained in 0.5, 1.0, 1.5% GEPP could be attributed to the presence of phenolic compounds in the samples (Lu et al., 2006). The TBARS values of cooked samples for all treatments were significantly higher ($P<0.05$) than that of the raw samples. This result agrees with the observation of Krinsky et al. (1994) who indicated that the increase in temperature associated with cooking activates some endogenous lipolytic enzymes such as lipase and phospholipase, which act on the unsaturated fats in the meat and promote oxidative rancidity. Another probable reason for the high TBARS value observed in the cooked samples could be due to the formation of hypervalent ferry myoglobin during cooking (Olorunsanya et al., 2010).

The TBARS values for all the treatments at storage day 6 and 9 were significantly higher ($P<0.05$) than those observed on storage days 0 and 3. This observation is similar to the findings of Adeyemi and Olorunsanya (2012). The TBARS values of meat samples on day 0 did not differ ($P> 0.05$) from day 3.

Table 2: Main effect of antioxidant treatment, state of meat and storage days on oxidative stability of broiler breast meat

Factor	TBARS (mg MDA/kg)					
Antioxidant	0%	0.5% GEPP	1.0% GEPP	1.5% GEPP	0.01% BHA	SEM
	0.61[b]	0.14[b]	0.12[b]	0.11[b]	0.08[b]	0.03
State of meat	Cooked	Raw				
	0.36[b]	0.06[b]				0.02
Storage days	0	3	6	9		
	0.03[b]	0.04[b]	0.34[b]	0.44[b]		0.04

[b,] means having same superscripts along the same row are significantly different ($p < 0.05$)

Table 3: Interaction effect of antioxidant and state of meat on oxidative stability of broiler breast meat

State of meat	Antioxidants	TBARS (mg MDA/kg)
Raw	0%	0.12[a]
	0.5% GEPP	0.03[b]
	1% GEPP	0.06[b]
	1.5% GEPP	0.07[b]
	0.01% BHA	0.04[b]
cooked	0%	1.10[a]
	0.5% GEPP	0.25[a]
	1% GEPP	0.19[a]
	1.5% GEPP	0.16[a]
	0.01% BHA	0.11[a]
	SEM	0.03

[a, b,] means having different superscripts along the same column are significantly different ($p < 0.05$).

There was a significant interaction ($P< 0.05$) between antioxidant treatments and state of meat on lipid oxidation of breast meat of broiler chickens (Table 3). In both cooked and raw meat samples, the control samples had significantly higher ($P< 0.05$) TBARS values than those treated with GEPP and BHA. The TBARS values of cooked meat samples treated with GEPP and BHA were significantly higher than those of the raw meat samples. This observation was in agreement with report by Olorunsanya et al. (2010), who observed that the lipid oxidation in broiler meat could be influenced by the state of meat.

Table 4: Interaction effect of antioxidant and storage days on oxidative stability of broiler breast meat

Antioxidant	Storage days	TBARS (mg MDA/kg)
0%		0.07[b]
0.5% GEPP		0.02[a]
1% GEPP	0	0.04[a]
1.5% GEPP		0.03[a]
0.01% BHA		0.04[a]
0%		1.01[b]
0.5% GEPP		0.02[a]
1% GEPP	3	0.03[a]
1.5% GEPP		0.03[a]
0.01% BHA		0.02[a]
0%		1.03[b]
0.5% GEPP		0.19[b]
1% GEPP	6	0.18[b]
1.5% GEPP		0.24[b]
0.01% BHA		0.09[b]
0%		1.33[b]
0.5% GEPP		0.35[b]
1% GEPP	9	0.20[b]
1.5% GEPP		0.15[b]
0.01% BHA		0.16[b]
SEM		0.01

[a, b,] means having superscripts along the same column are significantly different ($p < 0.05$)

On day 0, 3, 6 and 9, the control meat samples had higher ($P< 0.05$) TBARS values than meat samples treated with GEPP and BHA (Table 4). The TBARS values of BHA treated samples did not differ ($P> 0.05$) from those of meat samples treated with 0.5, 1.0, 1.5% GEPP on day 0 and 3. The BHA treated samples had significantly lower ($P< 0.05$) TBARS values compared with meat samples treated with GEPP. The TBARS values of GEPP and BHA samples on days 0 and 3 were less than 0.6mg/kg required to bring about an objectionable flavour related with lipid oxidation (Greene & Cumuze, 1982).

In the cooked samples, there were significant differences ($P< 0.05$) in the TBARS values at storage day 0 and 3. There was no significant difference ($P> 0.05$) between the TBARS values at storage day 6 and 9 for cooked samples. There were significant differences ($P<0.05$) in the TBARS value at different storage day 0 and 3 for raw samples. However, there was no significant difference ($P> 0.05$) between the TBARS values at storage day 6 and 9 for raw samples. This finding is in agreement with Olorunsanya et al., 2010

who suggested that the state of meat and storage days affects the oxidative stability of broiler chicken meat as post-mortem deterioration starts a day after the broiler chicken is slaughtered.

Table 5: Interaction effect of state of meat and storage days on oxidative stability of broiler meat

	TBARS (mg/MDA/kg)		
Storage days	State of meats		SEM
	Cooked	Raw	
0	0.04[a]	0.05[a]	
3	0.04[a]	0.02[a]	0.03
6	0.56[b]	0.12[b]	
9	0.81[b]	0.06[b]	

[a, b,] means having different superscripts along the same row are significantly different ($p < 0.05$)

At storage day 0 and 6 (Table 5), there was no significant difference (P>0.05) in the TBARS values in all the GEPP and BHA treatment samples. However, at day 6 and 9 in the cooked samples, the TBARS values were higher to that of raw meat samples at storage day 0 and 3. This result agrees with that of Olorunsanya et al. (2009) who observed that oxidative deterioration in cooked samples was due to induced release of protein bond ion after cooking.

In Table 6, there was no significant difference in the TBARS value (P>0.05) for the graded levels of antioxidants and BHA treatments at storage day 0 and 3 for the cooked samples. At days 6 and 9, the control treatment had a higher TBARS values than the GEPP and BHA treated cooked meat samples. At storage day 0 and 3 in the raw samples, there was no significant difference in the TBARS values (P>0.05) for all the GEPP and BHA treated samples. At day 6, the negative control (0%) has a higher TBARS values than the GEPP used and the BHA treated meat samples. However, there was no significant difference (P>0.05) in the TBARS values of garden egg treated at graded levels and BHA treatment. While at storage day 9, even though, the BHA treated meat samples had a higher TBARS value than the negative control (0%) and those treated with graded levels of garden egg peel powder, no significant effect (P>0.05) of this was observed.

The interactive effect of antioxidant treatment, state of meat and storage days on oxidative stability of broiler meat (Table 6), has been observed to show a normal trend as indicated by Pradhan et al. (2000) who demonstrated that antioxidant enzymes could maintain post-mortem oxidative stability of meat. Addition of antioxidant treatment and storage days were found to be significant (P<0.05) on oxidative stability of cooked and raw meat samples. The antioxidant effect of BHA and GEPP treated at graded levels of 0.5, 1.0 and 1.5% were more pronounced in raw meat samples than cooked meat samples. In raw meat samples at storage day 6 and 9, control samples were found to have the highest TBARS values. BHA had a lower TBARS values than the other did. Oxidation was observed to increase sharply at

Table 6: Interaction effect of antioxidants, state of meat and storage days on oxidative stability of broiler meat

	TBARS (mg/MDA/kg)				
State of meat	Antioxidants	Storage days			
		0	3	6	9
Cooked	Control (0%)	0.05[a]	0.02[a]	1.69[b]	2.65[b]
	0.05%	0.01[a]	0.02[a]	0.33[b]	0.66[b]
	1.0%	0.04[a]	0.09[b]	0.28[b]	0.3[b]
	1.5%	0.04[a]	0.04[a]	0.36[b]	0.19[b]
	BHA (0.01%)	0.04[a]	0.02[a]	0.17[b]	0.21[b]
Raw	Control (0%)	0.09[b]	0.01[a]	0.36[dc]	0.01[a]
	0.05%	0.04[a]	0.01[a]	0.04[a]	0.03[a]
	1.0%	0.04[a]	0.06[b]	0.08[b]	0.04[a]
	1.5%	0.03[a]	0.02[a]	0.12[b]	0.11[b]
	BHA (0.01%)	0.03[a]	0.02[a]	0.01[a]	0.12[b]
SEM		0.05			

[a, b,] means having different superscripts along the same row and column are significantly different ($p < 0.05$)

storage day 6 and 9 for all treatments of cooked samples. This observation is consistent with the findings reported during post-mortem chill storage of chevon (Sabow et al., 2016), lamb (Petron et al., 2007), emu (Naveena et al., 2015) and broiler meat (Olorunsanya et al., 2011). In raw samples, all the antioxidant treatments exhibited a higher antioxidant potential at day 0 and 3.

5. Conclusion

The garden egg peel powder (GEPP) contains flavonoid, phenols, terpenoids and tannins. Lipid oxidation was higher in cooked broiler meat than raw broiler meat. Broiler meat samples treated with garden egg peel powder and BHA had lower lipid oxidation than the control meat samples. Lipid oxidation increased as storage progressed. The application of garden egg peel powder in broiler meat is therefore recommended to reduce lipid oxidation for up to 3 days under refrigerated storage.

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