

Comparative Analysis of Phenolic Composition and Antioxidant Effect of Seed Coat Extracts of Four Cowpea (*Vigna unguiculata*) Varieties on Broiler Meat

Research Article

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Received on: 18 Jul 2011

Revised on: 20 Oct 2011

Accepted on: 21 Oct 2011

Online Published on: Dec 2012

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Online version is available on: www.ijas.ir

ABSTRACT

Seed coat crude extracts of white-coloured ITA-256, white and brown-coloured TVS-716, deep brown-coloured IT-90K and light brown-coloured Ife brown cowpea varieties were analyzed for the presence and amount of total phenol, tannin and flavonoid content. Crude extracts of the cowpea varieties were analyzed for antioxidant activity using the Thiobarbituric Acid (TBA) assay. The seed coat extracts were added separately to 200 g of minced broiler meat sample at the rate of 0.05% of the weight of the meat and their effect compared to that of the synthetic antioxidant, butylated hydroxyl anisole (BHA) added to the meat at the rate of 0.05%. Each sample was divided into 16 parts of 12.5 g each. Eight of these were cooked while the other 8 parts were left raw. Antioxidant effect of the seed coat extracts was determined during refrigerated storage of raw and cooked minced broiler meat. All the cowpea varieties studied except TVS-716 contained phenol, tannin and flavonoids. TVS-716 contained only phenol and tannin. Seed coats extract from Ife brown contained higher levels of total phenol, tannin and flavonoids than the corresponding samples of IT-90K, ITA-256 and TVS-716. IT-90K had higher tannin content than ITA-256 and TVS-716 but its phenol and flavonoids content are not significantly different ($P>0.05$) from that of ITA-256. TBA assay shows that all the additives and BHA were able to reduce lipid oxidation in broiler meat. This was shown by lower TBARS values in broiler meat samples with added additives compared to the control samples (meat without additives). Seed coat extracts of Ife brown, ITA-256 and IT-90k reduce lipid oxidation more than BHA in raw broiler meat samples but are less potent than BHA in cooked meat samples. Seed coat extracts of Ife brown was more effective than other cowpea varieties in reducing lipid oxidation in both cooked and raw meat samples. The antioxidant potential of IT-90K was not significantly different ($P>0.05$) from that of ITA-256 while TVS-716 exhibited the least antioxidant potency.

KEY WORDS antioxidant, butylated hydroxyl anisole, broiler meat, cowpea, phenolic.

INTRODUCTION

Lipids are important component of meat and contribute to several desirable quality characteristics of meat. Lipids are important in enhancing the flavour and aroma profile of meat and also increase the tenderness and juiciness of meat (Olorunsanya *et al.* 2009). However, lipid oxidation is responsible for quality deterioration of meat during storage.

Quality characteristics affected in meat by lipid oxidation include flavour, colour, texture and its nutritional value (Gray *et al.* 1996). The development of rancidity in meat by lipid oxidation begins at the time of slaughter and continues during storage. Storing meat at low temperature and packaging of meat in oxygen free containers retards the rapid development of rancidity. However, oxidation of lipids may continue even during frozen storage (Salih *et al.* 1989). The

need to curb reduction in nutritional quality, incidence of off-colour, off-odour and rancid taste or warmed over flavor caused by lipid oxidation necessitate the use of antioxidants in foods (Olorunsanya *et al.* 2009) Based on their source, antioxidant can be classified as synthetic and natural (Brannen, 1975; Kahl and Kappus, 1993). Synthetic antioxidants have been commonly used to suppress lipid oxidation in foods for a long period and are ascertained to be very effective (Brannen, 1975; Kahl and Kappus, 1993).

Examples are butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and tertiary butylhydroquinone (TBHQ) (Gray *et al.* 1996). However, despite the effectiveness of these chemicals, there are some problems associated with their use (Cao *et al.* 1997). Synthetic antioxidants are very scarce, expensive and pose health hazards to consumers (Olorunsanya *et al.* 2009). There is a concern about the safety and toxicity of synthetic antioxidants in relation to their metabolism and accumulation in the body organs and tissues (Cao *et al.* 1997).

Synthetic antioxidants cause impairment to blood clotting, lung damage and act as tumor promoters (Kahl and Kappus, 1993).

Because of these, consumers prefer natural additives and there is a growing interest in the potential use of antioxidants from natural sources. The need to find alternative sources of antioxidants brought about the use of spices such as ginger, tomato, and garlic. Phenolic extracts from herbs and spices (Abdallah and Roozen, 1999), cereals and legumes (Onyeneho and Hiettiarachy, 1992) have been reported to retard lipid oxidation in oils and fatty foods. Cowpea are processed and consumed extensively in developing countries and the large amount of seed coats discarded as waste may be considered potential sources of phenolic compounds for application as natural antioxidants in foods (Lethabo, 2006).

This will be of particular relevance to meat industry where there is currently a drive towards the use of natural food additive. The objective of this study was to determine the phenolic composition of seed coat extracts of four cowpea varieties and their effect on oxidative stability of broiler meat.

MATERIALS AND METHODS

Meat preparation

Ten broilers of 8 weeks old weighing 2 ± 0.35 kg were obtained from Animal Production Pavillon, University of Ilorin, Nigeria.

The broilers were slaughtered by cutting through the jugular vein with a sharp knife. They were scalded manually by dipping into boiled water for a minute, de-feathered, 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 together using a food processor (National MK-5080 M). The minced meat was mixed thoroughly to form a homogenous mix.

Cowpea processing

Four varieties of cowpea were obtained from National Agricultural Seed Council, Federal Ministry of Agriculture Ilorin, Kwara State. The four cowpea varieties are Ife brown (light brown colour), TVX-716 (white and brown colour), IT-90K (deep brown colour) and ITA-256 (white colour). The cowpeas were soaked in water for a minute and dehulled manually. The seed coats were air-dried at ambient temperature for forty-eight hours. Thereafter, the seed coats were extracted with methanol in a soxhlet apparatus.

Treatments

The minced meat was divided into twenty-four 200 g samples. An amount of 0.1 g of extract or BHA (positive control) was added to each 200 g of the minced meat. An untreated sample was used as negative control. Each 200 g of minced meat was divided into sixteen parts of 12.5 g each.

Eight of these were cooked for one minute thirty seconds using a microwave oven (National-NN-55WF) while the other eight parts were left raw. The cooked and raw minced meat samples were packaged in different foil papers corresponding to different treatments. The samples were stored in a refrigerator (HR-170T) at a temperature of 4 °C, for twelve days.

Analysis

Lipid oxidation in meat

The oxidative stability was monitored at two day-intervals. Lipid oxidation in the meat samples was evaluated using the 2-thiobarbituric acid (TBA) test. The thiobarbituric acid reactive substance (TBARS) values were measured on a duplicate 10 g samples at each storage day using the distillation method of Tarladgris *et al.* (1964). 10 g of the meat sample was homogenized with 47.5 mL of distilled water in a specimen bottle using glass pestle.

The homogenized mixture was rinsed with 50ml of distilled water into a round bottom flask. Thereafter, 2.5 mL of diluted Hydrochloric acid (1:2 solution) was added and the mixture was distilled through a condensing assembly to collect about 15 mL of the distillate. 5 mL of the distillate was mixed with 5 mL of Thiobarbituric acid (TBA) (0.02 M) and boiled for 35 minutes in boiled water. Then, the mixture was cooled for ten minutes under a running tap water for color development.

The duplicate absorbance readings were measured at a wavelength of 538 nm against a blank that contains 5 mL of

hydrochloric acid solution and 5 mL 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 milligram per malonaldehyde per kilogram of sample (mg/MDA/kg). Each treatment was replicated four times.

Qualitative analysis of phenolic compounds in cowpea tannin

1 mL of freshly prepared 10% w/v ethanolic KOH (0.01 M) was added to 1 mL of the extract. A dirty white precipitate indicates the presence of tannins (Sofowora, 1980).

Phenol

2 drops of 5% w/v FeCl₃ was added to 1 mL of the extract. A greenish precipitate indicates the presence of phenols (Awe and Shodipo, 2001).

Flavonoids

1 mL of 10% w/v NaOH (0.01 M) was added to 3 mL of extract. A yellow colouration indicates the presence of flavonoids (Awe and Shodipo, 2001).

Quantitative analysis of phenolic compounds in cowpea by spectrophotometric method

Phenol

5 mL of the extract was introduced into a 50 mL flask. Then, 10 mL of distilled water was added. 2 mL of ammonium hydroxide solution and 5 mL of concentrated Amyl alcohol were also added.

The samples were made up to mark and left for 30 minutes for colour development. This absorbance was measured at a wavelength of 503 nm using a spectrophotometer (CECILL-2000). The percentage phenol was calculated using the formula:

% Phenol= absorbance of sample x gradient factor x dilution factor / wt of sample x 10000
Dilution factor= 100.
Gradient factor= 18.41.

Tannin

50 g of the sample (cowpea seed coat) was weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken thoroughly for 30 minutes.

This was filtered into a 50 mL volumetric flask and made up to mark with water, then 5 mL of the filtrate was pipette out into a test tube and mixed with 2 mL of 0.1 M FeCl₃ in 0.1 M HCl and 0.008 M potassium ferrocyanide.

The absorbance was measured at 120 nm within 10 minutes using a spectrophotometer (CECILL-2000).

The percentage of tannin was calculated using the formula:

% Tannin= absorbance of sample x gradient factor x dilution factor / wt of sample x 10000
Dilution factor= 100.
Gradient factor= 17.84.

Flavonoids

For each cowpea, 1 mL of the cowpea's seed coat extract was introduced into 50 mL volumetric flask, 4 drops of concentrated HCl (0.01 M) was added with a dropping pipette after which 0.5 g of magnesium turnings was added to develop a magenta red colouration. The absorbance was measured on a spectrophotometer (CECILL-2000) at a wavelength of 520 nm. The percentage flavonoid was calculated using the following expression.

% Favonoid= absorbance of sample x average gradient factor x dilution factor / wt of sample x 10000
Dilution factor= 100.
Gradient factor= 15.19.

Statistical analysis

Completely randomized design was used for the quantitative analysis of phenolic compounds in cowpea. The difference between means was determined by Duncan multiple range test and significance was defined at significance level of P<0.05.

The analysis of meat conservation followed a 6*2*7 factorial design.

The 6, 2, and 7 represents the antioxidant treatments (control, Ife brown, ITA 256, IT-90K, TVS 716 and BHA), state of meat (cooked or raw) and storage days (0, 2, 4, 6, 8, 10, 12) respectively. The data obtained were analyzed using analysis of the variance model suitable for factorial design with the aid of a Genstat 5 program package (Payne, Lane and Genstat committee, 1987). Each treatment was replicated four times. The ANOVA model is as follow:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha_i \times \beta_j) + (\alpha_i \times \gamma_k) + (\beta_j \times \gamma_k) + (\alpha_i \times \beta_j \times \gamma_k) + e_{ijkl}$$

Where Y_{ijkl} denotes the i^{th} observation arising from level i of antioxidant treatment, level j of state of meat and level k of storage days.

μ = overall mean.

α_i = effect of level i of antioxidant treatment.

β_j = effect of level j of state of meat.

γ_k = effect of level k of storage days.

$(\alpha_i \times \beta_j)$ = interaction effect of antioxidant and state of meat.

$(\alpha_i \times \gamma_k)$ = interaction effect of antioxidant and storage days.

$(\beta_j \times \gamma_k)$ = interaction effect of state of meat and storage days.

$(\alpha_i \times \beta_j \times \gamma_k)$ = interaction effect of antioxidant, state of meat and storage days.

e_{ijkl} = random error term.

l = replicate.

RESULTS AND DISCUSSION

Every cowpea varieties had tannin and phenol (Table 1). Only TVS-716 lacks flavonoids. Various researches have shown that cowpea contains phenolic compounds in the three main groups namely, flavonoids (quercetin, myricetin and kaempferol) (Lattanzio, *et al.* 1997), phenolic acids (coumaric, ferulic, caffeic, hydroxybenzoic, syringic, sinapic and procatechuic acids) (Cai *et al.* 2003; Sosulski and Dabroski, 1984) and tannins (Morrison *et al.* 1995; Lattanzio *et al.* 1997; Egonlety and Aworh, 2003). These phenolic compounds are mainly concentrated in the seed coat (Preet and Punia, 2000). Cai *et al.* (2003) analyzed 17 varieties of cowpea and observed that protocatechuic acid was the major bound phenolic acid. Analyses of tannin in cowpeas have been done using specific methods for condensed tannin such as the main vanillin-HCL method (Chang *et al.* 1994 and Morrison *et al.* 1995). The seed coats extract of Ife brown (light brown in colour) contained greater concentration of total phenol and tannin than IT-90K (deep brown in colour) (Table 2). This observation differed from the generally observed trend in literature where darker-coloured legumes grains tend to contain higher concentration of phenolic compounds than lighter-coloured grains. Chang *et al.* (1994) reported higher concentration of phenolic compounds in coloured cowpea varieties than the white varieties. Dark coloured seed of lima beans, pigeon peas, African yam bean and jack bean were also found to contain significantly higher tannin content than the lighter coloured seed coat (Oboh *et al.* 1998).

The higher amount of total phenols in Ife brown than in IT-90K and in ITA-256 than TVS-716 could result from the presence of some phenolic compounds that may not necessarily contribute to seed coat colour in Ife brown and ITA-256. The levels of phenols found in the seed coat extracts of Ife brown and IT-90K were higher than reported levels for total phenols in whole cowpeas, which are of the order of 0.1-0.2% (Giami and Okwechime, 1993) and 0.03-0.1% (Cai *et al.* 2003).

The high phenol content in the seed coats was because phenolic compounds in legume are known to be concentrated in the seed coat (Preet and Prunia, 2000). Phenolic compound in seed coats play a major role in the physical and chemical defense system of the seeds when exposed to environmental factors such as oxidative damage and microbial infections thus contributing to antioxidant and antimicrobial activity (Troszynska *et al.* 2003).

Among the cowpea varieties, Ife brown exhibited the highest antioxidant capacity (Table 2). Its TBARS value (0.28) was close to that of BHA (0.29). Seed coat extracts of IT-90K and ITA-256 were equally potent while that of TVS-716 was the least in terms of antioxidant potency.

The relative antioxidant capacity of crude extracts may be related to the quality and quantity of phenolic compounds present. The antioxidant effect of the seed coat extracts may be attributed to the presence of phenolic compounds, which donate hydrogen atoms from their hydroxyl groups thus reducing the formation of hydroperoxides, the cause of lipid oxidation in meat.

Even though correlations were not done in this study, the results seemed to agree with reports in literature that indicated that levels of phenolic compounds can be positively correlated with antioxidant activity. In other words, the higher the levels of total phenols tannin and flavonoids, the higher the antioxidant activity (Veliogu, 1998 and Awika *et al.* 2003). More importantly however, structural-activity relationships are the main factors that determine the antioxidant activity of phenolic compounds measured by free radical scavenging capacity (Rice-Evans *et al.* 1996; Sroka and Cisowski, 2003). The ability of any particular phenolic compound to scavenge free radicals depends on its structure. Therefore, the types of phenolic compounds present in an extract would determine its resultant antioxidant activity (Rice-Evans, 1996; Larrauri, 1998; Sroka and Cisowski, 2003). Thus, the low performance of TVS-716 may be due to the absence of flavonoids in the crude extracts. Interaction effects of antioxidant treatments and storage days on the oxidative stability of broiler meat samples were observed (Table 4).

Table 1 Qualitative analysis of phenolic compounds in cowpea varieties

Cowpea varieties	Phenolic compounds		
	Tannin	Flavonoid	Phenol
Ife brown	+	+	+
IT-90K	+	+	+
TVS-716	+	-	+
ITA-256	+	+	+

+: means presence of constituent; -: means absence of constituent.

Table 2 Percentage composition of phenolic compounds in cowpea varieties

Cowpea varieties	Phenolic compounds		
	Flavonoids	Phenol	Tannin
Ife brown	0.264 ^{ns}	1.379 ^c	1.526 ^c
IT-90K	0.235 ^{ns}	0.512 ^b	0.998 ^b
TVS-716	-	0.196 ^a	0.298 ^a
ITA-256	0.279 ^{ns}	0.248 ^b	0.514 ^{ab}
SEM	0.067		

The means within the same column with at least one common letter, do not have significant difference ($P > 0.05$).

NS: non significant; SEM: standard error of the means.

As the storage days increases, lipid oxidation increases. This was brought about by increase in the formation of pro-oxidant compounds like peroxides that eventually led to increment in lipid oxidation. At storage days 0, 2, 4 and 6, no difference was detected ($P>0.05$) among the treatments. At storage days 8 and 10, BHA had the lowest TBARS value (0.264 and 0.303) respectively. The result obtained reflected the observed levels of total phenols obtained in Table 2. Phenolic compounds scavenge free radicals through hydrogen or electron donation from phenolic hydroxyl groups in the aromatic ring, which influence the ability of the phenolic compounds to act as radical scaveng-

ers. Thus, the higher levels of hydroxyl groups, the greater the antioxidant activity. At storage day 12, Ife brown and ITA-256 had lower TBARS values of 0.520 and 0.570 respectively which was very close to that of BHA (0.569). The cooked meat samples had higher TBARS value than the raw samples. The reason might 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). Another reason for the high TBARS value observed in the cooked samples might be as a result of the denaturation of the antioxidant compounds due to increase in tempe-

Table 3 Main effect of antioxidant treatments on oxidative stability of broiler meat

Antioxidant treatment	Control (0%)	Ife brown	IT-90K	TVS-716	ITA-256	BHA
TBARS (mg/MDA/kg)	0.53 ^c	0.28 ^a	0.34 ^b	0.40 ^{bc}	0.37 ^b	0.29 ^a
SEM	0.096					

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$). SEM: standard error of the means.

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

Antioxidant treatment	TBARS (mg/MDA/kg)						
	Storage days of sample						
	0	2	4	6	8	10	12
Control (0%)	0.119 ^a	0.272 ^a	0.287 ^a	0.362 ^{ab}	0.429 ^{ab}	0.444 ^{ab}	0.850 ^{yz}
Ife brown	0.190 ^a	0.350 ^{ab}	0.297 ^a	0.317 ^a	0.382 ^{ab}	0.322 ^{ab}	0.520 ^x
IT-90K	0.182 ^a	0.427 ^{ab}	0.289 ^a	0.360 ^{ab}	0.302 ^a	0.304 ^a	0.809 ^y
TVX-716	0.230 ^a	0.409 ^{ab}	0.317 ^a	0.305 ^a	0.306 ^a	0.481 ^z	0.809 ^y
ITA-256	0.225 ^a	0.395 ^a	0.362 ^a	0.397 ^a	0.419 ^a	0.335 ^{xy}	0.570 ^a
BHA	0.153 ^a	0.219 ^a	0.291 ^a	0.433 ^a	0.264 ^a	0.303 ^a	0.569 ^a
SEM	0.087						

a, b, c: the means within the same row with at least one common letter, do not have significant difference ($P>0.05$). x, y, z: the means within the same column with at least one common letter, do not have significant difference ($P>0.05$). SEM: standard error of the means; BHA: butylated hydroxyl anisole.

Table 5 Interaction effect of antioxidant treatments (TBARS, mg/MDA/Kg), storage days and state of meat on oxidative stability of broiler meat

Antioxidant	State	Storage days						
		0	2	4	6	8	10	12
Control (0%)	Raw	0.18 ^a	0.20 ^a	0.38 ^b	0.43 ^b	0.49 ^b	0.62 ^{bc}	1.33 ^c
Ife brown		0.18 ^a	0.29 ^b	0.22 ^a	0.26 ^a	0.30 ^b	0.27 ^b	0.38 ^b
IT90K		0.18 ^a	0.20 ^a	0.22 ^a	0.25 ^a	0.26 ^a	0.25 ^a	0.38 ^b
TVS-716		0.23 ^a	0.23 ^a	0.28 ^a	0.30 ^a	0.31 ^{ab}	0.31 ^{ab}	0.53 ^c
ITA-256		0.25 ^a	0.32 ^b	0.28 ^a	0.24 ^a	0.31 ^{ab}	0.48 ^c	0.38 ^c
BHA		0.22 ^a	0.23 ^a	0.35 ^{ab}	0.58 ^c	0.27 ^a	0.29 ^a	0.42 ^b
SEM		0.085						
Control (0%)	Cooked	0.15 ^a	0.24 ^{ab}	0.49 ^a	0.31 ^{ab}	0.37 ^{xy}	0.27 ^{ab}	0.38 ^b
Ife brown		0.20 ^a	0.41 ^b	0.37 ^{ab}	0.37 ^{ab}	0.47 ^b	0.38 ^{ab}	0.67 ^c
IT-90K		0.18 ^a	0.65 ^c	0.36 ^{ab}	0.47 ^b	0.34 ^{xy}	0.35 ^{ab}	0.76 ^c
TVS-716		0.22 ^a	0.56 ^{bc}	0.44 ^{ab}	0.50 ^{ab}	0.53 ^z	0.36 ^b	0.76 ^c
ITA-256		0.21 ^a	0.50 ^{yz}	0.36 ^{ab}	0.37 ^{xy}	0.41 ^b	0.48 ^b	1.09 ^c
BHA		0.09 ^a	0.21 ^b	0.24 ^b	0.29 ^{bc}	0.26 ^b	0.32 ^{xy}	0.35 ^c
SEM		0.085						

a, b, c: the means within the same row with at least one common letter, do not have significant difference ($P>0.05$). x, y, z: the means within the same column with at least one common letter, do not have significant difference ($P>0.05$). SEM: standard error of the means; BHA: butylated hydroxyl anisole.

perature associated with cooking. It was reported that increase in cooking temperature of meat, results in decrease in the moisture content and increase in the fat content of the cooked meat thus increasing the rate of oxidative rancidity (Smith *et al.* 1982). Increase in lipid oxidation in the cooked samples may also be due to increase in ionic iron concentration from heat induced release of protein bound iron after cooking and the formation of hypervalent ferryl-myoglobin (or activated metmyoglobin) during cooking (Olorunsanya *et al.* 2010). Other studies suggested that cooking disrupt the lipid membrane system (leading to loss of structural integrity) which caused the reaction of oxygen and molecular weight metal with unsaturated fatty acids resulting in the generation of oxidative reactions (Asghar *et al.* 1988).

This result however contradicted the report given by Olorunsanya *et al.* (2009) who asserted that cooked pork patties have lower TBARS value than raw pork patties due to the formation of Maillard reaction products (MRPs) during cooking. Maillard reaction products have been shown to have antioxidative activity (Salih *et al.* 1989). It was further reported that the mechanism for antioxidant activity of MRPs is that they reduce hydroperoxides to products that are unable to form free radicals and also deactivate free radicals that were formed during oxidative degradation of unsaturated fatty acids (Salih *et al.* 1989 and Olorunsanya *et al.* 2009). In all the storage days, all additives except BHA were observed to prevent lipid oxidation more ($P < 0.05$) in the raw meat samples than the cooked meat samples. In raw samples, at storage days 4 and 6, all cowpea varieties performed equally ($P > 0.05$) and better than ($P < 0.05$) BHA. At storage day 12, in the raw meat samples, Ife brown, IT90K and ITA 256 reduce lipid oxidation more than BHA ($P < 0.05$). In cooked meat samples, BHA performed better than other treatments. The ineffectiveness of the cowpea seed coat extracts in cooked meat samples may be because phenolic compounds in the seed coat extracts are in crude form occurring together with other compounds, which may be pro-oxidant in nature.

CONCLUSION

All additives and BHA reduced lipid oxidation in broiler meat. This was shown by lower TBARS values in meat samples with additives compared to the control samples (meat without additive). The presence and amount of phenolic compounds present in cowpea seed coat is independent of its' colour. Cowpea seed coats extracts of Ife brown had the highest amount of phenolic compounds followed by IT90K and ITA256 whose phenolic composition are not significantly different. Seed coat extracts of Ife brown, ITA-256 and IT-90k reduce lipid oxidation in raw broiler

meat samples than BHA but are less potent than BHA in cooked meat samples.

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