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ORIGINAL ARTICLE

Comparative antioxidant effect of BHT and water extracts of banana and sapodilla peels in raw poultry meat

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Abstract Antioxidant properties of banana (*Musa paradisiaca*) and Sapodilla/Chikoo (*Manilkara zapota*) peel extracts in chicken patties were evaluated. Four treatments viz., I. Control (meat+2% salt), II.BHT (meat+2% salt+0.1% BHT), III. BPE (meat+2% salt+2% banana peel extract) and IV. SPE (meat+2% salt+2% sapodilla/chikoo peel extract) were compared for changes in colour and lipid oxidation during 8 days refrigerated storage (4±°C). The average phenolic content was 550.2 and 550.8 mg gallic acid equivalent per 10 g peel in BPE and SPE respectively. Free radical scavenging activity was 66.9 and 67.8% in BPE and SPE respectively. Banana peel extract had significantly ($P<0.05$) higher reducing activity (1.6) as compared to sapodilla peel extract (0.91). During refrigerated storage period, all color parameters decreased significantly in all treatments. Observation on lipid oxidation showed a significantly ($P<0.05$) higher TBARS values in control than other three treatments. The increase in TBARS from initial day of storage to last day of storage was highest in control (514.3%) as compared to BHT (387.7%), BPE (370.6%) and SPE (383.7%). Both synthetic antioxidants and natural extracts significantly decreased the TBARS. The average decrease in TBARS values during 8 days of storage was 56.8%, 38.3% and 37.2% values in BHT, BPE and SPE treatments respectively. Therefore, it was concluded that water extracts obtained from banana and sapodilla peels could be explored as natural antioxidants in poultry meat and meat products.

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Introduction

Ground meat products provide convenience and variety to the consumers. Grinding and addition of salt are common processes used in the preparation of ground meat products. Grinding exposes lipid membranes to metal oxidation catalysts and salt has been demonstrated to accelerate lipid oxidation in chicken meat (O'Neill et al. 1999; Rhee and Ziprin 2001). Lipid oxidation is one of the major factors that determine quality and shelf-life of ground meat products. Synthetic antioxidants like BHT, BHA have been successfully used to prevent the oxidation problems in meat products. However, natural sources of antioxidants are considered to be safer than synthetic antioxidants. Hence there is a growing interest in natural antioxidants in meat products. Many research reports demonstrated antioxidant effects of grape seed extracts in poultry meat (Brannan 2008), tea catechins in chicken patties (Mitsumoto et al. 2005), rosemary and sage in chicken nuggets (O'Sullivan et al. 2004) and extracts of pomegranate and kinnow peels in chicken meat products (Devatkal et al. 2011).

Banana (*Musa paradisiaca*) is one of the most popular tropical fruits of the world. Banana peel constitutes up to 30% of the ripe fruit and it protects the inner fruit from sunlight and high temperature. It was reported that banana peel contains various antioxidant compounds such as gallocatechin and dopamine (Kanazawa and Sakakibara 2000; Someya et al. 2003; Mokbel and Hashinaga 2005). Since the banana fruits are widely available and peel could be a potential source of natural antioxidants.

Manilkara zapota is commonly known as sapodilla/ chikoo and grown in huge quantities in India, Pakistan and Mexico. The fruit and its peel contain high amounts of saponin, which has astringent properties similar to tannin. Jamuna et al. (2010) reported a significant higher reducing power and DPPH activity in aqueous extract of sapodilla. Isabelle et al. (2010) reported that sapodilla had the highest oxygen radical absorbance capacity and total phenolic content amongst many fruits tested.

Hence, an investigation was carried out to evaluate the antioxidant activities of water extracts from banana and sapodilla peels in ground chicken patties stored aerobically for 8 days at 4±1 °C.

Materials and methods

Meat and fruit by-products Fresh chicken meat (7–8 weeks vencobb broilers) was obtained from a local retail meat processing plant. Meat samples were chilled at 4 °C for 24 h before use. Fresh ripe banana (*Musa paradisiaca*) and sapodilla *(Manilkara zapota*) fruits were also obtained from retail fruit market. Standard gallic acid (SD Fine Chemicals, Mumbai, India), Thiobarbituric acid (MP Biomedicals Pvt. Ltd. Mumbai, India), and 1, 1, 3, 3- tetraethoxypropane, DPPH, phosphate buffer (Sigma Aldrich, New Delhi, India) were used in this study.

Preparation of extracts banana and sapodilla peel Bananas and sapodilla were washed, peeled off manually. The peels were cut into small pieces using a sharp knife. About 10 g of peel was mixed with 25 ml boiled distilled water and left for 1 h. The extract obtained by filtration was used for biochemical analysis and in further experiments.

Preparation of meat patties About 3 kg of chicken meat was minced twice (10 mm plate followed by 8 mm plates using a meat mince (Sirman, Italy). After mincing, the meat samples (500 g each) were assigned to one of the following four treatments : I) Control (meat without salt and natural antioxidant); II). BHT (meat with 2% w/w salt+0.1% BHT); III) BPE (meat with 2% w/w salt and 5% w/v banana peel extract); IV) SPE (meat with 2% w/w salt and 5% w/v sapodilla peel extract). Immediately after adding all ingredients, meat samples were thoroughly mixed and made into patties manually (75 g each). These patties were packed individually in low density polyethylene bags and stored at 4 ±1 °C for 8 days.

Estimation of total phenolics For total phenolics estimation, suitable aliquots of extracts were taken in a test tube and the volume was made to 0.5 ml with distilled water followed by the addition of 0.25 ml F–C (1 N) reagent and 1.25 ml sodium carbonate solution (20%). The tubes were vortexed and the absorbance recorded at 725 nm after 40 min. The amount of total phenolics was calculated as gallic acid equivalent from the calibration curve using standard gallic acid solution (0.1 mg/ml) (Escarpa and Gonzalez 2001)

Estimation of free radical scavenging activity The ability to scavenge 1, 1-diphenyl 1-2-picrylhydrazyl (DPPH) radical by banana and sapodilla extracts was estimated by the method Negi and Jayaprakasha (2003). Extract of banana and sapodilla peels (100 μg) diluted with 0.1 M Tris–HCl buffer (pH 7.4) was mixed with 1 ml of DPPH (250 μM) with vigorous shaking. The reaction mixture was stored in the dark at room temperature for 20 min and then absorbance was measured at 517 nm using a UV–VIS spectrophotometer (Model: Spectroscan 80 DV Biotech Eng. Management Company Ltd. U.K.). The scavenging activity was calculated by the following equation:

$$\text{Scavenging activity \%} = \frac{(\text{Absorbance Blank} - \text{Absorbance of Sample})}{\text{Absorbance Blank}} \times 100$$

Estimation of ferric reducing power The reducing power of the extracts was determined by the methods of Negi and Jayaprakasha (2003). A known concentration of extracts were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferric cyanide in a 10 ml test tube The mixtures were incubated for 20 min at 50 °C. At the end of the incubation 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged at 5000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 9.5 ml ferric chloride

Table 1 Antioxidant properties of aqueous extracts of banana and sapodilla peels(Mean±S.E)

Properties	Banana peel	Sapodilla peel
Total pehonolics (mg GAE in 10 g peel)	555±1.2	550±0.85
DPPH (free radical scavenging activity%)	66.9±4.8	67.8±4.0
Ferric reducing activity	1.6 ± 0.10^{b}	0.91 ± 0.07^{a}

GAE Gallic acid equivalent; *DPPH* 2, 2-diphenyl-1-picrylhydrazyl. Means within a row with different superscript differ significantly ($p<0.05$); $n=20$

Table 2 Overall treatment means of TBARS and colour values of different treatments during storage period (Mean±S.E)

Treatments	TBARS values (mg malonaldehyde/kg meat)	Lightness (L) value	Yellowness (b) value	Redness (a) value	Hue	Chroma
Control	0.53±0.02 [b]	52.8±0.30	18.4±0.23	9.6±0.21	62.4±0.66 [a]	18.4±0.25
BHT	0.29±0.01[a]	53.8±0.21	18.7±0.24	8.9±0.20	64.7±0.59[bc]	18.7±0.24
Banana	0.33±0.02 [a]	52.6±0.31	18.5±0.25	9.4±0.20	63.2±0.60[ab]	18.5±0.25
Sapodilla	0.34±0.01 [a]	53.1±0.30	18.9±0.24	8.7±0.20	65.2±0.59[c]	18.9±0.25

TBARS Thiobarbituric acid reacting substances. Means within a column with different superscript differ significantly ($p<0.05$); $n=6$

(0.1%) and absorbance was measured at 700 nm. Increase in absorbance indicated the reducing power of the extracts.

Instrumental colour evaluation The colour changes during storage was monitored by evaluating Hunter 'L', 'a', 'b', hue and chroma values at an interval of 2 days. Colourimetric analysis on surface of chicken patties was performed using a Hunter Lab Miniscan XE Plus colorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) with 25 mm aperture set for illumination D65, 10° standard observer angle. Hunter L (lightness), a (redness) and b (yellowness) values were measured on the outer surface of chicken patties from four randomly chosen spots. Hue angle (Tan -b/a) and chroma values (a2+b2)1/2 were calculated according to Hunter and Harold (1987).

Thiobarbituric acid reacting substances (TBARS) value Lipid oxidization was monitored by measuring thiobarbituric acid reactive substances at an interval of 2 days during storage. TBARS were determined using extraction method described by Witte et al. (1970). TBARS were extracted in chilled 20% trichloroacetic acid. Thiobarbituric acid extracts of each sample were used for measuring the absorbance at 532 nm. 1, 1, 3, 3, tetraethoxypropane was used as standard for TBARS assay. TBARS numbers were calculated as mg of malonaldehyde per kg of meat sample. The average increase in TBARS from 0 day to 8th day was also calculated and expressed in percentage. The percent reduction in TBARS by different treatments in comparison to control was also calculated arithmetically.

Statistical analysis Twenty samples were evaluated for total phenolics, DPPH activity and reducing power. Data presented is the average of twenty samples. The experiment on meat was replicated thrice and all parameters were measured in duplicate ($n=6$). All data were analyzed using statistical software, Agristat (Indian Agriculture Statistical Research institute, New Delhi, India). Storage data of Hunter colour and TBARS values were analyzed using two-way ANOVA with treatment and storage time as main effects. Statistical significance was identified at the 95% confidence level ($P<0.05$).

Results and discussion

Table 1 shows the antioxidant properties of banana and sapodilla peel extracts. Both extract showed higher phenolics and radical scavenging activity. However, no significant difference was observed between banana and sapodilla extracts for phenolic content and DPPH scavenging activity. But reducing power was significantly ($p<0.05$) higher in banana than sapodilla. Mokbel and Hashinaga (2005) evaluated the antioxidant effects of crude extracts of banana peels. They attributed higher total phenolics, DPPH activity and increased antioxidant activity to polyphenols, flavones and flavonoids. They reported 18 to 80% DPPH activity in methanol extracts of yellow banana peel. Kanazawa and Sakakibara (2000) examined the banana peel and identified dopamine, one of the catecholamine as a strong water-soluble antioxidant from peel. They found that diffusates of peel from banana showed maximum antioxidant and DPPH scavenging activity in a model linoleic acid emulsion and same was attributed to the catecholamine. They further observed that dopamine had greater antioxidative potency than glutathione, and food additives such as butylated hydroxyanisole and butylated hydroxytoluene, flavone, luteolin, flavonol,

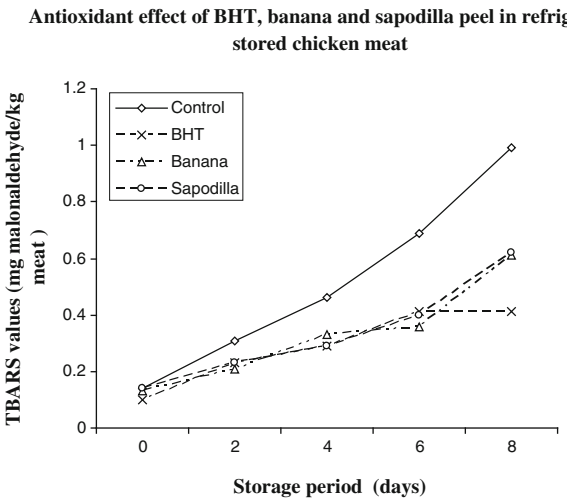

Fig. 1 Antioxidant effect of BHT, banana and sapodilla peel in refrigerated stored chicken meat

quercetin, and catechin. Similarly, Someya et al. (2003) reported the antioxidant compounds from commercial bananas, *Musa Cavendish*. They observed that gallocatechin was more abundant in peel (158 mg/100 g dry wt.) than in pulp (29.6 mg/100 g dry wt) and the antioxidant activity of the banana peel extract against lipid autoxidation was stronger than that of the banana pulp extract. Thus, the antioxidant capacity of the bananas may be attributed to their gallocatechin and dopamine.

Other studies have also suggested that phenolic compounds were the major contributors of antioxidant activity of sapodilla. The polyphenols catechin, epicatechin, leucocyanidin, leucodelphinidin, leucopelargonidin, chlorogenic acid, and gallic acid have been reported previously from unripe sapodilla fruit (Jun et al. 2003; Shui, et al. 2004; Isabelle et al. 2010). A value of 15.94 mg GAE/g in sapodilla had been reported by Isabelle et al. (2010) and they further observed a low hydrophilic oxygen radical absorbance capacity (H-ORAC) to total phenolics ratio. Jun et al. (2003) identified activity-guided fractionation of a methanol extract from the fruit of *Manilkara zapota* and isolated two new antioxidants, methyl 4-*O*-galloylchlorogenate and 4-*O*-galloylchlorogenicacid along with eight known polyphenolic antioxidants, namely, methyl chlorogenate, dihydromyricetin quercitrin, myricitrin, catechin, epicatechin and gallocatechin and gallic acid. Chanda and Nagani (2010) studied the antioxidant capacity of the *Manilkara zapota* L. leaves extracts, and observed that the acetone extract showed best DPPH radical scavenging activity. The high antioxidant capacity observed for acetone extract suggested that this plant could be used as an additive in the food industry providing good protection against oxidative damage.

Instrumental colour Mean hunter colour values, hue and chroma values are presented in Table 1. Overall treatment mean values of all color parameters were not significantly different. However, storage significantly ($P<0.05$) decreased L, a, b and chroma values in all treatments. A significant decease in 'L' value due to addition of salt to comminuted chicken patties had been reported by Swatland and Barbut (1999). In a study, Naveena et al. (2008) reported a reduction in 'L' value and an increase in 'a' values due to addition of pomegranate peel extract in chicken patties. Decrease in 'a' value indicates the change in colour from red to brown which could be due to the formation of metmyoglobin. It has been further reported that salt greatly accelerate the process of meat discolouration due to pro-oxidative activity which can be attributed to its ability to release iron from heme pigments and other heme binding molecules (Rhee and Ziprin 2001)

TBARS values Mean TBARS values (Table 2) were significantly ($P<0.5$) higher in control than treated samples. Treatments with BHT and natural extracts showed no significant difference for TBARS values. During all storage intervals TBARS was significantly higher in control and lower in treated samples (Fig. 1). After 8 days of storage TBARS significantly increased in all samples but the levels of increase were significantly lower treatments containing BHT, banana and sapodilla peel extracts as compared to control treatment. Thus treatments with fruit by-products extract and BHT showed a significant ($P<0.05$) lower TBARS values than control samples. Average percent increase in TBARS was maximum in control. BHT and extract treated samples showed a significant ($P<0.05$) lower percent increase in TBARS as compared to control. Among three treated sample there was no significant difference in percent increase in TBARS during storage period. Similar observations on% reduction of TBARS revealed that BHT, banana extract and sapodilla extract reduced TBARS by 56.8%, 38.3% and 37.2% respectively in comparison to control sample. Thus these extracts effectively delayed the formation of TBARS during refrigerated storage of chicken patties. These results clearly demonstrated that addition of banana and sapodilla extract significantly reduced the oxidation in control sample and the antioxidant effect was comparable to that of BHT. Banana contains high level of dopamine (500 mg/100 g) and a banana water extract successfully inhibited autoxidation of lineoleic acid by 70% after 5 days incubation in an emulsion system. (Kanazawa and Sakaibara Kanazawa and Sakakibara 2000). There are no studies on the effect of banana and sapodilla peel extract in oxidation in meat products. However earlier studies have indicated significant relation between phenolics content and antioxidant effect of natural extract (Negi and Jayaprakasha 2003; Devatkal et al. 2011).

Conclusion

Banana and sapodilla peel extracts significantly reduced the lipid oxidation in chicken meat during refrigerated storage and antioxidant effect was comparable to that of BHT .Therefore extracts of these fruits by-products could be successfully added to meat to function as antioxidant.

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