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A comparative study of bioactive compounds from apple peels and aloe vera gel and their effects on colour and oxidative stability of turkey meat

A K BISWAS[1], C K BEURA[2] and A K SACHDEV[3]

Central Avian Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

The aim of this study was to assess antioxidant activity of bioactive compounds of apple peel and aloe vera gel extracts and their effects on colour and oxidative stability of turkey meat sample stored at 4±1 °C. A total of 6 solvent extracts were prepared and the best extracts selected from each of apple peel and aloe vera gel were used at 0.5 % level in improvement of turkey meat quality. Results indicated that methanol extract of apple peel (MeAP) and ethanol extract of aloe vera gel (EhAG) had greatest ABTS$^+$, DPPH and superoxide anionic scavenging activities. The EhAG had the highest total phenolic contents and that was lowest for MeAG sample. Lovibond tintometer colour values indicated that addition of MeAP and EhAG in turkey meat sample significantly influenced redness (α-value) and yellowness (β-value) values during storage. The pH, TBARS, FFAs and PVs were higher in control sample than treatment groups. EhAG and MeAP showed greater antioxidant potential which can be used as functional ingredients in improvement of raw turkey meat quality with added health beneficial effects.

Key words: Antioxidants, Apple peels, Lipid oxidation, Meat, Turkey

Lipid oxidation is one of the major factors affecting the quality of muscle foods. Lipid oxidation is influenced by the composition of phospholipids, polyunsaturated fatty acids (PUFA), metal ions, oxygen, haeme pigments, addition of salt and many processing techniques or methods. Lipid oxidation is initiated when PUFA react with molecular oxygen via free radical chain mechanism form peroxides. Myoglobin oxidation caused discolouration, while oxidative process gave rise to production of malodorous compounds which are significantly affects human health. However, fatty acid compositions of phospholipid fractions of the muscle cells are especially important in determining the stability of meat, because of oxidative process initiates from the membrane components of muscle cells (Ahn *et al.* 1993). Turkey meat resembles like red meat but contains more white fibres and higher protein content. Turkey meat also contains high content of phospholipids that are oxidized very rapidly. Many research works have been carried out on lipid oxidation study in other muscle foods but limited literatures were found on turkey meat oxidative process though it has different lipid compositions either from red meat or meats from other species of poultry. Further, recent health claim reports on synthetic chemicals, it is necessary to search effective antioxidants in particularly from natural sources which are safe, healthy and also meet regulatory requirements.

Present address: [1]Senior Scientist (biswaslpt@gmail.com), [2,3]Principal Scientist (ckbeura@rediffmail.com, sachdevpht @rediffmail.com), Division of Post-Harvest Technology.

Apples (*Malus domestica* Borkh.) are rich source of phenolic compounds which pose very strong antioxidant activity in *in-vitro* food systems (Tow *et al.* 2011). Apple peel contained nearly 3 to 6 folds more phenolic compounds than the flesh (Tsao *et al.* 2003). Consumptions of apples are associated with prevention of chronic diseases such as lung cancers, cardio-vascular diseases and thrombotic stroke. *Aloe vera* (*Aloe barbadensis* Mill.) is an herb extensively used in many medicinal and therapeutic remedies. It has very good antioxidant activity which is comparable to that of the synthetic antioxidant like BHT (Miranda *et al.* 2009). However, antioxidant activity of apple peels and aloe vera gel extracts may vary depending on extraction methods, purity, types and quantity of active compounds present according to cultivar, maturity, geographic origin, growing season, post-harvest storage practices etc.

As apple peels and aloe vera gel are rich in bioactive compounds and many food processors are incorporating them in development of functional meat products. So, the present study was carried out to optimize the suitable solvent for extraction of major bioactive compounds from apple peels and aloe vera gel, and the best solvent extracts identified were applied in colour and lipid stability of turkey meat sample.

MATERIALS AND METHODS

Fresh turkey (Beltsville white, 14 months of age) meat samples were obtained from the experimental poultry

processing plant of this Institute following scientific slaughtering techniques. The dressed carcases (6 to 7kg) were chilled at 4±1 °C for overnight, deboned manually, and then frozen at –18 °C for subsequent use. Apples and *aloe vera* were collected from local market and garden respectively. All chemicals and reagents used in the study were of analytical grade.

Preparation of apple peel and aloe vera *gel extracts:* Extracts namely ethanol extract of apple peels (EhAP), methanol extract of apple peels (MeAP), acetone extract of apple peels (AcnAP), ethanol extract of *aloe vera* gel (EhAG), methanol extract of aloe vera gel (MeAG) and acetone extract of *aloe vera* gel (AcnAG) were prepared for antioxidant activity (AOA) study. For preparation of EhAP, fresh apple peels (*Malus domestica* Borkh.) were cleaned, dried, and finally pulverized into fine power. About 1 g of powder was accurately weighed, mixed with 10 ml of ethanol, held for 10 min at 4±1 °C, vortexed at high speed for 2 min, and then centrifuged at 5,000 rpm for 10 min. Supernatant was collected into a glass test tube, and re-extracted the compounds as above. Both of the supernatants were pooled together, passed through Whatman filter paper No. 42 and then condensed to 5 ml in rotary vacuum evapourator. The extract was stored at - 20 °C for further studies. Other extracts from apple peels were also prepared in similar manner as mentioned for EhAP. In preparation of *aloe vera* gel (AVG) extracts, about 10 g of AVG first dried up using anhydrous sodium sulphate and then extraction methods were followed as mentioned for apple peels. All the extracts were analyzed for antioxidant activity. The efficacy of the extracts was quantified on fresh weight basis.

Preparation of turkey meat samples: About 4.0 kg of turkey meat was minced through a 4 mm grinding plate in a meat mincer. After mincing, meat samples were divided into 4 different batches of 1.0 kg each. The first and second batches designated as control (meat without any extract) and butylated hydroxytoluene (BHT) treated samples (contained 100 ppm of BHT; T_1) while the other treatments contained MeAP (T_2) and EhAG (T_3) as selected best from 2 individual groups of extracts following determination of $ABTS^+$, DPPH, SA scavenging activity (SASA) and total phenolic contents. Accurately, 5 ml of each selected extract of apple peels and *aloe vera* gel was added respectively to T_2 and T_3 samples while control and T_1 samples contained 5 ml distilled water to make up the volumes of extracts used for treatments. Sodium chloride (2.5 %; w/w) was used as a pro-oxidant in all samples including control. All batches of minced meat samples were mixed separately in Hobart paddle type mixture for 2 min. After complete mixing, each sample was subdivided into 5 groups and then aerobically packaged in low density polyethylene bags. The samples were stored at 4±1 °C and drawn at 3 days interval (0, 3rd, 6th, 9th and 12th day) for evaluation of changes in colour, pH, TBARS value, free fatty acid (FFA) and peroxide value (PV).

Analytical methods: The antioxidant activity of extracts were determined by evaluating ABTS free radical scavenging activity, DPPH activity, SASA and total phenolic contents as per methodology of Khare *et al.* (2013). The quality changes of fortified meat samples were determined by evaluation of pH, lipid stability, free fatty acids (FFAs), peroxide value (PV) and instrumental colour. The pH was measured with a digital (Century make) pH meter (Trout *et al.* 1992) on 10 g of sample homogenized with 50 mL distilled water. The lipid stability was performed by measuring 2- thiobarbituric acid reacting substances (TBARS) following the method of Witte *et al.* (1970). The method as described by Koniecko (1979) was followed for quantification of FFAs and PV. The colour profiles of meat samples were measured using Lovibond tintometer. The 'Hue' and 'Chroma' values were determined by using formula, $(\tan^{-1})^{b/a}$ (Little 1975) and $(a^2 + b^2)^{1/2}$ (Froehlich *et al.* 1983) respectively, where a, red unit; b, yellow unit. Data were interpreted by using 'SPSS-12.0' software package as per standard methods of Snedecor and Cochran (1994). Means of DPPH, SASA, $ABTS^+$ and total phenolic contents were analyzed using one-way ANOVA. Storage data were analyzed using two-way ANOVA with Duncan's Multiple Range Tests. Three replicate experiments were carried out and statistical significance was expressed at $P<0.05$.

RESULTS AND DISCUSSION

Bioactive compounds of fruits, plants and herbs can be extracted using many solvents. But the ideal solvents should have good chemical properties, environmental friendly, non-toxic to human and have the ability to increase extraction yield. The antioxidant activity (AOA) of ethanol (Eh), methanol (Me) and acetone (Acn) extracts of apple peel and *aloe vera* gel extracts are shown in Table 1. Amongst all the extracts, MeAP and EhAG were showed greatest AOA in $ABTS^+$, DPPH and SASA assay than other extracts. The overall AOA were found in following order- EhAG>MeAP>AcnAP>AcnAG>MeAG>EhAP. Amongst different extracts of apple peels, MeAP exhibited greater DPPH and SA scavenging activity than other two extracts. MeAP was also contained the highest amount of TPC (Table 1). However, amongst the different extracts of aloe vera gel, EhAG had greater share in TPC showed highest AOA in terms of $ABTS^+$, DPPH and SA scavenging activity. MeAP exhibited greatest AOA which could be inferred to methanol could effectively be extracted major polyphenols (flavonols) present in apple peels. Similar findings were reported by Makris *et al.* (2007). But ethanol was effectively extracted major bioactive compounds responsible for AOA of aloe vera gel. This indicated that though methanol was effectively extracted bioactive compounds from apple peels, ethanol was inefficient. In other words, the bioactive compounds in the different extracts of plant and fruits were highly variable, and quantity was solely dependent on type and polarity of solvents. Similar findings were also reported in earlier studies (Hu *et al.* 2003, Thilakarathna *et al.* 2013 and Xingbin *et al.* 2013). Xingbin *et al.* (2013) reported

Table 1. Antioxidant activity and total phenolic content of apple peels and aloe vera gel extracts

Extracts	ABTS (%)	DPPH (%)	SASA (%)	TPC (mg/g)
EhAP	13.66±0.18^{a}	18.52±1.15^{a}	10.73±1.14^{b}	1.86±0.06^{c}
MeAP	67.18±2.15^{d}	53.13±1.71^{c}	77.56±3.40^{e}	2.16±0.10^{d}
AcnAP	38.78±2.13^{c}	25.18±2.14^{ab}	46.38±2.25^{c}	1.65±0.05^{b}
EhAG	74.35±3.19^{e}	65.43±2.18^{d}	89.57±3.17^{f}	2.86±0.08^{e}
MeAG	26.17±2.20^{b}	23.15±1.86^{ab}	18.33±1.35^{b}	1.51±0.06^{a}
AcnAG	32.53±2.36^{bc}	29.47±2.13^{b}	51.61±2.16^{d}	1.88±0.04^{c}

ABTS, 2-2-azinobis-3ethylbenthiazoline-6-sulphonic acid radical cation activity; DPPH, 1, 1 diphenyl-2picrylhydrazyl; SASA, superoxide anion scavenging activity; TPC, total phenolic content EhAP, ethanol extract of apple peels; MeAP, Methanol extract of apple peels; AcnAP, Acetone extract of apple peels; EhAG, Ethanol extract of aloe vera gel; MeAG, Methanol extract of aloe vera gel and AcnAG, Acetone extract of aloe vera gel. $^{a-f}$ Mean±SE with different small letter superscripts on the same column are significantly different (P<0.05).

Table 2. Effect of MeAP and EhAG on physicochemical characteristics of turkey meat during refrigeration storage (4±1°C)

	Storage period (days)				
Treatments	0	3	6	9	12
pH					
Control	6.12±0.03^{c}	6.17±0.01^{bcA}	6.24±0.01^{bA}	6.38±0.01^{abA}	6.50±0.02^{aA}
T_1	6.16±0.04^{c}	6.21±0.01^{cB}	6.25±0.02^{bAB}	6.27±0.02^{abB}	6.37±0.01^{aB}
T_2	6.14±0.03^{c}	6.17±0.04^{cA}	6.19±0.01^{bA}	6.28±0.01^{abB}	6.34±0.04^{aAB}
T_3	6.04±0.02^{c}	6.11±0.02^{cB}	6.13±0.01^{bB}	6.17±0.02^{abB}	6.22±0.01^{aAB}
TBARS values (mg malonaldehyde/kg)					
Control	0.34±0.03^{eA}	0.47±0.01^{dA}	0.88±0.01^{cA}	1.36±0.02^{bD}	2.11±0.01^{aA}
T_1	0.22±0.01^{eD}	0.45±0.01^{dD}	0.70±0.02^{cC}	1.13±0.02^{bB}	1.76±0.01^{aB}
T_2	0.24±0.01^{dC}	0.44±0.01^{cC}	0.62±0.01^{bC}	0.94±0.02^{aA}	1.62±0.03^{aC}
T_3	0.28±0.02^{eB}	0.46±0.02^{dB}	0.58±0.02^{cB}	0.86±0.01^{bC}	1.59±0.02^{aA}
Free fatty acids (%)					
Control	0.06±0.003^{e}	0.08±0.005^{dA}	0.09±0.007^{cA}	0.13±0.002^{bA}	0.23±0.009^{aA}
T_1	0.04±0.001^{e}	0.05±0.005^{dC}	0.06±0.004^{cBC}	0.09±0.002^{bB}	0.17±0.001^{aB}
T_1	0.05±0.005^{d}	0.06±0.008^{cB}	0.07±0.005^{cB}	0.10±0.008^{bAB}	0.16±0.010^{aBC}
T_3	0.05±0.004^{d}	0.07±0.006^{cA}	0.08±0.008^{bAB}	0.08±0.006^{bC}	0.14±0.02^{aC}
Peroxide value (meq/kg)					
Control	0.76±0.03^{e}	1.13±0.01^{dA}	1.47±0.01^{cA}	1.77±0.01^{bA}	2.20±0.02^{aA}
T_1	0.80±0.04^{e}	0.97±0.01^{dB}	1.25±0.02^{cB}	1.43±0.02^{bB}	1.84±0.01^{aB}
T_2	0.78±0.03^{e}	0.83±0.04^{dD}	1.07±0.01^{cC}	1.27±0.01^{bC}	1.60±0.04^{aC}
T_3	0.81±0.02^{e}	0.87±0.02^{dC}	1.03±0.01^{cD}	1.17±0.02^{bD}	1.41±0.01^{aD}

N, 6; Control, added salt only; T_1, salt + BHT; T_2, salt + methanol extract of apple peels (MeAP); and T_3, salt + ethanol extract of *aloe vera* gel (EhAG). $^{a-e}$ Mean±SE with different small letter superscripts on the same row are significantly different (P<0.05). $^{A-D}$ Mean±SE with different capital letter superscripts for a particular trait are significantly different (P<0.05).

that hot water extract of apple peels exhibited higher antioxidant activity than ethanol. But contradictory results were also reported. Hu *et al.* (2003) reported that radical scavenging activity of EhAG was significantly higher than the BHT and α-tocoferol. So, MeAP and EhAG were selected best from both the fruit and herb, and these extracts were used to assess their effect on colour and oxidative stability of turkey meat samples.

The effect of different extracts in physico-chemical changes of turkey meat pH is presented in Table 2. During initial days of storage, the pH values did not vary significantly (P>0.05) amongst the control and different treatment groups. However, on the day 3, pH was significantly (P< 0.05) increased in control sample. In general, pH value of T_3 sample was slightly lower that other treatments and control. Lower pH in T_3 sample could be attributed to lower pH of aloe vera gel. But increase of pH with the advancement of storage time could be attributed to greater numbers of aerobic bacterial multiplication. Treated samples showed least variation of pH due to inhibition of microbes by the essential oils present in added extracts (Wolfe *et al.* 2003).

The TBARS values were increased (P<0.05) rapidly in control sample than treatment groups (Table 2). In T_2 sample, significant increase of TBARS value was observed on the day 6. This indicated that treatment with aloe vera gel extract (T_2) showed comparatively lower (P<0.05) TBARS value than control, T_1 and T_3 samples. The control sample showed significantly (P<0.05) higher TBARS values as compared to all other samples because salt exhibited a

strong pro-oxidant effect on turkey meat samples. Pro-oxidant effect also noticed in all the treated samples but activity was less pronounced. Many research findings were reported that the addition of salt to meat and meat products resulted in an increase in the TBARS values. Rhee and Zipin (2001) demonstrated a pro-oxidant effect of salt during storage of chicken products. Possible reasons for salt promoted lipid oxidation are reduction in the activity of antioxidant enzymes like catalase and glutathione peroxidases, stimulation of lipid oxidation via iron activation by chloride ions, displacement of iron molecule from myoglobin structure by sodium ions thereby providing free iron for the catalysis of lipid oxidation. The antioxidant properties of phenolic compounds were very well documented. A significant relationship between phenolic content and antioxidant effect of apple peels extract was reported by Xingbin *et al.* (2013). Similarly, Hu *et al.* (2009) observed strong antioxidant effect of aloe vera gel extract. They also reported that the antioxidant potential of this extract was comparable to that of the synthetic antioxidant, BHT. Thilakarathna *et al.* (2013) found a positive correlation between phenolic content of apple peels extracts and reduction of in-vitro human LDL cholesterol oxidation.

In respect to FFA contents, T_2 showed lower value as compared to T_1 while that was least for T_3. In general, FFA in all products increased with the increase of storage time. The differences in FFA contents amongst all types of samples could be due to level of oxidation and decomposition of fat by bacterial multiplication. Modi *et al.* (2007) reported gradual increase of FFA contents in chicken products with increase of storage time.

As expected PV was lowest at day 0 and there was no significant difference amongst the control and treated samples (Table 2). However, at the end of the storage, the control sample exhibited highest PV. Amongst the treatments, T_3 exhibited lowest PV which could be due to the presence of bioactive compounds may suppress the chemical reactions. However, increase of PVs with the storage time could be attributed to presence of residual oxygen in the packages (Kong *et al.* 2010).

The colour co-ordinates of α- (redness) and β- (yellowness) values indicated that they were increased and decreased respectively with the increase of storage days (Table 3). But amongst the all treatments, T_2 showed greater colour stability than others. The *Hue* angle was significantly ($P<0.05$) decreased in control sample as compared to treatment groups. In T_1 sample, *Hue* angle was first increased up to day 3 and then decreased significantly ($P<0.5$) on the day 6 which indicating a dark discolouration. Significant ($P<0.05$) difference was also observed in *Hue* angles for T_2 and T_3 treatments. Chroma values were also followed similar trends to that of *Hue* angle. The significant increase of α-value indicated changes in colour from red to brown which could be attributed to the formation of metmyoglobin mostly observed in control sample because of salt greatly accelerated the process of myoglobin oxidation as a result of release of iron molecules from haeme and non-haeme pigments. Similar event might also occur

Table 3. Effect of MeAP and EhAG on Lovibond tintometer colour values of turkey meat during refrigeration storage (4±1°C) Storage period (days)

Treatment	Storage period (days)				
	0	3	6	9	12
α-value (redness)					
Control	5.63±0.11^{aA}	5.91±0.20^{aA}	6.24±0.12^{bA}	7.77±0.13^{bA}	8.52±0.22^{bA}
T_1	5.44±0.25^{abB}	5.22±0.24^{aB}	5.59±0.27^{aB}	6.16±0.33^{bB}	6.84±0.26^{bAB}
T_2	5.52±0.21^{aC}	4.83±0.34^{aC}	5.44±0.23^{aB}	5.98±0.19^{aC}	6.01±0.25^{aB}
T_3	5.39±0.16^{aC}	4.97±0.18^{aC}	5.66±0.11^{aC}	6.10±0.13^{aC}	6.18±0.22^{aB}
β-value (yellowness)					
Control	6.18±0.36^{aA}	6.51±0.42^{bA}	6.18±0.47^{cA}	5.88±0.61^{cA}	4.76±0.55^{cA}
T_1	6.92±0.51^{aAB}	6.22±0.61^{bAB}	6.45±0.43^{bA}	5.91±0.54^{bA}	5.66±0.48^{cA}
T_2	7.06±0.41^{aB}	7.18±0.39^{aB}	6.88±0.52^{bAB}	5.84±0.38^{bB}	5.96±0.27^{bA}
T_3	7.20±0.33^{aC}	7.24±0.41^{aC}	7.04±0.31^{abB}	6.26±0.28^{bC}	6.11±0.12^{abB}
Hue					
Control	47.67±1.22^{aA}	48.25±0.92^{aA}	44.72±1.17^{bA}	37.12±0.86^{bA}	29.19±0.47^{cA}
T_1	51.83±0.71^{abB}	49.99±0.66^{aB}	49.09±1.13^{bAB}	43.81±1.02^{bcAB}	39.61±0.81^{cB}
T_2	51.98±0.64^{aC}	56.07±0.88^{abC}	51.67±1.18^{bcB}	44.32±0.44^{cC}	44.76±0.62^{cdB}
T_3	53.18±1.02^{aD}	55.53±0.77^{aC}	51.20±0.55^{aB}	45.74±0.68^{aC}	44.67±0.39^{aB}
Chroma					
Control	8.36±0.21^{aA}	8.79±0.17^{aA}	8.78±0.39^{bA}	9.74±0.32^{bA}	9.76±0.26^{bA}
T_1	8.80±0.33^{abB}	8.12±0.20^{aB}	8.56±0.28^{aB}	8.54±0.24^{bB}	8.88±0.28^{bAB}
T_2	8.95±0.19^{aC}	8.71±0.33^{aC}	8.77±0.28^{aB}	8.36±0.18^{aC}	8.46±0.19^{aB}
T_3	8.99±0.18^{aC}	8.78±0.22^{aC}	9.03±0.15^{aC}	8.74±0.12^{aC}	8.69±0.16^{aB}

N, 18; Control, added salt only; T_1, salt + BHT; T_2, salt + methanol extract of apple peels (MeAP); and T_3, salt + ethanol extract of aloe vera gel (EhAG). $^{a-c}$ Mean±SE with different small letter superscripts on the same row are significantly different ($P<0.05$). $^{A-D}$Mean±SE with different capital letter superscripts on the same column are significantly different ($P<0.05$).

in all the treated samples but it was less intense due to antioxidant activity of treatments. Rojas and Brewer (2008) were reported an increase and decrease of *a*- and *b*-values with the addition of natural antioxidants in meat patties.

The study showed the antioxidant effect of MeAP and EhAG. These extracts significantly reduced auto-oxidation and salt promoted lipid oxidation in turkey meat during refrigeration storage. Among the two different extracts, EhAG was more effective in reducing manoladehyde formation, FFAs and PVs. Therefore, it is concluded that EhAG and MeAP could successfully be used in lipid stability of turkey meat with added health benefits and increasing consumer appeal.

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