Utilization of citrus waste as a source of natural antioxidant for shelf stable broiler meat and meat products

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ABSTRACT
Meat is a perishable food commodity and is usually at the higher risk of deterioration. The main spoilage of the meat is due to the oxidative rancidity. The dietary antioxidants are considered as agents to prevent lipid peroxidation in animal muscles and consequently increase the meat stability after slaughtering. Accordingly, this study was conducted in order to elucidate the effect of antioxidant enriched diet containing citrus peel on broiler meat quality. Different concentrations of dried citrus peel (4%, 8% and 12%) were added to the feed in order to determine their effects on the oxidative stability of meat. The birds were provided with standard conditions of rearing for 42 days. After 42 days the broilers were slaughtered and meat was analyzed for total phenolic contents, peroxide value, β-carotene bleaching assay and TBARS analysis. Results of the study demonstrated that treatment containing higher concentration of citrus (T4=12%) showed highest potential to control the oxidative deterioration of chicken meat during freeze storage without imparting negative impacts on the sensory attributes of product.

Keywords: Meat quality, Antioxidants, Citrus peel, Oxidative stability

INTRODUCTION
Citrus species have been studied of several origins because of their phenolic compounds and are significant antioxidants source such as flavonoids, carotenoids and ascorbic acid. Flavanones are found to be great important amid the phenolic compound groups in citrus. The manufacturing of juice by citrus fruit processing peels are the prime by-products. They converted to waste and a conceivable reason of ecological pollution if unused. These by-products have been conventionally has been used as molasses for fuel production, fiber and for animal feed. The antioxidant capability of citrus is linked both to phenolic and vitamin C (Barros et al., 2012).

Citrus fruits are the leading fruit trees grown-up all over the world and are well honored for their energizing and pleasing juice and fitness paybacks. Many healing characteristics have been endorsed to citrus fruits like antiviral, anti-inflammatory, anti-tumurcic, anticancer and special effects on capillary in stability in addition to capability to prevent aggregation of platelets. In recent times, curative advantages in relation to heart diseases and muscular deterioration in relation to age have been reported. Citrus fruits have abundant health benefits that are related to the increased values of bioactive compounds and phytochemical such as phenols, flavonoids, carotenoids, vitamins and minerals obtainable in citrus fruits. These nutrients stimulate the immune systems and may act as antioxidants. In citrus species phytonutrients and vitamins may be the reason for the antioxidant property (Fernandez-Lopez et al., 2005).

Meat quality can be described as the overall characteristics of meat such as chemical, physical, microbiological, nutritional, sensory and cooking properties. Some properties are important to consumer including color, flavor, odor, texture, juiciness and tenderness, which influence their judgment prior or after purchasing the meat. Whereas the quality of meat be influenced by numerous factors for example shelf life, drip loss, water holding capacity, pH, protein solubility, cooking loss, fat binding and shear force (Allen et al., 1998). The worldwide used poultry grading system is established on visual assigns such as carcass fault, missing parts and bruises. Meat quality is usually assessed by measuring its pH, color and water holding capacity, since these are three main attributes for fresh and further processed products (Mitchell and Kettlewell, 2009).

Lipid peroxidation in meat and meat products decreases its quality. Reactive oxygen species causes lipid peroxidation, which leads to severe health problems. Therefore, it is a persistent need to search behaviors to hinder the incidence of lipid peroxidation arising in meat (Sohaib et al., 2012). Broiler meat and its goods are becoming more popular and are broadly spread worldwide. Lipids oxidation is accountable for decrease in nutritional
Citrus fruits and juices are significant source of bioactive compounds together with antioxidants for example ascorbic acid, phenolic compounds, pectin and flavonoids that are necessary to humanoid nutrition. BHA/BHT and α-tocopherol presence in broilers diet did not notably control the fatty acid composition of the membrane phospholipids. On the other hand, oxidized sunflower oil in broilers diet decreases long chains of unsaturated fatty acids in neutral lipid fraction (Asghar et al., 1989).

MATERIALS AND METHODS

Procurement of raw material and sample preparation

Citrus peel was collected from local shops of juice extraction and dried in dryer giving standard conditions. Additionally, one day old chicks were purchased from local hatchery. Chicks were provided with uniform diet for 1st two weeks to acclimatize the conditions. After that they were divided into various groups and provided with different feed combinations according to treatment plan for 42 days. After 42 days birds were slaughtered by following the Halal Ethical Guidelines, eviscerated and meat samples were prepared and stored at -40°C for further analysis.

Chemical analysis of citrus peel

Moisture, crude protein, fat and ash percentages were estimated from citrus peel following the methods described by (AOAC, 2003).

Antioxidant activity of citrus peel

1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

The free radical scavenging activity of citrus peel extract was measured by spectrophotometer at 517 nm (El-Ghorab et al., 2007).

Total phenolic content (TPC) determination

The total phenolic compounds were estimated by Folin-Ciocalteu method (FCM) (Sun and Ho, 2005).

β-carotene bleaching assay

Antioxidant activity was determined by β-carotene method using spectrophotometer at 470 nm (Hinnebjerg et al., 2006).

Experimental Birds Rearing

A day old, (250) broiler chicks “Cobb 550 strain” were weighed individually and randomly divided into five experimental units with three replicates (5 birds in each replicate). The birds in each experimental unit were kept in separate disinfected pens (4 ft × 3 ft × 1.5 ft) (Rodenburg et al., 2004) a glucose solution (50 grams per 5 Liter) was given to the broilers after 1-2 hours of allocation in to their respective pens for the removal of waste from their body. Then feed along with fresh and clean water was given ad-libitum. Birds were acclimatized on commercial feed for two weeks. From third week, feeds supplemented different levels of citrus pulp was fed to boilers. Twenty four hour light and proper ventilation was provided in the experimental room throughout the study period.

Chemical analysis of broiler meat

Moisture, crude protein, fat and ash percentages were estimated from minced meat following the methods described by (AOAC, 2003).

Antioxidant assay of raw broiler meat

DPPH assay

The free radical scavenging activity of broiler meat extracts was measured by spectrophotometer at 517 nm (El-Ghorab et al., 2007). A methanol solution of DPPH was prepared immediately before the assay. Samples were taken in different test tubes using duplicates and then 1mL of DPPH solution was added in each test tube containing samples. The reaction mixtures were shaken vigorously and allowed to stand for 30 min at room temperature in dark place. The absorbance of the samples was measured by a spectrophotometer at 517 nm. In this assay, BHT was used as a standard antioxidant to validate the assay.
Total phenolic content (TPC) determination

The total phenolic compounds were estimated by Folin-Ciocalteu method (FCM) (Sun and Ho, 2005). From a known concentration of the sample solution 125 µL of sample was taken in test tube. Then 500 µL distilled water was added in it. After that 125 µL of Folin-Ciocalteu reagent was added in it and gave a stand of 6 minutes. Then 1.25 mL of 7% sodium carbonate was added in it and final volume of 3mL was made by adding 1mL distilled water. A rest of 90 min. was given to samples for completion of reaction and then absorbance was taken at 760 nm by using a UV-vis spectrophotometer. Gallic acid was run as a standard along with the samples and its absorbance was taken at 725 nm.

β carotene bleaching assay

Antioxidant activity was determined by β-carotene method using spectrophotometer at 470 nm (Hinneborg et al., 2006). 1 ml of chloroform solution of β-carotene (1 mg/ml), 40µL of linoleic acid, and 400µL of tween80 (water soluble vit. E) were placed in a round bottom flask. After chlorophorm was removed under a nitrogen stream, 100 ml of distilled water was added slowly to the residue in the flask which was subsequently agitated to give a stable emulsion. An aliquot of 4.5 ml of this emulsion was transferred to a 10 ml test tube, and then 500µL of appropriately diluted ginger and garlic samples (10-60µg/ml) was added. The tubes were in a water bath at 50 ºC and the absorbance was measured after 120 min. at 470 nm. A blank sample was prepared by the adding 500µL of distilled water to the controlled reaction mixtures and the absorbance was measured immediately after preparation at 470 nm.

Peroxide value

Peroxide value of broiler meat sample was determined as described by (Xu et al., 2007). Sample was weighed to 5 g and added into a 250ml glass stoppered flask. It was heated in a water bath for 3 min at 60°C in order for the fat to melt. A thorough mixing by agitation was carried out in order to dissolve the fat and homogenize the sample after addition of 30ml of acetic acid-chloroform solution (3:2 v/v). Filtration of this sample was carried out by Whatman filter paper in order to remove the meat particles. In this filtrate addition of saturated potassium iodide solution at a concentration of 0.5ml was done before transferring into a burette. This was titrated against a standard solution of sodium thiosulfate of concentration 25g/l. Starch solution was added as an indicator. POV was calculated and expressed in milliequivalent peroxide per kg of the meat sample:

\[
POV \left( \frac{\text{meq}}{\text{Kg}} \right) = \frac{S \times N}{W} \times 1000
\]

S= Volume of titration in mL
N= Normality of the sodium thiosulfate solution
W= Sample weight in kg

Product analysis

TBARS assay

Thiobarbituric acid reactive substances (TBARS) were determined as described by (Hossain et al., 2012). Broiler meat (5 g) was dispersed in 20 ml of thiobarbituric acid solution (0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 mol/l HCl). The mixture was heated in boiling water for 10 min, cooled with water and centrifuged at 3600 g for 20 min at room temperature. The absorbance of this sample was carried out under 532nm utilizing a spectrophotometer. The standard curve was prepared using malondialdehyde (MDA) and TBARS were expressed as mg MDA/kg sample. The lipid oxidation was determined as a TBARS value by a formula:

\[
TBA \text{ Value} \left( \frac{\text{MDA}}{\text{Kg}} \right) = \frac{50 \times (A - B)}{m}
\]

Where,

A = absorbance of the test solution
B = absorbance of blank reagent
m = mass of the test portion

50 = a valid factor if the volume of the volumetric flash is 25 mL and the cuvette width is 10 mm.

Sensory evaluation

Sensory evaluation, based on color, flavor, firmness and overall acceptability of meatballs were conducted using 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) according to the procedure of
(Fernandez-Lopez et al. (2005). Panelists of National Institute of Food Science and Technology (NIFSAT) were provided with the evaluation Performa. The panelists were asked to express their opinion about the product by giving score after every fifteen days interval.

Statistical Analysis
The data obtained for each parameter was subjected to statistical analysis to determine the level of significance and comparison of means were also carried out according to the methods as described by (Steel et al., 1997).

RESULTS AND DISCUSSIONS
Characterization of citrus peel
Results for compositional analysis and antioxidant activity of citrus peel are shown in Table 1. These results revealed that average moisture content of citrus peel is 58.67%. Moisture content of citrus peel is very sensitive parameter to determine because higher moisture content is favorable for microbial growth. The mean value for protein contents of citrus peel is also given in Table 1. The protein content in citrus peel is 8.13%. These results are in corroboration with the findings of Figuerola et al. (2005) who determined protein content 6.70±0.05%. The results regarding fat contents revealed that citrus peel contains about 2.23% crude fat. The result of fibre contents of citrus peel is also mentioned in the Table 1. The fibre content in citrus peel is 56.86%. The results of the present study are parallel to the findings of Chau and Huang (2003) who determined fibre content ranged between 57-61%. The results regarding ash contents of citrus peel showed that citrus feel has ash contents up to 2.1%.

Table 1 shows the value of DPPH radical scavenging activity of the ethanolic extracts of citrus peel. The value of DPPH radical scavenging activity for citrus peel is 43.01%. The results of the present study regarding DPPH radical scavenging activity of citrus peel are similar to the previous findings reported by Hayat et al. (2010), who analyzed phenolic compounds from citrus mandarin peels by microwave heating and study its effect on antioxidant activity and they calculated the values for DPPH radical scavenging activity for citrus peel is ranged between 34-57%. The mean value for the total phenolic content is given in Table 1. Antioxidant activity is directly correlates with total phenolic compounds. The mean value of total phenolic content for citrus peel is 134.5 mg GAE/100g. The result of the present study are in line with Li et al. (2006) who studied phenolic contents of citrus peel and observed that total phenolic contents of citrus peels is ranged from 134-161 mgGAE/100g. The antioxidant activity was also determined by using β-carotene bleaching assay. The results showed the average value of β-carotene bleaching assay in citrus peel which is 67.83%. The results of present study are in close collaboration with the result of Hegazy and Ibrahim (2012) who studied antioxidant activity of orange peel extracts and calculated the value of β-carotene bleaching assay ranged between 64-87% in citrus peel.

Compositional analysis of broiler meat
Chemical composition of broiler meat fed on various diet plans is depicted in Table 2. The mean values for moisture contents of chicken fillets were recorded as 72% (T1), 71.92% (T2), 71.97% (T3) and 71.86% (T4). The results of the instant investigation are parallel to the findings of Li et al. (2009) who studied the influence of dietary vitamin E supplementation on meat quality traits and determined moisture content ranged between 71.95-72.38%. Table 2 also shows that protein contents varied from 19.87-21.28% in all the four treatments. The present investigations are in corroboration with the findings of Yasin et al. (2012) who discovered protein contents in the range of 21-22%. Statistical results showed that fat percentage varied significantly due to the addition of citrus peel in broiler feed. Fat contents varied from 6.01-7.11% in all the four treatments. The outcomes of the investigation concluded that fat percentage decreased as concentration of citrus peel was increased in the feed. The results of the present study were enclosed proximity with the findings of Yasin et al. (2012) who discovered fat content of 4.7% in thigh meat and 3.6% in breast meat. The mean values for ash contents of chicken fillets are presented in Table 2. The ash contents varied from 1.37 to 1.43% in all treatments. The findings of instant investigations are in line with the previous work of Demirba, (2011) who stated that ash contents of meat varied non-significantly.

Antioxidant analysis of broiler meat
1, 1-diphenyl-2-picrylhidrazyl (DPPH) scavenging activity
It is well known that the antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. DPPH is a free radical and it is a powerful oxidant. It has been widely used as a free radical to evaluate antioxidant
Table 1. Chemical analysis and antioxidant activity of citrus peel

<table>
<thead>
<tr>
<th>Compositional analysis (%)</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>Total Phenolic (mgGAE/100g) 134.5</td>
</tr>
<tr>
<td>Fat</td>
<td>DPPH Scavenging activity (%) 43.01</td>
</tr>
<tr>
<td>Fibre</td>
<td>β-carotene bleaching assay(%) 67.83</td>
</tr>
<tr>
<td>Protein</td>
<td>8.13</td>
</tr>
<tr>
<td>Ash</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Table 2. Compositional analysis of chicken fillets

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Moisture (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>72</td>
<td>19.87</td>
<td>7.11</td>
<td>1.37</td>
</tr>
<tr>
<td>T2</td>
<td>71.92</td>
<td>20.01</td>
<td>6.74</td>
<td>1.41</td>
</tr>
<tr>
<td>T3</td>
<td>71.97</td>
<td>21.28</td>
<td>6.51</td>
<td>1.39</td>
</tr>
<tr>
<td>T4</td>
<td>71.86</td>
<td>20.84</td>
<td>6.01</td>
<td>1.43</td>
</tr>
</tbody>
</table>

T1 = 0% Citrus peel in broiler feed
T2 = 4% Citrus peel in broiler feed
T3 = 8% Citrus peel in broiler feed
T4 = 12% Citrus peel in broiler feed

activity of natural plant sources. DPPH analysis is also one of the tests used to prove the ability of the components of the citrus peel extract to act as donors of hydrogen atoms (Yen and Chen, 1995; Stoilova et al., 2007). The mean values of DPPH scavenging activity is given in Table 3. It can be seen that the samples of broiler meat showed slightly high antioxidant activity in treatments T3 and T4. Treatments means are 39.58%, 48.25%, 55.04% and 58.59% for T1, T2, T3 and T4 respectively. This high value of DPPH scavenging activity in treatment T4 broiler meat was due to the high intake of citrus peel by broiler birds. T1 showed the lowest inhibition effect on DPPH radical as compared to the T4. All the values showed increasing antioxidant action with increase in the concentration of sample. These results are in agreement with the findings of Stoilova et al. (2007) who studied the antioxidant activity of meat from Vietnam and found that the DPPH radical inhibition reached up to 58.35%.

Total Phenolics

Total phenolic compounds directly indicate the antioxidant activity. There are several health benefits of phenolics because of the free radical scavenging activities in biological systems (O’Keefe et al., 1995). Table 4 shows the results of statistical analyses for total phenolic content of broiler meat in mgGAE/100g of sample. Mean values of total phenolic content in the treated meat increased linearly against the amount of citrus peel in the feed. The total phenolic content of the group fed with 4%, 8% and 12% of citrus peel supplemented feed is 53.33±1.0 mgGAE/100g, 59.30±1.1 mgGAE/100g and 70.37±1.5 mgGAE/100g in broiler meat, respectively. It is shown that T4 at 0 day showed highest total phenolic content that reached 76.35 mgGAE/100g meat. These results agree to a previous study in which De Araujo, (2011) studied the antioxidant activity of 66.3 mg GAE/100g.

β-carotene bleaching assay

Statistical analysis showed the significant variation in β-carotene bleaching assay in broiler meat but their interaction showed highly significant results for broiler meat. Table 5 shows the mean values in % age of β-carotene bleaching assay for broiler meat. Mean value of T4 exhibited the highest activity of β-carotene bleaching assay which is 58.59%. It is due to the high percentage of citrus peel in broiler feed. The results of present study are in close collaboration with the result of O’Keefe et al. (1995).

Peroxide value

Peroxide value was significantly affected with the treatments and storage periods. Interactive effect of both factors affected the peroxide value significantly (Table 6). Results indicated that treatments with antioxidants addition gave comparatively high POV as compared to T1. Meat samples obtained from the birds fed on antioxidant enriched diet also inhibited lipid peroxidation the POV value of them were 57.46 at the end of 30 days. Time scale measurement of POV revealed that with the passage of time POV values tends to decrease and minimum POV value was recorded at the end of the study while highest was recorded at 0 days of storage that was lower than the values recorded in meat at the start of the study. This result was in accordance with that of Yang et al.
Table 3. Variations in DPPH values of broiler meat fed on different levels of citrus peel-enriched diet during storage

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 day</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>42.15</td>
<td>40.81</td>
<td>38.91</td>
<td>36.45</td>
<td>39.58</td>
</tr>
<tr>
<td>T₂</td>
<td>51.32</td>
<td>49.92</td>
<td>47.57</td>
<td>44.21</td>
<td>48.255</td>
</tr>
<tr>
<td>T₃</td>
<td>58.76</td>
<td>56.77</td>
<td>54.26</td>
<td>50.39</td>
<td>55.045</td>
</tr>
<tr>
<td>T₄</td>
<td>62.47</td>
<td>60.58</td>
<td>57.46</td>
<td>53.87</td>
<td>58.59</td>
</tr>
<tr>
<td>Means</td>
<td>53.675</td>
<td>52.02</td>
<td>49.55</td>
<td>46.23</td>
<td></td>
</tr>
</tbody>
</table>

T₁ = 0% Citrus peel in broiler feed; T₂ = 4% Citrus peel in broiler feed; T₃ = 8% Citrus peel in broiler feed; T₄ = 12% Citrus peel in broiler feed

Table 4. Changes in total phenolic contents (mg GAE/100g of meat) of broiler meat fed on antioxidant enriched diet during freeze storage

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 Day</th>
<th>15 Days</th>
<th>30 days</th>
<th>45 Days</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>45.21</td>
<td>42.67</td>
<td>41.07</td>
<td>39.69</td>
<td>42.16</td>
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<tr>
<td>T₂</td>
<td>56.28</td>
<td>53.38</td>
<td>52.63</td>
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<tr>
<td>T₃</td>
<td>62.74</td>
<td>59.73</td>
<td>58.01</td>
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<tr>
<td>T₄</td>
<td>76.35</td>
<td>71.96</td>
<td>68.56</td>
<td>64.62</td>
<td>70.37</td>
</tr>
<tr>
<td>Means</td>
<td>60.145</td>
<td>57.007</td>
<td>55.067</td>
<td>53.022</td>
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</tbody>
</table>

T₁ = 0% Citrus peel in broiler feed; T₂ = 4% Citrus peel in broiler feed; T₃ = 8% Citrus peel in broiler feed; T₄ = 12% Citrus peel in broiler feed

Table 5. Effect of antioxidant enriched diet on β-carotene bleaching assay of broiler meat during freezing storage

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 Day</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>41.15</td>
<td>37.81</td>
<td>34.91</td>
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<tr>
<td>T₂</td>
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<td>41.57</td>
<td>39.21</td>
<td>48.25</td>
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<tr>
<td>T₃</td>
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<td>48.26</td>
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<td>T₄</td>
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<td>58.59</td>
</tr>
<tr>
<td>Means</td>
<td>53.675</td>
<td>52.02</td>
<td>49.55</td>
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</table>
Table 6. Peroxide value of broiler meat during storage

<table>
<thead>
<tr>
<th>Treatments</th>
<th>0 Day</th>
<th>15 Days</th>
<th>30 days</th>
<th>45 Days</th>
<th>Means</th>
</tr>
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<tbody>
<tr>
<td>T₁</td>
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<tr>
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<td>T₃</td>
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<td>T₄</td>
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<td>58.59</td>
</tr>
<tr>
<td>Mean</td>
<td>53.675</td>
<td>52.02</td>
<td>49.55</td>
<td>46.23</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Effect of citrus peel enriched diet on TBA of meatballs during the storage

<table>
<thead>
<tr>
<th>Treatment/day</th>
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<th>21</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>T₁</td>
<td>0.14±0.01</td>
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<td>0.16±0.02</td>
<td>0.14±0.02</td>
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<tr>
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<td>0.17±0.05</td>
<td>0.15±0.02</td>
</tr>
<tr>
<td>Mean</td>
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<td></td>
</tr>
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</table>

Table 8. Sensory evaluation of meat balls during storage

<table>
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<tr>
<th>Treatment/Parameters</th>
<th>Appearance</th>
<th>Color</th>
<th>Flavor</th>
<th>Texture</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>7.11±0.22b</td>
<td>7.28±0.16a</td>
<td>7.38±0.16ab</td>
<td>7.31±0.18abc</td>
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</tr>
<tr>
<td>T₂</td>
<td>7.82±0.24ab</td>
<td>7.53±0.18a</td>
<td>7.79±0.17a</td>
<td>7.22±0.18a</td>
<td>7.69±0.26a</td>
</tr>
<tr>
<td>T₃</td>
<td>7.67±0.23ab</td>
<td>6.97±0.15b</td>
<td>6.90±0.15ab</td>
<td>6.87±0.17ab</td>
<td>7.12±0.24b</td>
</tr>
<tr>
<td>T₄</td>
<td>7.72±0.24a</td>
<td>6.66±0.14b</td>
<td>6.46±0.14b</td>
<td>6.36±0.16bc</td>
<td>6.78±0.22b</td>
</tr>
</tbody>
</table>

T₁ = 0% Citrus peel in broiler feed; T₂ = 4% Citrus peel in broiler feed; T₃ = 8% Citrus peel in broiler feed; T₄ = 12% Citrus peel in broiler feed
(1993) who noted that the antioxidant activity of different meat was concentration dependent.

**Product analysis**

**TBARS value**

The data of meatballs is presented in Table 7. It was obvious from the results that the treatments had significant effect on the TBA of meatballs. Whereas the storage days and their interactive also have significant impact on the TBA of meatballs. Results also indicated that treatments T2, T3 and T4 showed little increase in TBA value as compared to T1. Furthermore, the increase in the TBA value in meatballs of T4 is less than that of T1 and T2. Likewise, storage has profound effect on TBA value and it increased with increase in storage and least TBA value was observed at 0 day and while maximum TBA value was obtained at 45 days of storage. The results of present study are in lined with the findings of Sallam et al. (2004) who worked on the antioxidant and antimicrobial effect in chicken meat.

**Sensory evaluation**

Sensory evaluation is an important tool in a product development. Acceptance of a food product depends upon the consumer’s perception of the color, taste, texture, flavor and overall acceptability into overall impression of quality. Although chemical, physical and microbiological tests are employed to check the quality of a food product, but these tests can’t provide such kind of information whether consumer will accept it or not. The meatballs prepared, deep fried and then subjected to sensory evaluation by panelist from National Institute of Food Science & Technology for different attributes viz., color, flavor, taste, texture and overall acceptability and scores were recorded using a nine point hedonic scale after every 15 day to assess the liking and disliking of the panelists. The meatballs of treatment T1 spoiled due to microbial load.

Mean values for appearance of meatballs are presented in Table 8. The maximum appearance score 7.82±0.24 while minimum score 7.11±0.22 was observed for T1 (control). These results of present study are close with the findings of Fernandez-Lopez et al. (2004) who studied the effect of functional compounds in citrus by-products in meat products and determined that meatballs showed good appearance during 1st week of storage. Results also stated that color of meatballs was significantly affected by treatments and storage interval. The average scores of different treatments of meatballs were found as 7.53±0.18 for T2 and 6.66±0.14 for T4. The results of this study are well supported by the findings of Fernandez-Lopez (2000) who found the mincing effect on color properties in pork meat. The mean score for flavor varied from 7.79±0.17 to 6.46±0.14 among different treatments, T2 showed maximum mean score 7.79±0.17 followed by T1 (7.38±0.16), T3 (6.90±0.15) while minimum exhibited by T4 (6.46±0.14).

Mean scores for texture of meatballs showed significant differences for different treatments. The mean scores for T1, T2, T3 and T4 were 7.31±0.18, 7.22±0.18, 6.87±0.17 and 6.36±0.16, respectively. The results of instant study are in close agreement with the findings of Garcia-Esteban et al. (2004) who studied microbiological quality and effect of packaging of cured ham during long period storage. The means scores for overall acceptability showed significant differences in acceptability of meatballs due to different treatments. Maximum score for overall acceptability was assigned to T2 (7.69±0.26), while minimum score was recorded for T4 (6.78±0.22).

**CONCLUSION**

The overall evaluation of this study concludes that citrus peel has a good antioxidant. It showed appreciable amounts of antioxidant compounds showing good inhibition properties against the free radicals. Citrus peel exhibited good antioxidant and antimicrobial activity in broiler meat. During storage at freezing temperature broiler meat showed good shelf stability as compared to control. This study concludes that citrus peel as a feed ingredient in poultry feed has good antioxidant and antimicrobial potential.

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