Effect of α-Lipoic Acid and α-Tocopherol Acetate Enriched Broiler Diet on Oxidative Stability and Quality of Broiler Leg Meat and Meat Products

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Abstract

The present study was conducted to evaluate the effect of inclusion of two dietary antioxidants α-lipoic acid (ALA 25, 75 and 150 mg/kg diet) and α-tocopheryl acetate (ATA 200 mg/kg diet) on growth performance, susceptibility to oxidation and quality of leg chicken meat and meat products during storage. A total of 180 broiler birds were randomly divided into 6 groups with 3 replicates of 10 hens each and fed on different diet plan. Body weight gain of broiler birds was decreased with increasing α-lipoic acid supplementation in feed and Birds in (T4) containing minimum dose of alaphipoic acid (25 mg/kg feed gained more weight 1948.25g whereas T1 containing maximum dose of alpha lipoic acid (150 mg/kg of feed) gained minimum weight i.e. 1691.25 g. The inclusion of α-tocopheryl acetate improved body weight gain. The maximum deposition of ALA took place in T4 which contain maximum dose of ALA. The thiobarbituric acid reactive substances (TBARS) assay and DPPH assay of the broiler leg meat were as in T1 (0.15 ± 0.02 MDA/kg of meat, 76.69 ± 0.14%) and in T4 were (0.25 ± 0.08 MDA/kg of meat, 44.77 ± 0.09%) accordingly. The (HPLC) data revealed that ALA and ATA concentration were highest in T1 (96.54 ± 0.28 mg/g of meat, 159.40 ± 0.20 mg/g of meat) and lowest in T4 (17.19 ± 0.12mg/g, 35.86 ± 0.08 mg/g of meat). Sensory results showed that nuggets and patties made of T1 containing oxidize oil were least preferred and T4 got highest score. In a nutshell, 150 mg/kg level feed dietary supplementation of ALA with constant level of ATA can enhanced the antioxidant potential, lipid stability and nutritional qualities of broiler leg meat and meat products.

Keywords: Alphalipoic acid; Alpha tocopherol acetate; Leg meat; Nuggets; Patties

Abbreviations: MDA: Malondialdehyde; ALA: α-lipoic acid; ATA: α-tocopherolacetate; TBARS: Thiobarbituricacid reactive oxygen species; HPLC: High-performance liquid chromatography

Introduction

The major cause to decrease the quality of meat and meat products is auto-oxidation of lipid which generates off flavor and reduces its quality during storage. The resulting products of this process are reactive oxygen species, which leads to serious health problems [1]. The oxidative stability of meat and meat products depends upon the balance of anti- and pro-oxidants and the oxidation substrates including polyunsaturated fatty acids (PUFA), cholesterol, proteins and pigments [2]. Antioxidants can be used to retard lipid peroxidation in meat but the use of cocktails of antioxidants may have superior effects as compared to single antioxidants because two or more antioxidants together can act synergistically [3]. The Dietary supplementation of ALA and ATA enhanced the oxidative stability and improved the antioxidant potential of the broiler meat [4].

Alpha-lipoic acid (ALA) in its oxidized form is a coenzyme involved in the biooxidation of pyruvates. The reduced form of ALA, dihydrolipoic acid, is a powerful mitochondrial antioxidant [5]. ALA is considered an important antioxidant that may be of therapeutic value in some pathologic conditions related to the overproduction of oxidant radicals [6]. ALA is an essential cofactor for mitochondrial enzymes and a natural antioxidant that is used to quench free radicals [7]. ALA has valuable potential in clinical interests as a cellular thiol-replenishing and redox-modulating agent [8]. The reduced form of ALA, dihydrolipoic acid, reacts with oxidants such as superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and singlet oxygen and minimize the cellular damage effects [9].

Antioxidant potential of the broiler breast meat and meat products can be improved by feeding the broilers on diet supplemented with ALA and ATA [10]. Alpha-tocopherol acetate (ATA) is the most efficient chain-breaking, fat-soluble antioxidant in the cellular locations [11].

Unlike other fat-soluble vitamins, α-tocopherol is not accumulated in the body to toxic levels. Importantly, the body has the ability to eliminate “excess” α-tocopherol by increased metabolism and excretion to prevent excess accumulation of α-tocopherol, even when daily pharmacologic vitamin E doses are administered [12]. Dietary supplementation of ATA enhanced the lipid stability in muscle and improved the meat quality of Beijing-you chicken [13]. ALA and vitamin E have shown synergistic effects against lipid peroxidation by oxidant radicals in several pathological conditions such as a thromboembolic stroke model in rats for neurological functions, glial reactivity and neuronal remodeling [14]. In the present study, broiler birds were raised specifically for meat production, fed feed supplemented with α-lipoic acid and α-tocopherol acetate, and the antioxidant activity and component changes in the meat and meat products were investigated to assess the role of antioxidant supplements on the storage stability of broiler meat and meat products.

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Materials and Methods

Chickens, diet and management of birds

The composition of the control feed was 300 g of corn, 200.0 g of soybean meal of 44% protein, 174.0 g of sorghum, 100.0 g of sunflower meal, 80 g of barley, 58 g of rye, 50.0 g of beef tallow, 20 g of dicalcium phosphate, 3 g of sodium bicarbonate, 10 g of salt and 14.0 g of Sepiolite (total 1000 g). The metabolizable energy of the control feed was 2850 kcal/kg, crude protein (16.0%), crude fat (4.1%) fibre (5.1%) and ash content was (7.4%). One hundred and eighty broiler birds (1 day old), of Hubbard strain, were purchased from the Hi-Tech feeds private limited, Faisalabad, Pakistan. The birds in each experimental unit were kept in separate pens (L×W×H 4×3×1.5 ft), and the pens were disinfected by using fumigation method. The temperature of the experimental room was maintained at 95°F during the first week of the trial. It was then lowered by 5°F until it reached 75°F at the completion of the study. The birds were reared up to 6 weeks. During the first 2 weeks, the birds were fed with the control diet and after that feed was supplemented with ATA (200 mg/kg of feed) and ALA as indicated in the treatment plan (Table 1).

Slaughtering and sample collection

At the end of the 6 weeks trial, the birds were slaughtered by following Islamic ethical guidelines which are approved by the authorities of university and sampling of leg meat was done. Five birds from each feeding group were randomly selected and slaughtered at the farm by cervical dislocation and then the leg meat was vacuum packed in polythene bags and stored at -18°C in a freezer (Sanyo, Japan) for further analysis.

Sample preparation

Five gram meat sample was taken in 50 ml polypropylene tube having a cap and samples were homogenized by using phosphate (Potassium phosphate) buffer and glycerol (20%) pH (7.4) with the help of homogenizer. The homogenized leg meat sample was centrifuged at 1000rpm for ten minutes to remove the nuclear fractions. After the removal of the nuclear fractions, supernatant was collected in a separate tube and then it was used for further analysis.

Analysis of leg muscles of broiler meat

The antioxidant activity of leg meat was assessed by measuring their scavenging abilities to DPPH stable radicals as described by the method described by Ahn et al. [16]. An alpha-lipoic acid content of leg meat was measured as the TBARS value by using the method described by Arshad et al. [18].

Product development

The antioxidants enriched broiler leg meat was used in the preparation of nuggets and patties.

Preparation of nuggets

Nuggets were prepared by the method described by Perlo et al. [19]. The basic recipe of the nuggets is as (boneless chicken 625 g, fresh bread crumbs 90 g, onions 250 g, tomatoes 150 g, Chopped fresh coriander 45 g, Ginger grated 2.5 g, Clove 1 g, Ground cumin 15 g, Ground Spices powder 15 g, Egg 1, salt 50 g, vegetable oil 250 g). The raw material in the manufacturing of the nuggets was weighed and cleaned according to the recipe. Antioxidant enriched broiler leg meat was washed with tap water, deboned manually and was minced by using electric mincer to obtain highly minced meat for the preparation of high textured broiler meat nuggets. First of all leg meat and onion were mixed in a meat mixer for five minutes, then all other ingredients were added according to the recipe, then all these ingredients were mixed to provide a uniform blend. When all components were thoroughly mixed then the mixture was extended in a thin layer (10 mm thickness) and shaped into discs of 30 mm diameter (10 ± 1 g/ piece). When nuggets were prepared, these were dipped in plain flour and bread crumbs separately and frying of the nuggets was done in canola oil at 180°C till golden brown color was achieved.

Burger patties preparation

The burger Patties were made by the method as described by Mc Carthy et al. [20]. The basic recipe of burger patties is as (boneless chicken 625 g, fresh bread crumbs 90 g, onions 250 g, tomatoes 150 g, Chopped fresh coriander 45 g, Ginger grated 2.5 g, Clove 1 g, Ground cumin 15 g, Ground Spices powder 15 g, Egg 1, salt 50 g, vegetable oil 250 g). The raw material in the manufacturing of the burger patties were weighed and cleaned according to the recipe. Antioxidant enriched broiler leg meat was washed with tap water, deboned manually and was minced by using electric mincer to obtain highly minced meat for the preparation of the good textured broiler meat burger patties. Minced chicken meat and onion were boiled in water for 15 minutes in pressure cooker. First of all minced broiler meat and onion were mixed in a meat mixer for 5 minutes, and then all other ingredients were mixed by using meat mixer. After well mixing of all the ingredients, burger patties were made by using burger Patty maker of 50 g size. When patties were ready for frying, these were rested for some time, followed by frying in canola oil at 180°C till golden brown color achieved.

Analysis of nuggets and burger patties

Lipid peroxidation stability by thiobarbituric acid reactive substances (TBARS) assay: The oxidative stability of the leg meat nuggets and burger patties was assessed by measuring mg of malondialdehyde per kg of meat. Five gram sample of nuggets and burger patties was taken in test tube with 15 mL distilled water was homogenized at 1130rpm for 1 min. Sample homogenate (1 mL) was transferred to a test tube. Aftetr that, 50 mL butylated hydroxyanisol (7.2%) and 2 mL thiobarbituric acid (20 mM TBARS in 15% trichloroacetic acid (TCA) were added to the test tube. Tubes were heated at 90°C in a boiling water bath for 30 min, cooled, and then centrifuged at 2090 rpm for 15 min. Absorbance of the supernatant was measured at 532 nm with a spectrophotometer (Cecil, England). The lipid oxidation was determined as the TBARS value by using the method described by Ahn et al. [16].

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control feed</td>
</tr>
<tr>
<td>2</td>
<td>Control+200 mg vitamin E+25 mg α-lipoic acid/kg feed</td>
</tr>
<tr>
<td>3</td>
<td>Control+200 mg vitamin E+75 mg α-lipoic acid/kg feed</td>
</tr>
<tr>
<td>4</td>
<td>Control+200 mg vitamin E+150 mg α-lipoic acid/kg feed</td>
</tr>
<tr>
<td>5</td>
<td>Control+Containing oxidized oil*</td>
</tr>
<tr>
<td>6</td>
<td>Control+Oxidized oil+200 mg vitamin E+150 mg α-lipoic acid/kg feed</td>
</tr>
</tbody>
</table>

Table 1: Treatment plan of the diet fed to the experimental birds.

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was assessed by measuring their scavenging abilities to DPPH stable radicals. A sample of 125 μL was mixed with 0.0012 m DPPH solution followed by the addition of 95% MeOH up to a final volume of 4 mL. The absorbance of the resulting solution and the blank were recorded after 1 h at room temperature. The conversion the color of DPPH was read spectrophotometrically at 515 nm. Inhibition of free radicals by DPPH in percent (%) was calculated in according to the method of Bozin et al. [15].

\[
1 \% = 100 \times \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right)
\]

Physicochemical analysis of nuggets and patties

The pH of nuggets and patties was measured by using pH meter by the method described by Sallam et al. [21]. Ten gram of sample was homogenized with 50 mL distilled water and pH value was measured by a digital pH-meter. A hand held tristimulus colorimeter (Color Test Meter II) was used to determine the color of leg Nuggets and Patties at regular storage intervals (0, 30, 60 and 90 days) as described by El-Gasim [22]. Calibration of the colorimeter was done by using standards (54°C Tn for dark and 151°C Tn for light). Color was determined by placing nuggets and patties in petriplate under the photocell. The water activity of leg nuggets and patties was determined by using an electronic Hygropalm water activity meter (Model Aw-Win, Rotronic, equipped with a Karl-Fast probe) at regular storage intervals as described by Cosenza et al. [23]. The sample was taken in plastic cup and the water activity was measured. The textural characteristics of nuggets and patties were analyzed at different storage intervals by means of texture analyzer (Mod. TA-XT2, Stable Microsystems, surrey, UK). Texture was determined with the help of texture analyzer (model TA_ XT Plus, Stable Microsystems, Surrey, UK) as described by Carlos et al. [24]. The nuggets and patties were fried and compression test was performed to check the texture of the product.

Sensory evaluation of nuggets and patties

The sensory evaluation of fried nuggets and patties was carried out for the different attributes like appearance, flavor, taste and overall acceptability by using a nine point hedonic scale after 0, 30, 60 and 90 days, by trained panelists as the method described by Meilgaard et al. [3].

Statistical analysis

The data obtained for each parameter was subjected to statistical analysis to determine the level of significance according to the method described by Steel et al. [25] and by using the software package Statistic 8.1 (Analytical Software, Tallahassee, FL). The Duncan’s multiple range (DMR) test was used to estimate the level of significance that existed between the mean values.

Results and Discussion

Broiler growth performance

The results (Figures 1 and 2) revealed a highly significant (p<0.01) effect on weight gain of broilers among all the treatments. Birds in (T₄) containing minimum dose of aliphatic acid (25 mg/kg of feed) gained more weight 1948.25 g whereas T₅ containing maximum dose of alpha lipoic acid (150 mg/kg of feed) gained minimum weight i.e. 1691.25 g at the end of 42 day trial. The weight gain of the treatment (T₄) was 1783.63 grams which contain oxidized oil. The feed conversion ratio (FCR) of the treatment T₄ was 1.92 and the treatment T₅ was 2.07. It means that the group containing only oxidized oil had adverse effect on the performance of broiler chicks and the group containing maximum dose of alpha lipoic acid also reduces the overall weight of the broiler birds. The results of this study are in correlation with the studies of Arshad et al. [18] and Mielnik et al. [26] who also observed significantly p<0.05 lower weight gain of broilers fed on diets containing oxidized oil whereas antioxidant supplementation in feed improved the growth performance of the birds.

Analyses of fresh leg meat stored at -18°C

The lipid peroxidation is the measurement of MDA compound formed per kg of meat during autoxidation of lipids present in meat and meat products during storage. Higher MDA compounds revealed higher amount of lipids, which results in quality deterioration of the meat. T₄ containing (200 mg ATA and 150 mg ALA/kg of feed) exhibited less MDA (0.15 ± 0.02) as compared with T₂ (0.21 ± 0.063), and T₅ showed highest MDA (0.25 ± 0.08) formation in broiler leg meat. The treatments with antioxidants ALA and ATA supplemented showed less peroxidation by producing lower amounts of MDA. The results of current studies are agreed with the studies of Asghar et al. and Lin et al. [27,28] who also observed less MDA peroxidation with the supplementation of antioxidants through feed.

The antioxidant activity of specific compounds or extracts is calculated by reacting the compound chemically with a stable radical
of 2, 2-diphenyl-1-picrylhydrazyle (DPPH) in methanol solution. The results (Table 2) of DPPH free scavenging assay of leg meat showed that there is significant effect of ALA and ATA on all the treatments. Free radical inhibition percentage of leg meat ranged from 44.77 ± 0.09D to 78.69 ± 0.19A mg/g as showed in Table 2. Our findings are concurred with the agreement with Fasseas et al. [29] who reported that vitamin E containing ALA and ATA (antioxidant) maintained lower TBARS values throughout the storage period. The lowest TBARS values of leg nuggets were observed in T1 (0.15 ± 0.02) which contain maximum amount of alpha-lipoic acid and highest TBARS values were observed in T5 (0.36 ± 0.05) at 0 day which contain only oxidized oil. The same pattern was followed by the leg meat patties. The lowest TBARS values in T5 were due to the antioxidants addition of ALA and ATA. The findings of this study are concurred with Arshad et al. [18] who reported that vitamin E increased the antiradical power than control treatment respectively. A highly significant effect (P < 0.01) on DPPH assay was observed in the chicken nuggets and patties after ALA and ATA incorporation through feed. Chicken nuggets containing ALA and ATA (antioxidant) maintained higher DPPH percentage inhibition throughout the storage period. The DPPH percentage inhibition values.

### Analyses of broiler meat products

**Oxidative stability of nuggets and patties:** Cooking process and salt addition enhances lipid peroxidation and ultimately less stability enhancement is observed in nuggets and patties as compared to fresh meat.

**Effect on TBARS and DPPH value of nuggets and patties stored at -18°C:** The results (Table 3) revealed a highly significant effect of ALA and ATA (P < 0.01) on TBARS value observed in the leg chicken nuggets and patties after incorporation through feed. Chicken nuggets containing ALA and ATA (antioxidant) maintained lower TBARS values in T4 (0.15 ± 0.02) which contain maximum amount of alpha-lipoic acid and highest TBARS values were observed in T5 (0.36 ± 0.05) at 0 day which contain only oxidized oil. The same pattern was followed by the leg meat patties. The lowest TBARS values in T5 were due to the antioxidants addition of ALA and ATA. The findings of this study are concurred with Arshad et al. [18] who reported that vitamin E increased the antiradical power than control treatment respectively. A highly significant effect (P < 0.01) on DPPH assay was observed in the chicken nuggets and patties after ALA and ATA incorporation through feed. Chicken nuggets containing ALA and ATA (antioxidants) maintained higher DPPH percentage inhibition throughout the storage period. The DPPH percentage inhibition values.

### Table 2: TBARS Value, DPPH Assay, Alpha lipoic acid content and Alpha tocopherol acetate content in leg meat of broiler birds fed on supplemented diet.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TBARS values nmol of malondialdehyde/kg of meat</th>
<th>DPPH Assay Free radical scavenging activity (%)</th>
<th>ALA content mg/gram of meat</th>
<th>ATA content mg/gram of meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.21 ± 0.06&lt;sup&gt;C&lt;/sup&gt;</td>
<td>55.34 ± 0.11&lt;sup&gt;D&lt;/sup&gt;</td>
<td>22.69 ± 0.14&lt;sup&gt;C&lt;/sup&gt;</td>
<td>48.70 ± 0.10&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td>0.18 ± 0.04&lt;sup&gt;B&lt;/sup&gt;</td>
<td>64.89 ± 0.14&lt;sup&gt;C&lt;/sup&gt;</td>
<td>49.31 ± 0.18&lt;sup&gt;B&lt;/sup&gt;</td>
<td>96.38 ± 0.15&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>T3</td>
<td>0.17 ± 0.03&lt;sup&gt;B&lt;/sup&gt;</td>
<td>73.94 ± 0.17&lt;sup&gt;B&lt;/sup&gt;</td>
<td>60.23 ± 0.22&lt;sup&gt;B&lt;/sup&gt;</td>
<td>150.24 ± 0.18&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>T4</td>
<td>0.15 ± 0.02&lt;sup&gt;C&lt;/sup&gt;</td>
<td>78.69 ± 0.19&lt;sup&gt;B&lt;/sup&gt;</td>
<td>96.54 ± 0.28&lt;sup&gt;B&lt;/sup&gt;</td>
<td>159.40 ± 0.20&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>T5</td>
<td>0.25 ± 0.08&lt;sup&gt;B&lt;/sup&gt;</td>
<td>44.77 ± 0.09&lt;sup&gt;B&lt;/sup&gt;</td>
<td>17.19 ± 0.12&lt;sup&gt;B&lt;/sup&gt;</td>
<td>35.86 ± 0.08&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>T6</td>
<td>0.23 ± 0.07&lt;sup&gt;B&lt;/sup&gt;</td>
<td>48.98 ± 0.10&lt;sup&gt;E&lt;/sup&gt;</td>
<td>40.12 ± 0.17&lt;sup&gt;D&lt;/sup&gt;</td>
<td>70.25 ± 0.13&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data Values are expressed as mean ±SE.

Means in the same row have the same letter are not significantly different at 0.05.

### Table 3: Antioxidant potential of leg nuggets and patties performed by TBARS and DPPH assay method.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Storage days</th>
<th>TBARS Assay</th>
<th>DPPH Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Broiler meat Nuggets</td>
<td>Broiler meat Patties</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>0 day</td>
<td>0.22 ± 0.01</td>
<td>55.64 ± 2.80</td>
</tr>
<tr>
<td></td>
<td>30 day</td>
<td>0.30 ± 0.02</td>
<td>53.76 ± 2.65</td>
</tr>
<tr>
<td></td>
<td>60 day</td>
<td>0.33 ± 0.04</td>
<td>50.61 ± 2.23</td>
</tr>
<tr>
<td></td>
<td>90 day</td>
<td>0.39 ± 0.2</td>
<td>49.67 ± 2.12</td>
</tr>
<tr>
<td>T2</td>
<td>0 day</td>
<td>0.18 ± 0.02</td>
<td>58.30 ± 3.00</td>
</tr>
<tr>
<td></td>
<td>30 day</td>
<td>0.21 ± 0.02</td>
<td>56.42 ± 2.84</td>
</tr>
<tr>
<td></td>
<td>60 day</td>
<td>0.23 ± 0.03</td>
<td>55.00 ± 2.68</td>
</tr>
<tr>
<td></td>
<td>90 day</td>
<td>0.25 ± 0.01</td>
<td>52.06 ± 2.56</td>
</tr>
<tr>
<td>T3</td>
<td>0 day</td>
<td>0.17 ± 0.01</td>
<td>63.52 ± 3.57</td>
</tr>
<tr>
<td></td>
<td>30 day</td>
<td>0.24 ± 0.03</td>
<td>64.20 ± 3.46</td>
</tr>
<tr>
<td></td>
<td>60 day</td>
<td>0.27 ± 0.02</td>
<td>62.78 ± 3.38</td>
</tr>
<tr>
<td></td>
<td>90 day</td>
<td>0.35 ± 0.03</td>
<td>61.84 ± 3.04</td>
</tr>
<tr>
<td>T4</td>
<td>0 day</td>
<td>0.15 ± 0.02</td>
<td>67.08 ± 3.57</td>
</tr>
<tr>
<td></td>
<td>30 day</td>
<td>0.25 ± 0.02</td>
<td>64.34 ± 2.05</td>
</tr>
<tr>
<td></td>
<td>60 day</td>
<td>0.31 ± 0.01</td>
<td>49.22 ± 1.90</td>
</tr>
<tr>
<td></td>
<td>90 day</td>
<td>0.36 ± 0.01</td>
<td>41.98 ± 1.84</td>
</tr>
<tr>
<td>T5</td>
<td>0 day</td>
<td>0.36 ± 0.05</td>
<td>46.23 ± 2.40</td>
</tr>
<tr>
<td></td>
<td>30 day</td>
<td>0.51 ± 0.02</td>
<td>56.46 ± 2.98</td>
</tr>
<tr>
<td></td>
<td>60 day</td>
<td>0.54 ± 0.06</td>
<td>54.04 ± 2.87</td>
</tr>
<tr>
<td></td>
<td>90 day</td>
<td>0.65 ± 0.05</td>
<td>51.02 ± 2.69</td>
</tr>
<tr>
<td>T6</td>
<td>0 day</td>
<td>0.20 ± 0.02</td>
<td>58.35 ± 3.01</td>
</tr>
<tr>
<td></td>
<td>30 day</td>
<td>0.27 ± 0.03</td>
<td>56.48 ± 2.98</td>
</tr>
<tr>
<td></td>
<td>60 day</td>
<td>0.32 ± 0.03</td>
<td>54.04 ± 2.87</td>
</tr>
<tr>
<td></td>
<td>90 day</td>
<td>0.40 ± 0.02</td>
<td>51.02 ± 2.69</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SE.

Means in the same row have the same letter are not significantly different at 0.05.
of the leg nuggets were higher as compared to leg nuggets because it
contains less amount of fat. Highest DPPH percentage inhibition
values of leg nuggets were observed in T₅ (67.08 ± 3.57) which contain
maximum amount of alpha lipoic acid and highest and lowest DPPH
percentage inhibition values were observed in T₄ (67.08 ± 3.57) at 0
day which contain oxidized oil. The percentage inhibition of leg meat
ranged from (46.23 ± 0.02) in T₅ to (67.08 ± 3.57) in T₄. The production
of free radicals was also lowest at the start of the storage and increase
with the passage of the time. The findings of this study are in agreement
with the results reported by Fasseas et al. [29] and Mielnik et al. [26]
who reported that vitamin E and sage extract increased the antiradical
power than control treatment respectively.

**Physico chemical characteristics of nuggets and patties**

**pH and water activity nuggets and patties stored at -18°C:** The results
(Table 4) revealed that there is a highly significant effect of ALA and ATA treatments on the pH of broiler leg meat nuggets and
patties. The effect of days on the pH of broiler meat products was also
significant. It is obvious from results that pH value of leg nuggets varied
from 5.82-6.83 due to the fact of variation of antioxidants in treatments.
The pH range of leg patties was that of 5.89-6.55. The pH values of the
nuggets and patties increases with passages of the time. The increase in
pH value during storage period revealed that, there was significant
breakdown of meat protein on storage of the product. The findings of
this study are in agreement with that of Devendra and Tanwar [34]
who also observed increase in pH with the passage of the time in the
nuggets containing antioxidants.

The result showed that the different treatments of alpha lipoic acid,
alpha tocopherol acetate and oxidized oil have highly significant effect
on the water activity of antioxidant enriched broiler leg meat nuggets
and patties. Water activity of leg nuggets in T₅ (0.84 ± 0.04) and in T₆
was (0.80 ± 0.03) at 0 day storage interval. The water activity of the
nuggets and patties decreases and product move towards hardness with
the passage of the time. This result correlate with the result of Ruiz et
al. [35] who studied that water activity of crusted dry-cured loin and
non-crusted dry-cured loin decrease and the texture of the product go
towards hardness with advancement of the storage period.

**Color and texture of leg nuggets and patties stored at -18°C:** The results
(Table 5) showed that there is a highly significant effect of ALA and
ATA treatments on the color of leg meat nuggets and patties. The
color of the patties and nuggets was lighter at the start of the storage
period and with the advancement of the storage period, the color of the
meat nuggets and patties become dark. The color of nuggets containing
oxidized oil was darker as compared to the other treatment containing
ALA and ATA. The color of leg meat nuggets ranged from 94.33-115and
the color of leg meat patties ranged from 104 ± 1.00-116 ± 1.58. During
storage the values for the color decreased with the passage of time.
The decrease in value indicated that the nuggets and patties become
darker during storage. The results of this study are in accordance with
Sudesh et al. [32] who stated that color value of the chicken patties
decrease with passage of the time and color of the patties change from
red to brown which could be due to the formation of metmyoglobin
in salt containing treatments. The results revealed a significant effect
of ALA and ATA treatments on the texture values of leg meat nuggets
and patties at freezing conditions. However the storage days and their
interactive effect of treatments and storage days also have significant
effect on the texture properties of nuggets and patties. Actually the
addition of ALA improves the antioxidant capacity and also improves
the water retaining capacity of leg meat and meat products but with the
advancement in the storage period, the nuggets and patties becomes
harder due to loss of moisture content. The results of present study are
in close collaboration with Sohaib et al. [10] who stated that the texture
value varied significantly with the advancement of the storage period.

**Effect on sensory parameters of nuggets and patties stored at -18°C:** Sensory evaluation of food is a scientific method used to
measure, analyze and interpret responses to products as perceived
through the senses of sight, touch, smell, taste, and hearing. The results
(Table 6) showed that there is a highly significant effect of ALA and
ATA treatments on all the sensory parameters (appearance, flavor,
Taste and overall acceptability) of broiler meat nuggets and patties.
The leg chicken nuggets and patties containing ALA and ATA scored
significantly (P<0.05) higher for appearance, flavor, taste and overall
acceptability than all other preparation. T₅ nuggets and patties got least
score by sensory evaluation because of the rancid taste imparted by
the oxidized oil. The appearance of the nuggets and patties decreases with
passage of the time. The minimum value for flavor was observed in
T₅ (6.2 ± 0.3) at day 30 because it contain oxidized oil and it impart
a rancid flavor to the products. The decrease in flavor value is due to
the peroxidation of PUFA which results in rancid odors and flavors.
Decrease in flavor scores might be due to development of oxidative
rancidity and microbial deterioration in products during storage.
During storage the score for taste of nuggets and patties was also

![Table 4: pH and water activity of leg nuggets and patties at various storage intervals stored at -18°C.](image-url)
Means in the same row have the same letter are not significantly different at (0.05).
Values are expressed as mean ±SE

Table 5: Color and texture of nuggets and patties stored at -18°C for a storage period of 90 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 day</th>
<th>30 day</th>
<th>60 day</th>
<th>90 day</th>
<th>0 day</th>
<th>30 day</th>
<th>60 day</th>
<th>90 day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Color</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>112 ± 1.24</td>
<td>108 ± 1.20</td>
<td>106 ± 1.16</td>
<td>101 ± 1.10</td>
<td>121 ± 1.94</td>
<td>117 ± 1.88</td>
<td>114 ± 1.76</td>
<td>111 ± 1.72</td>
</tr>
<tr>
<td>T2</td>
<td>113 ± 1.27</td>
<td>110 ± 1.24</td>
<td>107 ± 1.19</td>
<td>104 ± 1.14</td>
<td>122 ± 1.95</td>
<td>118 ± 1.89</td>
<td>115 ± 1.77</td>
<td>111 ± 1.72</td>
</tr>
<tr>
<td>T3</td>
<td>115 ± 1.47</td>
<td>111 ± 1.40</td>
<td>108 ± 1.26</td>
<td>103 ± 1.19</td>
<td>124 ± 1.97</td>
<td>120 ± 1.90</td>
<td>117 ± 1.88</td>
<td>113 ± 1.76</td>
</tr>
<tr>
<td>T4</td>
<td>116 ± 1.58</td>
<td>112 ± 1.46</td>
<td>109 ± 1.35</td>
<td>105 ± 1.28</td>
<td>125 ± 2.00</td>
<td>122 ± 1.95</td>
<td>118 ± 1.89</td>
<td>114 ± 1.78</td>
</tr>
<tr>
<td>T5</td>
<td>104 ± 1.00</td>
<td>101 ± 0.97</td>
<td>99 ± 0.88</td>
<td>95 ± 0.96</td>
<td>115 ± 1.77</td>
<td>111 ± 1.72</td>
<td>108 ± 1.68</td>
<td>104 ± 1.60</td>
</tr>
<tr>
<td>T6</td>
<td>108 ± 1.14</td>
<td>103 ± 1.11</td>
<td>100 ± 1.07</td>
<td>97 ± 0.98</td>
<td>117 ± 1.88</td>
<td>113 ± 1.73</td>
<td>110 ± 1.70</td>
<td>106 ± 1.63</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SE
Means in the same row and the same letter are not significantly different at (0.05)

Table 6: Appearance, flavor, taste and overall acceptability of leg nuggets and patties stored at -18°C.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 day</th>
<th>30 day</th>
<th>60 day</th>
<th>90 day</th>
<th>0 day</th>
<th>30 day</th>
<th>60 day</th>
<th>90 day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appearance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>6.8 ± 0.45</td>
<td>6.8 ± 0.43</td>
<td>6.5 ± 0.38</td>
<td>6.4 ± 0.33</td>
<td>6.4 ± 0.4</td>
<td>6.0 ± 0.5</td>
<td>5.8 ± 0.5</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>T2</td>
<td>7.3 ± 0.50</td>
<td>7.2 ± 0.51</td>
<td>7.0 ± 0.45</td>
<td>6.7 ± 0.42</td>
<td>6.8 ± 0.3</td>
<td>6.2 ± 0.3</td>
<td>6.0 ± 0.3</td>
<td>5.8 ± 0.6</td>
</tr>
<tr>
<td>T3</td>
<td>7.5 ± 0.55</td>
<td>7.4 ± 0.56</td>
<td>7.2 ± 0.51</td>
<td>7.0 ± 0.45</td>
<td>7.5 ± 0.55</td>
<td>7.0 ± 0.5</td>
<td>6.8 ± 0.5</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>T4</td>
<td>7.9 ± 0.60</td>
<td>7.8 ± 0.58</td>
<td>7.5 ± 0.55</td>
<td>7.3 ± 0.50</td>
<td>7.9 ± 0.60</td>
<td>7.5 ± 0.5</td>
<td>7.3 ± 0.5</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td>T5</td>
<td>6.3 ± 0.32</td>
<td>5.9 ± 0.31</td>
<td>5.6 ± 0.26</td>
<td>5.1 ± 0.21</td>
<td>6.0 ± 0.3</td>
<td>5.7 ± 0.3</td>
<td>5.4 ± 0.3</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>T6</td>
<td>6.5 ± 0.36</td>
<td>6.1 ± 0.33</td>
<td>5.9 ± 0.31</td>
<td>5.5 ± 0.25</td>
<td>6.4 ± 0.3</td>
<td>6.1 ± 0.3</td>
<td>6.0 ± 0.3</td>
<td>5.5 ± 0.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SE
Means in the same row and the same letter are not significantly different at (0.05)

**decreased because with the passage of time lipid peroxidation of the nuggets and patties was enhanced and it decreases the taste of the products. The decreasing trend in taste of nuggets may be associated with the peroxidation of PUFA. The results of the study correlate with Yadav et al. [36] who reported a decrease in the taste value of the nuggets and patties stored at -18°C.**

The results of the mean tables of the nuggets and patties showed that T6 was highly acceptable by the panelists as it got highest score for overall acceptability while T5 got minimum score for the overall acceptability. The results are in correlation with Devendra [34] and Tanwar and Biswas et al. [37] who also reported that all the sensory attributes values were decreased significantly with the advancement of storage period.
Conclusion

The results from the present study revealed that the antioxidant potential and quality of the leg meat and meat products of the broiler can be enhanced by incorporating ALA and ATA through feed supplementation. The 150 mg/kg feed dietary level of ALA and ATA (200mg/kg feed) supplementation gives best results and improved the antioxidant potential and enhances the oxidative stability of meat and meat products. It is also evident from results that broiler leg meat nuggets and patties can also be stored (-18°C) ± 2°C up to 90 days.

Acknowledgement

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References
