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Research Article

Impact of Dietary α-Lipoic Acid on Antioxidant Potential of Broiler Thigh Meat

Muhammad Issa Khan,[1,2] Komal Shehzad,[2] Muhammad Sajid Arshad,[3] Amna Sahar,[2] Muhammad Asim Shabbir,[2] and Muhammad Saeed[2]

[1]*College of Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea*
[2]*National Institute of Food Science and Technology, University of Agriculture, Faisalabad 38040, Pakistan*
[3]*Institute of Home and Food Sciences, Government College University, Faisalabad 38000, Pakistan*

Correspondence should be addressed to Muhammad Issa Khan; drkhan@uaf.edu.pk

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The lipid oxidation depressed the meat quality and can be triggered during industrial processing. The current study was designed to assess the antioxidant activity of thigh meat and meat products enriched with natural antioxidants (α-lipoic acid and α-tocopherol acetate). Broilers (21 days) were fed on feed supplemented with varying α-lipoic acid and constant concentration of α-tocopherol acetate for 3 weeks. Birds were slaughtered at the age of 42 days and meat samples were collected and stored for further analysis and product preparation. TPC and DPPH value indicated that meat of broilers receiving 100 mg of α-lipoic acid with 200 mg of α-tocopherol acetate/kg of feed possessed the highest antioxidant activity. TBARS and peroxides values were found to be lower for meat of broilers fed on different levels of α-lipoic acid. The antioxidants (lipoic acid and tocopherol) enrichment in meat helps to reduce PUFAs. A similar lipid stability trend was observed in nuggets prepared from broiler thigh meat and maximum sensory evaluation scores for nuggets prepared from thigh meat of broilers having the highest dose of lipoic acid. The lipoic acid supplementation in feed enhances the antioxidant capacity of thigh meat and meat products.

1. Introduction

Meat is a vital product in human diet having balance chemical composition, high biological value, digestibility, and dietary potential. Broiler meat being low in fat and cholesterol is usually considered healthier than other animal protein sources, especially red meats of mammalian origin [1]. Health and nutritional aspects of broiler meat have led to an increase demand of broiler meat in the whole world. The household income and price of substitute meats relative to flavor and tastiness are other factors that contribute to rising demand of broiler meat. Likewise, awareness of consumers on the health and nutritional value of meat also increases with population growth and food consumption trends [2]. Meat quality is generally judged through its appearance, texture, color, aroma, and taste. The color and taste both significantly affect the purchasing behavior and eating preferences of consumers [3]. Raw broiler meat has little blood-like taste while containing a number of natural components which upon heating generate a large number of volatile compounds responsible for the cooked meat aroma and flavor through thermal degradation of lipids and thiamine, breakdown of peptides and amino acids, and interaction between amino acids and sugars. Besides, pre- and postslaughter factors also have significant effect on broiler meat texture and flavor [4]. Lipid oxidation is considered as one of the primary causes of quality deterioration and flavor defector in meats and other lipid containing food products [5]. Many times myoglobin pigment oxidation is positively correlated with lipid oxidation leading to color as well as odor defects of the fresh meat [6]. Broiler thigh muscles are considered oxidative with more mitochondria and a higher content of myoglobin compared to glycolytic breast muscles and utilize fatty acids as energy source more than glycogen indicating the presence of higher amount of fatty acids in this part of broiler meat [7]. Similarly, cooking is a significant factor that accelerates lipid

peroxidation and volatile production in meat by disrupting muscle cell structure, inactivating antioxidant enzymes and other antioxidant compounds, and releasing iron from heme pigments therefore enhancing fat oxidation in cooked meat [8]. Antioxidant feed supplementation is an effective method to control and preserve the oxidative changes in meat and meat products [9]. In broiler chickens, α-tocopherol feed supplementation not only increases vitamin E concentration in the tissue, but also reduces the rancidity levels in broiler meat and maintains redness of tissues [10]. While alpha lipoic acid (ALA) being a natural antioxidant acts as an NADH oxidase inhibitor to block oxidant production, diminishes hepatic fatty acid oxidation in broiler chickens, and reduces the ketone body production. Dietary ALA supplementation also recycles the vitamin E contents of the muscles and potentially reduces the incidence of PSE (pale, soft, and exudative) meat [11]. Whenever vitamin E and lipoic acid are used together, their antioxidant properties improve as lipoic acid recycles vitamin E and lowers the oxidative stress and protects immune cells [12]. The objective of the present research was to evaluate the effect of alpha lipoic acid and alpha tocopherol acetate as feed supplement in broiler diet and to assess the oxidative stability of raw and processed thigh meat and meat product.

2. Materials and Methods

2.1. Procurement of Birds and Feed Ingredients. 150 chicks (Hubbard) were purchased from Hi-Tech Feeds (Pvt.) Ltd., Pakistan. The birds were randomly selected having the same weight and were kept in room reserved for research at University of Agriculture, Faisalabad, Poultry Farm having pens of 4 × 3 × 1.5 feet. The room and the pens were cleaned thoroughly, white-washed, and disinfected. There were 5 groups and 3 replicates for each group. Each replicate carried 10 birds. The experimental birds were reared up to six weeks. For the first three weeks, the chicks were fed on basal feed and the following three weeks chicks were fed on feed supplemented with different levels of alpha lipoic acid (T_0: control; T_1: 25 mg/kg; T_2: 50 mg/kg; T_3: 75 mg/kg; T_4: 100 mg/kg) with constant level of alpha tocopherol acetate (200 mg/kg).

2.2. Sample Collection and Preparation. The birds were slaughtered according to Islamic halal ethical guidelines. The protocol for this method was approved in our institute. At the age of 6 weeks thigh meat samples of each treatment were kept in polyethylene plastic bags and stored at −18°C till further analysis. 5 gram thigh meat sample was taken in 50 mL polypropylene tube having a cap and sample was homogenized by using phosphate buffer and glycerol (20%) pH (7.4) in 1 : 10 ratio with the help of homogenizer. The homogenized thigh meat sample was centrifuged at 1000 ×g for ten minutes and 20-second rest was given to dissipate the heat generated during homogenization. Supernatant of each treatment was collected and stored at −18°C for further analysis [13].

2.3. Assessment of Antioxidant Status in Raw Thigh Meat. Total phenolic contents of thigh meat were determined by a procedure followed by Senevirathne et al. [14]. The antioxidant activity of thigh meat was assessed by measuring its DPPH stable radicals scavenging abilities by the method as described by Brand-Williams et al. [15]. Oxidative stability of broiler thigh meat sample was determined by measuring mg of malondialdehyde per kg of meat following the method described by Asghar et al. [16].

2.4. Quantification of Alpha Lipoic Acid by Using HPLC. The frozen sample (500 mg) was ground in mortar pestle for the isolation of the alpha lipoic acid from meat. The meat tissue was homogenized with 2 mL of m-phosphoric acid (20% w/v). After 2-minute vortex of sample, 3 mL of n-hexane and 250 μL isopropanol were added and then tube was shaken for 30 minutes for the extraction of alpha lipoic acid. The tube was centrifuged at 1500 rpm and was collected the upper n-hexane layer in vial. We repeated this step twice and pooled the n-hexane layer [17]. The mobile phase was prepared by using acetonitrile and water (80 : 20). The sample was eluted isocratically with 1 mL/minute of flow rate. The standards of alpha lipoic acid were prepared by dissolving alpha lipoic acid in n-hexane as a stock solution (30 μg/mL), where the further dilutions were made 10 μg/mL, 60 μg/mL, and 90 μg/mL, respectively. A Shimadzu (Kyoto, Japan) HPLC system consisting of two LC-10AD pumps, an SCL-10A system controller, a manual injector, and a degasser (DGU-12A) was used. The analytical column for reversed-phase chromatography was a 5 μm particle size Shim-Pack CLC (C18) column, 15 cm × 4.6 mm × 5 μm (Shinwa Chemicals, Kyoto, Japan). The columns were maintained at 25°C by a CTO-10A column oven (Shimadzu). The effluent was monitored by SPD-l0AVYP UV-Vis and RF-10AXL fluorescence detectors equipped with an 8 μL flow cell (Shimadzu). The fluorescence detector was set at 343 nm.

2.5. Quantification of Alpha Tocopherol by Using HPLC. One-gram sample was taken in centrifuge tube (30 mL) for alpha tocopherol extraction. Tissue was solubilized when 1.5 mL of urea (6 M) was added in tube. The tissue of meat was disintegrated when 1 mL of 0.1 M sodium dodecyl sulphate (SDS) solution was added in tube. The 4 mL of ethyl alcohol (EtOH 95%) containing 1% pyrogallol was added in tube for deproteination and freeing of alpha tocopherol from the meat tissue. The phase separation was facilitated by adding 10 mL of petroleum ether in tube and vortex for 2 minutes. Then the tube was centrifuged at 2000 rpm for 5 minutes. The upper solvent layer was transferred in a vial. This step was repeated two times for complete extraction of alpha tocopherol. Evaporate the pooled solvent phase under nitrogen to dryness. 500 μL EtOH (mobile phase) was added in tube; tight the screw and vortex the tube for 1 minute at 45°C (in water bath) to facilitate the solubilization of alpha tocopherol in mobile phase. The sample was filter, using Anspec H1056 microfilter and centrifugation at 2000 rpm for 5 minutes. The filtrate was collected in the vial and stored in the dark place to protect degradation of tocopherol in the sample. The

standard of alpha tocopherol was prepared by dissolving 1 μg of alpha tocopherol in 1 mL of methanol. Further dilutions of 2 μg/mL, 3 μg/mL, and 4 μg/mL were made, respectively. A Shimadzu (Kyoto, Japan) HPLC system consisting of two LC-10AD pumps, an SCL 10A system controller, manual injection syringe, and a degasser (DGU-12A) was used. The analytical column for reversed-phase chromatography was a 5 μm particle size Shim-Pack CLC (C18) column, 15 cm × 4.6 mm × 5 μm (Shinwa Chemicals, Kyoto, Japan). The columns were maintained at 25°C by a CTO-10A column oven (Shimadzu). The effluent was monitored by SPD-10AVvp UV-Visible detectors equipped with an 8 μL flow cell (Shimadzu). The UV-Vis detector was set at 290 nm. A linear gradient elution from methanol (100%) was adopted. The flow rate of the mobile phase was 1.0 mL/minute. The peak areas obtained from detector were calculated using Vstation chromatography software (GL Science, Tokyo, Japan). This method was modified form of Asghar et al. [18].

2.6. Peroxide Value. Oxidation was evaluated by peroxide value. Peroxide value of the oil recovered from thigh samples was determined by a procedure described by Shantha and Decker [19]. The lipid sample (5.0 g) was treated with 25 mL of organic solvent mixture (chloroform/acetic acid, 2/3: v/v). The mixture was shaken vigorously, followed by addition of 1 mL of saturated potassium iodide solution. The mixture was kept in the dark for 5 min before adding 75 mL of distilled water. The mixture was titrated against standardized 0.01 N sodium thiosulfate solution till yellow color almost disappeared. Then 0.5 mL of starch solution (1% w/v) was added to the mixture, as an indicator, shaken vigorously, and carefully titrated till blue color just disappeared.

2.7. Fatty Acids Profile of Thigh Meat. Free fatty acids contents in oil recovered from thigh samples were quantitatively measured through gas chromatography coupled with flame ionization detector (GC-FID) according to a method described by [20]. 100 mg of oil sample was saponified with 100 μL 2 N KOH, and 3 mL hexane was added to the mixture. The mixture was vigorously shaken with a vortex for 1 min and then centrifuged at 5000 rpm for 5 min and left overnight at room temperature for phase separation. The top hexane layer containing methylated fatty acids was analyzed for fatty acids composition using a GC. Gas Chromatograph (Agilent Technologies, 6890 N) equipped with an autosampler, flame-ionization detector (FID), and a HP-5 column (Silica 30 m × 0.25 film thickness, Hewlett Packard Co.) was used for fatty acids separation. A ramped oven temperature condition (180°C for 2.5 min, increased to 230°C at 2.5°C/min and then held at 230°C for 7.5 min) was used. Temperatures of both inlet and detector were 280°C. Helium was the carrier gas at linear flow of 1.1 mL/min linearly. Detector (FID) air, hydrogen gas, and make-up gas (He) flows were 350, 35, and 43 mL/min, respectively. Fatty acids were identified by the retention time of known standards using Agilent Chem. Station software. Relative quantities were expressed as weight % of total fatty acids.

2.8. Product Development. The raw meat samples stored at −18°C were used to prepare nuggets for the assessment of frying effect on the oxidative stability of processed products.

2.9. Analysis of Thigh Nuggets. Oxidative stability of nuggets samples was determined by measuring mg of malondialdehyde per kg of meat by following the method described by Asghar et al. [16]. Peroxide value of the oil recovered from nuggets samples was determined by a procedure described in Shantha and Decker [19].

2.10. Sensory Evaluation of Nuggets. The sensory evaluation of thigh nuggets for color, taste, flavor, appearance, and overall acceptability was carried on 9-point hedonic scale to anticipate the acceptability of meat products on quality bases.

2.11. Statistical Analysis. The data obtained from each treatment was subjected to statistical analysis to determine the level of significance by the factorial design (2-way interaction) described by Steel et al. [21] by using the software package (statistic 8).

3. Results and Discussion

3.1. Antioxidant Capacity and Lipoid Oxidation of Thigh Meat. The antioxidant activity of the tissue is assessed by its total phenolic acid; the higher the total phenolic contents, the higher the free radical scavenging activity. Supplementation of biological antioxidants, that is, different level of α-lipoic acid with constant α-tocopherol acetate, significantly increased the phenols content with the increase in α-lipoic acid (Table 1). The highest TPC (61.28 ± 2.1 mg of GAE/g) was observed in meat of broiler fed on maximum amount of supplemented α-lipoic acid (T_4) while the lowest TPC (49.03 ± 1.9 mg of GAE/g) in broiler meat fed o ncontrol feed (T_0). Arshad at al. [22] reported an increase in level of total phenolic contents in microsomal fraction of thigh meat with increase in α-lipoic acid concentration in feed. The total phenolic contents of thigh meat decreased during 60-day storage of meat from 59.1 ± 2.8 mg of GAE/g (0 day) to 50.73 ± 1.4 mg of GAE/g (60 days). It is evident from results of DPPH free radicals scavenging assay of thigh meat that free radical scavenging activity of broiler thigh meat is significantly influenced by the antioxidant supplementation of broiler feed (Table 1). The highest DPPH percent inhibition values of thigh samples were observed in T_4 (81.80 ± 3.6%) containing maximum amount of alpha lipoic acid and lowest inhibition (71.92 ± 2.4%) was observed for control samples (T_0). DPPH percent inhibition values were observed for broiler fed on control diet. These findings are in agreement with previous study of Fasseas et al. [23] who concluded that vitamin E and sage extract increased the antiradical power of meat more than control treatment. These findings are also in good agreement with Min et al. [24] who reported that DPPH radical scavenging activities of thigh meat did not decrease significantly during storage. Lipid peroxidation is measure of malondialdehyde compounds formed during autoxidation of lipid in meat tissue. It is obvious from results that the

Table 1: Effect of different groups on total phenolic contents (mg of GAE/g) and DPPH (%) activity in thigh meat.

Groups	Total phenolic contents (mg of GAE/g)				DPPH activity (%)			
	Day 0	Day 30	Day 60	Mean	Day 0	Day 30	Day 60	Mean
T_0	54.5 ± 2.4	49.8 ± 1.8	43.3 ± 1.4	49.03 ± 1.9^e	76 ± 3.4	71.9 ± 2.2	65.44 ± 2.6	71.92 ± 2.4^d
T_1	56.3 ± 2.5	53 ± 1.6	46.7 ± 1.6	52 ± 1.7^d	79 ± 3.2	73.6 ± 2.6	66.4 ± 2.8	73 ± 2.9^d
T_2	59 ± 2.6	55.2 ± 1.3	50.8 ± 1.8	55 ± 1.3^c	83.2 ± 2.5	76.9 ± 3.4	68 ± 2.4	76.03 ± 3.8^c
T_3	61.7 ± 2.4	58 ± 1.9	54.5 ± 1.4	58.06 ± 1.4^b	85.10 ± 3.7	77.9 ± 2.8	72.2 ± 2.6	78.4 ± 2.7^b
T_4	64.5 ± 2.3	61 ± 2.1	58.3 ± 2.1	61.28 ± 2.1^a	87.3 ± 3.9	81 ± 3.4	77.1 ± 2.6	81.8 ± 3.6^a
Mean	59.1 ± 2.8^a	55.40 ± 2.2^b	50.73 ± 1.4^c		82.12 ± 3.1^a	76.26 ± 3.1^b	69.82 ± 2.7^c	

Results are presented as mean ± SD ($n = 3$), whereas results in the same column with no superscripts in common differ significantly at $P < 0.01$.
T_0: control, T_1: control + 200 mg vitamin E + 25 mg α-lipoic acid/kg feed, T_2: control + 200 mg vitamin E + 50 mg α-lipoic acid/kg feed, T_3: control + 200 mg vitamin E + 75 mg α-lipoic acid/kg feed, and T_4: control + 200 mg vitamin E + 100 mg α-lipoic acid/kg feed.

highest TBARS value (0.284 ± 0.02 μg MDA/g) of thigh meat was recorded for meat sample of broilers fed on control diet (T_0) while the lowest TBARS value (0.208 ± 0.04 μg MDA/g) was found for meat sample of broilers fed on feed containing 100 mg ALA/Kg feed (T_4) (Table 2). The increase in TBARS value was observed with the progression of storage period. Botsoglou et al. [25] showed that dietary antioxidants supplementation significantly reduces lipid oxidation during frozen storage and subsequent refrigeration. The decrease in TBARS values was observed by Fernández et al. [26] and Cardenia et al. [27] if antioxidants are incorporated into broiler meat during storage. The peroxide value is used to detect the oxidation of unsaturated fatty acids or more accurately determining the oxidation state of fatty acids. It is evident from the data that peroxide values for different treatments during storage varied significantly ($P < 0.01$). The highest peroxide value (1.88 ± 0.04 meq O_2/kg of fat) was observed in sample after 2 months of storage while the lowest value (0.69 ± 0.06 meq O_2/kg of fat) was recorded at the start of experiment. As depicted in data the highest peroxide value (1.88 ± 0.04 meq O_2/kg of fat) was found in thigh meat of broiler fed on control diet (T_0) while the lowest peroxide value (0.69 ± 0.06 meq O_2/kg of fat) was observed in treatment T_4 (100 mg lipoic acid/Kg feed) at the end of storage (60 days) (Table 2). Levermore [28] reported that peroxide formation during storage depends on presence of antioxidants, length of storage, and quality of lipid and rate of formation increases with passage of time. The findings of research are in agreement with Gheisari [29] observations that peroxide value increased during refrigerated storage of meat.

3.2. Alpha Lipoic Acid and Alpha Tocopherol Contents. Alpha lipoic acid is a novel antioxidant having lipid lowering effect while exogenous supply of α-lipoic acid appears to impart a variety of significant positive effects in biological system including free radical scavenging potential [30]. Alpha lipoic acid and alpha tocopherol contents varied significantly ($P < 0.01$) among different treatments. The highest lipoic acid contents (49.1 ± 3.7 μg/g) were reported at the start of storage while the lowest contents (42.1±3.7 μg/g) were recorded at the end of storage period (60 days) (Table 3). The data explicated that the highest lipoic acid contents (69.40 ± 4.8 μg/g) of thigh meat were recorded in birds getting higher level of dietary lipoic acid (T_4) while the lowest lipoic acid contents (22.17 ± 1.5 μg/g) were observed in broilers fed on control diet. These results are in agreement with previous findings of Parveen et al. [31] who reported that the deposition of α-lipoic acid in thigh muscles increases with progressive increasing of the α-lipoic acid in the feed. The gradual increase in the α-lipoic acid concentration increases the deposition of α-tocopherol, thus improving the antioxidant status of meat as reported by Arshad et al. [22]. Decrease in lipoic acid concentration upon storage may be due to short-term incubation and rapid uptake by tissue or cells [32]. The tocopherol contents vary from 49.4±3.9 μg/g to 109.3±6.8 μg/g for different treatments. The highest tocopherol contents (79.28 ± 5.6 μg/g) for all treatments were reported in the first day of storage while the lowest value (74.93 ± 7.6 μg/g) for all treatments was recorded at 2nd month storage. Similarly, it is clear from data that the highest tocopherol value (111.84±4.9 μg/g) of thigh meat was recorded with treatment T_4 while the lowest tocopherol value (47.75 ± 4.2 μg/g) was found in treatment T_0 at the end of storage study (Table 3). It can be concluded from the above results that T_4 containing 200 mg α-tocopherol acetate + 100 mg α-lipoic acid exhibited more deposition of tocopherol in thigh muscles as compared with T_0 (control) during 2-month storage study. Alpha tocopherol contents also increase with an increase in the level of ALA broiler feed. Our results depicted the fact that alpha lipoic acid has positive impact on alpha tocopherol contents, which is also in line with Arshad et al. [22].

3.3. Fatty Acid Profile of Thigh Meat. The fatty acid profile (mg/100 g) results related to broiler thigh meat revealed lauric acid (C-12) varying from 0.027 to 0.021 (mg/100 g), myristic acid (C-14) varying from 0.85 to 0.80 (mg/100 g), and palmitic acid (C-16) varying from 29.79 to 26.14 (mg/100 g) in broiler thighs at different α-lipoic acid concentration with constant α-tocopherol acetate concentration indicating a decrease in SFAs values due to these antioxidants' supplementation. However, values of arachidonic acid (C-20:4) ranged from 0.78 to 0.75 (mg/100 g), eicosapentaenoic acid (C-20:5) ranged from 0.068 to 0.05 (mg/100 g), and docosahexaenoic acid (C-22:6) ranged from 0.35 to 0.31 (mg/100 g), respectively, showing a decrease in these PUFAs

Table 2: Effect of different groups on TBARS value (μg MDA/g) and peroxide value (meq O_2/kg of fat) in thigh meat.

Groups	TBARS value (μg MDA/g)				Peroxide value (meq O_2/kg of fat)			
	Day 0	Day 30	Day 60	Mean	Day 0	Day 30	Day 60	Mean
T_0	0.198 ± 0.009	0.28^{d} ± 0.01	0.375 ± 0.03	0.284 ± 0.02^{a}	0.98 ± 0.06	1.35 ± 0.07	1.88 ± 0.04	1.40 ± 0.04^{a}
T_1	0.179 ± 0.007	0.244 ± 0.02	0.334 ± 0.04	0.252 ± 0.03^{b}	0.90 ± 0.07	1.28 ± 0.09	1.67 ± 0.07	1.28 ± 0.09^{b}
T_2	0.165 ± 0.005	0.226 ± 0.01	0.306 ± 0.02	0.232 ± 0.01^{c}	0.84 ± 0.09	1.21 ± 0.07	1.54 ± 0.06	1.19 ± 0.04^{c}
T_3	0.154 ± 0.009	0.213 ± 0.01	0.292 ± 0.04	0.219 ± 0.03^{d}	0.76 ± 0.04	1.13 ± 0.02	1.42 ± 0.08	1.10 ± 0.01^{d}
T_4	0.147 ± 0.005	0.199 ± 0.01^{i}	0.278 ± 0.03	0.208 ± 0.04^{e}	0.69 ± 0.06	1.06 ± 0.01	1.33 ± 0.04	1.02 ± 0.05^{e}
Mean	0.168 ± 0.004^{c}	0.232 ± 0.02^{b}	0.317 ± 0.04^{a}		0.83 ± 0.04^{c}	1.20 ± 0.06^{b}	1.56 ± 0.06^{a}	

Results are presented as mean ± SD ($n = 3$), whereas results in the same column with no superscripts in common differ significantly at $P < 0.01$.
T_0: control, T_1: control + 200 mg vitamin E + 25 mg α-lipoic acid/kg feed, T_2: control + 200 mg vitamin E + 50 mg α-lipoic acid/kg feed, T_3: control + 200 mg vitamin E + 75 mg α-lipoic acid/kg feed, and T_4: control + 200 mg vitamin E + 100 mg α-lipoic acid/kg feed.

Table 3: Alpha lipoic acid content (μg/g) and alpha tocopherol content (μg/g) in thigh meat.

Groups	Alpha lipoic acid content (μg/g)				Alpha tocopherol content (μg/g)			
	Day 0	Day 30	Day 60	Mean	Day 0	Day 30	Day 60	Mean
T_0	25.8 ± 1.2	22.1 ± 1.4	18.6 ± 0.9	22.17 ± 1.5^{e}	49.4 ± 3.9	47.8 ± 4.5	45.5 ± 5.7	47.75 ± 4.2^{e}
T_1	39.2 ± 2.4	37 ± 2.5	34.7 ± 2.5	36.97 ± 2.9^{d}	63.6 ± 4.9	62 ± 6.3	59.7 ± 5.6	61.77 ± 5.1^{d}
T_2	47.9 ± 3.4	45.5 ± 3.5	39.2 ± 3.3	44.20 ± 2.1^{c}	74.9 ± 5.2	73 ± 4.1	70.2 ± 4.6	72.70 ± 6.8^{c}
T_3	59.4 ± 3.8	56 ± 4.1	52.7 ± 4.3	56.03 ± 5.7^{b}	94.8 ± 8.1	93.2 ± 2.3	90.1 ± 8.1	92.70 ± 9.4^{b}
T_4	73.2 ± 5.4	69.7 ± 5.3	65.3 ± 6.2	69.40 ± 4.8^{a}	113.7 ± 9.7	112.5 ± 3.4	109.3 ± 6.8	111.84 ± 4.9^{a}
Mean	49.1 ± 3.7^{a}	46.06 ± 3.9^{b}	42.1 ± 3.7^{c}		79.28 ± 5.6^{a}	77.7 ± 6.1^{b}	74.93 ± 7.6^{c}	

Results are presented as mean ± SD ($n = 3$), whereas results in the same column with no superscripts in common differ significantly at $P < 0.01$.
T_0: control, T_1: control + 200 mg vitamin E + 25 mg α-lipoic acid/kg feed, T_2: control + 200 mg vitamin E + 50 mg α-lipoic acid/kg feed, T_3: control + 200 mg vitamin E + 75 mg α-lipoic acid/kg feed, and T_4: control + 200 mg vitamin E + 100 mg α-lipoic acid/kg feed.

(Table 4). It is evident from the findings that concentration of PUFAs decreases with increase in lipoic acid concentration. The same trend of a decrease in PUFAs was observed by Çelik and Özkaya [33] using the same antioxidants. The decreasing trend of SFAs and MUFAs is in agreement with results of Cortinas et al. [34] who observed a decreasing trend in SFAs and MUFAs in thigh meat with α-tocopherol acetate supplementation in feed. The changing trend in fatty acids composition is in line with findings of Kolsarıcı et al. [35] who stored mechanically deboned chicken meat for 4 months and with results of Bou et al. [36] who observed the same trend in stored white and dark mixed meat for 5 months at −20°C. The same trend was found in the findings of Arshad et al. [13, 37] who found that using α-lipoic acid decreases the fatty acids profile in chicken thigh meat.

3.4. TBARS Values and POV of Thigh Meat Nuggets. The TBARS values of different treatments for broiler thigh nuggets varied significantly ($P < 0.01$). Highest TBARS value (0.515 ± 0.04 μg MDA/g) of broiler thigh nuggets was recorded with control samples (T_0) while the lowest TBARS value (0.296±0.01 μg MDA/g) occurred in the broiler thigh nuggets with T_4 samples. The highest TBARS mean value (0.482 ± 0.03 μg MDA/g) for broiler thigh nuggets was reported at 2 months of storage while the lowest value (0.290 ± 0.01 μg MDA/g) was recorded at the start of storage (Table 5). The combinations of α-lipoic acid with constant concentration of α-tocopherol acetate resulted in less MDA production as compared to control samples. The same trend of lowering TBARS values due to antioxidants was observed in previous study by Haak et al. [38] and Sohaib et al. [39] in cooked meat products. Soyer et al. [40] observed increase in TBARS values of the meat products with cooking. Similarly POVs of different treatments for broiler thigh nuggets varied significantly ($P < 0.01$). It is manifest from the data that the mean peroxide values of broiler thigh nuggets vary from 1.02 ± 0.04 meq O_2/kg of fat to 3.88 ± 0.4 meq O_2/kg of fat for different treatments during 2-month frozen storage (Table 5). The highest peroxide value (2.71 ± 0.1 meq O_2/kg of fat) of broiler thigh nuggets was recorded with control samples (T_0) while the lowest peroxide value (1.91 ± 0.2 meq O_2/kg of fat) occurred in the broiler thigh nuggets from T_4 samples. Peroxide values increase with storage days while frying of nuggets also results in an increase in POV as cooking process and salt addition enhance lipid peroxidation, Mohamed et al. [41]. Thus freezing with subsequent frying resulted in an increase in peroxide values of nuggets. These are in agreement with Teets and Wenk [42] who state that duration of cooked chicken patties storage had significant effect on POV. Thomas et al. [43] have a similar finding regarding storage of cooked meat products and increase in peroxide value.

3.5. Effect on Sensory Parameters of Nuggets. Sensory evaluation is generally used to predict the acceptability of a new product made through ingredient modification, Barbut [44]. In cooking procedure color serves as a cue for the acceptance of foods and is correlated with change in aroma and flavor. The results depicted that the color scores for different

Table 4: Effect of different groups on TBARS value (μg MDA/g) and peroxide value (meq O_2/kg of fat) in thigh chicken meat nuggets.

Groups	TBARS value (μg MDA/g)				Peroxide value (meq O_2/kg of fat)			
	Day 0	Day 30	Day 60	Mean	Day 0	Day 30	Day 60	Mean
T_0	0.389 ± 0.02^{ef}	0.512 ± 0.03^{b}	0.645 ± 0.04^{a}	0.515 ± 0.04^{a}	1.56 ± 0.02^{h}	2.71 ± 0.2^{e}	3.88 ± 0.4^{a}	2.71 ± 0.1^{a}
T_1	0.327 ± 0.01^{h}	0.401 ± 0.04^{de}	0.510 ± 0.02^{b}	0.412 ± 0.02^{b}	1.38 ± 0.1^{hi}	2.45 ± 0.3^{f}	3.51 ± 0.3^{b}	2.44 ± 0.2^{b}
T_2	0.289 ± 0.02^{i}	0.377 ± 0.02^{f}	0.456 ± 0.03^{c}	0.374 ± 0.01^{c}	1.25 ± 0.2^{ij}	2.13 ± 0.1^{g}	3.27 ± 0.2^{c}	2.05 ± 0.3^{c}
T_3	0.245 ± 0.01^{j}	0.342 ± 0.04^{g}	0.411 ± 0.02^{d}	0.332 ± 0.02^{d}	1.11 ± 0.01^{jk}	2.04 ± 0.1^{g}	3.01 ± 0.1^{d}	2.21 ± 0.1^{d}
T_4	0.201 ± 0.02^{k}	0.299 ± 0.01^{i}	0.389 ± 0.01^{ef}	0.296 ± 0.01^{e}	1.02 ± 0.04^{k}	1.93 ± 0.2^{g}	2.78 ± 0.2^{e}	1.91 ± 0.2^{e}
Mean	0.290 ± 0.01^{c}	0.386 ± 0.02^{b}	0.482 ± 0.03^{a}		1.26 ± 0.1^{c}	2.25 ± 0.1^{b}	3.29 ± 0.3^{a}	

Results are presented as mean ± SD ($n = 3$), whereas results in the same column with no superscripts in common differ significantly at $P < 0.01$.
T_0: control, T_1: control + 200 mg vitamin E + 25 mg α-lipoic acid/kg feed, T_2: control + 200 mg vitamin E + 50 mg α-lipoic acid/kg feed, T_3: control + 200 mg vitamin E + 75 mg α-lipoic acid/kg feed, and T_4: control + 200 mg vitamin E + 100 mg α-lipoic acid/kg feed.

Table 5: Fatty acids profile (mg/100 g of fat) of chicken thigh meat.

Groups	Fatty acids (mg/100 g of fat) of chicken thigh meat											Fatty acids profile (%)			
	12:0	14:0	16:0	16:1	18:0	18:1n9	18:2n6	18:3n3	20:4n6	20:5n3	22:6n3	SFA	MUFA	PUFA	UFA
T_0	0.027^{a}	0.85^{a}	29.79^{a}	1.46^{a}	3.28^{a}	34.29^{a}	22.72^{a}	35.48^{a}	0.78^{a}	0.068^{a}	0.35^{a}	33.95^{a}	95.15^{a}	35.75^{a}	59.398^{a}
T_1	0.026^{a}	0.84^{a}	28.42^{a}	1.45^{a}	3.28^{a}	33.85^{a}	22.01^{a}	34.14^{a}	0.77^{a}	0.07^{a}	0.34^{a}	32.57^{b}	92.63^{b}	35.3^{a}	57.33^{b}
T_2	0.024^{b}	0.82^{b}	27.64^{b}	1.43^{b}	3.27^{a}	33.14^{a}	21.14^{a}	32.69^{b}	0.76^{a}	0.06^{a}	0.32^{a}	31.75^{c}	89.54^{c}	34.57^{b}	54.97^{c}
T_3	0.023^{b}	0.81^{b}	27.21^{b}	1.42^{b}	3.25^{b}	32.19^{b}	20.22^{b}	31.47^{b}	0.76^{a}	0.06^{a}	0.34^{a}	31.29^{bc}	86.46^{d}	33.61^{b}	52.85^{d}
T_4	0.021^{c}	0.8^{b}	26.14^{b}	1.39^{c}	3.22^{c}	32.01^{b}	19.41^{c}	29.48^{c}	0.75^{a}	0.05^{a}	0.31^{a}	30.18^{d}	83.40^{e}	33.4^{b}	50^{e}

Results are presented as mean ($n = 3$), whereas results in the same column with no superscripts in common differ significantly at $P < 0.01$.
T_0: control, T_1: control + 200 mg vitamin E + 25 mg α-lipoic acid/kg feed, T_2: control + 200 mg vitamin E + 50 mg α-lipoic acid/kg feed, T_3: control + 200 mg vitamin E + 75 mg α-lipoic acid/kg feed, and T_4: control + 200 mg vitamin E + 100 mg α-lipoic acid/kg feed.

Table 6: Sensory evaluation of chicken thigh meat nuggets.

Parameters	T_0		T_1		T_2		T_3		T_4	
	0	60	0	60	0	60	0	60	0	60
Color	6.5	5.8	6.2	5.2	6.0	5.1	6.0	5.5	6.2	5.1
Taste	7.7	6.8	6.8	5.4	7.0	5.9	7.2	6.2	7.8	6.8
Flavor	7.9	7.6	7.1	6.6	7.0	6.4	7.3	6.8	7.9	7.6
Appearance	8.1	7.1	7.4	6.8	6.1	6.0	6.8	6.1	8.0	7.5
Acceptability	7.7	7.4	7.3	7.0	7.3	7.1	7.2	7.0	7.8	7.5

Results are presented as mean ($n = 3$). T_0: control, T_1: control + 200 mg vitamin E + 25 mg α-lipoic acid/kg feed, T_2: control + 200 mg vitamin E + 50 mg α-lipoic acid/kg feed, T_3: control + 200 mg vitamin E + 75 mg α-lipoic acid/kg feed, and T_4: control + 200 mg vitamin E + 100 mg α-lipoic acid/kg feed.

treatments vary significantly ($P < 0.01$). Yet, the interactive effect of treatments and storage of product showed significant ($P < 0.01$) effect on color scores of thigh nuggets. The highest mean score of color for broiler thigh nuggets was reported at the start of storage while the lowest mean score was recorded at the end of 60-day storage (Table 6). The results of study are in agreement with Naveena et al. [45] who stated that color value of the chicken patties decreases with passage of the time. Similar results were presented by Fasseas et al. [23] and Sohaib et al. [39] in their respective studies where storage has influence on color of meat products. The taste scores of product also decreased with the progression of storage periods and this decrease in taste scores may be attributed to peroxidation of PUFA. In earlier studies Biswas et al. [46] and Sohaib et al. [39] also reported the significant decrease in taste of the nuggets with the advancement of storage period. Flavor is an important parameter in sensory evaluation of a food product and it is the combined perception of smell, taste, and mouth feel. Flavor scores significantly decreased with progression of storage period. These results confirm the findings of Devendra and Tanwar [47] who reported that all the sensory quality values decreased significantly with the advancement of storage period. However, antioxidants have positive impact on the flavor of nuggets as nuggets prepared from meat having higher concentration of lipoic acid were liked more by consumers. The appearance and overall acceptability of nuggets decreased significantly with the advancement in storage period. The previous researchers also have a similar finding that sensory score decreased with passage of storage time [39, 48, 49].

4. Conclusion

Alpha tocopherol and lipoic acid both are strong natural antioxidants and their supplementation in broiler diet can enhance the antioxidant potential of thigh meat and meat

products. 100 mg/kg feed dietary level of α-lipoic acid and α-tocopherol acetate (200 mg/kg feed) supplementation gives best results and enhances the oxidative stability of meat and meat products. Future researches are required for investigating the impact on meat products flavor volatiles and the development of antioxidants-enriched meat, which would benefit the meat industry.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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