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Effects of Packaging Methods on the Meat Quality of α-Tocopherol Supplemented Broiler Chicks during Refrigerated Storage

Youn-Chul Ryu, Min-Suk Rhee, Moo-Ha Lee[1], Sung-Ki Lee[2], and Byoung-Chul Kim*

Division of Food Science, College of Life and Environmental Sciences, Korea University, Seoul 136-713, Korea
[1]Department of Food and Animal Biotechnology, Seoul National University, Seoul 151-742, Korea
[2]Department of Food Science and Technology in Animal Resources, Kangwon National University, Gangwon 200-701, Korea

Abstract This study examined the effects of vacuum packaging and modified atmosphere packaging (MAP) on the meat quality of α-tocopherol supplemented broiler chicks after prolonged refrigeration. The supplemented α-tocopherol levels had no effect on the body weight and feed efficiency. The dietary treatments and packaging methods had no significant effects on the level of microbial growth, lightness, and the metmyoglobin content. The vacuum packaging showed the highest level of purge loss. The broilers supplemented with either 200 or 400 IU of α-tocopherol had a lower level of malondialdehyde than the control group. The vacuum packaged meats from chicks fed the control diet showed the highest levels of lipid oxidation. The lipid oxidative stability was most improved in modified atmosphere (20% CO_2 + 80% N_2) packaged breast meat from the broilers supplemented with either 200 or 400 IU of α-tocopheryl acetate.

Keywords: α-tocopherol, broiler, color stability, lipid oxidation, packaging methods

Introduction

Lipid oxidation and discoloration are the major causes of the deterioration in meat quality during refrigeration. Poultry meat is sensitive to oxidative deterioration due to the high levels of polyunsaturated fatty acids (1, 2). Many studies have reported that lipid oxidation in meat products can be effectively controlled using antioxidants (3-6). The oxidative stability and shelf life of poultry meat can be easily improved by feeding poultry a higher level of dietary antioxidants (7).

It is well known that α-tocopherol supplementation in poultry diets increases the α-tocopherol concentration in the tissue with a concomitant increase in the stability of the meat (8-10). α-Tocopherol is a chain-breaking lipid antioxidant and a free radical scavenger in cell membrane and sub-cellular organs (11). The antioxidant effect of α-tocopherol on the breast and thigh muscles of chickens has been well documented (12, 13).

Vacuum and modified atmosphere packaging (MAP) has gained popularity in the past few years in the meat industry (14, 15). Oxygen, nitrogen, and carbon dioxide are the gases normally used in MAP. The elimination of O_2 from packaging as well as the introduction of different CO_2 and N_2 concentrations inhibits the growth of aerobic micro-organisms, proteolytic bacteria, yeast, and fungi (16). The effectiveness of MAP to extend the shelf life of chicken meat has been examined (17, 18). Generally, studies on rancidity have been performed using processed meats or after the addition of antioxidant supplements into the feed (4, 19). However, there are few studies on the effects of vacuum packaging or MAP on the lipid oxidation level in poultry meat over extended periods. This study investigated the effects of vacuum packaging and the MAP system on the quality change in meat obtained from α-tocopherol supplemented broiler chicks during extended refrigeration periods up to 24 days.

*Corresponding author: Tel: 82-2-3290-3052; Fax: 82-2-925-1970
E-mail: bckim@korea.ac.kr
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Materials and Methods

Birds and diets Two hundred and forty male broiler chicks (Arbor Acres, 1 day old) were randomly assigned to 5 pens containing 48 chicks each, which correspond to 5 dietary treatments. This experiment was repeated 3 times (total 720 chicks, 144 chicks/treatment, 48 birds/pen). Table 1 and 2 show the ingredients and composition of the starter and basal diets. Up to 3 weeks of age, the chicks were fed the same starter diet. From 3 to 6 weeks of age, 5 different α-tocopherol levels were supplied to the chicks as follows: The control (CON) received the basal diet consisting of α-tocopherol at a rate of 20 IU/kg feed; Treatments 1 (50), 2 (100), 3 (200), or 4 (400) were fed the basal diet supplemented with 50, 100, 200, or 400 IU of *all-rac*-α-tocopheryl acetate/kg feed, respectively. The chicks were maintained on a 24-hr constant lighting schedule and given access to feed and tap water *ad libitum* until slaughter at 42 days of age. The body weight and feed consumption for the individual pens were recorded at 1, 7, 14, 21, 32, and 42 days.

Packaging and storage After 42 days of feeding, all the broilers were slaughtered at a conventional slaughtering plant, and the carcasses were chilled in a spin chiller. The carcasses were then weighed and packed individually using the following 4 different packaging methods: MAP1 and MAP 2 were packed using BB4l pouches with a low gas permeability (7 cc/m^2/24 hr O_2 at 4°C and 80% RH, 150 cc/m^2/24 hr CO_2 at 23°C and 75% RH, water vapor transmission rate was 1.5 g/m^2/24 hr at 38°C and 100% RH) and flushed with either 100% N_2 (MAP1) or 80% N_2

Table 1. Ingredients and composition of the starter diets fed up to 3 weeks of age

Ingredient	%	Composition	Calculated analysis
Corn, yellow	37.51	Moisture	11.44
Wheat	25.00	Crude protein	19.00
Lupine-kernel	5.00	Crude fat	6.61
Soybean meal	20.61	Crude fiber	2.84
Rapeseed meal	1.30	Crude ash	4.64
Fish meal	4.86	Metabolizable energy (cal/g)	3,060
Yellow grease	3.00	Lysine	0.98
Lysine-HCl	0.34	Methionine	0.34
Limestone	0.29	Met + Cys	0.67
Tricalcium phosphate	1.00	Threonine	0.70
NaCl	0.13	Tryptophan	0.21
Wheat bran	0.23	Available lysine	0.90
Methionine	0.21	Available methionine	0.32
Threonine	0.05	Available Met + Cys	0.63
Mineral premix[1)]	0.10	Available threonine	0.65
Vitamin premix[2)]	0.10	Available tryptophan	0.20
Choline chloride	0.12	Calcium	0.80
Clinacox	0.05	Total phosphate	0.61
virginia-M	0.05	Available phosphate	0.37
Bio-plus	0.05	Sodium	0.14
		Chloride	0.20

[1)]Mineral premix provided per kg of ration with 50 mg Fe, 70 mg Mn, 50 mg Zn, 7 mg Cu, 0.4 mg Co, 0.17 mg Se, and 0.75 mg I.
[2)]Vitamin premix provided per kg of ration with 13000 IU A, 2600 IU D_3, 20 IU E, 2.5 mg K_3, 0.02 mg B_{12}, 4.0 mg riboflavin, 10 mg pantothenic acid, 40 mg niacin, 1.0 mg folic acid, 0.08 mg biotin, and 0.5 mg ethoxyquin.

Table 2. Ingredients and composition of the basal diets fed from 3 to 6 weeks of age

Ingredient	%	Composition	Calculated Analysis
Corn	35.00	Moisture	11.66
Corn gluten meal	1.83	Crude protein	20.00
Wheat	25.00	Crude fat	5.80
Lupine-Kernel	8.00	Crude fiber	2.82
Soybean meal	21.65	Crude ash	4.65
Fish meal	1.35	Metabolizable energy (cal/g)	3,031
Yellow Grease	4.00	Lysine	1.13
Lysine-HCl	0.07	Methionine	0.44
Limestone	0.48	Met + Cys	0.77
Tricalcium phosphate	1.36	Threonine	0.77
NaCl	0.24	Tryptophan	0.23
Wheat bran	0.34	Available lysine	1.04
Methionine	0.12	Available methionine	0.41
Mineral premix[1)]	0.10	Available Met + Cys	0.72
Vitamin premix[2)]	0.09	Available threonine	0.71
Choline chloride	0.12	Available tryptophan	0.21
Salinomycin	0.10	Calcium	0.80
Flavomycin	0.08	Total phosphate	0.64
Bio-plus	0.05	Available phosphate	0.41
		Sodium	0.14
		Chloride	0.20

[1)]Mineral premix provided per kg of ration with 50 mg Fe, 70 mg Mn, 50 mg Zn, 7 mg Cu, 0.4 mg Co, 0.17 mg Se, and 0.75 mg I.
[2)]Vitamin premix provided per kg of ration with 13000 IU A, 2600 IU D_3, 20 IU E, 2.5 mg K_3, 0.02 mg B_{12}, 4.0 mg rivoflavin, 10 mg pantothenic acid, 40 mg niacin, 1.0 mg folic acid, 0.08 mg biotin, and 0.5 mg ethoxyquin.

and 20% CO_2 (MAP2), respectively. VAC1 was vacuum-sealed in Cryovac B550T bags and subsequently heat shrunk in 82°C water according to the manufacturer's recommendations. VAC2 was packed in vacuum bags (Cryovac BB4L; oxygen permeability 30 $cm^3/m^2/24$ hr at 23°C, 0% RH and 1 bar) and sealed using a vacuum packaging machine (GSL-2000; Vac Star, Switzerland).

The packaged carcasses were then stored in a 4°C cold room for 6, 12, 18, or 24 days. After each time point, the individual carcasses were reweighed and the level of purge loss was reported as a percentage. The individual carcasses were then trimmed in order to obtain the breast (*pectoralis major*) muscles.

Shelf life evaluation Total mesophilic count, discoloration, and lipid oxidation of the samples were analyzed on days 6, 12, 18, and 24. For the total mesophilic count, a 10 ×10 cm area of the skin was swabbed with a sterile cotton swab (Fisher Scientific, Pittsburgh, PA, USA), and serially diluted (10^{-1} to 10^{-7}) with 0.2% sterile peptone water. After a 10-fold dilution, 1 mL of the diluents was transferred to a petri dish and pour-plated with Plate Count Agar (Difco Laboratories, Detroit, MI, USA) in duplicate. After mixing completely, the plates for the total mesophilic counts were incubated at 32°C for 48 hr. After incubation, the total mesophilic count was determined, and the total mesophilic counts were reported as colony forming units (log cfu)/cm^2 meat sample. The muscle color (CIE L*, a*, and b*) was measured using a Minolta chromameter (CR-300; Minolta Camera Co., Osaka, Japan). The percentage of metmyoglobin accumulation on the muscle surface was determined as described by Krzywicki (20). The lipid oxidation level was determined using the thiobarbituric acid (TBA) procedure reported by Salih *et al.* (21), which was modified by Ahn *et al.* (22). These results are expressed as milligrams of malondialdehyde (MDA) per kg of tissue.

Statistical analyses The data were analyzed by ANOVA using the GLM procedure in the SAS PC software (SAS Inst., Inc., Cary, NC). The model included the effects of the dietary treatment, packaging method, storage times, and their interactions. When a significant difference ($p<0.05$) was detected, the mean values were separated

using the probability difference (PDIFF) option at a predetermined probability of 5%. The results are presented as the least square means (LSM) for each treatment group along with the standard error.

Results and Discussion

Performance of broiler chicks The *all-rac*-α-tocopheryl acetate given to the broilers at 50, 100, 200, or 400 IU/kg feed from 3 to 6 weeks of age had no effect on the body weight and feed efficiency (Table 3). The α-tocopherol requirement of the broiler chicks, recommended by the National Research Council (USA), is 10 IU/kg of diet, but Combs and Scott (23) reported that at least 30 IU of vitamin E per kg of diet is needed to minimize the susceptibility of hepatic microsomes to peroxidation. Haq *et al.* (24) showed that a higher allowance of vitamin E had beneficial effects on chicks under commercial production conditions.

In this study, there were no beneficial effects of α-tocopherol supplementation on growth, similar to the findings of Guo *et al.* (11), who reported that the growth performance of broiler chicks was not influenced by vitamin E supplementation of 50 or 100 mg/kg from 4 to 6 weeks of age or from 0 to 6 weeks of age. Sheehy *et al.* (25) also reported that dietary α-tocopherol levels of 5, 25, 65, or 180 mg/kg did not affect chicken growth.

Table 3. Effect of dietary α-tocopheryl acetate supplementation on the body weight and feed efficiency in broiler chicks

Variable	Treatment[1)]				
	CON	50	100	200	400
Body weight (g)					
1 day	43 (0.2)[2)]	43 (0.2)	43 (0.2)	43 (0.2)	43 (0.2)
7 day	130 (3.1)	132 (3.1)	131 (3.1)	133 (3.1)	130 (3.1)
14 day	321 (7.2)	319 (7.2)	319 (7.2)	320 (7.2)	322 (7.2)
21 day	618 (20.5)	618 (20.5)	621 (28.4)	620 (18.1)	619 (28.4)
32 day	1261 (22.4)	1249 (22.4)	1253 (22.4)	1259 (22.4)	1251 (22.4)
42 day	1872 (34.3)	1874 (34.3)	1877 (34.3)	1871 (34.3)	1869 (34.3)
Feed efficiency (gain/feed)					
1~7 day	0.43 (0.03)	0.43 (0.03)	0.43 (0.03)	0.43 (0.03)	0.43 (0.03)
7~14 day	0.57 (0.01)	0.56 (0.01)	0.56 (0.01)	0.56 (0.01)	0.56 (0.01)
14~21 day	0.49 (0.02)	0.49 (0.02)	0.49 (0.02)	0.49 (0.02)	0.48 (0.02)
21~32 day	0.51 (0.01)	0.50 (0.01)	0.49 (0.01)	0.50 (0.01)	0.50 (0.01)
32~42 day	0.42 (0.01)	0.43 (0.01)	0.43 (0.01)	0.42 (0.01)	0.43 (0.01)

[1)]Treatment 50, 100, 200, or 400 were fed the basal diet (α-tocopherol at a rate of 20 IU/kg feed) supplemented with 50, 100, 200, or 400 IU of all-rac-α-tocopheryl acetate/kg feed, respectively.
[2)] Standard error of least square means.

Shelf life evaluation ***The effects of α-tocopherol supplementation and packaging methodology on microbial growth:*** The total plate count was measured to determine the level of microbial growth on broiler meat samples. The dietary treatments and packaging methods had no significant effects on the total plate count (Table 4). A similar level of microbial growth was observed in all 4 different packaging methods. Microbial growth increased with increasing storage time. After 18 days storage, the total viable counts reached high numbers ($p<0.001$). Despite the large microbial populations, the overall aspect throughout the storage period remained acceptable. Previous studies have shown that the effectiveness of the MAP system is due to nitrogen delaying the oxidative rancidity and inhibiting the growth of aerobic microorganisms by displacing oxygen in the package (26). The addition of carbon dioxide acts as an antimicrobial agent, and inhibits the growth of microorganisms during the logarithmic phase and extends the lag phase (26). In this study, the level of microbial growth was similar in the vacuum and modified atmosphere packed samples.

The effect of α-tocopherol supplementation and packaging methodology on purge loss: No differences in the purge loss attributable to α-tocopherol supplementation were noted (data not shown). This finding is consistent with those reported by Cannon *et al.* (27) where vitamin E supplementation had no real effect on the water holding capacity of poultry meat. However, the effect of the packaging methods on the purge loss measured after 6, 12, and 24 days was significant (Table 5, $p<0.001$). Samples packed with 100% N_2 (MAP1) or 80% N_2 + 20% CO_2 (MAP2) showed a similar purge loss during the storage period. However, poultry packaged under vacuum (VAC2) showed the highest level of purge loss until 24 days of storage. Therefore, modified atmosphere packaging decreased the level of purge loss more efficiently than

Table 4. Total mesophilic counts (log cfu/cm^2) in broiler chicken meat stored under extended refrigeration

Packaging methods[1)]	Days of storage				Level of significance[3)]
	6	12	18	24	
MAP1	4.46^a (4.20)[2)]	4.52^a (4.23)	5.04^b (4.11)	6.15^b (5.08)	***
MAP2	3.18^a (3.11)	4.79^a (4.66)	5.18^b (4.43)	6.00^b (5.28)	***
VAC1	3.11^a (3.23)	4.48^a (4.20)	5.20^b (4.20)	6.15^b (5.23)	***
VAC2	4.04^a (4.15)	4.51^{ab} (4.46)	5.15^{bc} (4.59)	6.32^c (5.66)	**

[a-c]Least square means within row with no common superscripts differ significantly ($p<0.05$).
[1)]MAP1, modified atmosphere package (N_2 100%); MAP2, modified atmosphere package (N_2 : CO_2 = 80 : 20); VAC1, heat-shrink vacuum package; VAC2, vacuum package.
[2)]Standard error of least-square means.
[3)]Level of significance: $^{**}p<0.01$; $^{***}p<0.001$.

Table 5. Effect of the packaging methods on purge loss (%) in broiler chicken meat stored under extended refrigeration

Days of storage	Packaging methods[1]				Level of significance[3]
	MAP1	MAP2	VAC1	VAC2	
6	3.86[a] (0.25)[2]	4.47[ab] (0.25)	5.15[b] (0.25)	5.88[c] (0.25)	***
12	4.66[a] (0.24)	5.03[ab] (0.24)	5.58[b] (0.24)	6.57[c] (0.24)	***
18	5.10 (0.25)	5.50 (0.27)	5.72 (0.25)	5.76 (0.25)	NS
24	5.09[a] (0.36)	5.45[a] (0.37)	4.98[a] (0.35)	7.86[b] (0.38)	***

[a-c]Least square means within row with no common superscripts differ significantly ($p<0.05$).
[1]MAP1, modified atmosphere package (N_2 100%); MAP2, modified atmosphere package (N_2 : CO_2, 80 : 20); VAC1, heat-shrink vacuum package; VAC2 = vacuum package.
[2]Standard error of least-square means.
[3]Level of significance: NS, not significant; ***$p<0.001$.

vacuum packaging. Bentley *et al.* (28) also reported that nitrogen atmosphere packages had the lowest level of purge loss while those packaged with no gas backflush had the highest levels.

The effect of α-tocopherol supplementation and packaging methodology on meat discoloration: Figure 1 shows the surface meat lightness (L*) and the level of surface metmyoglobin accumulation in the breast muscle during 24 days of refrigerated storage. The lightness of the breast muscle in the broiler chicks supplemented with different α-tocopherol levels showed large variations during storage (Fig. 1A). No significant differences in this parameter were observed in the samples packaged under either vacuum or under MAP (Fig. 1B). The metmyoglobin content increased in all the treatments with increasing storage time up to 24 days. The level of surface metmyoglobin accumu-lation showed a similar rate of discoloration, and this tendency was unaffected by the dietary α-tocopherol supple-ments (Fig. 1C) and the different packaging methods (Fig. 1D). The differences in the metmyoglobin content observed during the extended storage were related to the intensity of the oxidation process that occurs during the storage.

The formation of metmyoglobin is positively correlated to lipid oxidation (29). Mikkelsen *et al.* (30) reported that free radicals produced during lipid oxidation could alter the heme chemistry and initiate pigment oxidation. Lynch *et al.* (31) also reported that vitamin E-supplemented beef showed less metmyoglobin formation than the unsupple-mented beef. In this study, the dietary α-tocopherol supplements and the packaging methods showed no clear advantage in terms of meat color and discoloration.

The effect of α-tocopherol supplementation and packaging methodology on lipid oxidation levels in poultry meat: The influence of dietary supplementation with α-tocopherol on lipid oxidation in breast muscle is shown in Fig. 2A. The amount of TBA increased in all the treatments during storage for up to 24 days, and there were some differences ($p<0.05$) between the treatments. The broilers fed 400 IU of α-tocopherol showed the lowest level of malondialdehyde ($p<0.05$), and the lipid oxidative

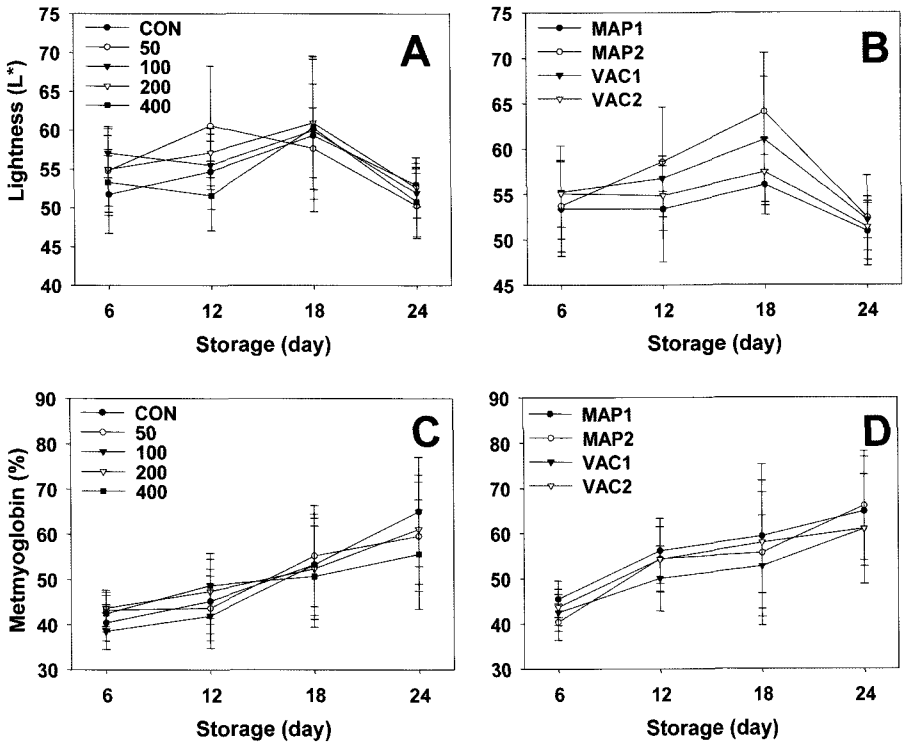

Fig. 1. Effects of dietary α-tocopherol levels (A, C) and packaging methods (B, D) on the lightness and metmyoglobin content in the breast muscle of broiler chicks during refrigerated storage. MAP1, modified atmosphere package (N_2 100%); MAP2, modified atmosphere package (N_2 : CO_2 = 80 : 20); VAC1, heat-shrink vacuum package; VAC2, vacuum package.

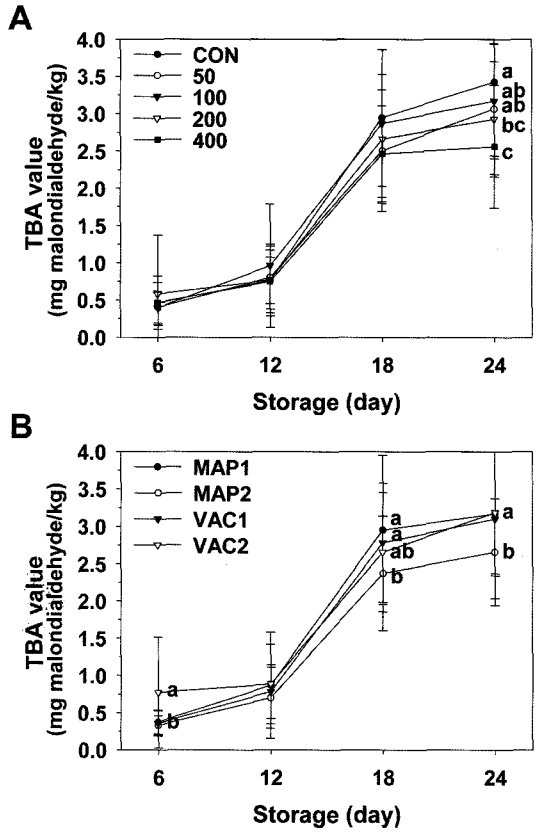

Fig. 2. Effects of the packaging methods (A) and dietary α-tocopherol levels (B) on the thiobarbituric acid values (mg malondialdehyde/kg) in the breast muscle of broiler chicks during refrigerated storage. Significance ($p<0.05$) was indicated by the letters. MAP1, modified atmosphere package (N_2 100%); MAP2, modified atmosphere package (N_2 : CO_2 = 80 : 20); VAC1, heat-shrink vacuum package; VAC2, vacuum package.

stability was improved by the supplementation with 200 IU α-tocopheryl acetate/kg feed even after 24 days of storage ($p<0.05$). Avanzo *et al.* (3) suggested that α-tocopherol affects the course of lipid peroxidation.

Our results demonstrate that supplementation with 200 IU of α-tocopherol significantly stabilized the rate of lipid oxidation compared with the control group fed only 20 IU of α-tocopherol/kg feed. Figure 2B shows the influence of the different packaging methods on lipid oxidation. Samples packed with MAP2 (80% N_2 + 20% CO_2) showed the lowest TBA value after 18 days of storage ($p<0.05$). This result showed that the MAP2 method was more effective in reducing the level of lipid oxidation compared to vacuum packaging. This is finding is consistent with those described by Wang *et al.* (32), who reported that the TBA and peroxide values were lower in MAP conditions compared with vacuum conditions. The interaction between the dietary α-tocopherol level and the packaging method was significant for the TBA values after 24 days of storage (Table 6). The TBA values were highest in the vacuum packaged meats from the control diet and lowest in the MAP (80% N_2 + 20% CO_2) breast meat from broilers fed 400 IU of α-tocopheryl acetate.

Our results show that the oxidative stability of meat

Table 6. Two-way interaction between α-tocopherol supplementation and the packaging methods on the TBA values (mg MDA/kg) in broiler chicken meat after 24 days of refrigerated storage

Packaging Methods[2)]	Treatments[1)]					SE
	CON	50	100	200	400	
MAP1	3.49^{abc}	3.15^{abcd}	3.39^{abc}	3.09^{abcd}	2.72^{bcd}	0.30
MAP2	3.11^{abcd}	2.53^{cd}	2.88^{abcd}	2.52^{cd}	2.24^{d}	
VAC1	3.27^{abc}	2.99^{abcd}	3.39^{abc}	3.21^{abcd}	2.64^{bcd}	
VAC2	3.82^{a}	3.58^{ab}	3.01^{abcd}	2.88^{abcd}	2.64^{bcd}	

[a-d]Least square means with different superscripts significantly differ ($p<0.05$).
[1)]Treatment 50, 100, 200, or 400 were fed the basal diet (α-tocopherol at a rate of 20 IU/kg feed) supplemented with 50, 100, 200, or 400 IU of all-rac-α-tocopheryl acetate/kg feed, respectively.
[2)]MAP1 = modified atmosphere package (N_2 100%); MAP2 = modified atmosphere package (N_2 : CO_2 = 80 : 20); VAC1 = heat-shrink vacuum package; VAC2 = vacuum package.

stored under refrigeration was consistently increased by increasing the level of dietary α-tocopherol supplementation in broiler chicks. Broiler meats packaged under vacuum or under modified atmospheres showed a similar color and microbiological stability. The MAP2 (80% N_2 + 20% CO_2) method was more effective in reducing the level of purge loss and lipid oxidation than vacuum packaging. The lipid oxidative stability was higher in the modified atmosphere (80% N_2 + 20% CO_2) packaged meat from the broilers fed 200 or 400 IU of α-tocopheryl acetate.

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