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Antioxidant Activity of Dietary Oregano Essential Oil and α-Tocopheryl Acetate Supplementation in Long-Term Frozen Stored Turkey Meat

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The effects of dietary oregano essential oil and α-tocopheryl acetate supplementation on the oxidative stability of long-term frozen stored turkey meat were investigated. Thirty 12-week-old turkeys, randomly divided into five groups, were given a basal diet or a basal diet supplemented with 200 mg of α-tocopheryl acetate kg^{-1}, or 100 or 200 mg of oregano oil kg^{-1}, or 100 mg of oregano oil plus 100 mg of α-tocopheryl acetate kg^{-1} for 4 weeks prior to slaughter. Lipid oxidation in breast and thigh meat was assessed after 1, 3, 6, and 9 months of frozen storage at −20 °C prior to or following 7 days of refrigerated storage at 4 °C. Results showed that oregano oil increased the oxidative stability of breast and thigh meat during the frozen storage. Dietary oregano oil at the inclusion level of 200 mg kg^{-1} feed was significantly ($p < 0.05$) more effective in delaying lipid oxidation compared to the level of 100 mg kg^{-1}, but equivalent to dietary α-tocopheryl acetate supplementation at 200 mg kg^{-1}, which in turn was inferior to dietary supplementation of 100 mg kg^{-1} oregano essential oil plus 100 mg kg^{-1} α-tocopheryl acetate that was significantly ($p < 0.05$) superior to all other treatments. Thigh meat was more susceptible to oxidation than breast meat, although the former contained α-tocopherol at markedly higher levels. Mean α-tocopherol levels in breast and thigh meat from all treatments decreased during the frozen storage, the decrease being sharper between 1 and 3 months of frozen storage for breast and between 3 and 6 months for thigh meat. Oregano oil supplementation increased ($p < 0.05$) the retention of α-tocopherol in meat, the increase being positively correlated with the supplementation level. However, the retention of α-tocopherol in meat could only partly elucidate the antioxidant activity exhibited by dietary oregano oil supplementation.

KEYWORDS: Dietary supplementation; oregano essential oil; α-tocopherol; antioxidant activity; oxidative stability; turkey meat; frozen storage

INTRODUCTION

Poultry meat products have excellent potential to achieve wider acceptance in the category of precooked-frozen entrees. Poultry meat has many desirable nutritional characteristics such as low lipid content and relatively high concentrations of polyunsaturated fatty acids (*1*) that can be further increased by specific dietary strategies. Reported benefits of long-chain polyunsaturated fatty acids, mainly eicosapentaenoic and docosahexaenoic acids, on human health (*2*, *3*) have attracted interest in animal products with high levels of these acids (*4*, *5*). However, when the degree of lipid unsaturation is increased by dietary manipulation, the susceptibility of poultry meat to oxidative deterioration during cooking and storage increases (*6*−*8*) and, as a consequence, flavor and nutritional value are reduced. The oxidative stability of poultry lipids has been found to be dependent on the α-tocopherol concentration present in cell membrane phospholipids, which in turn is dependent on the concentration of α-tocopheryl acetate in the feed (*9*, *10*). However, turkey meat is particularly susceptible to oxidation (*11*−*15*), due to its weak ability to store dietary α-tocopherol in cell membrane phospholipids (*10*, *16*).

A major preventive measure against lipid oxidation in meat and meat products is the addition of antioxidants. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) that function either by scav-

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enging chain-carrying peroxyl radicals or by diminishing the formation of initiating lipid radicals (*17*), have long been used to control lipid oxidation in foods. However, recent concern over their use (*18*, *19*) has created a need and prompted research for alternative antioxidants, particularly from natural sources.

Several aromatic plants have been studied as sources of natural antioxidants. Among them, oregano, a traditional Mediterranean spice obtained by drying the leaves and flowers of *Origanum vulgare* subsp. *hirtum* plants, is well-known for its antioxidative activity (*20*). The ground material and its essential oil, obtained by a steam distillation process, comprise >30 ingredients, most of which are phenolic antioxidants (*21*). Carvacrol and thymol, the two main phenols that constitute ~78−82% of the essential oil of oregano (*22*), are principally responsible for this activity (*23*), but other minor constituents such as γ-terpinene and *p*-cymene, two monoterpene hydrocarbons that constitute about 5 and 7% of the total oil (*22*), respectively, may also contribute to this activity (*24*). Recently, rosmarinic acid, an important antioxidant phytochemical, has also been identified as another main active ingredient in Greek oregano (*25*).

Oregano and its extracts have all been reported to inhibit lipid oxidation when added in various food systems (*26*, *27*), lard (*20*, *21*, *28*, *29*), and mackerel oil (*30*), respectively. Recent research has also shown that dietary supplementation of oregano oil to broilers could improve the oxidative stability of raw and precooked chicken meat during refrigerated storage (*31*, *32*). Dietary supplementation is a simple and convenient strategy to introduce a natural antioxidant into phospholipid membranes, where it can effectively inhibit the oxidative reactions at their localized sites (*33*−*36*).

The present study was designed to evaluate the effect of dietary oregano essential oil and α-tocopheryl acetate supplementation on susceptibility of raw breast and thigh turkey meat to lipid oxidation during long-term frozen storage.

MATERIALS AND METHODS

Chemicals. Analytical grade BHT, 2-thiobarbituric acid, α-tocopherol reference standard, and 1,1,3,3-tetraethoxypropane, the precursor of malondialdehyde (MDA), were obtained from Sigma Chemical Co. (St. Louis, MO), whereas trichloroacetic acid, pyrocatechol, hexane, methanol, acetonitrile, hydrochloric acid, and sodium hydroxide were from Merck (Darmstadt, Germany). α-Tocopheryl acetate was obtained from Roche Products Ltd. (Hertfordshire, U.K.), whereas oregano essential oil was from Ecopharm Hellas S.A. (Kilkis, Greece). Oregano oil was in the form of a powder called Orego-Stim [Meriden Animal Health Ltd. (Luton, U.K.) t]hat contains 5% essential oil of *O. vulgare* subsp. *hirtum* plants and 95% natural feed grade inert carrier.

Animals and Diets. Thirty 10-week-old female turkeys of the black strain of a local type purchased from a local poultry farm were used in this study. On arrival, turkeys were weighed and randomly assigned to five groups of six birds each. Ambient temperature was controlled, continuous lighting was provided, and conventional breeding and management procedures were employed throughout the experiment. All birds were allowed to acclimate for a period of 2 weeks. During the acclimatization period, all groups were fed ad libitum on a commercial turkey diet supplemented with a basal amount of 30 mg of α-tocopheryl acetate kg^{-1} of feed. The ingredients and the composition of the commercial basal diet are presented in **Table 1**. After the end of the acclimatization period, feeding of the commercial diet was discontinued to all but one of the groups. The birds within this group (control) were given the commercial diet for a further 4 weeks. The experimental diets given to the remaining four groups were based on the same commercial diet but contained either an additional 200 mg of α-tocopheryl acetate kg^{-1} of feed (VE200 group), or 100 mg of oregano oil kg^{-1} of feed (OR100 group) or 200 mg of oregano oil kg^{-1} of feed (OR200 group), or 100 mg of oregano oil plus 100 mg of α-tocopheryl acetate kg^{-1} of feed (ORVE100 group). Supplementation of the feed with the oregano oil was achieved by adding to each diet the appropriate amount of the Orego-Stim supplement. Feeding of the experimental diets to turkeys lasted 4 weeks.

Table 1. Composition of the Commercial Basal Diet for Turkeys

ingredient	composition, g/kg
corn, grains	505.8
herring meal	30.0
soybean meal	330.0
soybean oil	25.0
corn gluten feed	50
yeast	25
DL-methionine	1.0
biolysine	1.0
choline chloride	0.2
Avatec, Roche (15% in lasalocid)	0.05
dimetridazole	0.05
limestone, pulverized	17.0
dicalcium phosphate	10.0
sodium chloride, iodized	2.8
binder (methylcellulose)	1.4
Natuphos, BASF (phytase)	0.1
vitamin premix[a]	1.0
trace-mineral premix[b]	1.0

[a] Supplying, per kg of feed, 14000 IU of *all-trans*-retinol acetate, 5000 IU of cholecalciferol, 30 mg of α-tocopheryl acetate, 7 mg of menadione sodium bisulfite, 5 mg of thiamine hydrochloride, 10 mg of riboflavin, 10 mg of pyridoxine hydrochloride, 0.02 mg of cyanocobalamine, 85 mg of niacin, 25 mg of pantothenic acid, 2 mg of folic acid, 0.25 mg of biotin, and 10 mg of ascorbic acid. [b] Supplying, per kg feed, 100 mg of Zn, 120 mg of Mn, 20 mg of Fe, 20 mg of Cu, 0.2 mg of Co, 1 mg of I, and 0.3 mg of Se.

Sampling. All turkeys were slaughtered under commercial conditions at 16 weeks of age. Carcasses from all birds were immediately trimmed for breast and thigh meat by removing skin, bones, and connective tissue. Following trimming, breast and thigh meat from each bird within each group were individually sliced and sampled for lipid oxidation studies and analysis of α-tocopherol.

Frozen Storage-Induced Lipid Oxidation. For oxidative stability studies, collected breast and thigh samples were individually vacuum-packaged and placed in a freezer at −20 °C to be held for 9 months. At 1, 3, 6, and 9 months of frozen storage, subsamples from breast and thigh meat from each bird from each dietary treatment were removed from the freezer and thawed overnight at 4 °C. Part of the thawed subsamples was directly submitted to malondialdehyde analysis for estimation of the extent of lipid oxidation and to α-tocopherol analysis for evaluation of the effect of frozen storage on α-tocopherol content. To further accelerate the extent of lipid oxidation and make the effects of the dietary treatments clearer, the remaining subsamples were wrapped in transparent oxygen-permeable poly(vinyl chloride) film (6000−8000 cm^3 m^{-2} 24 h^{-1}), placed in a nonilluminated refrigerated cabinet at 4 °C, and analyzed for malondialdehyde content after 7 days of storage.

Measurement of Lipid Oxidation. Lipid oxidation was assessed on the basis of the malondialdehyde formed in treated samples. Malondialdehyde, the compound used as an index of lipid peroxidation, was determined by a selective third-order derivative spectrophotometric method previously developed by some of the authors (*37*). In brief, samples were homogenized (Polytron homogenizer, PCU) in the presence of 8 mL of aqueous trichloroacetic acid (50 g L^{-1}) and 5 mL of BHT in hexane (8 g L^{-1}), and the mixture was centrifuged. The top layer was discarded, and a 2.5-mL aliquot from the bottom layer was mixed with 1.5 mL of aqueous 2-thiobarbituric acid (8 g L^{-1}) to be further incubated at 70 °C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to conventional spectrophotometry (Shimadzu, model UV-160A, Tokyo, Japan) in the range of 400−650 nm. Third-order derivative spectra were produced by digital differentiation of the normal spectra using a derivative wavelength difference setting of 21 nm. The concentration of malondialdehyde (nanograms per gram of wet tissue) in analyzed samples was calculated

on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of standard calibration curve prepared using 1,1,3,3-tetraethoxypropane.

Determination of α-Tocopherol Concentrations. Extraction of α-tocopherol from meat samples was as described by Botsoglou et al. (*38*). In brief, samples (0.5 g) were homogenized (Polytron homogenizer, PCU) with 100 μL of pyrocatechol solution and 5 mL of a saturated methanolic solution of KOH and saponified by heating in a water bath at 80 °C for 15 min. Following saponification, 5 mL of hexane and 1 mL of water were added, the mixture was vortex-mixed and centrifuged at 2000*g*, and an aliquot of the upper phase was evaporated to dryness to be further reconstituted in methanol and injected into the liquid chromatograph (Shimadzu, model 6AV). Liquid chromatography was carried out using a Nucleosil C_{18}, 5 mm, 250 × 4.6 mm, column, and a mobile phase of methanol/water (97:3, v/v) that was delivered in the system at a flow rate of 2 mL min^{-1} (*39*). Monitoring of the column effluents was performed using a fluorometric detector set at excitation wavelength of 290 nm and emission wavelength of 330 nm. Detector signals were quantified using peak heights and a standard calibration curve.

Statistical Analysis. An individual turkey was the experimental unit for analysis of all data. Before statistical analysis, Bartlett's test was applied to test the homogeneity of the variances (*40*). To overcome the problem of variances correlated with the mean, all data were subjected to log transformation to stabilize the variances. The development of malondialdehyde in meat during storage was analyzed by a two-way analysis of variance, fixed-effects model, including main effects of dietary treatment (five levels) and time of storage (four levels), and interaction between the two factors. Data on α-tocopherol concentrations in meat were also subjected to two-way analysis of variance including main effects of dietary treatment (five levels) and time of storage (five levels), as well as interaction between the two factors. When the effect of factors was significant, Tukey's test was applied to determine statistical differences between least-squares means. A probability level of $p < 0.05$ was used in testing the statistical significance of all experimental data that were computerized using the SPSS 10.05 statistical package (SPSS Ltd., Woking, Surrey, U.K.).

RESULTS AND DISCUSSION

The influence of dietary supplementation with oregano oil and/or α-tocopheryl acetate on lipid oxidation in breast and thigh meat of turkeys frozen-stored for 1, 3, 6, or 9 months is illustrated in **Figures 1** and **3**, respectively. Frozen storage for 1 month increased the levels of malondialdehyde in breast and thigh meat samples, the increase being higher in the control group. This trend was maintained after 3, 6, and 9 months of frozen storage.

The extent of lipid oxidation, as measured by malondialdehyde formation, differed ($p < 0.05$) between the dietary treatments even at 0 time of frozen storage, the differences being already present just after slaughter (**Table 2**). Because neither mincing nor any other oxidation-inducing procedure had been applied to the samples, the malondialdehyde found in the fresh meat should be due to either the consumption and subsequent deposition of malondialdehyde that was already present in the diets or the in vivo production of malondialdehyde by the turkeys during digestion. If the former possibility was true, the levels of malondialdehyde should have been equal among treatments. Therefore, the in vivo production of different levels of malondialdehyde by the turkeys as a function of inclusion of oregano oil and/or α-tocopheryl acetate in their diet seems to be more reasonable.

Figure 1 shows that samples from the OR100 group had always significantly lower malondialdehyde values than the control group after 3, 6, and 9 months of refrigerated storage, a finding suggesting that dietary oregano oil exerted antioxidant

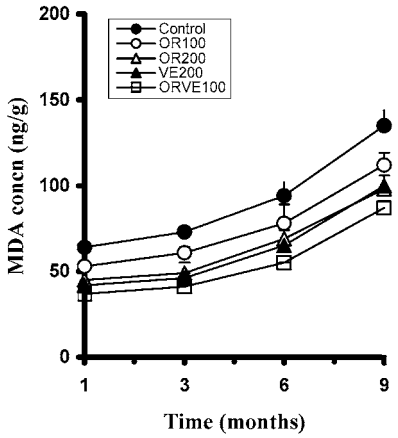

Figure 1. Effect of long-term frozen storage prior to refrigerated display on lipid oxidation of turkey breast meat as a function of the dietary oregano essential oil at 100 mg kg^{-1} (OR100 group) and 200 mg kg^{-1} (OR200 group), α-tocopheryl acetate supplementation at 200 mg kg^{-1} (VE200 group), and 100 mg kg^{-1} oregano essential oil plus 100 mg kg^{-1} α-tocopheryl acetate supplementation (ORVE100 group). Data points are mean malondialdehyde (MDA) concentrations from six samples analyzed ± standard deviation.

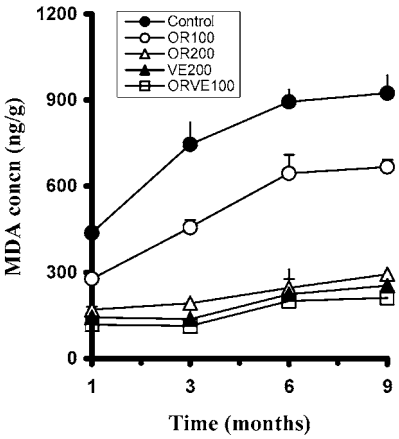

Figure 2. Effect of long-term frozen storage after 7 days of refrigerated display on lipid oxidation of turkey breast meat as a function of the dietary oregano essential oil at 100 mg kg^{-1} (OR100 group) and 200 mg kg^{-1} (OR200 group), α-tocopheryl acetate supplementation at 200 mg kg^{-1} (VE200 group), and 100 mg kg^{-1} oregano essential oil plus 100 mg kg^{-1} α-tocopheryl acetate supplementation (ORVE100 group). Data points are mean malondialdehyde (MDA) concentrations from six samples analyzed ± standard deviation.

activity even at its lower inclusion level of 100 mg kg^{-1} of feed. At the higher inclusion level of 200 mg kg^{-1} feed, dietary oregano showed much higher antioxidant activity after 3, 6, and 9 months of frozen storage, with malondialdehyde values in the OR200 group significantly ($p < 0.05$) lower than in the OR100 group. The activity of the OR200 group was almost equivalent to that exhibited by the group on 200 mg of α-tocopheryl acetate kg^{-1} of feed, as it is indicated by the comparable malondialdehyde values of the OR200 and VE200 groups at all time points during frozen storage. The group on 100 mg of oregano oil plus 100 mg of α-tocopheryl acetate kg^{-1} of feed also exhibited an antioxidant activity equivalent to the OR200 and VE200 groups after 3 months of frozen storage. However, this activity significantly ($p < 0.05$) increased after 6 and 9 months of frozen storage; hence, the dietary supplementation of 100 mg of oregano oil plus 100 mg of α-tocopheryl acetate kg^{-1} of feed was more effective in

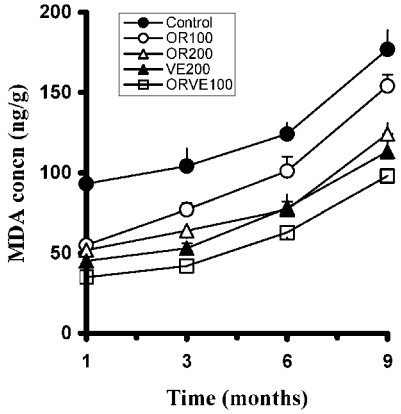

Figure 3. Effect of long-term frozen storage prior to refrigerated display on lipid oxidation of turkey thigh meat as a function of the dietary oregano essential oil at 100 mg kg^{-1} (OR100 group) and 200 mg kg^{-1} (OR200 group), α-tocopheryl acetate supplementation at 200 mg/kg (VE200 group), and 100 mg kg^{-1} oregano essential oil plus 100 mg kg^{-1} α-tocopheryl acetate supplementation (ORVE100 group). Data points are mean malondialdehyde (MDA) concentrations from six samples analyzed ± standard deviation.

Table 2. Mean Malondialdehyde Concentrations in Breast and Thigh Meat of Turkeys Fed Diets Supplemented with Oregano Essential Oil and/or α-Tocopheryl Acetate, Prior to and Following 1 Month of Frozen Storage

	mean malondialdehyde concn, ng/g			
	breast meat		thigh meat	
	prior to storage	1 month of storage	prior to storage	1 month of storage
control	55.1 ± 2.4	64.1 ± 2.7	63.9 ± 2.1	93.3 ± 4.0
OR100	45.7 ± 1.4	53.2 ± 1.6	49.6 ± 2.6	55.4 ± 3.1
OR200	36.6 ± 3.6	45.0 ± 2.4	40.4 ± 2.2	52.2 ± 2.1
VE200	37.2 ± 2.8	42.2 ± 2.1	39.8 ± 1.2	45.1 ± 3.3
ORVE100	33.4 ± 2.1	37.4 ± 4.0	37.3 ± 2.8	35.2 ± 4.0

retarding lipid oxidation during frozen storage than all other dietary treatments.

Lipid oxidation was significantly ($p < 0.05$) greater at day 7 of refrigerated display compared to their counterparts at day 0 after 1, 3, 6, or 9 months of frozen storage (**Figure 2**). Thus, following 7 days of refrigerated display, mean malondialdehyde level in the control breast samples was 437 ppb versus the level of 64 ppb monitored prior to refrigerated display after 1 month of frozen storage, whereas it reached the level of 987 ppb after 9 months of frozen storage. Breast meat samples from the OR100 group presented significantly ($p < 0.05$) lower malondialdehyde levels compared to the control group but higher compared to the OR200, VE200, and ORVE100 groups, which in turn exhibited equivalent antioxidant activity at all time points, with malondialdehyde values comparable each other.

The extent of lipid oxidation in turkey thigh meat samples frozen-stored for 1, 3, 6, or 9 months and then refrigerated at 4 °C for 0 and 7 days is illustrated in **Figures 3** and **4**, respectively. It can be seen that thigh meat was more susceptible to lipid oxidation during the frozen storage than breast meat. In addition, lipid oxidation in thigh meat was significantly ($p < 0.05$) greater at day 7 of refrigerated display (**Figure 4**) compared to day 0 (**Figure 3**). The OR100 treatment significantly ($p < 0.05$) retarded lipid oxidation compared to the control, but it was less ($p < 0.05$) effective compared to the OR200 and VE200 treatments, which presented an equivalent

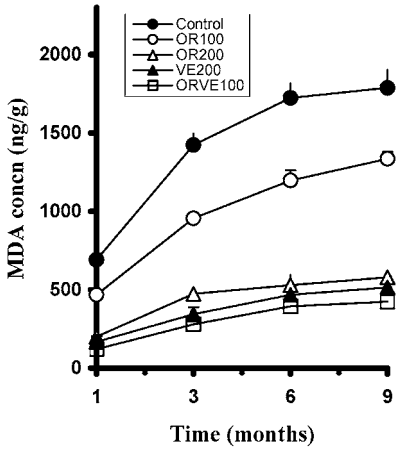

Figure 4. Effect of long-term frozen storage after 7 days of refrigerated display on lipid oxidation of turkey thigh meat as a function of the dietary oregano essential oil at 100 mg kg^{-1} (OR100 group) and 200 mg kg^{-1} (OR200 group), α-tocopheryl acetate supplementation at 200 mg kg^{-1} (VE200 group), and 100 mg kg^{-1} oregano essential oil plus 100 mg kg^{-1} α-tocopheryl acetate supplementation (ORVE100 group). Data points are mean malondialdehyde (MDA) concentrations from six samples analyzed ± standard deviation.

antioxidant activity after the frozen storage. More effective than all other dietary treatments in retarding lipid oxidation was again the combination of 100 mg of oregano oil plus 100 mg of α-tocopheryl acetate kg^{-1} of feed. This treatment presented after 1, 3, 6, and 9 months of frozen storage significantly ($p < 0.05$) lower malondialdehyde values than all other treatments prior to refrigerated display.

Figures 1 and **3** that refer to measurements on day 0 of refrigerated display after 1, 3, 6, or 9 months of frozen storage show that the lipid oxidation curves of all treatments exhibited a lag phase by month 6, followed by a rapid increase thereafter. In contrast, **Figures 2** and **4** that concern measurements on day 7 of refrigerated display after frozen storage show a rapid increase by month 6 but a rather stable level thereafter. Considering the nature of malondialdehyde, which is a secondary lipid oxidation product formed by hydrolysis of lipid hydroperoxides, the observed lag phase in **Figures 1** and **3** might be due to the initially low levels of malondialdehyde formed during the early stages of frozen storage, whereas the rapid increase thereafter to high production of malondialdehyde as hydrolysis of the lipid hydroperoxides formed during the early stages of frozen storage proceeds during the late stages of frozen storage. Also, the rapid increase of malondialdehyde during the 7-day refrigerated storage (**Figures 2** and **4**) by month 6 and the rather stable levels thereafter could be explained on the basis of the amounts of the lipid hydroperoxides expected to occur in these samples, because higher amounts of intact lipid hydroperoxides should occur in the frozen meat during the first months of frozen storage and lower thereafter, leading after hydrolysis during the 7-day refrigerated storage to higher and lower production of malondialdehyde, respectively.

Considering that the greater the amount of α-tocopherol deposited, the better protection the meat should have against oxidative attack, all breast and thigh meat samples after 0, 1, 3, 6, and 9 months of frozen storage and prior to refrigerated display were analyzed for their α-tocopherol content. The concentrations of α-tocopherol appear to be low (**Tables 3** and **4**); however, α-tocopherol is not efficiently absorbed and deposited in the cell membranes of turkeys (*11*). These results are consistent with the findings of a pertinent study (*41*) where,

Table 3. Effect of Frozen Storage on the α-Tocopherol Concentrations of Raw Breast Meat from Turkeys Fed Diets Supplemented with Oregano Essential Oil and/or α-Tocopheryl Acetate at Different Levels

months of frozen storage	concn[a] of α-tocopherol in breast meat, μg/g				
	control	OR100	OR200	VE200	ORVE100
0	1.05a ± 0.05	1.27b ± 0.05	1.43c ± 0.06	2.20d ± 0.41	1.80e ± 0.14
1	1.05a ± 0.08	1.25b ± 0.10	1.44c ± 0.15	2.22d ± 0.33	1.75e ± 0.12
3	0.65a ± 0.13	0.88a ± 0.08	1.08b ± 0.05	1.32c ± 0.47	1.39cd ± 0.15
6	0.63a ± 0.12	0.89b ± 0.05	1.05c ± 0.12	1.35d ± 0.15	1.35de ± 0.13
9	0.61a ± 0.05	0.87b ± 0.12	1.05c ± 0.05	1.28d ± 0.12	1.36de ± 0.24

[a] Mean of six analyses ± standard deviation. Mean values in the same row with a letter in common do not differ significantly ($p > 0.05$).

Table 4. Effect of Frozen Storage on the α-Tocopherol Concentrations of Raw Thigh Meat from Turkeys Fed Diets Supplemented with Oregano Essential Oil and/or α-Tocopheryl Acetate at Different Levels

months of frozen storage	concn[a] of α-tocopherol in thigh meat, μg/g				
	control	OR100	OR200	VE200	ORVE100
0	1.22a ± 0.10	1.45b ± 0.11	1.72c ± 0.33	5.82d ± 0.67	3.55e ± 0.15
1	1.23a ± 0.12	1.43b ± 0.10	1.70c ± 0.24	5.85d ± 1.05	3.47e ± 0.22
3	1.08a ± 0.06	1.36b ± 0.10	1.68c ± 0.18	5.80d ± 0.95	3.46e ± 0.44
6	0.68a ± 0.08	0.95b ± 0.06	1.15c ± 0.10	4.46d ± 0.42	2.78e ± 0.22
9	0.70a ± 0.05	0.87b ± 0.12	1.12c ± 0.10	4.32d ± 0.27	2.74e ± 0.12

[a] Mean of six analyses ± standard deviation. Mean values in the same row with a superscript in common do not differ significantly ($p > 0.05$).

after turkeys were fed with 250 mg of α-tocopheryl acetate kg^{-1} of diet for 3 weeks before slaughter, the difference in α-tocopherol levels between treated and control meat was only 1.2-fold. The accumulation of α-tocopherol in turkey appears to be low compared to other species such as broilers or pork and, therefore, dietary supplementation for longer periods is recommended (*16*, *42*). It has been reported (*43*) that the levels of α-tocopherol in turkey breast muscle are about one-third those in chickens fed the same dietary supplement of α-tocopheryl acetate for the same period of time. In the same study, the lower levels of α-tocopherol in turkey meat were attributed to decreased gastrointestinal absorption, although this was not determined. In another paper (*16*), the low α-tocopherol levels in turkey tissues were explained on the basis of a greater production and excretion of α-tocopheryl glucuronides.

Prior to frozen storage, mean α-tocopherol levels in breast and thigh meat samples in the control group differed significantly ($p < 0.05$), being 1.05 and 1.22 mg/kg, respectively. When the diet was supplemented with 200 mg of α-tocopheryl acetate kg^{-1}, the concentrations of α-tocopherol in breast and thigh samples increased to 2.20 and 5.82 mg kg^{-1}, respectively. These concentrations compare well with literature values (*10*, *12*, *13*). The higher accumulation of α-tocopherol in thigh meat has been associated with the more highly developed vascular system and greater lipid content of thigh than breast meat (*7*).

Although thigh meat contained higher amounts of α-tocopherol than breast meat, it tended to oxidize more quickly. The greater susceptibility of thigh meat to oxidation has been attributed to the higher absolute content of polyunsaturated fatty acids with more than two double bonds in this tissue (*44*). Although breast meat has a higher percentage of these acids in the fat, the absolute amount in thigh meat is 3 times higher than in breast meat regardless of the dietary treatment (*44*) because the total fat content in thigh meat is ∼5 times that of breast meat. In addition, the large amount of pro-oxidative agents originating from meat myoglobin and other iron-containing proteins in thigh meat may also reduce oxidative stability in this tissue (*45*).

Tables 3 and **4** show that all meat samples from the oregano oil supplemented groups had significantly ($p < 0.05$) higher levels of α-tocopherol compared with the control, the increase being positively correlated with the supplementation level. Oregano oil even at its higher inclusion level of 200 mg kg^{-1} could not offer to the diet more that 4 mg of endogenous α-tocopherol kg^{-1} (*46*, *47*), so the increased levels of α-tocopherol found in meat indicated that dietary oregano oil protected α-tocopherol in meat during the frozen storage. These results are consistent with a study (*48*) reporting that oregano could preserve α-tocopherol in sunflower oil. In another study (*49*), a mixture of α-tocopherol and an extract of rosemary was found to exert a stronger antioxidant effect than either α-tocopherol or rosemary extract alone, in a sardine model system. These workers proposed that the synergistic action of rosemary might be due to regeneration of α-tocopherol through donation of hydrogen atoms to the tocopheroxyl radicals. Other workers (*50*) studying the antioxidative activity of α-tocopherol versus δ-tocopherol in combination with ascorbyl palmitate in cooked, minced turkey reported that the synergistic action of ascorbyl palmitate was the result of a shielding of α-tocopherol by ascorbyl palmitate rather than a direct regeneration.

The hypothesis that oregano oil functions by stabilization, recycling, and retaining of α-tocopherol might possibly be considered strong enough to explain the antioxidant activity shown by the 100 or 200 mg of oregano oil kg^{-1} of diet treatments, because they presented significantly ($p < 0.05$) higher levels of α-tocopherol compared to controls. However, this hypothesis cannot explain the synergistic action exhibited by the dietary supplementation of 100 mg of oregano oil plus 100 mg of α-tocopheryl acetate kg^{-1} of diet, which, although containing significantly ($p < 0.05$) lower levels of α-tocopherol, presented higher antioxidant activity than the α-tocopheryl acetate treatment. Therefore, oregano oil should contribute to the antioxidant activity by additional mechanisms.

During the frozen storage, the levels of α-tocopherol in meat samples decreased with the time of storage (**Tables 3** and **4**). There have been several possible explanations for the depletion of α-tocopherol from meat during frozen storage. Muscle cytosol contains compounds that strongly inhibit mermbrane lipid peroxidation by the ferric ion and partially inhibit peroxidation by an iron redox cycle (*51*). In addition, muscle cytosol is the

area where the tocopheroxyl radicals formed during the early stages of lipid peroxidation in meat are re-reduced to their native form by other intracellular reductants (*34*). However, at −20 °C, the antioxidant activity of the cytosolic phase may no longer function optimally because some catalysts and antioxidants may be trapped in the frozen solid phase (*11*). Moreover, lipid free radicals that are soluble in the oil phase can diffuse to longer distances and spread the lipid oxidation reaction (*51*). Hence, free radicals may escape the antioxidants in the frozen aqueous phase and diffuse into the membrane cell phospholipids, where they initiate and promote lipid oxidation. Some workers (*11*) stated that if this occurs, the lipid-soluble α-tocopherol becomes the first line of antioxidant defense and thus may be rapidly depleted.

In breast meat, the greater rate of α-tocopherol depletion occurred between 1 and 3 months of frozen storage; afterward, it remained almost unchanged (**Table 3)**. However, the concentration of MDA during the same period was quite stable, whereas the main increase in MDA occurred during 3−9 months of storage (**Figure 1**), when the content of α-tocopherol was quite stable. These changes could be explained if malondialdehyde were considered to be a secondary lipid oxidation product formed by hydrolysis of lipid hydroperoxides. Thus, higher quantities of α-tocopherol could be consumed for protecting the polyunsaturated fatty acids of the membrane phospholipids from oxidation during the early stages of frozen storage, whereas the rapid increase in malondialdehyde observed between 3 and 9 months of storage should be due to hydrolysis of the lipid hydroperoxides already formed during the early stages of frozen storage. In thigh meat, the greater rate of α-tocopherol depletion occurred between 3 and 6 months of frozen storage (**Table 4**). This delayed α-tocopherol depletion might be attributed to the higher amount of polyunsaturated fatty acids present in thigh meat compared to the breast meat (*44*).

In conclusion, the results of the present investigation show that dietary oregano oil supplementation reduced lipid oxidation in turkey meat during frozen and subsequent refrigerated storage. A dose−response increase in the antioxidative capacity of turkey meat as a result of feeding oregano oil was observed. Dietary oregano oil at the inclusion level of 200 mg kg^{-1} of feed was more effective in delaying lipid oxidation compared to the level of 100 mg kg^{-1}, but equivalent to dietary α-tocopheryl acetate at 200 mg kg^{-1}, which in turn was inferior to the dietary combination of 100 mg kg^{-1} oregano essential oil plus 100 mg kg^{-1} α-tocopheryl acetate, which was superior to all other dietary treatments. The better oxidative stability of meat samples from turkeys receiving the diets supplemented with oregano oil was probably the result of antioxidant constituents of the oregano oil that entered the circulatory system and were distributed and retained in meat. Because there is no method available so far for the identification and quantification of the antioxidant constituents of oregano oil deposited in turkey meat, the bioavailability of these compounds cannot not be yet directly demonstrated.

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