

Effect of dietary olive leaves and rosemary on microbial growth and lipid oxidation of turkey breast during refrigerated storage

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Abstract

Forty turkeys were allocated to five groups of eight birds each. One (control) was fed a basal diet while the others were fed diets supplemented with either olive leaves at 10 g/kg, rosemary at 10 g/kg, α -tocopheryl acetate at 150 or at 300 mg/kg feed. Following slaughter, fillets from the breasts of the birds were stored at 4 °C in the dark for 12 days and their lipid oxidation and microbial growth rate were monitored. Results showed that dietary olive leaves were more effective in inhibiting lipid oxidation of the breast fillets compared to rosemary, but inferior to the dietary supplementation of 300 mg α -tocopheryl acetate/kg. In turn, lipid oxidation was more effectively inhibited when the birds received α -tocopheryl acetate supplementation at 150 mg/kg compared to when the birds received no supplementation, but inferior to rosemary supplementation. Total viable counts, lactic acid bacteria, *Enterobacteriaceae* and psychrotrophic bacterial counts were all increased in breast fillets of all groups throughout refrigerated storage. Diet supplementation with a basal level of α -tocopheryl acetate had no effect on the bacterial counts recorded in the control group, but diet supplementation with olive leaves or rosemary resulted in a decrease in all bacterial counts from day 2 of storage and thereafter. During this period olive leaves were more effective in inhibiting bacterial growth than rosemary.

Keywords: Antioxidant additives, α -tocopherol, turkey meat, herbs, spices, meat quality

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Introduction

Lipid oxidation is primarily initiated in the highly unsaturated fatty acid fraction of membrane phospholipids, leading to production of hydroperoxides, which are susceptible to further oxidation or decomposition to secondary reaction products that may adversely affect the overall quality of meat (Vercellotti *et al.*, 1992). Poultry muscle tissues are particularly susceptible to lipid oxidation during refrigerated storage due to their relatively high content in polyunsaturated fatty acids. The susceptibility to oxidative deterioration is higher for turkey compared to chicken tissues, although tissues from both species contain comparable fatty acid compositions. This is primarily due to a weaker ability of turkeys to accumulate dietary tocopherol in their tissues (Botsoglou *et al.*, 2003; Papageorgiou *et al.*, 2003). Dietary administration of α -tocopherol allows its incorporation into subcellular membranes where it can effectively inhibit oxidative reactions at their localized sites (Packer & Kagan, 1993).

In the last few years oxidative deterioration of muscle tissues is of increasing concern since novel feeding strategies have raised the levels of polyunsaturated fatty acids in the so-called functional meat. However, increasing levels of polyunsaturated fatty acids in tissues lead to faster lipid oxidation, with a consequent quality loss and lower consumer acceptability. To delay lipid oxidation, various additives especially of synthetic origin, are often added to meat products. However, consumers and health authorities increasingly dictate that the use of synthetic food additives should be phased out and, where possible, only natural products should be used. Hence, alternatives from natural sources with potential antioxidant activity are investigated.

In the search for natural antioxidant additives, much attention has been directed to herbs and spices. Rosemary, in particular, has been found to be a promising dietary supplement that contains a variety of

compounds including carnosol, carnosic acid, rosmanol, 7-methyl-epirosmanol, isorosmanol, rosmadial, caffeic acid, 1,8 cineol, camphor and α -pinene, which have substantial *in vitro* antimicrobial and antioxidant activities (Barrata *et al.*, 1998; Ibanez *et al.*, 2003). Rosemary is a spice obtained by drying the leaves and flowers of the plant, *Rosmarinus officinalis*. In a study assessing the antioxidant properties of some spices frequently used in the Mediterranean diet, rosemary was found to be the most effective scavenger of peroxy radicals in an ox brain phospholipid system. It was compared with oregano, annatto, paprika, cumin, saffron and common synthetic food additives (Martinez-Tome *et al.*, 2001). Carnosic acid, the most active antioxidant constituent of rosemary, has shown in *in vitro* studies an antioxidant activity approximately three times higher than carnosol and seven times higher than that of the common synthetic additives, butylated hydroxytoluene and butylated hydroxyanisole (Cuvelier *et al.*, 1996; Richheimer *et al.*, 1996; Offord *et al.*, 1997). In addition, 1,8 cineol, α -pinene and camphor have been identified as most active antimicrobial components (Pandit & Shelef, 1994; Barrata *et al.*, 1998). Obviously, due to its high content of bioactive components, ground rosemary has proven in *in vivo* studies to be an efficient dietary supplement that could inhibit microbial growth and lipid oxidation in turkey tissues during refrigerated storage (Botsoglou *et al.*, 2007; Govaris *et al.*, 2007).

Unlike rosemary that has to be cultivated before processing, there are several other potential sources of antioxidant phytochemicals such as the leaves of the olive tree (*Olea europea* L.), which are readily available and require minimal processing. Olive leaves, one of the by-products of olive tree farming, can be found in large quantities in the olive oil industries and can also accumulate in large volumes on farms during the pruning of the trees. Olive leaves are a good source of several antioxidants including oleuropeoside compounds such as oleuropein and verbascoside, and flavonoid compounds such as luteolin, luteolin-7-glucoside, apigenin-7-glucoside, diosmetin, diosmetin-7-glucoside, rutin and catechin, and simple phenolic compounds such as tyrosol, hydroxytyrosol, vanillin, vanillic acid and caffeic acid. Oleuropein, the most prominent compound, may reach concentrations of 60 - 90 mg/g dried leaves (Ryan *et al.*, 2002). Most of these phenolic compounds have been shown to possess substantial antimicrobial and antioxidant activities *in vitro* (Kubo *et al.*, 1995; Markin *et al.*, 2002), while other non-phenolic components such as aldehydes have also been studied for their antimicrobial properties (Kubo *et al.*, 1995; Bisignano *et al.*, 2001). However, *in vivo* investigations on the antioxidant and antimicrobial activities of olive leaves as a dietary supplement to food producing animals have not been conducted.

Use of nutritional strategies such as the incorporation of suitable phytochemicals to improve the oxidative stability of animal products can extend the shelf life and increase the acceptability of muscle food during retail display. However, a major concern with extending the shelf life of muscle food is associated with the extent and type of microbial growth that might occur during the additional time of product acceptability. Delayed lipid oxidation caused by the use of supplemental dietary antioxidants may mask microbial growth without warning consumers because some of the initial signs of meat spoilage such as off-flavours or discoloration may be hidden due to decreased lipid oxidation, although concentrations of bacteria might be high. This fact may be an adverse factor to the health of consumers of such meats. The present study was designed to evaluate and compare the inhibitory potentials of feed supplementation with olive leaves, rosemary and/or α -tocopheryl acetate on microbial growth and lipid oxidation of turkey breast fillets during refrigerated storage.

Materials and Methods

Forty female turkeys (black local breed), eight weeks old, were allocated to five experimental groups in this study. The birds were allowed to acclimatize for a period of two weeks. During the acclimatization period they were fed *ad libitum* a commercial maize-soyabean based turkey diet supplemented with 10 mg α -tocopheryl acetate/kg. The ingredients and the composition of the commercial diet are presented in Table 1. The lighting programme was set at 60 watt/10 m² during the first week with continuous light thereafter, and the temperature was set at 21 °C. The humidity was 65 - 75%, and conventional breeding and management procedures were employed throughout the experiment. Birds were cared for in accordance with the guide for the care and use of laboratory animals (Committee on Care and Use of Laboratory Animals, 1996).

At the end of the acclimatization period, feeding of the commercial diet was discontinued to all but one of the groups. The birds within this control group (CONT) were fed the commercial diet for a further

Table 1 Nutrients composition of commercial diet for turkeys

Ingredients	Composition (g/kg)	Ingredients	Composition (g/kg)
Maize grains	484.5	Biolysine	2.5
Soyabean meal	320.0	Choline chloride	0.2
Soyabean oil	18.0	Limestone pulverized	17.5
Herring meal	20.0	Monocalcium phosphate	14.0
Corn gluten meal	22.5	Sodium chloride, iodized	2.7
Wheat bran	40.0	Natuphos (phytase)	0.1
Corn gluten feed	40.0	Vitamin premix ^a	1.0
Yeast	15.0	Trace-mineral premix ^b	1.0
DL-methionine	1.0		

^a Content per kg feed: 10,000 IU all-trans retinol acetate; 5,000 IU cholecalciferol; 30 mg α -tocopheryl acetate; 6 mg menadione sodium bisulphite; 5 mg thiamine hydrochloride; 10 mg riboflavin; 10 mg pyridoxine hydrochloride; 0.02 mg cyanocobalamine; 85 mg niacin; 25 mg pantothenic acid; 2 mg folic acid; 0.25 mg biotin; 10 mg ascorbic acid.

^b Content per kg feed: 100 mg Zn; 120 mg Mn; 20 mg Fe; 20 mg Cu; 0.2 mg Co; 1 mg I; 0.3 mg Se.

four weeks. The experimental diets fed to the remaining four groups were based on the same commercial diet, but supplemented with 10 g olive leaves/kg (OLE), 10 g rosemary/kg (ROS), 150 mg α -tocopheryl acetate /kg (T150) or 300 mg α -tocopheryl acetate/kg (T300) of feed. Supplementation of the commercial diet with olive leaves or rosemary was achieved at the expense of wheat bran. The turkeys were fed the experimental diets for four weeks.

Olive leaves were collected from *Olea europaea* L. trees not treated with any chemical in the last six months, and rosemary consisted of flower leaves of cultivated *Rosmarinus officinalis* plants. Collected leaves were first air-oven dried at 45 °C for three consecutive days and then ground to pass a 2 mm screen. Proximate analysis of the dry olive leaves showed that they contained 70 g moisture, 85 g crude protein, 45 g fat, 165 g crude fibre, 172 g lignin and 51 g ash/kg. Steam distillation of the collected rosemary using a Clevenger type apparatus resulted in an essential oil yield of 1.0 mL/100 g. Analysis of this essential oil by gas chromatography (ThermoQuest Trace GC, Italy) on a fused silica column, Durabond DB-1, 60 m x 0.25 mm i.d., showed the major components were eucalyptol at 392 g/kg, vorneol at 131 g/kg and a-pinene at 46 g/kg. α -Tocopheryl acetate was obtained from Roche Products Ltd. (Hertfordshire, UK).

At 14 weeks of age the turkeys were weighed, slaughtered and their carcasses were trimmed for breast fillets by removing the skin, bones and connective tissues. Following trimming, the breast fillets from each bird were individually divided into two. One group was used for microbiological analysis and the other for oxidative stability studies

The concentrations of total phenolics in olive leaves and rosemary were determined using the Folin-Ciocalteu reagent according to a slightly modified procedure (Singleton *et al.*, 1999). In this analysis, the sample (0.5 g) was transferred to a 50 mL centrifugal tube where 25 mL of 80% aqueous methanol was added. The mixture was homogenized (Ultra-Turrax homogenizer) at high speed (20500 rpm) for 30 s and the homogenate was filtered. Filtrate aliquots of 0.05 mL were transferred to 15 mL tubes and mixed (Vortex apparatus, Scientific Industries, Inc., Model K-550-GE) with 6.45 mL distilled water and 0.5 mL of the Folin-Ciocalteu reagent (Sigma-Aldrich, GmbH, Sternheim, Germany). The mixture was allowed to stand for 3 min and, after the addition of 3 mL of a 7.5% aqueous Na₂CO₃ solution, was allowed to incubate for an additional 60 min at room temperature. Following incubation, the mixture was centrifuged at 2000 g for 5 min. The absorbance of the supernatant was measured at 760 nm (Spectrophotometer Shimadzu, Model UV-160A, Tokyo, Japan).

Since the concentration of total phenolics was calculated in gallic acid equivalents per gram of sample, the calibration curve was constructed using gallic acid as a reference standard. This standard solution was prepared by weighing 20 mg of gallic acid into a 50 mL volumetric flask and diluted to volume with 80% aqueous methanol. Volumes of 0.05 - 2.5 mL from this solution that corresponded to 20 - 100 μ g of gallic acid were placed in 15 mL tubes, diluted to 6.5 mL with water, mixed with 0.5 mL of the Folin-Ciocalteu

reagent and 3 mL of 7.5% Na₂CO₃. Their absorbance was measured at 760 nm. Total phenolics were calculated in mg of gallic acid equivalents per gram by referring to slope and intercept data of the computed least-squares fit of this standard calibration curve.

For the determination of the antioxidant capacity of olive leaves and rosemary, an improved version (Re *et al.*, 1999) of the trolox equivalent antioxidant capacity (TEAC) assay applicable to both lipophilic and hydrophilic antioxidants was applied. This improved version involves direct production of the blue/green ABTS radical cation chromophore through the reaction between a 7 mM aqueous solution of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS) obtained from Aldrich Chemical Co (Gillingham, Dorset, U.K.) and potassium persulphate with 2.45 mM (final concentration) aqueous solution of potassium persulphate in the dark for 12 - 16 h, at ambient temperature. The radical cation was stable in this form for more than two days when stored in the dark at room temperature. Addition of antioxidants to the preformed radical cation causes decolorization at an extent which depends on the antioxidant activity, and calculated relative to the reactivity of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as a standard, obtained from Aldrich Chemical Co, Gillingham, Dorset, UK. For the determination of the TEAC value, the ABTS radical cation solution was further diluted with methanol to an absorbance of 0.7 at 734 nm. Samples (0.5 g) were placed in 50 mL centrifugal tubes and 25 mL of 80% aqueous methanol were added. The mixtures were homogenized at high speed (20500 rpm) for 1 min and the homogenates were filtered. The aqueous methanol extracts were further diluted with methanol such that, after addition of a 50 µL aliquot to 2.45 mL of the diluted ABTS radical cation solution, they resulted in inhibition of the blank absorbance between 20 - 80%. Absorbance readings were taken at 734 nm exactly 1 min after initial mixing. Appropriate solvent blanks were run in each assay. In addition, a calibration curve was generated by running a series of Trolox standards (range of final concentrations, 0 - 15 µM) in methanol. The recorded percentage inhibition values of absorbance at 734 nm vs. the corresponding mass of the analyte were plotted, and the slope, intercept and least squared fit of standard curve were computed. TEAC values of samples (micromoles of Trolox equivalents per gram) were realized by reference to the standard curve, and multiplying with appropriate dilution factor.

To access the effect of the dietary treatments on the oxidative stability of turkey breast fillets during refrigerated storage, samples were sliced 0.5 cm thick, placed in open sterile polypropylene bags and stored at 4 °C in the dark. Oxidative changes were monitored on the basis of malondialdehyde (MDA) formed at day 0 and at 2-d intervals for up to 12 days of storage. MDA, the compound used as an index of lipid peroxidation, was determined by a selective third-order derivative spectrophotometric method (Botsoglou *et al.*, 1994). In brief, samples were homogenized in the presence of 8 mL of 5 g/100 mL aqueous trichloroacetic acid (Merck, Darmstadt, Germany) and 5 mL of 0.8 g/100 mL butylated hydroxytoluene (Sigma Chemical Co, St. Louis, MO) in hexane, 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 0.8 g/100 mL aqueous 2-thiobarbituric acid (Sigma Chemical Co, St. Louis, MO) and 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 MDA in the 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 (Sigma Chemical Co, St. Louis, M.O., USA.).

For microbiological analysis, fillets (25 g) were aseptically weighed, placed in sterile stomacher bags, wrapped and stored at 4 °C in the dark for 12 days. Sampling was carried out on day 0 and at 2-day intervals for up to 12 days of refrigerated storage. At each sampling day samples in stomacher bags were suspended in 225 mL sterile 0.1 g/100 g peptone water (Oxoid, Unipath, Basingstone, UK), and homogenized in a stomacher (Lab Blender 400, Seward Medical Ltd, London, England) for 90 s at room temperature. Serial decimal dilutions were prepared in 0.1 g/100 g peptone water and 1 mL of each diluent was poured in duplicate along with molten agar on plates utilizing the pour-plating technique. Bacterial counts were determined as follows: total viable count (TVC) on plate count agar (Oxoid), incubated at 25 °C for 72 h; lactic acid bacteria (LAB) on de Man, Rogosa, Sharpe (MRS) agar (Oxoid), overlaid with the same medium and incubated anaerobically at 25 °C for 96 h; *Enterobacteriaceae* (ENB) on violet red bile dextrose

(VRBD) agar (Oxoid) incubated at 37 °C for 24 h; psychrotrophic bacteria counts (PSY) on plate count agar (Oxoid), incubated at 7 °C for 10 days.

For the extraction of α -tocopherol, muscle samples (0.5 g) were homogenized with 5 mL of a saturated methanolic solution of KOH in the presence of 100 μ L pyrocatechol (Merck, Darmstadt, Germany) solution (200 mg/mL), and then immersed in a waterbath at 80 °C for 15 min (Botsoglou *et al.*, 1998). Following saponification, 5 mL hexane and 1 mL water were added, and the mixture was vortex-mixed and centrifuged at 2000 g. 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, Tokyo, Japan). Liquid chromatography was carried out, using a Nucleosil C₁₈, 5 mm, 250 x 4.6 mm column (Reading, U.K.), and a mobile phase of methanol/water (97:3, v/v) that was delivered to the system at a flowrate of 2 mL/min. A fluorimetric detector set at an excitation wavelength of 290 nm and an emission wavelength of 330 nm was used for monitoring column effluents. Detector signals were quantified on the basis of peak heights and a calibration curve using α -tocopherol (Sigma Chemical Co, St. Louis, M.O., USA) as reference standard.

Each individual turkey within each group served as an experimental unit in the statistical analysis of all data. Data were subjected to a two-way analysis of variance (ANOVA) with treatment and storage as fixed effects in the general linear model of the SPSS 10.05 statistical package (SPSS Ltd., Woking, Surrey, UK). The homogeneity of the variances was tested by the Levene's test. When significant treatment effects were disclosed at the significance level of $P < 0.05$, the Tukey's test was applied in order to determine statistical differences between means.

Results and Discussion

At the completion of the study, average body weights (4920 ± 213 g) and feed conversion ratios (2.16 ± 0.02) did not differ significantly ($P > 0.05$) between groups, indicating that the incorporation of olive leaves or rosemary to the diets had no adverse influences on the growth rate of the turkeys.

Figure 1 shows that the extent of lipid oxidation in raw turkey breast fillets stored at 4 °C for up to 12 days varied with the storage time and the dietary treatment. Refrigerated storage increased the levels of malondialdehyde (MDA), the compound used as an index of lipid oxidation, the increase being higher for the CONT group. The T150 group presented MDA values that were significantly ($P < 0.05$) lower than the

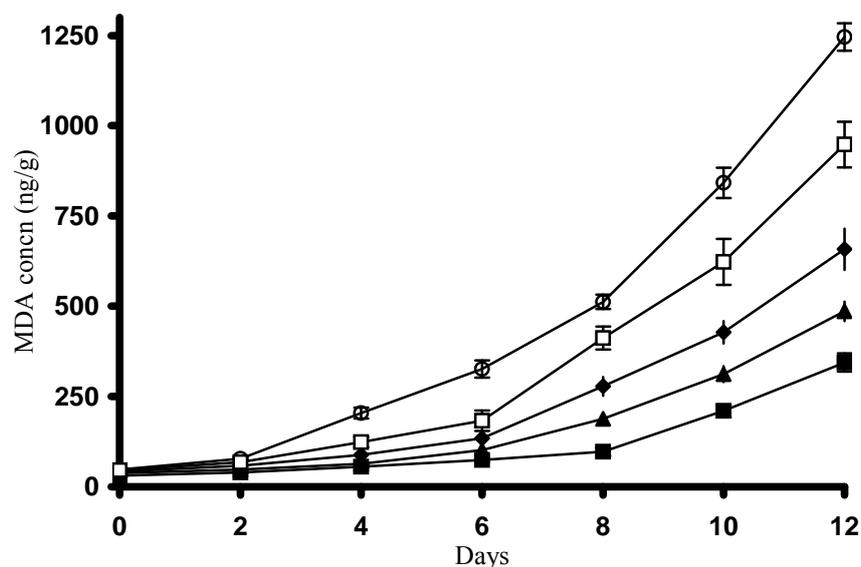


Figure 1 Effect of refrigerated storage on lipid oxidation of raw breast fillets as a function of storage time and diet; control diet (o), diet supplemented with 10 g rosemary//kg feed (◆), diet supplemented with 10 g olive leaves/kg feed (▲), diet supplemented with 150 mg α -tocopheryl acetate/kg feed (□), diet supplemented with 300 mg α -tocopheryl acetate/kg feed (■). All data points represent mean values accompanied by standard deviation bars some of which, however, lie within the data point regions.

CONT group but higher ($P < 0.05$) than the ROS group at all time points of refrigerated storage. On the other hand, the OL group had MDA values that were significantly ($P < 0.05$) lower than the ROS group but higher ($P < 0.05$) than the T300 group at all time points of refrigerated storage.

These results indicate that the incorporation of α -tocopheryl acetate at a level of 150 mg/kg into the turkey diet delayed lipid oxidation as compared to the control ($P < 0.05$). Rosemary incorporation was more effective ($P < 0.05$) in delaying lipid oxidation compared to α -tocopheryl acetate at 150 mg/kg, but less effective ($P < 0.05$) as compared to the incorporation of olive leaves. In turn, olive leaves delayed lipid oxidation at an extent lower than that of α -tocopheryl acetate at a level of 300 mg/kg feed.

The inhibition of lipid oxidation in the breast meat after diet supplementation with olive leaves or rosemary is probably the result of various constituents with antioxidant activity that entered the circulatory system, and distributed and retained in turkey tissues. Even though, in the present experiment, the active constituents of olive leaves and rosemary could not be individually quantified since their bioavailability could not be demonstrated directly due to the lack of adequate analytical methodology and equipment, the amount of total phenolics and their antioxidant activity toward the ABTS radical cation were determined.

Chemical analysis showed that the total phenol content of olive leaves was 23.8 mg of gallic acid equivalents/g, whereas the antioxidant activity expressed in TEAC values was 1.52 mM/g. This TEAC value compares well with that reported in a previous study where it was further found that olive leaves had an antioxidant activity higher than vitamins C and E, due to a synergy between flavonoids, oleuropeosides and substituted phenols (Benavente-Garcia *et al.*, 2000). Analysis results also showed that the total phenol content of rosemary was 29.4 mg of gallic acid equivalents/g, whereas its antioxidant activity expressed in TEAC values was 0.95 mM trolox equivalents/g.

The results obtained in this study are difficult to compare with previous reports since there have not been specific *in vivo* studies where both olive leaves and rosemary were supplemented. Supplementation of turkey diets with only rosemary at the level of 10 g/kg, although effective in delaying lipid oxidation in turkey tissues, has been reported to be inferior to the dietary supplementation of 300 mg α -tocopheryl acetate/kg feed (Botsoglou *et al.*, 2007). Other *in vitro* experiments with rosemary showed that the antioxidant activity of rosemary extracts was lower, comparable, or even higher than that of α -tocopherol (Kuzmenko *et al.*, 1999). On the other hand, *in vivo* studies with olive leaves components demonstrated that hydroxytyrosol, administered to rats as the only bioactive component of an olive mill waste, was able to increase plasma antioxidant capacity (Visioli *et al.*, 2001), whereas it could also protect human erythrocytes against oxidation (Manna *et al.*, 1999).

Moreover, *in vitro* experiments showed that olive leaf extracts and their main components, oleuropein and hydroxytyrosol, were much more effective than BHT or vitamin E in limiting the oxidation of methyl linoleate in model systems (Le Tutour & Guedon, 1992). The use of olive leaf juice has also been found suitable for extending the stability of sunflower oil during heating (Farag *et al.*, 2007).

Considering that the larger the amount of α -tocopherol in tissues the better the protection against oxidative attack, samples from all groups were analyzed for their α -tocopherol content at day 0 of refrigerated storage. Mean α -tocopherol level in the CONT group was found to be 0.96 ± 0.22 $\mu\text{g/g}$ tissue. Supplementing the diets with olive leaves or rosemary at 10 g/kg changed the levels of α -tocopherol in fillets to values of 0.86 ± 0.32 and 1.12 ± 0.38 $\mu\text{g/g}$, respectively. These values did not differ ($P > 0.05$) among each other or from the CONT group. Supplementing the diet with 150 and 300 mg α -tocopheryl acetate/kg increased the levels of α -tocopherol in the fillets to 1.92 ± 0.44 and 2.72 ± 0.26 $\mu\text{g/g}$, respectively. Values of α -tocopherol for the CONT and TOC breast samples compare well with literature data. However, the concentrations of α -tocopherol are low compared to that in chickens, substantiating the fact that α -tocopherol is not efficiently absorbed and deposited in the cell membranes of turkeys (Botsoglou *et al.*, 2003; Papageorgiou *et al.*, 2003).

The shelf life of stored muscle food is associated with the extent and type of microbial growth that may occur during the additional time of product storage. Therefore, TVC, LAB, ENB and PSY bacteria that constitute a major cause of poultry meat spoilage during refrigerated storage were determined. Figure 2 shows that TVC, LAB, ENB and PSY bacterial counts were all significantly increased ($P < 0.05$) in samples of all groups as the refrigerated storage period progressed. The CONT, T150 and T300 groups presented TVC, LAB, ENB and PSY counts that did not differ ($P > 0.05$) among each other during the whole storage period. However, the olive leaves- and rosemary-supplemented groups presented bacterial counts that were significantly lower ($P < 0.05$) than the CONT, T150 and T300 groups, at day 2 of storage period and

thereafter. During this period, ROS group presented TVC, LAB, ENB and PSY counts that were significantly higher ($P < 0.05$) than the OL group.

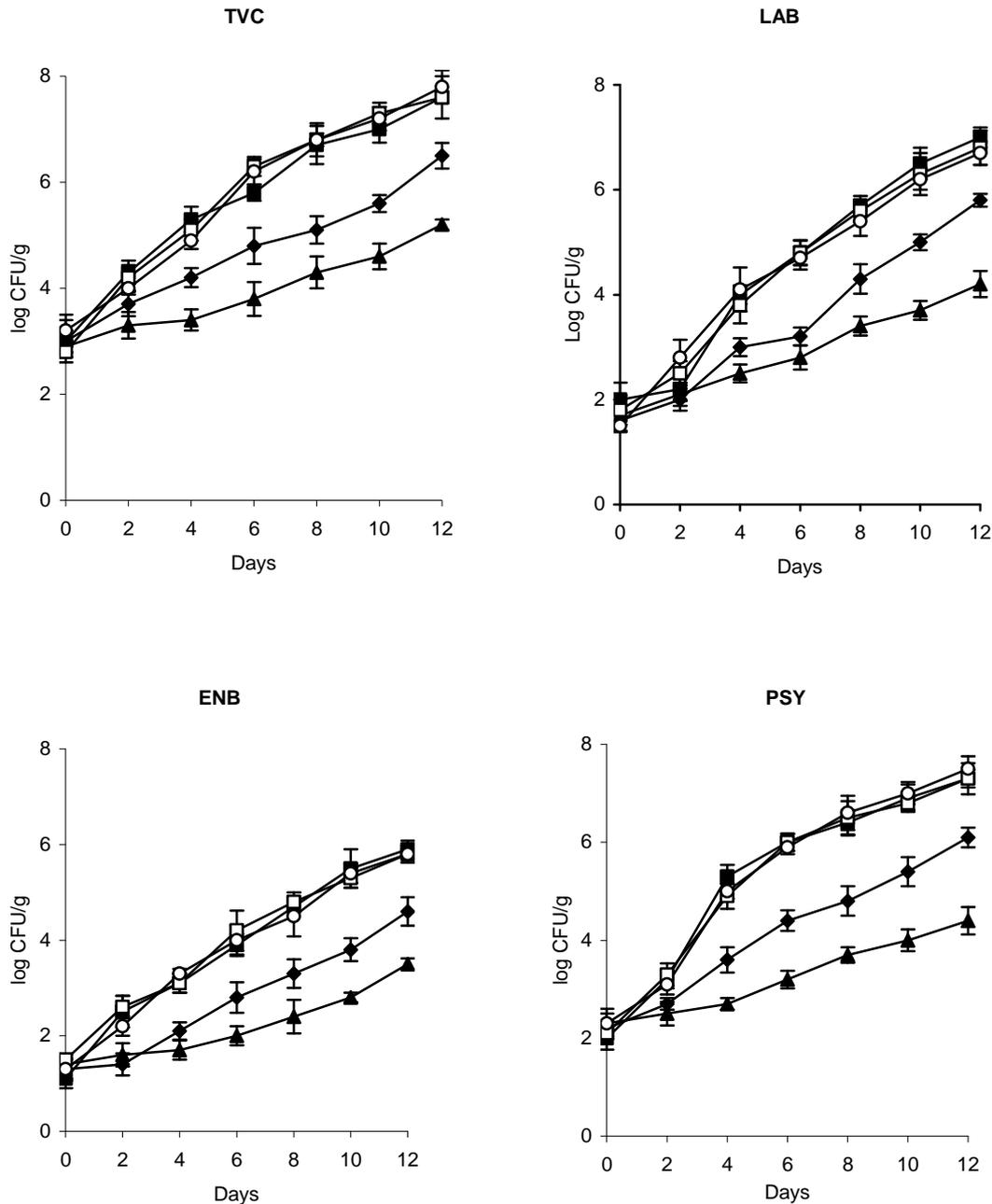


Figure 2 Changes in total viable counts (TVC), lactic acid bacteria (LAB), *Enterobacteriaceae* (ENB) and psychrotrophic bacteria (PSY) in turkey breast fillets stored at 4 °C in the dark for up to 12 days: Control diet (o), diet supplemented with 10 g rosemary//kg feed (◆), diet supplemented with 10 g olive leaves/kg feed (▲), diet supplemented with 150 mg α -tocopheryl acetate/kg feed (□), diet supplemented with 300 mg α -tocopheryl acetate/kg feed (■). All data points represent mean values accompanied by standard deviation bars some of which, however, lie within the data point regions.

At day 12 of storage, mean levels of TVC counts in the CONT, T150 and T300 groups were in the range of 7.6 - 7.8 log CFU/g, whereas in the ROS and OL groups at 6.5 and 5.2 log CFU/g, respectively. At this day of storage, mean levels of LAB counts in the CONT, T150 and T300 groups were in the range of 6.7 - 7.0 log CFU/g, whereas in the ROS and OL groups at 5.8 and 4.2 log CFU/g, respectively. At the same time, mean levels of ENB counts in the CONT, T150 and T300 groups were in the range of 5.8 - 5.9 log CFU/g, whereas in ROS and OL10 groups at 4.6 and 3.5 log CFU/g, respectively. On the same day, mean levels of PSY counts in the CONT, T150 and T300 groups were in the range of 7.3 - 7.5 log CFU/g, whereas in the ROS and OL groups counts were at 6.1 and 4.4 log CFU/g, respectively.

These results indicate that the dietary supplementation of both olive leaves and rosemary at 10 g/kg feed exerted an inhibitory activity on the growth of TVC, LAB, ENB and PSY bacteria in the stored turkey breast samples. This is in agreement with previous reports on the *in vitro* antimicrobial activity of olive leaves and its constituents (Capasso *et al.*, 1995; Markin *et al.*, 2002).

Olive leaf extracts and its constituents have shown an inhibitory effect against psychrotrophic bacteria such as *B. subtilis*, *Pseudomonas* spp. and *Proteus vulgaris* (Kubo *et al.*, 1995; Markin *et al.*, 2002). Oleuropein, the main active component of olive leaves, has been reported to act against lactic acid bacteria such as *Lactobacillus plantarum* (Ruiz-Barba *et al.*, 1991) and food-borne pathogens such as *Salmonella enteritidis* (Tassou & Nychas, 1995) and *Bacillus cereus* (Tassou *et al.*, 1991). In addition, oleuropein showed an inhibitory effect on the growth and enterotoxin production by *Staphylococcus aureus* (Tassou & Nychas, 1994), whereas both hydroxytyrosol and oleuropein exhibited an inhibitory effect against several bacterial strains (ATCC) such as *Staphylococcus aureus*, *Vibrio parahaemolyticus* or *Salmonella* spp., that are causal agents of intestinal or respiratory tract infections in humans (Bisignano *et al.*, 2001).

The results of this study also support previous reports on the *in vitro* antimicrobial activity of rosemary and its extracts. The *in vitro* antimicrobial activity of the essential oil of rosemary against several foodborne pathogens such as *Salmonella enteritidis*, *Salmonella typhimurium*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Aeromonas hydrophila* has been reported frequently (Smith-Palmer *et al.*, 1998; Ahn *et al.*, 2004). The essential oil of rosemary has been found to be bacteriostatic against strains of *Listeria monocytogenes* at concentrations of 0.02 - 0.05% (Smith-Palmer *et al.*, 1998), bacteriocidal at concentrations of 0.1 - 0.5% (Pandit & Shelef, 1994; Smith-Palmer *et al.*, 1998) and sporostatic against *Bacillus cereus* at 170 mg/kg (Chaibi *et al.*, 1997) in culture media. It has also been reported to be effective against several enterobacteria including *E. coli*, *Streptococcus faecalis*, *Alcaligenes faecalis* (Barrata *et al.*, 1998; Smith-Palmer *et al.*, 1998), *Shigella* spp. (Bagamboula *et al.*, 2003), certain lactic acid bacteria including *Lactobacillus plantarum*, *Leuconostoc cremoris* and *Lactobacillus carvatus* (Quattara *et al.*, 1997) and six psychrotrophic bacteria of meat spoilage including *Pseudomonas fluorescens*, *Serratia liquefaciens*, *Brochothrix thermoshacta*, *Carnobacterium piscicola*, *Lactobacillus carvatus* and *Lactobacillus sake* at levels of 0.01 - 0.1% in culture broths (Quattara *et al.*, 1997). Moreover, the essential oil of rosemary sprayed on beef steaks at a level of 1000 mg/kg reduced the microbial growth of psychrotrophic bacteria during refrigerated storage under a modified atmosphere (Djenane *et al.*, 2003), whereas its direct addition to ground beef at a level of 1% resulted in reduction of *Salmonella typhimurium*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* populations at about 1 log CFU/g after refrigerated storage for nine days (Ahn *et al.*, 2004).

The results of this study also showed that the dietary supplementation of 150 and 300 mg α -tocopheryl acetate/kg feed exerted no inhibitory effect on TVC, LAB, ENB and PSY bacterial counts in breast samples stored for up to 12 days. This is in line with previous studies, reporting no effect of dietary vitamin E on aerobic plate counts in raw pork (Cannon *et al.*, 1995) and beef meat (Chan *et al.*, 1995). However, other workers (Asghar *et al.*, 1991) reported that bacterial growth was greater in chops from pigs fed diets with supplemental vitamin E, which had lower drip losses. This difference in bacterial counts was ascribed to potentially higher water activity or reduced antimicrobial activity due to lack of lipid oxidation products because of the action of vitamin E.

At day 10 and thereafter, the CONT group showed initial signs of spoilage with the development of off-flavours and indication of slime formation; by this time, TVC counts of these samples were about 7 log CFU/g. This is in agreement with Sofos *et al.* (2000), who reported that meat spoilage is visible when bacteria counts reached about 7 log CFU/g. Other workers found similar changes in microbial growth and time for the onset of spoilage in turkey fillets under refrigerated storage (Mano *et al.*, 2000). However, T150 and T300 groups did not show initial signs of spoilage, although samples presented bacterial counts similar

to the CONT group. No signs of spoilage were also noticed for the other dietary groups up to the end of the storage period. This retardation on visible signs of spoilage of these samples might endanger consumers' health due to increased numbers of bacterial counts.

Conclusion

Results showed that the incorporation of olive leaves and rosemary in turkey diets delayed lipid oxidation in raw breast fillets during refrigerated storage for up to 12 days. The incorporation of olive leaves at the level of 10 g/kg was found to be more effective in delaying lipid oxidation than rosemary, but inferior to the dietary supplementation of 300 mg α -tocopheryl acetate/kg. Both olive leaves and rosemary also exerted an inhibitory effect on the growth of TVC, LAB, ENB and PSY bacteria in breast samples during storage. The rate of inhibition against microbial growth was higher in the case of olive leaves and lower in the case of rosemary. The dietary supplemented α -tocopheryl acetate did not show such an antimicrobial effect. Therefore, if shown clinically to be safe and having beneficial effects *in vivo*, rosemary and particularly olive leaves might be utilized in novel applications as nutritional supplements or as functional food components.

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