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Full Length Research Paper

Effect of dietary vitamin E supplementation on lipid and colour stability of chicken thigh meat

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Lipid and myoglobin oxidation are major causes of meat quality deterioration during storage of fresh chicken meat. Our objective is to determine the effects of dietary α-tocopherol supplementation on lipid and colour stability of fresh chicken thigh meat exposed in a supermarket shopwindow or stored in a refrigerator. Chickens were fed with diets containing 25 (control), 100, 200 or 300 mg vitamin E/kg of food for 20 days before slaughtering. Peroxide value (PV) and oxidation products specific extinctions (K232 and K270), chosen as markers for oxidative deterioration of lipids, were lower in chicken meat from animals supplemented with 200 or 300 mg vitamin E than that of the control meat upon storage during 5 days in a shopwindow ($p < 0.01$) or 9 days in a refrigerator ($p < 0.01$). Vitamin E dietary supplementation (200 mg vitamin E/kg of food) reduced oxidation products formation in fresh thighs meat but had no significant impact on colour analysed by value redness (a*) measurements.

Key words: Chicken, diet supplementation, vitamin E, lipid oxidation, meat colour.

INTRODUCTION

Lipid and myoglobin oxidation are major causes of meat quality deterioration during storage (Jensen et al., 1998). In addition, different animal species may be classified on the basis of meat sensibility to oxidation in the following order fish > turkey > chicken > pork > beef > lamb (Tichivangana and Morissey, 1985). The basic mechanism of lipid oxidative reactions can be characterised by 3 distinctive steps; initiation, propagation and termination reactions (Underland, 2001). The oxidation phenomenon depends on both intrinsic factors (unsaturated fatty acids concentration, myoglobin, enzymes and iron) and extrinsic factors (light and oxygen consumption) (Jensen et al., 1998; Underland, 2001; Andreo et al., 2003). During lipid oxidation, decompo-sition reactions occur simultaneously and generate a wide variety of different molecules, including aldehydes, ketones, alcohols, peroxides and hydrocarbons. These oxidation products are responsible essentially for the changes in meat flavour. As a result, such meat is not assumed to be fresh by the consumer.

To ensure optimum quality, it is necessary to consider the entire production chain from farm to fork. Many studies focused on the impact of many dietary supplemental components such as vitamin E (Jensen et al., 1998) or fatty acids (Wood et al., 2003) on *post-mortem* meat quality. Supplementation of vitamin E significantly improved the meat stability against oxidative deterioration in beef (Mitsumoto et al., 1993), pork (Phillips et al., 2001) and turkey (Guo et al., 2001) meat. Vitamin E cannot be synthesized by animals and therefore its presence in animal tissue reflects the dietary availability. Due to its lipophilic character, the vitamin E absorption is dependent on animals' fat digestion and absorption (Wiss et al., 1962). The most commonly used commercial form of vitamin E for animals' food supplementation is the acetate ester of all rac-α-tocopherol. The esters display no antioxidant activity and they are hydrolysed in the gut, releasing the native α-tocopherol that possesses an antioxidant activity. In fact, some studies have shown that dietary vitamin E supplementation significantly increased the α-tocopherol content of muscle membranes in many animals (Lauridsen et al., 1997) and functions as a lipid antioxidant and free radical scavenger (Guo et al., 2001).

It is important to note that *postmortem* addition of α-

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Table 1. Composition and nutrient levels (g/kg) of basal diet.

Nutrient	Level (g/kg)
Maize	670
Soya bean meal	290
Dicalcium phosphate	18
DL-Methionine	1.3
L-Lysine-HCl	0.3
Choline chloride	0.8
Flavomycin	1.6
Anticoccidien	14
Minerals premix[a]	2
Multi-vitamin premix[b]	2

[a]Minerals premix provided per kg of food with Fe 50 mg, Cu 1250 mg, Zn 50 mg, Mn 50 mg, Co 250 mg, Se 50 mg and I 300 mg.
[b]Multi-vitamin premix provided per kg of food with vitamin E 25 mg, K_3 500 mg, B_1 160 mg, B_2 1000 mg, B_3 5 mg, B_6 160 mg, B_{12} 5 mg, pp 5 mg and folic acid 140 mg.

tocopherol to meat is not as effective as dietary supplementation, since α-tocopherol is not incorporated directly into the membrane where lipid oxidation is initiated (Mitsumoto et al., 1993).

The objective of this work is to develop a better understanding of the effect of dietary vitamin E supplementation on lipid oxidation and colour quality of fresh chicken thigh meat during exposition or storage under sales conditions.

MATERIALS AND METHODS

Animals and diets

One day old chickens (*Gallus gallus*) (French race JV) were provided by Société d'Aviculture du Nord, Tunisia. Female chicken (n = 1600) were raised at a local hen house divided into 4 groups containing 400 chickens each. Animals were fed a basal diet (Table 1) containing 25 mg vitamin E/kg of food for 14 days. The supplemented vitamin E which represents the unique antioxidant in the diet, was an α-tocopherol acetate supplied by (Nutrisud, Tunisia). Animals of one group remained on the basal diet (25 mg/kg of food) and were considered as control animals. The other groups of animals were switched to the treatment diet containing respectively 100, 200 or 300 mg vitamin E/kg of food for 20 days before slaughtering. Feed added to each group and the amounts of residual feed remaining at the end of periods of feeding were recorded. The feed intake represents the average of the values determined during 20 days of feeding. Mortality of birds was also recorded. The average live body weight at slaughtering was 1.7 kg. All chickens were slaughtered at a local abattoir (Chahia, Tunisia).

Experimental conditions

Chicken thighs (n = 160) relative to each dietary treatment [control (25), 100, 200 or 300 mg vitamin E/kg of food] were stored under sales conditions either in a shopwindow (light for 12 h/day, 8°C) or in a refrigerator (dark, 4°C) without any packaging. We noticed that time needed for meat handling and delivering to the supermarket is around 24 h after slaughtering.

Fat extraction

Lipids were extracted from 5 thighs selected randomly from each treatment and for each storage condition, using a method adapted from that described earlier (Folsh et al., 1957). 50 g of thigh tissues were homogenised with 150 ml of chloroform/methanol (2/1: v/v) solution using a blender mixer (Waring, USA) (2 x 30 s). The mixture was filtered then added to 50 ml KCl solution (0.88%). After decantation, the organic phase (lower phase) was collected and reextracted twice with 100 ml methanol/KCl 0.88% (1/1:v/v) solution. The solvent was made to evaporate using a rotary evaporator at 35°C and the residual solvent was removed by flushing with nitrogen. Finally, the obtained oil was stored in a freezer (-20°C) for subsequent physico-chemical analysis. The total lipid content of chicken thigh was found to be around 14% (w/w).

Vitamin E detection on thigh fat

The presence of vitamin E in the fat extracted from the chicken thighs was determined using HPLC system from aplied biosystem (Roissy, France). The fat sample (50 µl) was solubilised in 1 ml of methanol. Then, 100 µl of fat solution was loaded on a hydrophobic column (C18 Eurospher 100 ; 250 x 4.6 mm) equilibrated in methanol/water solution (9/1: v/v). Elution was performed with the same solution at 1.5 ml/min and the absorbance of eluted fractions was measured at 292 nm (Pyka and Sliwiok, 2001).

Peroxide value measurement

Oxidation was evaluated by the peroxide value (PV) according to the analytical method described in the Regulation 2568/91 of the European Union Commission (EUC, 1991). The lipid sample (1.0 g) was treated with 25 ml of organic solvent mixture (chloroform/acetic acid, 2/3: v/v). The mixture was shaken vigorously, followed by addition of 1 ml of saturated potassium iodide solution. The mixture was kept in the dark for 5 min before adding 75 ml of distilled water. 0.5 ml of starch solution (1%, w/v) was added to the mixture, as an indicator. The PV was determined by titrating the iodine liberated from potassium iodide with standardised 0.01 N sodium thiosulfate solution and was expressed as milliequivalents (meq) of free iodine per kg of lipid.

Specific extinctions measurement

Specific extinction coefficients K232 and K270 were determined according to the analytical methods described in the regulation 2568/91 of the European Union Commission (EUC, 1991). These coefficients were calculated from absorbances at 232 and 270 nm, respectively, with UV spectrophotometer (Hitachi, Japan) using a 1% solution of fat in cyclohexane and a path length of 1 cm.

Surface discoloration analysis

The meat used for surface discoloration analysis was preliminary de-skinned and the apparent fat removed manually. The CIELAB coordinate (a*) was directly read on exposed surface samples of 13 mm thickness using a Minolta chromameter CR200 (Osaka, Japan). In those coordinates system, the (a*) value ranges from -100 (blueness) to +100 (redness). 10 readings were taken on each sample.

Table 2. Levels of the two factors studied.

Variable	Level			
V	25 mg/kg	100 mg/kg	200 mg/kg	300 mg/kg
S	Light 12h, 8°C	-	-	Dark, 4°C

V: vitamin E level; S: Storage condition.

Statistical analysis

Analytical determinations (PV, K232 and K270) were performed at least in triplicate. values of different parameters were expressed as the mean ± standard deviation (X ± SD). Significant differences between mean ($P < 0.01$) were determined by Fisher's test. Data were analysed by a crossed two-factor (Table 2) analysis of variance (ANOVA) using (Med calc. V. 9370 software) and according to the model,

$$Yijk = \mu + Vi + Sj + (V*S)ij + eijk$$

where Yijk are the observations of (PV, K232 or K270) for the k^{th} repetitions level i of V and j of S, Vi is the effect of level i of vitamin E, (i = 1 - 4), Sj is the effect of level j of storage condition, (j = 1, 2), (V*S)ij is the interaction effect between vitamin E level and storage condition, eijk is the error, and k = 3 number of analysis replications.

RESULTS AND DISCUSSION

Chickens performance

Vitamin E tissue accumulation has been shown to be correlated with the dietary level, the duration of the supplementing period, the animal race and sex as well as with the tissue type (Shehhy et al., 1991). In fact, it was shown that vitamin E saturation of the chicken thigh was reached after 3 weeks of dietary supplementation with 200 mg vitamin E/kg of food (Brandon et al., 1993).

In the present study, animals were supplemented with 4 different vitamin E concentrations (control (25), 100, 200 or 300 mg vitamin E/kg of food) for 20 days before slaughtering in order to preserve the highest meat quality. Live body weight was recorded during animal raising (Figure 1). Our results showed that no significant correlation existed between high dietary supplemental vitamin E levels and animals' growth. Furthermore, the feed intake values for control and treated groups were found to be 126.93 ± 0.65 g of dry matter/day. The mortality yield of studied groups was found to be 1.375% ± 0.14. This is in line with the findings of Guo et al. (2001), that feed intake and mortality of birds were not influenced by vitamin E supplementation. In addition, some studies showed that a 150 - 300 mg/kg of food vitamin E supplementation enforces the poultry immune response against newcastle disease (Franchini et al., 1995) and infectious bronchi (Mcknight, 1996).

Oxidative stability of thigh meat

It was shown that thigh meat has higher amounts of endogenous vitamin E and that at the same time it tends to oxidize faster than breast meat (Jensen et al., 1997). In fact, the higher susceptibility of thigh meat to oxidation is readily explained by its higher content of poly-unsaturated fatty acids and the high level of pro-oxidative species originating from myoglobin and other iron containing proteins (Rhee and Ziprin, 1987). The thigh lipids were extracted by organic solvents and the presence of vitamin E in these lipid samples was determined by HPLC analysis. Our results showed that vitamin E levels were higher in all chickens which received 100, 200 or 300 mg vitamin E/kg of food than the control group receiving only 25 mg vitamin E/kg of food. The thigh fat of animals supplemented with 200 mg/kg of food contained a vitamin E level 7 times higher than that of the control samples (Figure 2). This result indicates that dietary vitamin E supplementation increases the endogenous vitamin E level in *post-mortem* muscles.

A kinetic study of fat oxidation was carried out on chicken thighs stored in a supermarket shopwindow (light for 12 h/day, 8°C) or in a refrigerator (dark, 4°C). Peroxide value (PV) and oxidation products specific extinctions (K232 and K270) were chosen as markers for oxidative deterioration of lipids during meat storage. Our results showed that, as the allowance of dietary vitamin E increased, the PV and specific extinctions coefficients of thigh fat increased during the first 5 storage days in the shopwindow (Figure 3). However, their values remained lower than those corresponding to the control ($p < 0.01$). The lowest oxidation rate corresponds to the chickens supplemented with vitamin E at 200 mg/kg of food or 300 mg/kg of food. It is noteworthy that treatments corresponding to the control (25 mg vitamin E/kg of food) or 100 mg vitamin E/kg of food have been found to have also comparable effect on fat oxidation. After 3 days of storage, PV of control thighs reached 11.5 meq/kg (Figure 3A). Meanwhile, the thigh meat corresponding to the chicken supplemented with 200 mg/kg of food did not exceed 7 meq/kg. These results suggest that 200 mg/kg of food or 300 mg/kg of food dietary vitamin E improved the oxidative stability of thigh meat during it exposition at 8°C (shopwindow) which is due to accumulation of vitamin E in these tissues (Figure 2).

Thigh from chicken fed with vitamin E (200 mg/kg of food or 300 mg/kg of food) supplemented diets exhibited a significantly lower PV and specific extinctions coefficients than the control thigh ($p < 0.01$) following 9 days of refrigeration (dark, 4°C) (Figure 4). Within 3 days of storage in these conditions, PV of control thighs (8

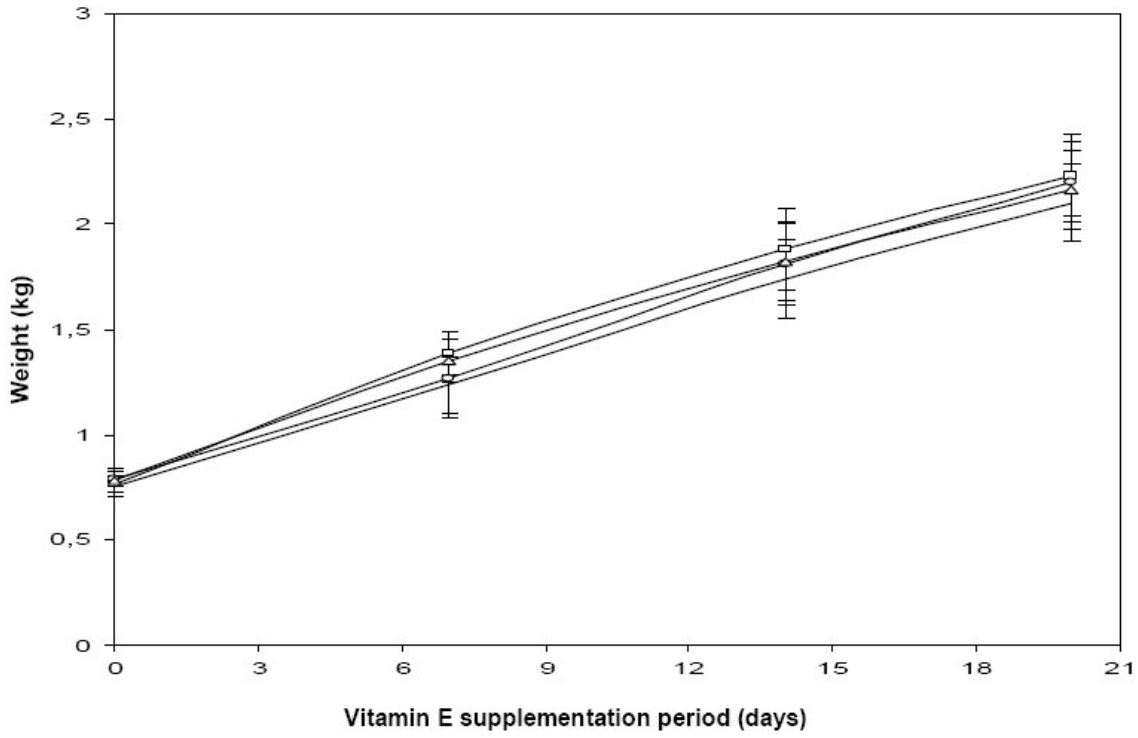

Figure 1. Changes of chicken weight during vitamin E supplementation period. Animals were fed with vitamin E: 100 mg/kg of food (□), 200 mg/kg of food (○) or 300 mg/kg of food (Δ) for 20 days before slaughtering. Control animals were fed with 25 mg vitamin E/kg of food. Every point represents the mean of 10 weights and standard error bars are indicated.

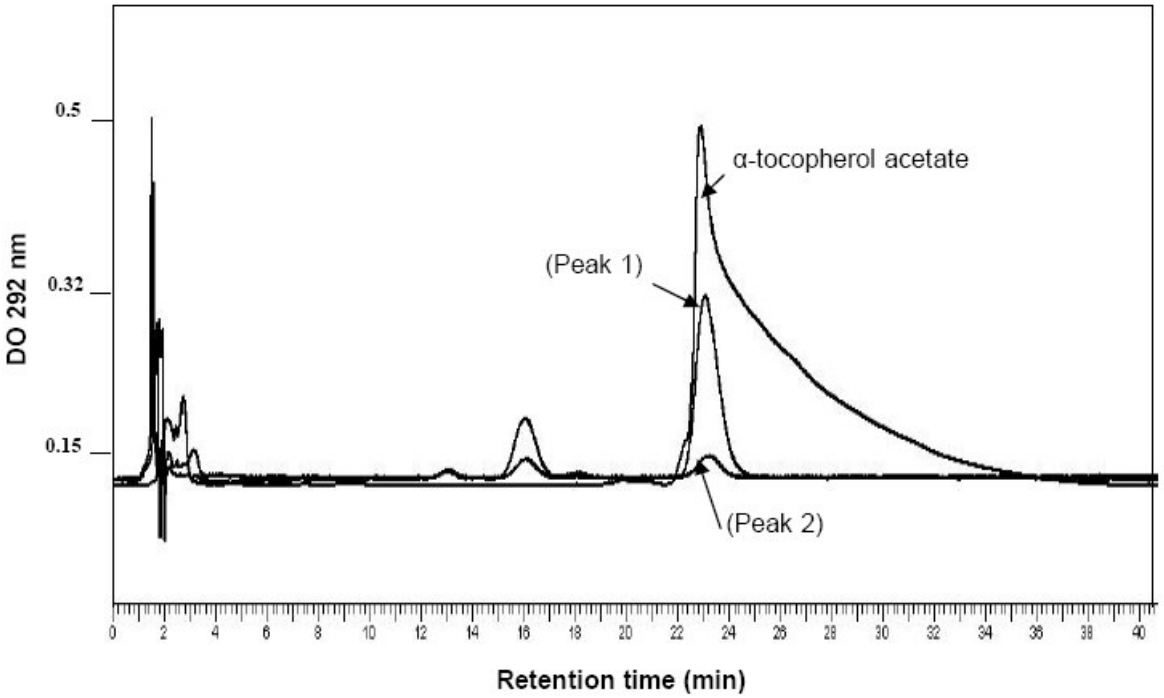

Figure 2. C18-HPLC chromatogram of α-tocopherol acetate (supplemented vitamin E) (23 min). Fat of animals fed with 200 mg vitamin E/kg of food (23 min) (peak 1) and fat of control animals fed only with the basal diet (25 mg vitamin E/kg of food) (23 min) (peak 2). Injected sample volume was 100 µl of fat solution. Elution was performed with methanol/water solution at 1.5 ml/min and the absorbance of eluted fractions was measured at 292 nm.

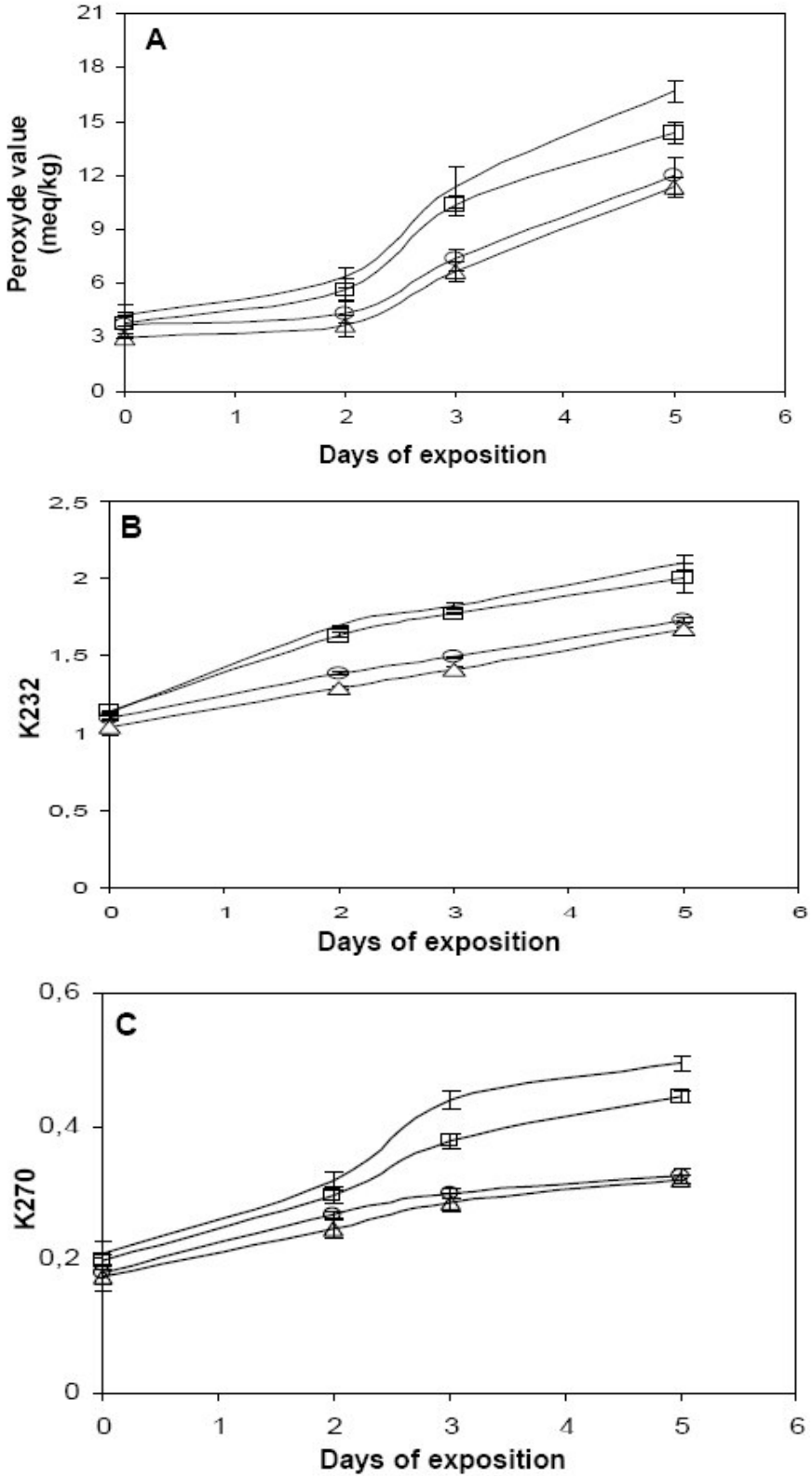

Figure 3. Lipid oxidation during meat exposition in shopwindow (light for 12 h/day, 8°C) in thigh fat of animals fed with vitamin E: 100 mg/kg of food (□), 200 mg/kg of food (○) or 300 mg/kg of food (Δ). Control animals were fed with 25 mg vitamin E/kg of food. A. Changes in peroxide values. B. Specific extinction coefficients K232 at 232 nm. C. Specific extinction coefficients K270 at 270 nm. Standard error bars are indicated.

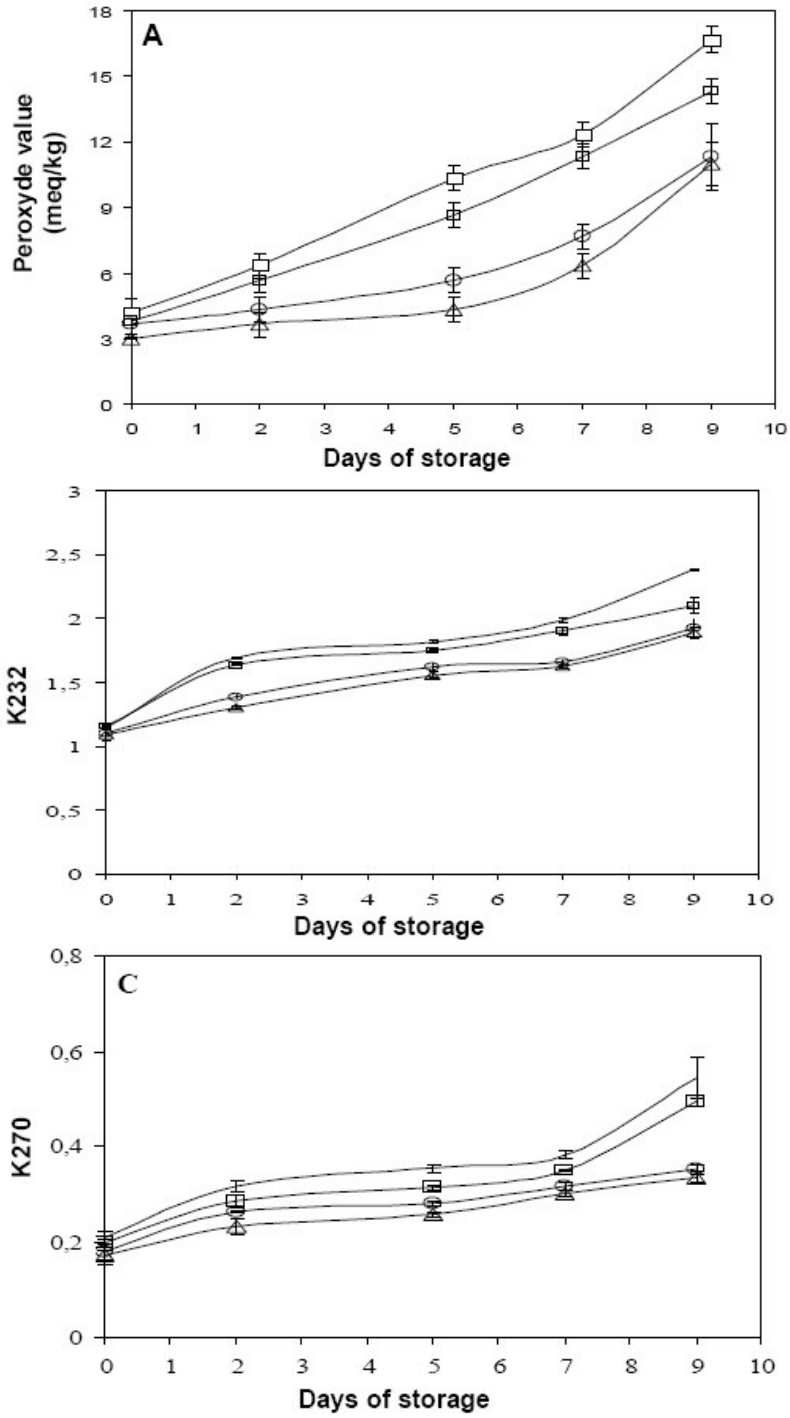

Figure 4. Lipid oxidation during meat storage in refrigerator (dark, 4°C) in thigh fat of animals fed with vitamin E: 100 mg/kg of food (□), 200 mg/kg of food (○) or 300 mg/kg of food (Δ). Control animals were fed with 25 mg vitamin E/kg of food. A. Changes in peroxide values. B. Specific extinction coefficient K232 at 232 nm. C. Specific extinction coefficient K270 at 270 nm. Standard error bars are indicated.

meq/kg) was higher than that of the chicken supplemented with 200 mg/kg of food (5 meq/kg). This indicates the effectiveness of vitamin E in reducing meat oxidation. As expected, in comparison with samples exposed in shop-window (light for 12 h/day, 8°C), thighs stored in the refrigerator (dark, 4°C) showed lower oxidation indicator (PV, K232 or K270) values (Figure 4). These results indicate the effect of light and temperature in enhancing

Table 3. Results of the analysis of the variance of lipid oxidation estimated by PV, K232 or K270 measurements.

Response	Source of variation		
	V	S	(V*S) interaction
PV	F-test = 95.33 P < 0.001	F-test = 577.6 P < 0.001	F-test = 1.07 P = 0.391
K232	F-test = 94.85 P < 0.001	F-test = 133.41 P < 0.001	F-test = 11.08 P <0.001
K270	F-test = 369.10 P < 0.001	F-test = 787.71 P < 0.001	F-test = 49.76 P < 0.001

V: vitamin E level; S: Storage condition.

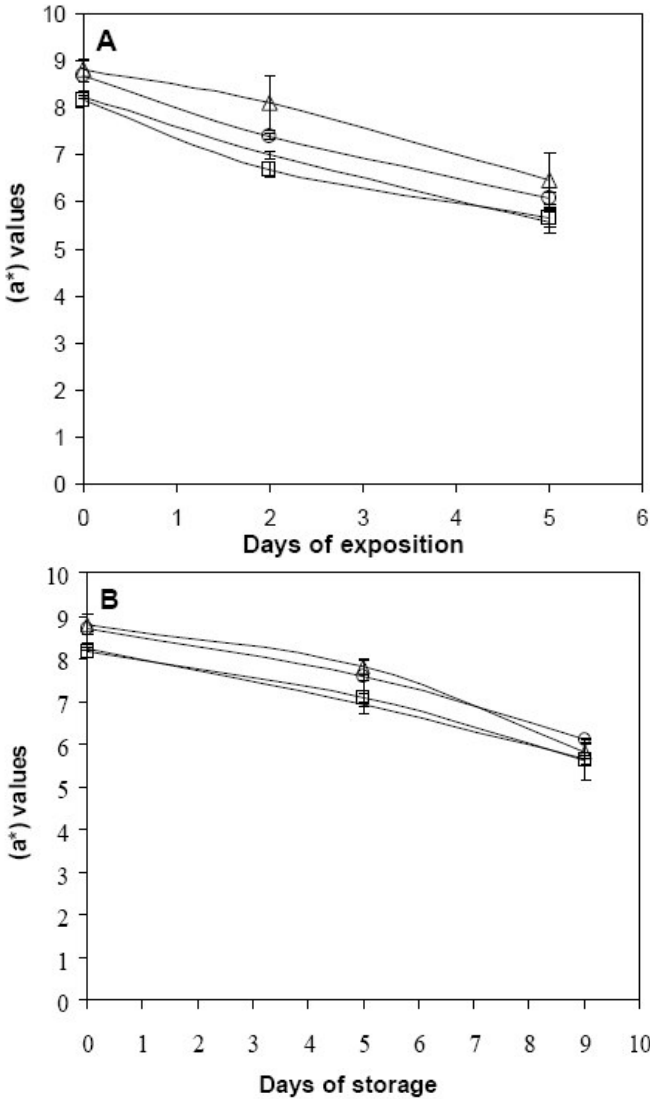

Figure 5. Changes in (a*) values in thigh meat of animals fed with vitamin E: 100 mg/kg of food (□), 200 mg/kg of food (○) or 300 mg/kg of food (Δ) during exposition in shopwindow (light for 12 h/day, 8°C) (A) or storage in refrigerator (dark, 4°C) (B). Control animals were fed with 25 mg vitamin E/kg of food. Standard error bars are indicated.

lipid oxidation as it was previously described (Andersen et al., 1990).

To study the effect of the vitamin E level and the storage condition on lipid oxidation, a two-factor analysis of variance with interaction was determined after 5 days of storage for the PV, K232 and K270 measurements (Table 3). This analysis showed that the two factors significantly affected the lipid oxidation for the determined responses ($P < 0.001$). Interaction was significant ($P < 0.001$) for K232 and K270, but not for PV measurements (Table 3).

Thigh meat colour stability

In general, meat colour is perceived by consumers as indicative of freshness, in that they discriminate against meat that has turned brown in colour. The rate of discolouration in fresh meat is related to the rate of pigment oxidation, oxygen consumption and to the effectiveness of the metmyoglobin reducing system (O'keefe and Hood, 1982; Ledward, 1991). In fact, discoloration and lipid oxidation are known to be related (Greene et al., 1971). In comparison to beef, few studies have been carried out on the discoloration of "white" meats such as chicken. In this work, we were interested in assessing the effect of diet supplementation on the chicken thigh meat colour, by measuring the meat (a*) value that is an indicator of redness. Our results showed that (a*) values decreased over time and were not significantly different between thigh meat from vitamin E-fed chicken or control ones upon refrigeration in shop-window or in refrigerator ($p < 0.01$) (Figure 5). In fact, vitamin E supplementation, did not improve the colour stability of the thigh meat. Similar results were reported by (Cannon et al., 1996) and Phillips et al. (2001) for pork meat. Nevertheless, Vitamin E was shown to be beneficial for sensory scores (freshness, tenderness and juiciness) of refrigerated pork chops (Dirinck et al., 1996). The effect of endogenous vitamin E on colour quality is more evident in species having higher levels of myo-globin and positive relationship between dietary vitamin E

and improved colour stability has been clearly demonstrated in beef (Chan et al., 1996) and lamb (Guidera et al., 1997).

Conclusion

Lipid oxidation is an important process of quality deterioration in poultry meat which manifested essentially adverse changes in flavour. Our results showed that there was no significant correlation between high dietary supplemental vitamin E levels (100 -300 mg/kg of food) and animals' growth rate.

Vitamin E dietary supplementation with 200 mg/kg of food or 300 mg/kg of food for 20 days before slaughtering has been found to be effective in reducing lipid oxidation in fresh thigh meat during refrigerated storage. However, no significant impact on meat colour analysed by value redness measurements was observed. Due to the cost and the comparable effect of vitamin E dietary supplementation with 200 and 300 mg vitamin E/kg of food, the quantity of 200 mg/kg of food may be recommended for chicken for 3 weeks before slaughtering to ensure protection against oxidative changes, thereby improving storage stability.

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