Evaluation of Lipid Ultraviolet Absorption as a Parameter To Measure Lipid Oxidation in Dark Chicken Meat

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The application of lipid UV absorption (235, 269, and 280 nm) to follow up lipid oxidation in dark chicken meat has been evaluated using raw and cooked samples with different α-tocopherol contents (modulated by dietary supplementation). To this purpose, when absorption was measured at 235 nm, second-derivative spectrophotometry did not show any significant advantage over nonderivative spectrophotometry. For absorption at 269 and 280 nm, nonderivative spectrophotometry more sensitively monitored lipid oxidation than second- and third-derivative spectrophotometry. In addition, only direct measurements at 235 and 269 nm and second-derivative measurements at 235 nm showed a limited usefulness to follow up lipid oxidation in our samples. However, these UV absorption parameters were much less effective than lipid hydroperoxide values measured through a ferrous oxidation−xylenol orange method and 2-thiobarbituric acid values determined by a third-derivative spectrophotometric method with acid aqueous extraction.

Keywords: *Lipid oxidation; dark chicken meat; derivative spectrophotometry; lipid UV absorption; xylenol orange; 2-thiobarbituric acid*

INTRODUCTION

Lipid peroxidation is a major cause of muscle food spoilage, resulting in the generation of off-odors/flavors as well as diminishing the nutritional value of the product (Chow, 1992; Igene et al., 1985). In addition, several biological effects, often detrimental, have been reported for numerous lipid oxidation products. More frequently reported effects are cytotoxicity, atherogenesis, mutagenesis, carcinogenesis, alteration of cellular membrane properties, and alteration of the activity of several enzymes (Brown and Jessup, 1999; Chow, 1992; Guardiola et al., 1996; Kubow, 1990; Smith and Johnson, 1989). Consequently, there is a need for accurate methods of screening to measure the extent of lipid oxidation in muscle foods. Methods measuring lipid hydroperoxides (LHP) and malondialdehyde (MDA) have been used for this purpose (Gray and Monahan, 1992; Melton, 1983). In previous works, we reviewed the advantages and drawbacks of such methods and studied the applicability of a ferrous oxidation−xylenol orange (FOX) method and a 2-thiobarbituric acid (TBA) assay to determine, respectively, LHP and TBA values in dark chicken meat (Grau et al., 2000a,b).

Another simple and rapid technique to assess lipid oxidation in meat could be the measurement of conjugated diene formation by lipid UV absorbance at 230−235 nm. This method has been extensively used to monitor lipid oxidation of polyunsaturated fatty acids (PUFA) in both vegetable and fish oils and in some biological samples, mainly low-density lipoproteins (Frankel et al., 1996; Jialal and Devaraj, 1996; Moore and Roberts, 1998; Puhl et al., 1994; Wanasundara and Shahidi, 1996; White, 1995). However, the usefulness of this method for absolute quantification of lipid oxidation is limited because the magnitude of the value obtained greatly depends on the fatty acid composition of the lipid fraction analyzed (Moore and Roberts, 1998; White, 1995). This drawback is due to the fact that the absorption peak appears as a shoulder on the strong absorption band due to other lipid components, especially the PUFA themselves (Corongiu et al., 1986; Corongiu and Banni, 1994; Corongiu and Milia, 1983; Defrancesco et al., 1980). This drawback seems to be overcome by the use of second-derivative spectrophotometry, which is particularly effective in extracting signals out of shoulders on absorption slopes. In this line, various authors have reported better resolution and higher sensitivity using this technique rather than direct spectrophotometry in vegetable oils (Defrancesco et al., 1980), experimental models with PUFA (Baron et al., 1997; Corongiu and Milia, 1983), and lipid extracts from biological samples (Corongiu et al., 1983, 1986; Sergent et al., 1993).

In addition, the measurement of UV absorbance between 265 and 280 nm has been less popular in samples other than vegetable oils. Absorption at these wavelengths is mainly due to secondary oxidation products of ketonic and aldehydic nature with conjugated double bonds (Galanos et al., 1968; White, 1995; Wolff, 1968).

Because PUFA content in chicken is higher than in other meats, the purpose of this work was to assess the usefulness of measuring (through nonderivative and derivative spectrophotometry) lipid UV absorption at

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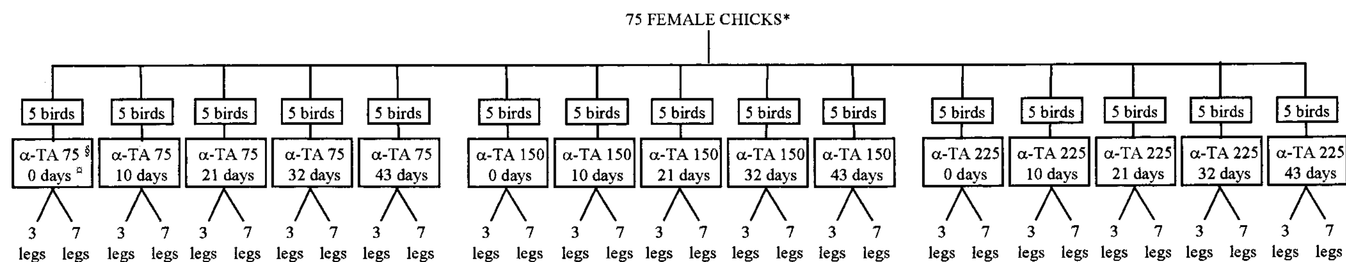

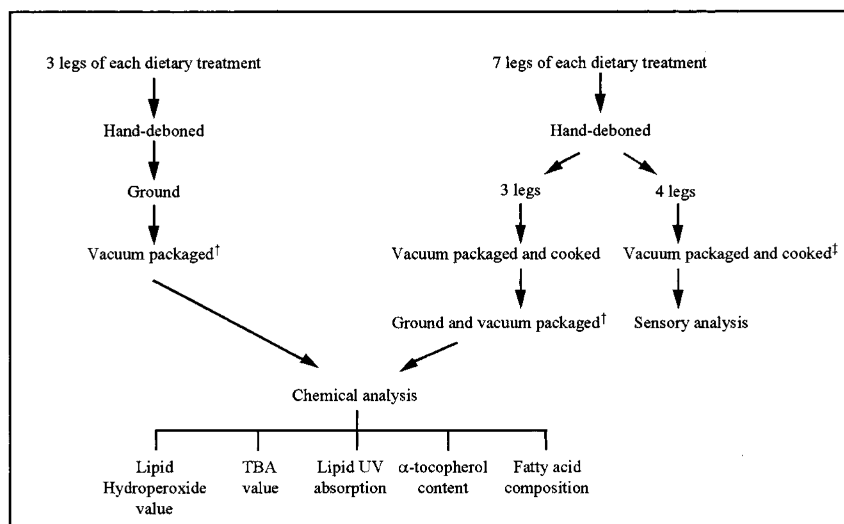

* This experimental design was conducted in triplicate (75 × 3 female broiler chicks randomly assigned to 15 × 3 treatments).
§ Dose of α-tocopheryl acetate supplementation expressed as mg/kg of feed.
¤ Days of α-tocopheryl acetate supplementation.
† Samples were stored at -80 °C until analysis.
‡ Samples were stored at -20 °C until analysis.
Abbreviations used: TA, tocopheryl acetate; TBA, 2-thiobarbituric acid.

Figure 1. Experimental design.

235, 269, and 280 nm as a simple and rapid method to monitor lipid oxidation in raw and cooked dark chicken meat with different α-tocopherol content (modulated by dietary supplementation). Results were contrasted to those from FOX and TBA methods, and the correlation between them and the α-tocopherol content of samples was studied.

MATERIALS AND METHODS

Reagents and Standards. Ethylenediaminetetraacetic acid (EDTA) disodium salt was from Sigma (St. Louis, MO) and cyclohexane (spectrophotometric grade) from Panreac (Barcelona, Spain). Methanol used in the FOX method and α-tocopherol analysis was of HPLC grade (SDS, Peypin, France), whereas that used in the UV absorption method and fatty acid determination was of analytical grade (Panreac). Distilled deionized water was used throughout.

Diets and Animals. A 3 × 5 factorial arrangement was planned and conducted in triplicate to study the influence of *dl*-α-tocopheryl acetate (α-TA) (dose and days of dietary supplementation prior to slaughter) on lipid oxidation in raw and cooked dark chicken meat. Fifteen dietary treatments were prepared from a basal diet (Table 1), which resulted from the combination of the different levels of the factors studied (3 × 5): dose of α-TA supplementation (75, 150, and 225 mg/kg of feed) and days of supplementation prior to slaughter (0, 10, 21, 32, and 43 days). Seventy-five female broiler chicks (Hubber, 1 day old) were randomly assigned to the 15 dietary treatments (Figure 1), and they were fed ad libitum throughout the experiment. α-TA (Rovimix E-50 Adsorbate) was supplied by Hoffmann-La Roche (Basel, Switzerland).

Table 1. Ingredients and Composition of the Basal Diet

ingredients	%	composition[a]	%
corn	54.0	dry matter	89.1
soybean meal, 48% protein	33.7	crude protein	21.8
full-fat extruded soybeans	4.7	crude fat	7.0
lard	3.4	ash	6.4
dicalcium phosphate	2.2	crude fiber	3.2
calcium carbonate	1.0		
salt	0.5		
DL-methionine	0.2		
trace mineral−vitamin mix[b]	0.4		

[a] Metabolizable energy, 3100 kcal/kg of feed. [b] Includes dl-α-tocopheryl acetate (20 mg/kg of feed).

Sample Preparation. At 44 days of age, broiler chickens were leg-ringed and slaughtered according to commercial procedures at the COPAGA slaughterhouse (Lleida, Spain). Legs from each treatment were divided into two groups and hand-deboned. One group (three legs with skin) was ground, vacuum packaged in 18.5 × 22 cm high-barrier multilayer bags, and stored at −80 °C until analysis. The other group (seven legs with skin) was vacuum packaged in 20 × 40 cm high-barrier multilayer bags and cooked in an oven (90 °C and 95% of relative humidity) to an internal temperature of 80 °C. Part of the cooked legs were then ground, vacuum packaged again in 18.5 × 22 cm high-barrier multilayer bags, and stored at −80 °C until analysis. LHP value, TBA value, lipid UV absorption, α-tocopherol content, and fatty acid composition were determined in raw ($n = 15 \times 3$) and cooked ($n = 15 \times 3$) samples (Figure 1).

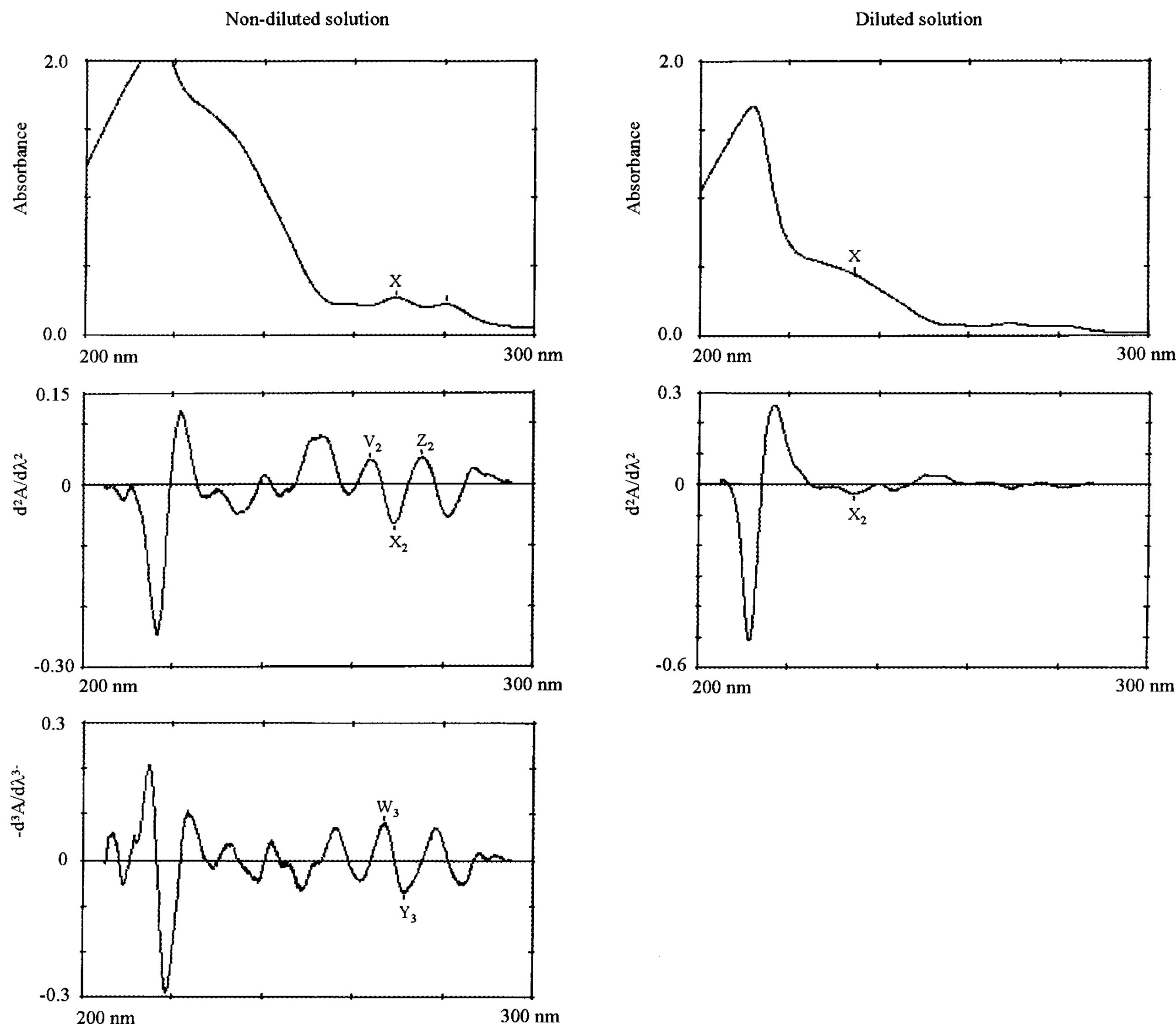

Figure 2. Direct and second- and third-derivative spectra of the lipid fraction extracted from a raw dark chicken meat sample. Parameters at 269 and 280 nm were determined in the nondiluted solution. Parameters at 235 nm were determined in the diluted solution.

Determination of LHP Values. LHP values were assessed according to the FOX method described by Grau et al. (2000a). Reaction mixtures containing 140 μL of sample extract were incubated for 80 h.

Determination of TBA Values. TBA values were determined by an acid aqueous extraction method with third-derivative spectrophotometry, as described by Grau et al. (2000b).

Determination of Lipid UV Absorption. Prethawed meat (1.5 g; raw or cooked) was weighed into a 32 × 210 mm tube, and 1.5 mL of 0.1% aqueous EDTA disodium salt was immediately added. Just before homogenization, 15 mL of chloroform/methanol (2:1) was added and the tube contents was homogenized for 40 s at 12 000 rpm using a Polytron PT 3000 (Kinematica, Lucerne, Switzerland). The extract was filtered through a Whatman No. 1 filter paper into a 50 mL screw-capped tube, and the residue was re-extracted twice with the same solvent: first with 15 mL (30 s at 14 000 rpm) and then with 7 mL (10 s at 15 000 rpm). Next, 10 mL of water was added to the tube and, after centrifugation (20 min at 400*g*), the chloroformic phase was filtered through anhydrous sodium sulfate (Whatman No. 1 filter paper), which was then washed twice with 5 mL of chloroform. The lipid extract thus obtained was concentrated to 1 mL in a vacuum rotatory evaporator at 35 °C, and the rest of the solvent was removed first by a slight nitrogen stream and then by keeping the flask in a vacuum desiccator at 10 mmHg overnight. The extracted lipid fraction was weighed and dissolved in 10 mL of cyclohexane. To determine the absorbance at 269 and 280 nm, 2, 5, or 7 mL (depending on the degree of sample oxidation) was pipetted into a 10 mL volumetric flask, and the volume was made up with cyclohexane. After that, this solution was diluted until the absorbance at 235 nm was between 0.2 and 0.8. Second- and third-derivative spectra of the nondiluted solution and second-derivative spectra of the diluted solution were recorded (Figure 2). A Shimadzu UV-160A double-beam spectrophotometer was used, and spectrophotometric conditions were as follows: spectrum range, 200−300 nm [as suggested by Baron et al. (1997)]; scan speed, 480 nm/min; and derivative difference setting ($\Delta\lambda$), 14 nm; 1-cm quartz cuvettes were used.

Specific absorbances (K_{235}, K_{269}, and K_{280}) were calculated using the formula

$$K_\lambda = E_{1\ \mathrm{cm}\lambda}^{1\%} = A_\lambda/(CW) \quad (1)$$

where C stands for the concentration of the cyclohexanic solution in grams of lipid extract per 100 mL, W for the width of the spectrophotometer cell in centimeters, and A_λ for the absorbance at a specific wavelength (235, 269, or 280 nm) of the direct spectrum.

In addition, some ways to express UV absorption through derivative spectrophotometry were assayed. In these cases, A_λ

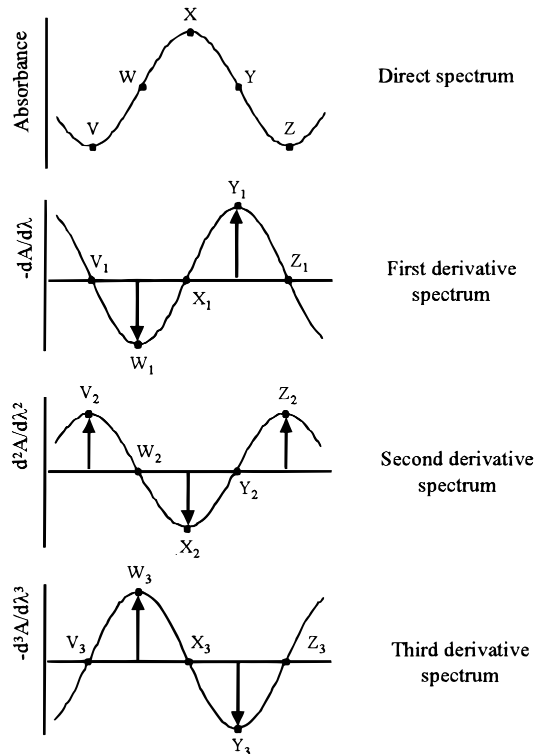

Figure 3. Peak transformation through derivative spectrophotometry. The spectrophotometer used in this study (Shimadzu UV-160A) calculated derivative spectra using the function $f^n_{(\lambda)} = (-1)^n \times (d^n\, A/d\, \lambda^n)$.

was substituted by data from the derivative spectra expressed in arbitrary units, as printed on the instrument chart:

$D2_{\lambda X}$: The data used was the value given by the minimum on the second-derivative spectrum (X_2 in Figure 3), which corresponds to the peak maximum at 235 or 269 nm on the direct spectrum (X).

$D3_{269W}$: The data used was the value given by the maximum on the third-derivative spectrum (W_3 in Figure 3), which corresponds to the inflection point of the ascendant part of the peak at 269 nm on the direct spectrum (W).

$D3_{269Y}$: The data used was the value given by the minimum on the third-derivative spectrum (Y_3 in Figure 3), which corresponds to the inflection point of the descendant part of the peak at 269 nm on the direct spectrum (Y).

$D2_{269f}$: The value used was obtained using the formula

$$A = (V_2 + 2X_2 + Z_2)/2 \tag{2}$$

where V_2 and Z_2 are the values given by the maximums on the second derivative, which correspond respectively to the beginning (V) and the ending (Z) of the peak at 269 nm on the direct spectrum (Figure 3).

$D3_{269f}$: The value used was obtained using the formula

$$A = W_3 + Y_3 \tag{3}$$

where W_3 and Y_3 are the values respectively given by the maximum and the minimum on the third-derivative spectrum, which correspond to the inflection points (W and Y) of the peak at 269 nm on the direct spectrum (Figure 3).

As previously commented, the applicability of the second-derivative spectrophotometry at 233−235 nm to follow up PUFA oxidation has been widely studied. Therefore, at this wavelength, we substituted A_λ in eq 1 for the value described above ($D2_{235X}$) and for the values previously described by Corongiu and Milia (1983) and Corongiu and Banni (1994). As results obtained followed very similar patterns, we report only the results coming from the value described above ($D2_{235X}$). On the contrary, only a few authors (Baron et al., 1997) have studied the usefulness of derivative spectrophotometry at 269 and 280 nm to follow up PUFA oxidation. For this reason, the substitution of A_λ in eq 1 for several values obtained from second- and third-derivative spectra was studied at these wavelengths. Results obtained for both wavelengths followed very similar patterns, and thus only results for derivative spectrophotometry at 269 nm ($D2_{269X}$, $D3_{269W}$, $D3_{269Y}$, $D2_{269f}$, and $D3_{269f}$) are reported in this paper.

FOX, TBA, and lipid UV absorption determinations were all performed under attenuated light conditions.

Determination of α-Tocopherol Content and Fatty Acid Composition. α-Tocopherol content and fatty acid composition were determined as described in Grau et al. (2000a).

Statistics. Pearson correlation coefficients were used to examine possible linear correlations between responses (LHP and TBA values, lipid UV absorption, and α-tocopherol content). Multifactor analyses of variance (MANOVAs) were performed to determine whether any significant effects were produced by the studied factors (dose and period of α-TA supplementation) on the responses (LHP and TBA values and lipid UV absorption). In all cases, P values ≤0.05 were considered to be significant. When the effect of factors was significant, the Scheffé test for a posteriori contrasts ($\alpha = 0.05$) was applied to determine statistical differences between least-squares means.

RESULTS AND DISCUSSION

In raw samples ($n = 45$), the LHP values determined by using the FOX method clearly decreased ($P < 0.0001$) when the α-TA dose increased (Table 2). For TBA values, this decrease was much less pronounced but almost significant ($P = 0.0552$). In addition, both oxidation parameters showed significant differences depending on the α-TA supplementation period ($P < 0.0001$). On the contrary, none of the lipid UV absorption parameters studied denoted any significant effect of the α-TA supplementation period and, in addition, when the α-TA dose increased, only a slight and nonsignificant tendency to decrease was observed for these UV absorption values (Table 2).

Concerning the correlation between these oxidation parameters in raw samples, LHP and TBA values were highly correlated between them and highly inversely correlated with α-tocopherol content (Table 3). However, UV absorption results did not correlate significantly with any of these parameters (Table 3) and, in addition, correlation coefficients for direct and derivative UV spectrophotometric parameters were similar.

In cooked samples ($n = 45$), both LHP and TBA values were clearly influenced by dose and period of α-TA supplementation ($P < 0.0001$). Oxidation was lower with higher α-TA doses and larger supplementation periods (Table 2). In contrast, UV absorption parameters were not able to statistically differentiate between the various α-TA doses. In addition, with regard to the supplementation period, only K_{235} ($P = 0.0019$), $D2_{235X}$ ($P = 0.0007$), and K_{269} ($P = 0.0389$) denoted a statistically significant effect of this factor (Table 2).

Concerning the correlation between results from these methods in cooked meat samples, TBA and LHP values and α-tocopherol content were well correlated, as in raw meat (Table 3). However, among all of the UV absorption parameters, only the ones above-mentioned (K_{235}, $D2_{235X}$, and K_{269}) were significantly correlated with LHP and TBA values and α-tocopherol content, although

Table 2. Effect of α-TA Supplementation on LHP, TBA, and Lipid UV Absorption Values in Raw and Cooked Dark Chicken Meat Samples[a]

	dose of α-tocopheryl acetate (mg/kg of feed)			days of supplementation				
	75	150	225	0	10	21	32	43
Raw Meat								
LHP value (mg of CHP/kg)	314.36^{a}	185.56^{b}	112.45^{c}	399.82^{a}	291.42^{b}	154.56^{c}	133.40^{c}	41.40^{d}
TBA value (μg of MDA/kg)	35.13	33.76	27.90	53.39^{a}	30.59^{b}	28.63^{b}	23.25^{b}	25.47^{b}
K_{235}	2.16	2.09	2.03	2.05	2.06	2.06	2.22	2.07
$D2_{235X}$	0.070	0.070	0.065	0.070	0.069	0.067	0.071	0.064
K_{269}	0.374	0.333	0.345	0.334	0.340	0.349	0.371	0.358
$D2_{269X}$	0.082	0.078	0.075	0.075	0.077	0.077	0.079	0.081
$D3_{269W}$	0.090	0.087	0.083	0.083	0.085	0.086	0.088	0.091
$D3_{269Y}$	0.103	0.099	0.094	0.095	0.097	0.096	0.099	0.103
$D2_{269f}$	0.136	0.130	0.126	0.126	0.131	0.129	0.132	0.136
$D3_{269f}$	0.193	0.185	0.182	0.179	0.191	0.183	0.188	0.194
K_{280}	0.310	0.277	0.290	0.278	0.283	0.291	0.312	0.299
Cooked Meat								
LHP value (mg of CHP/kg)	389.03^{a}	215.33^{b}	166.90^{b}	440.29^{a}	374.36^{a}	191.92^{b}	146.79^{b}	132.08^{b}
TBA value (μg of MDA/kg)	651.20^{a}	445.47^{b}	321.44^{b}	940.69^{a}	506.46^{b}	461.47^{bc}	267.00^{cd}	187.89^{d}
K_{235}	2.31	2.24	2.08	3.01^{a}	2.06^{b}	1.92^{b}	2.12^{ab}	1.93^{b}
$D2_{235X}$	0.082	0.079	0.071	0.127^{a}	0.070^{b}	0.062^{b}	0.066^{b}	0.062^{b}
K_{269}	0.369	0.360	0.335	0.418^{a}	0.347^{b}	0.328^{b}	0.347^{b}	0.332^{b}
$D2_{269X}$	0.078	0.075	0.073	0.071	0.079	0.074	0.076	0.079
$D3_{269W}$	0.084	0.082	0.080	0.077	0.086	0.080	0.083	0.084
$D3_{269Y}$	0.097	0.094	0.092	0.091	0.098	0.093	0.096	0.096
$D2_{269f}$	0.132	0.127	0.122	0.121	0.133	0.124	0.127	0.131
$D3_{269f}$	0.182	0.176	0.172	0.168	0.184	0.173	0.179	0.180
K_{280}	0.296	0.297	0.279	0.325	0.287	0.273	0.290	0.279

[a] Values correspond to least-squares means obtained from MANOVAs ($n = 45$) for raw or cooked samples. Values in the same row for a certain factor bearing a different superscript are statistically different ($P \leq 0.05$).

Table 3. Correlations between Various Oxidative Parameters and α-Tocopherol Content in Raw and Cooked Dark Chicken Meat

	LHP content	TBA value	K_{235}	$D2_{235X}$	K_{269}	$D2_{269X}$	$D3_{269W}$	$D3_{269Y}$	$D2_{269f}$	$D3_{269f}$	K_{280}
Raw Meat											
LHP value		0.6007[a]	0.0839	0.2505	0.0552	0.0111	0.0043	0.0380	−0.0194	−0.0330	−0.0410
		(<0.0001)	(0.6267)	(0.1406)	(0.7490)	(0.9486)	(0.9801)	(0.8257)	(0.9118)	(0.8483)	(0.8122)
TBA value			−0.1392	0.1919	−0.2363	−0.1880	−0.2062	−0.2099	−0.2016	−0.2138	−0.2565
			(0.3618)	(0.2066)	(0.1181)	(0.2162)	(0.1741)	(0.1664)	(0.1895)	(0.1585)	(0.0890)
α-tocopherol content	−0.7599	−0.6378	0.1119	−0.1374	0.1579	0.0606	0.0722	0.0526	0.0398	0.0239	0.1835
	(<0.0001)	(<0.0001)	(0.4641)	(0.3680)	(0.3003)	(0.6925)	(0.6373)	(0.7314)	(0.7974)	(0.8760)	(0.2276)
Cooked Meat											
LHP value		0.7981	0.3141	0.3852	0.2955	−0.0499	−0.0755	−0.0679	−0.0104	−0.0718	0.2175
		(<0.0001)	(0.0356)	(0.0098)	(0.0488)	(0.7479)	(0.6219)	(0.6576)	(0.9475)	(0.6391)	(0.1664)
TBA value			0.5186	0.5677	0.5418	−0.0342	−0.0536	−0.0480	0.0224	−0.0509	0.4312
			(0.0006)	(0.0002)	(0.0003)	(0.8362)	(0.7425)	(0.7687)	(0.8936)	(0.7551)	(0.0077)
α-tocopherol content	−0.8775	−0.8430	−0.3894	−0.4588	−0.3813	−0.0441	−0.0105	−0.0567	−0.0992	−0.0353	−0.2718
	(<0.0001)	(<0.0001)	(0.0090)	(0.0020)	(0.0107)	(0.7788)	(0.9462)	(0.7146)	(0.5321)	(0.8203)	(0.0856)

[a] Pearson correlation coefficient ($n = 45$). P value is stated in parentheses.

correlation coefficients were low. K_{280} was significantly correlated only with TBA values.

From these results (Tables 2 and 3), it can be concluded that derivative spectrophotometry was not a useful tool to express lipid UV absorption of dark chicken meat samples, as it did not improve results from direct spectrophotometry. In the case of absorption at 235 nm for cooked samples, the second-derivative measurement ($D2_{235X}$) showed correlation coefficients (Table 3) and discrimination power between periods of α-TA supplementation (Table 2) similar to those of the direct measurement (K_{235}). This is in disagreement with the results of several authors (Baron et al., 1997; Corongiu and Milia, 1983; Corongiu et al., 1983, 1986; Defrancesco et al., 1980; Sergent et al., 1993), which showed a higher selectivity and sensitivity when the second derivative was used. However, lipid fractions used in these studies showed a higher content of PUFA, from which conjugated dienes arise during oxidation. For absorption at 269 nm, second- and third-derivative spectrophotometries were applied because they are effective in eliminating background (matrix suppression effect), and increasing selectivity and sensitivity in other spectrophotometric determinations (Botsoglou et al., 1994; Corongiu and Banni, 1994; Özgür and Sungur, 1995). However, in this case, derivative spectrophotometry did not help to differentiate α-TA doses and supplementation periods (Table 2, cooked samples). In addition, among all of the parameters from absorption at 269 nm evaluated, only the one based on data from the direct spectra (K_{269}) showed a statistically significant correlation with LHP and TBA values and α-tocopherol content in cooked samples (Table 3, cooked samples). In fact, the absorption peak at 269 nm in the direct spectrum was well resolved (Figure 2) and, thus, the main effect of the derivative spectrophotometry in this case was background elimination (matrix suppression effect). In addition, as lipid fractions of different samples were very similar (broiler diets differed only in α-TA content), then we can assume that K_{269} yielded better results than derivative parameters because of some oxidation products that contribute to background

absorption and that are important to follow up lipid oxidation in our case. This is supported by the fact that Galanos et al. (1968) reported numerous secondary oxidation products from PUFA with absorption maximums between 265 and 280 nm. In addition, direct and derivative spectrophotometries at 280 and 269 nm showed similar patterns (Tables 2 and 3; data from derivative spectrophotometry at 280 nm is not shown). However, K_{280} was less sensitive than K_{269} to denote oxidative differences between samples (Table 2, cooked samples) and was significantly correlated only with TBA values (Table 3, cooked samples). The applicability of the absorption parameters at 269 or 280 nm to follow up lipid oxidation probably depends on the fatty acid composition of the sample, which determines the secondary oxidation products formed during oxidation.

On the other hand, in cooked samples, UV measurements at 235 nm showed a higher correlation with TBA values than with LHP values (Table 3), which seems to be inconsistent with the fact that absorbance at 230–235 mainly measures conjugated diene structures, which are principally primary oxidation products. However, the FOX method applied in this study was, to a certain extent, an induced method (Grau et al., 2000a). Moreover, high correlations between UV measurements at 230–235 nm and hydroperoxide content have been primarily reported when fats and oils are highly unsaturated and not submitted to high temperatures [reviewed by White (1995)]. In addition, some authors, depending on the heat treatment and the unsaturation degree of the lipid fraction, did not find correlation between UV measures at 230–235 nm and peroxide value [reviewed by White (1995)]. In addition, some studies (Halliwell and Gutteridge, 1985; Smith and Anderson, 1987) have shown that in some samples much of the diene-conjugated material does not contain the hydroperoxide functional group. Thus, in part, our findings might be related to the well-known fact that compounds formed during lipid oxidation greatly depend on fatty acid composition and on processing and storage conditions.

When MANOVA was performed to all samples ($n = 90$) introducing cooking as a new factor, LHP and TBA values clearly distinguished between raw and cooked meat, whereas UV absorption parameters did not. TBA values clearly differentiate ($P < 0.0001$) between raw (global mean = 32.26 μg of MDA/kg) and cooked samples (global mean = 472.70 μg of MDA/kg). For LHP values, the difference between raw (global mean = 206.56 mg of CHP/kg) and cooked (global mean = 257.09 mg of CHP/kg) samples was not so evident, but still statistically significant ($P = 0.0014$). The difference was more obvious in the case of the TBA values because the FOX assay was, to some extent, an induced method and, in addition, during cooking MDA and MDA-like substances are formed, whereas peroxide compounds are easily destroyed as they are thermolabile. In contrast, as commented above, UV absorption parameters did not differentiate between global means for raw and cooked samples. However, some of the UV absorption parameters (K_{235}, $D2_{235X}$, and K_{269}) distinguished between raw and cooked samples when there was no supplementation with α-TA (Figure 4), whereas LHP and TBA values could distinguish these samples even when there was supplementation with α-TA (Figure 5).

Because FOX and TBA methods used were able to find smaller oxidative differences between samples, we can conclude that, in dark chicken meat, these methods showed a much higher sensitivity to follow up lipid oxidation than parameters based on lipid absorption at 235, 269, or 280 nm. In addition, we can state that the LHP value is the best parameter to estimate differences between α-TA treatments (doses or supplementation periods) within raw samples, whereas the TBA value is the best parameter to assess differences between raw and cooked samples and between α-TA treatments (doses or supplementation periods) within cooked samples. This fact is clearly related to the well-known formation and accumulation profile of primary and secondary oxidation products during the evolution of lipid oxidation. In fact, the high capability of TBA values to show differences between α-TA treatments in cooked samples (Table 2) is due to the fact that MDA accumulates as lipid oxidation advances, especially if it is mediated by high temperatures and sample composition includes fatty acids with more than two double bonds (Esterbauer et al., 1991), as it does in our case (Table 4). In this connection, these authors reported that the formation of free MDA (determined by HPLC) in iron/ascorbate autoxidized linoleic, linolenic, and docosahexanoic acid was, respectively, 0.5, 4.5, and 7.6 mol %.

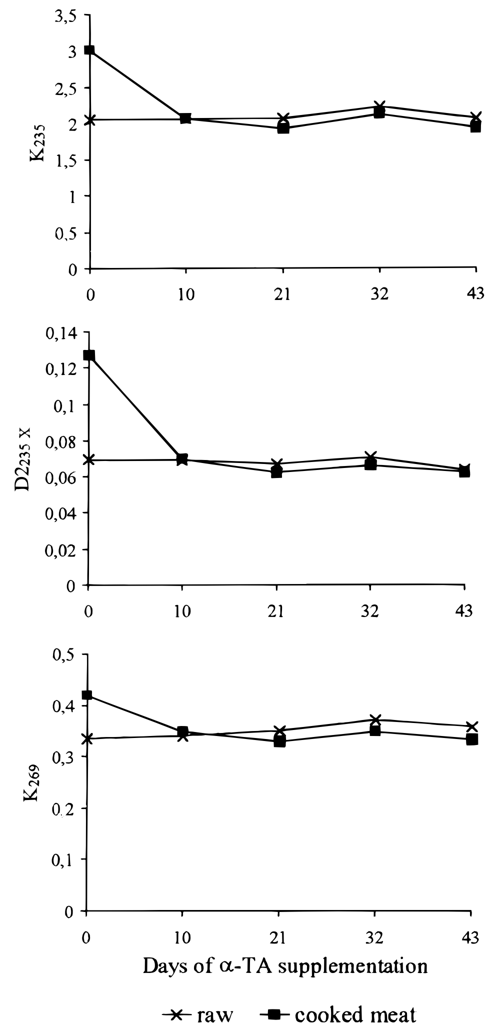

Figure 4. Effect of cooking and α-TA supplementation period on the values of various UV absorption parameters from dark chicken meat samples.

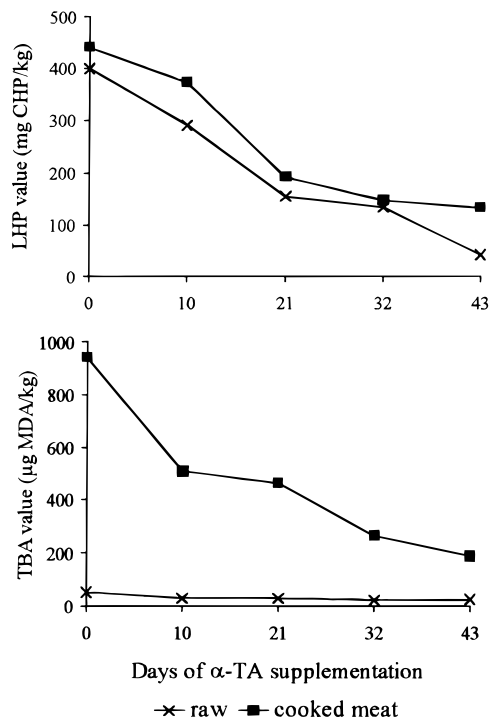

Figure 5. Effect of cooking and α-TA supplementation period on lipid hydroperoxide LHP and TBA values from dark chicken meat samples.

Table 4. Fatty Acid Composition of Raw Dark Chicken Meat Samples (Expressed as Area Normalization in Percent)

fatty acid	%
total SFA[a]	30.50
total MUFA	43.67
***n*−6 PUFA**	
C18:2 *n*−6	22.25
C18:3 *n*−6	0.24
C20:2 *n*−6	0.27
C20:3 *n*−6	0.22
C20:4 *n*−6	0.89
C22:4 *n*−6	0.24
C22:5 *n*−6	0.08
total	24.19
***n*−3 PUFA**	
C18:3 *n*−3	1.34
C18:4 *n*−3	0.04
C20:5 *n*−3	0.04
C22:5 *n*−3	0.12
C22:6 *n*−3	0.09
total	1.64
PUFA/SFA	0.85

[a] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

CONCLUSION

The suitability of an oxidation parameter to follow up lipid oxidation greatly depends on the fatty acid composition of the sample lipid fraction. Other factors that influence this suitability are processing and storage conditions and interactions between fatty acid oxidation and certain matrix components. In our case, lipid UV absorption at 235, 269, and 280 nm measured through both direct and derivative spectrophotometries showed a limited applicability to follow up lipid oxidation. In addition, we can assume that these UV absorption parameters will be less useful in meats more highly saturated than dark chicken meat.

ABBREVIATIONS USED

FOX, ferrous oxidation−xylenol orange; LHP, lipid hydroperoxides; MDA, malondialdehyde; PUFA, polyunsaturated fatty acids; TBA, 2-thiobarbituric acid; α-TA, *dl*-α-tocopheryl acetate; EDTA, ethylenediaminetetraacetic acid; CHP, cumene hydroperoxide.

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