Biochemical Survey on Episodic Localized Darkening in Turkey Deboned Thigh Meat Packaged in Modified Atmosphere

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ABSTRACT The color of food, especially meat and meat products, is a parameter that strongly influences consumer choice. In Italy, repeated cases of darkening in deboned thigh meat of male turkeys packaged in modified atmosphere (MAP; 80% O_2, 20% CO_2) have been reported. The pH, lipid oxidation (TBARS), heme proteins, and iron content were investigated in MAP samples of turkey males, females, and in oxygen-permeable film-packaged males. Furthermore, the absorbance spectrum (400 to 700 nm) of the meat extracts was analyzed to better delineate the evolution and characteristics of the darkening process. Results showed that darkening occurred only in males with higher content of total iron, independently of the content of heme proteins, which differs only between males and females. Furthermore, pH was higher in muscles taken as controls, with respect to muscles involved in the darkening, as well as in females. Finally, TBARS values were found to be higher in darkened regions than in not darkened ones, as well as in MAP samples with respect to oxygen-permeable film-packaged samples. These findings suggest that darkening occurrence might depend on kind of muscle, sex, and individual characteristics of the animals raised under the same breeding conditions.

Key words: color, darkening, modified atmosphere packaging, turkey meat

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INTRODUCTION

As is well known, the commercial success of meat products is due to many factors such as price, presentation, and organoleptic characteristics (i.e., odor, color, and tenderness). In particular, visual appearance seems to be the quality most influencing the consumer's purchase decision (Faustman and Cassens, 1990; Liu et al., 1995).

The color of meat is directly affected by conservation period, kind of muscle, storage temperature, packaging, and atmosphere composition. Nowadays, modified atmosphere packaging (**MAP**) has become common practice in the meat industry; it implies the use of impermeable films that guarantee maintenance of high CO_2 and O_2 levels. The goal is extending the shelf life of a product because CO_2 inhibits microorganism proliferation, whereas O_2 mainly preserves the original color (Gatellier et al., 2000; Sanchez-Escalante et al., 2001). Besides additives that may be included in processing, color alteration of meat is essentially determined by factors such as the action of microbiological agents (Ben Abdallah et al., 1999) or tissue oxidative stress, which are related to the alteration of the muscle proteic and lipidic fraction. These variations also provoke changes in flavor and texture, lead to the production of cytotoxic and genotoxic compounds, and determine the cooxidation of vitamins, resulting in a general loss of quality (Kanner, 1994; Lapidot et al., 2005).

Lipid oxidation is positively correlated with pigment oxidation (Liu et al., 1995), and heme proteins were recognized as crucial factors for the onset of oxidation in muscle, participating in the formation of radicals also in processes involving unsaturated fatty acids and transition elements, in particular iron (Baron and Andersen, 2002; Lapidot et al., 2005).

Fundamentally, there are few proteic species (heme-containing proteins) responsible for muscle color: cytochromes and ribonucleases, the very low concentration of which determines a minor contribution (Livingston and Brown, 1981), haemoglobin, the levels of which severely decrease during bleeding, and myoglobin, the main pigment in well-bled meat, the concentration of which does not change (Pegg and Shahidi, 1997; Liu and Chen, 2001). For this reason, the latter plays a primary role in the visible spectrum absorption capacity of meat. Myoglobin is a 17-kDa protein, present in large quantities in muscle. The capacity of this molecule to absorb different wavelengths depends on the oxidation state of the iron and its chemical interaction with small molecules, such as oxygen itself, thus determining the chromatic shades of the whole muscle. Myoglobin is present in different forms: deoxymyoglobin, with iron (II), deter-

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mines the purple color of the tissue. Oxygen released from blood binds to iron and forms a complex called oxymyoglobin, which does not change its own oxidation state. In this case the color of the meat turns to a bright fresh meat-like red. Further oxidation transforms the oxymyoglobin in metmyoglobin, with iron (III), conferring a brownish color and in general darkening the tissue (Cornforth, 1994). During life, notwithstanding the continuous physiologic activity of enzymatic reduction, metmyoglobin is present at 2 to 3% (Halliwell and Gutteridge, 1989). A further reaction of metmyoglobin with hydrogen peroxide results in the formation of ferrylmyoglobin, with iron (IV), (Harel and Kanner, 1985).

This work arises from a real problem in processed poultry meat products commercialized in Italy, i.e., repeated cases of chromatic alteration (browning) in male turkey deboned thigh meat, packaged in modified atmosphere. Since previous analyses excluded a microbiological etiology, any aspects of oxidative stress, which meat might undergo in MAP, have been considered by analyzing the spectrophotometric features of the proteic fraction of the muscle tissues and correlating the occurrence of the darkening with heme proteins and lipid oxidation and with total heme protein and iron content of the muscle.

MATERIALS AND METHODS

Samples Analysis

This work was carried out in 3 replications. Samples of packaged turkey thighs were received directly from the producer and stored at 4°C in the dark until analyzed. They were divided overall as follows: 43 males in MAP (80% O_2 and 20% CO_2), 21 males packaged with oxygen-permeable film, and 30 females in MAP. Slaughtered males and females were respectively 20 wk and 15 wk old. Analyses were performed at d 8 after packaging, except for darkened samples, which were analyzed as soon as darkening reached clearly noticeable spots of about 4 to 7 cm in diameter (between 6 and 8 d after packaging).

If not otherwise specified, analyzed samples are modified atmosphere packaged.

Reagents, if not differently indicated, were purchased from Sigma-Aldrich (St. Louis, MO).

pH Measurement

Tissue samples were divided as follows: darkened tissues of inner muscles contacting the femur of darkened males (♂$\mathbf{F_D}$), iliotrochantericus caudalis muscle of darkened ($\mathbf{C_D}$) and not darkened males (**C**) as control, inner muscles contacting the femur of not darkened males (♂**F**), inner muscles contacting the femur of females (♀**F**), and inner muscles contacting the femur of males packaged with O_2-permeable film. A Cyberscan pH meter (Eutech Instruments, Singapore) with direct immersion probe was used.

Lipid Oxidation

The method of Tarladgis et al. (1960) for thiobarbituric acid-reactive substance (**TBARS**) measurement was adapted to determine lipid oxidation.

One gram of thoroughly minced tissue was transferred to a 50-mL distillation tube with 10 mL of butylated hydroxytoluene 0.02%; 20 μL of silicone antifoaming agent and 250 μL of HCl 4 *N* were added. The suspension was then distilled with a Steam Distillation Unit UDK126A (VELP Scientifica s.r.l., Usmate, MI, Italy), and the first 10 mL of distillate was collected in a 50-mL volumetric flask. Next 1 mL of the distillate was added to 1 mL of 0.02 *M* thiobarbituric acid (Merck, Darmstadt, Germany) and heated in boiling water bath for 35 min for color development. Malonaldehyde (**MDA**) was distilled in duplicate from the turkey meat, and its absorbance was determined at 534 nm in a 1-cm glass cell against a blank containing a solution of 0.01 *M* thiobarbituric acid, using an Ultrospec 2000 UV/VIS spectrophotometer (Pharmacia Biotech, Piscataway, NJ). The results were reported as milligrams of MDA per kilogram of sample. Standard curves were prepared by making appropriate dilutions of the 2×10^{-4} *M* 1,1,3,3-tetramethoxy-propane standard solutions to give amounts ranging from 2×10^{-5} to 8×10^{-7} *M* MDA. Equations for the standard curves were obtained from the linear regression of the absorbance results against the concentration of the standards. Tissue samples were divided as described for pH measurement.

Total Iron Determination

Total iron was calculated according to the method described in the work of Carpenter and Clark (1995). Values are expressed in micrograms per gram of wet tissue. Tissue samples were taken from the inner muscles contacting the femur and divided as follows: not darkened males, darkened males, and females. The C and C_D were taken as controls.

Heme Proteins Extraction

Muscle tissues were treated by adapting the procedures proposed by Kranen et al. (1999). Tissue samples were weighed and placed at a weight per volume ratio of 1:4 in a tube containing 4°C prerefrigerated buffer (10 m*M* Tris (hydroxymethyl)-aminomethane, 1 m*M* EDTA, and 80 m*M* KCl, pH 8.0). The samples were then homogenized (Ultra Turrax T25, IKA, Stanfen, Germany), and the tubes were dipped in ice-cold water. Homogenization was carried out at 15,000 rpm in 3 steps of 10 s, at intervals of 30 s, to keep the temperature of the suspension low and avoid protein denaturation. At the end of the process, the particulate was accurately washed out of the homogenizer with a quantity of buffer corresponding to three-fourths of the initial volume and collected with the rest of the suspension, then kept at 4°C for 1 h with continuous shaking. The suspension was

then centrifuged for 30 min at 1,600 × *g* at 4°C. Supernatants were pooled, and fat was removed by filtration through filter paper. A further centrifugation at 100,000 × g for 30 min at 4°C was intended to remove cell organelles; the cytochrome fraction was considered removed with the mitochondria.

Total Heme Proteins Determination

The method described by Kranen et al. (1999) was adopted for heme proteins determination in muscle extracts. Tissue samples were taken from inner muscles contacting the femur and divided as follows: males, females, and darkened tissues. The C and C_D were taken as controls. Values were expressed in milligrams per gram of wet tissue.

Percentage of Met Forms

The proportion of Met forms among the heme proteins was calculated measuring the absorbance of the extracts at 630 nm before and after oxidation. Sodium azide determines the complete disappearance of Met forms and, consequently, of the peak at 630 nm. On the contrary, $K_3Fe(III)(CN)_6$ determines the oxidation of all the pigments to the Met form. The 100 μL of water (A) or sodium azide, 20 m*M* (B) or $K_3Fe(III)(CN)_6$, and 60 m*M* (D) were added to 900 μL of extract. The percentage of Met forms was calculated as follows: $(A_{630} - B_{630})/(D_{630} - B_{630}) \times 100$. Blanks were made of extraction buffer plus A, B, or D (9:1).

Spectrophotometric Analysis

The extracts were analyzed in the visible range (400 to 700 nm) at room temperature, using an Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Piscataway, NJ), against a blank made of the buffer used for the extraction. The samples were divided as follows: ♂F_D, not darkened tissues of inner muscles contacting the femur of darkened males (♂$\mathbf{F_{nD}}$), ♀F, and tissues from areas bordering with the darkened tissues. Reported data represent the average of the absorbance values obtained at each wavelength for every group of samples.

Statistical Analysis

All data are presented as means with relative standard deviations. Differences among groups and their significance were evaluated by Student's *t*-test (2-tailed). Paired *t*-test was used when comparing the same parameter in different areas of the same sample. Analyses were performed with SigmaPlot 8.0 software (Richmond, CA).

RESULTS AND DISCUSSION

Description of the Darkening Occurrence

Color alteration was observed only in the group of males in MAP, in which 50% of samples presented darkening within 8 d after packaging. The tissues concerned belonged only to muscles contacting the femur, which had undergone the deboning process. The onset of darkening involved small superficial, sometimes multiple spots in the cutting areas, broadening radially at a high rate, but never deepening beyond a few millimeters.

Several practices, among them de-boning, determine the loss of integrity of fibers, compromise cellular compartmentalization, and facilitate interaction of prooxidants with unsaturated fatty acids, consequently producing free radicals and the propagating oxidative reactions (Asghar et al., 1988). During observations, darkening was always generated from cut regions; moreover, the pattern of darkening progression suggests a typical radical-mediated process, supported constantly by oxygen availability in the shallow areas where the gas is easily accessible.

pH

Oxidation in meat and meat products may be induced or supported by several factors and postmortem processes, among which is pH fall (Buckley et al., 1995), which contributes to inactivation of reductive enzyme systems and stimulation of acid-catalyzed autoxidation of iron (II) state to iron (III) state of myoglobin, resulting in accumulation of MbFe (III). Moreover, it was found that these heme species show prooxidative activity at acidic pH (Baron and Andersen, 2002).

Table 1 shows the values of the measured pH. No differences were observed between ♂F_D and ♂F, but comparison between ♂F or ♂F_D and the relative controls (C and C_D) revealed a high significance ($P < 0.01$) by paired *t*-test. No differences between C and C_D were detected. A consistent gap was found also between ♂F and ♀F ($P < 0.01$), the higher pH of which could be considered one of the multiple factors contributing to the maintenance of the original color of female meat. Finally, no differences were found between inner muscles contacting the femur of males packaged with O_2-permeable film and ♂F.

Lipid Oxidation

Susceptibility of muscle tissues to lipid oxidation is strongly influenced by the relative concentration of unsaturated fatty acids (Allen and Foegeding, 1981), mostly located in subcellular membranes of mitochondria and microsomes (Gray and Pearson, 1987). Red fibers are characterized by the presence of numerous small mitochondria, increasing the total surface for respiration (Liu et al., 1995) and consequently a predisposition to tissue oxidative damage. As nonflying birds, turkeys have an elevated content of red fibers in lower limb muscles and, for this reason, a higher quantity of substrate to trigger and maintain oxidative reactions. Furthermore, turkey muscle is known to have a high polyunsaturated fatty acid content, mainly associated with phospholipids of the membrane (Batifoulier et al., 2002).

Table 1. The pH and thiobarbituric acid-reactive substance (TBARS) values (mean ± SD) of turkey thighs packaged in modified atmosphere (MAP) or with oxygen-permeable (OP) film[1]

	♂F_D	C_D	♂F	♀F	♂F-OP
pH	5.72 ± 0.2	5.98 ± 0.19	5.76 ± 0.18	6.15 ± 0.2	5.89 ± 0.09
TBARS (mg of MDA/kg)	8.40 ± 2.82	3.70 ± 2.3	3.97 ± 1.78	2.12 ± 0.68	1.40 ± 0.66

[1] ♂F_D = darkened tissues of inner muscles contacting the femur of darkened males in MAP; C_D = *iliotrochantericus caudalis* muscle of darkened males (♂F_D control); ♂F = inner muscles contacting the femur of not darkened males in MAP; ♀F = inner muscles contacting the femur of females in MAP; ♂F-OP = inner muscles contacting the femur of males packaged with oxygen-permeable film. Compared means, which showed significant differences, are as specified: pH: ♂F_D × C_D = means differ ($P < 0.01$, paired *t*-test); ♂F × ♀F = means differ ($P < 0.01$, *t*-test). TBARS: ♂F_D × C_D = means differ ($P < 0.001$, paired *t*-test); ♂F_D × ♂F = means differ ($P < 0.01$, *t*-test); ♂F × ♀F = means differ ($P < 0.01$, *t*-test); ♂F × ♂F-OP = means differ ($P < 0.001$, *t*-test).

To quantify tissue oxidative stress, the level of lipid peroxidation in muscle was measured. The TBARS assay measures hydroperoxides and aldehydes that increase as a result of the oxidation and breakage of the lipid chains, providing important information about free radical activity.

Table 1 reports TBARS values of the MAP groups: ♂F_D showed highest values of milligrams of MDA per kilogram of meat, revealing differences with high significance when compared with ♂F (0.01) or compared with controls ($P < 0.001$). The C and C_D presented overlapping mean values, and no statistical differences between ♂F and C were found (paired *t*-test). Furthermore, ♀F showed lower TBARS values than ♂F ($P < 0.01$). To our knowledge, data on the relative content of unsaturated lipids in turkey according to sex are not available, but the lower level of lipid oxidation in females agrees with the fact that darkening occurred only in males and might be correlated with a lower content of myoglobin and total iron in females.

Finally, inner muscles contacting the femur of males packaged with oxygen-permeable film showed TBARS values with an average almost 3 times lower than ♂F ($P < 0.001$), confirming the strong influence of MAP on oxidation processes because oxygen availability seems to be one of the limiting factor for lipid degradation, as already suggested by other authors (Ahn et al., 1998; Jo et al., 1999; Gatellier et al., 2001).

After slaughtering, what little oxygen remains is rapidly consumed by the residual activity of enzymes, but its supply is ensured by the normal or modified atmosphere of the packaging. Considering the low gas-diffusion coefficient in the fiber matrix, the hypothesis that oxygen is directly implied in the darkening of meat is supported by the fact that this kind of alteration occurs only at a shallow level, as it was always observed.

Some very reactive molecules are produced from metabolic reactions with oxygen, such as hydrogen peroxide, hydroxyl and superoxide anion radicals, active oxygen-iron complexes, and iron-mediated hemolytic cleavage of hydroperoxides. These aggressive molecules are easily able to attack either proteins or fatty acids, generating organic free radicals and modifying their structure (Hsieh and Kinsella, 1989).

Cells have developed shield mechanisms that strongly reduce the potential for extensive damage to biological substrates. They are based on enzymatic systems (superoxide dismutase, catalase, glutathione peroxidase, etc.) and on secondary antioxidants, such as substances with low molecular weight able to intercept and quench free radicals (glutathione, carnosine, vitamins A, C, and E, etc.; Burton et al., 1985; Kohen et al., 1988). Among antioxidants, vitamin E is surely the most widely studied. In particular, α-tocopherol, its most potent biological form, is able to dissolve into membranes and capture free radicals (Gray et al., 1996). This antioxidant activity is high during life, but the concentration of active molecules decreases rapidly after death. For this reason their action is unlikely to be effective for long after slaughtering (Buckley et al., 1995). More probably it can only delay degradation processes in meat. Turkey meat contains relatively low amounts of natural tocopherols and has a high oxidative potential (Mielnik et al., 2003). It was observed that integration of feed with vitamin E can stabilize the color of meat for a while after packaging (Liu et al., 1995; Faustman et al., 1998), although the outcome of this attempt was not satisfactory on the birds raised together with the ones considered in this work, probably because of the poor ability of turkey muscle to store vitamin E (Batifoulier et al., 2002).

Total Iron

Besides the ability of the different myoglobin forms to catalyze lipid oxidation in an acid environment, several studies have highlighted how transition elements, in particular iron, can catalyze decomposition of peroxides by generating free radicals which in turn oxidize unsaturated fatty acids, leading to deterioration of meat (Nawar, 1996; Mancuso et al. 1999). Although it has been suggested that this process is related either to free iron or to iron incorporated in proteins, conflicting evidence has been reported (Buckley et al., 1995).

Besides heme-iron, the total amount of iron was considered in every sample because free iron is known to catalyze lipid oxidation in processes often involving free radical production (Halliwell and Gutteridge, 1992). A significant difference was found between darkened and not darkened males ($P < 0.05$), and no differences were found between inner muscles contacting the femur and the relative controls. Moreover, iron amounts in females resulted even lower, with a high significance ($P < 0.01$) compared with the average of darkened males (Table 2).

Table 2. Total Fe (μg/g; mean ± SD) of inner muscles contacting the femur of turkey thighs packaged in modified atmosphere

Not darkened males	Darkened males	Females
21.73 ± 3.75[ac]	26.79 ± 5.71[b]	19.56 ± 2.45[cd]

[a,b]Means with no common superscript differ ($P < 0.05$, *t*-test).
[c,d]Means with no common superscript differ ($P < 0.01$, *t*-test).

Table 3. Content of heme proteins (mg/g) and percentage of Met-forms (mean ± SD) of inner muscles contacting the femur of turkey thighs, in darkened and not darkened tissues

	Male	Female	Darkened tissue
Heme proteins concentration (mg/g)	1.96 ± 0.28[a]	0.91 ± 0.26[b]	0.85 ± 0.18[b]
Percentage of Met forms	17 ± 3[a]	12 ± 4[a]	67 ± 12[b]

[a,b]Means within the same row with no common superscript differ ($P < 0.001$, *t*-test).

Heme Proteins

As mentioned previously, the major contribution to meat color is from myoglobin, mainly present in 3 convertible forms (deoxymyoglobin, oxymyoglobin, and metmyoglobin). With death of tissues, a progressive extinction of enzyme activity and proteic denaturation or degradation occurs, often promoted by oxidation or pH decrease, enhancing the formation of metmyoglobin, accumulation of which provokes a darkening of muscle color.

Alteration of the tertiary structure of the proteins may facilitate the exposure of the heme iron to unusual ligands, such as water (Baron and Andersen, 2002), forming metmyoglobin (III). Although it was observed that in vitro low O_2 pressure accelerates the oxidation of oxymyoglobin (II) to metmyoglobin (III; George and Stratmann, 1952), a high oxygen concentration in the meat environment increases oxidation processes and might support protein destabilization or denaturation with the possible formation of metmyoglobin and, consequently, darkening. Among the different substances generated by lipid oxidation, the most copious are aldehydes, which were proved to hamper metmyoglobin reduction, increasing its catalytic activity toward lipids and determining structural changes of the protein itself, forming covalent bonds. From this, myoglobin oxidation, which results in the formation of metmyoglobin, can be positively correlated with lipid oxidation (Lynch and Faustman, 2000).

Concentration of total heme species in the analyzed samples was calculated. No significant differences were found between the inner muscles contacting the femur and the control muscles (data not shown). No correlation between higher total heme protein content and darkening occurrence was found. The average for males was 1.96 ± 0.28 mg/g of fresh tissue and 0.91 ± 0.26 for females; there was a proportion of Met forms with an average of 17% (± 3) and 12% (± 4), respectively (Table 3). This marked difference in heme proteins concentration ($P < 0.001$), associated with the comparison of browning only in males and with the higher lipid oxidation in darkened areas, is not in contrast with the findings of other authors who correlated the prooxidative activity of metmyoglobin (III) to a high lipid/heme proteins ratio at physiological pH (Baron and Andersen, 2002). The lower this ratio is, the longer is the induction period of peroxidation. This was explained by the denaturation of proteins probably due to interaction with lipids and direct exposure of the heme-group to the fatty acids. The hemicrome state of metmyoglobin (III) implies the coordination of distal histidine to the iron, which is thought to hinder access of lipid hydroperoxides in catalytic cavity. Yet at acidic pH, histidine does not bind iron and the cavity is free. Moreover, even at physiological pH, ferrylmyoglobin (IV) proved to be able to have prooxidant activity as well (Baron et al., 2002), but at acidic pH (5.5–5.8) iron (IV) rapidly autoreduces to iron (III; Baron and Andersen, 2002). This means that in the characteristic microenvironment of meat, high levels of heme proteins may promote or sustain lipid oxidation, even though small differences in concentrations do not seem to be relevant, as suggested by the results of this study.

The concentration of total heme proteins in darkened areas was found to be reduced by more than 50% with respect to not darkened tissues (0.85 ± 0.32 mg/g of fresh tissue), according to the method used, which needs structurally intact proteins. Metmyoglobin represented 67% (± 12) of total heme proteins (Table 3). This evidence suggests a diffuse degradation of the proteic component of muscle, determining the detachment of heme from the globins, as confirmed by the spectrophotometric analyses reported here.

The $♂F_{nD}$ and ♀F (Figure 1) showed spectra with similar patterns but with lower intensity at each wavelength for females (due to the lower content of heme proteins), with the Soret band peak at 416 nm and the 2 characteristic peaks of oxy-heme proteins at 544 and 582. On the contrary, samples from the $♂F_D$ group had quite different features: absorbance at 544 and 582 had almost completely lost its original intensity, suggesting, however, a residual presence of oxymyoglobin. The characteristic peak at 630 of metmyoglobin began to appear, but with a low intensity, probably because of the high amount of denatured or structurally altered heme proteins. The other typical peak at 505 nm was slightly outlined. The Soret band can be distinctly observed around 409 nm but with a lower intensity compared with one expected with a full content of structurally intact proteins.

The spectrum of tissues from areas bordering with the darkened tissues begins to change slightly, the 548 and 582 peaks being of lower intensity as well as the Soret band, which show a faint enlargement toward the peak of metmyoglobin (409 nm). Moreover, a peak at 630 nm of metmyoglobin is slightly outlined, suggesting the

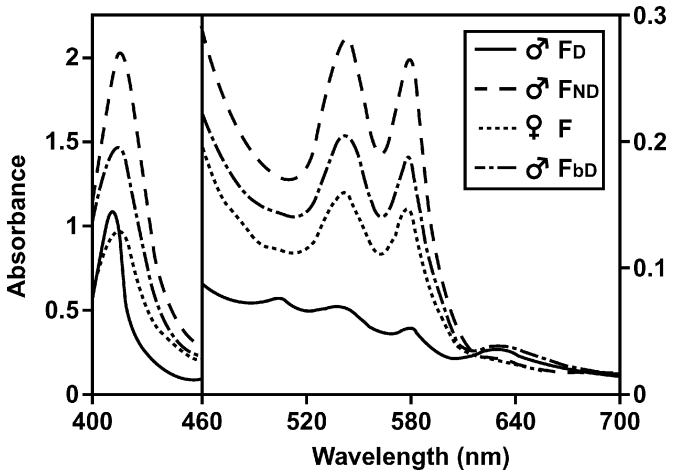

Figure 1. Visible absorption spectrum of meat extracts from inner muscles contacting the femur of turkey thighs, packaged in modified atmosphere. $♂F_D$ = darkened tissues of darkened males; $♂F_{nD}$ = not darkened tissues of darkened males; $♂F_{bD}$ = areas bordering with the darkened tissues of darkened males; ♀F = females.

detectable onset of metmyoglobin formation in areas surrounding already darkened tissues.

This seems to confirm that strong oxidation processes take place at an exponential rate some time after packaging, soon compromising the structure of proteins that only partially maintain the native conformation, allowing the prostetic group to stay in the correct position and interact with the surrounding amino acids to determine the characteristic absorbance spectrum of heme proteins. This implies that when the human eye starts to perceive the darkening occurrence, proteins are already in an advanced state of decomposition. Thus, the metmyoglobin (III) status may be a transitory condition.

These results confirm that the darkening process of some male turkey thigh meat is associated with lipid oxidative degradation and is stimulated or sustained by the high oxygen concentration of MAP. Heme proteins are also involved, rapidly changing their oxidation state and partially losing their original structure soon after the onset of the process.

Other parameters appeared to be involved in the occurrence, such as hydrogen ion concentration and iron content, which were found to be significantly higher in darkened samples, whereas differences in content of heme proteins were associated only with sex.

Taken altogether results suggest that, other than by the kind of packaging, color alteration is strongly affected by sex, the kind of muscle, and the individual characteristics of animals raised under the same breeding conditions.

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