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Utilization of fluorescence spectroscopy as a novel approach to evaluate the oxidative stability in beef retail displayed

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ABSTRACT

Beef samples from grazing steers finished with different seed-supplemented diets were vacuum packaged for 3, 14 and 56 days (VC) and subsequently exposed to aerobic conditions (AE) for 0 and 5 days. Different fluorescent compounds of interest in the oxidation process were detected in meat, namely tryptophan residues, Schiff bases and porphyrins. Tryptophan intensity fluorescence increased with 14 days of VC; while Schiff bases intensity increased ($P < 0.05$) in beef samples stored under VC-56 and in all samples after AE-5 days. Porphyrins increased ($P < 0.05$) gradually with the extension of vacuum storage time, but were degraded in beef with long vacuum storage and 5 days of AE. Higher levels of porphyrins in beef under VC were correlated ($P < 0.05$) with lower redness and higher TBARS after AE-5. This study revealed the potential of fluorescence signals to detect oxidative changes in beef under different storage conditions using a fast and nondestructive method such as fluorescence spectroscopy.

1. Introduction

Oxidative damage is the major non-microbiological factor involved in quality deterioration of meat during refrigerated storage (Faustman, Sun, Mancini, & Suman, 2010). Oxidation induces modifications of muscle lipids and proteins and, therefore, affects the organoleptic and nutritional properties of meat and meat products. This is reflected in economic losses and human health disorders (Sample, 2013). Storage conditions play an important role in oxidative damage of meat that could influence the extent of oxidation (Xiao, Zhang, Lee, Ma, & Ahn, 2011). Moreover, the type of diet consumed by animals during the production phase has a great influence on the susceptibility of meat for *postmortem* oxidation. Feed supplementation with flaxseed has been proposed as a strategy to increase the concentration of beneficial fatty acids, especially highly unsaturated n-3 fatty acids in muscle of several ruminant species (Juárez et al., 2012; Nute et al., 2007). Flaxseed is unique among oilseeds because of its exceptionally high content of α-linolenic acid (45 to 52%) (Singh, Mridula, Rehal, & Barnwal, 2011). In a recent publication (Pouzo, Descalzo, Zaritzky, Rossetti, & Pavan, 2016) it was observed that it is possible to increase the total n-3 PUFA concentration and reduce the n-6:n-3 ratio in muscle by supplementing grazing cattle with increasing amounts of whole flaxseed, thus improving the nutritional value of the beef. However, such changes in fatty acid profile could have negative effects on the appearance and shelf-life of meat (via oxidative processes).

A wide range of biological tissues contain naturally-occurring fluorophores (Lakowicz, 2013); the emission generated from these compounds is named intrinsic fluorescence (or autofluorescence) which can be detected by fluorescence spectroscopy. This method has the advantage of being highly sensitive, rapid, non-destructive and relatively low cost. It can be applied both in fundamental research as well as in industry as on-line sensors for monitoring food products. Meat contains a series of relatively strong autofluorophores; some of them have been related to oxidative stability in meat and meat products (Veberg, Olsen, et al., 2006a). The presence of the aromatic ring in the amino acid tryptophan is responsible for its natural fluorescence emitted at 350 nm when it is excited at around 290 nm. Changes in the intrinsic fluorescence of tryptophan have been used to monitor physicochemical changes in proteins (Vivian & Callis, 2001), including those derived from oxidative stress (Giessauf, Steiner, & Esterbauer, 1995). On the other hand, the compounds formed as a result of the reactions between lipid oxidation products (aldehydes) and amino groups from proteins (Schiff bases) are conjugated fluorophores with spectral properties, which may be detected by recording fluorescence at 450 nm

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when excited at 350 nm, and therefore used as a protein oxidation index (Heinonen et al., 1998; Viljanen, Kylli, Kivikari, & Heinonen, 2004). This fluorescence has been positively correlated with measurements of protein carbonyl compounds in meat products (Armenteros, Heinonen, Ollilainen, Toldrá, & Estévez, 2009) and TBARS value in poultry meat (Gatellier et al., 2007). However, the suitability of using this technique in beef under retail conditions has not been well studied.

Porphyrins are other compounds with spectral properties which could be detected by recording fluorescence between 500 and 750 nm, after excitation at 420 nm (Durek, Bolling, Knorr, Schwägele, & Schlüter, 2012; Schneider et al., 2008). These are a large group of organic compounds and they play very important roles in various biological processes. Porphyrins consist of four pyrrole rings joined by methane bridges and can bind to metals such as magnesium, iron, zinc, nickel, and cobalt (Grimm, 2003; Labbé, Vreman, & Stevenson, 1999) and it has been pointed out as a possible color contributor in Parma ham (Wakamatsu, Okui, Ikeda, Nishimura, & Hattori, 2004). Fresh meat also contains porphyrins. Since porphyrins are significant photosensitizers (compounds able to produce singlet oxygen upon light exposure) (Dolmans, Fukumura, & Jain, 2003) and may be involved in the process of photo-oxidation in foods it is of high interest to investigate these compounds in meat stored under retail conditions.

The aim of this research was: 1) to evaluate the potential of measuring fluorescence signals in beef (tryptophan residues, products of interactions protein–oxidized lipids and porphyrins) as indicators of oxidative changes concerning shelf life of beef using a fast and non-destructive method such as fluorescence spectroscopy, 2) to establish the relationship between fluorescent compounds and shelf life parameters (oxidative stability of color and lipid), and 3) to analyze the influence of utilizing diets rich in PUFA as dietary supplement of grazing cattle on the development of fluorescent compounds related to oxidative stability of beef.

2. Materials and methods

2.1. Animals and experimental design

In order to evaluate oxidative stability of beef enriched in PUFAn-3, steers from grazing systems supplemented with corn grain and increasing levels of flaxseed were analyzed in the present study. The animals and treatments used in this study were previously described in full (Pouzo et al., 2016). Briefly, twenty four Angus steers (458 ± 42.8 kg of average live weight) from the same herd and backgrounded on a rotational grazing system without supplementation were randomly assigned to four dietary treatments (no-supplement, *CNTRL*; supplemented: 0.7% on live weight basis (LW) of cracked corn grain plus no flaxseed, *FLAX-0*, plus 0.125% LW of whole flaxseed, *FLAX-1*, or plus 0.250% LW of whole-flaxseed, *FLAX-2*). Throughout the study, steers from the four dietary treatments grazed as one group, but individually received 0.5 kg (as-fed) of wheat bran in addition to their individual dietary treatment (supplement), so that each animal was considered an experimental unit (n = 6 per treatment). Animals were harvested at a commercial slaughter house after 70 days on trial with an average of 508 kg BW.

Sections of beef samples containing 6–8 ribs were collected from the left carcass sides after 24 h postmortem. The *longissimus thoracis* muscle was removed and cut into six 2.5 cm thick steaks. Steaks obtained from each section were individually vacuum-packaged and randomly distributed among six treatments, generated by the combination of three vacuum storage periods at 2 °C (VC; 3, 14 and 56 days) and two aerobic exposure periods (AE; 0 and 5 days).

For aerobic exposure (AE) simulating retail display, steaks were placed on Polyfoam trays, overwrapped with an oxygen-permeable polyvinylchloride film and stored under simulated retail display conditions of illumination (Halogen light; 2000 lx) and temperature (2 °C). After completing their assigned vacuum storage and aerobic exposure

Table 1
Levels of significance (*P* values) for main effects, i.e. diet (CNTRL, FLAX-0, FLAX-1 and FLAX-2), vacuum storage time (VC-3, VC-14 and VC-56) and aerobic exposition time (AE = 0 and AE = 5) and their interactions (Diet × VC; Diet × AE; VC × AE; Diet × VC × AE) on meat fluorescent compounds, detected by spectroscopy of fluorescence of *longissimus thoracis* muscle.

Effect	Residues of tryptophan (341 nm emission/290 nm excitation)	Oxidized lipid–protein products (440 nm emission/350 nm excitation)	Protoporphyrins 1° peak; (597 nm emission/432 nm excitation)	Protoporphyrins 2° peak; (650 nm emission/432 nm excitation)
Diet	0.711	0.451	0.104	0.319
Vacuum storage time	0.037	<0.0001	<0.0001	<0.0001
Aerobic exposition time	0.093	<0.0001	0.0002	0.0001
Diet by vacuum storage interaction	0.576	0.183	0.183	0.150
Diet by aerobic exposition interaction	0.867	0.195	0.830	0.812
Vacuum storage by aerobic exposition time interaction	0.343	0.395	0.0004	<0.0001
Diet by vacuum storage by aerobic exposition time interaction	0.053	0.245	0.240	0.468

periods, steaks were vacuum packaged and stored at −25 °C until further analysis.

2.2. Determination of the oxidative stability: meat color and lipid oxidation

Meat color was measured during retail display conditions using a Minolta CR 310 Chroma meter (Minolta Corp., Ramsey, NJ). Redness (a* value) was recorded. The instrumental conditions were: large area aperture (5 cm diameter), D65-artificial and 10° standard observation angle. The instrument was calibrated against a white plate. After opening of the vacuum packs, samples were allowed to bloom for 30 min prior to color determination. The values from three scans were averaged for each color determination.

The lipid stability of muscle samples was measured in each VC period (3, 14 and 56 days) at the beginning (AE-0) and the end (AE-5) of 5 day display (days 0 and 5 respectively), on the same samples analyzed for color. To assess lipid peroxidation, TBARS assays were performed following the procedure described by Pouzo et al. (2016).

2.3. Front face fluorescence measurements

Fluorescence spectra were recorded using an LS55 fluorescence spectrometer (PerkinElmer GmbH, Rodgau-Jügesheim, Germany) equipped with a pulsed xenon lamp. A front surface accessory (Perkin–Elmer Plate Reader) was installed for the direct measurement of the solid sample. Different emission spectra were performed varying excitation wavelengths in order to find the precise excitation wavelength of the maximum intensity value reached in the peak in the emission range. Emission spectra in the ranges 300–460 nm, 400–550 nm and 500–750 nm were recorded with excitation wavelengths 290, 350 and 432 nm, respectively; excitation and emission slits were set at 10 nm. Before each measurement, sample temperature was equilibrated for 30 min at room temperature (18 °C) before recording fluorescence at the same temperature. Fluorescence spectra were recorded in duplicated for each VC period (3, 14 and 56 days) at the beginning (AE-0) and the end (AE-5) of display (days 0 and 5 respectively).

2.4. Statistical analysis

Data were analyzed using the Proc Mixed procedure (SAS Inst. Inc., Cary, NC), with the main effects of DIETS, VC and AE, as well as their interactions, included in the statistical model; each individual animal was used as the experimental unit (n = 6). Least squares means were computed for main and interactive effects and were separated statistically using F-protected ($P < 0.05$) t-tests (PDIFF option).

The CORR procedure of SAS was used to compute the Pearson coefficient for the correlation between oxidative parameters (a* value and TBARS value) and the intensity of fluorescent compounds.

3. Results and discussion

Table 1 shows the effects of the principal factors and their interactions on the fluorescent compounds detected in the present study. As is reported in Figs. 1 to 3 the mean values for significant differences ($P < 0.05$) for compounds detected were: 566.7 AU vs 588.8 and 586.2 for VC-3 vs VC-14 and VC-56, respectively (Fig. 1B); 7.53 and 7.52 AU vs 9.34 AU for VC-3 and VC-14 vs VC-56, respectively (Fig. 2B) and 7.56 vs 8.97 AU for AE = 0 vs AE = 5 (Fig. 2C). Finally the compounds observed in Fig. 3 show significant differences across all vacuum storage periods studied within AE = 0 (3.74, 5.68 and 10.93 AU for VC-3, 14 and 56 respectively) and within AE = 5 (3.86, 5.29 and 8.36 AU for VC-3, 14 and 56, respectively). Moreover significant differences were observed across AE just only for VC-56 with mean values of 10.93 and 8.36 AU for 0 and 5 days of aerobic exposition (AE), respectively (Fig. 3B). The compound reported in Fig. 3C, had similar behavior than the previous one reported, but with lower mean values.

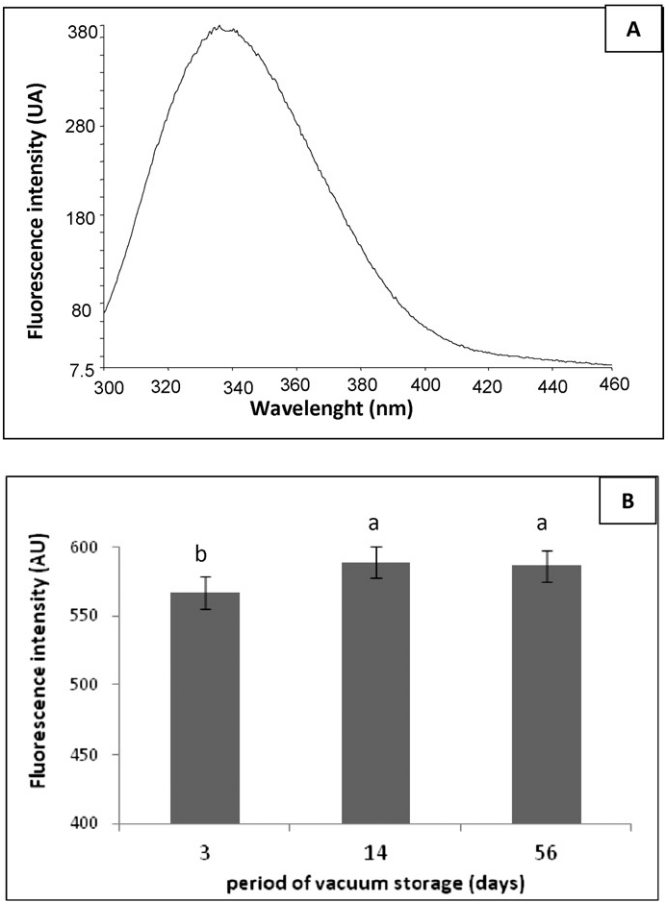

Fig. 1. A) Emission spectra of beef samples recorded between 300 and 460 nm with excitation wavelength set at 290 nm. B) Fluorescence intensity (maximum intensity reached by the peak; arbitrary units, AU) corresponding to tryptophan residues measured in beef samples vacuum packaged and stored during 3, 14 and 56 days (significant differences between periods of storage on vacuum are denoted by different letters).

The fluorescence spectra of beef, recorded following excitation at 290 nm (Fig. 1A) showed a maximum about 340 nm; this peak could be originated from tryptophan residues in proteins, as it was observed by other authors using fluorescence spectroscopy in meat (Dufour, Frencia, & Kane, 2003; Sahar, Boubellouta, Lepetit, & Dofour, 2009; Skjervold et al., 2003). Only the effect of vacuum storage (VC) time was significant for this parameter, showing a slight increase ($P = 0.04$) in fluorescence intensity of tryptophan after 14 days of storage under vacuum above the initial level (VC-3) (Fig. 1B). Tryptophan fluorescence provides information about the structure of the protein. It has been observed that changes in proteolysis and increases in the pH level during ripening in other systems, such as cheese, can cause conformational changes in the molecular proteins and to tryptophan molecule and therefore in its fluorescence spectra (Dufour, Devaux, Fortier, & Hebert, 2001). Therefore, probably pH changes during proteolysis of meat through ageing as well as, modifications derived from the oxidative damage could change the conformational structure of proteins (Omana, Xu, Moayedi, & Betti, 2010; Xiong, 2000) modifying the exposition of tryptophan to the three-dimensional configuration of the proteins changing, therefore, its intensity of fluorescence which probably explain our results.

The period of aerobic exposure did not affect significantly fluorescence intensity of tryptophan in beef; however its fluorescence intensity tended ($P = 0.09$) to be reduced by about 3% after 5 days in oxidative conditions (586.9 and 571.0 AU for AE = 0 and AE = 5 respectively). This could indicate initial degradation of this amino acid due to its

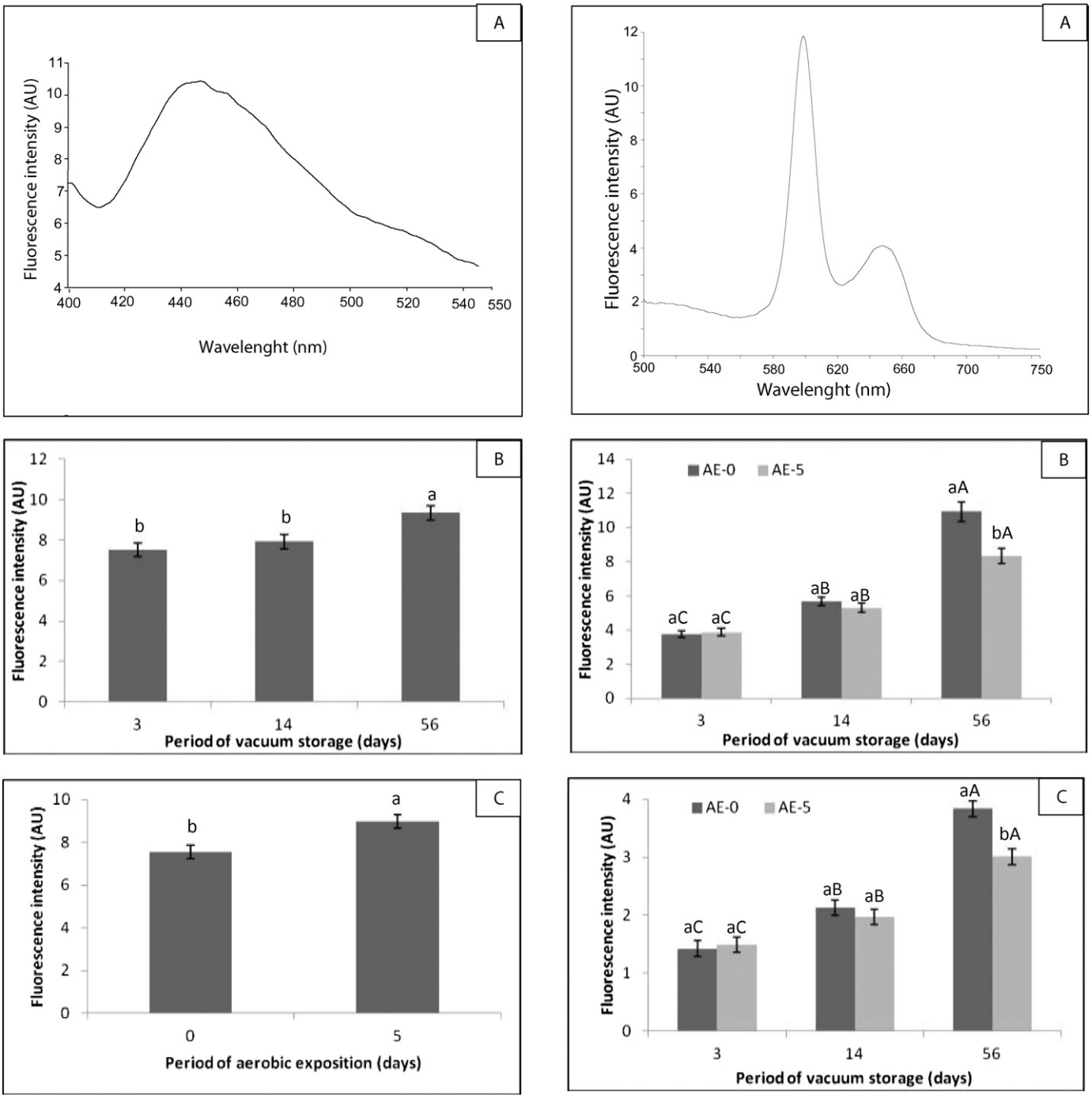

Fig. 2. A) Emission spectra of beef samples recorded between 400 and 550 nm with excitation wavelength set at 350 nm. B) Fluorescence intensity (maximum intensity reached by the peak; arbitrary units, AU) corresponding to Schiff bases in beef samples vacuum packed and stored during 3, 14 and 56 days (significant differences between periods of storage on vacuum are denoted by different letters). C) Fluorescence intensity (maximum intensity reached by the peak; arbitrary units, AU) corresponding to Schiff bases in beef samples with or without aerobic exposure (0 and 5 days of EA, respectively) (significant differences between periods of storage under AE are denoted by different letters).

Fig. 3. A) Emission spectra of beef samples recorded between 500 and 750 nm with excitation wavelength set at 432 nm. B) Interactive effect of vacuum storage period (VC) and days of aerobic exposure (AE) on fluorescence intensity (maximum intensity reached by the peak; arbitrary units, AU) corresponding to the peak of porphyrins with maximum emission at 597 nm. Significant differences between vacuum storage treatments within each day of aerobic exposition (AE) are denoted by different capital letters. Significant differences between aerobic exposition periods (AE) within each day of vacuum storage (VC) are denoted by different non-capital letters. C) Interactive effect of period of vacuum storage (VC) and days of aerobic exposure (AE) on fluorescence intensity (maximum intensity reached by the peak; arbitrary units, AU) corresponding to the peak with maximum emission at 650 nm. Significant differences between vacuum storage treatments within each day of aerobic exposition (AE) are denoted by different capital letters. Significant differences between aerobic exposition periods (AE) within each day of vacuum storage (VC) are denoted by different non-capital letters.

relatively high sensitivity to oxidative stress (Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008). Moreover, this small decrease in emission fluorescence intensity could also be explained by small changes in tryptophan residue environment. The emission of tryptophan is highly sensitive to its local environment, and is therefore used as a reporter group for protein conformational change (Lakowicz, 2013). As it was suggested by Andersen and Mortensen (2008) the fluorescence of proteins depends on how tryptophan is exposed in the three-dimensional configuration of the proteins.

Table 2
Effect of diet, vacuum storage time and aerobic exposition time on lipid oxidation (TBARS) and redness (a* value) of *longissimus thoracis* muscle.

Variable	Aerobic exposition(days)	Diet[1]					Vacuum storage (days)				Significance[2]				
		CNTRL	FLAX-0	FLAX-1	FLAX-2	SE	3 days	14 days	56 days	SE	D	V	A	D A	V A
TBARS (mg MDA/kg)	0	0.10^{aB}	0.11^{aB}	0.09^{aB}	0.11^{aB}	0.02	0.10^{aB}	0.10^{aB}	0.10^{aB}	0.03	***	***	***	***	***
	5	0.35^{bA}	0.38^{bA}	0.26^{cA}	0.46^{aA}		0.23^{cA}	0.30^{bA}	0.56^{aA}						
Redness (a* value)	0	16.33^{aA}	16.61^{aA}	17.22^{aA}	17.20^{aA}	0.52	16.40^{aA}	17.25^{aA}	16.87^{aA}	0.45	ns	***	***	ns	**
	5	14.53^{aB}	14.23^{aB}	14.78^{aB}	13.66^{aB}		14.62^{aB}	15.76^{aB}	12.52^{bB}						
	Interactive effect of treatment D × V; D × V × A not significant; P > 0.05														

D: diet; V: vacuum storage; A: aerobic exposition; D ∗ A: interaction diet ∗ aerobic exposition; V ∗ A: interaction vacuum storage ∗ aerobic exposition; D ∗ V: interaction diet ∗ vacuum storage; D ∗ V ∗ A: interaction diet ∗ vacuum storage ∗ aerobic exposition.
Means with different capital letters within the same column (Aerobic exposition effect) and different non-capital letters within the same row (dietary treatment or vacuum storage effect) indicate significant differences (P < 0.05).
[1] CNTRL, not supplemented; FLAX-0, supplemented with 0.7% BW of corn grain; FLAX-1, supplemented with 0.7% BW of corn grain plus 0.125% BW of flaxseed; FLAX-2, supplemented with 0.7% of corn grain plus 0.250% BW of flaxseed.
[2] Ns, no significant; *, ** and *** refer to P < 0.05, P < 0.01, and P < 0.001 respectively.

The peak with a maximum of emission around 440–450 nm (Fig. 2A) detected in the present study following excitation at 350 nm with a maximum of emission around 440–450 nm (Fig. 2A) could be attributed to oxidized lipid–protein interaction products, namely, Schiff bases in meat. Similar peaks have been observed by Armenteros et al. (2009) in experiments to assess protein oxidation in ground meat and different meat products. These authors found a high positive correlation between carbonyl content (DNPH-method) and fluorescence spectroscopy, and used both methods as indices of protein oxidation.

In the present study, two factors "vacuum storage periods at 2 °C (VC)" and "aerobic exposure periods (AE)" had significant effects (P < 0.0001) on the peak attributed to Schiff bases. Diet as a factor as well as two-way (Diet × AE; Diet × VC and VC × AE) and three-way interactions (Diet × VC × AE), were not significant (P > 0.05; Table 1).

Regardless of aerobic exposure time (AE) a significant increase in the fluorescence intensity was observed at 56 days of vacuum storage which exceeded the levels observed in meat stored for 3 and 14 days under the same conditions (Fig. 2B). Moreover, regardless of diet or vacuum storage, the fluorescence intensity of this peak was 23% higher (P < 0.05) in beef subsequently stored for 5 days under retail display conditions (AE = 5) than in beef without extended aerobic exposure (AE = 0) (Fig. 2C).

Table 2 reports the effect of diet and storage conditions on lipid stability as previous reported by Pouzo et al. (2016) and color stability of beef; TBARS levels increased between 3 and 4-fold (P < 0.05) when the period of vacuum storage was followed by 5 days of exposure to oxygen. When the overall association between fluorescent compounds attributed to Schiff bases and TBARS was analyzed, there was a positive correlation (r = 0.32; P < 0.0001; data not shown). These data were consistent with those reported by Veberg, Olsen, et al. (2006a) to assess turkey burgers vacuum packed or stored in packages with high oxygen permeability. Gatellier et al. (2007) also detected a direct relationship between the lipid oxidation of meat chicken and the intensity of the fluorescence peak assigned to Schiff bases.

Protein oxidation (measured by protein carbonyl group content and/or by Western blotting) as well as lipid oxidation (TBARS) has been previously reported in beef under retail display conditions (Fu et al., 2015; Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010), supporting therefore, our findings.

The lack of effect (P > 0.05) of dietary treatments on the fluorescence intensity of Schiff bases (oxidized lipid–protein products) or amino acid residues (tryptophan) could be due to the relatively lower rate of oxidation of proteins with respect to lipids (Insani et al., 2008), whereby small differences in lipid oxidation between dietary treatments would not have been sufficient to be reflected in protein oxidation.

Similarly, Haug et al. (2007) did not observe any change in the front-face fluorescence spectra obtained chicken meat feed with diets formulated with selenium and oils rich in omega-3 fatty acids (canola and flaxseeds), whereas TBARS levels in the same samples increased in oilseed enriched meat.

The emission fluorescence spectra recorded between 500 and 750 nm showed two consecutives peaks with maxima at 597 and 650 nm, with an optimum excitation at 432 nm (Fig. 3A). These peaks could be attributed to porphyrins, mainly protoporphyrins, present in meat (Durek et al., 2012; Schneider et al., 2008). The usual wavelength used for excitation of this fluorophore is 420 nm, but as was suggested by Sahar et al. (2009) protoporphyrin may be excited at different wavelengths (432 nm in this case) due to the large width of the fluorophore excitation spectrum.

Table 1 shows that the intensity of the fluorescence peaks attributed to protoporphyrins in meat was affected by the period of vacuum packaging (VC), aerobic exposure (AE) and their interaction (VC × AE), (P < 0.05), while an effect of the dietary treatment was not observed (P > 0.05) on the level of this fluorophore. This result could be explained by the fact that the level of myoglobin (the main contributor of porphyrins) is mainly affected by the animal age, the type of muscle, but not by the diet.

The extension of storage time under vacuum led to a gradual increase in the level of both porphyrin peaks (VC-3 < VC-14 < VC-56; P < 0.05) in beef with or without aerobic exposure (AE = 0 and AE = 5) (Fig. 3B and C). Moreover a significant reduction in fluorescence intensity of porphyrins was observed in the present work in beef samples after 56 days of vacuum packaging storage and subsequent aerobic exposure under light (AE = 5), probably due to the high photosensitivity of these compounds. Porphyrins are highly labile to light exposure, decreasing its content when tissues are exposed to light conditions; in this way Durek et al. (2012) observed that fluorescence intensity of protoporphyrins was almost completely bleached in pork meat stored under halogen light for 18 days.

Some researchers have used fluorescence spectroscopy to investigate the behavior of protoporphyrins during storage in products like pork meat and cured Parma hams (Durek et al., 2012; Veberg, Sørheim, et al., 2006b; Wakamatsu, Nishimura, & Hattori, 2004). In accordance with our results, Durek et al. (2012) found that two consecutive emission fluorescence peaks attributed to protoporphyrins (592 ± 3 nm and 638 ± 2 nm) increased in intensity as storage time increased on slices of pork meat packed under vacuum. Similar results were obtained by Veberg et al. (2006b) and Wakamatsu et al. (2004). The authors noted that protoporphyrins are mainly produced under anaerobic conditions, while oxygen inhibits their formation; the authors suggested that the formation of protoporphyrins is caused by the action of endogenous enzymes in meat. A similar mechanism could be present in our study carried out on beef samples stored under anaerobic conditions (VC). It is well known that porphyrins and chlorins are photolabile during light exposure, but at the same time they are strong photosensitizers. They produce singlet oxygen upon light exposure. Wold et al. (2005) reported that photoinduced breakdown of porphyrin and

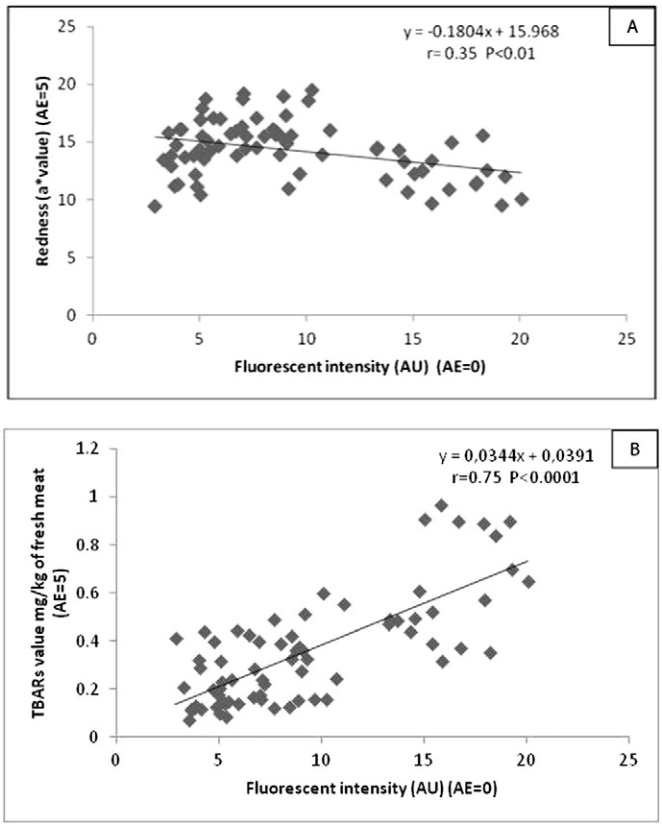

Fig. 4. A) Relationship between redness (a* value) during 3, 14 and 56 days of vacuum storage with 5 days of subsequent aerobic exposition (AE = 5) and fluorescent signal (maximum intensity reached by the peak; AU, arbitrary units) obtained as the sum of both porphyrin peaks during 3, 14 and 56 days of vacuum storage without subsequent aerobic exposition (AE = 0). B) Relationship between TBARS value during 3, 14 and 56 days of vacuum storage with 5 days of subsequent aerobic exposition (AE = 5) and fluorescent signal (maximum intensity reached by the peak; AU, arbitrary units) obtained as the sum of both porphyrin peaks during 3, 14 and 56 days of vacuum storage without subsequent aerobic exposition (AE = 0).

chlorin compounds could induce photooxidative deterioration in dairy products where these molecules are abundant. Therefore, according to the present study, porphyrin compounds could provide a significant contribution to photo-oxidation in the beef stored under light conditions, as indicated by its high TBARS levels and Schiff bases products.

As shown in Table 2 the a* value corresponding to the redness of meat was reduced while lipid oxidation (expressed as TBARS value) increased as vacuum storage of beef was extended from 3 to 56 days in beef after 5 days of aerobic retail display; however differences in a* value were not significant ($P > 0.05$) for beef stored under anaerobic conditions and in darkness (AE-0). When the redness and TBARS values of beef after 5 days of aerobic exposition (AE = 5) were correlated with fluorescence signal of both peaks attributed to porphyrins in beef stored under anaerobic conditions (AE = 0), a slightly inverse relationship between redness and porphyrins ($r = -0.35$; $P < 0.01$; Fig. 4A) and a strong direct relationship between TBARS and porphyrins ($r = 0.75$; $P < 0.0001$; Fig. 4B) were observed in the present study.

These findings suggest that it could be possible to use fluorescence signals of porphyrins as an early index of oxidative degradation in beef under vacuum storage that will be subsequently displayed in the retail market (aerobic exposure).

4. Conclusions

This study revealed the potential of fluorescence signals to detect tryptophan residues, Schiff bases and porphyrins as indicators of oxidative changes in relation to the shelf life of beef, using a fast and non-destructive method such as fluorescence spectroscopy. Correlations observed between fluorescent signals of porphyrins with redness and TBARS indicate that this technique could be used as a predictor of oxidative stability of beef stored under retail conditions. Lipids seem to be more prone to oxidation than proteins mainly due to the lower rate of oxidation of proteins than that of lipids. The addition of flaxseed to the diet of animals on grazing used as a strategy to increase the PUFAn-3 content in beef did not affect the formation of fluorophores related to oxidative stability in beef.

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