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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