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Effect of dietary supplementation on lipid photooxidation in beef meat, during storage under commercial retail conditions

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ABSTRACT

The effects of feeding composition on the photosensitized oxidation of lipids from beef meat, were evaluated during storage under commercial retail conditions. Feeding was enriched with linseed oil (LO), Dl-α tocopheryl acetate (vE) and conjugated linoleic acid (CLA) at different doses and provided for diverse periods, resulting in 7 diet groups (A–G). After slaughtering and 2 weeks of holding period, meat slices were packed in vessels with transparent shrink film and exposed to white fluorescent light for 8 h at 8 °C. Total cholesterol oxidation products (COPs) level varied from 4.0 to 13.0 μg/g of lipids, which corresponded to 0.1–0.6% oxidized cholesterol. The lowest peroxide value (PV) was found in the diet added with vE and LO for 90 days. Light exposure only had a significant impact on thiobarbituric acid reactive substances (TBARs). In general, Dl-α tocopheryl acetate supplemented for 90 days improved the oxidative stability of beef meat stored under commercial retail conditions.

1. Introduction

Over the past few years, the consumer needs have driven the beef meat market towards the production of leaner and healthier meat. Several strategies have been undertaken to modulate the lipid fraction of beef meat, as it influences its nutritional and sensory quality. To enable the differentiation of beef meat based on quality, the definition of the latter has become increasingly complex as it encompasses the physical intrinsic (color, shape, appearance, tenderness, juiciness, flavor) and extrinsic quality traits (brand, quality mark, origin, healthiness, production environment, etc.) (Scollan et al., 2006).

Diet formulation with vegetable oils that contain an elevated percentage of unsaturated fatty acids (UFA), should result in healthier meat products (Mitchaothai et al., 2007). In fact, it is recommended that total fat, saturated fatty acids (SFA), n − 6 polyunsaturated fatty acids (n − 6 PUFA), n − 3 PUFA and *trans* fatty acids (TFA) should contribute <15–30%, <10%, <5–8%, <1–2% and <1% of total energy intake, respectively (EFSA, 2010). Reduction of the SFA intake (which are known to raise total and low-density lipoprotein (LDL) cholesterol) and increase of the n − 3 PUFA consumption are particularly encouraged (Simopoulos, 2006). On the other hand, beef meat is also a dietary source of conjugated linoleic acid (CLA) (Ritzenthaler et al., 2001). The dominant CLA in beef is the *cis*-9,*trans*-11 isomer, which has been found to display several health promoting biological properties, including antitumoral and anticarcinogenic activities (De la Torre et al., 2006). CLA *cis*-9,*trans*-11 isomer is mainly associated with the triacylglycerol lipid fraction and, therefore, is positively correlated with the level of fatness. The content of CLA *cis*-9,*trans*-11 isomer in beef is related to the amount of this isomer produced in the rumen and the one synthesized in the tissue, by delta-9 desaturase, from ruminally produced vaccenic acid (18:1 *trans*-11). The latter is the major *trans* 18:1 isomer in beef and, as the precursor of tissue CLA in both animals and man, it should be considered as a neutral or beneficial *trans*-isomer (Wood et al., 2004).

However, a higher degree of FA unsaturation is known to favor meat oxidation (Boselli, Cardenia, & Rodriguez-Estrada, 2012; Boselli et al., 2005). Lipid oxidation has a great impact on the overall quality of muscle foods, since meat color, texture, nutritional value and safety are negatively affected (Williams et al., 1992). In addition, aldehydes, ketones and carboxylic acids are generated by this degradation process, thus leading to undesirable odors and flavors (Mottram, 1987).

Oxidation in muscle originates at the cell membrane, where a large amount of PUFA is present (Wood et al., 2004). However, the cell

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membrane contains other unsaturated lipophilic molecules, such as cholesterol, that can also oxidize. A wide range of cholesterol oxidation products (COPs) can be generated by chemical, photosensitized and enzymatic oxidation (Lercker & Rodriguez-Estrada, 2002; Smith, 1996). Photosensitized oxidation could be critical in beef meat due to its high content of heme pigments (mainly myoglobin and hemoglobin) (Boselli et al., 2012), which act as photosensitizers thus promoting lipid oxidation. When assessing lipid oxidation in beef meat, however, it is very important to consider that it is usually subjected to a holding period for few days at 3–6 °C to improve its tenderness and promote the formation of aroma compounds; this may lead to a higher initial cholesterol oxidation degree in the untreated meat slices, even before being subjected to photooxidation (Boselli, Rodriguez-Estrada, Fedrizzi, & Caboni, 2009). Large attention has been focused on COPs as they are likely to be involved in lipid metabolism, various chronic and degenerative diseases, and disturbance of cell functionality (Garcia-Cruset, Carpenter, Codony, & Guardiola, 2002; Osada, 2002; Otaegui-Arrazola, Menendez-Carreno, Ansorena, & Astiasaran, 2010; Schroepfer, 2000). Although COPs are usually present in low amounts in raw muscle food, their concentrations tend to dramatically increase after exposure to prooxidant agents or after being highly processed (Hur, Park, & Joo, 2007; Kerry, Gilroy, & O'Brien, 2002; Otaegui-Arrazola et al., 2010). Under this situation, dietary supplementation or addition of antioxidants (such as vitamin E) could be an important strategy to extend their shelf-life, by reducing or preventing lipid peroxidation (Williams et al., 1992). To the best of our knowledge, no study has been performed on the photosensitized oxidation of beef meat, as related to the dietary supplementation.

The aim of this work was to evaluate the effect of dietary supplementation on lipid oxidation in beef meat, during storage under commercial retail conditions. Particular attention was addressed to oxidation of fatty acids and cholesterol.

2. Materials and methods

2.1. Reagents and solvents

Ammonium thiocyanate (NH_4SCN, ≥97.5%), barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$, ≥99%), ethylenediamine-tetraacetic acid (EDTA) disodium salt (100% ± 1%), iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, ≥99.0%), trichloroacetic acid (≥99%) and diethyl ether, were supplied by Carlo Erba Reagenti (Rodano, Italy). Chloroform, *n*-hexane, methanol and ethanol were purchased from Merck (Darmstadt, Germany). Silylating agents (pyridine, hexamethyldisilazane and trimethylchlorosilane) and double distilled water were supplied by Carlo Erba (Milan, Italy). Anhydrous sodium sulfate and potassium hydroxide were purchased from BDH (Poole, England) and Prolabo (Fontenay, France), respectively. The standard mixture of fatty acid methyl esters (GLC 463) was supplied by Nu-Chek (Elysian, MN, USA). Tridecanoic acid methyl ester (purity: 99%), cholest-5-en-3β,19-diol (19-hydroxycholesterol, 19-HC) (purity: 99%) and cholest-5-en-3β,7α-diol (7α-hydroxycholesterol, 7α-HC) (purity: 99%) were purchased from Steraloids (Newport, Rhode Island, USA). (24*S*)-ethylcholest-5,22-dien-3β-ol (stigmasterol) (purity: 95%), β-sitosterol (purity: 60%), campesterol (purity: 37.5%), cholest-5-en-3β,7β-diol (7β-hydroxycholesterol, 7β-HC) (purity: 90%), 5α,6α-epoxy-cholestan-3β-ol (α-epoxycholesterol, α-EC) (purity: 87%), 5β,6β-epoxy-cholestan-3β-ol (β-epoxycholesterol, β-EC) (purity: 80%), cholestan-3β,5α,6β-triol (cholestanetriol, triol) (purity: 99%), cholest-5-en-3β-ol-7-one (7-ketocholesterol, 7-KC) (purity: 99%) and cholest-5-en-3β-ol (cholesterol) (purity: 99%), were purchased from Sigma (St. Louis, MO, USA). N°1 filters (70 mm diameter) were used (Whatmann, Maidstone, England). Aminopropyl solid-phase extraction (SPE) cartridges (Strata NH_2-55 mm, 70A, 500 mg/3 mL) from Phenomenex (Torrence, CA, USA) were utilized for sterol oxide purification.

The phosphate buffer used for the TBARs determination was prepared by adding 65.8 mL of 0.5 M NaH_2PO_4 and 111 mL of 0.5 M $Na_2HPO_4 \cdot H_2O$ (water solutions) in a 500 mL volumetric flask. pH was controlled, taken to neutrality (either with the acid or the basic solution), and then taken to volume with water. To delay oxidation and prevent the prooxidative effect of metals, proper amounts of EDTA and ascorbic acid were added to the buffer to reach a final concentration of 0.1% (w/v) for both of them.

The silylation mixture was prepared with dried pyridine, hexamethyldisilazane and trimethylchlorosilane (all from Sigma) at a ratio of 5:2:1 by volume.

2.2. Sampling, packaging and set-up of the photosensitized oxidation experiment

Fifty-six male heifers of race Charolaise, approximately 10 month old and 400 kg weight, were randomly separated in seven groups. Each group received corn silage (8 kg/head/day), beet pulp silage (5 kg/head/day), corn meal (2.5 kg/head/day) and straw (0.8 kg/head/day). Feeding was enriched with various ingredients (linseed oil, Dl-α tocopheryl acetate, conjugated linoleic acid ((CLA) *cis*-9, *trans* 11 and *trans*-10, *cis*-12 isomers) at various doses, leading to 7 independent diet groups: control (A), linseed oil/ Dl-α tocopheryl acetate for 180 days (B), linseed oil/ Dl-α tocopheryl acetate for 90 days (C), CLA/ Dl-α tocopheryl acetate for 180 days (D), CLA/ Dl-α tocopheryl acetate for 90 days (E), linseed oil/CLA/ Dl-α tocopheryl acetate for 180 days (F) and linseed oil/CLA for 180 days (G), as shown in Table 1.

Heifers were slaughtered six months later, after reaching approximately 650 kg weight. The carcasses were refrigerated for 24 h before muscle samples were taken. The *longissimus lumborum* (LL) muscle was excised from the carcass and freed from the superficial lipids; the meat sample was divided longitudinally and the terminal parts were excluded, since the diameter was not homogeneous. The muscle samples were vacuum-packed and wet-aged for 2 weeks at 4 °C. At the end of the aging period, the LL muscle was then cut into three pieces, trimmed of surface adipose tissue and 168 subsamples were obtained; the meat slice was 1 cm thick and had a weight ranging 100–150 g (thin slices).

Each thin slice was packed in a polyethylene vessel, which was wrapped with a transparent shrink film (14 μm thickness) with 10,445 mL/m^2/24 h of oxygen permeability. The packed slices were subjected to the following storage conditions:

(a) Fifty-six vessels were immediately frozen (−20 °C), which represented T0;
(b) Fifty-six vessels were stored in the dark at 8 °C for 8 h (T8D) in a bench refrigerator;
(c) Fifty-six vessels were stored at 8 °C under a daylight lamp for 8 h (T8L), in a bench refrigerator. The daylight lamp had a temperature and power of 3800 K, 1200 lx and 36 W (Osram, Milan, Italy), respectively. The lamps were located 1.5 m above the samples.

2.3. Lipid extraction

Lipids were extracted according to a modified version (Boselli et al., 2005) of the method described by Folch, Lees, and Sloane-Stanley (1957). The frozen samples were minced and 15 g were homogenized with 200 mL of a chloroform:methanol solution (1:1, v/v) in a glass bottle with screw-cap. The bottle was kept in an oven thermostated at 60 °C for 20 min before adding 100 mL chloroform. After 3 min of homogenization, the content of the bottle was filtered through filter paper to eliminate the solid residue, which consisted mostly of proteins. The filtrate was mixed thoroughly with 100 mL of a 1 M KCl solution and left overnight at 4 °C in order to obtain phase separation. The lower phase