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Cholesterol photosensitised oxidation of beef meat under standard and modified atmosphere at retail conditions

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ABSTRACT

The effect of the fluorescent light exposure and type of packaging (normal atmosphere and oxygen-rich atmosphere) was evaluated on the oxidation parameters (peroxides and cholesterol oxidation products) of raw beef slices placed in packed vessels and refrigerated. The concentration of COPs in meat treated under modified atmosphere ranged from 0.15 to 0.52 mg/100 g meat (average value of 0.27 mg COPs/100 g meat), which was twice as much as the average COPs content (0.14 mg/100 g) of meat packed under air (0.04–0.27 mg COPs/100 g meat). The main cholesterol oxide was 7k, which represented about one third of the total cholesterol oxides, followed by 7β-OH (20–25% of total COPs), 7α-OH (about 20%) and β-epoxy (12–18%). In normal atmosphere, photoxidation was a superficial process, since an inverse correlation between meat slice weight and COPs content on a lipid basis was observed, unlike in a high oxygen (32%) atmosphere.

1. Introduction

Cholesterol is a monounsaturated lypophilic biomolecule, which is arranged with the 3-hydroxyl group and the A ring exposed to the outer side of the double phospholipid layer in the biological membrane and the side chain located among the alkylic chain of phospholipids (Cercaci, Rodriguez-Estrada, Lercker, & Decker, 2007; Smith, 1987). It is, therefore, prone to both enzymic and chemical oxidation with the formation of compounds potentially harmful to human health, such as cholesterol oxidation products (COPs) (Garcia-Cruset, Carpenter, Codony, & Guardiola, 2002; Osada, 2002; Schroepfer, 2000). COPs are likely to be involved in lipid metabolism, various chronic and degenerative diseases (such as cancer, aging and atherosclerosis) and disturbance of cell functionality (Garcia-Cruset et al., 2002; Leonarduzzi, Sottero, & Poli, 2002; Osada, 2002; Schroepfer, 2000). COPs contain an additional hydroxy, ketone or epoxide group on the sterol nucleus and/or a hydroxyl group on the side chain of their molecules (Lercker & Rodriguez-Estrada, 2002).

Cholesterol oxides are present in quite low amount in raw food of animal origin (meat, milk, eggs, sea products), but their concentration increases dramatically in high-temperature treated food, after exposure to light, metals, natural sensitizers and oxygen, as well as in highly processed food products cooked meat (Badiani et al., 2002; Ferioli, Caboni, & Dutta, 2008; Galvin, Lynch, Kerry, Morrissey, & Buckley, 2000; Rodriguez-Estrada, Penazzi, Caboni, Bertacco, & Lercker, 1997) irradiated (Ahn, Nam, Du, & Jo, 2001) and dry-cured meat products (Vestergaard, & Parolari, 1999). The extent of meat oxidation is also related to the content of natural antioxidants and the unsaturation degree of the fatty acids (Bou, Grimpa, Baucells, Codony, & Guardiola, 2006; Decker, & Xu, 1998; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998).

Unlike previous studies, the aim of the current work was to evaluate the influence of both fluorescent light exposure and packaging under modified atmosphere, on lipid oxidation of beef slices stored in a bench refrigerator of a retail shop. Markers of the total primary lipid oxidation (peroxide value) and cholesterol oxidation products were used to monitor lipid oxidation. Storage time was chosen according to the typical shelf-life of beef slices packed in a closed vessel: 8 h storage under light exposure was selected as the maximum for the meat slices packed in normal atmosphere, whereas storage lasted 8 days for meat packed in a high oxygen partial pressure. For meat irradiation, a warm-tone lamp emanating red light with low emission in the blue region was used.

2. Materials and methods

2.1. Sampling

Meat samples were obtained from the half-carcase of a *Garronese* breed cow slaughtered at 165 kg weight and stored for 5 days at 3 °C. The inside round was excised from the half-carcase and trimmed from the superficial lipids and connective tissue; the

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meat sample was divided longitudinally and the terminal parts were excluded. The inside round was then cut with an automated cutter and 26 slices were obtained: the meat was 5–6 mm thick and had a weight ranging from 100 to 120 g (thin slices). In addition, 6 meat slices were cut with a thickness of 1 cm with a weight that varied between 135 g and 187 g (heavy slices). A total of 32 slices were, thus, obtained.

Of the 32 slices, fourteen thin slices and three heavy slices were packed individually in polyethylene vessel, wrapped with a transparent shrink film (14 μm thickness) with 10445 ml/m^2/24 h of oxygen permeability. The packed slices were subjected to the following storage conditions:

(a) Two vessels were immediately frozen (−18 °C) and represented the control (t001 and t002);
(b) three vessels were stored in the dark at 4 °C overnight (t01, t02 and t03);
(c) three vessels were stored at 4 °C in the dark (wrapped with tin foil) for 8 hours (8hD1, 8hD2 and 8hD3);
(d) three vessels were stored at 4 °C under a daylight lamp for 4 hours (4h1, 4h2 and 4h3);
(e) three vessels (8h1, 8h2 and 8h3) and the three heavy slices (8hH1, 8hH2 and 8hH3) were stored at 4 °C under a daylight lamp for 8 h. The remaining 12 thin slices and 3 heavy slices were packed in vessels under modified atmosphere (oxygen 32%, nitrogen 30%, carbon dioxide 38%) using a sealing film by means of a Tetra Laval Food Tiromat Compact packaging machine (Pully, Switzerland). The packed slices were subjected to the following storage conditions under modified atmosphere;
(f) three vessels were immediately frozen (−18 °C) and represented the control (MAt01, MAt02 and MAt03);
(g) three vessels were stored at 4 °C under a daylight lamp for 4 days (MA4d1, MA4d2 and MA4d3);
(h) three vessels were stored at 4 °C in the dark (wrapped with tin foil) for 8 days (MA8dD1, MA8dD2 and MA8dD3);
(i) three vessels (MA8d1, MA8d2 and MA8d3) and the three heavy slices were stored at 4 °C under a daylight lamp for 8 days (MA8dH1, MA8dH2 and MA8dH3).

The storage at 4 °C was in a bench refrigerator as usually happens in a retail shop. The warm-tone lamps had a colour temperature of 3000 K (low emission in the blue radiation) and a power of 36 W (Osram, Milan, Italy). The lamps were located 1.5 m above the samples, as in a retail shop.

2.2. Reagents and solvents

Analytical grade solvents and reagents were utilised. The standards supplied by Sigma Chemical Co. (St. Louis, MO, USA), are listed as follows: 19-hydroxycholesterol (19-OH), cholesterol, dihydrocholesterol, 3β-hydroxycholest-5-en-7-one (7k), 5,6α-epoxy-5α-cholestan-3β-ol (α-epoxy), 5,6β-epoxy-5β-cholestan-3β-ol (β-epoxy), cholest-5-en-3β,20α-diol (20-OH) and 5α-cholestan-3β,5,6β-triol (triol). The standards cholest-5-en-3β,7α-diol (7α-OH) and cholest-5-en-3β,7β-diol (7β-OH) were purchased from Steraloids (Wilton, NH, USA).

2.3. Extraction procedure

The lipids were extracted according to a modified version of the method described by Folch, Lees, & Sloane-Stanley (1957), as reported previously (Boselli et al., 2005). The frozen samples were minced and 60 g were homogenised with 500 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 250 ml chloroform. After 3 min of homogenisation, the contents of the bottle were filtered. The filtrate was mixed thoroughly with a 1 M KCl solution and left overnight at 4 °C in order to obtain phase separation. The lower phase containing the lipids was collected and dried with a rotary evaporator. The fat content was determined gravimetrically.

2.4. Determination of total cholesterol and cholesterol oxidation products (COPs)

A 250 mg lipid subfraction of the Folch extract was treated with a known amount of the internal standard solutions (12.5 μg of 19-OH and 5 mg dihydrocholesterol, for the determination of COPs and total cholesterol, respectively). Subsequently, the sample was dried under nitrogen and treated with 10 ml of 1 N KOH solution in methanol, for saponification at room temperature for 18 h (Sander, Addis, Park, & Smith, 1989). For the extraction of the unsaponifiable matter, 10 ml of water and 10 ml of diethyl ether were added to the samples, which were vigorously shaken. The diethyl ether fraction was then separated; the extraction with 10 ml of diethyl ether was repeated twice. The three portions of diethyl ether were pooled, treated with 5 ml of a 0.5 N KOH solution and extracted. The resulting ether extract was washed twice with 5 ml of water. The ether solution was finally evaporated in a rotary evaporator, after elimination of excess water by addition of anhydrous sodium sulphate. One tenth of the unsaponifiable matter was used for the determination of total cholesterol and the remainder for COPs analysis.

The determination of total cholesterol (sum of free and esterified) was achieved by means of capillary gas chromatography (CGC) after silylation (Sweeley, Bentley, Makita, & Wells, 1963). The gas chromatograph (HRGC Mega2 Series, Fisons, Rodano, Italy) was equipped with a split/splitless injector and a flame ionisation detector. A fused-silica capillary column (30 m × 0.32 mm i.d. × 0.25 μm film thickness) coated with 100% dimethyl-polysiloxane (DB-1, J&W Scientific, Folsom, CA, USA), was used. The oven temperature was programmed from 250 to 325 °C at 3 °C/min; the injector and detector temperatures were both set at 325 °C. Helium was used as carrier gas at a flow rate of 2 ml/min; the split ratio was 1:15. COPs purification was performed with nine tenths of the unsaponifiable matter by solid phase extraction (SPE) using a NH_2 cartridge, according to Rose-Sallin, Hugget, Bosset, Tabacchi, and Fay (1995). After silylation, the derivatised COPs were injected into a CGC under the same conditions as reported for the determination of total cholesterol. The identification of COPs was confirmed by comparison with the retention time and mass spectra of the COP standards. The mass spectra were obtained using the same column mounted in a 3400 GC from Varian (Palo Alto, CA, USA) coupled to an ion trap mass spectrometer (Magnum, Finnigan, San José, CA, USA).

2.5. Determination of peroxide value (PV)

The peroxide value was determined in 50 mg of lipid extract, according to Takagi, Mitsuno, and Masumura (1978), a iodometric procedure suitable for small lipid samples. The peroxides present in the meat lipids oxidised iodide to iodine and, after 5 min, the excess of iodide ion was immediately converted to a cadmium complex under a nitrogen atmosphere. The iodine was measured at 358 nm with a double beam UV/visible spectrophotometer Jasco model UVIDEC-430 (Tokyo, Japan). The PV was calculated from the absorbance. For the quantitative determination of PV, a calibration curve was prepared by adding solutions of potassium dichromate at different concentrations to the KI solution. The same procedure was used for the measurement of the released iodine absorbance (358 nm), which was plotted against the active oxygen content.