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Evaluation of cholesterol and lipid oxidation in raw and cooked minced beef stored under oxygen-enriched atmosphere

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ABSTRACT

Oxygen-enriched modified atmosphere packaging (MAP) represents an important means to stabilize meat colour but may lead to an increase in lipid oxidation, influencing the acceptability and safety of the product. In this work, the effect on cholesterol and lipid susceptibility to oxidation was investigated in commercial minced beef held under MAP (80% O_2/20% CO_2). Cholesterol oxidation products (COPs), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were determined, before and after pan frying, at 1, 8 and 15 days since packaging under refrigerated storage (3–4 °C). 7α-Hydroxycholesterol, 7β-hydroxycholesterol and 7-ketocholesterol were the more abundant COPs identified. COPs significantly increased in raw beef during storage: after 1, 8 and 15 days since packaging COPs were at the levels of 10.4, 30.7 and 60.5 μg/g of fat, respectively. Cooking did not affect cholesterol oxidation in freshly packaged minced beef but led to a rise in COPs amount with respect to raw muscle after 8 and 15 days of storage. The trend in cholesterol oxidation reflected the progressive increase in lipid peroxidation rate brought by MAP conditions.

1. Introduction

Meat packaging has been a matter of major interest to meat traders, health officials and scientists for several years. Packaging is not only the material immediately surrounding meat or meat products but also the synthesis of product, processing, labour and machines, for addressing specific functional and marketing requirements (Bell, 2001).

Modified atmosphere packaging (MAP) requires the use of films with low gas permeabilities and, after air evacuation, employs a gas or a mixture of gases (i.e. carbon dioxide, nitrogen, and oxygen) to control the microbial growth or to prevent colour deterioration (Seideman & Durland, 1984; von Elbe & Schwartz, 1996). The trend to self-service merchandising of fresh meat requires a high standard of colour presentation since consumers' judgement about meat freshness is related to the bright-red colour of oxymyoglobin (Bell, 2001). The colour of meat is influenced by the chemistry of the heme pigment myoglobin (von Elbe & Schwartz, 1996). While reduced myoglobin (Mb) is the predominant muscle pigment in fresh cut and in vacuum packaged meat, the binding of molecular oxygen yields oxymyoglobin (MbO_2) and the colour of meat changes to the customary bright-red. With time, the loss of oxygen and the oxidation of hemic iron from Fe^{2+} (ferrous ion) to Fe^{3+} (ferric ion) lead to the undesirable brown metmyoglobin (MMb) (Fox, 1966; von Elbe & Schwartz, 1996). Saturation levels of oxygen in the packaging environment of meat are useful, favouring myoglobin oxygenation and delaying the formation of MMb (von Elbe & Schwartz, 1996). For red meat, high-oxygen MAP systems typically utilize atmospheres containing from 70% to 80% of oxygen and from 20% to 30% of carbon dioxide. In this way, the high oxygen concentration enhances the bright-red colour, as well as carbon dioxide inhibits the growth of aerobic spoilage microorganisms, ensuring a long shelf-life (5–10 days) (Bell, 2001).

As a main drawback, this kind of MAP may lead to a faster development of lipid oxidation that can negatively affect the sensorial and nutritional quality of meat. Meat oxidation has been extensively reviewed (Gray, Gomaa, & Buckley, 1996; Kanner, 1994; Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998) as one of the major causes of fat-containing food spoilage (Nawar, 1996).

Another detrimental consequence of lipid oxidation is the generation of toxic compounds such as cholesterol oxidation products (COPs). The occurrence of COPs in foods has arisen a great concern amongst nutritionist and food scientists for their biologically adverse effects, especially regarding the onset of degenerative diseases like atherosclerosis and cancer (Garcia-Cruset, Carpenter, Codony, & Guardiola, 2002; Osada, 2002; Schroepfer, 2000).

Some papers have recently dealt with the effect of packaging conditions on the development of lipid peroxidation and the

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formation of COPs in a variety of meat during storage, comparing aerobic and vacuum packaging (Ahn, Nam, Du, & Jo, 2001; Du, Nam, & Ahn, 2001; Galvin, Lynch, Kerry, Morrissey, & Buckley, 2000; Nam, Du, Jo, & Ahn, 2001) but not taking into account MAP. Only a few reports illustrated the effects of MAP both on lipid and cholesterol oxidation during storage, in particular in pork and chicken meat (Cayuela, Gil, Bañón, & Garrido, 2004; Conchillo, Ansorena, & Astiasarán, 2005).

The aim of the current work was a preliminary investigation on the consequences of an oxygen-enriched MAP on cholesterol oxidation and lipid stability in commercial minced beef, before and after pan frying, after different periods of refrigerated storage. The analytical parameters evaluated were COPs, peroxide value (PV) and thiobarbituric acid reactive substances (TBARS).

2. Materials and methods

2.1. Samples

Commercial samples of minced beef (400 g per tray) coming from the same batch were purchased from a local supermarket located in Uppsala, Sweden. Minced beef was stored under a modified atmosphere packaging (MAP) with a high oxygen content (80% O_2/20% CO_2) and wrapped using a low permeable oxygen film (shelf life: 8 days). Samples were kept in the dark at a temperature ranging from 3 to 4 °C and analyzed after 1, 8 and 15 days since packaging. Burgers (100 g, thickness ≈0.5 cm) were prepared from raw beef after mixing the meat and pan fried without the addition of any fat or vegetable oil. The heating device was kept at 150–160 °C; each burger was fried for 15 min (7.5 min per side) until a core temperature of 71–80 °C was reached. The core temperature was controlled with the aid of a digital thermometer. The cooked burgers were not stored in order to follow the widespread consumers' practise to store raw meat at low temperatures and cook it just before consumption. The plan of storage and sample names are summarized in Fig. 1.

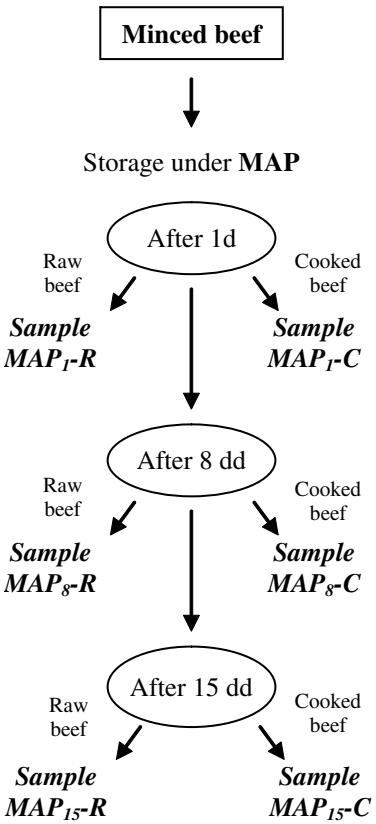

Fig. 1. Flow chart of storage plan and sample names. In each name the subscript number refers to storage time (1, 8 or 15 days) while R and C stand for raw and cooked minced beef, respectively.

2.2. Reagents and chemicals

All chemicals and solvents, unless specified, were of analytical grade and purchased from Merck (Darmstadt, Germany), Sigma (St. Louis, MO, USA) and Solveco Chemicals AB (Rosersberg, Sweden). Tetraethoxypropane (TEP) employed in the TBARS assay was from Merck. 19-Hydroxycholesterol (19-HC), 7α-hydroxycholesterol (7α-HC), 7β-hydroxycholesterol (7β-HC), cholesterol-α-epoxide (α-CE), cholesterol-β-epoxide (β-CE), 7-ketocholesterol (7-kC), cholestanetriol (CT), 20-hydroxycholesterol (20-HC) and 25-hydroxycholesterol (25-HC) were from Sigma and Steraloids (Newport, RI, USA). Aqueous solutions were prepared in deionized water.

2.3. Lipid extraction

Lipid extraction was performed according to Hara and Radin (1978) with some modifications. About 25 and 15 g of raw and cooked beef, respectively, were weighed into a screw-cap plastic centrifuge bottle, added to 140 ml of *n*-hexane/*i*-propanol 3/2 (v/v) and homogenized (5 × 30 s at maximum speed) using an Ultra-Turrax T25 Basic homogenizer fitted with a S25N-18G dispersing tool (IKA-Werke, Staufen, Germany). The dispersing tool was washed with 10 ml of *n*-hexane/*i*-propanol 3/2 (v/v). Subsequently, 60 and 70 ml, for raw and cooked beef, respectively, of a 6.67% (w/v) water solution of sodium sulphate was added to the homogenate, that was then gently shaken for about 30 s and centrifuged at 4000 rpm for 3 min at a temperature of 7–13 °C to obtain a better separation between the organic and water phases. The supernatant organic layer was siphoned off and collected in a 250 ml conical-bottom glass flask which was previously weighed. The residue was extracted for second time with 80 ml of *n*-hexane, following the same procedure. The fat matter was dried under a nitrogen flow for 45–60 min, weighed and stored at −18 °C until further analyses.

2.4. Spectrophotometric determination of peroxide value (PV)

PV was determined according to the IDF Standard Method (1991) on 50–100 mg of fat. This method is based on the spectrophotometric determination of ferric ions (Fe^{3+}) formed by the oxidation of ferrous ions (Fe^{2+}) by hydroperoxides in the presence of ammonium thiocyanate (NH_4SCN). To quantify PV, a calibration curve was constructed every week (absorbance at 500 nm *vs.* Fe(III) amount expressed in μg) over a calibration range from 1 to 50 μg. PV was expressed as meq of O_2/kg of fat.

2.5. Spectrophotometric determination of thiobarbituric acid reactive substances (TBARS)

TBARS were determined according to the spectrophotometric method described by Miller (1998). About 5 g of muscle were used for this analysis. This method is based on the ability of malondialdehyde (MDA) to form a pink-coloured chromogen that absorbs at 450, 530 or 538 nm. To quantify TBARS, a calibration curve was constructed every week (absorbance at 530 nm *vs.* amount of MDA expressed in nmol) employing TEP as precursor of MDA. TBARS were expressed as mg of MDA/kg of muscle.