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# Fat content has a significant impact on protein oxidation occurred during frozen storage of beef patties

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ABSTRACT

This study examined the relationship between protein and lipid oxidation and the impairment of water holding capacity (WHC), colour and texture after frozen storage (20 weeks/−18 °C) and subsequent processing (cooking, chilled storage) of beef patties with increasing fat content (3, 20 and 35%). Various manifestations of protein oxidation were found to occur during frozen storage and processing of patties including, loss of tryptophan fluorescence, carbonylation and formation of Schiff bases structures (SB). Patties with higher fat content underwent the more intense protein oxidation as assessed by formation of protein carbonyls and SB, highlighting the timely interaction between proteins and oxidizing lipids. Protein oxidation occurred concomitantly with loss of WHC and discolouration of beef patties. Mechanisms and consequences of the chemical modifications induced by oxidative stress in meat proteins are thoroughly discussed.



## 1. Introduction

Currently, intense socio-economical changes, rising incomes in developed countries and rapid urbanization have led to a great increase in consumer demands for ready-to-eat products, generally frozen (FAO, 2012; MacKenzie, 2011). Therefore, the consumption of ground beef based products such as meat balls, hamburgers or fillers, has increased considerably (MacKenzie, 2011). These products are characterized by a high fat content; up to 20% according to European regulations or up to 30% in U.S.A. (BOE, 1998, pp. 1086–1101; Code of Federal Regulations, 2012). Fat content plays critical functional and sensory roles in muscle foods. It is involved in the development of flavour compounds and in the perception of tenderness, juiciness and mouthfeel (Berry, 1992). However, recent studies have linked a high dietary intake of meat and saturated fat with important health problems, such as coronary heart disease, obesity and diabetes (Webb & O'Neill, 2008). In addition, lipids are also known as one of the most chemically unstable food components and readily undergo oxidative reactions, responsible for loss of quality and nutritional value of meat (Faustman, Sun, Mancini, & Suman, 2010).

Different meat processing treatments promote lipid oxidation, including frozen storage. Since the formation of ice crystals during freezing causes cell disruption and concentrates prooxidant solutes, such as iron, in the unfrozen phase leading to an increased formation of reactive oxygen species (ROS) and oxidative reactions (Zaritzky, 2012). On the other hand, meat proteins are also known to be susceptible to oxidative reactions (Estévez, 2011; Stadtman, 2004), but little is known about the effects of frozen storage on protein oxidation. Oxidation of proteins is affected by many environmental factors such as temperature, processing conditions and the presence of catalysts or inhibitors (Estévez, 2011). Moreover, oxidative reactions can easily transfer from lipids to proteins (Viljanen, Kivikari, & Heinonen, 2004). Oxidative damage to proteins include the oxidation of amino acid residue side chains, formation of protein carbonyls, cleavage of the peptide bonds, and formation of some covalent protein–protein cross-linked derivatives (Lund, Heinonen, Baron, & Estévez, 2011). These modifications can significantly alter the properties of meat proteins and may ultimately influence the quality of meat products (Xiong, 2000). Therefore, it is the great importance to shed light on the possible relationship between lipid and protein oxidation, and the quality changes in muscle foods related to these oxidative modifications.

In the present paper, the effect of increasing fat contents on the susceptibility of meat proteins of frozen beef patties to undergo particular oxidative changes was studied. The impact of the oxidative damage on particular quality traits was also assessed.

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## 2. Materials and methods

### 2.1. Chemicals and materials

All chemicals were supplied from Panreac (Panreac Quimíca, S.A., Barcelona, Spain), Merck (Merck, Darmstadt, Germany), and Sigma Chemicals (Sigma–Aldrich, Steinheim, Germany). Water used was purified by passage through a Milli-Q system (Millipore Corp., Bedford, MA, USA). Beef (longissimus dorsi muscle) and beef ribs belonged to industrial genotypes and were purchased from a local slaughterhouse in Cáceres (Spain). Meat was obtained from the longissimus dorsi muscle, freed from visible fat, immediately chopped into pieces (2 $cm^3$), kept at 4 °C during 8 h and used as such for the manufacture of the patties. Fat was obtained from beef ribs and freed from connective tissue and lean.

### 2.2. Manufacture of patties

Depending on the fat content, three different types of burger patties were produced, namely, low, medium and high fat content patties (LF, MF and HF, respectively). LF patties were manufactured according to the basic recipe as follows (g/kg raw batter): 732 g beef muscle, 244 g distilled water and 24 g sodium chloride. In MF and HF patties, 178 and 390 g of beef muscle, respectively, were replaced by fat.

All ingredients were minced in a cutter until a homogeneous raw batter was obtained (9 min). Patties were formed using a conventional burgermaker (~80 g/patty), to give average dimensions of 10 cm diameter and 1 cm thickness. In total, 20 patties per fat level were prepared in four independent manufacturing processes. Four patties per group were subjected to further analyses the day after the manufacture (I: unfrozen patties). The other patties were stored in individual oxygen permeable polyethylene bags, dispensed in trays and subsequently stored for 20 weeks at −18 °C in the dark, in order to simulate in-home storage conditions. After frozen storage, patties were thawed at 4 °C during 10 h. Upon thawing, four patties per group were subjected to analysis (II: frozen patties), while the other eight were placed on trays and cooked at 170 °C for 18 min in a forced-air oven. After cooking, four samples were allowed to cool down at room temperature and subjected to further analyses (III: cooked patties), while the other four were dispensed in individual oxygen permeable polyethylene bags and subsequently stored for 15 days at 4 °C in a refrigerator under white fluorescent light (620 lx), simulating retail display conditions (IV: cooked and chilled patties). Upon chilled storage, these samples were as well analysed.

### 2.3. Proximate composition of patties

Moisture and protein contents (g/100 g sample) were determined using official methods (AOAC, 2000). The method of Folch, Lees, and Sloane Stanley (1957) was used for determining fat content in patties (g/100 g sample). Haem-iron content was determined by spectrophotometric quantification of myoglobin at 640 nm after extraction with acidified 80% acetone and results were expressed as ppm of haem-iron (Hornsey, 1956).

### 2.4. Determination of thiobarbituric acid-reactive substances (TBARS)

TBARS were assessed as described by Salih, Smith, Price, and Dawson (1987) with some modifications. Samples (5 g) were homogenised with 15 mL perchloric acid (3.86%) and 0.5 mL butylated hydroxytoluene (4.2% in ethanol). The slurry was filtered and centrifuged (1509 g for 4 min) and 2 mL aliquots were mixed with 2 mL thiobarbituric acid (0.02 M) in test tubes. The test tubes were placed in a boiling water bath (100 °C) for 45 min. After cooling, absorbance was measured at 532 nm. TBARS content was calculated from a standard curve of 1,1,3,3-tetraethoxypropane (TEP) solution (ranging from $0.28 \times 10^3$ to $1.13 \times 10^3$ mg/mL) in 3.86% perchloric acid. Results were expressed as mg malondialdehyde (MDA) per kg of sample.

### 2.5. Fluorescence measurements of tryptophan and Schiff base structures (SB)

The natural fluorescence of tryptophan and the emission of fluorescence by protein oxidation products (SB) were assessed by using fluorescence spectroscopy (Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008). Patties were ground and homogenized according to the process described by Utrera, Rodríguez-Carpena, Morcuende, and Estevez (2012). A 1 mL aliquot of the homogenates was redissolved in 20 mL of the 20 mM sodium phosphate buffer and then dispensed in a 4 mL quartz spectrofluorometer cell. Emission spectra of tryptophan were recorded from 300 to 400 nm with the excitation wavelength established at 283 nm (LS 55 Perkin–Elmer luminescence spectrometer, MA, USA). Emission spectra of Schiff bases were recorded from 400 to 500 nm with the excitation wavelength set at 350 nm. Excitation and emission slit widths were set at 10 nm and data were collected at 500 nm per minute in both measurements. Tryptophan content was calculated from a standard curve of N-acetyl-L-tryptophan amide (NATA). A reliable standard curve was obtained between NATA concentration (ranged from 0.1 to 0.5 μM) and fluorescence intensity ($R^2 = 0.9955$; $p < 0.05$). Results were expressed as μmoles NATA equivalents per 1 g of sample. The content of Schiff base structures was expressed as fluorescence intensity units emitted at 460 nm. Values were expressed relative to the sample weight.

### 2.6. HPLC-FLD analysis of α-aminoadipic semialdehyde (AAS)

5 mg of protein were derivatized with 50 mM aminobenzoic acid (ABA) and subsequently hydrolyzed with 6 N HCl (Utrera, Rodríguez-Carpena, et al., 2012). Hydrosylates were dried *in vacuo*, reconstituted with 200 μL of Milli-Q water, and filtered through a PVDF syringe filter (0.45 μm pore size, Pall Corp., New York, USA). Samples were injected in an HPLC using a Cosmosil (Nacalai, USA) C18-AR-II RP-HPLC column (5 μm, 150 × 4.6 mm) and a guard column (10 × 4.6 mm) filled with the same material. The Shimadzu "Prominence" HPLC apparatus (Shimadzu Corp., Kyoto, Japan) was equipped with a quaternary solvent delivery system (LC-20AD), a DGU-20AS online degasser, an SIL-20A autosampler, an RF-10A XL fluorescence detector, and a CBM-20A system controller. Fifty mM sodium acetate buffer (pH 5.4, eluent A) and acetonitrile (ACN, eluent B) were used as eluents. A low-pressure gradient program was used, varying eluent B concentration from 0% (min 0) to 8% (min 20). The injection volume was 1 μL, the flow rate was kept at 1 mL/min, and the temperature of the column was maintained constant at 30 °C. Excitation and emission wavelengths were set at 283 and 350 nm, respectively. Identification of the derivatized semialdehydes in the FLD chromatograms was carried out by comparing its retention time with that from a standard compound injected and analyzed under the above-mentioned conditions (Utrera, Rodríguez-Carpena, et al., 2012). Results were expressed as nmol of AAS per g of protein.

### 2.7. Moisture losses

Moisture losses of patties were calculated as percentage of weight loss after each process stage (freezing/thawing, cooking loss