Meat Science 96 (2014) 812–820

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Protein oxidation during frozen storage and subsequent processing of different beef muscles

Mariana Utrera, Vita Parra, Mario Estévez *

Food Technology, Department of Animal Production and Food Science, University of Extremadura, 10003 Cáceres, Spain

ARTICLE INFO

Article history:
Received 21 May 2013
Received in revised form 5 September 2013
Accepted 6 September 2013

Keywords:
Beef
Muscles
Heme-iron
Antioxidant enzymes
Carbonylation
Freezing

ABSTRACT

This study examined the relationship between protein and lipid oxidation and the impairment of the water holding capacity (WHC), redness and instrumental hardness occurring during 20 weeks of frozen storage (−18 °C) and subsequent processing (cooking, chilled storage) of psoas major, quadriceps femoris and longissimus dorsi beef patties. Patties were analyzed at sampling times upon thawing (weeks 0, 4, 8, 12 and 20) for lipid (TBARS, hexanal) and protein oxidation products (α-aminoadipic and γ-glutamic semialdehydes, α-aminoadipic acid, Schiff bases). A significant impact of frozen storage on protein oxidation was found, which occurred concomitantly with a loss of WHC, redness and significant changes in the hardness of cooked patties. Heme-iron, endogenous antioxidant enzymes and to a lower extent, fatty acid composition, played a role in the oxidative stability of patties. Plausible mechanisms by which particular protein oxidation changes may lead to loss of WHC and impaired quality traits were discussed.

1. Introduction

Foods are exposed to reactive oxygen species (ROS) that are formed enzymatically, chemically, photochemically, and by irradiation (Choe & Min, 2006). ROS-mediated oxidative stress on lipids and proteins is a major cause of chemical deterioration in foods, mainly in meat and meat products (Choe & Min, 2006). Mechanisms and consequences of lipid oxidation have been deeply studied. Consequently, it is known that oxidation of meat lipids leads to discoloration, off-odor and off-flavor development, texture defects and the production of potentially toxic compounds (Ladikos & Lougovois, 1990). In contrast, the impact of protein oxidation on meat quality is still the subject of multiple studies but it is generally accepted that it involves the loss of essential amino acids, changes in texture, alteration in protein functionality and impaired digestibility (Lund, Heinonen, Baron, & Estévez, 2011). Numerous ROS are able to trigger the oxidative damage to proteins and oxidizing lipids are known to be influential (Stadtman & Levine, 2003). In such manner, many oxidative modifications on proteins may occur such as gain of carbonyl groups, oxidative degradation of essential amino acids (e.g. histidine, tryptophan, methiodine, cysteine), loss of sulfhydryl groups and formation of intra and/or intermolecular cross-links (Stadtman & Levine, 2003).

Despite the potential connection between lipid and protein oxidation, it is well-known that both processes are governed by different factors and mechanisms (Estévez, 2011). The understanding of the scientific and technological significance of protein oxidation in muscle foods requires the study of the specific chemistry behind the oxidative damage to muscle proteins. In order to fulfill this objective, the analysis of specific oxidation products from particular amino acids is considerably more valuable than the application of unspecific procedures such the dinitrophenylhydrazine (DNPH) method (Estévez, 2011). Many are the factors reported to have an influence on the occurrence and extent of protein oxidation, including various processing factors such as meat aging, cooking and prolonged storage (Estévez, 2011; Leygonie, Britz, & Hoffman, 2012). Frozen storage, in particular, has been found to have a significant impact on protein carbonylation and that, in turn, on particular meat quality traits such as water-holding capacity, color and texture (Estévez, Ventanas, Heinonen, & Puolanne, 2011; Utrera, Armenteros, Ventanas, Solano, & Estévez, 2012; Utrera, Rodríguez-Carpena, Morcuende, & Estévez, 2012; Xia, Kong, Liu, & Liu, 2009). In addition to these external factors, the susceptibility of meat to suffer protein oxidation is closely related to endogenous factors such as the composition of proteins and lipids, iron content, metabolism of the muscle and antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase–reductase system (Estévez et al., 2011; Mercier, Gatellier, & Renerre, 2004; Utrera, Armenteros, et al., 2012; Utrera, Rodríguez-Carpena, et al., 2012; Xia et al., 2009). The knowledge on the effect of the abovementioned endogenous factors on the occurrence, extent and consequences of protein oxidation during frozen storage of meat is limited. Owing to the high content of iron, the main promoter of protein carbonylation in meat systems (Estévez, 2011), oxidative muscles may have a lower oxidative stability than glycolitic muscles. In this regard, a previous study devoted to study porcine meat subjected to frozen storage linked the high susceptibility to carbonylation of oxidative muscles with their large iron content (Estévez, Kylli, Puolanne,

* Corresponding author. Tel.: +34 927257100x51390; fax: +34 927257110.
E-mail address: mariovet@unex.es (M. Estévez).

0309-1740/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2013.09.006

Kivikari, & Heinonen, 2008; Utrera, Armenteros, et al., 2012; Utrera, Rodríguez-Carpena, et al., 2012). However, there are other factors depending on the metabolic profile of muscles that may be influential such as the lipid content and characteristics, the content of reducing sugars and the presence of antioxidant enzymes. The study of the influence of these factors on the occurrence, extent and nature of protein oxidation may deserve specific studies. Additionally, further understanding of the consequences of protein oxidation during frozen storage of meat would clarify the actual technological relevance of such reactions and would urge meat scientists to develop antioxidant strategies to control the potential negative impact of oxidative protein damage on muscle food quality.

The aim of this study was to determine the oxidative stability of proteins during frozen storage of different beef muscles through the analysis of novel protein oxidation markers and assess the impact of such oxidation damage on particular quality traits in subsequently processed patties.

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 quadriceps femoris (QF), longissimus dorsi (LD) and psoas major (PM) muscles belonged to industrial genotypes slaughtered and purchased in Cáceres (Spain). Meat was 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.

2.2. Manufacture of burger patties

Three types of beef burger patties were prepared depending on the muscle used, namely, psoas major (PM), quadriceps femoris (QF) and longissimus dorsi (LD). The basic recipe was as follows (g/kg raw batter): 732 g meat, 244 g distilled water and 24 g sodium chloride. 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, 28 burger patties per type were prepared in four independent manufacturing processes. The chemical composition of the burger patties was determined the day of the manufacture. Patties were stored in individual oxygen permeable polyethylene bags, dispensed in trays and subsequently stored for 20 weeks at −18 °C in the dark. At each sampling time (weeks 0, 4, 8, and 12), four patties per muscle were taken out, thawed at 4 °C for 10 h and subjected to subsequent analysis. After the 20 weeks of frozen storage, the 12 remaining patties were thawed at 4 °C for 10 h and subjected to further processing. Four patties were subjected to analysis (sampling at week 20, Raw patties — R), 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 analysis were performed (Cooked patties — C), the other four were dispensed and stored 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 (Cooked and chilled patties — C&C). Upon chilled storage, these samples were as well analyzed.

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). Heme-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 heme-iron (Hornsey, 1956).

2.4. Assays of antioxidant enzyme activities

Samples (5 g) were homogenized in 25 mL of phosphate buffer (0.05 M, pH 7) and centrifuged at 4 °C for 2 min at 3200 g. The supernatant fraction was filtered through gauze and used to determine catalase, glutathione reductase (GR) and superoxide dismutase (SOD) activities. Catalase activity assay was performed as described by Hernández, Zomeño, Ariño, and Blasco (2004). The supernatant (50 μL) was reacted at room temperature with 1.95 mL of 11 mM H_2O_2 in phosphate buffer, and the reaction (H_2O_2 loss) was monitored by measuring the absorbance at 240 nm during 2 min. One unit (U) of catalase was defined as the amount of extract needed to decompose 1 mmol of H_2O_2 per min. SOD activity was measured according to the procedure described by Mercier et al. (2004) using inhibition of pyrogallol autoxidation in a basic medium. 50 μL of pyrogallol (10 mM) were added to 1.9 mL of 50 mM Tris–HCl buffer pH 8.2. The rate of pyrogallol autoxidation in presence of 50 μL of muscle extract was compared to a blank (with 50 μL of the phosphate extraction buffer) by measuring the increase of absorbance at 340 nm during 2 min. One unit was taken as the activity that inhibits the pyrogallol autoxidation by 50%. GR activity was assessed by monitoring the oxidation of NADPH initiated by oxidized glutathione (glutathione sulphide, GSSG) addition at 37 °C (Cribb, Leeder, & Spielberg, 1989). To measure GR, the following reagents, in 0.1 M sodium phosphate buffer, pH 7.5, with 1 mM ethylenediaminetetraacetic acid (EDTA), were added in quadruplicate wells of a 96-well plate: 150 μL of 0.1 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), 10 μL of nicotinamide adenine dinucleotide phosphate (NADPH) (12 mM) and 20 μL of reductase standard (0.025 to 0.50 U/mL) or the sample to be assayed. The reaction was initiated by the addition of 10 μL of GSSG (3.25 mM). For blank wells, no GSSG was added. Absorbance at 415 nm was measured every 30 s for 3 min at room temperature. Samples were measured in quadruplicate. GR was determined through a standard curve. Results are expressed as U/mL of sample.

2.5. Quantification of reducing sugars

Reducing sugars were extracted from patties (5 g) with 20 mL of warm (60 °C) distilled water using an Ultra-Turrax homogeniser. Homogenates were centrifuged for 3 min at 805 *g* and filtered through Whatman No. 4 paper. Volumes were made up to 25 mL in volumetric flasks after cooling. Reducing sugars were determined by the 3,5-dinitrosalicylic acid (DNS) method (Freitas et al., 2012). Briefly, 0.5 mL of the extract were added to 0.5 mL of 1% DNS placed in a boiling water bath (100 °C) for 5 min. After cooling, 5 mL of distilled water were added to the reaction mixture and absorbance was measured at 540 nm. Reducing sugar content was calculated from a standard curve of glucose at a range of 0–1 mg/mL. Results were expressed as mg of glucose per g of sample.

2.6. Determination of thiobarbituric acid-reactive substances (TBARS)

TBARS were assessed as described by Ganhão, Estévez, and Morcuende (2011) with some modifications. Samples (5 g) were homogenized with 15 mL perchloric acid (3.86%) and 0.5 mL BHT (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. TBA-RS content was calculated from a standard curve of 1,1,3,3-tetraethoxypropane (TEP) solution (ranging from 0.28×10^3