Meat Science 85 (2010) 506–514

Contents lists available at ScienceDirect

Meat Science

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

Lipid and protein oxidation and sensory properties of vacuum-packaged dry-cured ham subjected to high hydrostatic pressure

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ARTICLE INFO

Article history:
Received 3 December 2009
Received in revised form 19 February 2010
Accepted 21 February 2010

Keywords:
Dry-cured ham
High pressure treatment
Lipid oxidation
Protein oxidation
Sensory traits
Vacuum packaged format

ABSTRACT

The effect of HHP treatment (600 MPa) on the oxidative stability of lipids and proteins of vacuum-packaged Iberian dry-cured ham and the impact on the sensory characteristics of the product was investigated. In order to assess how different commercial presentations are affected by HHP treatment, three different presentations of vacuum-packaged Iberian dry-cured ham were considered, namely, (i) intact format (IF) corresponding to non-sliced vacuum-packaged dry-cured ham, (ii) conventional-sliced format (CSF) corresponding to dry-cured ham slices placed stretched out in the package and (iii) alternative-sliced format (ASF) corresponding to dry-cured ham slices piled up horizontally. The oxidation of dry-cured ham lipids and proteins was enhanced by HHP-treatment with the presentation being highly influential on these oxidative reactions. Pre-slicing dry-cured ham results in a more susceptible product to oxidative reactions during pressurisation and subsequent refrigerated storage. Possible mechanisms, by which HHP-induced oxidative reactions would affect particular sensory traits in vacuum-packaged Iberian dry-cured ham such as colour, texture and flavour attributes, are discussed.

1. Introduction

Iberian dry-cured ham is a traditional processed Spanish meat product highly appreciated by consumers from Mediterranean countries and of increasing interest amongst emerging markets such as those in Northern Europe, Japan, USA, Australia and South Africa. Dry-cured meats from Iberian pigs are high quality products mostly due to their sensory features achieved after about two-years of ripening (Ventanas, Ventanas, Ruiz, & Estévez, 2005). Post-processed operations including deboning, slicing and packaging are critical steps since they can contribute to cross-contamination which affects the shelf life and safety of the product. In addition, Iberian dry-cured ham is commonly exhibited in refrigerated display cabinets and this has an impact on its quality since the physico-chemical and thus, the sensory characteristics of the product are modified (Andrés, Adamsen, Møller, Ruiz, & Skibsted, 2006). In recent years, slicing followed by vacuum or modified atmosphere packaging are the most used methods to avoid unpleasant sensory changes and safety problems (Adamsen, Hansen, MØller, & Skibsted, 2003; García-Esteban, Ansorena, & Astiasarán, 2004).

High-pressure (HHP) processing at 600 MPa has been confirmed as an efficient non-thermal technology for the preservation of sliced dry-cured ham. This emerging technology enables the reduction of microorganisms linked to spoilage while keeping the surviving microbiota at low levels during subsequent storage (Garriga, Grébol, Aymerich, Monfort, & Hugas, 2004). The application of HHP to sliced dry-cured ham is necessary to meet the demanding safety regulations in countries such as USA and Japan. However, studies have confirmed that HHP promotes lipid oxidation and volatile formation and induces colour changes in sliced dry-cured ham (Andrés, Adamsen, Møller, and Skibsted, 2004; Andrés et al., 2006; Rivas-Cañedo, Fernández-García, & Nuñez, 2009). Moreover, HHP is known to affect the sensory characteristics of meat products (Mor-Mur & Yuste, 2003) but the influence of HHP on the sensory properties of dry-cured ham is unknown.

Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and meat products (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Muscle proteins are susceptible to oxidative reactions initiated by oxidizing lipids, metal ions and other pro-oxidants generated during processing (Xiong & Decker, 1995; Estévez, Killy, Puolanne, Kivikari, & Heinonen, 2008a). The oxidation of muscle proteins involves the loss of essential amino acids and decreases protein digestibility affecting its nutritional value (Xiong, 2000). Moreover, colour and texture deterioration of meat has been related to protein oxidation phenomenon (Estévez, Ventanas, & Cava, 2005). The formation of carbonyl compounds is one of the most marked changes occurring during the oxidation of proteins, with the quantification of the total protein carbonyls using the 2,4-dinitrophenylhydrazine (DNPH) method

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doi:10.1016/j.meatsci.2010.02.024

being routinely used for the assessment of protein oxidation in muscle foods (Oliver, Alin, Moerman, Goldstein, & Stadtman, 1987; Lund, Lametsch, Hviid, Jense, & Skibsted, 2007; Estévez et al., 2008a). The DNPH method is, however, non-specific since it measures the total amount of a variety of carbonyl derivatives formed through unspecific pathways during the oxidative process. More recently, Estévez, Ollilainen, and Heinonen (2009) developed a specific method to detect particular protein carbonyls namely, α-aminoadipic and γ-glutamic semialdehydes (AAS and GGS, respectively) using liquid-chromatography–electrospray ionisation-mass spectrometry (LC–ESI-MS) in myofibrillar proteins. This method has been successfully applied to various meat products confirming the potential of both semialdehydes as indicators of protein oxidation (Armenteros, Heinonen, Ollilainen, Toldrá, & Estévez, 2009). The impact of HHP on the oxidative degradation of myofibrillar proteins is poorly understood and the specific oxidation products AAS and GGS have never been detected in meats subjected to HHP. The aim of the present study was to investigate the effect of HHP (600 MPa) on the oxidative stability of lipids and proteins of vacuum-packaged Iberian dry-cured ham and its impact on the sensory characteristics of the product. To study the effect of different commercial presentations on the effectiveness and consequences of HHP treatment, three different presentations of sliced and vacuum-packaged dry-cured ham were considered.

2. Materials and methods

2.1. Sampling, packaging and pressure treatment

Dry-cured hams (7.5 kg average) were obtained from free-range reared Iberian pigs fed on grass and commercial concentrates during the fattening period (60 days prior to slaughter) (Ventanas, Ventanas, Tovar, García, & Estévez, 2007) and slaughtered at 160 kg live weight and 12 months of age. Green hams were processed traditionally, including two defined steps: salting/post-salting and ripening (Estévez, Morcuende, Ventanas, & Ventanas, 2008b). During the first period (4–6 months), low temperatures (0–3 °C) were combined with high relative humidity (80–90%) to reduce the risk of bacterial spoilage. Then, the hams were ripened for 15 months in a cellar at temperatures ranging from 10 to 27 °C and relative humidity 58–80%. Once the ripening process was finished (~30% weight loss), the hams were deboned and the *biceps femoris* extracted. Afterwards, muscles were sliced (1 mm thickness) and vacuum-packaged (HF100 in the upper film with 34.0 cm^3/m^2 permeability to O_2 at 23 °C and 85% HR; HF200 in the lower film with 18.0 cm^3/m^2 permeability to O_2 at 23 °C and 85% HR) (Mobepack company, Salamanca, Spain) using three different presentations: (i) intact format (IF) corresponding to non-sliced vacuum-packaged dry-cured ham, (ii) conventional-sliced format (CSF) corresponding to dry-cured ham slices stretched out in the package and (iii) alternative-sliced format (ASF) corresponding to dry-cured ham slices piled horizontally. A total of 104 packages (90 g) were obtained and half of the packages were subjected to HHP treatment at the CENTA Institute (Monells, Girona, Spain). Packages of dry-cured ham were pressurized at 600 Mpa for 6 min in a bath set at 12 °C (NC Hyperbaric Wave 6500/120; 120 l and 6500 bars). Subsequently, all batches (pressurized and control) were refrigerated (4 °C) under white fluorescent light (620 lux) following a light/dark cycle of 12 h for one month reproducing conditions during retail display.

2.2. Physico-chemical analysis

Proximate composition of Iberian dry-cured hams for IF and non-pressurized samples was (means ± standard deviation): pH 5.7 ± 0.03, moisture 48.04% ± 0.42 (AOAC, 1984), aw 0.9 ± 0.002, intramuscular fat (IMF) 12.8% ± 1.64 (Folch, Lees, & Sloane Stanley, 1957), chloride content 3.5% ± 1.04 (AOAC, 1984), protein 37.4% ± 0.56 (Kjeldahl, ISO, 1978) and myoglobin 1.8% ± 0.41 (Hornsey, 1956).

2.3. Colour analysis

Instrumental colour (CIE L^*, a^*, b^*; CIE, 1976) was measured on the surface of all dry-cured hams using a Minolta chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ). All measurements were made in triplicate. Three colour indices were obtained: L^* (lightness), a^* (redness) and b^* (yellowness) values. Before each measurement the equipment was standardised against a white tile. D65 illuminant and 0° standard observer angle were used. Measurements were performed on the first, second and the last slice for the CSF and ASF batches. Analyses were carried out at 18 °C before the sensory analysis of the same samples. A numerical total colour difference (ΔE) between treated (t) and control (c) was calculated as:

$$\Delta E_{t-c} = [(L^*_t - L^*_c)^2 + (a^*_t - a^*_c)^2 + (b^*_t - b^*_c)^2]^{1/2}$$

2.4. Analysis of AAS and GGS using LC–ESI-MS

Standard AAS and GGS were synthesised in vitro from N-acetyl-L-lysine and N-acetyl-L-ornithine using lysyl oxidase activity from egg shell membrane as described by Akagawa et al. (2006). AAS and GGS were analysed in Iberian dry-cured ham samples following a derivatisation procedure and a LC–ESI-MS technique described by Akagawa et al. (2006) and Estévez et al. (2009), respectively. Samples were cut, minced and subsequently homogenized 1:10 (w/v) in 10 mM phosphate buffer containing 0.6 M NaCl using an ultraturrax homogenizer for 30 s. Aliquots of 200 μL were dispensed in Eppendorf tubes and precipitated with 2 mL of 10% TCA and centrifuged at 670*g* for 30 min. The supernatants were removed and the resulting pellets treated with 2 mL of 5% TCA and subsequently centrifuged at 4200*g* for 5 min. Then, protein carbonyl groups were derivatized as follows: first, 500 μL of 250 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer containing 1% sodium dodecyl sulfate (SDS) and 1 mM diethylenetriaminepentaacetic acid (DTPA) were added to each sample. In addition, 500 μL of 250 mM MES buffer containing 50 mM of para-amino benzoic acid (ABA) and 500 μL 250 mM MES buffer containing 100 mM $NaCNBH_3$ were added. The mixture was incubated at 37 °C for 1 h and stirred regularly. Afterwards, the protein was again precipitated by addition of 500 μL of 50% TCA, and centrifuged for 10 min at 16,770*g*. The supernatants were removed and the pellets washed with 1 mL of 10% TCA and then washed twice with 1 mL of ethanol/diethyl ether 1:1 (v/v), shaken, and centrifuged for 5 min at 16,770*g*. Then, the precipitates were hydrolysed with 6 N HCl at 110 °C for 18 h. The protein hydrolysates were dried using a rotatory evaporator at 40 °C and the dried extracts were redissolved in 200 μL Milli-Q water. Samples (2 μL) were injected into an Agilent 1100 series HHPLC (Agilent Technologies, Palo Alto, CA, USA) equipped with a Luna reversed-phase (RP) column (5 μm C18 II column, 150 × 1.00 mm i.d. Phenomenex Torrance, CA, USA) eluted at a flow rate of 50 μL/min with isocratic water – 2.5% acetic acid (solvent A; 95%) and methanol – 2.5% acetic acid (solvent B; 5%). The column was operated at a constant temperature of 30 °C. MS analysis was carried out on an Esquire-LC quadrupole ion trap mass spectrometer equipped with an ESI interface (Bruker Daltonics, Bremen, Germany) and LC–MSD Trap software, version 5.2 (Bruker Daltonics). Capillary voltage was 3500 V, capillary exit offset 25 V, skimmer potential 15 V, and the trap drive value was 36. Conventional ESI-MS data were re-