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# Impact of high pressure treatment and intramuscular fat content on colour changes and protein and lipid oxidation in sliced and vacuum-packaged Iberian dry-cured ham

Verónica Fuentes, Mariana Utrera, Mario Estévez, Jesús Ventanas, Sonia Ventanas *

*Department of Animal Production and Food Science, Faculty of Veterinary Sciences, University of Extremadura, 10003 Cáceres, Spain*

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ABSTRACT

The effect of high hydrostatic pressure (HHP) (600 MPa) and intramuscular fat content (IMF) on colour parameters and oxidative stability of lipids and proteins in sliced vacuum-packaged Iberian dry-cured ham during refrigerated storage (120 days at 2 °C) was investigated. Several studies have investigated the influence of HHP on lipid oxidation of meat products. However, its effects on protein carbonylation, as also the influence of IMF content on this carbonylation are poorly understood. HHP treatment had a significant effect on lean lightness after 0 and 120 days of storage while IMF content increased lightness and yellowness over time. Regarding oxidative stability, the effect of HHP treatment depended on IMF content samples with a high IMF having greater lipid instability while samples with a low IMF underwent more protein carbonylation.



## 1. Introduction

Intramuscular fat content (IMF) plays a major role in consumer's acceptability of dry-cured products owing to its influence on the appearance, juiciness, and flavour of these muscle foods (Ventanas, Ventanas, & Ruiz, 2007). However, the influence of the application of innovative technologies (i.e. high-hydrostatic pressure, HHP), and subsequent storage on colour, flavour, and texture traits requires further investigation. Recent studies have related protein oxidation and colour changes in meat products (Fuentes, Ventanas, Morcuende, Estévez, & Ventanas, 2010; Utrera, Armenteros, Ventanas, Solano, & Estévez, 2012). The formation of carbonyl compounds is one of the most marked changes occurring during the oxidation of proteins (Estévez, 2011). Estévez, Ollilainen, and Heinonen (2009) applied a 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). In addition to the potential impact of protein carbonylation on particular meat quality traits, the formation of protein carbonyls is also responsible for a loss of nutritional value and impaired digestibility (Estévez, 2011).

In recent years, as a result of new consumer concerns, industry demands the use of preservation methods which increase the shelf life of manufactured foods ensuring food safety. Amongst these innovative technologies, vacuum packaging in combination with HHP, has been found to reduce microbial counts and prevent products from contamination (Hugas, Garriga, & Monfort, 2002). Nevertheless, intense HHP treatments (>400 MPa) induce undesirable changes in meat and meat products, modifying their texture and colour and increasing lipid oxidative reactions (Cheftel & Culioli, 1997). However, its impact on the oxidative degradation of myofibrillar proteins and the potential impact on particular traits are poorly understood. On the contrary, the impact of lipid oxidation on quality traits of meat products has been well studied (Gandemer, 2002). Overall, the IMF content in meat and meat products is closely related to lipid oxidation (Ventanas, Estévez, Andrés, & Ruiz, 2008). However, the effect of IMF content on protein carbonylation has been little studied. Furthermore, it is unknown, whether IMF content has an effect or not on the oxidative changes induced by HHP.

The aim of this study was to evaluate the combined effect of HHP treatment and IMF content on colour changes (external fat and lean) and oxidative stability (lipid oxidation and protein carbonylation) and their evolution during chilled storage and the implications of each factor on these traits in sliced and vacuum-packaged Iberian dry-cured ham.

* Corresponding author at: Facultad de Veterinaria (Food Technology). Avd/Universidad s.n., 10003 Cáceres, Spain. Tel.: +34 927257100x51390; fax: +34 927257110.
*E-mail address:* sanvenca@unex.es (S. Ventanas).

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## 2. Materials and methods

### 2.1. Sampling, packaging and pressure treatment

A total of 18 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) and slaughtered at 160 kg live weight and 12 months of age (Campo, DOP Dehesa de Extremadura). Green hams were processed as described by Fuentes et al. (2010). Once the ripening process was finished (~30% weight loss), the hams were deboned and two different sections (Flank and Hip) varying in IMF content were extracted. Afterwards, muscles were sliced (1 mm thickness) and vacuum-packaged (polyamide and polyethylene in the upper film with 34.0 $cm^3/m^2$ permeability to $O_2$ at 23 °C and 85% relative humidity (HR), polyamide and polyethylene 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). Approximately 108 packages (54 of flank samples and 54 of hip samples) containing 100 g of sliced Iberian dry-cured ham were obtained. Half of the packages of each group were subjected to HHP treatment at the CENTA Institute (Monells, Girona, Spain). Packages of sliced dry-cured ham were pressurised 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 vacuum packages of Iberian dry-cured ham slices, namely, (i) HHPHip (samples subjected to HHP with high IMF content), (ii) HHPFlank (samples subjected to HHP with low IMF content), (iii) CHip (non-pressurised samples with high IMF content), (iiii) CFlank (non-pressurised samples with low IMF content) were stored in darkness at 2 ± 1 °C. The samples of each group were opened for subsequent analysis after 0, 30 and 120 days of storage.

### 2.2. Physico-chemical analyses

The proximate composition of sliced Iberian dry-cured hams was determined as follows: moisture content, protein content and chloride content were determined using official methods (AOAC, 2000). The Folch method (Folch, Lees, & Sloane Stanley, 1957) was employed for determining IMF. A total of 12 replicates per ham section were performed.

### 2.3. Instrumental colour measurements

Instrumental colour (CIE L*, a*, b*; CIE, 1976) was measured on the surface and external fat of sliced dry-cured hams using a Minolta chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ). The following colour coordinates were determined: lightness (L*), redness (a*, red ± green) and yellowness (b*, yellow ± blue). Before each measurement the equipment was standardised against a white tile. D 65 illuminant and 0° standard observer angle were used. Analyses were carried out at 18 °C on randomly selected slices (within each group of samples) at 0, 30 and 120 days of storage. All measurements were made in triplicate on the surface of the external fat and the lean of vacuum-packaged slices. The packages were opened prior to colour measurements.

### 2.4. TBARS analysis

Thiobarbituric acid-reactive substances (TBARS) were assessed as described by Salih, Smith, Price, and Dawson (1987). The standard curve was prepared using a 1,1,3,3-tetraethoxypropane (TEP) solution (0.2268 g) in 3.86% perchloric acid. TBARS are expressed as mg of malondialdehyde (MDA) per $kg^{-1}$ of sample.

### 2.5. Volatile aldehydes analysis

Volatile aldehydes were analysed by headspace-SPME and GC/MS (gas chromatograph Hewlett–Packard 5890 series II coupled to a mass selective detector Hewlett–Packard HHP-5791A). One gramme of minced sample was weighed into a 4 mL vial. All vials were closed with a teflon/silicone septum. An SPME fibre (50/30 μm divinylbenzene–carboxen–polydimethylsiloxane coating) was inserted through the septum and exposed to the headspace of the vial. Vials were preconditioned for 15 min at 37 °C. Extraction was carried out at 37 °C for 30 min in a water bath. After extraction, the SPME fibre was immediately transferred to the injector of the chromatograph which was in splitless mode at 270 °C. The separation of volatile compounds was performed on a 5% phenyl–methyl silicone (HHP-5) bonded phase fused silica capillary column (Hewlett–Packard, 50 m × 0.32 mm i.d., film thickness 1.05 lm), operating at 6 psi of column head pressure. Oven programme was: 40 °C for 10 min, 5 °C $min^{-1}$ to 200 °C, 15 °C $min^{-1}$ to 250 °C, and held at 250 °C for 10 min. The transfer line to the mass spectrometer was maintained at 270 °C. The mass spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1756 V; data was collected at 1 scan $s^{-1}$ over the m/z range 30–500. Volatile compounds such as pentanal, hexanal, heptanal, nonanal, octanal, and 1-octen-3-ol as indicators of lipid oxidation, and 2-methylbutanal, and 3-methylbutanal compounds as the main branched aldehydes derived from Strecker degradation reactions of amino acids were measured. All volatile compounds were tentatively identified by comparing their mass spectrum and linear retention index with that of the standards. Results are given in area units (AU). Ten repetitions per group of samples were performed.

### 2.6. Protein oxidation measurements

The protein oxidation markers, α-aminoadipic and γ-glutamic semialdehydes (AAS and GGS, respectively) were analysed using the method described by Utrera, Morcuende, Rodríguez-Carpena, and Estévez (2011) by derivatisation with p-amino benzoic acid (ABA) and analysis by HPLC. 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 Estévez et al. (2009). A Shimadzu 'Prominence' HPLC (Shimadzu Corporation, Kyoto, Japan), equipped with a quaternary solvent delivery system (LC-20AD), a DGU-20AS on-line degasser, a SIL-20A auto-sampler, a RF-10A XL fluorescence detector, and a CBM-20A system controller, was used. An aliquot (1 μL) from the reconstituted protein hydrolysates was injected and analysed in the above HPLC equipment. AAS-ABA and GGS-ABA were eluted in a Cosmosil 5C18-AR-II RP-HPLC column (5 μm, 150 × 4.6 mm) equipped with a guard column (10 × 4.6 mm) packaged with the same material. The flow rate was kept at 1 mL/min and the temperature of the column was maintained at 30 °C. The eluate was monitored with excitation and emission wavelengths set at 283 and 350 nm, respectively. Standards (0.1 μL) were run and analysed under the same conditions. Identification of both derivatised semialdehydes in the FLD (fluorescence detector) chromatograms was carried out by comparing their retention times with those of the standard compounds. The peaks corresponding to AAS-ABA and GGS-ABA were manually integrated from FLD chromatograms and the resulting areas plotted against an ABA standard curve with known concentrations that ranged from 0.1 to 0.5 mM. Results are expressed as nmol of carbonyl compound per mg of protein.

### 2.7. Data analysis

The effects of HHP treatment and IMF content on colour, lipid and protein oxidation during chilled storage of sliced and vacuum-packaged dry-cured ham were analysed by two-way ANOVA with HHP treatment (treated and control) and IMF content (Flank and Hip) as main factors. To analyse the effect of storage time a one-way ANOVA was performed. The Tukey test was used at the 5% level to make comparisons between sample means when pertinent. All statistical analyses were conducted using the software SPSS (v 15.0) for windows.