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Multiple spectroscopic approach to elucidate water distribution and water–protein interactions in dry-cured ham after high pressure processing

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ABSTRACT

High Pressure Processing (HPP) influences water–protein interactions inducing sensory changes in dry-cured ham such as an increase in hardness and paleness. The effect of three pressure levels (200, 400 and 600 MPa applied for 5 min) on the water distribution and the protein arrangement of dry-cured ham differing in raw meat pH on *Semimembranosus* muscle 24 h after slaughtering (pH_{24SM}) was studied using nuclear magnetic resonance relaxometry (NMR), time domain reflectometry (TDR) and multispectral imaging (MI). HPP induced a reallocation of the water populations and a new arrangement of the proteins, particularly between 200 and 400 MPa treatments. The pH of the dry-cured ham affected the distribution of intrinsic water populations, even though samples were similarly affected when subjected to HPP.

1. Introduction

High Pressure Processing (HPP) is currently being used in industry to eliminate pathogenic microorganisms (especially *Listeria monocytogenes*), to extend product shelf-life and to improve the safety of commercial processed meat products (Aymerich et al., 2008; Bajovic et al., 2012). Pressure levels applied for the pasteurization of meats and meat products range from 400 to 600 MPa with short processing times of 3–7 min at room temperature (Cheftel and Culioli, 1997). Nevertheless, HPP can influence meat protein conformation and induce protein denaturation, aggregation or gelation, which can affect the appearance and the quality of the products. The means whereby HHP treatment exerts effects on meat protein structure are through the rupture of non-covalent interactions within protein molecules, and a subsequent re-formation of intra- and inter-molecular bonds within or among protein molecules (Cheftel and Culioli, 1997; Sun and Holley, 2010). Pressurization also causes a loosening of the meat protein matrix, alterations in the water distribution in meat (Bertram et al., 2006) and can also modify non-covalent interactions between muscle protein and sodium ions or water molecules in cured products (Picouet et al., 2012).

Different spectroscopic technologies may provide information regarding the effects produced on dry-cured ham by HPP. Proton nuclear magnetic resonance (NMR) relaxometry provides direct information about the compartmentalization and mobility of water in meat and has been widely applied in meat science (Bertram and Andersen, 2004). NMR relaxometry has been used in meat to determine fat and water contents (Sørland et al., 2004), study the mechanisms determining the development in sensory attributes during pork cooking (Bertram et al., 2005), evaluate water migration and water-binding within the meat pork matrix upon salting (Bertram et al., 2008; McDonnell et al., 2013), evaluate water properties during cooking of pork (Bertram et al., 2004), predict water holding capacity in pork (Bertram et al., 2002) and study changes in distribution and mobility of water as a result of high pressure processing of sausages (Møller et al., 2011). Other technologies such as microwave spectrometry and time domain reflectometry (TDR), by means of the determination of the dielectric properties of biological tissues, can also provide information about the linking state of water and salt content. Microwave

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dielectric spectra has been used to determine the presence of added water in pork products (Kent et al., 2002), to control pork salting process (Castro-Giráldez et al., 2010a) and to identify pork quality (Castro-Giráldez et al., 2010b). Dielectric TDR has also been used to develop models for fast estimation of water and salt content in dry-cured ham (Fulladosa et al., 2013) and the effect of temperature, HPP and freezing/thawing on the TDR response have been studied (Rubio-Celorio et al., 2015). Structural changes caused by HPP may also be elucidated using multispectral imaging since light reflection at different wavelenghts depends not only on the composition but also on the structure of the protein matrix (Hughes et al., 2014). This technology is useful when evaluating changes in meat color during storage (Christiansen et al., 2012) and the color of minced cured restructured ham subjected to HPP (Bak et al., 2012).

Currently, countries like United States, Japan, Canada and Australia applied the policy of 'zero tolerance' in relation to the presence of *L. monocytogenes* in dry-cured ham (Hereu et al., 2011). Since dry-cured ham is a non-cooked meat product, HPP is a recommended technology to obtain products meeting this requirement while having a minimal sensory impact. During the last decade, the effect of HPP on color (Andrés et al., 2004; Cava et al., 2009; Fuentes et al., 2014), volatile compounds (Rivas-Cañedo et al., 2009), protein and lipid oxidation (Fuentes et al., 2010), saltiness perception (Fulladosa et al., 2009; Serra et al., 2007) and texture and microstructure (Garcia-Gil et al., 2014) have been studied. Nevertheless, the interaction between the raw meat pH postmortem after 24 h (pH_{24SM}) and the pressure level used has not been determined. The *Semimembranosus* muscle pH_{24SM} has a large impact on the quality and technological parameters of both fresh meat and processed meat products as pH influences the three-dimensional structure of proteins (Anfinsen, 1973) and thus the allocation and mobility of water (Bertram et al., 2003). This results in changes in the structural conditions of muscles and influences the texture of dry-cured ham products (Ruiz-Ramírez et al., 2006).

The main objective of this work was to elucidate the impact of HPP processing (200, 400 and 600 MPa for 5 min) and of raw material (low and high pH_{24SM}) on the intrinsic water populations and water–protein interactions in dry-cured ham. For this purpose a multiple spectroscopic approach including NMR relaxometry, dielectric TDR, and multispectral imaging, combined with physicochemical analyses (water, salt content and water loss determination) was applied.

2. Materials and methods

2.1. Sampling and HPP treatments

In experiment 1, the effect of the pressure level was first determined in 10 dry-cured hams with similar pH_{24SM} value (5.63–5.76) were obtained from a commercial dry-cured ham producer (Pernils Llémena S.A., Sant Esteve de Llémena, Spain). In experiment 2, 20 additional dry-cured hams were grouped into two classes according to their pH_{24SM}, namely 10 hams with low pH_{24SM} (5.42–5.54) and 10 hams with high pH_{24SM} (6.09–6.40). In both experiments the dry-cured hams came from animals which were crosses of Large White and Landrace breeds elaborated using a traditional procedure (Arnau et al., 2001) until reaching a similar weight loss (~30%). Hams from experiment 1 and 2 originated from different batches. As several factors can influence the analyzed parameters, results from the different experiments are not comparable.

In all cases, *Biceps femoris* (BF) muscle was excised (Fig. 1) and vacuum packaged in plastic bags of multilayer polyamide/polyethylene (oxygen permeability of 50 cc/m^2/24 h at 23 °C and water permeability of 2.6 g/m^2/24 h at 23 °C and 85% RH, Sacoliva® S.L., Spain) for one month to reach homogenous salt and water content throughout the muscle. Sampling was performed as specified in Fig. 1. In brief, four 40 mm thick slices *a* were obtained from the central part of the dissected muscle for NMR, TDR and MI analyses. Between them, 3 mm thick slices *b* were taken for water loss analyses. The two edges *c* of the muscle were minced together and used for salt and water as described in section 2.2. All samples were individually vacuum packed. For both experiments, one slice *a* and one slice *b* were kept as a control (non-pressurized) and the other slices were pressurized at 200, 400 or 600 MPa for 5 min with water at 10 °C as pressure-transmitting medium and an initial sample temperature of 20 °C (Hyperbaric Wave 6500/120, N.C. Hyperbaric, S.A., Burgos, Spain). Pressurization rate was 220 MPa/min and the time for decompression was ≤10 s. To minimize the position effect of the slice within the muscle, the different slices obtained were subjected to the different high pressure treatment randomly. All measurements described below were performed 7 days after pressurization.

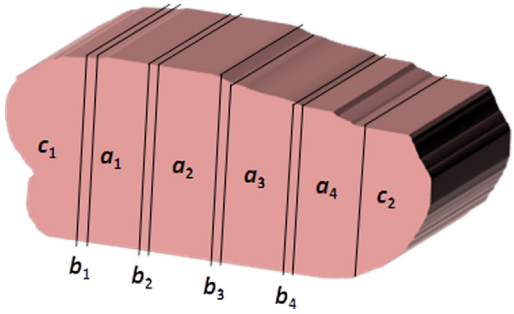

Fig. 1. Sampling procedure of *Biceps Femoris* in dry-cured hams.

2.2. Physicochemical analyses

NaCl content with an analytical standard deviation of 0.05% was determined according to ISO 1841-2 (1996) using a potentiometric titrator 785 DMP Titrino (Metrohm AG, Herisau, Switzerland). Water content with an analytical standard deviation of 0.25% was determined by drying at 103 °C ± 2 °C until reaching constant weight (AOAC, 1990). The pH of the dry-cured ham at the end of the elaboration process was directly measured on the *B. femoris* muscle with a pH penetration electrode (Crison 52-32) and a portable pH meter (Crison PH 25, Crison Instruments, SA, Alella, Spain) with an analytical standard error of 0.05. Water loss determinations were carried out following the procedure used by Picouet et al. (2012). In brief, squares of 10 × 40 mm^2 from 3 mm thick slices were weighed and then centrifuged at 17,400 g for 1 h at 4 °C (Beckman J2-MC, Beckman Instruments Inc., Palo Alto, California). Supernatants were decanted and the ham pieces were weighed again. Supernatant volume was expressed as the sample weight loss percentage. All analyses were performed in duplicate.

2.3. NMR relaxation measurements

Proton NMR T2 relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyzer (Resonance Instruments, Witney, UK) operating at 23.2 MHz and equipped with an 18 mm variable temperature probe, applying a CPMG sequence (Carr and Purcell, 1954; Meiboom and Gill, 1958). Samples (approx. 40 mm long and 10 mm in diameter) were analyzed in triplicate at 25 °C. The T2 relaxation data were analyzed using distributed exponential fitting analysis according to the regularization algorithm by Butler et al. (1981). Distributed exponential fitting analysis was carried out