Innovative Food Science and Emerging Technologies 22 (2014) 63–69

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

High pressure induces changes in texture and microstructure of muscles in dry-cured hams

Núria Garcia-Gil [a,1], Eva Santos-Garcés [a,1], Elena Fulladosa [a], Janine Laverse [b,c], Matteo Alessandro Del Nobile [b,c], Pere Gou [a,*]

[a] IRTA, XaRTA, Food Technology, Finca Camps i Armet, E-17121 Monells, Spain
[b] Department of Food Science, University of Foggia, Via Napoli 25, 71100 Foggia, Italy
[c] Istituto per la Ricerca e le Applicazioni Biotecnologiche per la Sicurezza e la Valorizzazione dei Prodotti Tipici e di Qualità, Università degli Studi di Foggia, BIOAGROMED, Via Napoli 52, 71100 Foggia, Italy

ARTICLE INFO

Article history:
Received 7 October 2013
Accepted 9 January 2014
Available online 22 January 2014

Editor Proof Receive Date 10 February 2014

Keywords:
High pressure processing
Dry-cured ham
Texture
Microstructure
X-ray microcomputed tomography
Scanning electron microscopy

ABSTRACT

The objective of this paper was to study the changes in the microstructure of *Semimembranosus* and *Biceps femoris* muscles of dry-cured hams induced by high pressure (HP) processing and their relationship with the changes in texture. The HP treatment (500 MPa) decreased softness (Y_{90}) in both muscles. Both X-ray microcomputed tomography (μCT) and scanning electron microscopy (SEM) analysis showed differences in microstructure between muscles. Some μCT geometric parameters were negatively (structure thickness of fatty objects, structure separation of lean tissue) and positively (surface/volume and fragmentation index of fatty objects) correlated with hardness, but no one was significantly modified by HP treatment. SEM analysis showed that pressurization had a higher effect on BF muscle than on SM muscle.

Industrial relevance: In ham industry, high pressure (HP) processing is used to achieve the microbial safety of hams and to improve texture, especially in those hams with a reduced salt content. The data presented in this study contributes to the understanding of HP effect on textural traits at microstructural level.

1. Introduction

Water content and proteolysis level are the main determinants of texture in dry-cured ham lean tissue (Ruiz-Ramírez, Arnau, Serra, & Gou, 2006; Serra, Ruiz-Ramírez, Arnau, & Gou, 2005). Several studies have demonstrated that high proteolysis level can result in soft and/or pasty texture (García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 1999; Parolari, Virgili, & Schivazappa, 1994; Virgili, Parolari, Schivazappa, Bordini, & Borri, 1995). This effect is related with the fact that proteolysis causes disruption of the meat proteins (Sárraga, Gil, Arnau, Monfort, & Cussó, 1989). Larrea et al. (2007) demonstrated that proteolysis during dry-cured ham processing occurs at microstructural level and affects especially myofibrillar proteins.

Since NaCl is a powerful inhibitor for most of the proteinases involved in the proteolytic breakdown of meat proteins (Rico, Toldrà, & Flores, 1990; Sárraga et al., 1989), the incidence of hams with excessive softness and pastiness increases when NaCl is reduced (Arnau, Guerrero, & Sárraga, 1998). High fat content hinders salting and drying during processing (Frøystein, Sörheim, Berg, & Dalen, 1989; Garcia-Gil et al., 2012; Ruiz-Cabrera, Gou, Foucat, Renou, & Daudin, 2004; Sánchez, Albarracin, Grau, Ricolfe, & Barat, 2008), which could affect the texture.

The muscles that constitute dry-cured ham show different water, salt and fat contents and proteolysis level (Boadas, Gou, Valero, & Arnau, 2001; Ruiz, Ventanas, Cava, Timón, & García, 1998). The most important and representative muscles in dry-cured ham are the *Biceps femoris* (BF) and the *Semimembranosus* (SM) muscles. Usually, BF muscle shows higher water content, intramuscular fat content and proteolysis than SM muscle (Boadas et al., 2001; Candek-Potokar, Monin, & Zlender, 2002; Ruiz-Ramírez, Arnau, Serra, & Gou, 2005; Ruiz-Ramírez et al., 2006). Therefore, the major incidence of softness and/or pastiness defects in BF muscle with respect to SM muscle can be attributed either to its higher proteolysis level or to its higher moisture content.

High pressure (HP) treatments are currently being used to eliminate pathogenic microorganisms (especially *Listeria monocytogenes*) and extend the shelf-life (Aymerich, Picouet, & Monfort, 2008; Jiménez-Colmenero & Borderías, 2003). Cheftel and Culioli (1997) reviewed many studies dealing with the specific effects of HP on muscle and meat products. One of the most widely described effect is the denaturation of the myofibrillar proteins which leads to changes in meat texture (Bouton, Harris, Macfarlane, & O'Shea, 1978; Hatae, Yoshimatsu, & Matsumotto, 1984; Kim, Lee, Lee, Kim, & Yamamoto, 2007; Macfarlane, McKenzie, & Turner, 1986; Macfarlane, McKenzie, Turner, & Jones, 1980; Picouet et al., 2012; Serra et al., 2007; Sun & Holley, 2010; Suzuki, Watanabe, Iwamura, Ikeuchi, & Saito, 1990). The HP treatment increases hardness, gumminess and fibrousness and decreases adhesiveness and pastiness in dry-cured hams with 30% weight loss

* Corresponding author. Tel.: +34 972630052; fax: +34 972630980.
E-mail address: pere.gou@irta.cat (P. Gou).
[1] Both authors contributed equally to this work.

1466-8564/$ – see front matter
http://dx.doi.org/10.1016/j.ifset.2014.01.004

(Fulladosa, Serra, Gou, & Arnau, 2009), but the effect is lower when HP treatment is applied to dry-cured hams which have reached 50% weight loss (Fulladosa, Sala, Gou, Garriga, & Arnau, 2012). The degree of the myofibrillar protein denaturation varies according to pressure, processing time, temperature, and pH (Huppertz, Fox, & Kelly, 2004). The sensory changes increased when pressure level increases (Macfarlane et al., 1986; Suzuki et al., 1990). Various studies conducted in meat have demonstrated that hardness increases at pressure over 300 MPa due to alterations in water distribution (Bertram, Wu, Straadt, Aagaard, & Aaslyng, 2006; Picouet et al., 2012). Therefore, sensory changes due to HP treatment depend on the product composition as well as the HP level applied to the product. Despite the effect on texture, the studies on microstructure of dry-cured hams produced by HP processing are limited. Thus, the objective of this paper was to study the changes in the microstructure of *Biceps femoris* and *Semimembranosus* muscles of dry-cured hams induced by HP treatment, by using microcomputed tomography and scanning electron microscopy, and their relationship with the changes in texture.

2. Material and methods

2.1. Ham processing and sampling

Six raw hams were obtained from a commercial slaughterhouse at 24 h *post-mortem*. The hams were cured with a mixture consisting of 0.15 g KNO_3, 0.15 g $NaNO_2$, 1.0 g dextrose, 0.5 g sodium ascorbate, and 10 g of fine NaCl per kg of ham applied to the surface by rubbing. In order to obtain hams showing softness defect, a reduced salting procedure was conducted by covering the hams with coarse NaCl for 0.4 days/kg of raw ham. After salting, the hams were washed with water at 12 °C to remove the excess of salt from the surface and hung in a cold room at 3–5 °C and 80–90% relative humidity during 60 days. Afterwards, the temperature was gradually raised (from 10 to 20 °C) to accelerate the drying process and develop the characteristic sensory properties of dry-cured hams. When hams achieved a weight loss on average of 34%, 2 consecutive slices 15 mm thick were cut from each ham at 100 mm from the aitch bone in the distal direction (Fig. 1). The slices were individually vacuum-packed and stored at 3 ± 1 °C. One of the slices from each ham was assigned as control and the other one was subjected to high-pressure (HP) treatment.

2.2. HP treatment

The HP treatment was performed in a NC Hyperbaric WAVE 6000/120 equipment (NC Hyperbaric, Burgos, Spain) located at IRTA centre of Monells (Spain). The pressure applied was 500 MPa (pressurization time: 3 min; pressure holding time: 7 min; pressure release time: nearly instantaneous (<2 s), initial temperature of the pressurization water: 7 °C) and the initial temperature of the samples was 3 °C. *Semimembranosus* (SM) and *Biceps femoris* (BF) muscles from each slice (control and HP treated) were sampled for microcomputed tomography (μCT), instrumental texture and scanning electron microscopy (SEM) analyses.

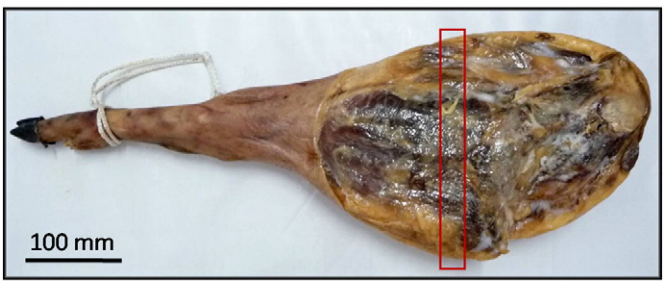

Fig. 1. The scheme shows the location where the slices were obtained (at 100 mm from the aitch bone in the distal direction, at the widest part of the ham).

2.3. X-ray microcomputed tomography (μCT)

μCT analysis was performed as described by Santos-Garcés et al. (2013). Two specimens per muscle (BF and SM) and treatment (control and HP) were accurately carved with a scalpel into cubes of $15 \times 15 \times 15$ mm^3 (n = 48). Specimens were wrapped with parafilm (PARAFILM®) to avoid drying and imaged at 20 ± 2 °C in a Skyscan 1172 high-resolution desktop X-ray microcomputed tomography equipment (Skyscan 2005, Skyscan N.V., Vluchtenburgstraat, Aartselaar, Belgium). Power settings were 100 kVp and 100 μA. The transmission of the conical X-ray beam through the sample was recorded with a CCD camera with 2000 × 1048 pixels. The pixel size was 17.13 μm. Four-frame averaging, a rotation step of 0.60°, and an exposure time of 1475 ms were used, covering a view of 180°. Scan time, on average, required 37 min. During acquisition, an aluminium filter was used to reduce the beam hardening artefact. The images acquired from each specimen were reconstructed using the Skyscan NRecon reconstruction software and a set of 2D cross-section images was obtained. NRecon software was also used to correct noise, beam hardening and ring artefact. Three-dimensional (3D) reconstructions from each set of 2D cross-section images were created by effectively stacking 146 slice images with a slice spacing of 0.069 mm.

For image processing and analysis, Skyscan software CTAn was used. A 10×10 mm^2 region of interest (ROI) was selected from the centre of the scanned slice in view and then copied to all the slices in our volume of interest (VOI). The original grey-scale cross-sectional images were converted into binary images (black and white) by an automatic threshold (Sahoo, Soltani, Wong, & Chen, 1988).

Prior to 3D reconstruction, a component-labelling algorithm available within CTAn was used to isolate the largest 3D connected structures. All reconstructions were created using an adaptive rendering (locality 10 and tolerance 0.25). The following geometric parameters for each constituent (intramuscular fat and muscular tissues) were measured using the CTAn software: (i) the percent object volume (POV) which is the percentage of volume for each constituent present within the VOI of the sample; (ii) the object surface/volume ratio (OSVR) which is the surface area of all the objects of a constituent divided by the volume of these objects; it is a basic parameter used to characterise the complexity of the structures (i.e. the spatial distribution); (iii) the fragmentation index (FI), developed and defined by Hahn, Vogel, Pompesius-Kempa, and Delling (1992) as the index of the structural connectivity; it calculates the relative convexity or concavity of the surface of objects, based on the principle that concavity indicates connectivity and convexity indicates isolated disconnected structures (Lim & Barigou, 2004); (iv) the degree of anisotropy (DA) which measures the preferential alignment of the structures; (v) the structure thickness (ST) for a point in solid which was defined by Hildebrand and Ruegsegger (1997) as the diameter of the largest sphere which fulfils two conditions: the sphere encloses the point (but the point is not necessarily the centre of the sphere) and the sphere is entirely bound within the solid surfaces; (vi) the structure separation (SS) which is the thickness of the spaces between structures; and (vii) the structure model index (SMI) which estimates the shape of the structure (i.e. intramuscular fat) (0 = ideal flat, 3 = cylindrical and 4 = spherical). For each parameter, the average of the two specimens per sample was used for statistical analysis.

2.4. Instrumental texture

The same specimens used in the μCT analysis were unwrapped and subjected to instrumental texture analysis. A Stress Relaxation test was performed in a Texture Analyser (Zwick/Roell, testXpert II, V3.2, Copyright © 1996–2010, Zwick GmbH & Co. KG, Ulm, Germany) with