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Feasibility of X-ray microcomputed tomography for microstructure analysis and its relationship with hardness in non-acid lean fermented sausages

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ABSTRACT

X-ray microcomputed tomography (μCT) was used for microstructure analysis on four different types of non-acid pork lean fermented sausages, three of them supplemented with 5% pork back fat, sunflower oil or diacylglycerols (DAGs). The data from the μCT analysis were related to instrumental texture (hardness). Although μCT analysis identified fat particles and air holes, the technique was not accurate enough to distinguish between pork lean and fat when these constituents were emulsified. Only μCT geometrical parameters related to the meat matrix (emulsion of pork lean and fat) provided useful information on the microstructure of the product. Parameters such as percent object volume (POV), object surface/volume ratio (OSVR), degree of anisotropy (DA), structure thickness (ST) and number of objects (NO) were correlated with instrumental hardness.

1. Introduction

Health problems such as coronary heart diseases and obesity have been associated with high fat ingestion from meat products (Department of Health, 1994; Fernández-Ginés, Fernández-López, Sayas-Barberá, & Pérez-Alvarez, 2005; Higgs, 2000); consequently the reduction of fat content and the simultaneous addition of more healthy oil-based fat substitutes have been widely studied in different dry-cured meat products. Replacement of pork back fat by an oil substitute in the production of Chorizo de Pamplona yields an acceptable product from a sensory point of view. The use of around 25% of pre-emulsified olive oil (Muguerza, Gimeno, Ansorena, Bloukas, & Astiasarán, 2001) and pre-emulsified soy oil (Muguerza, Ansorena, & Astiasarán, 2003) in Chorizo de Pamplona has been proved to be suitable alternatives. More recently, Mora-Gallego, Serra, Guàrdia, Miklos, Lametsch, and Arnau (2011) reported that sunflower oil is a promising ingredient to reduce the negative effects on sensory properties caused by the fat reduction in non-acid pork lean fermented sausages, such as the increase of hardness or the reduction of tenderness and juiciness. Recent studies have also focused on the use of diacylglycerols (DAGs) as an innovative fat substitute strategy for achieving more healthy products since DAGs have been reported to result in a lower fat accumulation in the human body compared to TAGs (Flickinger & Matsuo, 2003). Besides, sausages containing this substitute had beneficial effects on the technological characteristics of fat reduced products and showed a satisfactory overall sensory quality (Miklos, Xu, & Lametsch, 2011; Mora-Gallego et al., 2011), thus representing an improvement in both technological and health aspects of meat products.

The reduction of fat content or its replacement by fat substitutes modifies some product characteristics (Wirth, 1988). One of these characteristics is the internal microstructure, which determines the desirable properties of the products (i.e. physical, textural and sensory). Understanding how the reduction of fat content and the use of different types of fat influence the final microstructure, as well as the texture properties, could help to improve the formulation of fat reduced minced meat products.

Microstructure can be studied by X-ray microcomputed tomography (μCT). This technology is based on the differences in X-ray attenuation values produced by differences in the density of the constituents within a sample, giving a reconstruction of the internal microstructure of the scanned samples (Kerckhofs, Schrooten, Van Cleynenbreugel, Lomov, & Wevers, 2008). The best μCT images are obtained from objects in which microstructure coincides with a contrast in X-ray absorption of the sample constituent tissues. In the case of meat products, the main meat constituents (lean and fat) can be distinguished using μCT because of their different densities and therefore, their different attenuations (Jørgensen, 1998; Kalender, 2005). The accuracy of μCT to determine fat content and its spatial distribution in salami has been previously demonstrated by Frisullo, Laverse, Marino, and Del Nobile (2009) but

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the μCT parameters were not related to texture. Moreover, no studies focused on minced dry-cured meat products elaborated with fat substitutes have been found.

The objective of this study was to evaluate the applicability of μCT for the microstructure analysis of non-acid pork lean fermented sausages elaborated with the addition of 5% of different types of fat, and their relationship with hardness.

2. Material and methods

2.1. Product formulation and elaboration process

The samples used in this study were part of samples obtained from the study done by Mora-Gallego et al. (2011). Three batches of non-acid pork lean fermented sausages were manufactured using pork lean ham (95%) and different types of fat (5%) (pork back fat, sunflower oil or DAGs). A further additional batch was elaborated using pork lean with no added fat. The pork lean had an average water content of $70.7 \pm 0.1\%$.

The pork lean was trimmed of fat and minced at Ø 8 mm. For each batch, 1 kg of the pork lean was mixed in a bowl chopper machine (with 0.6 kg of back fat or oils in batches with added fat) until a paste was formed and was subsequently mixed with 10.4 kg of additional lean meat. All mixtures were minced at Ø 3 mm. The following additives per kilogramme of pork lean were added to the mixtures: NaCl 20 g, black pepper 1.50 g, lactose 20 g, potassium lactate (78% purity) 20 g, sodium ascorbate 0.5 g, sodium nitrite 0.15 g and potassium nitrate 0.15 g. The meat paste was stuffed into Ø 50 mm Fibran casings, immersed in a bath of water and mould (*Penicillium candidum*) and then hung up to dry for two months with an increasing temperature from 3 °C to 18 °C and a decreasing relative humidity from 90% to 70%. The sausages were periodically weighed until reaching an estimated final water content referred to defatted dry matter of 55%. This estimated final water content was calculated with the average initial water content of the pork lean, the percentage of added fat and the weight losses. Four sausages per batch were packaged in polyamide-polyethylene bags (Sacoliva, Sabadell, Spain) in a modified atmosphere (80% N_2: 20% CO_2) and stored at 3 °C for one month.

2.2. Sample preparation

From each fermented sausage, four 15 mm thick slices were obtained. From each slice, a specimen was accurately carved with a scalpel into a cube of $15 \times 15 \times 15$ mm^3.

2.3. X-ray microcomputed tomography analysis

All the specimens ($n = 64$) were wrapped in parafilm (PARAFILM ® M) to avoid moisture loss and were imaged at 20 °C ± 2 °C using the Skyscan 1172 high-resolution desktop X-ray microcomputed tomography system (Skyscan 2005, Skyscan N.V., Vluchtenburgstraat, Aartselaar, Belgium). Power settings were 100 kVp and 100 μA. A CCD camera with 2000×1048 pixels was used to record the transmission of the conical X-ray beam through the sample. 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°. During acquisition, an aluminium filter was used to reduce the beam hardening artefact. Scan time, on average, required 37 min. A set of 2D flat cross-section images was obtained for each sample after tomographical reconstruction by Skyscan NRecon reconstruction software. Noise, beam hardening and ring artefact corrections of NRecon software were applied. Three-dimensional (3D) reconstructions of the specimens were created by effectively stacking all the two-dimensional tomographs, a total of 146 slice images with a slice spacing of 0.069 mm.

2.4. Image processing and analysis

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). Due to the fact that the samples consisted of three constituents (fat, meat matrix and air holes) two different segmentations were carried out, obtaining a binary image for each constituent.

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 (fat, meat matrix or air holes) 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; (vii) the number of objects (NO) which reports the total number of discrete objects within the VOI.

2.5. Instrumental texture

A Texture Analyser (Zwick/Roell, testXpert II, V3.2, Copyright © 1996–2010, Zwick GmbH & Co.KG, Ulm, Germany) with a 10 kN load cell and a 60 mm diameter compression plate was used. Following the μCT analysis, the specimens were unwrapped and compressed to 75% of their total height, at a crosshead speed of 1 mm/s and at ambient temperature (20 °C ± 2 °C). Hardness (N), defined as the maximum peak force during the compression (Bourne, 1978), was recorded.

2.6. Chemical analysis

After the texture analysis, the specimens were minced and homogenized. Water content was analyzed by drying at 103 °C ± 2 °C until a reaching constant weight (AOAC, 1990). The total fat content was measured by Soxtec extraction (SoxCap 2047 and Soxtec 2055) according to ISO 1443 (1973). Analyses were performed in triplicate.

2.7. Statistical analysis

Analyses of variance were done using the General Linear Model (GLM) procedure of the statistical SAS package (SAS, 2001). The average of the specimens obtained for each sausage was used for the analyses of variance. The type of added fat was included in the model as a fixed effect. Pearson correlation coefficients between μCT parameters of the individual specimens and hardness were calculated using the CORR procedure of SAS package.