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Effect of prefreezing hams on endogenous enzyme activity during the processing of Iberian dry-cured hams

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ABSTRACT

The use of frozen/thawed raw material in the processing of Iberian dry-cured ham has been studied to determine its effect on the sensory quality of the final product. The proteolysis and lipolysis processes were measured by the proteolytic and lipolytic enzyme activities and free amino acids and free fatty acids. The thawed Iberian hams had lower salt contents throughout the process. The use of thawing raw material did not affect the proteolytic enzymes, cathepsins, aminopeptidases and dipeptidylpeptidases, only the activity of dipeptidylpeptidase III was reduced due to thawing. Moreover, there were no differences in the content of free amino acids between fresh and thawed hams during the whole process. However, the use of thawing hams affected the lipolytic activity. The activity of phospholipase and neutral lipase were significantly higher in the thawed hams and also the content of free fatty acids, at all the stages analyzed. Consumer sensory analysis showed thawed Iberian hams had the lowest hardness, probably due to an intense proteolysis. The acceptability of the Iberian hams was similar between fresh and thawed hams.

1. Introduction

Dry-cured ham processing is generally very long and it is responsible for the final quality of the product (Toldrá & Flores, 1998). The process consists of different stages; salting, where the salt containing nitrite and nitrate is rubbed onto the surface and hams are maintained at refrigeration temperatures; postsalting, where the hams are cleaned of salt and kept at refrigeration temperatures for salt equalization or distribution; and finally, drying/ripening, where the temperature of the chambers is progressively increased while the relative humidity is decreased to dry the hams and develop the flavor.

In the processing of dry-cured ham, fresh hams are used as the raw material but recently the use of frozen hams has allowed producers to store products, homogenise batches, control transport and market prices (Arnau, 1998).

The freezing and thawing of hams produces numerous changes in the meat structure and it is necessary to reduce salting time since salt penetrates faster (Barat, Grau, Pagán-Moreno, & Fito, 2004; Flores, 1989; Poma, 1989). However, freezing did not affect sensory quality (Bañón, Cayuela, Granados, & Garrido, 1999; Motilva, Toldrá, Nadal, & Flores, 1994,1993) except for an increase in the number of tyrosine crystals that can affect consumer acceptance (Arnau, Gou, & Guerrero, 1994). In addition, Bañón, Gil, Granados, and Garrido (1998) indicated that the use of prefreezing accentuates the proteolytic and exudative nature of the meat during the curing process.

In comparison to industrial genotype pigs, the Iberian breed is characterized by high intramuscular fat levels (>8%) and high percentage of oleic acid (Cava, Estevez, Ruiz, & Morcuende, 2003) and different muscle enzyme activities (Toldrá, Flores, Aristoy, Virgili, & Parolari, 1996). Both fatty acid composition and enzyme activity are responsible for the development of the characteristic flavor. In addition, the prolonged dry-cured processing of Iberian hams over 18–24 month may also be important to the sensory characteristics of the final product.

In 2008, Grau, Albarracín, Toldrá, Antequera, and Barat, fund that Iberian raw material is less affected by the freezing/thawing process than hams from industrial genotype pigs. However, it is necessary to determine how the proteolytic and lipolytic endogenous systems of Iberian hams are affected by the prefreezing treatment in order to elucidate its effect on the sensory properties of the final product.

Due to the absence of information about the use of frozen raw material in the Iberian industry the objective of the present study was to determine the effect of prefreezing and thawing on the proteolytic and lipolytic systems of Iberian hams and its effect on the

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generation of free fatty acids and amino acids, and finally, to determine the effect of freezing and thawing on the sensory properties of Iberian dry-cured hams.

2. Materials and methods

2.1. Samples

Thirty-six Iberian fresh hams with an average weight of 11.6 ± 0.3 kg were selected from the same batch as described by Grau, Albarracín, Toldrá, Antequera, and Barat (2008). Eighteen hams were manufactured using traditional fresh ham salting. The remaining 18 hams were frozen in an industrial freezer at −40 °C and stored for at least 30 days at −20 °C they were then thawed at 3 °C for 4 days and submitted to the salting process.

Salting and postsalting were carried out under usual Iberian ham manufacture practices as described by Grau et al. (2008). Salting was performed in piles by covering the hams with solid salt and left in the chamber at 3 °C; for fresh hams the time taken was 1.1 day/kg of ham while in the case of thawed Iberian hams the time was 0.78 day/kg. The postsalting stage was carried out at 4 °C and the relative humidity was progressively reduced to 75% during 60 days (Grau et al. 2008). Finally the hams were ripened for a total time of 23 months.

In both treatments, six hams were taken at the end of the salting, postsalting stage and finally after 23 months of ripening. All hams were sampled by removing approximately 100 g of muscle *Biceps femoris* which was immediately frozen at −80 °C for chemical, enzyme activity, free amino acid and free fatty acid analyses.

For sensory analysis, three dry-cured hams from both the fresh and thawed hams were selected. Each ham, once removed from the vacuum package, was cleaned and sliced. The sample was presented as a 3 g roll prepared from the slice of the ham containing the muscles *Biceps femoris, Semitendinosus* and *Semimembranosus*. Then, two rolls were placed in a petri dish and allowed to reach room temperature for 30 min before panellists presentation.

2.2. Chemical analyses

Sodium chloride was determined in a chloride analyzer while moisture was performed by oven drying to constant weight as described by Grau et al. (2008).

2.3. Assay of enzyme activities

Biceps femoris muscle (5 g) were homogenised in 25 ml of 50 mM sodium citrate buffer containing 1 mM EDTA and 0.2% (v/v) Triton X-100 at pH 5.0 (for cathepsins and lipases) or 4 g of muscle in 20 ml of 50 mM disodium phosphate buffer, pH 7.5, containing 5 mM EGTA (for peptidases and lipases). In both cases, the extracts were homogenised (3 × 10 s at 27.000 rpm while cooled on ice) with a polytron (Kinematica, Switzerland), centrifuged at 12,000*g* for 20 min at 4 °C and the supernatants filtered through glass wool and used for enzyme assays.

Cathepsins B and B + L were assayed as described by Toldrá and Etherington (1988) using N-CBZ-L-arginyl 7-amido (4-methyl)coumarin (AMC) at pH 6.0, N-CBZ-L-phenylalanyl-L-arginine-AMC at pH 6.0 as substrates, respectively.

Muscle aminopeptidase and dipeptidylpeptidase activities were measured by fluorometric assays using 7-amido-4-methyl coumarin derivatives as substrates (AMC) (Sigma Chemical Co., St. Louis, MO and Bachem, Switzerland) (Toldrá & Flores, 2000). Alanine-AMC was used for the measurement of alanyl aminopeptidase (AAP) and methionyl aminopeptidase (MAP) (Flores, Marina, & Toldrá, 2000), arginine-AMC for arginyl aminopeptidase (RAP), leucine-AMC for leucyl aminopeptidase (LAP), and pyroglutamic-AMC for pyroglutamyl aminopeptidase (PGAP). Dipeptidyl peptidases (DPP) I, II, III, and IV were assayed as previously described by Sentandreu and Toldrá (2001). DPP I was measured using Gly-Arg-AMC; DPP II: Lys-Ala-AMC; DPP III: Arg-Arg-AMC; and DPP IV: Gly-Pro-AMC.

Lipases were assayed using 4-methylumbelliferyl oleate as substrate (Martin, Ruiz, Flores, & Toldrá, 2006).

Then 50 μl of each enzyme extract were added to 250 μ l of the respective substrate solution. In all cases, the reaction was incubated at 37 °C and the fluorescence continuously monitored at 355 nm and 460 nm as excitation and emission wavelengths, respectively, for AMC derivatives using a Fluoroskan Ascent fluorimeter (Thermo Electron Co., Finland). Four replicates were measured for each experimental point. One unit of enzyme activity was defined as the amount of enzyme hydrolysing 1 μmol of substrate per minute (cathepsin, aminopeptidases and dipeptidylpeptidases) or per hour (lipase) at 37 °C.

2.4. Analysis of free amino acids

Samples for free amino acid analysis were extracted and deproteinized following the method described by Aristoy and Toldrá (1991). Ham samples (*Biceps femoris* muscle) were homogenised with 0.01 M HCl (dilution 1:5) in a Stomacher for 8 min at 4 °C and centrifuged in the cold at 10,000*g* for 20 min. Supernatant was filtered through glass wool and stored at −20 °C until use. Thawed samples (100 μl) plus 50 μl of an internal standard (10 mM norleucine) were deproteinized with 400 μl of acetonitrile. The supernatant was derivatized to its phenylthiocarbamyl derivatives according to the method of Bidlingmeyer, Cohen, Tarvin, and Frost (1987). Derivatized amino acids were analyzed in an Agilent 1100 liquid chromatograph equipped with a diode array detector (Agilent, Palo Alto, CA, USA). The column used was a Nova-Pack C-18 (3.9 × 300 mm) (Waters Co., MA, USA). The separation was achieved in a gradient between two solvents, 70 mM sodium acetate, pH 6.55 ± 0.05 containing 5% acetonitrile (solvent A), and acetonitrile–water–methanol, 45:40:15 v/v (solvent B). The gradient between 100% of solvent A and 60% of solvent B were as described in Flores, Aristoy, Spanier, and Toldrá (1997). Twenty minutes of column equilibration with 100% of solvent A before and 15 min of 100% of solvent B for washing the column, after each injection were achieved. Flow rate was 1 mL/min and column temperature was maintained at 52 °C. Separated amino acids were detected at 254 nm.

2.5. Free fatty acid analysis

Lipids were extracted from ham samples (*Biceps femoris* muscle) with methylene chloride/methanol (2:1) according to the method of Folch, Lees, and Sloane Stanlye (1957). The free fatty acid composition was determined as described by Needs, Ford, Owen, Tuckley, and Anderson (1983) by means of gas chromatography of the corresponding methyl esters. The analysis was carried out in a Fisons 816 gas chromatograph equipped with a split injector, a flame ionisation detector in a DB-225 capillary column (J&W Scientific, Barcelona, Spain), 30 m in length, with an internal diameter of 0.25 mm and 0.25 μm film thickness, as described by Navarro, Nadal, Izquierdo, and Flores (1997). The individual fatty acids were identified by comparing their retention times with those of standard fatty acids. The internal standard used was C20:0.

2.6. Sensory analysis

A consumer sensory test (n = 100) was performed on Iberian dry-cured hams. All the panellists evaluated all the samples. The