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Analysis of raw hams using SELDI-TOF-MS to predict the final quality of dry-cured hams

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ABSTRACT

The relationship between protein profiles of *Gluteus medius* (GM) muscles of raw hams obtained from 4 pure breed pigs (Duroc, Large White, Landrace, and Piétrain) with the final quality of the *Semimembranosus* and *Biceps femoris* muscles of dry-cured hams was investigated. As expected, Duroc hams showed higher levels of marbling and intramuscular fat content than the other breeds. Piétrain hams were the leanest and most conformed, and presented the lowest salt content in dry-cured hams. Even if differences in the quality traits (colour, water activity, texture, composition, intramuscular fat, and marbling) of dry-cured hams were observed among the studied breeds, only small differences in the sensory attributes were detected. Surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF-MS) was used to obtain the soluble protein profiles of GM muscles. Some associations between protein peaks obtained with SELDI-TOF-MS and quality traits, mainly colour (b*) and texture (F_0, Y_2, Y_{90}) were observed. Candidate protein markers for the quality of processed dry-cured hams were identified.

1. Introduction

The quality of dry-cured ham is affected by raw ham characteristics and the biochemical changes occurring during processing. Research efforts to study the influence of raw ham attributes on the quality of dry-cured hams have focused on meat quality traits (Arnau, Gou, & Guerrero, 1994; Maggi, Bracchi, & Nardelli, 1987) and the composition and content of fat (Antequera et al., 1992; Ruiz-Carrascal, Ventanas, Cava, Andrés, & García, 2000), that are susceptible to being affected by genotype among other factors (Čandek-Potokar, Monin, & Zlender, 2002; Oliver et al., 1994; Plastow et al., 2005).

Dry-cured hams show a high variability, a detrimental factor for product quality and a major concern for the industry. Thus, it is essential to provide methods to facilitate the assurance, control, and optimization of product quality. Recent high throughput proteomic approaches can assist research towards this goal.

Surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF-MS) combines chromatographic techniques and mass spectral measurements by using special chromatographic-like probe surfaces (protein chip arrays). It combines chromatographic separation and mass spectral measurement. The SELDI chip contains chromatographic coatings of selected type (i.e. hydrophobic, ion-exchange, metal-binding, etc.), that bind protein molecules with complementary physicochemical properties on their surface (O'Gorman et al., 2006). Unbound compounds are washed off, thus contaminants are removed and sample complexity is reduced. After application of a proper energy-absorbing matrix, proteins bound to stationary phase are analysed for MS profiling (Bodzon-Kulakowska et al., 2007). SELDI-TOF-MS proteomic approach can identify protein expression patterns or single protein markers in muscle tissue.

Because it is not necessary to know the identities of the proteins for the purpose of differential classification, this technology is a suitable approach to identify multiple potential markers (Mach et al., 2010).

Identification of protein markers in raw hams able to predict the quality of dry-cured hams would help the industry to select raw material of appropriate quality to reduce costs and improve the overall quality of dry-cured ham. In a previous work, Mach et al. (2010) detected potential protein markers from GM muscle that could be used to classify raw hams by breed type (Duroc, Large White, Landrace, and Piétrain). The animals from Mach et al. (2010) were used in the present study to produce dry-cured hams with the objective to assess the differences between breeds on dry-cured ham quality. Besides, the work also aimed to investigate the relationships between protein fingerprinting in GM muscle of raw hams and the final quality of dry-cured hams.

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2. Materials and methods

2.1. Animals and carcass measurements

One hundred and twenty entire male pigs from four pure breeds were studied. Duroc (DU, n = 21), Landrace (LR, n = 43), Large White (LW, n = 43) and Piétrain (PI, n = 13) pigs were fattened under identical conditions in the Pig Testing Station (IRTA-CAP) in Monells (Girona, Spain). The four pig genetic types (DU, LR, LW, PI) were reared under the same conditions of housing, environment and feeding, and the ante mortem handling was performed under low stress conditions. Therefore, the experiment design allowed the comparison between breeds with minimum interference from external influences.

The animals were weighed the day before slaughter. The average body weight (kg) was 117.5 ± 9.8 for DU, 116.2 ± 11.2 for LR, 118.5 ± 10.2 for LW, and 103.4 ± 11.9 for PI. The pigs were fasted on-farm for 9 h and transported for 1.5 h to a commercial slaughterhouse in Vic (Spain). Animals from different pens were not mixed. The animals from different breeds were slaughtered alternately on two different days (slaughtering batch) using CO_2 stunning at 90% of concentration for 2 min.

The subcutaneous backfat and loin thickness at 6 cm of the midline between the third and fourth last ribs were predicted using the Autofom ultrasonic automatic carcass grading probe (Carometec A/S, Herlev, Denmark). Minimum fat depth at the level of *Gluteus medius* (subcutaneous fat GM) was measured over the muscle using a ruler. Then, left sides from each carcass were commercially cut and all primal cuts were weighed. The average ham weight (kg) was 13.11 ± 1.03 for DU, 12.55 ± 1.13 for LR, 12.61 ± 1.00 for LW, and 12.75 ± 1.23 for PI.

2.2. Dry-cured ham processing and sampling

Hams were processed according to the specifications of the quality system of the Serrano Ham (European Commission, 1998), as a Traditional Speciality Guaranteed (European Commission, 2006). The salting of hams was carried out at 48 h post-mortem. Hams were weighed and measured (length, width, thickness) before processing. Hams were purged for blood residues and then pre-salted with 36.3 g/kg ham of a mixture of dextrose (5 g), sodium nitrite (0.5 g), potassium nitrate (0.3 g), sodium ascorbate (0.5 g), fine salt (15 g), and coarse salt (15 g). After 4 days the hams were manually salted with 20 g fine salt and 16.5 g coarse salt per kg of ham and allowed to rest for 9 days at 3 ± 2 °C. After washing with cold water, the hams were hung at 3 ± 2 °C and a relative humidity of 75–80% for 2 months. During drying, the temperature and the relative humidity were gradually increased up to 25 °C and decreased to 60%, respectively. Hams were weighed periodically, until 35% of weight losses were obtained. Processing time, final weight and weight losses were recorded after processing.

Dry-cured hams of each breed were boned and sampled according to Sánchez-Molinero and Arnau (2010). Samples for physical, chemical and sensory analysis were vacuum packed and stored at 4 °C until analysis. Samples for chemical analysis were homogenised, vacuum packed and kept in darkness at −20 °C until analysis.

2.3. Physical measurements

Colour measurements were carried out with a colorimeter Minolta Chroma Meter CR-200 (illuminant D65, 2° standard observer and the specular component included) in the CIELAB space: lightness (L*), redness (a*) and yellowness (b*). Colour measurements of *Semimembranosus* (SM) and *Biceps femoris* (BF) muscles were carried out on the slice surface, and averaged over five zones.

Texture was assessed using the Stress Relaxation (SR) test. Five specimens per sample (BF and SM muscles) were accurately carved with a scalpel into parallelepipeds of 20 mm × 20 mm × 15 mm. The specimens were wrapped in plastic film to avoid drying and stored for 24 h at 4 °C. The SR test was performed using a Universal Texture Analyser TA.XT2 (Stable Microsystems Ltd., Surrey, UK) with a 25 kg load cell and a 50 mm diameter compression plate. The specimens were compressed to 25% of their original height, perpendicular to the fibre bundle direction at a crosshead speed of 1 mm/s. The force versus time after the compression was recorded at a speed of 50 points per second during 90 s (relaxation time). The relaxation curves obtained for each specimen were normalised, i.e., the force decay Y(t) was calculated as follows:

$$Y(t) = \frac{F_0 - F(t)}{F_0}$$

where F_0 (kg) is the initial force and F(t) is the force recorded after t seconds of relaxation. The force decay at 2 s (Y_2) and 90 s (Y_{90}) was calculated (Morales, Guerrero, Serra, & Gou, 2007). The average of the five specimens per sample was used for statistical analysis.

2.4. Chemical analysis

The pH was measured on minced SM and BF muscles with a pH penetration electrode (Crison 52-32) on a portable pH-meter (Crison pH 25, Crison Instruments, SA, Alella, Spain). Water activity (a_w) measurement of SM and BF muscles was carried out at 25 °C with a Novasina AW SPRINT — TH 500 instrument (Axair Ltd., Pfäffikon, Switzerland) that allows temperature control during a_w measurement. Intramuscular fat and protein were measured in BF muscle by near infrared transmittance spectroscopy FoodScan® (FOSS Electric A/S, Denmark). Water content was determined in BF and SM muscles by drying the samples at 103 ± 2 °C until a constant weight was achieved (AOAC, 1990). Chloride content was measured in BF and SM muscles with a potentiometric titrator (785 P Titrino, Metrohm Ltd., Herisau, Switzerland) by using a standard silver nitrate titrant (0.1 M) according to ISO (1996). Results were expressed as percentage of NaCl on a dry-matter basis.

2.5. Quantitative descriptive analysis (QDA)

Quantitative descriptive analysis was carried out to assess the appearance, texture and flavour of dry-cured hams. Seven trained assessors (ASTM, 1981; ISO, 1993, 1994) undertook the sensory analysis on slices of dry-cured ham obtained as described by Sánchez-Molinero and Arnau (2010). The generation of the descriptors was carried out in open discussion during two previous sessions. The descriptors retained for visual, flavour and texture assessment are described in Table 1. A non-structured scoring scale (Amerine, Pangborn, & Roessler, 1965) was used, where 0 meant absence of the descriptor and 10 meant high intensity of the descriptor.

Sensory evaluation was undertaken in 25 sessions. Five samples per session were analysed in 20 sessions and 4 samples in the other 5 sessions. During each session at least three samples from different breeds and a maximum of two samples per breed were analysed. Samples were coded with three-random numbers and were presented to the assessors balancing the first-order and the carry-over effects according to Macfie, Bratchell, Greenhoff, and Vallis (1989) when possible. The average score of the seven experts for each sample was recorded and used in the statistical analysis.

2.6. Preparation of protein extracts for SELDI-TOF analyses

After 24 h of carcass chilling, a sample of *Gluteus medius* (GM) muscle was removed from each animal, frozen in liquid nitrogen, and stored at −80 °C until protein extraction.

Muscle samples were weighed (30 to 50 mg), placed in 1.5 mL of lysis buffer [10 mM Tris–HCl, pH 7.25, 10 mM KCl, 2% (vol/vol) Triton X-100, 1 mM PMSF], and homogenised (Ultraturrax T25, IKA Labortechnik,