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 on 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 on 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.

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 be 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, Keuning, Kruijt, Hortós, Arnau & te Pas, 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 of the relationships between protein fingerprinting in GM muscle of raw hams and the final quality of dry-cured hams.

Materials and methods

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 during 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 in 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.

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 (5g), sodium nitrite (0.5g), potassium nitrate (0.3g), sodium ascorbate (0.5g), fine salt (15g), and coarse salt (15g). After 4 days the hams were manually salted with 20g fine salt and 16.5 g coarse salt per kg of ham and allowed to rest for 9 days at 3±2ºC. After washing with cold water, the hams were hung at 3±2ºC and a relative humidity of 75-80% for 2 months. During drying, the temperature and the relative humidity were gradually increased up to 25ºC 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 4ºC until analysis. Samples for chemical analysis were homogenised, vacuum packed and kept in darkness at -20ºC until analysis.

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 x 20 mm x 15 mm. The specimens were wrapped in plastic film to avoid drying and stored for 24h at 4ºC. 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 normalized, 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}) were calculated (Morales, Guerrero, Serra & Gou, 2007). The average of the five specimens per sample was used for statistical analysis.

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 25ºC 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 ± 2ºC 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) following (ISO, 1996). Results were expressed as percentage of NaCl on a dry-matter basis.

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 et al. (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.

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 homogenized (Ultraturrax T25, IKA Labortechnik, Staufen, Germany) in ice to avoid mechanical heating of the samples. The resulting sample homogenates were centrifuged (20 min, 4°C, 12,000 × *g*) to remove insoluble debris. The supernatant was then analysed for total protein content using a commercial protein assay kit with BSA as standard (Bio-Rad, Veenendaal, The Netherlands).

SELDI-TOF-MS Analyses

For the SELDI-TOF analyses, all samples were analyzed in duplicate. The strong anion exchanger (Q10), weak cation exchanger (CM10), and immobilized metal affinity capture (IMAC30) protein arrays and binding buffer combinations were prepared according to the manufacturer's instructions (Bio-Rad Laboratories Inc., Hercules, CA). The Q10, CM10 and IMAC30 protein arrays were especially selected because they produce good quality proteome patterns with an optimal numbers of peaks (te Pas, Jansen, Broekman, Reimert & Heuven, 2009). The different protein arrays were equilibrated with the respective binding buffers containing 0.1% Triton. The binding/ washing buffer for the Q10 contained 0.1 M sodium acetate (pH 6.0), and that for the CM10 contained 0.1 M sodium acetate (pH 5.0). Before applying the samples to the IMAC30 protein array, the active spots of the array were preactivated with 100 μL of 0.1 M copper sulphate solution according to the manufacturer's instructions (Bio-Rad Laboratories Inc.). Twenty micrograms of protein were suspended in a 200-μL volume of binding buffer. Then 100 μL of sample was loaded to each well of the array and allowed to bind (60 min, room temperature, and on a platform shaker) to the array. After the binding step, the entire array was washed 3 times with the respective binding buffers (5 min, room temperature, with agitation) and then twice with deionised water. After briefly drying the arrays, 0.8 μL of a saturated solution of 4-hydroxy-3, 5-dimethoxy- cinnamic acid (sinapinic acid, Sigma, St. Louis, MO) dissolved in 50% (vol/vol) acetonitrile and 0.5% (vol/vol) trifluoracetic acid, was applied twice to each of the active spots of the array, and was allowed to thoroughly dry. The different protein arrays were then placed in the SELDI protein arrays Biology System Reader 4,000 (Bio-Rad Laboratories Inc.). The laser intensity was 3,000 nJ. The SELDI protein array spectra were further normalised and analysed as explained by Mach et al. (2010).

Statistical Analyses

Analysis of variance was carried out with the GLM procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA). The model for carcass measurements, physicochemical data, and sensory analysis included breed as fixed effect and slaughtering batch as a block effect. Animal weight was included as a covariable in

the model for carcass measurements. Differences were assessed using the Tukey test. The level of significance was set at $p<0.05$.

Pearson correlation analysis was used to investigate the relationship among dry-cured ham quality variables and protein peak profiles. Correlation coefficients were calculated with the CORR procedure of SAS 9.2 for each protein array. A multiple testing correction consisting of a modification of the effective number (Cheverud, 2001) was performed as suggested by Li & Ji (2005).

Regression models of the colour and texture parameters of dry-cured hams on peak intensities and ham characteristics (raw ham properties, processing yields and dry-cured ham characteristics) were fitted for each protein array (CM10, Q10, & IMAC30) with the REG procedure of SAS 9.2, using the stepwise regression method. Significant levels were set at $p=0.15$ to enter variables in the model and at $p= 0.05$ to be retained in the model.

Results and discussion

Influence of breed on quality characteristics

Table 2 shows dry-cured ham quality parameters presenting significant differences among breeds. As expected, PI carcasses showed the lowest subcutaneous fat thickness over the GM muscle. PI pigs have proved to show less fat depth and, accordingly, the highest muscular area and lean content in the carcass than other breeds (Plastow et al., 2005).

Hams from PI breed also showed significantly different conformation characteristics compared to the other studied breeds. PI hams were the shortest, but the thickest ($p<0.001$). Hams from PI also needed shorter processing times than other breeds in order to reach the weight losses fixed at 35%, being only significantly shorter than for LR hams (Table 2). The faster dehydration observed in PI hams seems to be related with the thinner subcutaneous fat cover and lower marbling observed over PI hams. As suggested by Guerrero, Gou, Alonso & Arnau (1996), the extend of water loss is related to the barrier effect of the fat on water diffusion and evaporation during processing.

Duroc hams showed higher values of intramuscular fat content (IMF) in BF ($p<0.001$). Similarly, Plastow et al. (2005) reported the highest values of IMF in Duroc lines compared with LR, LW and PI. Visual inspection of ham slices in SM, BF and ST muscles confirmed higher marbling in DU hams ($p<0.001$) in all studied muscles and the lowest scores for PI hams although these were not significantly different from LW. The highest marbling and IMF content of pure DU and DU sired pigs has been widely reported in the literature (Gou, Guerrero & Arnau, 1995; Oliver et al., 1994). Marbling and intramuscular fat has been accepted as a key quality trait for long aged dry-cured ham manufacturing.

DU hams tended to show higher L* values than other breeds, being only significantly higher in BF muscle ($p<0.001$). Similarly, Gou et al. (1995) also observed higher L* values for hams obtained from Duroc pigs, what has been partly related to high intramuscular fat levels in DU hams (McGloughlin, Allen, Tarrant, Joseph, Lynch & Hanrahan, 1988).

In relation to water activity values, PI hams showed higher values ($p<0.01$) than those from LR and LW breeds. This result could be related with the lower salt content ($p<0.01$) and the higher moisture ($p<0.001$) observed in PI compared to other breeds (Table 2). The lower levels of subcutaneous fat and IMF observed in PI pigs would explain the higher moisture observed at the fixed weight loss.

The degree of NaCl absorption into hams has been related with the thickness of the fat cover and the IMF content, being higher and faster as the fat levels are lower (Gou et al., 1995. Considering that PI hams were the leanest, other factors influencing salt absorption should be considered. The most plausible cause of the lower salt absorption in PI hams seems to be related to their higher conformation. In this sense, Gou et al. (1995) stated that heavily muscled hams such as PI, due to their higher thickness, presented a low salt content in BF muscle. Together with the lower salt absorption observed, these hams also resulted in higher sweetness scores ($p<0.01$) in the sensory analysis (Table 2).

Instrumental texture of dry-cured hams was measured by means of the Stress Relaxation (SR) test. The SR test has been successfully used to distinguish normal and soft texture in dry-cured hams (Morales et al., 2007). Hams from PI pigs showed the softest textures as reflected by the higher values of Y_{90} and lower values of F_0 in SM muscles. It has been reported that the salt level has an influence on texture

characteristics of dry-cured hams. Softness has been observed to increase with decreasing content of NaCl (Arnau, Guerrero & Sárraga, 1998; García-Garrido, Quiles-Zafra, Tapiador & Luque de Castro, 1999; García-Rey, García-Garrido, Quiles-Zafra, Tapiador & Luque de Castro, 2004) due to increased proteolitic activity. In this sense, the lower NaCl content reported in PI hams could be responsible in part for the lower F_0 and higher Y_{90} values observed. Morales et al. (2007) also reported a similar relationship between the NaCl content and SR parameters. Mechanical resistance has also been negatively correlated with the moisture content (Virgili, Parolari, Schivazappa, Soresi & Borri, 1995). Therefore, the higher moisture of PI hams could also be responsible for its softer texture. Even if some differences on the quality traits of dry-cured hams were observed among the different breeds, only small differences on the sensory attributes were detected (Table 2).

Relationship between proteome profiles and ham quality traits

The number of proteomic studies on dry-cured hams are limited and mainly focused on proteolytic changes produced during ham processing (Di Luccia et al., 2005; Mora, Sentandreu & Toldrá, 2011). Škrlep et al. (2011) studied the relationship between the sarcoplasmic protein profiles of dry-cured hams and soft texture. Other authors have studied the differences on protein expression between *Biceps femoris* and *Semimembranosus* muscles during dry-cured ham processing (Théron, Chevarin, Robert, Dutertre, Santé-Lhoutellier, 2009; Théron et al., 2011). However, to our knowledge, no studies relating the proteomic profiles of raw hams with the final quality characteristics of dry-cured hams have been done.

Tables 3-5 show significant correlations between quality parameters of dry-cured hams and protein peak profiles. Proteins significantly correlated with quality traits were in a range of 3,000 to 34,000 m/z ratio. Proteome profiles obtained with CM10 protein array presented more protein peaks showing associations with the final quality of dry-cured ham than the other arrays tested. Among the protein peaks detected with CM10 array, 11 peaks were correlated with b* value and instrumental texture of the hams ($p<0.005$). For Q10 array, we observed 7 protein peaks significantly correlated with b* values and

sensory parameters (metallic, sweet, and salty flavour; $p<0.005$). Finally, 5 protein peaks obtained with IMAC30 array were correlated with b* values ($p<0.009$). The quality traits of dry-cured ham associated with protein peaks obtained from raw hams were mainly colour and texture parameters. In spite of accounting for about 30% of total muscle protein, the role of sarcoplasmic proteins on functional properties of meat has received less attention compared to myofibrillar proteins (Miyaguchi, Nagayama & Tsutsumi, 2000). In this sense, some authors have highlighted the role of sarcoplasmic proteins on meat texture and colour parameters (Hwang, Park, Kim, Cho & Lee, 2005; Laville et al., 2007; Marcos, Kerry & Mullen, 2010; Sayd et al., 2006; te Pas et al., 2009). Moreover, Škrelep et al. (2011) observed that a number of sarcoplasmic proteins, mainly metabolic enzymes, were soluble in the myofibrillar fraction extracted from dry-cured hams. The authors suggested that the conditions during processing caused their denaturation and loss of solubility and therefore affecting texture.

To further confirm the implication of these protein peaks in the final quality of dry-cured hams, regression models for sensory and technological quality traits were obtained including peak intensities and other quality and processing parameters as independent variables (Table 6). For CM10 array, we obtained significant regression models for b* and instrumental texture parameters. It should be highlighted that peaks 6,158 and 12,223 m/z were retained in the models for both b^*_{SM} and b^*_{BF}. Similarly, all the models for texture parameters included peak 8,126 m/z. Mach et al. (2010) presented this protein peak as a potential protein marker for muscle type. The authors found that this protein was more over-represented in GM muscles. These relationships are interesting because texture is one of the main factors influencing consumer acceptability of dry-cured hams. No significant models were obtained with Q10 protein arrays. Finally, models for b* values were built with data obtained with IMAC30 protein arrays. Peaks 6,653 and 7,545 m/z were included in the model for both b^*_{SM} and b^*_{BF}. Apart from the reported protein peaks, other quality parameters included in the models for colour and texture were mainly moisture, pH_{24h}, and subcutaneous fat.

The obtained models relating muscle proteome of raw hams to the final quality of dry-cured hams are very promising. Identification and validation of these protein markers in other datasets considering other environmental and processing factors that contribute to the quality variability would be needed.

Conclusions

Although some quality differences were found among the studied pure breeds (DU, LR, LW, PI), only some small differences were detected in the sensory analysis at 35% weight losses.

Candidate soluble protein markers for the quality of dry-cured hams were obtained. The detection of these markers in the raw material would help to predict the final quality of hams and would provide us with a tool for raw material quality control and selection. However, further validation of the involvement of these proteins in the quality of dry-cured hams is needed before considering them as protein markers.

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Table 1. Definition of the sensory parameters used in the Quantitative Descriptive Analysis.

Parameter	Description
Visual[1]	
Marbling	Intermingling of fat with lean on the surface of the slice evaluated in the SM, BF and ST muscles.
Red rings	Visual assessment of the presence of colour rings due to a lack of nitrite in the core of the ham.
White film	Intensity of white colour that appears on the cut surface of the vacuum packaged product evaluated on 8 cm slices that were previously vacuum packaged and stored for 15 days at 2–4 °C.
Flavour[2]	
Metallic	Flavour similar to a solution of $FeSO_4 \bullet 7\ H_2O$.
Sweetness	Fundamental taste sensation elicited by sugar.
Saltiness	Fundamental taste sensation elicited by NaCl.
Umami	Fundamental taste sensation elicited by sodium glutamate.
Piquantness	Stinging sensation in the mouth and throat.
Bitterness	Fundamental taste sensation elicited by caffeine and L-tryptophan.
Aged	Pleasant flavour related to aged fat which is characteristic of long aged Spanish dry-cured ham partially skinned leaving the typical V shape.
Cured	Set of pleasant nuances characteristic of dry-cured meat products, not described by the other flavour attributes.
Texture[2]	
Adhesiveness	Degree to which the surface of the ham slice adheres to the palate when compressed with the tongue.
Hardness[3]	Amount of pressure required to completely compress the sample.
Chewiness	Facility to break ham into pieces in order to be swallowed.
Pastiness[3]	Feeling of paste detected in hams with a high proteolytic index.
Crumbliness[3]	Measures the ease with which a sample can be broken into smaller particles during mastication.
Fibrousness	Perception of muscle fibres during mastication.

[1]Visual inspection performed on ham slices. [2]Performed on BF muscle. [3]The references used to illustrate their maximum intensity were those described by and Arnau, Guàrdia, Guerrero & Claret (2011). BF: *Biceps femoris*, SM: *Semimembranosus*, ST: *Semitendinosus*.

Table 2. Quality parameters of green and dry-cured hams by breed.

Parameter	DU	LR	LW	PI	RMSE	Breed	Batch
Green hams							
Subcutaneous fat GM (mm)[1]	12.06^{a}	12.49^{a}	14.45^{a}	10.04^{b}	3.258	<0.001	NS
Length (cm)	57.45^{a}	56.83^{a}	57.11^{a}	54.05^{b}	1.902	<0.01	NS
Thickness (cm)	15.28^{b}	14.91^{b}	14.82^{b}	17.04^{a}	1.214	<0.001	NS
Dry-cured hams							
Processing time (days)	313.7^{ab}	329.1^{a}	313.4^{ab}	278.1^{b}	54.306	<0.05	<0.05
L^*_{ST}	42.55^{a}	40.97^{b}	41.32^{ab}	40.98^{ab}	2.117	<0.05	NS
L^*_{SM}	37.66^{a}	36.43^{ab}	36.20^{b}	37.08^{ab}	1.976	<0.05	<0.05
L^*_{BF}	41.86^{a}	39.96^{b}	39.61^{b}	39.46^{b}	1.643	<0.001	<0.05
a^*_{BF}	14.58^{ab}	15.53^{a}	15.71^{a}	15.03^{b}	1.351	<0.05	<0.05
$a_{w\ SM}$	0.913^{ab}	0.910^{b}	0.911^{b}	0.917^{a}	0.006	<0.01	<0.001
$a_{w\ BF}$	0.913^{ab}	0.911^{b}	0.912^{b}	0.919^{a}	0.006	<0.001	<0.001
$F_{0\ BF}$ (kg)	1.50^{b}	1.54^{ab}	1.94^{a}	1.28^{b}	0.699	<0.01	NS
$F_{0\ SM}$ (kg)	5.03^{ab}	5.37^{a}	5.29^{a}	3.61^{b}	1.583	<0.01	NS
$Y_{2\ SM}$	0.358^{b}	0.355^{b}	0.356^{b}	0.379^{a}	0.022	<0.01	<0.05
$Y_{90\ SM}$	0.629^{ab}	0.625^{b}	0.626^{b}	0.650^{a}	0.025	<0.05	<0.05
Protein $_{BF}$ (%)	28.67^{c}	29.47^{b}	30.13^{a}	28.69^{bc}	1.035	<0.001	NS
Moisture $_{BF}$ (%)	59.05^{b}	59.83^{b}	59.36^{b}	61.30^{a}	1.463	<0.001	<0.05
IMF $_{BF}$ (%)	2.56^{a}	1.39^{b}	1.21^{b}	0.99^{b}	0.693	<0.001	NS
MDDH (%)	63.77^{ab}	63.66^{b}	63.08^{b}	64.93^{a}	1.257	<0.001	<0.05
NaCl $_{BF}$ (%)	4.81^{a}	4.82^{a}	4.74^{a}	4.54^{b}	0.182	<0.001	<0.001
Marbling $_{SM}$	2.5^{a}	1.7^{b}	1.6^{bc}	1.1^{c}	0.672	<0.001	NS
Marbling $_{BF}$	3.7^{a}	3.0^{b}	2.6^{bc}	1.9^{c}	0.904	<0.001	NS
Marbling $_{ST}$	4.3^{a}	3.3^{b}	2.9^{bc}	2.0^{c}	1.112	<0.001	NS
Sweetness	1.5^{ab}	1.3^{b}	1.5^{b}	1.9^{a}	0.481	<0.01	NS
Cured flavour	2.7^{ab}	3.1^{a}	3.0^{ab}	2.3^{b}	0.870	<0.05	NS

Only quality variables showing significant differences among breeds ($p<0.05$) are presented. [1]minimum fat depth over the GM measured with a ruler. DU: Duroc, LR: Landrace, LW: Large White, PI: Piétrain. RMSE: root mean square error. Batch: slaughtering batch. L* (lightness), a* (redness): colour measurements with CIELAB scale. F_0, Y_2, Y_{90}: texture measurements with stress relaxation test. IMF: intramuscular fat. MDDH: moisture on defatted desalted ham (MDDH= moisture/ (100-IMF-NaCl)*100). GM: *Gluteus medius* ST: *Semitendinosus*, SM: *Semimembranosus*, BF: *Biceps femoris*.

Table 3. Correlation coefficients between quality parameters and protein peak intensities obtained with the CM10 protein chip array in *Gluteus medius* muscles.

	Peak (m/z)										
Parameter	3,098	4,338	4,525	6,158	6,651	7,095	8,126	8,464	9,811	10,268	12,223
b^*_{SM}	**0.255**	0.168	**-0.254**	**0.390**	**0.370**	**0.393**	**-0.266**	-0.021	**-0.267**	**0.324**	**-0.254**
p	**0.0048**	0.0656	**0.0050**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0031**	0.8157	**0.0031**	**0.0003**	**0.0049**
b^*_{BF}	**0.280**	0.148	-0.180	**0.347**	**0.342**	**0.395**	-0.185	0.048	-0.188	**0.332**	-0.186
p	**0.0019**	0.1048	0.0479	**<0.0001**	**0.0001**	**<0.0001**	0.0421	0.6001	0.0393	**0.0002**	0.0415
$Y_{2\ BF}$	0.145	**0.260**	0.123	-0.095	-0.012	0.007	0.132	**0.267**	0.038	-0.051	0.001
p	0.1117	**0.0040**	0.1786	0.2987	0.8944	0.9429	0.149	**0.0030**	0.6811	0.5786	0.9997
$F_{0\ SM}$	0.127	-0.139	-0.052	-0.068	0.064	0.067	-0.228	-0.131	-0.113	0.019	**-0.267**
p	0.1642	0.1285	0.5678	0.4597	0.4882	0.4637	0.0118	0.1505	0.2189	0.8314	**0.0027**
$Y_{2\ SM}$	-0.182	0.102	0.178	-0.030	-0.076	-0.156	**0.268**	0.103	0.188	-0.057	**0.271**
p	0.0456	0.2653	0.0511	0.7436	0.4058	0.0873	**0.0029**	0.2589	0.0388	0.5374	**0.0026**
$Y_{90\ SM}$	-0.155	0.174	0.203	-0.028	-0.086	-0.150	**0.311**	0.178	0.213	-0.028	**0.303**
p	0.0893	0.0565	0.0256	0.7634	0.3492	0.1009	**0.0005**	0.0501	0.019	0.7626	**0.0007**

Only quality variables and protein peaks showing significant correlations are shown. Significant correlations considering a multiple testing correction (Cheverud, 2001) are marked in bold ($p<0.005$). m/z: mass-to-charge ratio. b* (yellowness): colour measurements with CIELAB scale. F_0, Y_2, Y_{90}: texture measurements with stress relaxation test. SM: *Semimembranosus*, BF: *Biceps femoris.*

Table 4. Correlation coefficients between quality parameters and protein peak intensities obtained with the Q10 protein chip array in *Gluteus medius*.

	Peak (m/z)							
Parameter	3,817	4,507	5,147	6,554	9,311	16,526	22,290	33,689
b^*_{SM}	0.141	0.182	**0.261**	0.183	0.152	0.050	0.062	0.065
p	0.1298	0.0509	**0.0046**	0.0486	0.1031	0.5962	0.5081	0.4901
Metallic flavour[1]	-0.191	-0.180	-0.253	-0.257	-0.161	**-0.302**	-0.152	-0.139
p	0.0401	0.0534	0.0062	0.0053	0.0836	**0.001**	0.1038	0.1371
Sweetness[1]	0.072	0.084	0.078	**0.260**	**0.287**	0.223	**0.266**	**0.266**
p	0.4432	0.3700	0.4048	**0.0048**	**0.0018**	0.0163	**0.0039**	**0.0039**
Saltiness[1]	**0.268**	**0.304**	0.216	**0.265**	0.198	-0.167	0.038	0.048
p	**0.0036**	**0.0009**	0.0196	**0.0041**	0.0335	0.0731	0.6845	0.6062

Only quality variables and protein peaks showing significant correlations are shown. Significant correlations considering a multiple testing correction (Cheverud, 2001) are marked in bold ($p<0.005$). m/z: mass-to-charge ratio. b* (yellowness): colour measurements with CIELAB scale. [1]sensory analysis.SM: *Semimembranosus.*

Table 5. Correlation coefficients between quality parameters and protein peak intensities obtained with the IMAC30 protein chip array in *Gluteus medius*.

	Peak (m/z)				
Parameter	4,468	4,987	6,653	7,545	14,847
b^*_{SM}	**0.274**	**0.373**	**0.373**	**-0.329**	**-0.327**
p	**0.0024**	**<0.0001**	**<0.0001**	**0.0002**	**0.0003**
b^*_{BF}	**0.321**	**0.327**	**0.331**	**-0.252**	-0.236
p	**0.0003**	**0.0003**	**0.0002**	**0.0052**	0.0091

Only quality variables and protein peaks showing significant correlations are shown. Significant correlations considering a multiple testing correction (Cheverud, 2001) are marked in bold ($p<0.009$). m/z: mass-to-charge ratio. b* (yellowness): colour measurements with CIELAB scale. SM: *Semimembranosus*, BF: *Biceps femoris.*

Table 6. Lineal models for colour and texture parameters of dry-cured hams.

Protein array	Parameter	r^2	RMSE	SD	Variables included in the model	
					peaks (m/z)	quality parameters
CM10	b^*_{SM}	0.382	0.961	1.160	4,525 6,158 12,223	Moisture $_{SM}$ Ham thickness
	b^*_{BF}	0.3126	0.932	1.030	6,158 12,223	Processing time pH_{24h}
	$F_{0\ SM}$	0.523	1.160	1.649	8,126	Ham thickness Processing time Moisture $_{SM}$
	$Y_{2\ SM}$	0.352	0.019	0.023	8,126	Subcutaneous backfat[1] pH_{24h} Processing time
	$Y_{90\ SM}$	0.424	0.020	0.026	8,126	Subcutaneous backfat[1] pH_{24h} Processing time
IMAC30	b^*_{SM}	0.260	1.006	1.160	6,653 7,545	Moisture $_{SM}$
	b^*_{BF}	0.300	0.880	0.729	6,653 7,545	pH_{24h} Processing time

[1] measured with autofom. m/z: mass-to-charge ratio. r^2: coefficient of determination. RMSE: root mean square error. b* (yellowness): colour measurements with CIELAB scale. F_0, Y_2, Y_{90}: texture measurements with stress relaxation test. SM: *Semimembranosus*, BF: *Biceps femoris*