Surface-Enhanced Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry: a Fast Method to Assess Pork Quality

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Abstract— **The objective of this study was to evaluate the potential of proteomic markers to predict the quality of pork loin. With this purpose 120 pigs from 4 different pure breeds (Duroc, Landrace, Large White and Piétrain) were sampled. After processing, the technological and sensory characteristics of cooked loins were assessed. Surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF MS) with three different ProteinChip arrays (CM10, Q10, IMAC30) was used to obtain the proteomic profiles of water soluble proteins. This study showed some associations between protein peaks obtained with SELDI-TOF-MS and quality traits.**

Keywords— **SELDI-TOF MS, pork quality, proteomic markers**

I. INTRODUCTION

From a consumer point of view, meat quality is defined as the eating quality or palatability of meat. This concept covers quality attributes such as texture, mainly tenderness, meat colour, water holding capacity (WHC), and flavour. Variation in meat quality is a detrimental factor for meat acceptability and causes appreciable economic losses for meat producers. Thus, it is essential for the meat industry to have methods to facilitate the assurance, control, and optimization of product quality. The application of proteome platforms can assist research toward this goal [1].

Surface-enhanced laser desorption/ ionisation time-of-flight mass spectrometry (SELDI-TOF-MS) uses special chromatographic-like probe surfaces (ProteinChip arrays) to bind proteins with complimentary physicochemical properties [2]. 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.), on which sample components of a given type are captured. Unbound compounds are washed off, thus contaminants are removed and sample complexity is markedly reduced. After application of a proper energy-absorbing matrix proteins bound to stationary phase are analysed for MS profiling [3]. A proteomic approach using SELDI-TOF-MS can identify protein expression patterns or single protein biomarkers in muscle tissue. Because it is not necessary to know the identities of the proteins for the purpose of differential classification, this technology provides an alternative platform for the differential display of multiple potential biomarkers [4].

The objective of this study was to evaluate the potential of proteomic markers to predict the quality of cooked pork loin.

II. MATERIALS AND METHODS

A. Animals

One hundred twenty pure breed entire male pigs were studied: Duroc (n=21), Landrace (n=43), Large White (n=43) and Piétrain (n=13). The average body weight at slaughter was 117.5 ± 2.9.8 for DU, 116.2 ± 11.17 for LS, 118.5 ± 10.2 for LW, and 103.4 ± 2.98 for PI. Animals were slaughtered individually using CO_2 stunning at 90% of concentration for 2 min. Animals from different breeds were slaughtered alternatively. Carcass quality measurements were performed as explained below.

B. Carcass measurements

Fat and muscle depth were recorded for each carcass within 1 h *post mortem* using the Fat-O-Meat'er probe (SFK Technology, Denmark). Back fat

and loin muscle depths were measured at 60 mm from the mid-line between the 3rd and 4th last ribs. Carcass lean percentage was predicted using the Spanish official equation [5]. Then, left sides from each carcass were commercially cut and all primal cuts were weighed to obtain cut yields [6].

C. Meat quality measurements

Meat quality was assessed on the left side of each carcass. After dissection, the weight of *Longissimus thoracis* muscle was determined.

Muscle pH was measured at 24 h *post mortem* (pHu) using a Crison portable meter equipped with a xerolyt electrode.

Instrumental colour measurements were recorded at 24 h *post mortem* for L, a*, and b* on the exposed cut surface of the *Longissimus thoracis* muscle at the last rib level, using a Minolta Chromameter (CR-400, Minolta Inc., Japan) in the CIELAB space using illuminant C and 2°.

Drip losses were determined at 24 h *post mortem*, following the reference method supported by OECD [7] to assess the water holding capacity (WHC).

At 24 h of carcass chilling, samples of *Longissimus thoracis* muscle were vacuum-packed in aluminium bags and frozen at − 20 °C until meat quality analysis. Before analysis samples were thawed for 24 h at 4 ºC.

Intramuscular fat content (IMF) was analysed by NIT spectroscopy (Infratec® 1265, Tecator, Sweden).

Samples for texture and sensory analysis were cooked in a convection oven pre-heated to 110 °C to an internal temperature of 70 °C.

Instrumental texture was determined with the Warner Bratzler shear force test (WB). Six pieces of 2×1 cm cross section per chop were cut parallel to muscle fibre direction. All pieces were sheared using a MTS Aliance RT/5 texture analyzer (MTS System Corp., USA) equipped with a WB blade with crosshead speed set at 2 mm/s. Texture profile analysis (TPA) was performed on meat cubes (20×20×15 mm) compressed to 75% of their height using a crosshead speed of 5 mm/s. The Texture Analyser TA-XT2 (Stable Micro Systems, UK) was used.

Sensory analysis was performed through a Quantitative Descriptive Analysis (QDA) using 6 selected trained assessors. The retained attributes after open discussion were: odour intensity, skatole flavour, sweetness, metallic flavour, off-flavours, hardness, crumbliness, juiciness, and stringiness. A non-structured scoring scale was used, where 0 meant absence and 10 meant high intensity of the descriptor. Sensory evaluation was undertaken in 30 sessions in a sensory panel room equipped with red fluorescent lighting to mask the meat colour. Appearance attributes on raw meat were evaluated under white lighting. A complete block design was used where each taster assessed one sample of each breeds in each session. Samples were coded with three random numbers and were presented to the assessors balancing the first-order and the carry-over effects.

D. Preparation of Protein Extracts for SELDI-TOF Analyses

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

Muscle samples were taken from the freezer, and the water soluble fraction of proteins was isolated. 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% (v/v) Triton X-100, 1 mM PMSF], and homogenized (Ultraturrax T25, IKA, Germany) under ice to avoid mechanical heating of the samples. The resulting sample homogenates were briefly centrifuged (20 min, 4°C, 12,000×g) to remove insoluble debris. The supernatant was then assayed for total protein content using a commercial protein assay kit with BSA as standard (Bio-Rad, the Netherlands).

E. 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) ProteinChip arrays and binding buffer combinations were prepared according to the manufacturer's instructions (Bio-Rad Lab. Inc., Hercules, CA). The different ProteinChip 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), and that for the CM10 contained 0.1 M sodium acetate (pH 5). Before

applying the samples to the IMAC30 array, the active spots of the array were preactivated with 100 µL of 0.1 M copper sulfate solution according to the manufacturer's instructions (Bio-Rad). Twenty µg of protein was 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 to the array. After the binding step, the entire array was washed 3 times with the respective binding buffers (5 min with agitation) and then twice with deionized water. After briefly drying the arrays, 0.8 µL of a saturated solution of 4-hydroxy-3, 5-dimethoxy- cinnamic acid dissolved in 50% (v/v) acetonitrile and 0.5% (v/v) trifluoracetic acid, was applied twice to each of the active spots of the array, and was allowed to thoroughly dry. The different ProteinChip arrays were then placed in the SELDI ProteinChip Biology System Reader 4,000 (Bio-Rad). The laser intensity was 3,000 nJ. The SELDI ProteinChip spectra were pre-processed and analysed as explained by Mach et al. [3].

F. Statistical Analyses

Pearson correlation coefficients among meat quality traits and peak intensities were calculated with SAS 9.2for each ProteinChip. Regression models for meat quality traits were obtained for each ProteinChip with the SAS Analyst using a stepwise regression model including peak intensities and meat quality measurements in the models.

III. RESULTS AND DISCUSSION

Expression of water soluble proteins of fresh pork *Longissimus thoracis* was studied in an effort to identify candidate protein markers for quality traits.

Correlation analysis highlighted stronger correlations among protein peak intensities and quality parameters for those peaks obtained with CM10 ProteinChip than those obtained with the other two arrays. Significant correlations ($p<0.001$) were observed between cook loss, total work measured with WB shear force test, and sensory hardness with intensity of peak 4,338 m/z (r=0.318, -0.492, and -0.446, respectively), as well as 8,464 m/z peak (r=-0.370, 0.426, and 0.399, respectively) and 8,485 m/z peak (r=0.339, -0.432, and -0.443, respectively). These results indicate that increased peak intensity would be related with increased cook loss, while higher tenderness, suggesting that these protein peaks could be indicators of the state of meat structure. Increased peak intensities, especially for these traits, could also indicate that the peaks are protein degradation products [8]. Additionally, peaks 12,223 and 12,434 m/z showed positive correlations with drip loss and cook loss values (r=0.334 to 0.399). On the contrary, on Q10 ProteinChip, the peak 12,119 m/z showed a negative correlation with drip loss and cook loss (r=-0.340 and -0.355, respectively). From these results it can be extracted that peaks around of 12,000 m/z would be related to some extend with water holding capacity in pork loin.

Regression models for sensory and technological quality traits of cooked loin were obtained including peak intensities obtained with CM10, Q10 and IMAC30 ProteinChip arrays and quality data measured after slaughter in the models (Table 1). Regression model for drip loss obtained on CM10 ProteinChip array included peak 12,434 m/z, estimated lean (measured with the Fat'o'Meter), pH_u and L* as independent variables (r^2= 0.437). The regression model obtained for cook loss included the same peak, 12,434 m/z, and loin yield (loin weight/ carcass weight). The peak 4,338 among others was included in the models for both instrumental texture (total work measured with WB) and sensory texture (hardness). Another interesting result was the inclusion of the same peak, 9,398 m/z in the models for skatole, both measured by chemical and sensory analysis. Although the models only explained a very small proportion of the variation (r^2= 0.132 and 0.156, respectively), the inclusion of this peptide/ protein peak in the regression models of both skatole measurements would suggest a certain role of this peak on skatole content of loins.

Regression models for drip loss obtained on Q10 ProteinChip included peaks 4,507 m/z, 6,554 m/z, and 12,119 m/z, fat (measured with Fat'o'Meter), and pH_u (r^2= 0.4484); while models for cook loss included peaks 5,147 m/z, and 12,119 m/z, and % loin (r^2= 0.3126).

From the models obtained for cooked loin with both CM10 and Q10 ProteinChips, we can highlight the

relationship between peak intensities in the range of 12,000 m/z with drip loss and cook loss.

Table 1 Regression models for cooked loin quality traits.

Protein-Chip		R^2	Root MSE	SD	Variables included model: peaks (m/z)	Variables included model: quality parameters
CM10	Drip loss	0.437	1.226	1.607	12,434	estimated lean
						pH_u
						L*
	Cook loss	0.266	3.172	3.669	12,434	loin yield
	Cohesiveness (TPA)	0.365	0.022	0.026	8,677	cook loss
					10,056	IMF
	Total work (WB)	0.31	9.688	11.512	4,338	
					6,156	
					16,935	
	Skatole	0.132	0.111	0.118	8,126	estimated lean
					9,398	
	Skatole (sensory)	0.156	0.568	0.613	2,891	
					9,398	
	Hardness (sensory)	0.441	0.691	0.908	4,338	cook loss
					6,651	
					10,056	
Q10	Drip loss	0.4484	1.219	1.61	4,507	backfatthickness
					6,554	pH_u
					12,119	
	Cook loss	0.3126	3.083	3.672	5,147	loin yield
					12,119	
	Total work (WB)	0.2326	10.215	11.512	3,201	
					5,729	
					12,119	

MSE: mean square error; SD: standard error; TPA: texture profile analysis; WB: Warner Bratzler

It should be noted that the obtained models showed residual variability (RMSE) values similar to the own variability of each sensory and technological quality trait (SD). These models would explain a small proportion of variability.

IV. CONCLUSIONS

This study showed some associations between protein peaks obtained with SELDI-TOF-MS and quality traits, suggesting that deeper studies which consider other environmental factors contributing to the variability of quality parameters would be needed before considering these peptide/ protein peaks as candidate protein markers for cooked loin and dry-cured ham quality.

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