COMPARATIVE PROTEOMIC PROFILING BETWEEN SPANISH BOVINE BREEDS

R. Rodríguez-Vázquez[1*], A. Mato[1], M.A. Sentandreu[2], M. Oliván[3], D. Franco[4], C. Zapata[1]

[1]Department of Zoology, Genetics and Physical Anthropology, University of Santiago de Compostela, Santiago de Compostela – 15782, Spain.
[2]Agrochemistry and Food Technology Institute (CSIC), Valencia – 46980, Spain.
[3]Servicio Regional de Investigación y Desarrollo Alimentario (SERIDA), Villaviciosa - 33300, Spain.
[4]Meat Technology Centre of Galicia, San Cibrao das Viñas - 32900, Spain.

*Corresponding author email: raquel.rodriguez.vazquez1@rai.usc.es

Abstract – The Rubia Gallega (RG) and the Asturiana de los Valles (AV) are two important bovine breeds in the Spanish meat industry. In this study, we address for the first time the identification of diagnostic meat biomarkers of RG and AV breeds. For this purpose, proteome profiling of *longissimus dorsi* (LD) muscle from RG and AV breeds was assessed at 2 h post-mortem by two-dimensional electrophoresis (2-DE) and tandem mass spectrometry (MALDI-TOF/TOF MS). A total of 36 protein spots were found to have statistically significant differential abundance between sample groups, including metabolism enzymes and heat-shock proteins. These proteins can be candidate biomarkers for meat traceability and meat quality variations in RG and AV breeds.

Key Words – Bovine breed biomarkers, Muscle proteins, Rubia Gallega, Asturiana de los Valles.

I. INTRODUCTION

Proteomics is a powerful tool for unravelling the biological processes contributing to meat quality variations among bovine breeds as well to identify breed-specific meat biomarkers [1, 2]. To our knowledge, no comparative study has been carried out between proteomic profiles of RG and AV, two of the most important bovine breeds in the Spanish meat industry [3]. In this study, proteome profiling of LD muscle from RG and AV breeds was assessed at 2 h post-mortem by 2-DE coupled to MALDI-TOF/TOF MS. In addition, comparative proteomics was used to assess the extent of proteomic differentiation between breeds as well as the identification of breed-diagnostic and traceability biomarkers. In last term, differentially represented proteins in the proteomes of RG and AV immediately post-mortem can be candidate proteins linked to their meat quality variations.

II. MATERIALS AND METHODS

Six biological replicates of LD muscle from male calves of RG and AV breeds at 2 h post-mortem were used in this study. Muscle proteins were separated by 2-DE as described previously [4]. First dimension was performed using 24-cm-long IPG Strips (Bio-Rad) with linear pH gradient of 4-7. Second dimension was run on 12% SDS-PAGE gels. The 2-DE gels were stained with SYPRO Ruby stain (Lonza) and image analysis of digitalized gels was performed using PDQuest software (Bio-Rad). Protein identification was performed by MALDI-TOF/TOF MS according to Franco *et al*. [4]. Peptide fragmentation spectra data of each sample were combined through the GPS Explorer Software using Mascot software (Matrix Science) to search against the *Bos taurus* UniProt/SwissProt database. Statistical differences of spot volumes between sample groups were tested by the

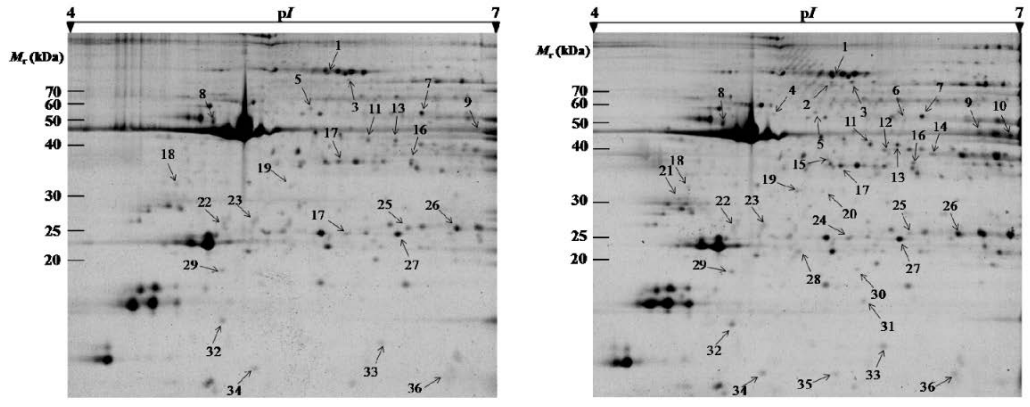

Figure 1. Representative 2-DE gel proteomic profiles in meat samples from the *longissimus dorsi* muscle of RG (left) and AV (right) bovine breeds. Protein spots with significantly difference abundance between breeds are marked and numbered.

Mann-Whitney U non-parametric test using the IBM SPSS Statistics. Quantitative changes in spot volumes from RG to AV samples were estimated using the measures "fold change" (*FC*) and "relative change" (*RC*) [4].

III. RESULTS AND DISCUSSION

Representative 2-DE gel images of protein profiles for RG and AV breeds are shown in Fig. 1. In total, 205 protein spots were reproducibly resolved over replicates. We found that 18% of protein spots (36 out of 205 spots) showed statistically significant differential abundance between sample groups (Mann-Whitney U test, p-value < 0.05). A number of 13 proteins spots (spots 2; 6; 8; 9; 10; 15; 16; 20; 21; 22; 28; 30 and 35) presented only qualitative changes, while the remaining 23 spots showed quantitative differences (Fig. 1). The measures *FC* and *RC* were computed for those 23 quantitatively-changed protein spots. However, *RC* is a more useful measure than *FC* because it always ranges from -1.0 to +1.0 and takes a value of zero if there is no volume change. Values of *RC* for each protein spot are given in Fig. 2. There can be seen that all spots with the exception of spot 7 showed *RC*-values with positive sign. It means that most spots are overrepresented in AV meat samples. In addition, protein identification by MALDI-TOF/TOF MS revealed that a diversity of proteins were associated with differentially

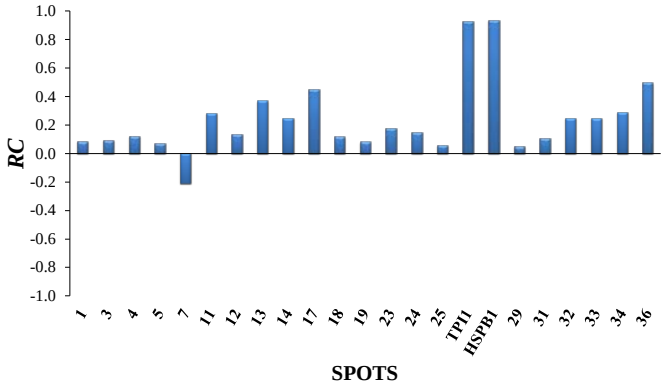

Figure 2. Relative change (*RC*) in the volume of protein spots with significantly different abundance between meat samples from *longissimus dorsi* muscle of AV and RG bovine breads.

abundant spots in sample groups, including structural-contractile proteins, metabolism enzymes and heat-shock proteins. Spots with highest *RC*-values, i.e. spots 26 and 27, were identified as triosephosphate isomerase 1 (TPI1) and heat shock protein beta 1 (HSPB1), respectively. These findings suggest that proteins linked to qualitatively-changed protein spots together with proteins TPI1 and HSPB1 could be used both as biomarkers of RG and AV meats and their traceability.

IV. CONCLUSION

The present study revealed marked proteomic differences between meat samples from LD muscle of RG and AV bovine breeds at the quantitative and qualitative level. A total of 36 functionally diverse proteins were found to have statistically significant differential abundance in sample groups. These results allowed us the identification of proteins that could be used as diagnostic biomarkers of RG and AV breed meats and traceability biomarkers. In addition, differentially represented proteins can be monitored in follow-up studies as candidate proteins underlying meat quality variations between breeds.

ACKNOWLEDGEMENTS

This work was supported by grant RTA2014-00034-C04-01 from the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, Spain).

REFERENCES

1. Timperio, A.M., D'Alessandro, A., Pariset, L., D'Amici, G. M., Valentini, A. & Zolla, L. (2009). Comparative proteomics and transcriptomics analyses of livers from two different *Bos taurus* breeds: "Chianina and Holstein Friesian". Journal Proteomics 73: 309-322.
2. de Souza Rodrigues, R. T., Chizzotti, M. L., Vital, C. E., Baracat-Pereira, M. C., Barros, E., Busato, K. C., Gomes, R. A., Ladeira, M. M. & da Silva Martins, T. (2017). Differences in beef quality between Angus (*Bos taurus taurus*) and Nellore (*Bos taurus indicus*) cattle through a proteomic and phosphoproteomic approach. PloS One 12: e0170294: 1-21.
3. Mapama.gob.es. (2017). Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente, http://mapama.gob.es/es/alimentacion/temas/calidad-agroalimentaria/calidad-diferenciada/dop/default.aspx.
4. Franco, D., Mato, A., Salgado, F.J., López-Pedrouso, M., Carrera, M., Parrado, M., Gallardo, J. M. & Zapata, C. (2015). Tackling proteome changes in cattle *longissimus thoracis* muscle under short-term stress prior to slaughter. Journal Proteomics 122: 73-85.