MEAT PROTEOMIC BIOMARKERS IN TWO DIFFERENT SPANISH BOVINE BREEDS

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Abstract – Proteomic technologies are a powerful tool to identify diagnostic biomarkers of meat from different *Bos taurus* breeds. In the present study, proteome profiles of *longissimus dorsi* (LD) muscle from male calves of Retinta (RE) and Rubia Gallega (RG) bovine breeds were assessed at 2 h *post-mortem* by two-dimensional electrophoresis (2-DE) and tandem mass spectrometry (MALDI-TOF/TOF MS). Seven non-redundant sarcoplasmic and myofibrillar proteins with statistically significant differential abundance between breeds were identified. Bioinformatic analysis of high level Gene Ontology (GO) slim terms using the AmiGO software and the web-based QuickGO tool revealed that differentially represented proteins were involved in diverse biological processes (e.g. skeletal muscle development and contraction, carbohydrate metabolism), molecular functions (e.g. calcium ion binding, catalytic activity, structural activity), and cellular locations. These findings provide specific biomarkers to monitor meat traceability as well as candidate proteins/genes underlying physiological differences and meat quality variations in the RE and RG breeds.

Key Words – Retinta, Rubia Gallega, Bovine breed biomarkers, Meat, Proteomics, Bioinformatics.

I. INTRODUCTION

Proteomics can be used to identify specific biomarkers of meat from different bovine breeds and contribute to a better understanding of the molecular processes determining variations in complex phenotypic traits such as meat quality [1-3]. In addition, differentially expressed proteins in bovine breeds can provide molecular evidence about their physiological differences linked to meat quality [4]. This study is a first attempt to pinpoint differentially represented proteins in meat samples of LD muscle from RE and RG, two Spanish bovine breeds very appreciated by consumers because of their excellent meat quality.

II. MATERIALS AND METHODS

Total protein was extracted from six biological replicates of LD muscle collected at 2 h *post-mortem* from male calves of RE and RG breeds. The 2-DE was carried out using 24-cm-long IPG strips (Bio-Rad Laboratories) with linear pH gradient of 4-7 and 12% SDS-PAGE gels. The 2-DE gels were subsequently stained with SYPRO Ruby fluorescent stain (Lonza) and digitalized. The detection and quantitation of spot volumes was performed with PDQuest software (Bio-Rad). Spots with statistical differences in volume between sample groups were identified using MALDI-TOF/TOF MS and Mascot software (Matrix Science) as previously reported [5]. Classification of proteins grouped into biological process, molecular function and cellular component categories was performed from high level Gene Ontology (GO) slim terms using the GO Slimmer tool of AmiGO software and the fine-grained information for each protein was retrieved by means of web-based QuickGO tool [5].

III. RESULTS AND DISCUSSION

A total of 18 protein spots on 2-DE gels showed qualitative or quantitative significant volume changes between RE and RG meat samples (P-value < 0.05, Mann-Whitney U test). These spots were excised from gels and analyzed by MALDI-TOF/TOF MS. A total of seven non-redundant sarcoplasmic and myofibrillar proteins were successfully identified: creatine kinase M-type (CKM), cyclin-G1 (CCNG1), myosin binding protein (MYBPH), myosin regulatory light chain 2 (MYLPF), pyruvate dehydrogenase (PDHB), thioredoxin-dependent peroxide

reductase (PRDX3) and 14-3-3 protein epsilon (YWHAE). Functional categorization (i.e. biological process, molecular function and cellular component) of differentially abundant proteins according to the GO terms is shown in Figure 1. GO analysis indicated that proteins were mainly involved in biosynthetic and structure development processes; molecular functions such as ion binding, structural activity, oxidoreductase activity and enzyme binding; and diverse locations (intracellular, mitochondria and extracellular space).

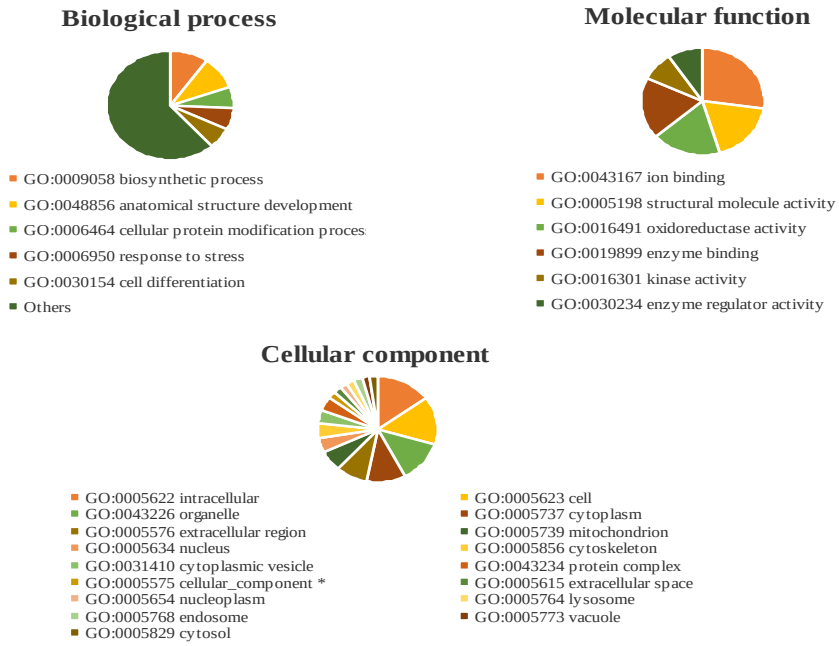

Figure 1. Biological process, molecular function and cellular component classification of differentially represented proteins in meat of LD from RE and RG bovine breeds.

IV. CONCLUSIONS

Seven non-redundant sarcoplasmic and myofibrillar proteins were found to be differentially represented in meat samples of LD muscle from RE and RG cattle breeds at 2 h *post-mortem*. Therefore, these proteins could be used as diagnostic biomarkers of RE and RG meat. Bioinformatic analysis provided functional information on the identified proteins that can help to explain the physiological differences linked to meat quality variations between RE and RG breeds.

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