# PROTEOME CHANGES INVOLVED IN MEAT AGEING OF RUBIA GALLEGA CALVES

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## I. INTRODUCTION

Meat quality is a complex trait influenced by multiple factors including genetic variability, type and properties of the muscle and rearing conditions [1]. The biochemical and structural changes involved in the muscle-to-meat conversion also play a critical role in tenderization and other meat quality parameters. However, *post-mortem* biochemical changes are highly variable within and among bovine breeds and are not fully understood [2,3]. Here, we examine the proteome changes associated with the conversion of LD muscle into meat with prolonged maturation time in the RG bovine breed. It is the most numerous autochthonous bovine breed in Spain, very appreciated by its high quality meat [4].

## II. MATERIALS AND METHODS

Four biological replicates of LD muscle of RG at 24 h and 12 days *postmortem* were used in this study. Protein extraction was performed by Clean-Up kit (GE Healthcare) and subsequently separated by 2-DE. First dimension was carried out with IPG strips (pH 4-7, 24 cm) in a PROTEAN IEF cell system (Bio-Rad Laboratories). For the second dimension, 12% SDS-PAGE gels were used and stained with SYPRO Ruby stain (Lonza). 2-DE gels, spot volumes were quantified by PDQuest software (Bio-Rad) and identified by MALDI-TOF/TOF MS as previously [5]. Statistical differences of spot volumes between sample groups were assessed tested by the Mann-Whitney U non-parametric test. Search for the retrieval of protein-protein interaction networks was carried using the STRING v10.5 software.

## III. RESULTS AND DISCUSSION

2-DE protein profiles of LD muscle from RG breed at 24 h and 12 days *postmortem* are shown in Figure 1. A total of 13 different protein spots showed reproducible and significant changes in volume between groups (*P*-value < 0.05). Seven myofibrillar and sarcoplasmic non-redundant proteins with altered abundance were identified by MALDI-TOF/TOF MS: phosphoglucomutase-1 (PGM1), malate dehydrogenase-1 (MDH1), superoxide dismutase-1 (SOD1), peroxiredoxin-6 (PRDX6), myosin regulatory light chain 2 (MYL2), fast skeletal myosin light chain 2 isoform (MYLPF) and 14-3-3 protein epsilon (YWHAE). These potential protein biomarkers of meat ageing in the RG breed differed markedly from those previously reported for prolonged maturation time of LD in the Charolaise cattle breed [5].

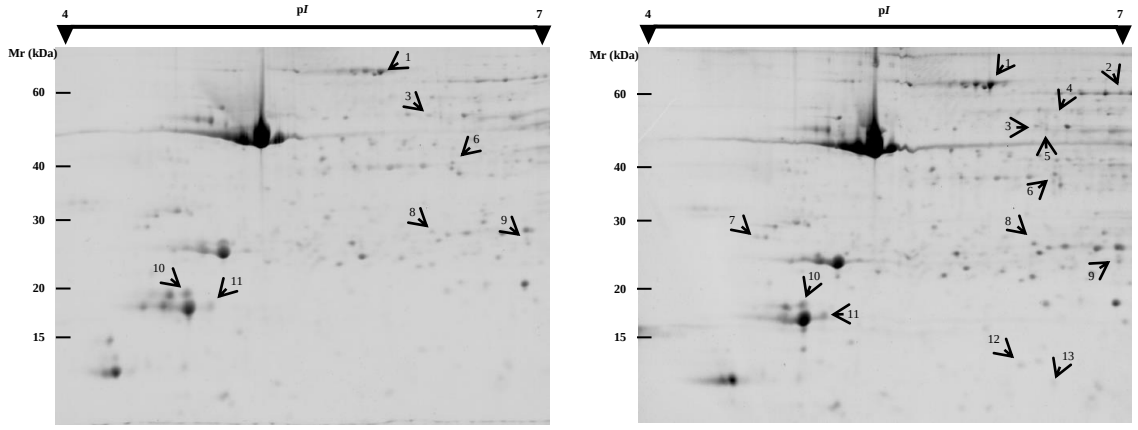


**Figure 1**. Representative images of 2-DE gels obtained from samples of LD muscle of the RG breed at 24 h (left) and 12 days *pm* (right). Spots showing statistically different protein abundance in sample groups are numbered.

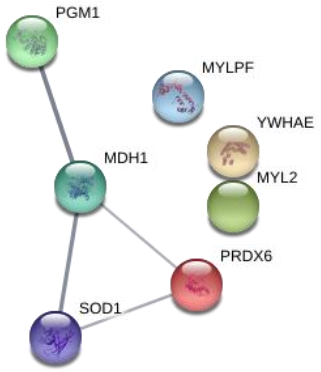


**Figure 2**. Protein-protein interaction network of differentially represented proteins in samples of LD with different maturation time according to STRING database. The degree of confidence prediction for each interaction is showed by the thickness of each line.

It is noteworthy that one group of four differentially represented proteins showed direct interaction according to STRING database: PGM1, MDH1, PRDX6 and SOD1 (Figure 2). PGM1 is connected with synthesis and catabolism of glucose, MDH1 is a oxidoreductase, SOD1 belongs to a group of enzymes which reacts with radicals, protecting cells from oxidation as well as PRDX6 involved in redox regulation as protection against oxidative injury.

## IV. CONCLUSIONS

Proteomic changes of LD bovine muscle of the RF breed were detected during the transition from early (24 h) to long maturation time (12 days). In total, seven non-redundant proteins showed statistically significant differential abundance in sample groups. Therefore, these proteins can be candidate biomarkers of tenderization and other meat quality parameters associated with prolonged maturation time. In addition, a network of four functionally related proteins (i.e. PGM1, MDH1, PRDX6 and SOD1) might be involved in a major metabolic pathway underlying *postmortem* ageing.

## ACKNOWLEDGMENTS

This research was supported by fellowship of the Xunta de Galicia (Spain) and the European Union (ESF) to R. Rodríguez-Vázquez.

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