USE OF LIQUID ISOELECTRIC FOCUSING (OFFGEL) TO STUDY CHANGES IN MUSCLE PROTEOME DURING BOVINE MEAT AGEING

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Abstract – In this work, *post mortem* evolution of the myofibrillar protein fraction from bovine *Longissimus thoracis* muscle was studied. Myofibrillar fraction from samples obtained at different *post mortem* times was fractionated by liquid isoelectric focusing in the pH range 4 to 7, and further analyzed by SDS-PAGE. Spots of interest were identified by liquid chromatography coupled to tandem mass spectrometry. Profiles were highly reproducible, so variations over the different ageing times were easily identified. The fraction showing the most significant changes along the process (P<0,01) mainly comprised metabolic proteins, which may appear in the myofibrillar fraction because of changes in solubility. This promising technique is a step forward in the design of more efficient and easily automated methodologies in proteomics.

Key Words – Ageing, protein fractionation, *post mortem* evolution, meat proteomics.

I. INTRODUCTION

Meat tenderness is ranked as one of the most important traits regarding consumer satisfaction, so the study of *post mortem* changes that give rise to meat tenderization is an active focus in meat science. Degradation of myofibrillar proteins lead to improved tenderness during meat ageing. On the other hand, changes in sarcoplasmic proteins, involved in metabolism and signal transduction, may also provide useful information as markers of *post mortem* proteolysis.

Traditional approaches consist on fractionation of muscle proteome by two-dimensional gel electrophoresis followed by spot identification using mass spectrometry. Despite its utility and resolving power, this technique is time-consuming and difficult to automate, so new strategies need to be developed. Liquid isoelectric focusing (OFFGEL) separates proteins or peptides according to their isoelectric points in a multiwell device, with the advantage to allow compounds to be directly recovered in liquid fractions [1]. The goal of this study was to follow up *post mortem* myofibrillar proteome changes using OFFGEL fractionation.

II. MATERIALS AND METHODS

Longissimus thoracis (LT) muscle samples (triplicate) were obtained from a Limousine veal carcass immediately after slaughter and preserved at 4ºC. Then, 100 g subsamples were obtained at 2 hours, 8 h, 24 h, 3 days, 7 d, 14 d and 22 d *post mortem*, and stored at -80ºC until further analysis.

The extraction procedure was performed as described in Sentandreu *et al.* [1]. From each extract, 1 mg of total protein was fractionated by OFFGEL in the pH range 4 to 7 using an Agilent 3100 OFFGEL fractionator and obtaining 12 protein fractions. The profile of each fraction was further assessed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 12% polyacrylamide gels. Gels were revealed by colloidal Coomassie stain and digitalization was carried out using Un-Scan it v6.1 software. Selected bands were excised, trypsin digested and characterized by liquid chromatography coupled to tandem mass spectrometry (LC-ESI-MS/MS). For the interpretation of the fragmentation mass spectra, MASCOT search engine, Uniprot KB and NCBInr protein databases were employed.

III. RESULTS AND DISCUSSION

Figures 1a and 1b are representative examples of the fractionation profiles obtained at 2 h and 22 d *post mortem*, respectively. The distribution of the myofibrillar sub-proteome was highly reproducible along the whole assayed samples, and the most evident changes in abundance (P<0.01) were observed in lane 12, which mainly contained proteins with isoelectric point values above 7. Over time, in general, the intensity of these proteins decreased until disappearance at 22 d. Despite of their occurrence in the same lane, good separations were accomplished and spots could be effectively identified (Table 1). As it can be observed, proteins related to cell metabolism, which theoretically occur in the sarcoplasmic fraction, were identified in the myofibrillar extract. In fact, during early *post*

mortem periods a decrease in protein solubility may be related to a decrease of the intramuscular pH that could be associated to anaerobic glycogenesis. The effect of pH fall in the loss of solubility of some glycolytic enzymes have been already reported by others [2].

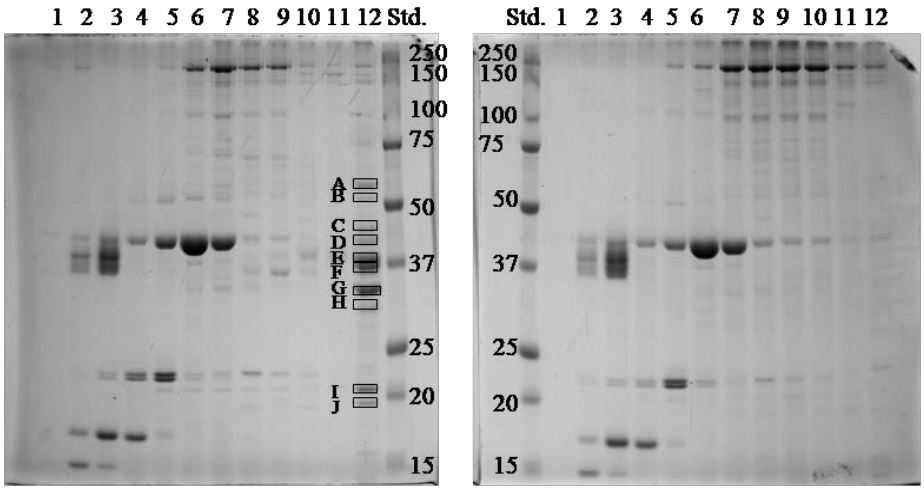

Figure 1. 12% SDS-PAGE of fractions obtained after OFFGEL isoelectric focusing of myofibrillar extracts from bovine meat obtained at a) 2 h and b) 22d *post mortem* in the pH range 4 to 7. Std: Molecular mass standards (kDa).

Table 1. Identification and biological function of selected protein spots.

Spot	Identified proteins	pI	Biological function
A	Pyruvate kinase PKM isoform X1	7,62	Cell metabolism
B	ATP synthase subunit alpha	9,21	Cell metabolism
C	Beta enolase	7,60	Cell metabolism
D	Creatine kinase M-type	8,45	Cell metabolism
E	Fructose bisphosphate aldolase A	8,45	Cell metabolism
F	Troponin T Fructose bisphosphate aldolase A	5,99 8,66	Muscle contraction Cell metabolism
G	Glyceraldehide 3 phosphate dehydrogenase	8,50	Cell metabolism
H	Malate dehydrogenase (fragment) LIM domain binding protein 3 (fragment)	8,88 9,34	Cell metabolism Cell metabolism
I	Troponin I, fast skeletal muscle	8,88	Muscle contraction
J	Alpha crystallin B chain	6,76	Stress response

pI: Isoelectric point.

IV. CONCLUSION

The comparison of bovine myofibrillar sub-proteomes at different ageing times using OFFGEL fractionation has been successfully achieved. Lane 12, mainly composed of metabolic enzymes, could provide relevant information about the ageing process which is less evident without OFFGEL fractionation. The developed approach constitutes promising research alternative to traditional methodologies in meat proteomics.

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