USE OF LIQUID ISOELECTRIC FOCUSING (OFFGEL) IN THE STUDY OF MUSCLE PROTEOME AS RELATED TO MEAT TENDERNESS

Diego M. Fernández[1], Brigitte Picard[2], Enrique Sentandreu[1], Luis Izquierdo[1] and Miguel A. Sentandreu[1]

[1] IATA-CSIC, 46980 Paterna (Valencia), Spain

[2] UMRH, INRA, 63122 Saint Genes Champanelle, France

Abstract – This work shows liquid isoelectric focusing (OFFGEL) as a useful tool in the fractionation, enrichment and characterization of muscle proteins. Here we present a comparative study, using this approach, of the muscle proteome from two different bovine meats that had been previously classified as tough and tender, respectively. Myofibrillar proteins were selectively extracted, then fractionated by liquid isoelectric focusing in the pH range 4-7. Proteins were focused according to their isoelectric point along a total of 12 liquid fractions. The reproducibility and general quality of the fractionation was assessed by SDS-PAGE. Comparison of the protein profiles obtained in each case allowed us to see some differences according to the type of meat, thus opening new ways in the discovery of new and reliable biomarkers of meat tenderness. The fact to obtain fractions in liquid state after isoelectric focusing allows for a faster subsequent analysis of proteins and peptides using mass spectrometry, thus being a step forward in the design of new, more efficient protocols of proteomic analysis.

Key Words – Protein fractionation, meat quality, myofibrillar proteins.

I. INTRODUCTION

The use of Proteomics in the study of meat and meat products has mainly relied on the previous fractionation of muscle proteome by two-dimensional gel electrophoresis (2-DE) followed by selection/excision of the spots of interest, in-gel trypsin digestion and identification of the generated peptides by mass spectrometry. Despite its utility and resolving power, 2-DE is a demanding technique with respect to the amount of time and manual work, being also difficult for automation. For these reasons, there is need to develop new analytical approaches allowing a more efficient study of muscle proteome without necessarily implying the use of 2-DE.

Liquid isoelectric focusing (OFFGEL) is a fractionation technique based on the separation of proteins and peptides according to their isoelectric point. Contrary to gel electrophoresis, the separated compounds remain in liquid fractions after fractionation [1]. Using this principle, in the present work we carried out a new approach for the comparative study between the muscle proteome of two types of bovine meat, previously classified as tough and tender. The final objective is to allow a easier and more efficient study of the muscle proteome.

II. MATERIALS AND METHODS

Half a gram of muscle (*Longissimus thoracis*) obtained at 24 h post-mortem from six Maine Anjou bovine animals that were previously classified into tender and tough meat groups, were homogenized in 5 ml of 50 mM Tris buffer, pH 8.0 by the use of a Polytron®. The homogenate was then centrifuged at 10,000 g for 20 min at 4 °C. The obtained precipitate was redissolved in 50 mM Tris buffer, pH 8.0 containing 6M urea and 1M thiourea. From each extract, the volume containing 1 mg of total protein was fractionated separately by liquid isoelectric focusing in the pH range 4-7 using an Agilent 3100 OFFGEL fractionator. The total time for fractionation was 16 hours, giving rise to 12 protein fractions covering the mentioned pH range. The protein composition of each one of these fractions was further assessed by SDS-PAGE on 12% polyacrylamide gels. After complete run, gels were further revealed by silver staining. Gel digitalization and densitometry analysis were carried out using un-scan it v6.1 software.

III. RESULTS AND DISCUSSION

Samples analyzed in this work corresponded to meat cuts from six different animals that had been previously classified into tender or tough meats according to the obtained Warner-Bratzler shear force values (data not shown). The main goal of the present study was to give an overview of the distribution of myofibrillar sub-proteome all along the assayed pH range (pH 4-7) using liquid isoelectric focusing. At the end of fractionation, the 12 fractions obtained for each sample were easily recovered by pipetting and transferred to Eppendorf tubes and stored at -20ºC until SDS-PAGE analyses were carried out.

Figures 1 and 2 are representative examples of the fractionation profile obtained for the myofibrillar proteins of tender and tough meats, respectively. It is worth highlighting that the protein distribution profile after OFFGEL fractionation is highly reproducible, thus allowing the possibility to analyze differences due to the type of meat (tender vs. tough).

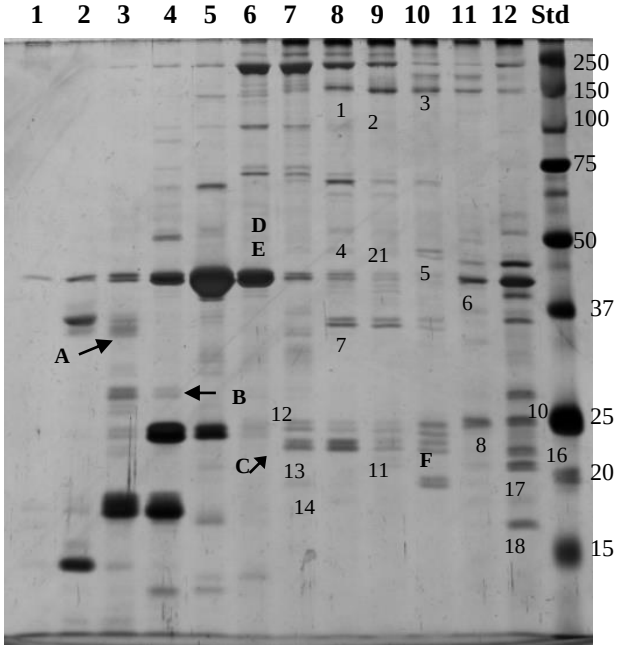

Figure 1. 12% SDS-PAGE of fractions obtained after fractionation by OFFGEL isoelectric focusing of a myofibrillar extract of bovine (Tender) meat in the pH range 4-7.
Std: molecular mass standards.

The main differences found between fractions obtained from the two different types of meats are indicated by either letters or numbers on both **Figure 1 and 2** and summarized in **Table 1**.

Spots represented by letters A to F constitute the main differences between the two sub-proteomes, since they are exclusive of one type of meat. In this sense, can observe that spots A, B and F are present only in the tender meat example (**Fig. 1**), whereas spots C, D and E are exclusive of the tough meat.

The spots indicated by numbers correspond to bands that are present in the two samples but with a significant difference in the degree of intensity, as assessed by densitometry analysis. Of a total of 17 quantitatively different proteins, 13 of them were significantly less abundant in the tender meat, whereas in only 4 of them their presence was more abundant in the tender meat with respect to its tough counterpart (spots 11, 6, 8 and 10).

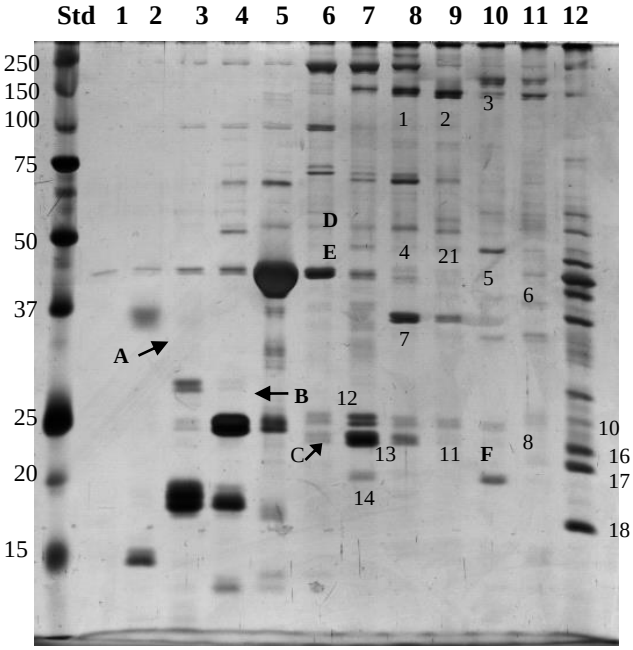

Figure 2. 12% SDS-PAGE of fractions obtained after fractionation by OFFGEL isoelectric focusing of a myofibrillar extract of bovine (Tough) meat in the pH range 4-7.
Std: molecular mass standards.

Table 1. Summary of the main spot differences found between the two representative examples of tender and though meats as analyzed by liquid isoelectric focusing and SDS-PAGE.

Fraction	Spot	Variation in tender sample
3	**A**	Only in tender
4	**B**	Only in tender
6	**C**	Not present in tender
7	**D**	Not present in tender
	E	Not present in tender
	12	(↓) Decreased
	13	(↓) Decreased
	14	(↓) Decreased
8	1	(↓) Decreased
	4	(↓) Decreased
	7	(↓) Decreased
9	2	(↓) Decreased
	21	(↓) Decreased
	11	(↑) Increased
10	3	(↓) Decreased
	5	(↓) Decreased
	F	Only in tender
11	6	(↑) Increased
	8	(↑) Increased
12	10	(↑) Increased
	16	(↓) Decreased
	17	(↓) Decreased
	18	(↓) Decreased

The next step in this new approach, to be done in near future, will be the identification of the differential spots (**Table 1**) by tandem mass spectrometry (MS/MS). This will allow us to compare our findings with previous studies dealing with the identification of biomarkers of meat tenderness. The use of Proteomics as state-of-the-art technology for searching biomarkers capable to explain changes responsible for the development of meat tenderness can be found in the literature since more than a decade ago. The work carried out by Bendixen and co-workers are between the pioneer contributions in this field. In 2002, these authors already reported the use of 2-DE for separating the muscle proteome following the identification of spots of interest by MALDI-TOF MS [2]. By this way, they were able to observe changes in the pig muscle proteome as consequence of post-mortem meat storage. Proteome changes during meat ageing in previously defined tender and tough bovine meats have also been studied by Laville [3] using a similar approach. In line with our work, they performed a selective extraction of the muscle proteins according to their solubility in Tris buffer. The two sub-proteomes were subsequently analysed following the classical approach reported by [2] comprising separation on 2-DE followed by identification using MALDI-TOF MS. In line with our results, these authors were able to see differences between tender and tough meats at early postmortem times. However, the approach shown in the present work would be more feasible to carry out, implying less manual work and with more possibilities for automation and discovery of new and reliable markers of tenderness. Once the different OFFGEL fractions have been characterized (**Figures 1 and 2**), the direct analysis of the 12 fractions by means of liquid chromatography coupled to mass spectrometry (LC-MS) would be feasible without requiring the gel fractionation step. After biomarker discovery and characterization, there is need to develop techniques for reliable and specific quantification analyses on a large number of meat samples. The development of a Dot-Blot quantitative technique for previously selected markers of tenderness is a good example of this [4].

IV. CONCLUSION

Fractionation of muscle proteome or sub-proteome using liquid isoelectric focusing constitutes a promising and interesting alternative to classical proteomic approaches based on two-dimensional gel electrophoresis. The fractionation profile is highly reproducible, allowing the comparison between different types of meat such as those presented in this work having different tenderness values. Using this approach, a total of 23 significant changes were observed between the two myofbrillar sub-proteomes. Research is in progress concerning the identification of the spots of interest for the discovery of new and reliable markers of tenderness. The fact to obtain fractions in liquid state after isoelectric focusing allows for less manual work, lower risk of sample contamination and the possibility to automate the protein/peptide identifications using mass spectrometry.

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