FUNCTIONAL PROTEOMIC ANALYSIS PREDICTS TENDERNESS IN BULL NELLORE MEAT

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Abstract - This paper aims to study the protein profile of beef cattle at different storage times. We analyzed samples (*Longissimus dorsi*) of feedlot bull Nellore cut between the 12th and 13th ribs and packaged in vacuum for 24 hours, after 7 and 14 days of storage. One gram samples were collected at predetermined time (24 hours, 7 and 14 days of storage) and stored at -80°C. Six pools of samples from animals previously selected were formed to perform two-dimensional electrophoresis. The gels were made in triplicate and analyzed using specific software. Statistical analyses were done in Statistical Analysis System. There were 25 spots with significant difference in the proteolysis of the times studied.

Key Words – Beef cattle, Tenderness, Two-dimensional electrophoresis.

I. INTRODUCTION

The cattle industry is one of the highlights of Brazilian agribusiness, with the second largest herd in the world, about 200 million livestock units [1]. Which, approximately 176 million of it are considered beef cattle, represented mostly by *Bos indicus* [2]

In the tropics *Bos indicus* animals have been preferred due of disease resistance and tolerance to higher temperatures [3]. Among the *Bos indicus* Nellore is the most commonly breed due to hardiness, prolificacy and precocity. However, *Bos indicus* have a disadvantage when compared to *Bos taurus*, in relation to meat tenderness [4].

Studies on meat quality show, it is possible to relate proteomic analysis to the *post-mortem* changes in meat tenderness [5]. Furthermore, proteomic analysis has been used to show the possible link between inherited traits and their interaction with the environment in the control of cellular and physiological functions. Thus, proteomics analyses are an important tool with regard to changes in protein and meat quality [6].

Though none study describes the protein profile of meat from *Bos indicus*. Therefore, this study has an innovative aspect, considering that the enzymatic profile have a specific way of the action over time in different peptides.

The objective of this work was to verify, through proteomic analysis, the protein profile of meat aged for 24 hours, 7 and 14 days of Nellore bulls.

II. MATERIALS AND METHODS

We used 17 Nellore bulls, grown on pasture and feedlot. The animals were slaughtered at 19 months of age in the Slaughterhouse School Campus Coordinator Pirassununga, following the humane slaughter techniques.

Steaks were removed from the *Longissimus dorsi* muscle at 24 hours *post mortem* between the 12th and 13th rib, two inches thick each. They were vacuum packaged and aged at 2°C for 24 hours, 7 and 14 days. The steaks were removed from the bags to Warner-Bratzler Shear Force (WBSF) determination. Simultaneously, one gram of sample was collected to be used in proteomic analysis. The samples were wrapped in aluminum foil and immediately frozen in liquid nitrogen and then transferred to the -80°C freezer.

The samples were divided into six pools and the process of protein extraction was carried out according Lametsch et al. [7]. The 2-D Quant Kit (GE Healthcare) was used for protein quantification. The protein extracts obtained were stored at -80°C.

To perform the 2-DE, IPG strips (13cm length pH 4-7; GE Healthcare), were used. The isoelectric focusing was performed with Ettan IPGphor (GE Healthcare) for separation of proteins according to isoelectric point. 12.5% SDS- PAGE gels were used for the electrophoresis. After that, the gels were stained with Coomassie R-250 dye and then destained in acetic acid and methanol. The gels were performed in triplicate.

The gels were scanned (Image Scanner III, GE Healthcare) and the images were stored for later analysis in the Image Master 2D Platinum program, version 7.0 (GE Healthcare).

Statistical analyzes were performed using the Statistical Analysis System, version 9.1.3 with the PROC MIXED.

III. RESULTS AND DISCUSSION

A descriptive statistical analysis showed an tenderness mean of 8.54 kg with standard error of 1.97.

To analyze the gel images, the mean of 382, 463, 372 spots in gels at times of 24 hours, 7 and 14 days were used. After a comparative study (match) between times, 256 common spots were found. 179 spots were subjected to analysis of variance to assess the effects of time of storage (24 hours, 7 and 14 days of maturation) on spot expression intensities.

The results indicated that 25 spots (Figure 1) showed significant differences ($P<0.05$). The regression analysis of the spots pattern showed that the expression of 3 spots have increased linearly over maturation, whereas 6 spots had the expression reduced linearly over the time. However, 16 spots indicated non-linear (quadratic) effects of the time on expression intensities. One spot (19) presented a maximum point at 9.06 days. The others spots showed minimum points in the range of 7.20 to 14.00 days *post-mortem*. The tenderness was evaluated as dependent variable and the significative spots as independent variables in multiple regression analysis, considering repeated measures model of the same animal. These analysis showed that 13 spots explained 73% of the variation of tenderness during the maturation, with a statistical $C(p) = 13.1$.

Kjaersgard et al. [8], studying proteolysis in fish meat stored for 11 different times, found that 89 of 504 protein spots appeared consistently in all gel analyzed. In pigs, 103 of 345 spots evaluated significant change in expression intensity between zero and 72 hours *post-mortem* [7]. Jia et al. [9], working with 12 cattle in two different times of storage, found significant proteomic changes between 0 and 24 hours *post-mortem*. According to these authors, of the 923 spots analyzed in *Longissimus dorsi* samples, 13 of them had significant differences, and 2 spots showed increases and 11 decreases in the expression intensities, respectively.

Mass spectrometry analyses allow inferring about these most important 13 peptides involved in process maturation in beef Nellore.

IV. CONCLUSION

The result obtained in this study suggests a possible relationship between the protein intensity and maturation process. The evaluation of these proteins with differential expression pattern may help in understanding the beef tenderness process in Nellore beef cattle.

ACKNOWLEDGEMENTS

To Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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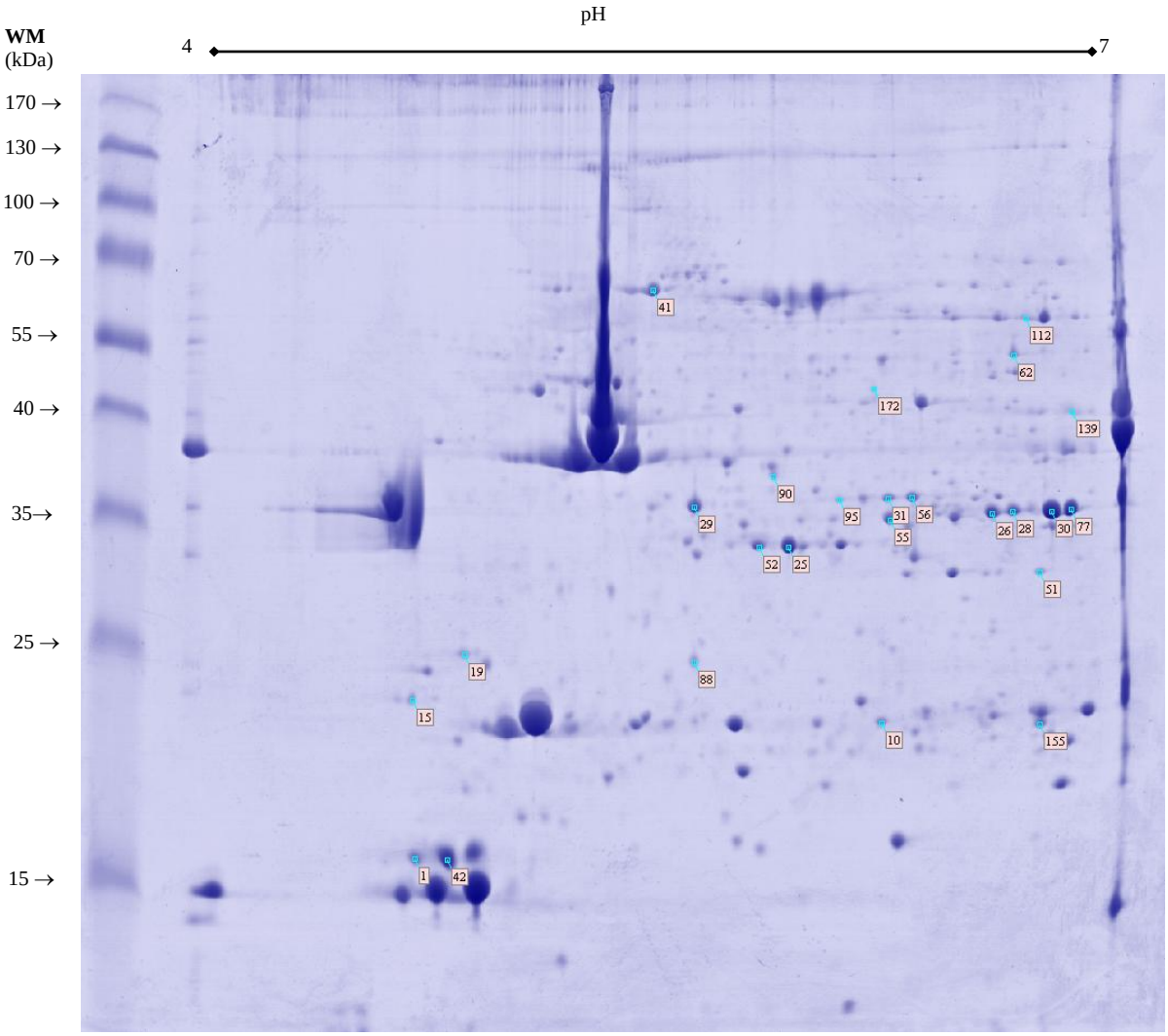

Figure 1. 2DE gels of cattle *Longissimus dorsi*. Arrows show the 25 spots significant differences (P<0.05)