PROTEOME CHANGES OF DIFFERENT MEAT TENDERNESS ON BEEF CATTLE DURING AGING PERIOD

Alessandra Rosa[1,*], Cristina Moncau[1], Mirele Poleti[2], Júlio Balieiro[3], Roberta Fonseca[1], and Joanir P. Eler[1]

[1] College of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil

[2] Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil

[3] College of Medicine Veterinary and Animal Science, University of São Paulo, São Paulo, São Paulo, Brazil

[*] Corresponding author email: afrosa@usp.br

Abstract – The aim of this work was evaluated the proteome changes between extreme groups of beef meat tenderness during aging. Were evaluated 303 F1 immunocastrated steers cross cattle (Nellore x South African Simmental), with 18.0 ±2.0 months years old and live weight at the slaughter of 500 kg. The meat was aging for 2 and 14 days after these the warner Bratzler Shear Force (WBSF) values were determined (unfrozen) according to AMSA (1995) and were used for classifying the meat like Tough (WBSF > 5.0 kg) or Tender (WBSF <4.0 kg). The proteins were separated by 1D gel electrophoresis and the identification was made by mass spectrometry (LC-MS/MS). A total of 1921 proteins were identified in these analyzes and 96 proteins were found in common among all groups (Tough, Tender, Day 2, and Day 14). Statistical analysis demonstrated that 4 proteins had differential expression between tender and tough groups, one protein was affected by aging increasing expression; and 2 proteins had interactions effect (P<0.05) between meat tenderness and aging period. The findings of this study revealed that two structural proteins (Desmin and Actin) and four metabolic proteins (PGAM2, F16P2, GPDA and LDHA) appear to be participating in the mechanism of meat tenderness.

Key Words – tenderness, beef, proteomics

I. INTRODUCTION

Solving the problem of inconsistent meat tenderness is a top priority of the meat industry. The consumers can distinguish between tough ad tender meat and they are willing to pay more for tender meat [1]. The meat tenderness process is complex and involves proteins in different biological pathways. Understanding the complex biochemical processes that occur during post-mortem period when the muscle is converted to meat, have a great importance [2].

Proteomic studies have aimed to identify the proteins of muscle to determine which are affecting meat quality. Although the post-mortem degradation of a series of structural proteins has been studied extensively in recent years has not been possible to establish whether this fact in itself, is directly responsible for the tenderization of the meat. According to Carvalho et al. [3], the strategy to compare animals with extreme Warner Bratzler Shear Force values resulted in the identification of structural, chaperones and metabolic proteins, associated with meat tenderness. Others studies showed the effect of biological processes, molecular makers and intricate pathways leading to tenderization on meat [4, 5, 6]. Although many efforts have been undertaken to minimize variability in tenderness, the causes for that are still not fully understood.

So, the aim of this study was evaluated the proteome changes between extreme groups of beef meat tenderness during aging.

II. MATERIALS AND METHODS

A. Animals and Experimental Procedure

For these study were evaluated 303 F1 immunocastrated steers cross cattle (Nellore x South African Simmental), with 18.0 ±2.0 months years old and live weight at the slaughter of 500 kg. The animals were raised on pasture and finished in feedlots receiving the same high-grain diets for all period (120 days). Animals were slaughtered according to standard humane procedures. After 48 hours post mortem were collect two steaks of 2.5 cm each at 12th and 13th ribs of Longissimus muscle. The steaks were

individually identified, vacuum packaged and aged for 2 and 14 days after slaughter.

B. Meat Tenderness and sampling

In each aging time, the vacuum packaging was opened and one piece of meat was cut and immediately frozen in liquid nitrogen for further proteomic analysis. Subsequently, the Warner Bratzler Shear Force (WBSF) measurements were determined according to AMSA (1995).

The WBSF values from 14 days of aging were used to classify the meat samples from 2 groups: tough (WBSF > 5.0 kg) and tender meat (WBSF <4.0 kg).

C. Muscle protein extraction and Gel Electrophoresis

Proteomic analysis was carried out on the muscle samples referred as tough, tender, Day 2 and Day 14. Three biological replicates were used per group, a total of 12 protein extracts. The meat samples were homogenized in a lysis buffer containing 8 M Urea, 2 M Thiourea, 1% DTT, 2% CHAPS and protease inhibitor and centrifuged at 10.000 x g for 30 min at 4ºC. The supernatant was harvested and protein concentration was determined with Plus One 2-D Quant Kit (GE Healthcare).

Prior to gel electrophoresis, samples aliquots containing 50 µg of protein were mixed with equal volume of loading buffer containing 0.5 M Tris-HCL, 85% glycerol, 10% SDS, 1% 2-mercaptoethanol and 1% bromophenol blue and incubated for 4 min at 100ºC.

Gel electrophoresis was performed under denaturing conditions in polyacrylamide precast gels (Amersham ECL Gel 10%, 10 wells) using ECL horizontal Gel electrophoresis system (GE healthcare) with constant 160v on Tris buffer. The run was stopped after 15 min of protein migration.

Prior to protein digestion, the gel lane resulting from each biological replicate was cut into 5 slices of approximately equal size. Each slice was cut into 1 mm cubes and transferred into polypropylene tubes for in gel digestion. In gel trypsin digestion was carried out according to Shevchenko et al. [2006]. Upon in gel digestion, gel pieces were saturated with 700µL of extraction buffer 5% formic acid (FA) / acetonitrile (1:2, v/v) and incubated for 30 min at 37ºC.

Supernatants were collected, dried down in a vacuum centrifuge and kept at -80ºC until LC-MS/MS analyzes.

D. Mass spectrometry analysis

For protein analysis, an aliquot of 4.5 ul of proteins resulting from peptide digestion was separated by C18 (100 mm6100 mm) RP-nanoUPLC (nanoAcquity, Waters) coupled with a Q-Tof Premier mass spectrometer (Waters) with nanoelectrospray source at a flow rate of 0.6 ml/min. The gradient was 2–90% acetonitrile in 0.1% formic acid over 50 min. The nanoelectrospray voltage was set to 3.5 kV, a cone voltage of 30 V and the source temperature was 100ºC. The instrument was operated in the 'top three' mode, in which one MS spectrum is acquired followed by MS/MS of the top three most-intense peaks detected. After MS/MS fragmentation, the ion was placed on the exclusion list for 60 s and for the analysis of endogenous cleavage peptides, a real time exclusion was used [7].

E. Data Analysis

The spectra were acquired using software MassLynx v.4.1 and the raw data files were converted to a peak list format (mgf) without summing the scans by the software Mascot Distiller v.2.3.2.0, 2009 (Matrix Science Ldt.) and searched against UniProt *Bos taurus* (36,948 sequences) using Mascot engine v.2.3.01 (Matrix Science Ltd.), with carbamidomethylation as fixed modifications, oxidation of methionine as variable modification, one trypsin missed cleavage and a tolerance of 0.1 Da for both precursor and fragment ions.

F. Statistical Methods

The spectral counts were analyzed with a mixed model that included fixed effects of ageing (one or 14 days), tenderness levels (tenderness or not tenderness), double interaction between the ageing and tenderness levels, as well as, random effects of error. The data were analyzed by the using PROC MIXED procedure of Statistical Analysis System (SAS), version 9.1.3 [8]. When significant ($P<0.05$) results were detected for *F* test, the least squares means were considered significantly different.

G. RESULTS AND DISCUSSION

A total of 1921 proteins were identified in these analyzes (Table 1). The Venn Diagram (Figure 1) shows 96 proteins found in common among all groups (Tough, Tender, Day 2 and Day 14).

Table 1. Number of proteins identified in Longissimus muscle from cattle

Aging	Tenderness		Total
	Tender	Tough	
2 days	509	436	945
14 days	475	501	976
Total	984	937	1921

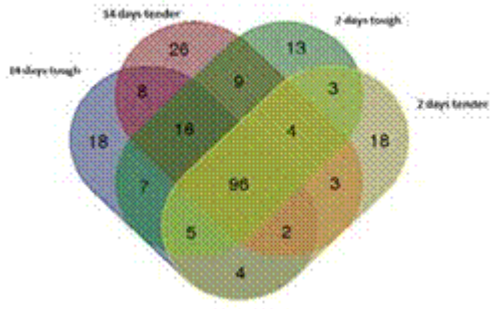

Figure 1. Venn Diagram with all proteins detected

The Statistical analysis demonstrated that 3 proteins had differential expression between tender and tough groups (Figure 2). In general, the samples from Tough Group presented a greater level of expression than those from Tender Group. These, it was found one structural protein (Desmin), which has activity on control of the spatial distribution of intermediate filaments or other structures, as by cross-linking; and three metabolic proteins (Fructose 1,6 bisphosfatase isozyme, Glycerol-3-phosphate dehydrogenase and Lactate dehydrogenase A) involved in glycolytic metabolism. Glycerol 3 phosphate dehydrogenase (GPDA) works with positive regulation of glycolytic process and Lactate Dehydrogenase A (LDHA) is involved in a glucose catabolic process to lactate via pyruvate. The anaerobic enzymatic chemical reactions of this protein and pathways resulting in the breakdown of glucose to lactate, via canonical glycolysis, yielding energy in the form of adenosine triphosphate (ATP).

In addition, Fructose 1,6 bisphosfatase isozyme (F16P2) had an effect ($P<0.05$) of aging time and tenderness group (Figure 3). F16P2 had higher expression in a Tough group and at 14 days post mortem. This is a metabolic protein involved in gluconeogenesis and is highly sensitive toward inhibition by AMP and calcium ions [9].

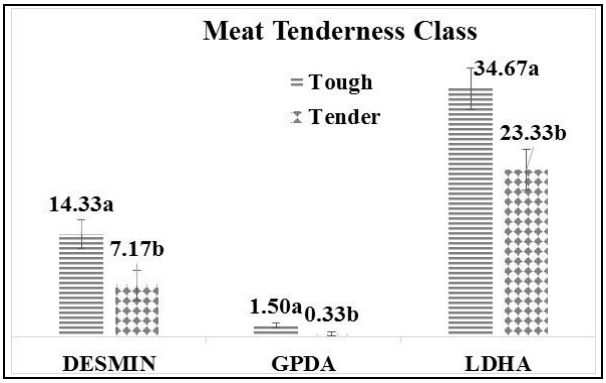

Figure 2. Total Spectrum Count of differentially expressed proteins: Desmin, Glycerol-3-phosphate dehydrogenase (GPDA) and Lactate dehydrogenase A (LDHA), in bovine *Longissimus* muscle between tenderness groups (tender and tough).

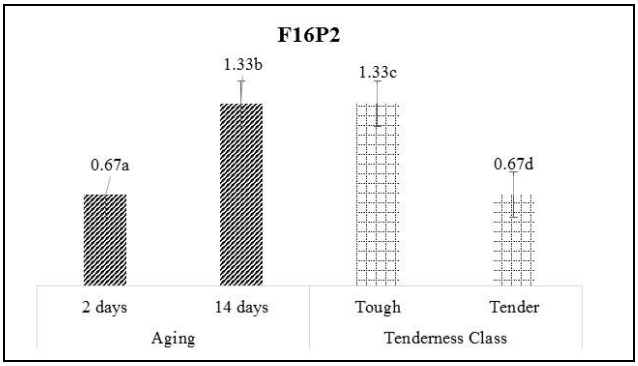

Figure 3. Total Spectrum Count of Fructose 1,6 bisphosfatase isozyme (F16P2_BOVIN), in bovine *Longissimus* muscle between aging times and tenderness groups (tough and tender).

Actin (ACTN3) and Phosphoglycerate mutase (PGAM2) were observed with interaction effect ($P<0.05$) between aging time and tenderness group (Figure 4). ACTN3 presented the highest expression values on day 14 inside the tender group. Actin is a molecule that contributes to the

structural integrity of a cytoskeletal and interacts selectively with calcium ions (Ca^{2+}). Whereas the Phosphoglycerate Mutase (PGAM2), had differences inside and between tenderness group. The expression highest values were observed at 2 days of aging on the tough Group and at 14 days of ageing on tender group. Also, was observed differences at 2 days of aging between tenderness groups. The PGAM2 is a metabolic protein involved in gluconeogenesis, glycolytic process and regulation of pentose-phosphate shunt.

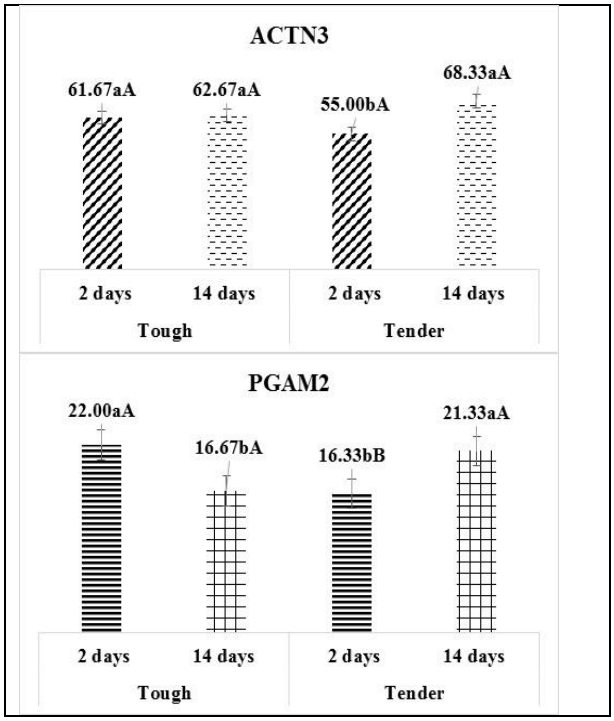

Figure 4. Total Spectrum Count of differentially expressed proteins, Actin (ACTN3) and Phosphoglycerate Mutase (PGAM2), in bovine *Longissimus* muscle with interaction effect between tenderness groups (tough and tender) and aging time (2 and 14 days).

H. CONCLUSION

The findings of this study revealed that two structural proteins (Desmin and Actin) and four metabolic proteins (PGAM2, F16P2, GPDA and LDHA) appear to be participating in the mechanism of meat tenderness. Apparently, the glycolytic process and gluconeogenesis are more important pathways involved in the meat tenderization process.

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