PROTEOME CHANGES OF BOVINE MUSCLE BASED ON ULTIMATE pH DURING AGING

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Abstract – The present study aimed to characterize the proteomic profile of the *Longissimus dorsi* (LD) beef samples during *post mortem* aging between two ultimate pH groups. Were evaluated 12 samples of bovine LD muscle, aged until 14 days and classified according final pH into two groups: normal (pHu_N: <5.80) and intermediate (pHu_I: 5.80-6.19). A total of 13 differentially expressed proteins responsible for metabolic, structural and stress-related functions were identified by the *GeLC-MS/MS* analysis. Only tropomyosin beta chain and malate dehydrogenase (cytoplasmic) were more abundant in intermediate pHu. This study allowed the identification of possible muscular biomarkers involved in several functions that affecting the meat quality during aging.

Key Words – Beef Quality, Biomarkers, Proteomics.

I. INTRODUCTION

The ultimate pH (pHu) is an indicator largely used in evaluating meat quality. When pHu ≥ 5.80 the muscle proteins associated with the water leaving the fibers a higher bond strength, featuring DFD meat - dark, firm and dry [1]. Proteomic approaches have been used for detected muscle changes during the post mortem aging and understanding how these changes affect the meat quality. Thus, the aim of this work was characterized the proteomic profile of Longissimus dorsi (LD) beef samples according to the different pHu ranges, in order to explore the role of differentially expressed proteins and to contribute to the discovery of biomarkers.

II. MATERIALS AND METHODS

Twelve samples from Nellore x South African Simmental beef cattle. After 48 hours *post mortem* the pH was measured (pHu) and were collect two steaks of 2.5 cm each between 12th and 13th ribs of LD. The steaks were aged for 1 and 14 days. Based on data from pHu, the beef samples were classified into two groups: Normal pH (pHu <5.80) and Intermediate pH (pHu 5.80-6.19) and were collected 6 samples from each group. Proteomic analysis was carried out on 6 muscle samples from normal and intermediate pH groups. Three biological replicates were used per group, a total of 18 protein extracts. The meat samples extracts were obtained according Carvalho et al [2]. For LC- MS/MS analyzes 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 [3].

III. RESULTS AND DISCUSSION

Thirteen differentially expressed proteins were identified between the normal and intermediated pH gropus (Table 1). Enzymes of the glycolytic pathway like TPI1 and PGAM2 were less expressed at intermediate pHu a 1 and 14 aging days, PKM2 was less expressed just at 1 aging day and AK1 was less expressed at 14 aging days, bough for intermediate pHu group, all these proteins are mainly responsible for the power supply. Also, MDH1, an enzyme present in gluconeogenesis, had higher expression in intermediate pHu only with 1 aging day, and it could be probably because there was a lower number of NAD+ binding enzymes due to the restricted access to the lactate substrate, necessary for the reaction.

TNNI2 and TNNC2 were less abundant in the intermediate pHu at 1 and 14 aging days and only 14 days, respectively. In normal pHu, the binding of Troponin to calcium ions prevents the inhibitory action of the troponin complex on actin filaments [4]. TPM2 had a higher expression in intermediate pHu at 14 days, possibly due to the degradation of this myofibrillar protein being higher in normal pHu meat samples. It was also found that CFL2, which regulates the reversibly polymerization and depolymerization of actin on pH influence [4], was more abundant in normal pHu at 14 aging days. The identified proteins related to the stress responses presented a similar behavior, with reduction of expression in the intermediate pHu at 1 aging day. CRYAB and HSP27 play a protective role in the cell and through their phosphorylation the actin-myosin complex is interrupted and this mechanism is related to meat tenderness [5].

Table 1. Differentially expressed proteins from bovine *Longissimus dorsi* muscle changed between intermediate ultimate pH (pHu_I) and normal ultimate pH (pHu_N) samples at 1 and 14 aging days by *GeLC-MS/MS* analysis.

Protein Name	Gene	MW[1] (kDa)	Uniprot ID	NUP[2]/ % SC[3]	1 day		14 days		Protein Function
					FC[4]	P-value	FC[4]	P-value	
Troponin I type 2 (skeletal, fast)	TNNI2	23	A5PJM2	4/16.00	-2.38	0.0330	-3.11	0.0270	Structural
Triosephosphate isomerase	TPI1	27	Q5E956	14/68.70	-1.93	0.0110	-1.81	0.0140	Metabolic
Phosphoglycerate mutase 2	PGAM2	29	F1N2F2	9/37.90	-1.79	0.0280	-1.51	0.0083	Metabolic
Protein deglycase DJ-1	PARK7	20	Q5E946	3/27.50	-4.20	0.0180	-	-	Defense/Stress
Alpha (B)-crystallin	CRYAB	20	P02510	10/56.60	-1.92	0.0190	-	-	Defense/Stress
HSP27	HSP27	22	Q3T149	10/60.70	-1.74	0.0170	-	-	Defense/Stress
Peroxiredoxin-1	PRDX1	22	Q5E947	3/15.60	-4.33	0.0270	-	-	Defense/Stress
Tropomyosin beta chain	TPM2	33	Q5KR48	15/42.60	+1.73	0.0320	-	-	Structural
Malate dehydrogenase (cytoplasmic)	MDH1	36	Q3T145	4/19.20	+4.75	0.0180	-	-	Metabolic
Pyruvate kinase	PKM2	58	A5D984	21/49.50	-1.32	0.0044	-	-	Metabolic
Troponin C type 2 (Fast)	TNNC2	18	Q148C2	6/24.30	-	-	-2.30	0.0480	Structural
Cofilin-2	CFL2	19	Q148F1	2/24.30	-	-	-2.50	0.0310	Structural
Adenylate kinase isoenzyme 1	AK1	22	P00570	8/47.40	-	-	-1.98	0.0450	Metabolic

[1]Molecular Weight; [2]Number of Unique Peptides; [3]Sequence Coverage; [4]Fold Change: ratio of pHu_I/pHu_N.

IV. CONCLUSION

There are some differences on proteomic profile of Longissimus muscle from Nellore x South African Simmental beef cattle based on ultimate pH values. The complementary studies with metabolic pathways characterization and protein-protein interaction will contribute to elucidate the importance of these proteins as biomarkers.

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