CHARACTERIZATION OF PROTEOLYSIS DURING MEAT AGING IN NELLORE CASTRATED CATTLE

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Abstract – This work aimed to verify the differences of protein expression among meat samples from Nellore castrated cattle with one, 7 and 14 days *post mortem* ageing, using two-dimensional electrophoresis (2-DE) and to evaluate explained variations between meat tenderness at 14 days *post mortem* and spots intensity. The 2-DE was performed in triplicate. A total of 18 gels for each time were done. The analysis of 54 gels detected 254 spots in common to the three times. Forty-three spots were removed of the analysis because they were not found in all periods or showed no change. Thus, 211 spots were submitted to ANOVA. Forty-one spots presented significant effects among the times of aging. Six spots explained 57% of the variability of meat tenderness at 14 days of aging.

Key Words – Meat tenderness, two-dimensional electrophoresis, shear force.

I. INTRODUCTION

The *Bos indicus* cattle have been preferred in tropical regions due to their disease resistance and high heat tolerance. However, meat tenderness problems have been attributed to the use of this breed. Among the beef quality traits, the tenderness has been considered the most important organoleptic trait by the consumers [1]. Consumers would be willing to pay more for a tender meat [2]. A study of Killinger et. al [3] showed that 65.4% of the consumers cite as preference the tenderness.

The degradation of myofibrillar proteins is an important factor in the process of meat aging. In this sense, the proteomic analysis seems to be a promising tool and is already being applied to describe modifications of these proteins during *post mortem* [4, 5, 6].

However, studies evaluating protein degradation are not completely established, nor which proteins are directly related to the meat tenderness process. In this context, two-dimensional electrophoresis (2-DE) is a relevant technique to identify potential predictors for meat tenderness.

The objective of this work was to verify the differences of expression among the meat samples from Nellore castrated cattle with one, seven and 14 days *post mortem*, using the two-dimensional electrophoresis (2-DE) and to evaluate explained variations between meat tenderness at 14 days *post mortem* and spots intensity.

II. MATERIALS AND METHODS

We used 16 Nellore castrated cattle, raised in pasture, feedlot and slaughtered at 19 months of age at the University´s experimental slaughterhouse belonging to the Coordinator of Campus Pirassununga, according to the humane slaughter techniques required by Brazilian law. Steaks were removed from the *Longissimus dorsi* muscle between the 10th and 12th rib, which were vacuum-packaged and aged at 2°C for one, seven and 14 days for the analyses of tenderness and proteomics. The steaks were removed from the packages and one gram of meat was collected to perform the 2-DE analysis. These samples were wrapped in aluminum and frozen in liquid nitrogen. Then, the samples were stored at –80°C. Meat samples were separated into six pools containing two or three animals per pool for the extraction of protein. Proteins were extracted as described by Lametsch et. al [4] and determined using 2-D Quant Kit (GE Healthcare). The 2-DE was performed in triplicate, a total of 18 gels for each time of aging. For isoelectric focus were used

strips of 13 cm pH 4-7 (Immobiline DryStrip a GE Healthcare), and the Ettan IPGphor (GE Healthcare).
The electrophoretic run was performed using SDS-12.5% PAGE. At the end of the run, the gels were stained with a solution of Coomassie R-250 and destained in methanol and acetic acid solution. The gels were scanned (ImageScanner III-GE Healthcare) and analyzed using the program ImageMaster 2D Platinum version 7.0 (GE Healthcare).
The spots were compared among the different times of aging through the means of the integrated intensity values of the spot.
The shear force was determined at the end of each time of aging. The steaks were roasted and stored overnight at −4ºC. In the next day, six cores with 13mm of diameter were taken for the shear force analysis using Warner-Bratzler equipment. The values were expressed in kgf.
Statistical analysis was performed using the the PROC MIXED procedure of the Statistical Analysis System (SAS), version 9.1.3.

III. RESULTS AND DISCUSSION

Differences in protein profiles were found based on a comparative study (match) of the gels within and among the times of aging. An average number of 384 spots were found for the 54 gels (among the times of aging), which 254 spots were common to the three times. From these 254 spots, 43 spots were not found in all the periods analyzed or showed no changes. Thus, a total of 211 spots were submitted to ANOVA.
The performance of multiple comparisons increases the chances of type I error and due to the high number of spots evaluated, the Bonferroni correction was used to control [7].
During the analysis of variance, effect of the time of aging ($P<0.0002$) was observed for the intensities of 41 spots. The means and standard errors of intensities for the 41 spots are shown in Table 1.
Regression analysis was conducted to investigate these 41 spots in function of the three times *post mortem*. It was verified that eight spots showed linear functions and 33 spots showed quadratic functions. For eight spots with linear functions, it was observed that the spot intensities reduced across the time of aging. For 33 spots with quadratic functions, 18 spots presented maximum points for expression intensities. In this case, the maximum points occurred at 8.81, 6.59, 8.37, 8.10, 1.24, 8.31, 4.55, 5.52, 8.28, 6.89, 8 25, 7.81, 1.08, 4.25, 7.67, 7.09, 8.03 and 7.39 days for the spots 3, 5, 9, 11, 22, 25, 49, 60, 64, 77, 79, 93, 109, 132, 143, 166, 175 and 182, respectively. For the others spots with quadratic functions were observed minimum points for expression intensities that occurred at 11.51, 10.34, 8.80, 13.61, 9.25, 10.58, 9.21 , 11.25, 11.38, 9.98, 9.24 10.34, 8.20, 11.03 and 9.67 days for the spots 21, 40, 68, 82, 99, 112, 121, 130, 138, 147, 149, 153, 160, 173 and 180, respectively.
Multiple regression analysis was performed for all spots that had significant effect in the analysis of variance. Table 2 presents the regression constant, regression coefficients, F values and their respective probability for each regression coefficient obtained in the analysis of the samples across the times.
The equation described in Table 2 showed a determination coefficient (R^2) of 0.5749, indicating moderate adjustment of the function in relation to the data on meat tenderness at 14 days *post mortem*.
Bjarnadóttir et al. [6] worked with eight cattle evaluating changes of expression intensities in two moments: one and 48 hours *post mortem*. The two-dimensional electrophoresis was performed in duplicate totaling 32 gels, where 300 spots were detected. From these, 35 spots showed significant changes during the first 48 hours *post mortem*. Jia et al. [8] evaluated proteomic changes in the first 24 hours *post mortem* in beef. These authors detected 105 spots, which 47 spots showed significant changes. Lametsch et al. [4], in a study using pigs, evaluated the differences in the intensity of protein expression at zero and 72 hours *post mortem*. In this study, it was identified 345 spots, which 103 spots had significant changes. No works were found using regression analysis to determine the standard changes that occurred across the times of aging for each peptide.

Table 1. Estimates of means (MEAN) and its standard error (SE) for the spot intensities significant spots in the samples evaluated with one, seven and 14 days of aging

	DAY 1			DAY 7			DAY 14		
SPOT	MEAN	SE		MEAN	SE		MEAN	SE	
3	0,634	0,04536	B	0,9364	0,04536	A	0,8126	0,04536	A
5	1,2417	0,05566	B	1,4927	0,05566	A	1,0519	0,05566	C
7	0,4984	0,01787	A	0,4209	0,01787	B	0,2822	0,01787	C
9	0,1109	0,007574	C	0,1771	0,007574	A	0,1397	0,007574	B
11	0,1406	0,009504	B	0,2085	0,009504	A	0,162	0,009504	B
15	0,4437	0,02376	A	0,2976	0,02376	B	0,1571	0,02376	C
18	0,835	0,04899	A	0,6065	0,04899	B	0,3327	0,04899	C
21	0,4211	0,02074	A	0,2901	0,02074	B	0,2694	0,02074	B
22	0,3447	0,02214	A	0,3017	0,02214	A	0,1331	0,02214	B
25	2,2098	0,1137	C	3,3204	0,1137	A	2,6631	0,1137	B
40	0,4191	0,01817	A	0,08655	0,0187	B	0,09649	0,01817	B
42	0,2316	0,01273	A	0,1321	0,01273	B	0,07164	0,01309	C
49	0,1047	0,00774	A	0,1082	0,00774	A	0,06222	0,007965	B
50	0,3506	0,01564	A	0,2815	0,01474	B	0,2043	0,01474	C
60	0,2327	0,0169	A	0,2684	0,01642	A	0,1315	0,0169	B
64	0,3518	0,01756	C	0,4788	0,01656	A	0,4027	0,01656	B
68	0,1127	0,00727	A	0,0543	0,007711	C	0,07836	0,00727	B
77	0,14	0,008364	B	0,1953	0,007635	A	0,1138	0,007635	C
79	2,8605	0,2575	B	4,6334	0,2649	A	3,5418	0,2731	B
82	0,4835	0,01932	A	0,3599	0,01763	B	0,3132	0,01763	B
93	0,1201	0,01108	B	0,2171	0,00977	A	0,1377	0,00977	B
97	0,5498	0,03373	A	0,335	0,03471	B	0,1991	0,03695	C
99	0,4529	0,02658	A	0,1866	0,03014	B	0,2607	0,02658	B
103	0,3866	0,02256	A	0,3542	0,0206	A	0,2051	0,02119	B
109	0,1978	0,01467	A	0,1672	0,01426	A	0,05184	0,01616	B
112	0,1244	0,006845	A	0,08429	0,006211	B	0,08376	0,006036	B
121	0,4442	0,01949	A	0,2668	0,02083	B	0,3179	0,01837	B
130	1,3818	0,08118	A	0,5622	0,1038	B	0,4636	0,08118	B
132	0,2265	0,01631	A	0,231	0,01585	A	0,09569	0,01942	B
137	0,1094	0,008405	A	0,06564	0,01128	B	0,04812	0,008649	B
138	0,3564	0,01584	A	0,09796	0,01796	B	0,06183	0,01864	B
143	0,05692	0,006809	B	0,09403	0,004955	A	0,06111	0,004815	B
147	0,7067	0,04043	A	0,2506	0,06483	B	0,297	0,04043	B
149	0,3225	0,01379	A	0,1782	0,0178	B	0,2185	0,01259	B
153	0,2261	0,01606	A	0,1227	0,02272	B	0,1257	0,01514	B
160	1,5237	0,05771	A	0,2276	0,09423	C	1,0546	0,05441	B
166	0,103	0,01371	B	0,2134	0,01939	A	0,07096	0,01371	B
173	0,1405	0,01103	A	0,05322	0,01306	B	0,04556	0,01103	B
175	0,04317	0,0062	B	0,0801	0,004384	A	0,05267	0,004101	B
180	0,311	0,02102	A	0,1204	0,02973	B	0,1529	0,02031	B
182	0,2234	0,03705	B	0,5434	0,06051	A	0,2011	0,03961	B

Table 17. Estimates of the regression constant, the regression coefficient F values and respective probability of each regression coefficient for the samples evaluated immediately to the period of aging

Coefficients	Estimates (bi)	SE	F Value	Pr > F
Intercept	-4,71437	3,84457	1,5	0,2277
SPOT_5	5,36661	2,10522	6,5	0,015
SPOT_15	60,65962	12,5276	23,45	<.0001
SPOT_18	-15,48662	5,70921	7,36	0,01
SPOT_21	-12,59449	4,1406	9,25	0,0042
SPOT_22	-21,47729	5,68001	14,3	0,0005
SPOT_25	2,63407	0,81277	10,5	0,0025

IV. CONCLUSION

Our results indicate that changes in protein profile may be a relevant mechanism related to the proteolysis process. Studies evaluating physico-chemical characteristics of beef for the spots that changed across the times will enable a better understanding of the mechanisms involved in the meat aging.

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