

# INFLUENCE OF ULTIMATE pH ON THE MEAT TENDERNESS AND HEAT SHOCK PROTEIN EXPRESSION FROM BEEF CATTLE

Pereira A. Mikaele<sup>1</sup>, Rosa F. Alessandra \*, Fonseca D. Leidyana<sup>1</sup>, Moncau T. Cristina † Poleti, D. Mirele<sup>2</sup> and Eler P. Joanir<sup>1</sup>

<sup>1</sup>Faculty of Animal Science and Food Engineering, University of São Paulo, Pirassununga-SP, 13635-900, Brasil

<sup>2</sup>Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil

\*Corresponding author email: afrosa@usp.br

**Abstract** – The aim of this study was evaluate the influence of ultimate pH on the meat quality characteristics and Heat Shock Proteins expression from beef cattle. Were evaluated 303 F1 immuno-castrated 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. After 48 hours *post mortem* de pH was measured (pHu) and were collect two steaks of 2.5 cm each between 12<sup>th</sup> and 13<sup>th</sup> ribs of Longissimus muscle for Warner Bratzler Shear Force (WBSF) and Heat Shock Proteins 27 and 70 expressions. Beef samples were classified into two groups according to pHu: Normal (pHu ≤5,8) and High (pHu ≥5,80) and were collected 19 samples from each group. There were observed differences for WBSF values (14 days) between Normal and High groups, an increase of HSP 27 and 70 expression values during the aging time and higher values from HSP27 for High pH group than Normal pH group. In conclusion, there are some differences on HSP 27 expression between different pH ranges and during the aging time, so more studies are necessary for elucidating the relation of HSP and meat quality.

**Key Words** – Heat Shock Protein, pH, tenderness, beef meat quality

## I. INTRODUCTION

Meat tenderness is the most important criteria for the consumer acceptance and the use of the ultimate pH (pHu) as a meat quality predictor, is widely used in the meat quality assessment. In particular, the speed and the rate of pH decline have directly influence on the denaturation of proteins, arrangement of myofibrillar proteins and on the muscle shrinkage, being therefore had fundamental importance in the determination of meat quality. Some studies showed that pH ranges between 5.8 and 6.2 can affect negatively the meat

tenderness [1, 2]. If the pHu be high ≥5,80, the fisical state of proteins will be higher than their isoelectric point and the proteins will have a strong association with water holding capacity. Through proteomic studies, some researchers found differential expression of small Heat Shock Proteins in muscle with variable meat quality characteristics like pH and tenderness [4,5,6]. Also, others studies reported that some variations on the ultimate pH can affect HSP expression in the muscle and consequently meat quality. The HSP principal function is to preserve cellular proteins against denaturation and possible loss function [7].

So, the aim of this study was to evaluate the influence of ultimate pH on the meat quality characteristics and Heat Shock Proteins expression beef cattle.

## II. MATERIALS AND METHODS

### A. Animals and Experimental Procedure

Were evaluated 303 F1 immuno- castrated 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* de pH was measured (pHu) and were collect two steaks of 2.5 cm each between 12<sup>th</sup> and 13<sup>th</sup> ribs of Longissimus muscle. The steaks were individually identified, vacuum packaged and aged for 1 and 14 days after slaughter.

### B. pH groups

The pH was measured at 48 hours post mortem with pHmeter (Hanna Instruments), at 13<sup>th</sup> ribs of Longissimus muscle. From 303 carcasses, were detected 19 carcasses with ultimate pH  $\geq 5,80$ , so, beef samples were classified into two groups: normal pH (pH<sub>N</sub>: pH<sub>u</sub>  $\leq 5,8$ ) and high pH (pH<sub>H</sub>: pH<sub>u</sub>  $\geq 5,80$ ) and were collected 19 samples from each group.

### C. Meat Tenderness and sampling

In each aging time (1 and 14 days), the vacuum packaging was opened and one piece of meat was cut and immediately frozen in liquid nitrogen for further Heat Shock Protein analysis. Subsequently, the Warner Bratzler Shear Force (WBSF) measurements were determined according to AMSA (1995).

### D. Heat Shock Protein Quantification

The heat shock protein expression quantification was determined with Bovine Heat Shock Protein (HSP 27 and HSP 70) ELISA kits (Mybiosource). The total protein content was calculated by Bradford method.

### E. Statistical Analysis

The data were subjected to verification of the data analysis assumptions (normality and homogeneity of variances) using the PROC UNIVARIATE then the data will go through analysis of variance by PROC MIXED adopting the significance level of 5% ( $p < 0.05$ ) using the following statistical model:  $Y_{ij} = \mu + pHu_i + T_j + (pHu_i * T_j) + e_{ij}$ , where:  $Y_{ij}$  = observed value of the dependent variable studied;  $\mu$  = general average;  $pHu_i$  = effect of  $pHu_u$ , where  $i$  = normal pH<sub>u</sub> and high pH<sub>u</sub>;  $T_j$  = effect of time of maturation,  $j = 1$  and 14 days;  $pHu_i * T_j$  = effect of the interaction between the factors pH<sub>u</sub>  $i$  and  $T_j$ ;  $e_{ij}$  = residual effect associated with the trait, normally distributed with mean 0 and variance  $\sigma^2$ . All procedures were performed with statistical software SAS 9.3.

## III. RESULTS AND DISCUSSION

There were differences for WBSF values (14 days) between Normal and High groups (Table 1). The WBSF values were higher from High pH group than the Normal pH group.

Table 1. Mean values of ultimate pH and Warner Bratzler Shear force (WBSF).

Trait	pHu		EPM	p-value
	Normal	High		
Ultimate pH	5.52	5.87	0.030	< 0.0001
WBSF (kg) – 1 day	9.46	8.25	0.475	0.2060
WBSF (kg) – 14 days	5.23	7.31	0.429	0.0129

There was an increase of HSP 27 and 70 expression values during aging time (Table 2). However, other studies related that the highest concentrations of sHSPs during age time was positively correlated with meat tenderness, what may be due to increased fragmentation of actin in the meat maturation process, facilitating the action of proteases during the *post mortem* [4].

Table 2. HSPs 27 and 70 expression values (pg/ mg protein) by aging time (1 and 14 days).

	Aging time (days)		EPM	p-value
	1	14		
HSP27	125.55	184.46	8.351	0.0009
HSP70	20.85	29.30	1.421	0.0008

Also, it was observed higher values of HSP27 for High pH group than Normal pH group (Table 3).

Normally, the HSPs are present at low levels in the muscle until the occurrence of a stressful event, which triggers increase their synthesis [8].

Table 3. HSPs 27 and 70 expression values (pg/ mg protein) pHu group (Normal and High).

	pHu		EPM	p-value
	Normal	High		
HSP27	138.39	174.42	8.351	0.0485
HSP70	23.14	26.67	1.421	0.3461

The total of HSP 27 expression values didn't differ between 1 and 14 days from normal pH group but in high pH, the values increased from 1 to 14 days of aging (Figure 1). On the other hand, there were

differences ( $P < 0.05$ ) between samples from normal and high pH<sub>u</sub> at 1 day of aging.

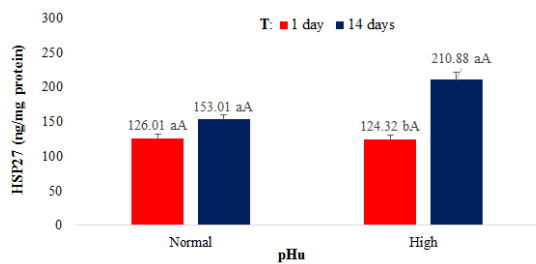


Figure 1. HSP 27 values (pg/mg protein) by aging time and pHu group. Different Single or Capital letter shows the differences between aging time and pH class, respectively.

The high sHSPs concentration may be related to the delayed of aging, affecting directly and negatively meat tenderness [10].

For HSP70 was observed higher values only from 14 days of aging, inside the High pH group (Figure 2). The HSP70 use energy in the form of ATP to restructure the myofibrillar proteins that have undergone denaturation [11].

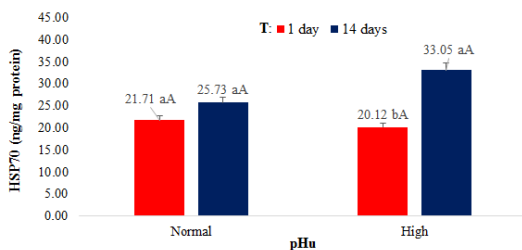


Figure 2. HSP 70 values (pg/mg protein) by aging time and pHu group. Different Single or Capital letter shows the differences between aging time and pH class, respectively.

#### IV. CONCLUSION

In conclusion, there are some differences on HSP 27 expression between different pH ranges and during the aging time. However, the expression of HSPs determined in this work, can not be considered conclusive, since it is only part of the large study. Further exploration of the cellular mechanisms involved in the synthesis and expression of sHSPs can show more concise results.

#### ACKNOWLEDGEMENTS

We acknowledge to São Paulo Research Foundation (FAPESP – Proc. N 2014/12492-8) and National Counsel of Technological and Scientific Development - (CNPQ – Proc. N. 454546/2014-9 and 150358/2015-6) - Brazil, for financial support

#### REFERENCES

- Balan, P., Kim, Y.H.B., Blijenburg, R. (2014). Small heat shock protein degradation could be an indicator of the extent of myofibrillar protein degradation. *Meat Science*, 97:220-222.
- Lomiwes, D., Farouk, M. M., Frost, D. A., Dobbie, P.M., Young, O. A. (2013). Small heat shock proteins and toughness in intermediate pHu beef. *Meat Science*, 95:472–479.
- Lomiwes, D., Farouk, M. M., Wiklund E., Young, O. A. (2014). Small heat shock proteins and their role in meat tenderness: A review. *Meat Science*, 96:26-40.
- Carvalho, M. E., Gasparin, G., Poleti, M., Rosa, A. F., Balieiro, J. C. C., Labate, C. A., Nassu, R. T., Tullio, R. R., Regitano, L. C. A., Mourão, G. B. & Coutinho, L. L. (2014). Heat shock and structural proteins associated with meat tenderness in Nellore beef cattle, a *Bos indicus* breed. *Meat Science*. 96: 1318-1324.
- Pulford, D. J., Fraga Vazquez, S., Frost, D. F., Fraser-Smith, E., Dobbie, P. & Rosenvold, K. (2008). The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. *Meat Science*. 79: 623-630.
- Hollung, K., Veiseth, E., Jia, X., Faergestad, E. M. & Hildrum, K. I. (2007). Application of proteomics to understand the molecular mechanisms behind meat quality. *Meat Science*. 77: 97-104.
- Ouali, A., Herrera-Mendez, C. H., Coulis, G., Becila, S., Boudejallal, A., Aubry, L. & Sentandreu, M. A. (2006). Revisiting the conversion of muscle into meat and the underlying mechanisms. *Meat Science*. 74: 44-58.
- Sugiyama, Y., Suzuki, A., Kishikawa, M., Aktsu, R., Hirose, T., Waye, M. M. Y., Tsui, S. K. W., Yoshida, S. & Ohno, S. (2000). Muscle develops a specific forma of small heat shock protein complex composed of MKBP/HSPB2 and HSPB3

druing myogenic differentiation. *Journal of Biological Chemistry*. 275: 1095-1104.

9. Lomiwes, D., Hurst, S. M., Dobbie, P., Frost, D. A., Hurst, R. D., Young, O. A. & Farouk, M. M. The protection of bovine skeletal myofibrils from proteolytic damage post mortem by small heat shock proteins. *Meat Science*. 97: 548-557.
10. Wang, K. & Spector, A. (2001). ATP causes small heat shock proteins to release denatured protein. *European Journal of Biochemistry*. 268: 6335-6345.
11. Conteras-Catillo, C. J., Lomiwes, D., Wu, G., Frost, D. & Farouk, M. M. (2016). The effect electrical stimulation on post mortem myofibrillar protein degradation and small heat shock protein kinetics in bull beef. *Meat Science*. 113: 65-72.