Universidade de São Paulo
Escola Superior de Agricultura "Luiz de Queiroz"

Influência do pH final nos parâmetros físico-químicos, bioquímicos e sensoriais associados à qualidade da carne de bovinos *Bos indicus*

Iliani Patinho

Tese apresentada para obtenção do título de Doutora em Ciências. Área de concentração: Ciência e Tecnologia de Alimentos

Piracicaba
2023

Iliani Patinho
Tecnóloga em Alimentos

Influência do pH final nos parâmetros físico-químicos, bioquímicos e sensoriais associados à qualidade da carne de bovinos *Bos indicus*

versão revisada de acordo com a Resolução CoPGr 6018 de 2011

Orientadora:
Profª. Drª. **CARMEN JOSEFINA CONTRERAS-CASTILLO**

Tese apresentada para obtenção do título de Doutora em Ciências. Área de concentração: Ciência e Tecnologia de Alimentos

Piracicaba
2023

Dados Internacionais de Catalogação na Publicação
DIVISÃO DE BIBLIOTECA - DIBD/ESALQ/USP

Patinho, Iliani
Influência do pH final nos parâmetros físico-químicos, bioquímicos e sensoriais associados à qualidade da carne de bovinos *Bos indicus*/ Iliani Patinho - - versão revisada de acordo com a resolução CoPGr 6018 de 2011 - - Piracicaba, 2023.

161 p.

Tese (Doutorado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”

1. Referencial teórico 2. Proteômica 3. Maciez 4. Perfil sensorial I. Título

2 PROTEOME OF HIGH AND NORMAL pH BEEF: FIRST INSIGHTS EMPHASIZING THE DYNAMIC PROTEIN CHANGES OF THE MUSCLE OVER DIFFERENT *POST-MORTEM* TIMES

[2] Artigo submetido em periódico internacional, 2023.
Paper under review

2.1. Introduction

Brazil has the largest bovine herd in the world and is the largest beef exporter, with a beef livestock agribusiness movement of approximately US$ 169.29 billion. Most of the Brazilian herd has influence of zebu cattle (*Bos taurus indicus*), mainly Nellore breed, which combine productivity, disease resistance and heat tolerance once animals are predominantly finished on pasture (ABIEC, 2022; Carvalho et al., 2014). However, although beef cattle have adapted well to tropical conditions in Brazil (Carvalho et al., 2014; Rodrigues et al., 2017; Malheiros et al., 2020), beef cattle finished in extensive systems (pasture-based) is usually darker in color appearance and produces a less tender cooked product than those cattle finished in feedlot systems (grain-based) (Antonelo et al., 2022; Gómez et al., 2022) which may be directly related to the rate and extent of muscle pH decline. In fact, ultimate pH (pHu) is associated with muscle glycogen and *postmortem* energy metabolism. Several factors influence muscle pHu, including increased physical activity, feed deprivation, season, and pre-slaughter stress. These events are well-known to rapidly reduce muscle glycogen stores (Zhao et al., 2022; Terlouw et al., 2021; García-Torres et al., 2021) which leads to a high pHu (Poleti et al., 2018; Kiyimba et al., 2021) that negatively affect the final beef quality (Wu et al., 2020; England et al., 2016; Franco et al., 2015). As a result, carcasses with darker lean are generally downgraded during the process of grading, leading to significant economic losses globally for the beef sector (Gagaoua, Warner, et al., 2021; Ponnampalam et al., 2017). Thus, it is essential to avoid failures in the categorization of carcasses according to the pHu and develop early *postmortem* tools for their monitoring.

Gagaoua, Warner, et al. (2021) reported that proteomics techniques together with mass spectrometry (MS) and bioinformatics approaches have been widely used to show the mechanisms that drive the beef quality development in *postmortem* muscle, in addition to investigating the dynamic changes and modifications that occur in the muscle proteome and search for candidate biomarkers (Picard & Gagaoua, 2020). Proteomics was successfully applied to the discovery of biomarkers of dark-cutting beef, however a strong disparity among the studies was revealed using a meta-analysis integromics approach (Gagaoua, Warner, et al., 2021). Although certain consistent pathways were reported, the authors evidenced a very weak overlap in the putative biomarkers among the eight dark-cutting beef proteomics studies (< 8%) with only 10 common protein biomarkers reported across no more than two studies. From the ten common proteins, eight were related to dark-cutting beef conditions in different directions (positive and negative). It was suggested that the molecular signatures underpinning dark-cutting beef development are more complex than expected, and exploratory studies of the muscle proteomes of the same animals at both early *postmortem* and at the usual sampling times used to categorized carcasses based on their high pHu (24 or 48h) would allow further insights on the biochemical mechanisms and discovery of more appropriate biomarkers. Foreseeing the above, the current study used a shotgun proteomics approach to fill the gap in the early *postmortem* muscle metabolism in *Bos indicus* cattle, aiming to evaluate for the first time in these animals and by following the temporal dynamic changes of high and normal muscle pHu at three different times these being 30 min, 9 h and 44 h *postmortem*. The study further aimed to reveal the unknowns behind the protein changes and the possibility of proposing a more robust list of biomarkers of dark-cutting beef condition.

2.2. Conclusion

The temporal shotgun proteomics coupled with an in-depth bioinformatics analysis allowed for the first time to evidence the dynamic time-course changes and molecular signatures underpinning the dark-cutting beef condition

development. The pivotal role of energy metabolism, cellular response to stress, muscle system process and oxidoreductase activity were significantly revealed throughout the early *postmortem* metabolism. Twenty-three proteins overlap among *postmortem* times (30 min, 9 h and 44 h *postmortem*) and may be suggested as candidate biomarkers to monitor the proteome changes in the dark-cutting development.

References

ABIEC. (2022). Beef Report–Perfil da Pecuária no Brasil*. Associação Brasileira das Indústrias Exportadoras de Carnes.* Retrieved from https://www.abiec.com.br/publicacoes/beef-report-2022/

Antonelo, D. S., Gómez, J. F. M., Silva, S. L., Beline, M., Zhang, X., Wang, Y., … Balieiro, J. C. C. (2022). Proteome basis for the biological variations in color and tenderness of longissimus thoracis muscle from beef cattle differing in growth rate and feeding regime. *Food Research International*, *153*, 110947. https://doi.org/10.1016/j.foodres.2022.110947

Antonelo, D. S., dos Santos-Donado, P. R., Ferreira, C. R., Colnago, L. A., Ocampos, F. M. M., Ribeiro, G. H., … Balieiro, J. C. C. (2022). Exploratory lipidome and metabolome profiling contributes to understanding differences in high and normal ultimate pH beef. *Meat Science*, *194*, 108978. https://doi.org/10.1016/j.meatsci.2022.108978

Baylor, S. M., & Hollingworth, S. (2012). Intracellular calcium movements during excitation-contraction coupling in mammalian slow-twitch and fast-twitch muscle fibers. *Journal of General Physiology*, *139*(4), 261–272. https://doi.org/10.1085/jgp.201210773

Bendall, J. R. (1973). The structure and function of muscleG. H. Bournes (Ed.). In Post mortem changes in muscle.

Bouley, J., Chambon, C., & Picard, B. (2004). Mapping of bovine skeletal muscle proteins using two-dimensional gel electrophoresis and mass spectrometry. *Proteomics*, *4*, 1811–1824. https://doi.org/10.1002/pmic.200300688

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/https://doi.org/10.1016/0003-2697(76)90527-3

Brohée, S., & van Helden, J. (2006). Evaluation of clustering algorithms for protein-protein interaction networks. *BMC Bioinformatics*, *7*(488). https://doi.org/10.1186/1471-2105-7-488

Carafoli, E. (2002). Calcium signaling: A tale for all seasons. *PNAS*, *99*(3), 1115–

1122. https://doi.org/10.1073/pnas.032427999

Carvalho, M. E., Gasparin, G., Poleti, M. D., Rosa, A. F., Balieiro, J. C. C., Labate, C. A., … Coutinho, L. L. (2014). Heat shock and structural proteins associated with meat tenderness in Nellore beef cattle, a Bos indicus breed. *Meat Science*, *96*(3), 1318–1324. https://doi.org/10.1016/j.meatsci.2013.11.014

Coe, H., & Michalak, M. (2009). Calcium binding chaperones of the endoplasmic reticulum. *Gen Physiol Biophys*, *28*, F96–F103.

Dang, D. S., Buhler, J. F., Davis, H. T., Thornton, K. J., Scheffler, T. L., & Matarneh, S. K. (2020). Inhibition of mitochondrial calcium uniporter enhances postmortem proteolysis and tenderness in beef cattle. *Meat Science*, *162*, 108039. https://doi.org/10.1016/j.meatsci.2019.108039

Daugaard, M., Rohde, M., & Jäättelä, M. (2007). The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. *FEBS Letters*, *581*, 3702–3710. https://doi.org/https://doi.org/10.1016/j.febslet.2007.05.039

Dubinsky, W. P. (1987). The Study of Transport and Enzymatic Processes in Reconstituted Biological Systems. In Andreoli, T.E., J. F. Hoffman, D. D. Fanestil, & S. G. Schultz (Eds.), *Membrane Physiology* (Second, pp. 167–173). https://doi.org/https://doi.org/10.1007/978-1-4613-1943-6_10

Elahian, F., Sepehrizadeh, Z., Moghimi, B., & Abbas, S. (2014). Critical Reviews in Biotechnology Human cytochrome b5 reductase: structure , function , and potential applications. *Critical Reviews in Biotechnology*, *34*(2), 134–143. https://doi.org/10.3109/07388551.2012.732031

England, E. M., Matarneh, S. K., Scheffler, T. L., Wachet, C., & Gerrard, D. E. (2015). Altered AMP deaminase activity may extend postmortem glycolysis. *Meat Science*, *102*, 8–14. https://doi.org/10.1016/j.meatsci.2014.11.009

England, E. M., Matarneh, S. K., Oliver, E. M., Apaoblaza, A., Scheffler, T. L., Shi, H., & Gerrard, D. E. (2016). Excess glycogen does not resolve high ultimate pH of oxidative muscle. *Meat Science*, *114*, 95–102. https://doi.org/10.1016/j.meatsci.2015.10.010

Ertbjerg, P. (2022). Current understanding on the role of proteolysis on meat quality. In *New Aspects of Meat Quality: From Genes to Ethics, Second Edition* (pp. 95–114). Elsevier Ltd. https://doi.org/10.1016/B978-0-323-85879-3.00022-2

Franco, D., Mato, A., Salgado, F. J., López-Pedrouso, M., Carrera, M., Bravo, S., … Zapata, C. (2015). Tackling proteome changes in the longissimus thoracis bovine muscle in response to pre-slaughter stress. *Journal of Proteomics*, *122*, 73–85. https://doi.org/10.1016/j.jprot.2015.03.029

Fuente-Garcia, C., Aldai, N., Sentandreu, E., Oliván, M., García-Torres, S., Franco, D., … Sentandreu, M. A. (2019). Search for proteomic biomarkers related to bovine pre-slaughter stress using liquid isoelectric focusing

(OFFGEL) and mass spectrometry. *Journal of Proteomics*, *198*, 59–65. https://doi.org/10.1016/j.jprot.2018.10.013

Fuente-Garcia, C., Sentandreu, E., Aldai, N., Oliván, M., Ángel, M., & Sentandreu. (2020). Characterization of the myofibrillar proteome as a way to better understand differences in bovine meats having different ultimate pH values. *Proteomics*, *20*(12), 2000012. https://doi.org/https://doi.org/10.1002/pmic.202000012

Gagaoua, M., Bonnet, M., Ellies-Oury, M.-P., Koning, L. De, & Picard, B. (2018). Reverse phase protein arrays for the identi fi cation / validation of biomarkers of beef texture and their use for early classi fi cation of carcasses. *Food Chemistry*, *250*, 245–252. https://doi.org/10.1016/j.foodchem.2018.01.070

Gagaoua, M., Bonnet, M., & Picard, B. (2020). Protein Array-Based Approach to Evaluate Biomarkers of Beef Tenderness and Marbling in Cows: Understanding of the Underlying Mechanisms and Prediction. *Foods*, *9*(9), 1180. https://doi.org/10.3390/foods9091180

Gagaoua, M., Terlouw, E. M. C., Boudjellal, A., & Picard, B. (2015). Coherent correlation networks among protein biomarkers of beef tenderness: What they reveal. *Journal of Proteomics*, *128*, 365–374. https://doi.org/10.1016/j.jprot.2015.08.022

Gagaoua, M., Hafid, K., Boudida, Y., Becila, S., Ouali, A., Picard, B., & Boudjellal, A. (2015). Caspases and Thrombin Activity Regulation by Specific Serpin Inhibitors in Bovine Skeletal Muscle. *Appl Biochem Biotechnol*, *177*, 279–303. https://doi.org/https://doi.org/10.1007/s12010-015-1762-4

Gagaoua, M., Hughes, J., Terlouw, E. M. C., Warner, R. D., Purslow, P. P., Lorenzo, J. M., & Picard, B. (2020). Trends in Food Science & Technology Proteomic biomarkers of beef colour. *Trends in Food Science & Technology*, *101*, 234–252. https://doi.org/10.1016/j.tifs.2020.05.005

Gagaoua, M., Schilling, W. M., Zhang, X., & Suman, S. P. (2022). *Applications of proteomics in meat research*. *Reference Module in Food Science* (Third Edit). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-323-85125-1.00123-X

Gagaoua, M., Terlouw, E. M. C., Richardson, I., Hocquette, J.-F., & Picard, B. (2019). The associations between proteomic biomarkers and beef tenderness depend on the end-point cooking temperature, the country origin of the panelists and breed. *Meat Science*, *157*, 107871. https://doi.org/10.1016/j.meatsci.2019.06.007

Gagaoua, M., Terlouw, E. M. C., Micol, D., Boudjellal, A., Hocquette, J.-F., & Picard, B. (2015). Understanding Early Post-Mortem Biochemical Processes Underlying Meat Color and pH Decline in the Longissimus thoracis Muscle of Young Blond d ' Aquitaine Bulls Using Protein Biomarkers. *Journal of Agricultural and Food Chemistry*, *63*, 6799–6809. https://doi.org/10.1021/acs.jafc.5b02615

Gagaoua, M., Terlouw, E. M. C., Mullen, A. M., Franco, D., Warner, R. D., Lorenzo, J. M., … Picard, B. (2021). Molecular signatures of beef tenderness : Underlying mechanisms based on integromics of protein biomarkers from multi-platform proteomics studies. *Meat Science*, *172*, 108311. https://doi.org/10.1016/j.meatsci.2020.108311

Gagaoua, M., Terlouw, E. M. C., & Picard, B. (2017). The study of protein biomarkers to understand the biochemical processes underlying beef color development in young bulls. *Meat Science*, *134*, 18–27. https://doi.org/10.1016/j.meatsci.2017.07.014

Gagaoua, M., Troy, D., & Mullen, A. M. (2021). The Extent and Rate of the Appearance of the Major 110 and 30 kDa Proteolytic Fragments during Post-Mortem Aging of Beef Depend on the Glycolysing Rate of the Muscle and Aging Time: An LC − MS/ MS Approach to Decipher Their Proteome and Associated Pathway. *Journal of Agricultural and Food Chemistry*, *69*, 602–614. https://doi.org/https://doi.org/10.1021/acs.jafc.0c06485

Gagaoua, M., Warner, R. D., Purslow, P., Ramanathan, R., Mullen, A. M., López-Pedrouso, M., … Terlouw, E. M. C. (2021). Dark-cutting beef: A brief review and an integromics meta-analysis at the proteome level to decipher the underlying pathways. *Meat Science*, *181*, 108611. https://doi.org/10.1016/j.meatsci.2021.108611

García-Torres, S., Cabeza de Vaca, M., Tejerina, D., Romero-Fernández, M. P., Ortiz, A., Franco, D., … Oliván, M. (2021). Assessment of stress by serum biomarkers in calves and their relationship to ultimate ph as an indicator of meat quality. *Animals*, *11*, 2291. https://doi.org/10.3390/ani11082291

Giorgi, C., Bonora, M., Sorrentino, G., Missiroli, S., Poletti, F., Suski, J. M., … Pinton, P. (2015). p53 at the endoplasmic reticulum regulates apoptosis in a Ca2 + -dependent manner. *PNAS*, *112*(6), 1779–1784. https://doi.org/10.1073/pnas.1410723112

Gómez, J. F. M., Cônsolo, N. R. B., Antonelo, D. S., Beline, M., Gagaoua, M., Higuera-Padilla, A., … Silva, S. L. (2022). Impact of Cattle Feeding Strategy on the Beef Metabolome. *Metabolites*, *12*, 640. https://doi.org/10.3390/metabo12070640

Green, D. R., Galluzzi, L., & Kroemer, G. (2014). Metabolic control of cell death. *Science*, *345*(6203). https://doi.org/10.1126/science.1250256

Hughes, J., Clarke, F., Li, Y., Purslow, P., & Warner, R. (2019). Differences in light scattering between pale and dark beef longissimus thoracis muscles are primarily caused by differences in the myofilament lattice, myofibril and muscle fibre transverse spacings. *Meat Science*, *149*, 96–106. https://doi.org/https://doi.org/10.1016/j.meatsci.2018.11.006

Hughes, J. M., Clarke, F. M., Purslow, P. P., & Warner, R. D. (2019). Meat color is determined not only by chromatic heme pigments but also by the physical structure and achromatic light scattering properties of the muscle. *Comprehensive Reviews in Food Science and Food Safety*, 1–20.

https://doi.org/10.1111/1541-4337.12509

Ishii, H., Shirai, T., Makino, C., & Nishikata, T. (2014). Mitochondrial inhibitor sodium azide inhibits the reorganization of mitochondria-rich cytoplasm and the establishment of the anteroposterior axis in ascidian embryo. *Development, Growth & Differentiation*, *56*, 175–188. https://doi.org/10.1111/dgd.12117

Jiang, B., Liang, P., Deng, G., Tu, Z., Liu, M., & Xiao, X. (2011). Increased stability of Bcl-2 in HSP70-mediated protection against apoptosis induced by oxidative stress. *Cell Stress and Chaperones*, *16*, 143–152. https://doi.org/10.1007/s12192-010-0226-6

Kiyimba, F., Hartson, S. D., Rogers, J., Vanoverbeke, D. L., Mafi, G. G., & Ramanathan, R. (2022). Dark-cutting beef mitochondrial proteomic signatures reveal increased biogenesis proteins and bioenergetics capabilities. *Journal of Proteomics*, *265*, 104637. https://doi.org/10.1016/j.jprot.2022.104637

Kiyimba, F., Hartson, S. D., Rogers, J., VanOverbeke, D. L., Mafi, G. G., & Ramanathan, R. (2021). Changes in glycolytic and mitochondrial protein profiles regulates postmortem muscle acidification and oxygen consumption in dark-cutting beef. *Journal of Proteomics*, *232*, 104016. https://doi.org/10.1016/j.jprot.2020.104016

Lamri, M., della Malva, A., Djenane, D., Albenzio, M., & Gagaoua, M. (2023). First insights into the dynamic protein changes in goat Semitendinosus muscle during the post-mortem period using high-throughput proteomics. *Meat Science*, *202*, 109207. https://doi.org/10.1016/j.meatsci.2023.109207

Lamri, M., della Malva, A., Djenane, D., López-Pedrouso, M., Franco, D., Albenzio, M., … Gagaoua, M. (2023). Towards the discovery of goat meat quality biomarkers using label-free proteomics. *Journal of Proteomics*, *278*, 104868. https://doi.org/10.1016/j.jprot.2023.104868

Lee, S. M., Kim, J. H., Cho, E. J., & Youn, H. D. (2009). A nucleocytoplasmic malate dehydrogenase regulates p53 transcriptional activity in response to metabolic stress. *Cell Death and Differentiation*, *16*, 738–748. https://doi.org/10.1038/cdd.2009.5

Lek, M., Quinlan, K. G. R., & North, K. N. (2009). The evolution of skeletal muscle performance: gene duplication and divergence of human sarcomeric a-actinins. *BioEssays*, *32*, 17–25. https://doi.org/https://doi.org/10.1002/bies.200900110

Lièvremont, J.-P., Rizzuto, R., Hendershot, L., & Meldolesi, J. (1997). BiP, a Major Chaperone Protein of the Endoplasmic Reticulum Lumen, Plays a Direct and Important Role in the Storage of the Rapidly Exchanging Pool of Ca2+. *The Journal of Biological Chemistry*, *272*(49), 30873–30879. https://doi.org/10.1074/jbc.272.49.30873

Lomiwes, D., Farouk, M. M., Wu, G., & Young, O. A. (2014). The development of

meat tenderness is likely to be compartmentalised by ultimate pH. *Meat Science*, *96*, 646–651. https://doi.org/10.1016/j.meatsci.2013.08.022

Longo, V., Lana, A., Bottero, M. T., & Zolla, L. (2015). Apoptosis in muscle-to-meat aging process: The omic witness. *Journal of Proteomics*, *125*, 29–40. https://doi.org/10.1016/J.JPROT.2015.04.023

López-Pedrouso, M., Lorenzo, J. M., Cittadini, A., Sarries, M. V, Gagaoua, M., & Franco, D. (2022). A proteomic approach to identify biomarkers of foal meat quality: A focus on tenderness, color and intramuscular fat traits. *Food Chemistry Journal*, *405*, 134805. https://doi.org/10.1016/j.foodchem.2022.134805

Mahmood, S., Turchinsky, N., Paradis, F., Dixon, W. T., & Bruce, H. L. (2018). Proteomics of dark cutting longissimus thoracis muscle from heifer and steer. *Meat Science*, *137*, 47–57. https://doi.org/10.1016/j.meatsci.2017.11.014

Malheiros, J. M., Enriquez-Valencia, C. E., Silva, J. A. I. de V., Curi, R. A., de Oliveira, H. N., de Albuquerque, L. G., & Chardulo, L. A. L. (2020). Carcass and meat quality of Nellore cattle (Bos taurus indicus) belonging to the breeding programs. *Livestock Science*, *242*, 104277. https://doi.org/10.1016/j.livsci.2020.104277

Matarneh, S. K., Beline, M., Luz, S. De, Shi, H., & Gerrard, D. E. (2018). Mitochondrial F1-ATPase extends glycolysis and pH decline in an in vitro model. *Meat Science*, *137*, 85–91. https://doi.org/10.1016/j.meatsci.2017.11.009

Matarneh, S. K., England, E. M., Scheffler, T. L., Yen, C. N., Wicks, J. C., Shi, H., & Gerrard, D. E. (2017). A mitochondrial protein increases glycolytic flux. *Meat Science*, *133*, 119–125. https://doi.org/10.1016/j.meatsci.2017.06.007

Matarneh, S. K., Yen, C., Bodmer, J., El-kadi, S. W., & Gerrard, D. E. (2021). Mitochondria influence glycolytic and tricarboxylic acid cycle metabolism under postmortem simulating conditions. *Meat Science*, *172*, 108316. https://doi.org/10.1016/j.meatsci.2020.108316

Mato, A., Rodríguez-Vázquez, R., López-Pedrouso, M., Bravo, S., Franco, D., & Zapata, C. (2019). The first evidence of global meat phosphoproteome changes in response to pre-slaughter stress. *BMC Genomics*, *20*(590). https://doi.org/https://doi.org/10.1186/s12864-019-5943-3

Ojima, K., Kawabata, Y., Nakao, H., Nakao, K., Doi, N., Kitamura, F., … Kawahara, H. (2010). Dynamic distribution of muscle-specific calpain in mice has a key role in physical-stress adaptation and is impaired in muscular dystrophy. *The Journal of Clinical Investigation*, *120*(8). https://doi.org/10.1172/JCI40658.2672

Okamoto, K., Matsuzaka, Y., Yoshikawa, Y., Takaki, A., Kulski, J. K., Tamiya, G., & Inoko, H. (2003). Identification of NAD+ -dependent isocitrate dehydrogenase 3 γ-like (IDH3GL) gene and its genetic polymorphisms. *Gene*, *323*, 141–148. https://doi.org/10.1016/j.gene.2003.09.014

Ouali, A., Gagaoua, M., Boudida, Y., Becila, S., Boudjellal, A., Herrera-mendez, C. H., & Sentandreu, M. A. (2013). Biomarkers of meat tenderness: Present knowledge and perspectives in regards to our current understanding of the mechanisms involved. *Meat Science*, *95*, 854–870. https://doi.org/10.1016/j.meatsci.2013.05.010

Pescador, N., Villar, D., Cifuentes, D., Garcia-Rocha, M., Ortiz-Barahona, A., Vazquez, S., … Peso, L. del. (2010). Hypoxia Promotes Glycogen Accumulation through Hypoxia Inducible Factor (HIF)-Mediated Induction of Glycogen Synthase 1. *PLoS One*, *5*(3), e9644. https://doi.org/10.1371/journal.pone.0009644

Picard, B., & Gagaoua, M. (2017). Proteomic Investigations of Beef Tenderness. *Proteomics in Food Science*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-804007-2.00011-4

Picard, B., & Gagaoua, M. (2020). Meta-proteomics for the discovery of protein biomarkers of beef tenderness: An overview of integrated studies. *Food Research International*, *127*, 108739. https://doi.org/10.1016/j.foodres.2019.108739

Pillar, N., Pleniceanu, O., Fang, M., Ziv, L., Lahav, E., Botchan, S., … Dekel, B. (2017). A rare variant in the FHL1 gene associated with X‐linked recessive hypoparathyroidism. *Human Genetics*, *136*, 835–845. https://doi.org/10.1007/s00439-017-1804-9

Poleti, M. D., Moncau, C. T., Silva-Vignato, B., Rosa, A. F., Lobo, A. R., Cataldi, T. R., … de Carvalho Balieiro, J. C. (2018). Label-free quantitative proteomic analysis reveals muscle contraction and metabolism proteins linked to ultimate pH in bovine skeletal muscle. *Meat Science*, *145*, 209–219. https://doi.org/10.1016/j.meatsci.2018.06.041

Ponnampalam, E. N., Hopkins, D. L., Bruce, H., Li, D., Baldi, G., & Bekhit, A. E. (2017). Causes and Contributing Factors to “Dark Cutting” Meat: Current Trends and Future Directions: A Review. *Comprehensive Reviews in Food Science and Food Safety*, *16*(3), 400–430. https://doi.org/10.1111/1541-4337.12258

Purslow, P. P., Gagaoua, M., & Warner, R. D. (2021). Insights on meat quality from combining traditional studies and proteomics. *Meat Science*, *174*, 108423. https://doi.org/10.1016/j.meatsci.2020.108423

Ramanathan, R., & Mancini, R. A. (2018). Role of Mitochondria in Beef Color: A Review. *Meat and Muscle Biology™*, *2*(1), 309–320. https://doi.org/10.22175/mmb2018.05.0013

Rodrigues, R. T. D. S., Chizzotti, M. L., Vital, C. E., Baracat-Pereira, M. C., Barros, E., Busato, K. C., … Martins, T. D. S. (2017). Differences in beef quality between Angus (Bos taurus taurus) and Nellore (Bos taurus indicus) cattle through a proteomic and phosphoproteomic approach. *PLoS ONE*, *12*(1), 1–21. https://doi.org/10.1371/journal.pone.0170294

Schulte, M. D., Hochmuth, K. G., Steadham, E. M., Lonergan, S. M., Hansen, S. L., & Huff-lonergan, E. J. (2022). Early postmortem muscle proteome and metabolome of beef longissimus thoracis muscle classified by pH at 6 hours postmortem. *Journal of Proteomics*, *271*, 104756. https://doi.org/https://doi.org/10.1016/j.jprot.2022.104756

Sedoris, K. C., Thomas, S. D., & Miller, D. M. (2010). Hypoxia induces differential translation of enolase / MBP-1. *BMC Cancer*, *10*(157). https://doi.org/https://doi.org/10.1186/1471-2407-10-157

Sentandreu, E., Fuente-García, C., Pardo, O., Oliván, M., León, N., Aldai, N., … Sentandreu, M. A. (2021). Protein Biomarkers of Bovine Defective Meats at a Glance: Gel-Free Hybrid Quadrupole-Orbitrap Analysis for Rapid Screening. *Journal of Agricultural and Food Chemistry*, *69*, 7478–7487. https://doi.org/10.1021/acs.jafc.1c02016

Shathasivam, T., Kislinger, T., & Gramolini, A. O. (2010). Genes, proteins and complexes: the multifaceted nature of FHL family proteins in diverse tissues. *Journal of Cellular and Molecular Medicine*, *14*(12), 2702–2720. https://doi.org/10.1111/j.1582-4934.2010.01176.x

Shen, Q. W., Gerrard, D. E., & Du, M. (2008). Compound C , an inhibitor of AMP-activated protein kinase , inhibits glycolysis in mouse longissimus dorsi postmortem. *Meat Science*, *78*, 323–330. https://doi.org/10.1016/j.meatsci.2007.06.023

Shojapour, M., Fatemi, F., Farahmand, S., & Shasaltaneh, M. D. (2021). Investigation of Cyc1 protein structure stability after H53I mutation using computational approaches to improve redox potential. *Journal of Molecular Graphics and Modelling*, *105*, 107864. https://doi.org/10.1016/j.jmgm.2021.107864

Sierra, V., González-Blanco, L., Diñeiro, Y., Díaz, F., García-Espina, M. J., Coto-Montes, A., … Oliván, M. (2021). New Insights on the Impact of Cattle Handling on Post-Mortem Myofibrillar Muscle Proteome and Meat Tenderization. *Foods*, *10*, 3115. https://doi.org/https://doi.org/10.3390/foods10123115

Stambergova, H., Skarydova, L., Dunford, J. E., & Wsol, V. (2014). Biochemical properties of human dehydrogenase/reductase (SDR family) member 7. *Chemico-Biological Interactions Journal*, *207*, 52–57. https://doi.org/10.1016/j.cbi.2013.11.003

Suman, S. P., & Joseph, P. (2013). Myoglobin chemistry and meat color. *Annual Review of Food Science and Technology*, *4*, 79–99. https://doi.org/10.1146/annurev-food-030212-182623

Suman, S. P., Wang, Y., Gagaoua, M., Kiyimba, F., & Ramanathan, R. (2023). Proteomic approaches to characterize biochemistry of fresh beef color. *Journal of Proteomics*, *281*, 104893. https://doi.org/10.1016/j.jprot.2023.104893

Tang, J., Faustman, C., Hoagland, T. A., Mancini, R. A., Seyfert, M., & Hunt, M. (2005). Postmortem Oxygen Consumption by Mitochondria and Its Effects on Myoglobin Form and Stability. *Journal of Agricultural and Food Chemistry*, *53*, 1223−1230. https://doi.org/10.1021/jf048646o

Tarrant, P. V. (1989). Animal Behaviour and Environment in the Dark-Cutting Condition in Beef - A Review. *Irish Journal of Food Science and Technology*, *13*(1), 1–21. https://doi.org/http://www.jstor.org/stable/25580937

Tasdemir, E., Maiuri, M. C., Galluzzi, L., Vitale, I., Djavaheri-mergny, M., Amelio, M. D., … Blomgren, K. (2008). Regulation of autophagy by cytoplasmic p53. *Nature Cell Biology*, *10*(6), 676–687. https://doi.org/https://doi.org/10.1038/ncb1730

Terlouw, E. M. C., Picard, B., Deiss, V., Berri, C., Hocquette, J. F., Lebret, B., … Gagaoua, M. (2021). Understanding the determination of meat quality using biochemical characteristics of the muscle: Stress at slaughter and other missing keys. *Foods*, *10*, 84. https://doi.org/10.3390/foods10010084

Ubaida-Mohien, C., Lyashkov, A., Gonzalez-freire, M., Tharakan, R., Shardell, M., Moaddel, R., … Ferrucci, L. (2019). Discovery proteomics in aging human skeletal muscle finds change in spliceosome , immunity , proteostasis and mitochondria. *ELife*, *8*(e49874). https://doi.org/DOI: https://doi.org/10.7554/eLife.49874.001 Introduction

Wiedemann, N., Frazier, A. E., & Pfanner, N. (2004). The Protein Import Machinery of Mitochondria. *Journal of Biological Chemistry*, *279*(15), 14473–14476. https://doi.org/https://doi.org/10.1074/jbc.R400003200

Wu, S., Luo, X., Yang, X., Hopkins, D. L., Mao, Y., & Zhang, Y. (2020). Understanding the development of color and color stability of dark cutting beef based on mitochondrial proteomics. *Meat Science*, *163*, 108046. https://doi.org/10.1016/j.meatsci.2020.108046

Wu, W., Gao, X., Dai, Y., Fu, Y., Li, X., & Dai, R. (2015). Post-mortem changes in sarcoplasmic proteome and its relationship to meat color traits in M . semitendinosus of Chinese Luxi yellow cattle. *Food Research International*, *72*, 98–105. https://doi.org/10.1016/j.foodres.2015.03.030

Wulff, T., Jokumsen, A., Højrup, P., & Jessen, F. (2012). Time-dependent changes in protein expression in rainbow trout muscle following hypoxia. *Journal of Proteomics*, *75*, 2342–2351. https://doi.org/10.1016/j.jprot.2012.02.010

Yang, X., Wu, S., Hopkins, D. L., Liang, R., Zhu, L., & Zhang, Y. (2018). Proteomic analysis to investigate color changes of chilled beef longissimus steaks held under carbon monoxide and high oxygen packaging. *Meat Science*, *142*, 23–31. https://doi.org/10.1016/j.meatsci.2018.04.001

Yu, Q., Wu, W., Tian, X., Jia, F., Xu, L., Dai, R., & Li, X. (2017). Comparative proteomics to reveal muscle-specific beef color stability of Holstein cattle

during post-mortem storage. *Food Chemistry*, *229*, 769–778. https://doi.org/10.1016/j.foodchem.2017.03.004

Zhai, C., Djimsa, B. A., Prenni, J. E., Woerner, D. R., & Belk, K. E. (2020). Tandem mass tag labeling to characterize muscle-specific proteome changes in beef during early postmortem period. *Journal of Proteomics*, *222*, 103794. https://doi.org/10.1016/j.jprot.2020.103794

Zhao, Y., Kong, X., Yang, X., Zhu, L., Liang, R., Luo, X., … Zhang, Y. (2022). Effect of energy metabolism and proteolysis on the toughness of intermediate ultimate pH beef. *Meat Science*, *188*, 108798. https://doi.org/10.1016/j.meatsci.2022.108798

Zhu, Y., Gagaoua, M., Mullen, A. M., Kelly, A. L., Sweeney, T., Cafferky, J., … Hamill, R. M. (2021). A Proteomic Study for the Discovery of Beef Tenderness Biomarkers and Prediction of Warner–Bratzler Shear Force Measured on Longissimus thoracis Muscles of Young Limousin-Sired Bulls. *Foods*, *10*, 952. https://doi.org/10.3390/foods10050952

3 DECLÍNIO DO pHf E SUA RELAÇÃO COM A MACIEZ DA CARNE EM BOVINOS DA RAÇA NELORE (*Bos taurus indicus*)

[3] Artigo em preparação para ser submetido em periódico internacional, 2023.

3.1. Introdução

A taxa e extensão do declínio do pH *postmortem* é um importante fator da qualidade da carne bovina, influenciando sobretudo a maciez. Alguns estudos, como Pereira et al. (2015) demonstraram que bovinos (*Bos taurus indicus*), principalmente raça Nelore, têm apresentado carne com grande variabilidade de maciez atribuída à diferentes enzimas proteolíticas encontradas nos músculos dos animais.

A maciez é uma das principais características da qualidade da carne bovina, sendo considerada prioridade pelo consumidor. A inconsistência na maciez pode influenciar as decisões de compra dos consumidores (Ferrinho et al., 2021). É conhecido que a maciez da carne é afetada por diferentes fatores, como sistema de produção, dieta, idade do animal no momento do abate, efeito do pH, entre outros. Segundo Gagaoua et al. (2021), essas mudanças são inicialmente acompanhadas por uma queda do pH e temperatura, descritos como os primeiros fatores do amaciamento da carne.

Assim, o pH merece atenção especial, visto que, a degradação de proteínas musculares está ligada ao metabolismo energético (aparecimento de fragmentos de enzimas glicolíticas e oxidativas), morte celular programada, proteínas de choque térmico, estresse oxidativo, etc. Os estudos recentes que abordaram esses aspectos sugeriram que os mecanismos subjacentes estão ligados ao pH inicial e à extensão do declínio do pH durante o processo de glicólise (Gagaoua et al., 2021). Em um estudo anterior, Lomiwes et al. (2014) utilizaram técnicas sofisticadas acoplada à espectrometria de massas e indicaram que o amaciamento da carne bovina provavelmente será categorizado pelo pH final, sendo pH final baixo ou normal (≤ 5,79), intermediário (5,80-6,19) e alto (≥ 6,20).

Portanto, o objetivo desse estudo foi avaliar o declínio do pH em diferentes faixas de pH *versus* tempo *postmortem,* e quantificar através de SDS-PAGE e Western Blotting a intensidade relativa de proteínas envolvidas na proteólise do músculo *postmortem*, bem como avaliar os parâmetros de maciez da carne bovina.

3.2. Conclusão

O presente estudo evidenciou que o declínio nas faixas de pH normal e intermediário foram significativos (P<0,01), mostrando redução dos valores de pH ao longo do tempo *postmortem*. O estudo quantificou por meio de SDS-PAGE os pesos moleculares das proteínas com destaque para 0,5 h; 6 h e 44 h *postmortem* que apresentaram diferença significativa (P<0,05) entre as faixas de pHf. Além disso, o pHf alto apresentou o menor valor de força de cisalhamento e parece que a associação da desmina com menores valores de força de cisalhamento no pHf alto às 44 h *postmortem* podem contribuir para melhorar a maciez da carne bovina.

Para HSP90, o pH normal e intermediário foram associados à níveis de expressão maiores de HSP90, especificadamente às 44 h *postmortem* no pHf intermediário. Na HSP27 houve interação entre os tempos *postmortem* com maior percentual de intensidade relativa para 9 h *postmortem.* Em nossas observações, o equilíbrio entre Bax e Bcl-2 ainda não foram totalmente compreendidos, mas os resultados sugerem que uma das vias representativas para induzir a apoptose pode ser a liberação de citocromo C da mitocôndria que pode ter sido promovida por Bcl-2 no pH normal em 0,5 h e 9 h *postmortem*. Em relação a caspase-3, foi observado que a intensidade relativa das bandas foi maior em pH normal, tempo 0,5 h, seguido de pH alto, tempo 6 h e suportam a teoria que as caspases são ativadas imediatamente após a sangria do animal, apresentando níveis de expressão mais elevados nos primeiros momentos do período *postmortem* e diminuindo ao longo do tempo.

Referências

Anderson, S. (2007). Determination of fat, moisture, and protein in meat and meat

products by using the FOSS FoodScan near-infrared spectrophotometer with FOSS artificial neural network calibration model and associated database: collaborative study. Journal of AOAC International, 90(4), 1073-1083.

Barón, C. L. C., dos Santos-Donado, P. R., Ramos, P. M., Donado-Pestana, C. M., Delgado, E. F., & Contreras-Castillo, C. J. (2021). Influence of ultimate pH on biochemistry and quality of Longissimus lumborum steaks from Nellore bulls during ageing. *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.14955

Bendall, J. R. (1973). *Postmortem* changes in muscle. The structure and function of muscle, 2(Part 1), 243-309.

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/https://doi.org/10.1016/0003-2697(76)90527-3

Chardulo, L. A. L., Baldassini, W. A., Curi, R. A., Pereira, G. L., Machado Neto, O. R., Dal-Pai, M., Vechetti-Júnior, I. J., Malheiros, J. M., & Enriquez-Valencia, C. E. (2019). Gene and protein expression of myosin heavy chain in Nellore cattle comparing growth or meat tenderness traits, Animal Biotechnology, 32:3, 300-309, DOI: 10.1080/10495398.2019.1688168

Cramer, T., Penick, M.L., Waddell, J.N., Bidwell, C.A., Kim, Y.H.B. (2018). A new insight into meat toughness of callipyge lamb loins – The relevance of anti-apoptotic systems to decreased proteolysis. Meat Science, 140, pp. 66-71, 10.1016/j.meatsci.2018.03.002

de Nadai Bonin, M., Pedrosa, V. B., da Luz e Silva, S., Bünger, L., Ross, D., da Costa Gomes, R., … Ferraz, J. B. S. (2021). Genetic parameters associated with meat quality of Nellore cattle at different anatomical points of longissimus: Brazilian standards. *Meat Science*, *171*(August 2020), 108281. https://doi.org/10.1016/j.meatsci.2020.108281

Ding, Z., Wei, Q., Zhang, C., Zhang, H., Huang, F. (2021). Influence of oxidation on heat shock protein 27 translocation, caspase-3 and calpain activities and myofibrils degradation in postmortem beef muscles. Food Chemistry, 340, p. 127914, 10.1016/j.foodchem.2020.127914

Ding, Z. J., Wei, Q. C., Zhang, C. J., et al. (2020). Influence of oxidation on heat shock protein 27 translocation, caspase-3 and calpain activities and myofibrils degradation in postmortem beef muscles. *Food Chemistry, 340(15)*, 127914. https://doi.org/10.1016/j.foodchem.2020.127914.

Dunne, P. G., Keane, M. G., O'Mara, F. P., Monahan, F. J., & Moloney, A. P. (2004). Colour of subcutaneous adipose tissue and M. longissimus dorsi of high index dairy and beef × dairy cattle slaughtered at two liveweights as bulls and steers. *Meat Science*, *68*(1), 97–106. https://doi.org/10.1016/j.meatsci.2004.02.010

Ferguson, D. M., Daly, B. L., Gardner, G. E., & Tume, R. K. (2008). Effect of glycogen concentration and form on the response to electrical stimulation and rate of post-mortem glycolysis in ovine muscle. *Meat Science*, *78*(3), 202–210. https://doi.org/10.1016/j.meatsci.2007.06.003

Ferrinho, A. M., de Moura, G. V., Martins, T. da S., Muñoz, J., Mueller, L. F., Garbossa, P. L. M., … Pereira, A. S. C. (2021). Rubia Gallega x Nelore crossbred cattle improve beef tenderness through changes in protein abundance and gene expression. *Livestock Science*, *251*. https://doi.org/10.1016/j.livsci.2021.104634

Franco, D., Mato, A., Salgado, F. J., López-Pedrouso, M., Carrera, M., Bravo, S., … Zapata, C. (2015). Tackling proteome changes in the longissimus thoracis bovine muscle in response to pre-slaughter stress. *Journal of Proteomics*, *122*, 73–85. https://doi.org/10.1016/j.jprot.2015.03.029

Fuente-García, C., Aldai, N., Sentandreu, E., Oliván, M., Franco, D., García-Torres, S., … Sentandreu, M. Á. (2021). Caspase activity in post mortem muscle and its relation to cattle handling practices. *Journal of the Science of Food and Agriculture*, *101*(15), 6258–6264. https://doi.org/10.1002/jsfa.11293

Gagaoua, M., Troy, D., Mullen, A. M. (2021). The extent and rate of the appearance of the major 110 and 30 kDa proteolytic fragments during post-mortem aging of beef depend on the glycolysing rate of the muscle and aging time: An LC-S/MS approach to decipher their proteome and associated pathways, *Journal of Agricultural and Food Chemistry*, 69(1), pp. 602-614, 10.1021/acs.jafc.0c06485

Gagaoua, M., Couvreur, S., Le Bec, G., Aminot, G., & Picard, B. (2017). Associations among Protein Biomarkers and pH and Color Traits in Longissimus thoracis and Rectus abdominis Muscles in Protected Designation of Origin Maine-Anjou Cull Cows. *Journal of Agricultural and Food Chemistry*, *65*(17), 3569–3580. https://doi.org/10.1021/acs.jafc.7b00434

Guo, B., Zhang, W., Tume, R. K., Hudson, N. J., Huang, F., Yin, Y., & Zhou, G. (2016). Disorder of endoplasmic reticulum calcium channel components is associated with the increased apoptotic potential in pale, soft, exudative pork. *Meat Science*, *115*, 34–40. https://doi.org/10.1016/j.meatsci.2016.01.003

Holman, B. W. B., Kerr, M. J., Refshauge, G., Diffey, S. M., Hayes, R. C., Newell, M. T., & Hopkins, D. L. (2021). Post-mortem pH decline in lamb semitendinosus muscle and its relationship to the pH decline parameters of the longissimus lumborum muscle: A pilot study. *Meat Science*, *176,* 108473. https://doi.org/10.1016/j.meatsci.2021.108473

Huang, F., Huang, M., Zhang, H., Zhang, C.J., Zhang, D.Q., Zhou, G.H. (2016). Changes in apoptotic factors and caspase activation pathways during the postmortem aging of beef muscle. Food Chemistry, 190, pp. 110-114. https://doi.org/10.1016/j.foodchem.2015.05.056

ISO 936:1998. Meat and meat products determination of total ash.

Li, X., Hu, L., Zhu, X., Guo, X., Deng, X., Zhang, J. (2022). The effect of caspase-3 in mitochondrial apoptosis activation on degradation of structure proteins of Esox lucius during postmortem storage. Food Chemistry, Volume 367, 130767, https://doi.org/10.1016/j.foodchem.2021.130767

Liu, R., Wu, G. Y., Li, K. Y., Ge, Q. F., Wu, M. G., Yu, H., … Bao, W. Bin. (2021). Comparative study on pale, soft and exudative (PSE) and red, firm and non-exudative (RFN) pork: Protein changes during aging and the differential protein expression of the myofibrillar fraction at 1 h postmortem. *Foods*, *10*(4). https://doi.org/10.3390/foods10040733

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*(3), 472–479. https://doi.org/10.1016/J.MEATSCI.2013.05.022

Lomiwes, D., Farouk, M. M., Wu, G., & Young, O. A. (2014). The development of meat tenderness is likely to be compartmentalised by ultimate pH. *Meat Science*, *96*(1), 646–651. https://doi.org/10.1016/j.meatsci.2013.08.022

Lonergan, E.H., Zhang, W., Lonergan, S.M. (2010). Biochemistry of postmortem muscle—Lessons on mechanisms of meat tenderization. Meat Science, 86 (1)

(2010), pp. 184-195, 10.1016/j.meatsci.2010.05.004
Ma, Y., Yuan, Y., Bi, X. et al. (2019). *Tenderization of Yak Meat by the Combination of Papain and High-Pressure Processing* Treatments. *Food Bioprocess Technol* 12**,** 681–693. https://doi.org/10.1007/s11947-019-2245-3
Maciel, I. C. F., Schweihofer, J. P., Fenton, J. I., Hodbod, J., McKendree, M. G. S., Cassida, K., Rowntree, J. E. (2021). Influence of beef genotypes on animal performance, carcass traits, meat quality, and sensory characteristics in grazing or feedlot-finished steers, *Translational Animal Science*, Volume 5, 4, txab214, https://doi.org/10.1093/tas/txab214
Martins, M. M., Saldaña, E., Teixeira, A. C. B., Selani, M. M., & Contreras-Castillo, C. J. (2021). Going beyond sensory and hedonic aspects: A Brazilian study of emotions evoked by beef in different contexts. *Meat Science, 180*, Article 108536. https://doi.org/10.1016/j.meatsci.2021.108536
Oh, E., Lee, B., Choi, Y. M. C. (2019). Associations of Heat-Shock Protein Expression with Meat Quality and Sensory Quality Characteristics in Highly Marbled Longissimus Thoracis Muscle from Hanwoo Steers Categorized by Warner Bratzler Shear Force Value. Foods.8(12)638 https://doi.org/10.3390/foods8120638
Ouali, A., Gagaoua, M., Boudida, Y., Becila, S., Boudjellal, A., Herrera-mendez, C. H., & Sentandreu, M. A. (2013). Biomarkers of meat tenderness: Present knowledge and perspectives in regards to our current understanding of the mechanisms involved. *Meat Science*, *95*, 854–870. https://doi.org/10.1016/j.meatsci.2013.05.010
Picard, B., & Gagaoua, M. (2020). Muscle Fiber Properties in Cattle and Their Relationships with Meat Qualities: An Overview. *Journal of Agricultural and Food Chemistry*, *68*(22), 6021–6039. https://doi.org/10.1021/acs.jafc.0c02086
Pereira, A. S. C., Baldi, F., Sainz, R. D., Utembergue, B. L., Chiaia, H. L. J., Magnabosco, C. U., Manicardi, F. R., Araujo, F. R. C., Guedes, C. F., Margarido, R. C., Leme, P. R., Sobral, P. J. A. (2015). Growth performance, and carcass and meat quality traits in progeny of Poll Nellore, Angus and Brahman sires under tropical conditions. *Animal Production Science*, 55, 1295-1302.
Poleti, M. D., Moncau, C. T., Silva-Vignato, B., Rosa, A. F., Lobo, A. R., Cataldi, T. R., Negrão, J. A., Silva, S. L., Eler, J. P., & Balieiro, J. C. C. (2018). Label-free quantitative proteomic analysis reveals muscle contraction and metabolism proteins linked to ultimate pH in bovine skeletal muscle. *Meat Science*, *145*, 209–219. https://doi.org/10.1016/j.meatsci.2018.06.041
Pulford, D. J., Dobbie, P., Fraga Vazquez, S., Fraser-Smith, E., Frost, D. A., & Morris, C. A. (2009). Variation in bull beef quality due to ultimate muscle pH is correlated to endopeptidase and small heat shock protein levels. *Meat Science*, *83*(1), 1–9. https://doi.org/10.1016/J.MEATSCI.2008.11.008
Ramanathan, R., Hunt, M. C., Mancini, R. A., Nair, M. N., Denzer, M. L., Suman, S. P., & Mafi, G. G. (2020). Recent Updates in Meat Color Research: Integrating Traditional and High-Throughput Approaches. *Meat and Muscle Biology*, *4*(2), 1–24. https://doi.org/10.22175/mmb.9598
Ramos, P. M.; Santos-Donado, P. R.; Oliveira, G. M.; Contreras-Castillo, C. J.; Scheffler, T. L.; Silva, S. L.; Martello, L. S.; Delgado, E. F. (2021). Beef from Nellore cattle have limited tenderization despite the *Longissimus lumborum* pH decline. Scientia Agricola. https://doi.org/10.1590/1678-992X-2020-0340
Rodrigues, R. T. de S., Chizzotti, M. L., Vital, C. E., Baracat-Pereira, M. C., Barros,

E., Busato, K. C., Gomes, R. A., Ladeira, M. M., & Martins, T. da S. (2017). Differences in beef quality between Angus (Bos taurus taurus) and Nellore (Bos taurus indicus) cattle through a proteomic and phosphoproteomic approach. *PLoS ONE*, *12*(1), 1–21. https://doi.org/10.1371/journal.pone.0170294

Silva, L. H. P., Rodrigues, R. T. S., Assis, D. E. F., Benedeti, P. D. B., Duarte, M. S., & Chizzotti, M. L. (2019). Explaining meat quality of bulls and steers by differential proteome and phosphoproteome analysis of skeletal muscle. *Journal of Proteomics*, *199*, 51–66. https://doi.org/10.1016/j.jprot.2019.03.004

Shackelford, S. D., M. Koohmaraie, M. F. Miller, J. D. Crouse, and J. O. Reagan. 1991. An evaluation of tenderness of the *longissimus* muscle of Angus by Hereford versus Brahman crossbred heifers. *Journal of Animal Science, 69*, 171-177. https://doi.org/10.2527/1991.691171x

Wang, L. L., Yu, Q. L., Han, L., Ma, X. L., Song, R. De, Zhao, S. N., & Zhang, W. H. (2018). Study on the effect of reactive oxygen species-mediated oxidative stress on the activation of mitochondrial apoptosis and the tenderness of yak meat. *Food Chemistry*, *244*(September 2017), 394–402. https://doi.org/10.1016/j.foodchem.2017.10.034

Whipple, G., Koohmaraie, M., Dikeman, M. E., & Crouse, J. D. (1990). Predicting beef-longissimus tenderness from various biochemical and histological muscle traits. *Journal of Animal Science*, *68*(12), 4193–4199. https://doi.org/10.2527/1990.68124193x

Zapata, I., Zerby, H. N., & Wick, M. (2009). Functional proteomic analysis predicts beef tenderness and the tenderness differential. *Journal of Agricultural and Food Chemistry*, *57*(11), 4956–4963. https://doi.org/10.1021/jf900041j

Zhang, M., Wang, D., Geng, Z., Bian, H., Liu, F., Zhu, Y., & Xu, W. (2014). The level of heat shock protein 90 in pig Longissimus dorsi muscle and its relationship with meat pH and quality. *Food Chemistry*, *165*, 337–341. https://doi.org/10.1016/j.foodchem.2014.05.111

Zhang M, Wang D, Geng Z, et al. (2017). Differential expression of heat shock protein 90, 70, 60 in chicken muscles postmortem and its relationship with meat quality. *Anim Biosci,* 30(1):94-99. https://doi.org/10.5713/ajas.16.0132

Zhang, J., Yu, Q., Han, L., Chen, C., Li, H., & Han, G. (2017b). Study on the apoptosis mediated by cytochrome c and factors that affect the activation of bovine longissimus muscle during postmortem aging. *Apoptosis*, *22*(6), 777–785. https://doi.org/10.1007/s10495-017-1374-2

Zhao, Y., Kong, X., Yang, X., Zhu, L., Liang, R., Luo, X., Zhang, L., Hopkins, D. L., Mao, Y., Zhang, Y. (2022). Effect of energy metabolism and proteolysis on the toughness of intermediate ultimate pH beef. Meat Science, 188, https://doi.org/10.1016/j.meatsci.2022.108798.

4 ASSESSMENT OF BEEF SENSORY ATTRIBUTES AND PHYSICOCHEMICAL CHARACTERISTICS: A COMPARATIVE STUDY OF INTERMEDIATE *VERSUS* NORMAL ULTIMATE pH STRIPLOIN CUTS

[4] Artigo publicado no periódico *Food Research International*, 113778, 2023. https://doi.org/10.1016/j.foodres.2023.113778

4.1. Introduction

Animal protein, particularly bovine meat, is a fundamental part of the human diet, especially in Western countries where beef is a major source of protein. Consumer preferences for beef vary across different markets and are influenced by diverse factors including (i) cultural and psychological aspects of consumers (Troy & Kerry, 2010), (ii) the sensory properties of the products (Saldaña et al., 2020a), and (iii) marketing considerations such as price, regulation, quality standards, and distribution (Font-i-Furnols & Guerrero, 2014).

Texture is a key sensory attribute of beef, offering a range of mouthfeel experiences during consumption, such as tenderness, juiciness, and chewiness (Zhu et al., 2021). The aroma and flavour are also critical, arising from chemical compounds like amino acids, peptides, organic acids, and adenine nucleotide metabolism by-products (Spanier et al., 1997). The Maillard reaction and lipid degradation in meat generate various volatile compounds, contributing to the distinctive aromas (Kosowska et al., 2017). Recent research suggests that the chemical properties of muscle fibres (type I and II) vary and significantly influence the formation of volatiles and beef flavour (Li et al., 2023).

The sensory quality of beef is largely determined by its ultimate pH (pHu), which is linked to the animal's ability to store glycogen in muscles and the differing amounts of mitochondria in muscle fibres (Picard & Gagaoua, 2020; Poleti et al., 2018). Variations in pHu between intermediate and normal beef can result from factors like animal diet, exercise, pre-slaughter stress, and processing conditions, all impacting the final quality, particularly in terms of colour, tenderness, and flavour (Gagaoua et al., 2021a; Loudon et al., 2018; Ponnampalam et al., 2017).

This presents a challenge for food scientists and the industry in aligning consumer perception with variations in beef pHu (Warner et al., 2021).

The meat industry aims to consistently produce high-quality cuts while reducing consumer dissatisfaction (Gagaoua & Picard, 2020). Various sensory evaluation methods have been deployed, including both analytical (trained panels) and holistic (consumer) approaches (Saldaña et al., 2021). According to Silva et al. (2012), a product's sensory profile can be established using the Optimised Descriptive Profile (ODP) by a trained panel, where product attributes are selected and agreed upon by the assessors (Aaslyng et al., 2014). Conversely, affective methods rely on consumer reactions, such as hedonic measures, and can be augmented with techniques like Check-all-that-apply (CATA) questions, a versatile tool for capturing perceptions, feelings, and attitudes (Jaeger et al., 2015).

Considering the prominence of beef in Brazilian diets and the preference for this animal protein, this study aimed to develop a comprehensive sensory profile and deepen our understanding of the acceptance and perception of beef cuts within two pHu ranges: intermediate and normal. This innovative study combined the expertise of trained assessors for consistent data, alongside the views of 135 regular beef consumers. Additionally, instrumental measurements taken at 3, 14, and 28 days *post-mortem* were analysed. The hypothesis was that individual reactions to sensory and physicochemical properties of beef could predict consumer perception of beef quality, providing valuable insights to the meat industry about consumer expectations and purchasing decisions.

4.2. Conclusion

This study provides valuable insights into assessing beef quality attributes and consumer perceptions based on variations in beef muscle pHu levels. We observed that beef with an intermediate pHu showed increased juiciness compared to normal pHu beef. This heightened juiciness is likely due to increased water-holding capacity. These findings align with consumer preference

assessments that identified steaks with intermediate pHu as tougher and those with normal pHu as drier. Consumer feedback indicates that attributes like tenderness and juiciness significantly influence overall beef appreciation. Other important factors include roast beef flavour, chewability, saltiness, and juiciness. The study also showed that steaks with intermediate pHu had higher deoxymyoglobin concentrations on the third day compared to the twenty-eighth day of ageing. Instrumental colour measurements, such as *L**, *b**, chroma, and oxymyoglobin, were significantly affected by both pHu category and ageing time (3, 14, and 28 days). This research enhances our understanding of pHu variations in beef muscle and their impact on sensory attributes and consumer preferences. Further studies with larger sample sizes are essential to support and expand these findings, aligning with consumer expectations for premium-quality beef.

References

Aaslyng, M. D., Bejerholm, C., Ertbjerg, P., Bertram, H. C., & Andersen, H. J. (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food Quality and Preference, 14(4)*, 277-288. https://doi.org/10.1016/S0950-3293(02)00086-1

Aaslyng, M. D., Meinert, L., Bejerholm, C., & Warner, R. (2014). Sensory assessment of meat. In Encyclopedia of Meat Sciences (pp. 272-279). Elsevier Inc. https://doi.org/10.1016/B978-0-323-85125-1.00042-9

AMSA. (2012). Guidelines for meat color measurement. American Meat Science Association. http://www.meatscience.org/publications-resources/printedpublicat ions/amsa-meat-color-measurement-guidelines.

AMSA American Meat Science Association (2016). Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat (2 ed, version 1.02). American Meat Science Association, Champaign, IL, USA.

Anne, D., Thierry, A., Keisuke, S., & Michiyo, M. (2022). Transformation of highly marbled meats under various cooking processes. Meat Science, 189, Article 108810. https://doi.org/10.1016/j.meatsci.2022.108810

Antonelo, D. S., Gómez, J. F., Silva, S. L., Beline, M., Zhang, X., Wang, Y., Pavan, B., Koulicoff, L. A., Rosa, A. F., Goulart, R. S., Li, S., Gerrard, D. E., Suman, S. P., Wes Schilling, M., & Balieiro, J. C. (2022). Proteome basis for the biological variations in color and tenderness of *longissimus thoracis* muscle from beef cattle differing in growth rate and feeding regime. *Food Research International*, *153*, Article 110947. https://doi.org/10.1016/j.foodres.2022.110947

AOAC. (1995). Official Methods of Analysis. Association of Official Analytical

Chemists International. Washington D.C.
Ares, G., & Jaeger, S. R. (2023). Check-all-that-apply (CATA) questions with consumers in practice: Experimental considerations and impact on outcome. In Rapid sensory profiling techniques (pp. 257-280). Woodhead Publishing. https://doi.org/10.1016/B978-0-12-821936-2.00013-3
Bendall, J. R. (1973). *Postmortem* changes in muscle. The structure and function of muscle, 2(Part 1), 243-309.
Berger, J., Kim, Y. H. B., Legako, J. F., Martini, S., Lee, J., Ebner, P., & Zuelly, S. M. S. (2018). Dry-aging improves meat quality attributes of grass-fed beef loins. *Meat Science, 145*, 285-291. https://doi.org/10.1016/j.meatsci.2018.07.004
Canto, A. C. V. C. S., Costa-Lima, B. R. C., Suman, S. P., Monteiro, M. L. G., Viana, F. M., Salim, A. P. A. A., Mahesh, N. N., Silva, T. J. P., & Conte-Junior, C. A. (2016). Color attributes and oxidative stability of *longissimus lumborum* and *psoas major* muscles from Nellore bulls. *Meat Science, 121*, 19-26. https://doi.org/10.1016/j.meatsci.2016.05.015
Christensen, M., Purslow, P. P., & Larsen, L. M. (2000). The effect of cooking temperature on mechanical properties of whole meat, single muscle fibres and perimysial connective tissue. *Meat Science, 55(3)*, 301-307. https://doi.org/10.1016/S0309-1740(99)00157-6
Corcoran, L. C., Schlich, P., Moloney, A. P., O'Riordan, E., Millar, K., Botinestean, C., Gallagher, E., O'Sullivan, M. G., & Crofton, E. C. (2023). Comparing consumer liking of beef from three feeding systems using a combination of traditional and temporal liking sensory methods. *Food Research International, 168*, Article 112747. https://doi.org/10.1016/j.foodres.2023.112747
Corlett, M. T., Pethick, D. W., Kelman, K. R., Jacob, R. H., & Gardner, G. E. (2021). Consumer perceptions of meat redness were strongly influenced by storage and display times. *Foods, 10(3)*, Article 540. https://doi.org/10.3390/foods10030540
della Malva, A., Gagaoua, M., Santillo, A., De Palo, P., Sevi, A., & Albenzio, M. (2022). First insights about the underlying mechanisms of Martina Franca donkey meat tenderization during aging: A proteomic approach. *Meat Science, 193*, Article 108925. https://doi.org/10.1016/j.meatsci.2022.108925
Djekic, I. V., Ilic, J. G., Sołowiej, B. G., Djekić, R. I., & Tomasevic, I. B. (2022). Application of Food Mechanics and Oral Processing in Modelling First Bite of Grilled Meat. *Journal of Food Quality, 2022*, Article ID 9176628. https://doi.org/10.1155/2022/9176628
Duarte, T. L., Bolkenov, B., Klopatek, S. C., Oltjen, J. W., King, D. A., Shackelford, S. D., Wheeler, T. L., & Yang, X. (2022). Evaluating the Shelf Life and Sensory Properties of Beef Steaks from Cattle Raised on Different Grass Feeding Systems in the Western United States. *Foods, 11(14)*, Article 2141. https://doi.org/10.3390/foods11142141
Faustman, C., & Suman, S. P. (2017). The Eating Quality of Meat: I—Color. Lawrie´s Meat Science (Eight Edition), 329-356. https://doi.org/10.1016/B978-0-08-100694-8.00011-X
Ferguson, D. M., & Warner, R. D. (2008). Have we underestimated the impact

of pre-slaughter stress on meat quality in ruminants? *Meat Science, 80(1)*, 12-19. https://doi.org/10.1016/j.meatsci.2008.05.004

Feiner, G. (2006). Definitions of terms used in meat science. *Meat Products Handbook*, 46-71. https://doi.org/https://doi.org/10.1533/9781845691721.1.46

Font-i-Furnols, M., & Guerrero, L. (2014). Consumer preference, behavior and perception about meat and meat products: An overview. *Meat science, 98(3)*, 361-371. https://doi.org/10.1016/j.meatsci.2014.06.025

Gagaoua, M., Picard, B., & Monteils, V. (2018). Associations among animal, carcass, muscle characteristics, and fresh meat color traits in Charolais cattle. *Meat Science, 140,* 145-156. https://doi.org/10.1016/j.meatsci.2018.03.004

Gagaoua, M., & Picard, B. (2020). Current advances in meat nutritional, sensory and physical quality improvement. *Foods, 9(3)*, Article 321. https://doi.org/10.3390/foods9030321

Gagaoua, M., Terlouw, E. M. C., Mullen, A. M., Franco, D., Warner, R. D., Lorenzo, J. M., Purslow, P. P., Gerrard, D., Hopkins, D. L., Troy, D., & Picard, B. (2021a). Molecular signatures of beef tenderness: Underlying mechanisms based on integromics of protein biomarkers from multi-platform proteomics studies. *Meat Science, 172*, Article 108311. https://doi.org/10.1016/j. meatsci.2020.108311

Gagaoua, M., Warner, R. D., Purslow, P., Ramanathan, R., Mullen, A. M., López-Pedrouso, M., Franco, D., Lorenzo, J. M., Tomasevic, I., Picard, B., Troy, D., & Terlouw, E. M. C. (2021b). Dark-cutting beef: A brief review and an integromics meta-analysis at the proteome level to decipher the underlying pathways. *Meat Science*, *181*, Article 108611. https://doi.org/10.1016/j.meatsci.2021.108611

Gomes, C. L., Pflanzer, S. B., Cruz, A. G., De Felício, P. E., & Bolini, H. M. A. (2014). Sensory descriptive profiling and consumer preferences of beef strip loin steaks. *Food Research International, 59*, 76-84. https://doi.org/10.1016/j.foodres.2014.01.061

Grau, R., & Hamm, R. (1953). Eine einfache methode zur bestimmung der wasserbindung im muskel. *Naturwissenschaften, 40(1)*, 29-30.

Grayson, A. L., Shackelford, S. D., King, D. A., McKeith, R. O., Miller, R. K., & Wheeler, T. L. (2016). The effects of degree of dark cutting on tenderness and sensory attributes of beef. *Journal of Animal Science, 94(6)*, 2583-2591. https://doi.org/10.2527/jas.2016-0388

Gruber, S. L., Tatum, J. D., Engle, T. E., Chapman, P. L., Belk, K. E., & Smith, G. C. (2010). Relationships of behavioral and physiological symptoms of preslaughter stress to beef *longissimus* muscle tenderness. *Journal of Animal Science, 88(3)*, 1148-1159. https://doi.org/10.2527/jas.2009-2183

Hoffmann, H., Hamm, R., & Bluchel, E. (1982). Neus uber die bestimung der wasserbinding des nut hielf filter paper premethods. *Fleishwirtchaft, 62(1)*, 87-94.

Holman, B. W., van de Ven, R. J., Mao, Y., Coombs, C. E., & Hopkins, D. L. (2017). Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science, 127*, 57-62. https://doi.org/10.1016/j.meatsci.2017.01.005

Honikel, K. O. (1998). Reference methods for the assessment of physical characteristics of meat. *Meat science, 49(4)*, 447-457. https://doi.org/10.1016/S0309-1740(98)00034-5

Hopkins, D. L. (1996). Assessment of lamb meat colour. *Meat Focus International*, *5*(11), 400-401.

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science, 71(1)*, 194-204. https://doi.org/10.1016/j.meatsci.2005.04.022

Hunt, M. C., Sørhelm, O., & Slinde, E. (1999). Color and heat denaturation of myoglobin forms in ground beef. *Journal of Food Science, 64(5)*, 847-851. https://doi.org/10.1111/j.1365-2621.1999.tb15925.x

Hwang, Y-H., Kim, G-D., Jeong, J-Y., Hur, S-J., & Joo, S-T. (2010). The relationship between muscle fiber characteristics and meat quality traits of highly marbled Hanwoo (Korean native cattle) steers. *Meat Science, 86(2)*, 456-461. https://doi.org/10.1016/j.meatsci.2010.05.034

Jaeger, S. R., Beresford, M. K., Paisley, A. G., Antúnez, L., Vidal, L., Cadena, R. S., Giménez, A., & Ares, G. (2015). Check-all-that-apply (CATA) questions for sensory product characterization by consumers: Investigations into the number of terms used in CATA questions. *Food Quality and Preference, 42*, 154-164. https://doi.org/10.1016/j.foodqual.2015.02.003

Jeong, J. Y., Hur, S. J., Yang, H. S., Moon, S. H., Hwang, Y. H., Park, G. B., & Joo, S. T. (2009). Discoloration Characteristics of 3 Major Muscles From Cattle During Cold Storage. *Journal of Food Science, 74(1)*, C1-C5. https://doi.org/10.1111/j.1750-3841.2008.00983.x

Jeremiah, L., Dugan, M., Aalhus, J., & Gibson, L. (2003). Assessment of the chemical and cooking properties of the major beef muscles and muscle groups. Meat Science, 65(3), 985-992. https://doi.org/10.1016/S0309-1740(02)00308-X

Jia, W., Wang, X., Zhang, R., Shi, Q., & Shi, L. (2022). Irradiation role on meat quality induced dynamic molecular transformation: From nutrition to texture. *Food Reviews International, 1*, 1-23. https://doi.org/10.1080/87559129.2022.2026377

Joseph, P., Suman, S. P., Rentfrow, G., Li, S., & Beach, C. M. (2012). Proteomics of muscle-specific beef color stability. *Journal of Agricultural and Food Chemistry, 60(12)*, 3196-3203. https://doi.org/10.1021/jf204188v

Kannan, G., Kouakou, B., & Gelaye, S. (2001). Color changes reflecting myoglobin and lipid oxidation in chevon cuts during refrigerated display. *Small Ruminant Research, 42(1)*, 67-74. https://doi.org/10.1016/S0921-4488(01)00232-2

Kerth, C. R., & R. K. Miller. 2015. Beef flavor: A review from chemistry to consumer. *Journal of the Science of Food and Agriculture, 95*, 2783-2798. https://doi.org/10.1002/jsfa.7204

Kim, Y. H., Keeton, J. T., Smith, S. B., Berghman, L. R., & Savell, J. W. (2009). Role of lactate dehydrogenase in metmyoglobin reduction and color stability of different bovine muscles. *Meat Science, 83(3)*, 376-382. https://doi.org/10.1016/j.meatsci.2009.06.009

Kiyimba, F., Hartson, S. D., Rogers, J., Vanoverbeke, D. L., Mafi, G. G., & Ramanathan, R. (2022). Dark-cutting beef mitochondrial proteomic signatures reveal increased biogenesis proteins and bioenergetics capabilities. *Journal of Proteomics*, *265*, Article 104637. https://doi.org/10.1016/j.jprot.2022.104637

Kosowska, M., Majcher, M. A., & Fortuna, T. (2017). Volatile compounds in meat and meat products. *Food Science and Technology (Brazil), 37(1)*, 1-7. https://doi.org/10.1590/1678-457X.08416

Li, X., Zhang, D., Ijaz, M., Tian, G., Chen, J., & Du, M. (2020). Colour characteristics of beef *longissimus thoracis* during early 72 h postmortem. *Meat Science*, *170*, 108245. https://doi.org/10.1016/j.meatsci.2020.108245

Li, Z., Ha, M., Frank, D., Hastie, M., & Warner, R. D. (2023). Muscle fibre type composition influences the formation of odour-active volatiles in beef. *Food Research International*, *165*, Article 112468. https://doi.org/10.1016/j.foodres.2023.112468

Lindahl, G., Lundström, K., & Tornberg, E. (2001). Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. *Meat Science, 59(2)*, 141-151. https://doi.org/10.1016/S0309-1740(01)00064-X

Liu, J., Ellies-Oury, M. P., Stoyanchev, T., & Hocquette, J. F. (2022). Consumer perception of beef quality and how to control, improve and predict it? Focus on eating quality. *Foods, 11(12)*, Article 1732. https://doi.org/10.3390/foods11121732

Lomiwes, D., Farouk, M. M., Wu, G., & Young, O. A. (2014). The development of meat tenderness is likely to be compartmentalised by ultimate pH. *Meat Science, 96(1)*, 646-651. https://doi.org/10.1016/j.meatsci.2013.08.022

Loudon, K. M. W., Lean, I. J., Pethick, D. W., Gardner, G. E., Grubb, L. J., Evans, A. C., & McGilchrist, P. (2018). On farm factors increasing dark cutting in pasture finished beef cattle. *Meat science, 144*, 110-117. https://doi.org/10.1016/j.meatsci.2018.06.011

Lyford, C., Thompson, J., Polkinghorne, R., Miller, M., Nishimura, T., Neath, K., Allen, P., & Belasco, E. (2010). Is willingness to pay (WTP) for beef quality grades affected by consumer demographics and meat consumption preferences? *Australasian Agribusiness Review, 18*, 1-17.

Madruga, M. S., & Mottram, D. S. (1995). The effect of pH on the formation of maillard-derived aroma volatiles using a cooked meat system. *Journal of the Science of Food and Agriculture, 68(3)*, 305-310. https://doi.org/10.1002/jsfa.2740680308

Malheiros, B. A., Spers, E. E., Silva, H. M. R. D., & Contreras Castillo, C. J. (2022). Southeast Brazilian Consumers' Involvement and Willingness to Pay for Quality Cues in Fresh and Cooked Beef. *Journal of Food Products Marketing, 28(6)*, 276-293. https://doi.org/10.1080/10454446.2022.2129539

Martins, M. M., Saldaña, E., Teixeira, A. C. B., Selani, M. M., & Contreras-Castillo, C. J. (2021). Going beyond sensory and hedonic aspects: A Brazilian study of emotions evoked by beef in different contexts. *Meat Science, 180*, Article 108536. https://doi.org/10.1016/j.meatsci.2021.108536

McKenna, D., Mies, P., Baird, B., Pfeiffer, K., Ellebracht, J., & Savell, J. (2005).

Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science, 70(4)*, 665-682. https://doi.org/10.1016/j.meatsci.2005.02.016

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of animal science, 79(12)*, 3062-3068. https://doi.org/10.2527/2001.79123062x

Mitacek, R. M., Ke, Y., Prenni, J. E., Jadeja, R., VanOverbeke, D. L., Mafi, G. G., & Ramanathan, R. (2019). Mitochondrial degeneration, depletion of NADH, and oxidative stress Decrease color stability of wet-aged beef *Longissimus* steaks. *Journal of Food Science, 84(1)*, 38-50. https://doi.org/10.1111/1750-3841.14396

Obuz, E., & Dikeman, M. E. (2003). Effects of cooking beef muscles from frozen or thawed states on cooking traits and palatability. *Meat Science, 65(3)*, 993–997. https://doi.org/10.1016/S0309-1740(02)00314-5

O'Quinn, T. G., Brooks, J. C., Polkinghorne, R. J., Garmyn, A. J., Johnson, B. J., Starkey, J. D., Rathmann, R. J., & Miller, M. F. (2012). Consumer assessment of beef strip loin steaks of varying fat levels. *Journal of Animal Science, 90(2)*, 626-634. https://doi.org/10.2527/jas.2011-4282

Patinho, I., Saldaña, E., Selani, M. M., Camargo, A. C., Merlo, T. C., Menegali, B. S., Silva, A. P. S., & Contreras-Castillo, C. J. (2019). Use of *Agaricus bisporus* mushroom in beef burgers: Antioxidant, flavor enhancer and fat replacing potential. *Food Production, Processing and Nutrition, 1*, 1-15. https://doi.org/10.1186/s43014-019-0006-3

Patinho, I., Selani, M. M., Saldaña, E., Bortoluzzi, A. C. T., Rios-Mera, J. D., Silva, C. M., Kushida, M. M., & Contreras-Castillo, C. J. (2021). *Agaricus bisporus* mushroom as partial fat replacer improves the sensory quality maintaining the instrumental characteristics of beef burger. *Meat science, 172*, Article 108307. https://doi.org/10.1016/j.meatsci.2020.108307

Picard, B., & Gagaoua, M. (2020). Muscle fiber properties in cattle and their relationships with meat qualities: An overview. *Journal of Agricultural and Food Chemistry, 68(22)*, 6021-6039. https://doi.org/10.1021/acs.jafc.0c02086

Poleti, M. D., Moncau, C. T., Silva-Vignato, B., Rosa, A. F., Lobo, A. R., Cataldi, T. R., Negrão, J. A., Silva, S. L., Eler, J. P., & Balieiro, J. C. C. (2018). Label-free quantitative proteomic analysis reveals muscle contraction and metabolism proteins linked to ultimate pH in bovine skeletal muscle. *Meat science, 145*, 209-219. https://doi.org/10.1016/j.meatsci.2018.06.041

Ponnampalam, E. N., Hopkins, D. L., Bruce, H., Li, D., Baldi, G., & Bekhit, A. E. (2017). Causes and contributing factors to “dark cutting” meat: Current trends and future directions: A review. *Comprehensive Reviews in Food Science and Food Safety, 16(3)*, 400-430. https://doi.org/10.1111/1541-4337.12258

Powell, L., Nicholson, K. L., Huerta-Montauti, D., Miller, R. K., & Savell, J. W. (2011). Constraints on establishing threshold levels for Warner–Bratzler shear-force values based on consumer sensory ratings for seven beef muscles. *Animal production science, 51(10)*, 959-966. https://doi.org/10.1071/AN10267

Purslow, P., Oiseth, S., Hughes, J., & Warner, R. (2016). The structural basis of cooking loss in beef: Variations with temperature and ageing. *Food Research International, 89*, 739-748. https://doi.org/10.1016/j.foodres.2016.09.010

Ramanathan, R. & Mancini, R. A. (2018). Role of Mitochondria in Beef Color: A Review, *Meat and Muscle Biology 2(1)*, 309-320. https://doi.org/10.22175/mmb2018.05.0013

Saldaña, E., Martins, M. M., Behrens, J. H., Valentin, D., Selani, M. M., & Contreras-Castillo, C. J. (2020a). Looking at non-sensory factors underlying consumers' perception of smoked bacon. *Meat science, 163*, Article 108072. https://doi.org/10.1016/j.meatsci.2020.108072

Saldaña, E., Serrano-León, J., Selani, M. M., & Contreras-Castillo, C. J. (2020b). Sensory and hedonic impact of the replacement of synthetic antioxidant for pink pepper residue extract in chicken burger. *Journal of food science and technology, 57*, 617-627. https://doi.org/10.1007/s13197-019-04093-x

Saldaña, E., Merlo, T. C., Patinho, I., Rios-Mera, J. D., Contreras-Castillo, C. J., & Selani, M. M. (2021). Use of sensory science for the development of healthier processed meat products: A critical opinion. *Current opinion in food science, 40*, 13-19. https://doi.org/10.1016/j.cofs.2020.04.012

Selani, M. M., Ramos, P. H. B., Patinho, I., França, F., dos Santos Harada-Padermo, S., Contreras-Castillo, C. J., & Saldaña, E. (2022). Consumer's perception and expected liking of labels of burgers with sodium reduction and addition of mushroom flavor enhancer. *Meat Science, 185*, Article 108720. https://doi.org/10.1016/j.meatsci.2021.108720

Shackelford, S. D., M. Koohmaraie, M. F. Miller, J. D. Crouse, and J. O. Reagan. 1991. An evaluation of tenderness of the *longissimus* muscle of Angus by Hereford versus Brahman crossbred heifers. *Journal of Animal Science, 69*, 171-177. https://doi.org/10.2527/1991.691171x

Sharedeh, D., Gatellier, P., Astruc, T., & Daudin, J. D. (2015). Effects of pH and NaCl levels in a beef marinade on physicochemical states of lipids and proteins and on tissue microstructure. *Meat Science*, *110*, 24-31. https://doi.org/10.1016/j.meatsci.2015.07.004

Silva, R. C. S. N., Minim, V. P. R., Simiqueli, A. A., Moraes, L. E. S., Gomide, A. I., & Minim, L. A. (2012). Optimized Descriptive Profile: A rapid methodology for sensory description. *Food Quality and Preference*, *24*(1), 190-200. https://doi.org/10.1016/j.foodqual.2011.10.014

Spanier, A. M., Flores, M., McMillin, K. W., & Bidner, T. D. (1997). The effect of post-mortem aging on meat flavor quality in Brangus beef. Correlation of treatments, sensory, instrumental and chemical descriptors. *Food Chemistry*, *59(4)*, 531-538. https://doi.org/10.1016/S0308-8146(97)00003-4

Suman, S. P., Nair, M. N., Joseph, P., & Hunt, M. C. (2016). Factors influencing internal color of cooked meats. *Meat Science, 120*, 133-144. https://doi.org/10.1016/j.meatsci.2016.04.006

Suman, S. P., Wang, Y., Gagaoua, M., Kiyimba, F., & Ramanathan, R. (2023). Proteomic approaches to characterize biochemistry of fresh beef color. *Journal of Proteomics, 281*, Article 104893. https://doi.org/10.1016/j.jprot.2023.104893

Torres Filho, R. D. A., Cazedey, H. P., Fontes, P. R., Ramos, A. D. L. S., & Ramos, E. M. (2017). Drip loss assessment by different analytical methods and their relationships with pork quality classification. *Journal of Food Quality, 2017*, Article 9170768. https://doi.org/10.1155/2017/9170768

Troy, D. J., & Kerry, J. P. (2010). Consumer perception and the role of science in the meat industry. *Meat science, 86(1)*, 214-226. https://doi.org/10.1016/j.meatsci.2010.05.009

Ueda, Y., Watanabe, A., Higuchi, M., Shingu, H., Kushibiki, S., & Shinoda, M. (2007). Effects of intramuscular fat deposition on the beef traits of Japanese Black steers (Wagyu). *Animal Science Journal, 78(2)*, 189-194. https://doi.org/10.1111/j.1740-0929.2007.00424.x

Wakeling, I. N., & MacFie, H. J. (1995). Designing consumer trials balanced for first and higher orders of carry-over effect when only a subset of k samples from t may be tested. *Food Quality and Preference, 6(4)*, 299-308. https://doi.org/10.1016/0950-3293(95)00032-1

Wang, F., Holman, B. W., Zhang, Y., Luo, X., Mao, Y., & Hopkins, D. L. (2020). Investigation of colour requirements of frozen beef rolls by Chinese consumers for hot pot. *Meat Science, 162*, Article 108038. https://doi.org/10.1016/j.meatsci.2019.108038

Warner, R. & Miller, R. & Ha, M. & Wheeler, T. L. & Dunshea, F. & Li, X. & Vaskoska, R. & Purslow, P. (2021). Meat Tenderness: Underlying Mechanisms, Instrumental Measurement, and Sensory Assessment. *Meat and Muscle Biology 4(2)*, 1-25. https://doi.org/10.22175/mmb.10489

Watanabe, G., Motoyama, M., Orita, K., Takita, K., Aonuma, T., Nakajima, I., Tajima, A., Abe, A., & Sasaki, K. (2019). Assessment of the dynamics of sensory perception of Wagyu beef strip loin prepared with different cooking methods and fattening periods using the temporal dominance of sensations. *Food Science & Nutrition, 7(11)*, 3538-3548. https://doi.org/10.1002/fsn3.1205

Watson, R., Polkinghorne, R., & Thompson, J. M. (2008). Development of the Meat Standards Australia (MSA) prediction model for beef palatability. *Australian Journal of Experimental Agriculture, 48(11)*, 1368-1379. https://doi.org/10.1071/EA07184

Wicklund, S. E., Homco-Ryan, C., Ryan, K. J., Mckeith, F. K., Mcfarlane, B. J., & Brewer, M. S. (2005). Aging and Enhancement Effects on Quality Characteristics of Beef Strip Steaks. *Journal of Food Science, 70(3)*, S242-S248. https://doi.org/10.1111/j.1365-2621.2005.tb07164.x

Wu, S., Han, J., Liang, R., Dong, P., Zhu, L., Hopkins, D. L., Zhang, Y., & Luo, X. (2020). Investigation of muscle-specific beef color stability at different ultimate pHs. *Asian-Australasian Journal of Animal Sciences, 33(12)*, 1999-2007. https://doi.org/10.5713/ajas.19.0943

Yang, X., Wang, J., Holman, B. W., Liang, R., Chen, X., Luo, X., Zhu, L., Hopkins, D. L., & Zhang, Y. (2021). Investigation of the physicochemical, bacteriological, and sensory quality of beef steaks held under modified atmosphere packaging and representative of different ultimate pH values. *Meat Science, 174*, Article 108416. https://doi.org/10.1016/j.meatsci.2020.108416

Zhang, Y., Kim, Y., Puolanne, E., & Ertbjerg, P. (2022). Role of freezing-

induced myofibrillar protein denaturation in the generation of thaw loss: A review. *Meat Science, 190*, Article 108841. https://doi.org/10.1016/j.meatsci.2022.108841

Zhu, Y., Gagaoua, M., Mullen, A. M., Viala, D., Rai, D. K., Kelly, A. L., Sheehan, D., & Hamill, R. M. (2021). Shotgun proteomics for the preliminary identification of biomarkers of beef sensory tenderness, juiciness and chewiness from plasma and muscle of young Limousin-sired bulls. Meat science, 176, Article 108488. https://doi.org/10.1016/j.meatsci.2021.108488