Shotgun proteomics and chemometrics to discriminate normal and dark-cutting beef muscles

Iliani Patinho, Daniel Silva Antonelo, Eduardo F. Delgado, Julio C.C. Balieiro, Laura Alessandroni, Carmen J. Contreras-Castillo, Mohammed Gagaoua

2023 PADOVA, ITALY

ICoMST

69th INTERNATIONAL CONGRESS of MEAT SCIENCE AND TECHNOLOGY

Proceedings of the 69th International Congress of Meat Science and Technology

from Tradition to Green Innovation

August 20-25, 2023

Padova, Italy

Edited by:

Antonella Dalle Zotte

Alberto Brugiapaglia

Marco Cullere

www.icomst2023.com

SHOTGUN PROTEOMICS AND CHEMOMETRICS TO DISCRIMINATE NORMAL AND DARK-CUTTING BEEF MUSCLES

Iliani Patinho [1*], Daniel S. Antonelo [2], Eduardo F. Delgado [3], Julio C.C. Balieiro [2], Laura Alessandroni [4], Carmen J. Contreras-Castillo [1] and Mohammed Gagaoua [5]

[1] Department of Agri-food Industry, Food and Nutrition, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP 13418-900, Brazil

[2] School of Veterinary Medicine and Animal Science, University of Sao Paulo, Pirassununga, SP 13635-900, Brazil

[3] Department of Animal Science, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP 13418-900, Brazil

[4] Chemistry Interdisciplinary Project (CHIP), School of Pharmacy, University of Camerino, Via Madonna delle Carceri, 62032 Camerino, Italy

[5] PEGASE, INRAE, Institut Agro, 35590 Saint-Gilles, France

*Corresponding author email: ilianipatinho@usp.br

I. INTRODUCTION

Proteins are the main constituents of muscle and they regulate myriad metabolic changes during the conversion of muscle into meat [1]. Dark-cutting beef is generally defined as high pHu beef (≥ 6.0) as a consequence of various factors before and after slaughter [2]. For example, the low glycogen content in muscles affects glycolytic metabolism after slaughter, which can lead to high pHu resulting in Dark-cutting beef compared to normal pHu beef [2,3]. To understand the underlying mechanisms and identify protein biomarkers, proteomics approaches together with mass spectrometry (MS) and bioinformatics have been applied in meat research [4]. This trial aimed to apply a shotgun proteomics approach using high-resolution mass spectrometry (LC-MS/MS) in combination with chemometrics to ass the suitability of the *post-mortem* muscle proteome to discriminate high pHu (dark-cutting) and normal pHu beef samples from pasture-finished Nellore (*Bos indicus*) bulls.

II. MATERIALS AND METHODS

Ten carcasses classified as normal (pHu < 5.8; n = 5) or high (pHu ≥ 6.2; n = 5) pHu beef from pasture-finished Nellore (*Bos indicus*) bulls, ranging from 30 to 35 months of age (4 to 6 permanent incisors teeth) and 349 ± 31 kg of hot carcass weight, were obtained from a commercial meat processor. *Longissimus thoracis* (LT) muscle samples (~ 30 g) were taken from the carcasses (between the 10th and 11th ribs) at 44 h *post-mortem*, immediately snap frozen in liquid nitrogen and stored at -80 °C for further proteomics analysis. The shotgun proteomics was carried out as described recently by Lamri *et al.* [5]. The relative abundances of the quantified proteins for each pHu group were first log-transformed and Pareto-scaled prior to analysis using partial least square discriminant analysis (PLS-DA). In the PLS-DA model, a variable importance in projection (VIP) plot was used to rank the proteins based on their relative importance of variation in discriminating groups. Protein features with VIP > 2.0 were uploaded to Metascape® (https://metascape.org/) to identify the enriched Gene Ontology (GO) terms (P ≤ 0.05, minimum overlap of 3, and enrichment factor > 1.5).

III. RESULTS AND DISCUSSION

The PLS-DA clearly discriminated normal and high pHu beef at 44 h *post-mortem* (Fig. 1A). Based on VIP scores, 19 proteins are the main contributors in the explanation residuals in the PLS-DA plot across pHu classes (Fig. 1B). Five top putative biomarkers can be proposed: 60S acidic ribosomal protein P0 (RPLP0), Synemin (SYNM), Myozenin-3 (MYOZ3), Protocadherin-10 (PCDH10) and NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12 (NDUFA12). The GO analysis of the 19 proteins revealed six enriched molecular signatures (Fig. 1C). GO analysis indicated that high and normal pHu groups had distinct metabolic patterns and the key proteins discriminating high from normal pHu meat were associated with cellular stress and intercellular protein transport and their regulation.

Moreover, mitochondrion organization was confirmed in agreement to the available knowledge [4,6] as pivotal pathways underlying dark-cutting beef development.

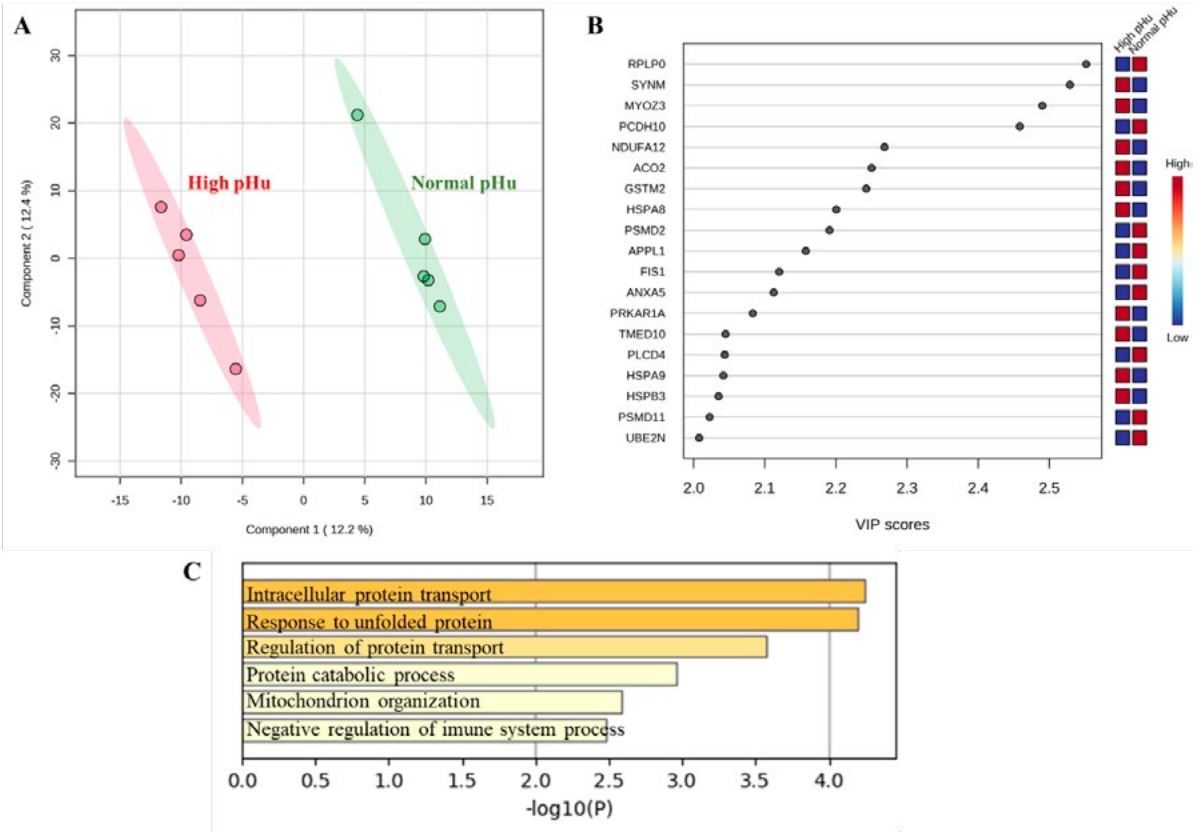

Figure 1. Chemometrics analysis to discriminate normal and high pHu beef. A) Partial least square discriminant analysis (PLS-DA); B) Variable importance in projection (VIP) plot; C) Gene Ontology analysis.

IV. CONCLUSION

The present study indicated that proteins related to cellular interconnected pathways contribute to the development of high pHu beef. Putative biomarkers are proposed for validation from which certain were previously identified as candidate biomarkers. Shotgun proteomics and chemometrics is a useful way to decipher the unknowns and help identifying meat quality defects such as dark-cutting beef.

ACKNOWLEDGEMENTS

This work was supported by the Foundation for Research Support of the State of Sao Paulo FAPESP (grant # 2017/26667-2; 2019/26026-2).

REFERENCES

1. Rodrigues, R. T. dS., Chizzotti, M. L., Vital, C. E., Baracat-Pereira, M. C., Barros, E., Busato, K. C., Gomes, R. A., Ladeira, M. M. & da Silva Martins, T. (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: 1-21.
2. Ponnampalam, E. N., Hopkins, D. L., Bruce, H., Li, D., Baldi, G., & Bekhit, A. E. D. (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: 400-430.
3. 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. & 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.
4. 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. (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.
5. Lamri, M., della Malva, A., Djenane, D., López-Pedrouso, M., Franco, D., Albenzio, M., Lorenzo, J. M. & Gagaoua, M. (2023). Towards the discovery of goat meat quality biomarkers using label-free proteomics. Journal of Proteomics 278: 104868.
6. 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.