Meat species identification and classification by MALDI-TOF mass spectrometry





Newton Valério Verbisck^{1*}, Larissa Bortoli de Souza², Marita Vedovelli Cardozo³, Nilton Gabriel Paiva Guimarães¹, Gelson Luís Dias Feijó¹

¹EMBRAPA Gado de Corte, ²Universidade Federal de Mato Grosso do Sul, ³Universidade Estadual Paulista,

* newton.verbisck@embrapa.br

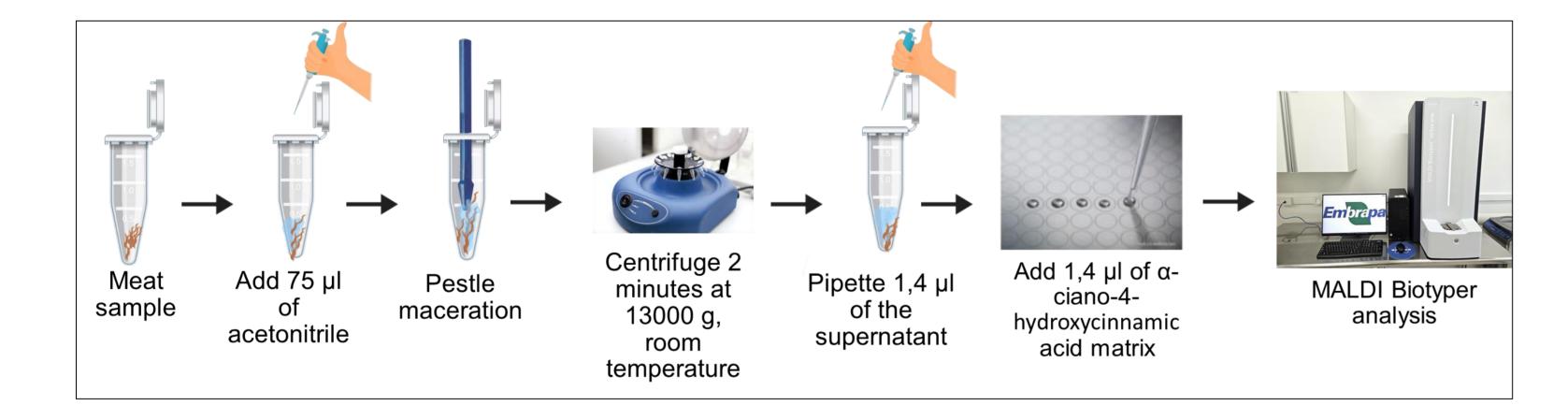
Brasil

Introduction

- MALDI-TOF mass spectrometry allows obtaining protein profiles for identification and classification of samples (Pham & Jimenez, 2014);
- In recent years, this approach has been used to study cells, tissues and whole organisms. In this context, it has been demonstrated that it is possible to distinguish meat from different animal species, including mammals, birds and reptiles (Rau *et al.*, 2021);
- Here we use the MALDI-TOF protein profiling and machine learning to distinguish and classify meat, in natura and processed by freezing and cooking, from different species of livestock animals, with the aim of establishing a methodology useful for certifying meat species.

Methods

- Samples of beef (fillet mignon and sirloin), pork (loin), chicken (breast fillet) and tilapia fish (fillet) purchased from a supermarket in Campo Grande, MS, Brazil, were analyzed;
- Approximately 3 cubic mm were excised from meat pieces, without apparent fat, with 24 hours of shelf time;
- Proteins were extracted after maceration with a sterile plastic pestle, with a mixture of acetonitrile:water:trifluoroacetic acid, 50:49.9:0.1 v/v, followed by centrifugation at 13,000 g for 2 minutes;
- Mass spectra were acquired with alpha-cyano-4-hydroxycinnamic acid as matrix in a MALDI Biotyper Sirius One (Bruker Daltonics), with external calibration;
- For the analysis of mass spectra and identification of peaks for meat classification, the MALDI Biotyper Compass Explorer 4.1 and ClinProTools 3.0 (Bruker Daltonics) software were used, respectively.



Conclusions

The preliminary data obtained allow us to conclude that different meat species can be correctly classified with the MALDI-TOF mass spectrometry methodology used for protein profile analysis, and may be used in the future for meat certification. The new aspect presented here comes from the possibility of distinguishing cattle breeds, which will be confirmed with larger sampling.

References

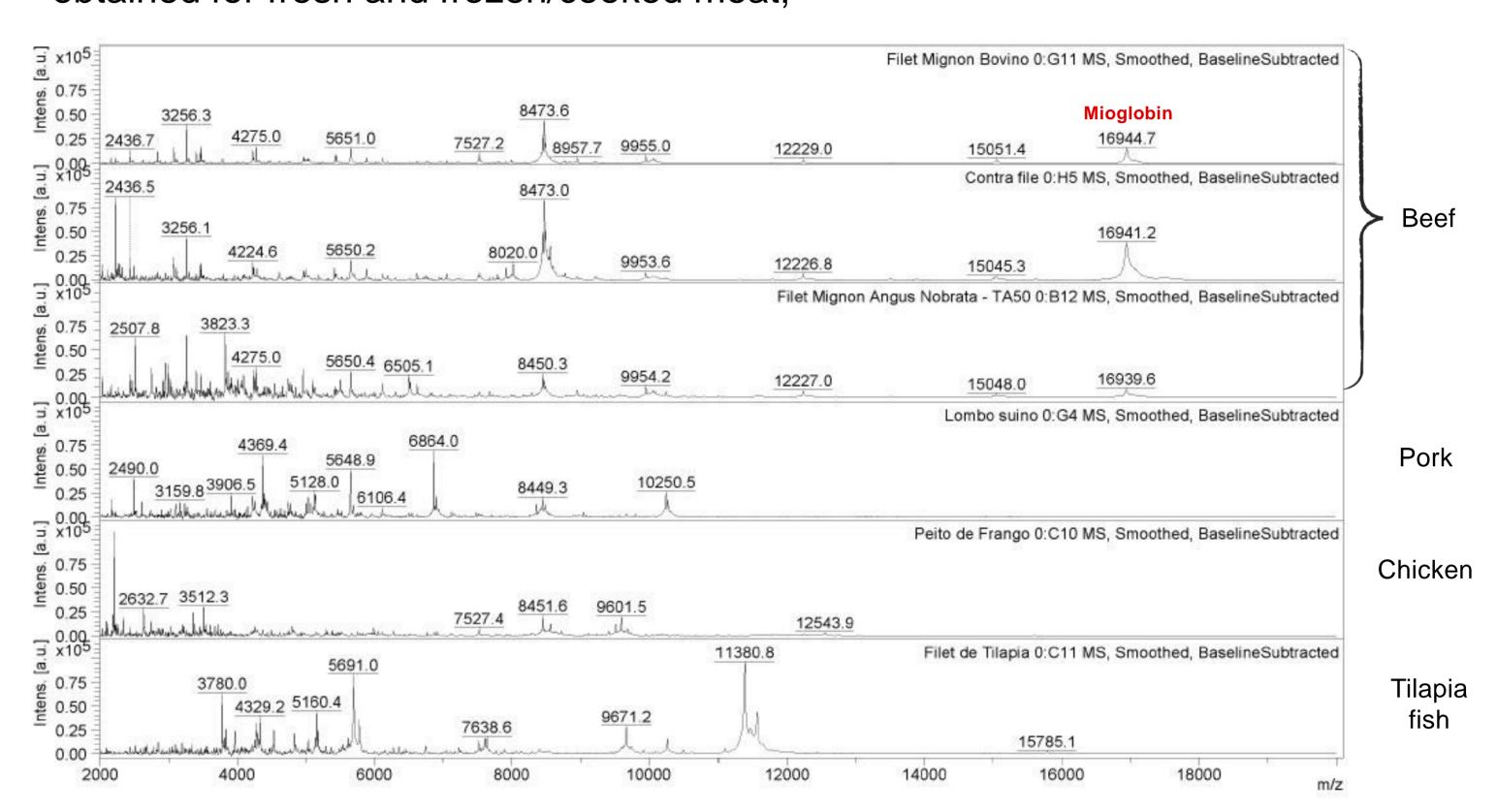
Pham TV, Jimenez CR. Computational analysis of high-throughput MALDI-TOF-MS-based peptide profiling. In: Hillenkamp F, Peter-Katalinic J, editors. MALDI MS: a practical guide to instrumentation, methods and applications. 2nd ed. Weinheim: Wiley Blackwell; 2014. p.411-30.

Rau J, Hiller E, Männig A, Dyk M, Wenninger O, Stoll P, et al. Animal Species Identification of Meat Using MALDI-TOF Mass Spectrometry. ChemRxiv. 2021; doi:10.26434/chemrxiv.14229413.v1 This content is a preprint and has not been peer-reviewed.

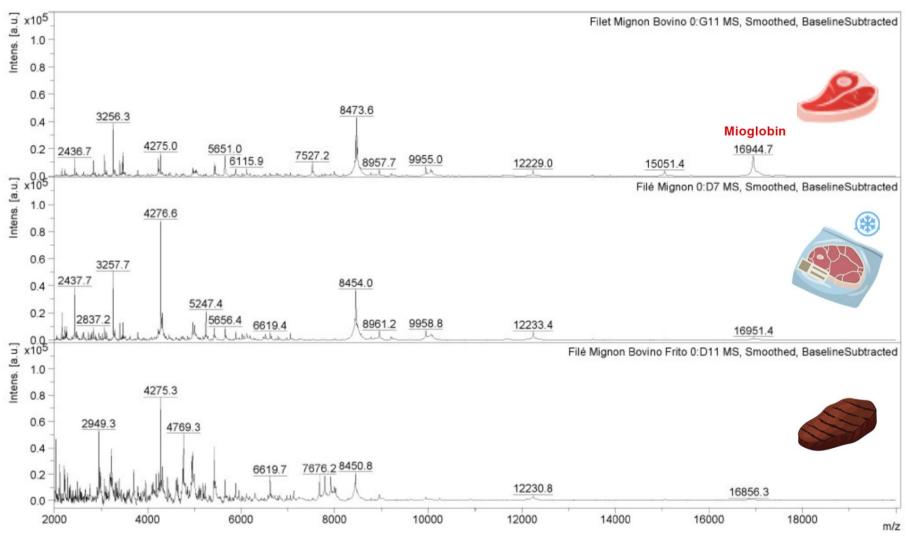
Funding

Results

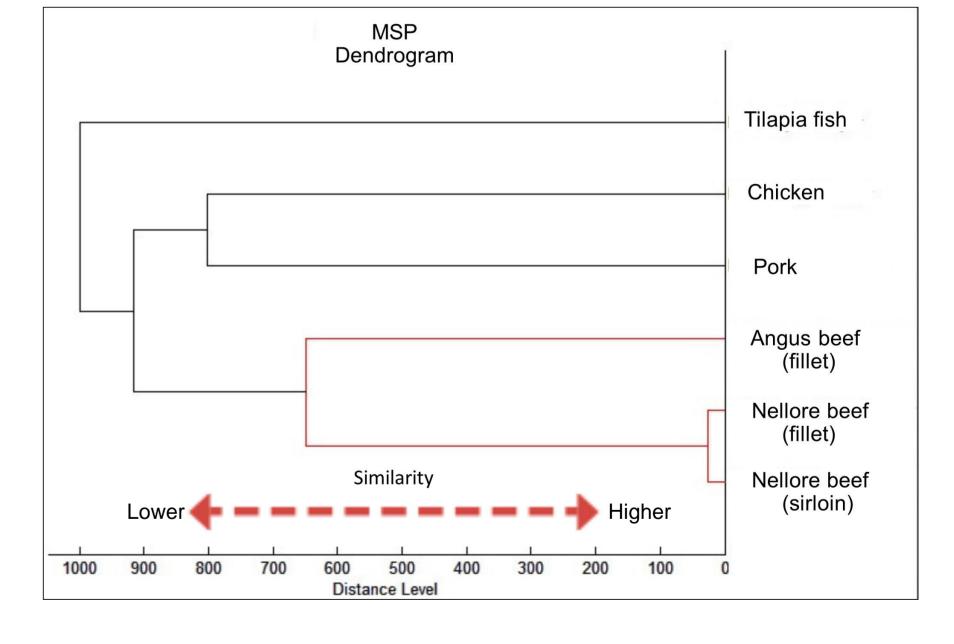
• The meat protein extraction methodology was successful, and good spectra were obtained for fresh and frozen/cooked meat;



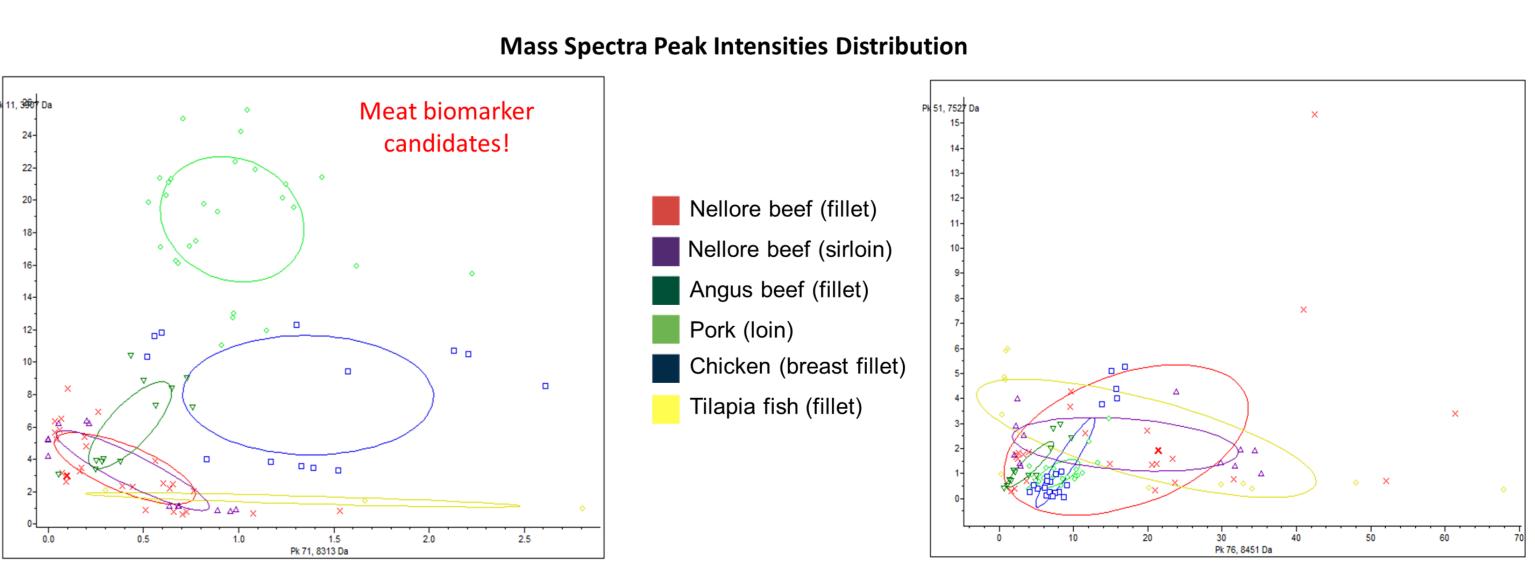
- Fresh samples of beef (n=6), pork (n=6), chicken (n=4) and tilapia (n=2) were analyzed to date, and clearly distinct peaks were observed in the spectra among those four types of meat;
- Considering peaks with signal/noise equal to or greater than 5, the mean numbers
 of peaks obtained were 30, 26, 17 and 41, respectively for beef, pork, chicken and
 tilapia;
- Analysis with the Biotyper algorithm allowed correct identification with an average score equal to or greater than 2,0 for beef after freezing or cooking;



The mass spectra obtained also allowed the distinction between Nellore and Angus breeds, but so far they have not differentiated between the fillet mignon and sirloin cuts;



• PCA classification revealed peaks that may be candidates for biomarkers of the meat types.



Graphic points = peak intensity in each mass spectrum

Ellipses = standard deviation of the mean intensities