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Proceeding Paper

Meat Species Identification and Classification by MALDI-TOF Mass Spectrometry †

Newton Valerio Verbisck [1,*], Larissa Bortoli de Souza [2], Marita Vedovelli Cardozo [3], Nilton Gabriel Paiva Guimarães [1] and Gelson Luis Dias Feijó [1]

[1] Embrapa Beef Cattle, Campo Grande 79106-550, Brazil
[2] Institute of Biosciences, Federal University of Mato Grosso do Sul, Campo Grande 79070-900, Brazil
[3] Department of Pathology, Reproduction and One Health, School of Agricultural and Veterinarian Sciences, São Paulo State University, Jaboticabal 14884-900, Brazil
* Correspondence: newton.verbisck@embrapa.br
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Abstract

Protein profiling generated by MALDI-TOF (matrix-assisted laser desorption ionization-time-of-flight) was used to distinguish meat from different livestock species and identify meat species. Meat proteins from fresh beef (n = 12), pork (n = 7), chicken (n = 5) and tilapia fish (n = 3) were extracted and analyzed in a MALDI Biotyper mass spectrometer (Bruker Daltonics, Bremen, Germany) with alpha-cyano-4-hydroxycinnamic acid as the matrix. Mass spectra allowed the distinction of meat species, including between Nellore and Angus bovine breeds, and PCA classification revealed possible biomarkers for meat types. Our results corroborate MALDI-TOF mass spectrometry as an interesting tool for meat identification, which is useful for quality control and the certification of meat products.

Keywords: meat; protein profiling; MALDI-TOF; quality control; certification

1. Introduction

Species identification in meat is an important subject for quality control, food safety and consumer protection [1]. Furthermore, determining the authenticity of meat allows for the detection of fraud in high-value commercial meats, which can occur through substitution with meats from lower-value species, nutritionally inferior meats, or even vegetable proteins, such as soy [2,3]. Meat species identification can be reliably performed using molecular techniques, through the analysis of proteins and nucleic acids [4], especially through the use of immunological methods or the amplification and sequencing of species-specific genes [5,6]. However, these methodologies are costly and relatively technically complex, as well as time-consuming, which often hinders their implementation and routine use by regulatory bodies, particularly in low-income countries.

Mass spectrometry is the analytical technique of choice for determining the mass-to-charge ratio (m/z) of atoms or molecules in an ionized state, thus obtaining molecular mass values with high accuracy and resolution [7]. MALDI-TOF mass spectrometry, an acronym for matrix-assisted laser desorption ionization–time-of-flight, is an analytical methodology for m/z determination and molecular identification, which stands out for its extremely high sensitivity, high resolution, high accuracy and scalability, ease of execution and relatively low cost [8,9]. MALDI-TOF mass spectrometry allows for the obtaining of protein mass profiles for identification and classification of biological samples [8,10], and MALDI-TOF

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has shown potential for the characterization and identification of plants [11] and animal species, including birds, reptiles and mammals [12] has been demonstrated. Here, we use MALDI-TOF protein mass profiling to distinguish meat from different livestock species, with the aim of demonstrating its usefulness for meat species identification.

2. Materials and Methods

2.1. Samples

Beef (tenderloin and sirloin, n = 12), pork (loin, n = 7), chicken (breast filet, n = 5), and tilapia (filet, n = 3) samples were acquired from a hypermarket (Comper chain) in Campo Grande-MS, Brazil, with less than 12 h of shelf time. In a laminar flow chamber at room temperature, samples of approximately 3 mm^3 of muscle tissue without apparent fat were collected using a scalpel with a disposable blade. Internal parts of the meat pieces were analyzed to avoid possible oxidation and contamination of the surface tissue. The collected tissues were deposited in sterile Eppendorf plastic tubes for protein extraction immediately afterward. The effect of freezing meat was evaluated by comparing the mass spectra of fresh meat with those of the same sample after freezing at −18 °C for approximately 24 h. For the analysis of the meat after heat processing, the pieces were cut into portions approximately 50 g and 0.5 cm thick and fried for 2 min on each side, using a small amount of canola oil in a frying pan. After cooling to room temperature, part of the inner portion was removed for MALDI-TOF analysis.

2.2. Protein Extraction

Proteins were extracted as described in [12], with modifications. A total of 100 µL of solvent (50% acetonitrile, 42.5% MilliQ (Merck Millipore, Burlington, MA, USA) ultrapure water, and 2.5% trifluoroacetic acid) were added to meat tissues for maceration with a sterile plastic micropestle, without silica beads. After centrifugation at 16,000× *g* for 2 min at room temperature, the supernatants containing the extracted protein fraction were collected for mass spectra acquisition. For this purpose, 1 µL of each protein extract was applied to a MALDI-TOF target (Bruker Daltonics, Bremen, Germany), followed by the addition of 1 µL of α-cyano-4-hydroxycinnamic acid (5 mg/mL) in a solution of 50% acetonitrile, 49.9% MilliQ ultrapure water, and 0.1% trifluoroacetic acid. After drying, the crystallized mixture was analyzed using a Biotyper Sirius One MALDI-TOF mass spectrometer (Bruker Daltonics).

2.3. Mass Spectra Acquisition and Analysis

Mass spectra acquisition was performed as described by Bier et al. [13]. Mass spectrometer parameters were IS1 source voltage 20 kV, IS2 source voltage 18.55 kV, lens voltage 8.80 kV, and an ion extraction delay time of 240 ns, in positive linear mode and with a mass-to-charge ratio (m/z) in the range between 2000 and 20,000 Daltons. Mass spectra were randomly acquired and summed to 1×10^5 per sample, after external calibration with the BTS calibrant mixture (Bruker Daltonics). For the analysis of mass spectra and protein-peak-based meat identification, the computer programs FlexAnalysis v.3.4 and MALDI Biotyper Compass Explorer v.4.1 (Bruker Daltonics) were used. Reference mass spectra (MSP) for each type of meat were generated using the standard parameters of the MALDI Biotyper Compass Explorer v.4.1, from a set of 20 mass spectra selected from at least three different samples of each meat type. The meat dendrogram was produced using the standard parameters of the MALDI Biotyper computer program, with the distance measurement determined by correlation and complete linkage, and not score-oriented. The score values for frozen and fried meats were determined using the standard parameters of the MALDI Biotyper program. The mean and standard deviation of the intensity of peaks

with a signal-to-noise ratio greater than or equal to 2 were calculated in sets of 20 mass spectra acquired for each meat species, in a comparative statistical analysis of MALDI-TOF mass profile variance using the ClinProTools v.3.0 software (Bruker Daltonics).

3. Results

The meat protein extraction protocol was successful for the beef, pork, chicken and tilapia fish samples analyzed so far, as can be seen from the representative mass spectra shown (Figure 1). Abundant peaks were found at the 2 to 10 kilodaltons (kDa) mass range in all meat types. Notably, it was also possible to identify the bovine myoglobin peak at ~16,944 Da.

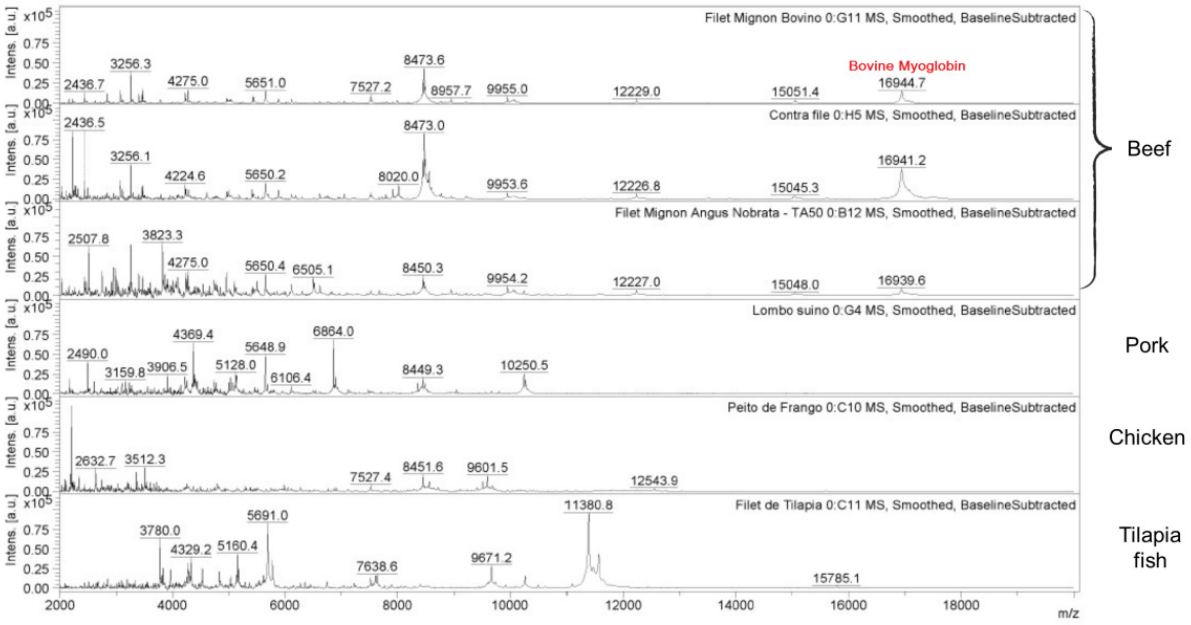

Figure 1. Representative MALDI-TOF mass spectra for commercial cuts of beef, pork, chicken and tilapia fish. Fresh samples were analyzed, and clearly distinct protein peaks were observed among these four types of meat. The bovine myoglobin peak (~16.944 Daltons) is highlighted in red.

Considering peaks with a signal-to-noise ratio greater than or equal to 5, the average number of peaks obtained was 30, 26, 17, and 41, respectively, for beef, pork, chicken, and tilapia fish (Table 1), denoting that our protein extraction protocol supports the mass profiling of meat types.

Table 1. Quantitative and qualitative evaluation of MALDI-TOF mass spectra of meat types.

Meat Species	Sample Number	MALDI-TOF Mass Spectra Number	Average Number of Peaks with Signal/Noise $\geq$ 5
Beef	12	63	30
Pork	7	33	26
Chicken	5	27	17
Tilapia Fish	3	20	41

Representative mass spectra obtained for frozen and fried beef are also presented (Figure 2). Analysis with the Biotyper algorithm allowed for correct identification, with an average score value equal to or greater than 2.0, indicating that beef can be correctly identified after freezing or frying.

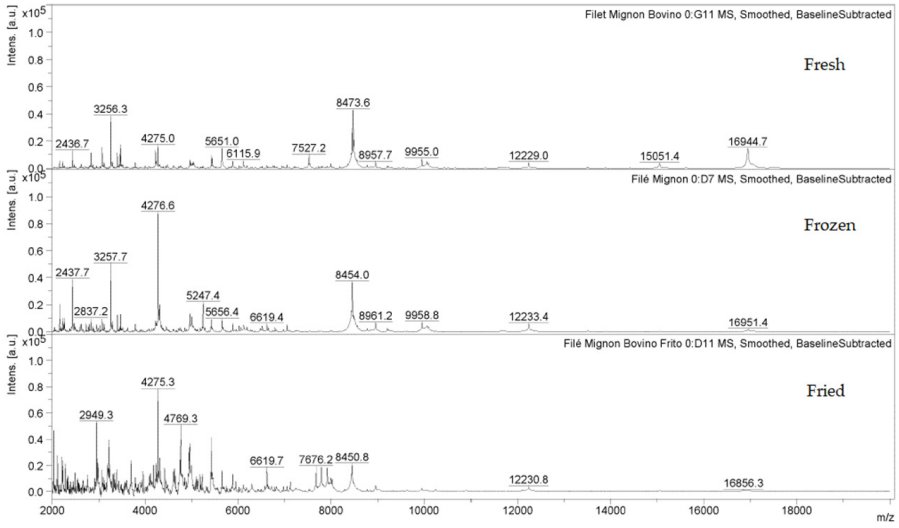

Figure 2. MALDI-TOF mass spectra of fresh, frozen and fried beef (tenderloin).

The protein mass profiles obtained also allowed for the distinction between the Nellore and Angus bovine breeds after a dendrogram analysis, which takes into account all observed peaks. Conversely, it was not possible to differentiate between the tenderloin and sirloin beef cuts (Figure 3).

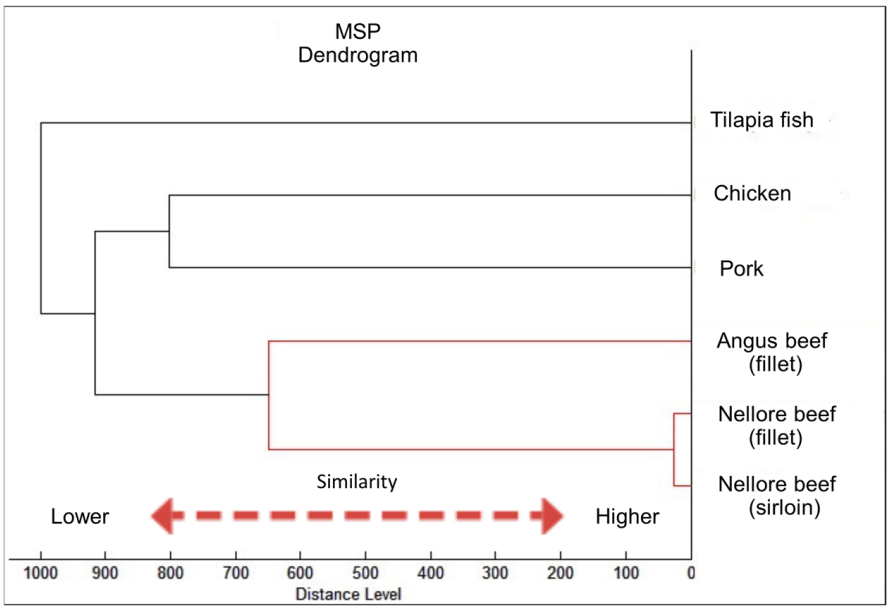

Figure 3. Dendrogram of MALDI-TOF mass profiles for beef, pork, chicken and tilapia fish. Reference mass spectra (MSP) were obtained with 20 mass spectra for each type of meat, and dendrogram analysis was performed with MALDI Biotyper software (Bruker Daltonics). Distances were measured by correlation with complete linkage, not score-oriented.

Furthermore, meat classification was evaluated by statistical analysis of variance for protein peak intensities, which revealed peaks that may be candidates for biomarkers of meat types (Figure 4). For instance, peaks of 3907 and 8313 kDa were found to be good classifiers for the meat types tested, including beef from Nellore and Angus bovine breeds, corroborating the results obtained in our previous dendrogram analysis.

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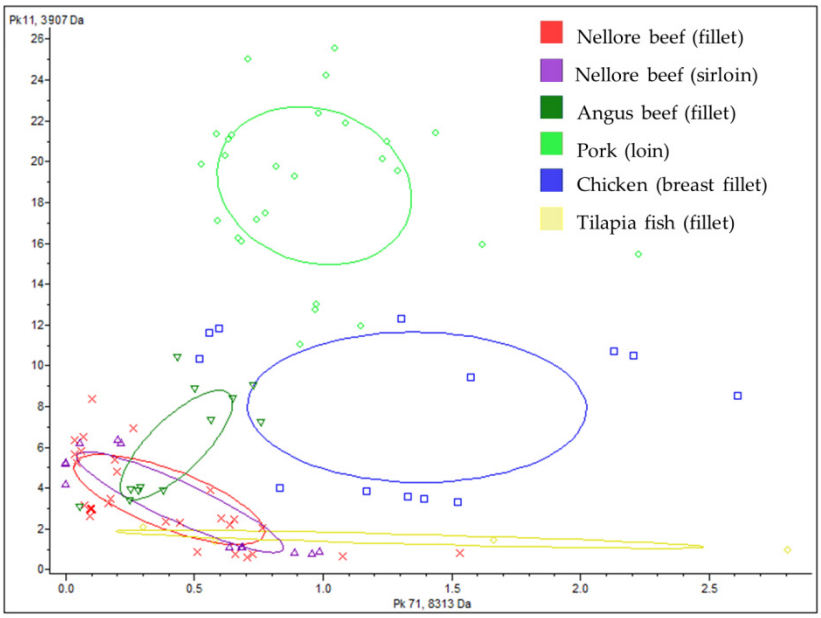

Figure 4. Classification of beef, pork, chicken and tilapia fish meats. The 2D PCA plot illustrates peak intensity distribution and variation for the 3907 and 8313 kDa peaks in 20 mass spectra from at least three technical replicates for each meat type. Graphic points represent peak intensities in each mass spectrum and ellipses represent standard deviation of the mean intensities, calculated with ClinProTools V.3.0 software (Bruker Daltonics).

Finally, the MSP for beef, pork, chicken and tilapia fish were generated with the MALDI Biotyper software and deposited at the Embrapa Research Data Repository (Redape) [14].

4. Discussion

The use of mass spectrometry for meat species determination is relatively recent and was also motivated by a fraud scandal in Europe, reported in 2013, in which horse meat was detected in beef hamburgers in Ireland [15]. The first report of the use of MALDI-TOF for determining the animal species from which meat originates was by Flaudrops et al. [16]. In this work, the authors analyzed beef (young and adult), pork, horse, and chicken meat in raw or cooked in various ways, and were able to correctly group them according to species [16].

A few years ago, a group of researchers from the Chemical and Veterinary Analysis Agency Stuttgart (CVUAS), Germany, conducted a study published in the ChemXRiv repository, demonstrating that it is possible to distinguish meats from different animal species [12]. These authors analyzed 1088 meat samples, 719 from mammals, 348 from poultry, and 21 from reptiles, and concluded that for the main meat species available on the market, MALDI-TOF allowed for the reliable identification of all samples [12].

In our work, it was possible to demonstrate the principle of the method for identifying fresh and processed meats by freezing and frying, through the analysis of protein mass profiles obtained by MALDI-TOF. Our protein extraction protocol was simplified compared to that recommended by Rau et al. [12], since we did not use silica beads for meat maceration, making the procedure slightly faster and less expensive, while obtaining quite informative mass spectra. Furthermore, it was possible to distinguish beef from Nellore (*Bos indicus*) zebu cattle and Angus (*Bos taurus*) taurine cattle, which is interesting due to the higher commercial value of beef from European breeds, something that, to our knowledge, had not yet been demonstrated.

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5. Conclusions

The preliminary data presented here allow us to conclude that different meat species can be correctly classified with the MALDI-TOF mass spectrometry methodology based on protein mass profile analysis, and this may be used in the future for meat quality control and certification. The new aspect presented here comes from the possibility of distinguishing cattle breeds, and further analyses will need to be carried out to validate and compare these results with those of other established methodologies for identifying meat species.

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Data Availability Statement: The reference mass spectra generated in this work are deposited at the Embrapa Research Data Repository (Redape) and are available upon request.

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Conflicts of Interest: Author N.V.V., G.L.D.F. and N.G.P.G. are employed by the company Embrapa, L.B.d.S. was a graduate student from Federal University of Mato Grosso do Sul, and M.V.C. is employed by Sao Paulo State University. The authors declare that this study received funding from Embrapa and Fundect. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

Abbreviations

The following abbreviations are used in this manuscript:

MALDI-TOF	Matrix-Assisted Laser Desorption Ionization–Time-Of-Flight
PCA	Principal Component Analysis

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