APPLYING FT-MIR SPECTROSCOPY FOR PROTEIN CONTENT ANALYSIS IN DEFATTED AÇAÍ PULP: ADVANCING BIOECONOMY AND CIRCULARITY

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ABSTRACT

This study aims to conduct spectral evaluation for subsequent use in protein quantification in defatted and dried açaí pulp using FT-MIR spectroscopy. Currently, the determination of proteins in this pulp involves laborious and potentially toxic methods. The research explored specific protein vibrational frequencies in the MIR region, identifying the amide I (~1650 cm^{-1}) and amide II (~1550 cm^{-1}) bands as promising for predicting protein content. A total of 61 spectra were analyzed, although some exhibited noise and other technical issues. These advancements have significant implications for nutritional analysis and can benefit food research with more efficient and environmentally friendly methods.

Keywords: Açaí. Spectral evaluation. FT-MIR. Spectral bands.

1 INTRODUCTION

The açaí (*Euterpe oleracea*) is a species of palm tree that plays a significant role in the flora of streams, upland areas, and floodplain regions. Mostly distributed in the eastern portion of the Amazon, it spans the states of Pará, Amapá, Maranhão, Tocantins, Lower Amazonas, and Piauí, and is also found in the Guianas and Venezuela. Açaí fruit formation occurs in clusters, and this process begins in the plant's third year of life. Açaí stands as the most significant fruit of the socio-biodiversity within the Amazon region. Nutritionally, açaí boasts lipids (32.5 to 50.5% of total solids - TS), total dietary fiber (20.9 to 21.8% TS), and proteins (8.1 to 12% TS) as its main constituents. However, it is renowned as a superfruit due to its high content of antioxidants such as anthocyanins (1,365.2 mg/kg fruit) and α-tocopherol (45 mg/100g TS). The booming açaí industry, driven by global demand, highlights the crucial need for quality control to maintain product authenticity and integrity.[1] Advanced analytical methods are essential for consistent quality and nutritional assurance in açaí-based products.

A circular economy reduces dependency on new resources by extending resource use through alternate cycles, complemented by the bioeconomy, which uses biological products and processes. This "circular bioeconomy" is promising for sustainability but still developing.[2] Traditional protein determination methods like Kjeldahl, UV absorption, and HPLC are laborious. Mid-infrared spectroscopy (MIR) offers a faster, affordable alternative for food analysis, but lacks a specific method for açaí pulp.[3] The study identified protein vibrational frequencies in the MIR region, with amide bands promising for protein content prediction.

Integrating the principles of circular bioeconomy with the açaí industry can enhance sustainability by promoting efficient use of resources and reducing waste. By utilizing advanced techniques such as MIR spectroscopy for protein analysis, the industry can improve product quality while adhering to environmentally friendly practices. This approach not only supports the bioeconomy by leveraging biological resources but also aligns with the circular economy by ensuring the prolonged and efficient use of these resources.

2 MATERIAL & METHODS

2.1 Samples Preparation

Açaí fruits (9 kg) were sourced from local farmers in twenty-six municipalities in the state of Pará, Brazil, and transported under refrigeration (4°C) to the Centre for Valorization of Amazonian Bioactive Compounds (CVACBA). The processing involved washing, softening, pulping, and freeze-drying for 48 hours, followed by Kjeldahl analysis for protein determination. Additionally, all samples underwent oven drying at 105°C for 16 hours to minimize water content and avoid interference in spectroscopic analyses. The total number of samples was sixty-one (61).

2.2 Spectral Acquisition by Fourier Transform Mid-Infrared Spectroscopy.

Instrumentation and spectral acquisition utilized the Agilent Cary 630 FTIR-ATR equipment with a ZnSe crystal. Spectral acquisition employed an optical resolution of 4 cm^{-1} with a 10-second acquisition time, and 32 scans were conducted for each spectrum within a spectral range of 4000 to 650 cm^{-1}, generating 900 variables. The standard data acquisition software (MicroLab) facilitated crystal cleaning configuration and validation of cleaning efficiency. The first step to conduct this analysis involved

subjecting the defatted açaí pulp samples to a drying process to avoid interference in spectrum readings due to hydrophilic interactions. The moisture content of the samples was standardized in an oven at 105°C until reaching a constant weight, followed by storage in a desiccator until the reading time. After placing the sample on the crystal, a reading was performed, presenting the spectrum result.

3 RESULTS & DISCUSSION

3.1 Sample statistics

The data presented in Table 1 show a protein variation with range from 9.69% to 15.72%, with an average of 13.27% and a median also at 13.27%. The data have been previously analyzed by Principal Component Analysis (PCA), and spectral outlier samples have been removed to enhance model performance. The standard deviation of approximately 0.78% indicates a relatively modest dispersion around the mean, while the variance of about 0.61% quantifies the magnitude of this dispersion. The prediction set, on the other hand, demonstrates lower dispersion compared to the calibration set, as evidenced by the lower standard deviation and variance. Furthermore, the skewness close to zero suggests that all sets exhibit an approximately symmetric distribution. These values provide a comprehensive view of the distribution and variability of percentage data, contributing to a more detailed analysis of its nature and trends.

Table 1. Statistical summary of protein content in defatted and freeze-dried açai in calibration and prediction sets.

Parameters*	Geral	Calibration set	Predition set
Number of samples	61	43	18
Mean, %	13.28	13.04	13.12
Max., %	15.72	15.72	15.01
Min., %	9.69	9.69	12.04
Median, %	13.27	13.19	13.23
Range, %	6.03	5.32	2.97
Standard deviation, %	0.78	1.06	0.72
Variance, %	0.61	1.12	0.51
Skewness	0.23	-0.11	0.26

* Max, maximum; Min, minimum.

The data presented in Table 1 show protein variation ranging from 9.69% to 15.72%. The data were previously analyzed by Principal Component Analysis (PCA), and spectral outlier samples were removed to enhance model performance. The standard deviation of approximately 0.78% indicates modest dispersion around the mean, while the variance of about 0.61% quantifies this dispersion's magnitude. The prediction set demonstrates lower dispersion compared to the calibration set, as evidenced by the lower standard deviation and variance. Furthermore, the skewness close to zero suggests that all sets exhibit an approximately symmetric distribution. These values provide a comprehensive view of the distribution and variability of the percentage data, contributing to a more detailed analysis of its nature and trends.

3.2 Spectral characteristics

In a comprehensive manner, the MIR spectrum displays a division into four prominent regions, as depicted in Figure 1, highlighting distinct absorption peaks in the fingerprint region (1500–600 cm^{-1}). These regions include double bond stretching (2000–1500 cm^{-1}), triple bond stretching (2500–2000 cm^{-1}), and X–H stretching (4000–2500 cm^{-1}), each playing a significant role in characterizing the average spectrum. This spectral pattern has been supported by previous studies that emphasized the presence of these spectral ranges as fundamental for the analysis and identification of proteins.[4]

The sharpness of absorption peaks in the fingerprint region is particularly relevant as it reflects the unique contribution of different functional groups, providing valuable insights into the molecular composition and chemical structure of the sample in question. These observations underscore the importance of MIR as a powerful analytical tool for investigating complex samples, as evidenced by the characteristic markers present in the various spectral regions mentioned.

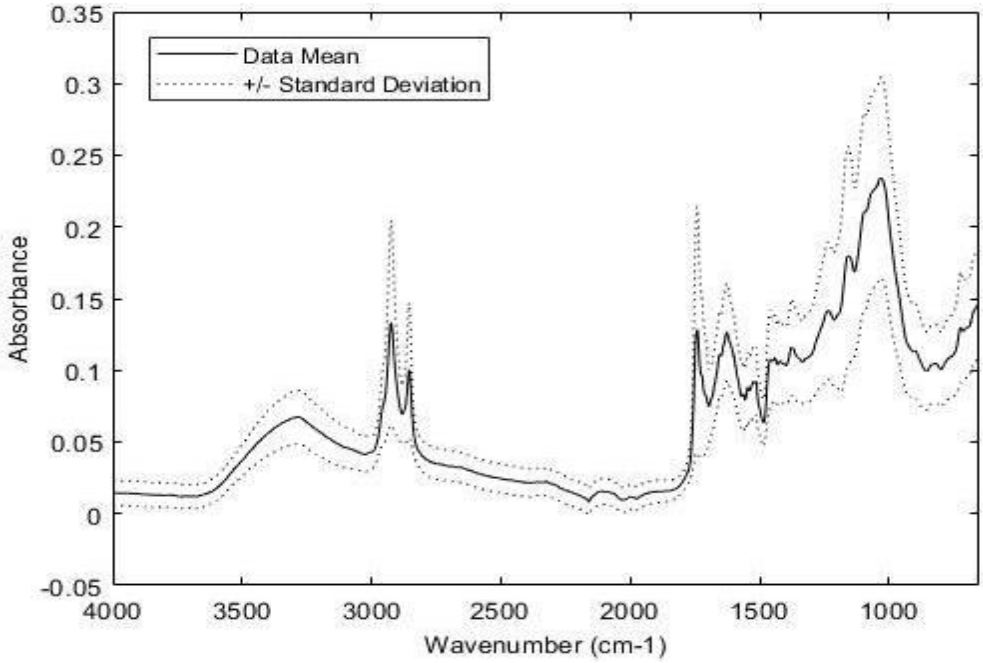

Fig. 1. FT-MIR spectral of freeze-dried açai.

In the extended MIR spectral region, protein molecules reveal vibrational frequencies, with the amide I (~1650 cm $^{-1}$) and amide II (~1550 cm $^{-1}$) bands standing out as the two most prominent and informative. The uniqueness of these bands makes them instrumental in protein prediction and identifying variations in protein secondary structure.[5] The amide I region originates from the C=O stretching vibration in the amide group, coupled with the stretching of the C-N bond and the in-phase bending of the N-H bond, recognized as the most sensitive region for assessing complex compositions of secondary structures.[6] In contrast, amide II is predominantly derived from in-plane N-H bending and C-N stretching vibration, presenting a higher complexity than amide I and therefore is rarely employed in the detailed analysis of proteins.[5]

4 CONCLUSION

In a comprehensive analysis of the MIR spectrum, two prominent regions were identified, with distinct absorption peaks highlighted in the fingerprint region. The sharpness of these peaks reflects the unique contribution of different functional groups, providing valuable insights into the molecular composition and chemical structure of the sample. Additionally, the amide I and amide II bands in the extended MIR spectrum emerged as instrumental in predicting proteins and identifying variations in protein secondary structure. These observations underscore the importance of MIR as a powerful analytical tool for investigating complex samples, delivering precise and reliable results. Integrating this analytical approach with the concepts of the circular economy and bioeconomy can further enhance sustainability in the investigation and utilization of biological resources. In this way, the combination of MIR's analytical capabilities with the principles of the circular bioeconomy can lead to significant advancements in scientific research and industrial application, contributing to a more sustainable and resilient future.

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