UFSM

CIÊNCIAeNATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, e92511, v. 48, 2026 • https://doi.org/10.5902/2179460X92511
Submitted: 06/17/2025 • Approved: 09/03/2025 • Published: 08/11/2026

Chemistry

Application of mid-infrared spectroscopy and nuclear magnetic resonance to assess the quality of processed Amazon nuts

Aplicação da espectroscopia no infravermelho médio (*MIR*) e Ressonância Magnética Nuclear (*RMN*) para avaliação da qualidade de Castanhas-da-amazônia processadas

Tiago Bratilieri dos Santos[I], Ladyslene Chrísthyns de Paula[I], Luiz Alberto Colnago[II], Lúcia Helena de Oliveira Wadt[III], Samir Carvalho Buzaglo Pinto[IV], Ariane Mendonça Kluczkovski[IV], Anthonioni Peron Dal Sasso[V], Gabrieli Oliveira Folador[I]

[I] Universidade Federal de Rondônia, Ariquemes, RO, Brazil
[II] Embrapa Instrumentation, São Carlos, SP, Brazil
[III] Embrapa Rondônia, Porto Velho, RO, Brazil
[IV] Universidade Federal do Amazonas, Manaus, AM, Brazil
[V] Instituto Federal de Rondônia, Ariquemes, RO, Brazil

ABSTRACT

The Amazon nut exhibits considerable variation in its nutritional composition, influenced by geographic origin, genetic characteristics of the plant, and production environments. To characterize the composition of almonds from various processed Amazon nut samples, mid-infrared spectroscopy (*MIR*) and Nuclear Magnetic Resonance (*NMR*) were employed. *MIR* analysis identified functional groups of proteins and lipids, offering valuable insights into the molecular composition of the samples. In contrast, NMR was used to evaluate transverse relaxation times (T_2), indicating a high presence of unsaturated fatty acids in the almonds. These methodologies can expedite sample characterization for the industry, aiding in the selection of nutritionally superior products. This study underscores the significance of advanced instrumental methods in food analysis, presenting an efficient and accurate alternative for nutritional characterization. *MIR* spectroscopy, *NMR*, and traditional analytical methods together provide consistent and complementary information on the nutritional variability of Amazon nuts and can be used jointly to analyze and characterize different samples.

Keywords: Nutritional quality; *Bertholletia excelsa* Bonpl, Instrumental analytical methods

RESUMO

A castanha-da-amazônia pode apresentar variações significativas em sua composição nutricional, ligados a origem geográficas, características genéticas da planta e aos ambientes de produção. Nesse sentido a espectroscopia no infravermelho médio (MIR) e a Ressonância Magnética Nuclear (RMN) foram empregadas para caracterizar a composição de amêndoas de diferentes amostras de castanha-da-amazônia beneficiadas. As análises MIR identificaram grupos funcionais de proteínas e lipídios, fornecendo informações relevantes sobre a composição molecular das amostras, enquanto a RMN foi utilizada para avaliar os tempos de relaxamento transversal (T_2), sugerindo a presença de ácidos graxos insaturados em grandes quantidades nas amêndoas. A aplicação dessas metodologias pode acelerar a caracterização de amostras para a indústria, contribuindo para a seleção de produtos de alta qualidade nutricional. Este estudo destaca a importância da utilização de métodos instrumentais avançados na análise de alimentos, proporcionando uma alternativa eficiente e precisa para a caracterização nutricional, pois a espectroscopia MIR, RMN e os métodos analíticos tradicionais aliados fornecem informações consistentes e complementares sobre a variabilidade nutricionais da castanha-da-amazônia e podem ser usados em conjunto para analisar e caracterizar diferentes amostras.

Palavras-chave: Qualidade nutricional; *Bertholletia excelsa* Bonpl; Métodos analíticos instrumentais

1 INTRODUCTION

The Amazon nut, commonly known as the "Pará nut" or "Brazil nut," originates from the fruit of the chestnut tree (*B. excelsa Bonpl,* 1808). This large tree belongs to the *Lecythidaceae* family and is an endemic species of the Amazon rainforest. It naturally occurs in Brazil, Bolivia, Peru, and to a lesser extent, in Suriname, the Guianas and southern Venezuela (Maldonado et al., 2020). The fruit is a hard wooden sphere called a hedgehog, which contains 10 to 25 seeds that protect the nuts. The chestnut comprises the seed, known as the almond, covered by a shell. The term seeds refers to the whole nut (shell plus almond), while walnuts or almonds refer exclusively to the edible part (Botelho et al., 2019).

The Amazon nut is high nutritious, consisting of 60 and 70% of lipids, primarily polyunsaturated fatty acids. It contains an average 38% oleic acid and 35–37% of linoleic acid, together accounting for approximately 75% of total fatty acids. Additionally, it contains 24% saturated fatty acids, including palmitic (13%), stearic (10%), and arachidonic (1%) acids, as well as tocopherols, which may help prevent cancer, atherosclerosis, and inflammatory diseases (Cardoso et al., 2017; Danielski et al., 2021).

According to Maestri et al. (2020), variations in oil content and fatty acid compositions in Amazon nuts are linked to geographic origin and production environments. These nuts exhibit high levels of polyunsaturated fatty acids, particularly linoleic acid, along with smaller amounts of linolenic acid (<0.2%).

The Amazon nut is considered a functional food due to its composition, which includes various bioactive substances such as essential amino acids, minerals, and lipids. These components interact with the human body to promote health (Birch & Bonwick, 2019).

Even a small addition of almonds can help meet the intake requirements for essential amino acids. The nuts composition includes 15 to 20% high-quality proteins, rich in essential amino acids such as methionine and cysteine (Woolf et al., 2011). These levels can meet the theoretical standards recommended by the Food and Agriculture Organization of the United Nations (FAO, 2006). The proteins in the Amazon nut are closely associated with Se, with 96% of the selenium present being bound to these proteins, primarily in the form of selenomethionine and its metabolites, including selenocystathionine and γ-glutamyl-selenomethionine (Cardoso et al., 2017).

Almonds also contain significant concentrations of carotenoids and vitamin E, which act as antioxidants, combating free radicals, and helping to prevent premature aging. They are also rich in calcium, phosphorus, magnesium, and B vitamins (Kluczkovski & Scussel, 2015).

Despite their nutritional potential, the quality of Amazon nuts can be compromised, affecting both the sensory characteristics of the almonds and the health benefits derived from their consumption. This is particularly due to oxidative rancidity and contamination by fungi and aflatoxins (AFLs). Aflatoxins (AFB1, AFB2, AFG1, and AFG2) are mycotoxins produced by fungi of the genus Aspergillus, especially Aspergillus *flavus*, though other species such as A. *parasiticus* and A. *nomius* can also produce AFLs. AFB1 is considered the most toxic aflatoxin and is classified as a Group-1 carcinogen for humans (FIB 2009; Casagrande et al., 2019).

Given these concerns, evaluating the quality of these almonds is essential. However, as we well know, conventional chemical analysis methods can be time-consuming and labor-intensive, even when the procedures are relatively straightforward (Silva et al., 2023).

Rapid, non-destructive characterization has become essential for quality control, traceability, and authentication of food products. Instrumental methods such as Mid-Infrared Spectroscopy (MIR) and Nuclear Magnetic Resonance (NMR) offer effective, fast and accurate alternatives, streamlining and enhancing the reliability of the characterization process (Mendes & Duarte, 2021; Sobolev et al., 2022).

MIR spectroscopy provides information based on the molecular vibrations of chemical bonds (C=O, C–H, N–H, O–H), revealing physicochemical properties in the spectral region between 4000 and 400 cm^{-1}. This technique allows the identification of structural changes in proteins, lipids, and polysaccharides, which are essential macronutrients (Dufour, 2009; Karoui et al., 2010). While NMR provides contrasts based on magnetic properties, the Free Induction Decay (FID), signal is processed to extract parameters such as relaxation times (T_1 and T_2), which are associated with molecular mobility, chemical structure, and the environment of atomic nuclei. This makes NMR widely applicable in oils and oilseeds (Constantino et al., 2014; Ancora et al., 2021; Sobolev et al., 2022).

In almonds and oilseeds, recent studies demonstrate the usefulness of Fourier Transform Infrared Spectroscopy (FT-IR), in the characterization of nutritionally relevant functional groups, cultivar recognition, geographic traceability, determination of fatty acid profiles, and almond differentiation (Cortés et al., 2019; Scappaticci et al., 2023; Lamas et al., 2025). Additionally, NMR has been used to assess the quality of raw and processed almonds, inspect nuts, and classify oils (olive, hazelnut, peanut), according to origin, quality, and potential adulteration. In oils and oilseeds, T_2 values increase with greater chain unsaturation and lower viscosity, while lower T_2 values indicate greater saturation, crystallization, or restricted mobility (Parker et al., 2014; Santos et al., 2022; Gianferri et al., 2023; Li et al., 2025).

Taken together, these analyses allow the identification of relevant chemical characteristics, such as oxidative and protein variations, oil mobility and viscosity, and factors sensitive to unsaturation, crystallization, and thermal processing. These physicochemical aspects are crucial for almond quality, justifying the application of MIR and NMR in assessing the quality of Amazon nuts.

It is important to note that data on the quality of Amazon nuts can enrich scientific information, diversify food industry processing techniques, and contribute to export growth, as Brazil is the world's largest producer of this nut. Therefore, the objective of this study was to apply MIR and NMR instrumental methodologies to evaluate the quality characteristics of processed Amazon nuts.

2 MATERIAL AND METHODS

2.1 Sampling

The almond samples from processed Amazon nuts were donated by five industries in the state of Rondônia, each providing 4 kg of almonds for the research. The sampling was conducted by the processing industry, with the almonds identified and coded as A01, A02, A03, A04, and A05. The samples were stored under vacuum and kept refrigerated until the time of analysis.

2.2 Determination of the physicochemical characteristics and quality parameters of Amazon nut kernels

MIR and NMR analyses were conducted at Embrapa Instrumentation in São Carlos-SP. Near infrared spectroscopy was performed using a Bruker spectrometer (model: Vertex) equipped with Fourier Transform (FT-TIR). Three slices from five almonds of each sample were placed directly on the ATR crystal. Measurements were taken between 4000 and 400 cm^{-1} with a resolution of 2 cm^{-1}.

NMR analyses were performed on a Bruker spectrometer (Mq 20) with a static magnetic field (B_0) of 0.21 T (8.5 MHz for ^{1}H). The spectrometer had a useful area of 10 cm in diameter and 10 cm in length. The ROSE sequence was used to generate an exponential decay with the transverse relaxation time (T_2).

For reference analyses, moisture, proteins, and lipids were measured following the official methodologies of the Adolf Lutz Institute (IAL, 2008). Moisture was determined by oven drying at 105 °C until constant weight. Protein content was measured by the Kjeldahl method, using a nitrogen-to-protein conversion factor of 5.46, specific for Amazon nuts. Lipids were quantified by the Soxhlet extraction method, employing n-hexane as the extraction solvent. All analyses were carried out at the Food Science and Technology Laboratory of the Federal University of Rondônia Foundation (UNIR), Campus Ariquemes-RO. The samples were first crushed in an industrial blender and then further ground in an analytical mill (Brand: Lucadema Mod. Multiuso). All analyses were performed in triplicate.

Lipid oxidation was assessed using Peroxide Index (PI) analysis, following IAL (2008) guidelines. Approximately 50 mL of oil was cold extracted using a hydraulic press (Brand: Bovenau, Model: P10300 10T), and stored away from light. For the analysis, 5 g of oil was weighed and diluted in 30 mL of an acetic acid-chloroform solution in a 3:2 ratio. Then, 0.5 ml of saturated potassium iodide (KI) solution was added, and the mixture was kept away from light for one minute. Afterwards, 30 mL of water and 0.5 mL of a starch indicator solution were added, followed by titration with a 0.1 M sodium thiosulfate solution until the blue color completely disappeared. The result was expressed in milliequivalents of active oxygen.

$$I.P\left(\mathrm{mEq\ O_2\ kg^{-1}}\right) = \frac{(V_{TA-}V_{TB}) \times N_t \times 1000}{m_a} \tag{1}$$

Where: Peroxide index (PI); $\mathbf{V^{TA}}$ = volume of sodium thiosulfate solution used in the sample (mL); $\mathbf{V^{TB}}$ = volume of sodium thiosulfate solution used in blank titration (mL); $\mathbf{N_t}$ = normality of sodium thiosulfate solution (0.1M); $\mathbf{m_a}$ = mass of the sample (g).

The samples were also analyzed for the presence and concentration of isolated and total AFLs, as well as water activity (A_w), at the Center for Studies in Food Composition and Toxicology (NECTA) at the Federal University of Amazonas (UFAM). AFLs levels were quantified according to AOAC methodology [991.31] (2016), using solid-phase extraction and High-Performance Liquid Chromatography for quantification, with recovery values and Relative Standard Deviation (RSDr) for AFLs. Water activity (A_w) was determined using AquaLab equipment (DECAGON, Mod. 4TE) at 25^{o}C, applying the dew point method in accordance with AOAC methodology [978.18] (2016).

2.3 Statistical design

The experiment followed a Completely Randomized Design, with three replications for each sample batch, and all analyses were performed in triplicate. The results for the variables were subjected to Analysis of Variance and compared using the Tukey test ($p > 0.05$) in the statistical program SAS® software (version 9.4) SAS (2013). Principal Component Analysis (PCA) was conducted using the Past® software (version 4.03), Hammer et al. (2001) and graphs were processed with OriginPro® Graphing Analysis (Version 10.1), Originlab (2024).

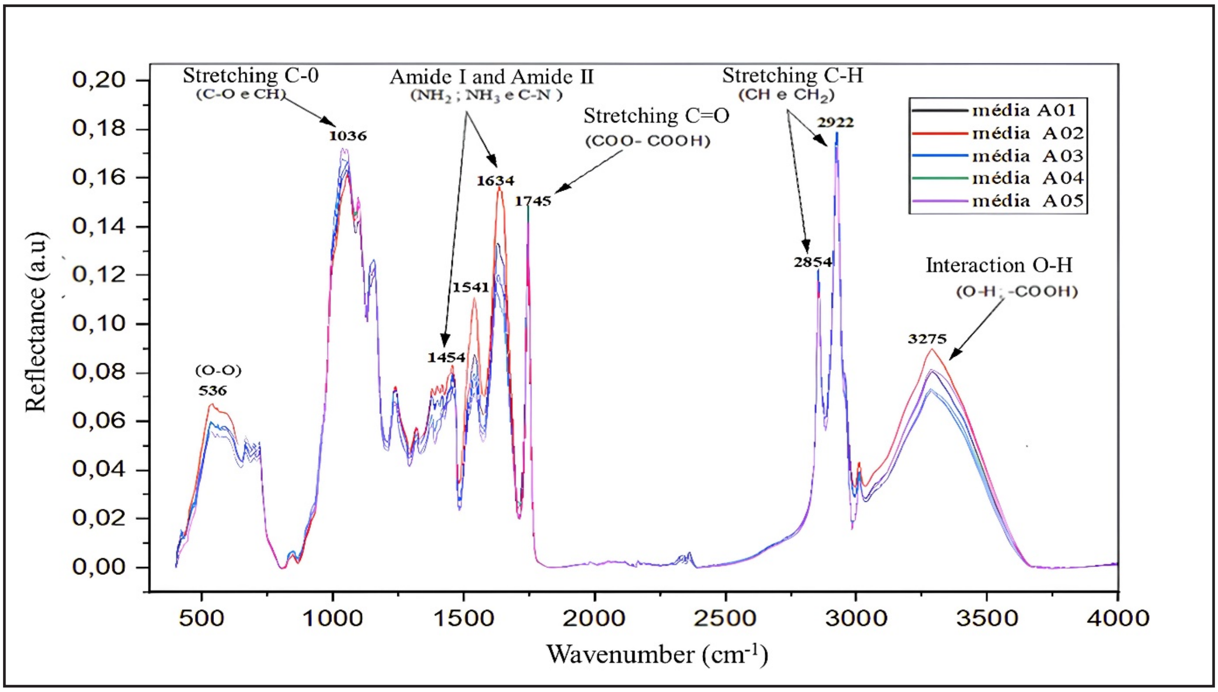
3 RESULTS AND DISCUSSION

3.1 Physicochemical quality of processed Amazon nut almonds

The *MIR* spectral range can be divided into two key regions: the functional group region (4000–1450 cm^{-1}) and the fingerprint region (1450–500 cm^{-1}). These regions are crucial for the identification and characterization of samples, as they are closely associated with the vibrations and interaction behaviors of the functional groups within the sample composition (Joshi et al., 2021).

The *MIR* spectra (Figure 1) displays the typical vibration patterns of organic material constituents, such as proteins, lipids, and vibrational distortions due to water content. These patterns reflect the composition of Amazon nut kernels and their variations.

Figure 1 – Fourier transform mid-infrared (*FT-MIR*) spectra of five processed Amazon nut kernel samples



Source: Authors private collection (2024)

In addition to the complexity of the chemical composition, other components in the samples, such as the curvature bands of adsorbed water, can interfere with the spectra. The spectral region between 3000 and 3800 cm^{-1} is strongly related to the presence of water, even in dehydrated samples, as it corresponds to strong interactions (OH). The location and shape of these bands can be influenced by factors such as the presence of solutes, hydrogen bonding, and temperature (Karoui et al., 2010; Derksen et al., 2023).

Sample A02 exhibited the highest moisture content (2.99 $g.100g^{-1}$) and a wave peak at 3275 cm^{-1}, slightly higher than the other samples. Similar OH stretching bands in regions of 3322 and 3280 cm^{-1} have also been reported for dehydrated cocoa beans (Batista et al., 2016; Escobar et al., 2023).

The regions between 2800 and 3000 cm^{-1} are commonly associated with lipid content, as these peaks are characteristics of fatty acids with long alkyl chains, which are prevalent in triglycerides (Karoui et al., 2010). It is important to note that in the dehydrated Amazon nut samples, lipids were the predominant component. Among the samples, A03 and A04 had the highest concentrations (Table 1). However, when evaluating the spectral profile, particularly in the highlighted region between 2922 cm^{-1} and 2854 cm^{-1} (related to group stretching (CH) and the 1745 cm^{-1} region (common to methyl esters, C=O), no clear distinction could be observed between the samples. The peaks were either very close or overlapping across all five samples.

Table 1 – Average values and standard deviation for moisture, protein, and lipid contents in different samples of processed Amazon nut kernels

Samples	Composition (g 100 g −1)		
	Moisture	**Proteins**	**Lipids**
A01	2.95 ± 0.07^{a}	13.34 ± 0.47^{ab}	64.40 ± 0.12^{b}
A02	2.99 ± 0.04^{a}	13.97 ± 0.57^{a}	60.79 ± 1.20^{c}
A03	2.26 ± 0.27^{b}	12.48 ± 0.84^{ab}	65.76 ± 0.79^{a}
A04	2.48 ± 0.01^{b}	12.10 ± 0.84^{b}	66.91 ± 0.18^{a}
A05	2.43 ± 0.04^{b}	12.57 ± 0.18^{ab}	61.48 ± 0.66^{c}
Overall average	2.62 ± 0.33	12.88 ± 0.75	63.82 ± 2.57

*Data are reported as mean ± standard deviation. Means followed by the same lowercase letter in the same column are not significantly different from each other, according to the Tukey test ($p > 0.05$).
Source: Organized by the authors (2024)

Adiamo et al. (2021) made relevant observations while investigating the nutritional composition of Australian acacia seed species using mid-infrared spectroscopy. The MIR spectra of these seeds displayed specific bands at 2852 and 2926 cm^{-1}, associated with lipids and fatty acids resulting from CH_2 stretching. Similarly, in another study using MIR to evaluate the quality of different cocoa bean varieties, bands at 2922 cm^{-1} and 2855 cm^{-1} were linked to CH_2 stretching, while bands at 1743 cm^{-1} were associated with C=O stretching in the triglyceride group adjacent to the CO group in esters (Escobar et al., 2023).

Vibrational distortions with peaks at 1634 cm^{-1} and 1541 cm^{-1} were also identified in the spectral profile. These regions correspond to the vibrational stretching of the NH group, characteristic of amide groups I and II, respectively, which are present in proteins. The band at 1541 cm^{-1} is associated with secondary amines, while the band at 1454 cm^{-1} is likely related to the vibrational stretching of peptide bonds (CN) originating from bioactive amides and free amino acids (Meutter & Goormaghtigh, 2021; Chiang et al., 2023).

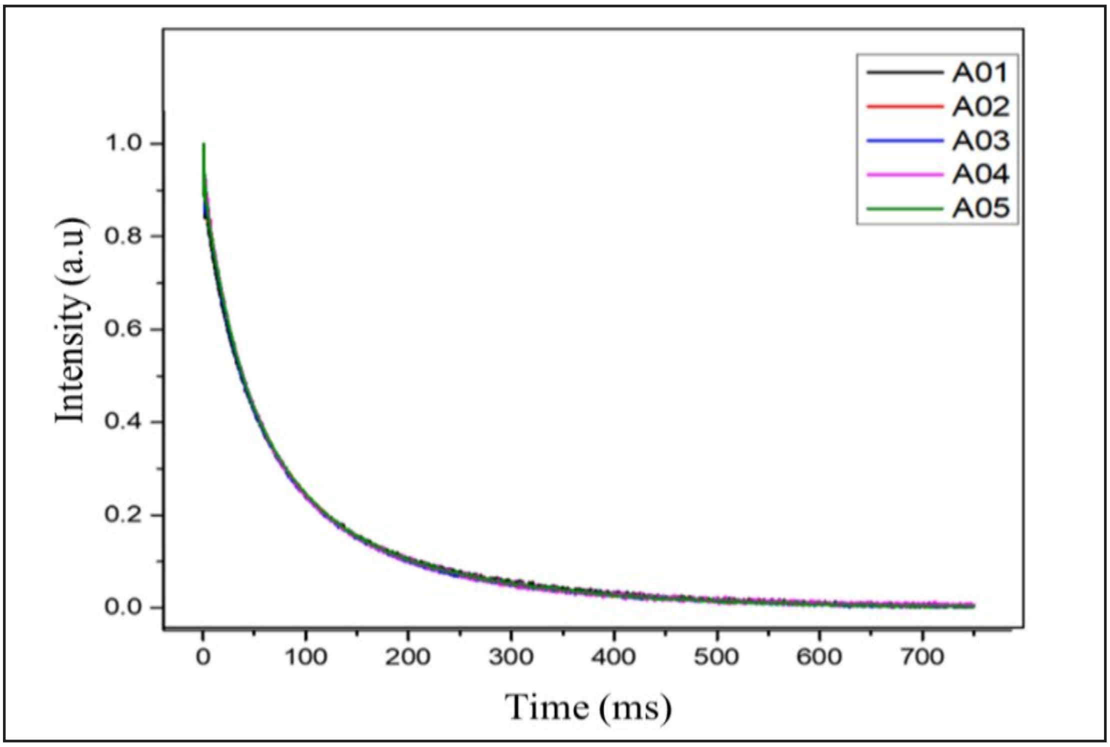
The spectra of the processed Amazon nut samples revealed that sample A02 presented the most prominent peaks in the region characteristic of nitrogenous compounds, specifically at 1634, 1541, and 1454 cm^{-1}. When comparing these results with the chemical composition findings obtained through reference methods, it was noted that A02 had the highest protein concentration (13.97 g.100 g^{-1}), although it did not show a significant difference from samples A01 and A05. This highlights the sensitivity of the mid-infrared region in detecting subtle distinctions between samples.

The regions associated with nitrogenous compounds identified in this study have been reported by other researchers. Mandrile et al. (2019) observed wave bands at 1560 cm^{-1} and 1525 cm^{-1}, with lower intensity bands at 1690 cm^{-1}, which are characteristic of amide regions (I and II) in their studies on cocoa bean authentication using *MIR*. In research on the nutritional parameters of common beans, Carbas et al. (2020), identified strong bands around 1540 cm^{-1} and 1636 cm^{-1}. Additionally, Adiamo et al. (2021), associated bands around 1744 cm^{-1} and 1641 cm^{-1} with nitrogenous compounds in Australian acacia seeds.

3.2 Spectroscopic profile in Nuclear Magnetic Resonance

The decay of an NMR signal over time is typically analyzed from an NMR spectrum or a Free Induction Decay (*FID*) plot. The decay curves for the Amazon nut samples exhibited a similar pattern, suggesting that the magnetic properties relax in a comparable manner. The overlapping of these curves indicates homogeneity in the composition of the almonds (Figure 2). These observations can be attributed to the physicochemical properties of lipids, the predominant component in Amazon nut kernels, particularly unsaturated fatty acids, which directly impacts the viscosity and quality of the oil.

Figure 2 – Free Induction Decay (FID) measurements from NMR analysis of five processed Amazon nut kernel samples



Source: Authors private collection (2024)

Based on the data reported (Table 2), higher T_2 values were observed for samples A01 and A05. This is likely due to the high content of unsaturated fatty acids (oleic and linoleic) in these Amazon nut kernels, which results in longer relaxation time and suggests a less viscous oil. The decay times are closely related to the oil's viscosity and are influenced by the primary fatty acids present. Typically, viscosity increases with the length of the carbon chain due to stronger intermolecular interactions (Van der Waals forces), which causes greater friction between molecules, making flow more difficult and increasing viscosity. Conversely, viscosity decreases with increased unsaturation for fatty acids with the same number of carbons. Thus, the decay (T_2) is inversely proportional to the oil's viscosity (Prestes et al., 2007).

Table 2 – Transverse relaxation times, T_2 long and T_2 short, for samples of processed Amazon nut kernels

Samples	Times in relaxation			
	A_1	T_2 long (ms)	A_2	T_2 short (ms)
A01	0.39	148.06	0.50	38.22
A02	0.45	131.27	0.48	34.83
A03	0.44	133.24	0.49	35.74
A04	0.44	131.89	0.50	35.90
A05	0.40	145.40	0.52	39.28

* ms = milliseconds; A_1 and A_2 = amplitude.
Source: Organized by the authors (2024)

According to Constantino et al. (2014), saturated fatty acids tend to increase oil viscosity due to their rigid and linear molecular structure. An increase in saturated fatty acids results in shorter relaxation time T_2, indicating a more viscous oil. In contrast, monounsaturated and polyunsaturated fatty acids have double bonds that prevent molecules from aligning closely, thereby reducing oil viscosity and affecting relaxation time.

The higher number of unsaturated fatty acids observed in the samples A01 and A05 correlates with their higher PI values (7.27 and 6.96 mEq O_2 kg^{-1}), suggesting greater oxidative degradation (Table 3). Double bonds in unsaturated fatty acids are more reactive compared to the single bonds in saturated fatty acids, making them more susceptible to oxidative degradation. This process, particularly affecting linoleic acid, leads to the formation of byproducts such as peroxides. and hydroperoxides, which can alter the sensory and physicochemical characteristics of foods, contributing to rancidity Alves et al. (2020b).

Table 3 – Average values and standard deviation of the Peroxide Index for processed Amazon nut almonds

Peroxide Index (mEq O_2 kg^{-1})					
A01	A02	A03	A04	A05	Average
7.27 ± 0.67[a]	6.86 ± 0.95[a]	6.48 ± 0.82[a]	6.43 ± 0.89[a]	6.96 ± 0.87[a]	6.80 ± 0.78

* Data are reported as mean ± standard deviation. Means followed by the same lowercase letter in the same row are not significantly different from each other according to the Tukey test ($p > 0.05$).
Source: Organized by the authors (2024)

NMR is highly sensitive for characterizing molecular behavior and can detect minor changes in chemical and structural composition that might not be reflected in the final quality of samples (Parlak & Güzeler, 2016). The higher presence of unsaturated fatty acids in samples A01 and A05 can alter molecular mobility, affecting relaxation times, as shown by Constantino et al. (2014). Their study demonstrated that vegetable oil samples with higher fatty acid content exhibited longer relaxation times, indicating greater molecular mobility.

3.3 Sanitary quality of processed Amazon nut samples

Most aflatoxigenic fungi require a relative air humidity above 85% and an A_w between 0.78 and 0.80 for ideal growth conditions. However, *AFL's* are generally produced only when A_w exceeds 0.85. High temperatures, high humidity, and irregular storage are the primary physical factors affecting fungal development and *AFL* contamination (Reis et al., 2012; Alves et al., 2020a).

Table 4 – Average value of water activity (A_w) and aflatoxin concentrations in samples of processed Amazon nut kernels

Samples	**A_w**	**Aflatoxins (μg kg⁻¹)**				
		AFB1	**AFB2**	**AFG1**	**AFG2**	**AFLs totals**
A01	0.66	0.65	0.30	0.72	1.18	2.85
A02	0.62	nd	nd	nd	nd	nd
A03	0.56	0.6	0.25	0.69	0.99	2.53
A04	0.61	nd	nd	nd	nd	nd
A05	0.55	0.33	nd	0.46	nd	0.79

nd: Not detected at the limit of quantification according to AOAC (2016).
Source: Organized by the authors (2024)

All samples exhibited water activity values below 0.7 and humidity below 5%, which ensures a longer conservation time and meets the recommendations of *Codex Alimentarius* (2006). Regarding *AFL* contamination, the samples compiled with Brazilian legislation limits (≤10 μg kg^{-1}) for total *AFL's* in unshelled Amazon nuts intended for direct consumption

(Table 4). They also adhered to regulation no. 165/2010 of the European Union, which specifies a maximum of 5 μg kg^{-1} for *AFB1* and 10 μg kg^{-1} for total *AFL's* in shelled Amazon nuts intended for direct consumption (FAO, 2006; EU, 2010; Brasil, 2022).

The Association of Food Industries (AFI) recommends that the moisture content for Amazon nuts should be no less than 1.5% and no more than 6% for dehydrated almonds. This range is crucial to ensure sensory quality, preserving the product's characteristic flavor, odor, and crunchiness (AFI, 2024).

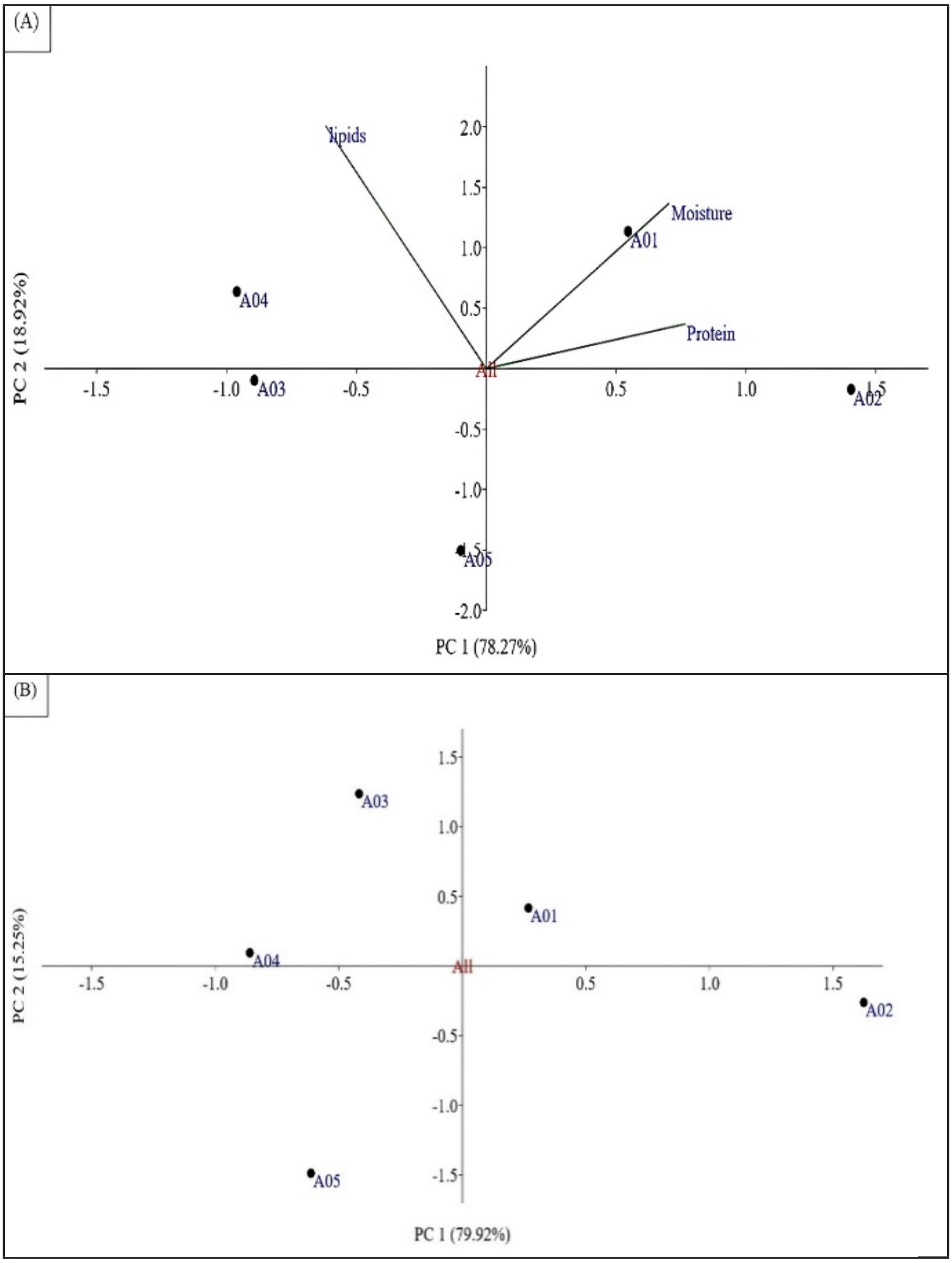
Kluczkovski et al. (2020) observed that drying effectively controls aflatoxins, with dehydrated samples showing compliant A_w values (0.53 to 0.23) compared to higher values in non-dehydrated samples (0.99 to 0.88). For *AFL* contamination, they reported values between 0.59 and 0.77 μg kg^{-1} for total *AFL's* in non-dehydrated almonds, while dehydrated almonds had no detectable *AFL's*. The authors attributed these findings to effective extraction and quality control practices, despite non-dehydrated almonds having A_w values above the recommended threshold.

In another study by Kluczkovski et al. (2023) on Amazon nut almonds sold in Manaus-AM, no contamination by *AFB1* and *AFB2* was observed, though concentrations of 2.65 μg kg^{-1} and 1.28 μg kg^{-1} for AFG1 and AFG2, respectively, were detected. This study noted that humidity and A_w values exceeded recommended safety limits, potentially contributing to fungal proliferation and *AFL* production, even in products available for sale. Alves et al. (2020) found 11 samples (13.1%) positive for *AFL's* in their analysis of 84 Amazon nut samples from retail establishments in São Luís-MA, with *AFB1* being the most commonly identified mycotoxin (n = 6; 7.2%) and three samples exceeding the allowed limit for total *AFL's* (13, 20, and 28 μ g kg^{-1}), respectively.

It is important to note that, unlike Brazilian legislation, the EU limit of 5 μg kg^{-1} specifically refers to contamination by *AFB1* due to its toxicity and does not consider the total *AFL* concentration, even though it adheres to the acceptable limit. (≤10 μg kg^{-1}). Therefore, batches with *AFB1* values exceeding 5 μg kg^{-1} are rejected for export to European Union countries (EU, 2010).

3.4 Principal Component Analysis

Figure 3 – Comparison of Principal Component Analysis (PCA) for Amazon nut samples: (A) chemical composition and (B) MIR spectra



Source: Authors private collection (2024)

The qualitative analysis of the samples was conducted using Principal Component Analysis (*PCA*), comparing the chemical composition variables obtained through traditional analytical methods with the MIR spectra of the five processed Amazon nut samples (Figure 3 A and B).

According to Rosenthal (1999), an adequate result should account for at least 70 and 80% of the total variation in the samples. In this study, the cumulative contributions of the principal components in the two PCA analyses were 96.56% and 95.17%, respectively, indicating that nearly all sample variations are explained.

Samples A01 and A02 exhibit the highest moisture and protein contents, as indicated by their positions on the positive side of PC1. Although the values for these components do not differ significantly ($p > 0.05$), sample A02 is more pronounced toward the positive end in both PCA analyses, indicating that the analysis detected a higher protein content in A02. The spectra are sensitive in molecule differences, conformational structures, and intermolecular interactions, which affect the spectral bands (Burns & Ciurczak, 2009; Joshi et al., 2021).

Samples A03 and A04 are positioned close to the negative side of PC2 in both the PC of chemical components and the PC of the *MIR* spectra. This suggests a chemical similarity between these samples. Analysis of their chemical composition reveals that they contain the highest concentrations of lipids, further demonstrating the sensitivity of the spectroscopic method in distinguishing between the samples.

5 FINAL CONSIDERATIONS

The instrumental *MIR* and *NMR* methods used to evaluate the quality of the Amazon nuts produced profiles without anomalies, allowing for the identification of the main components in the samples. These results indicate that the faster and more accurate methods for assessing qualitative parameters related to almond quality can serve as an alternative to traditional analytical techniques. In this study, mid-infrared spectroscopy (*MIR*) was proved effective for characterizing and identifying functional groups associated with proteins and lipids, thus reflecting the composition and variations in the nutritional profile of Amazon nut almonds. Conversely, the *NMR* profile revealed a higher presence of unsaturated fatty acids in the samples. The similarities in sample distribution across the *PCA* analyses suggest that traditional methods and MIR spectroscopy provide consistent and complementary information about the variability and nutritional characteristics of the Amazon nut, making their combined use advantageous for analyzing and characterizing different samples. Given the product's rich nutritional composition and global market presence, ongoing research on this raw material is crucial. Further studies should employ sophisticated techniques, particularly in conjunction with multivariate calibration, to quantitatively predict chemical composition and expedite market readiness.

ACKNOWLEDGMENTS

The authors thank the Brazilian Agricultural Research Corporation (EMBRAPA), especially the units in Rondônia and Embrapa Instrumentation in São Carlos-SP, for facilitating the connection between researchers and the Brazil nut production chain, as well as for all the technical and technological support provided.

REFERENCES

Adiamo, O. Q., Sultanbawa, Y., & Cozzolino, D. (2021). Mid-infrared spectroscopy as a rapid tool to qualitatively predict the effects of species, regions, and roasting on the nutritional composition of Australian acacia seed species. Molecules, 26(7), 1879. https://doi.org/10.3390/molecules26071879

Ancora, D., Milavec, J., Gradišek, A., Cifelli, M., Sepe, A., Apih, T., & Domenici, V. (2021). Sensitivity of proton NMR relaxation and proton NMR diffusion measurements to olive oil adulterations with vegetable oils. Journal of agricultural and food chemistry, 69(41), 12081-12088. https://doi.org/10.1021/acs.jafc.1c00914

AFI (Association of Food Industries). (2024). Shelled Brazil nuts AFI specifications for Brazil nuts https://prokcssmedia.blob.core.windows.net/sys- master images/h09/h16/9548514131998/Microsoft%20Word%20-%20Brazil%20Nuts.doc.pdf

Alves, C. M. S., Sousa, W. R., Cândido, C. J., Neto, J. A. B., Sampaio, R. M., Santana, A. A., & Monteiro, S. C. M. (2020a). Occurrence of aflatoxins in Brazil nuts commercialized in the northeast of Brazil. Brazilian Journal of Health Review, 3(4), 8129– 8145. https://doi.org/10.34119/bjhrv3n4-073

Alves, A. L., Izoton, T. M., Velloso, M. H. R., Partelli, F. L., Pessoa, M. S., & Moscon, P. S., (2020b). A espectroscopia de ressonância magnética nuclear na determinação de ácidos graxos em genótipos de cafés. Sustentabilidade em ciência e tecnologia de alimentos, (2),1-9. https://doi.org/10.22533/at.ed.6692014121

Association of official analytical chemists (AOAC). (2016). Official methods of analysis of the Association of Official Analytical Chemists. Official methods of analysis. (Latimer, GW., 18th) Maryland/USA.https://pdfcoffee.com/aoac- method- 99131- aflatoxins- esp- 3-pdf- free.html

Birch, C. S.; & Bonwick, G. A. (2019). Ensuring the future of functional foods. International Journal of Food Science and Technology, 54 (5), 1467-1485. https://doi.org/10.1111/ijfs.14060

Burns, D. A., & Ciurczak, E. W. (2009). Handbook of near-infrared analysis, 3rd ed. 3. ed. Boca Raton: Taylor & Francis Group. v. 393. ISBN 978- 0- 8493- 7393- 0. https://books.google.com.br/books?id=C8opEAAAQBAJ&printsec=frontcover&hl=pt-BR

Batista, N. N., Andrade, P. D., Ramos, L. C., Dias, R. D., & Schwan, F. R. (2016). Antioxidant capacity of cocoa beans and chocolate assessed by FTIR. Food Research International, 90, 313-319. http://dx.doi.org/10.1016/j.foodres.2016.10.028

Botelho, S. D. C., Baldoni, A. B., Tonini, H., Botelho, F. M., Hoogerheider, E. S. S., Wobeto, C., Botin, A. A., & Taffarel, C. (2019). Fruits, seeds, and oil of Brazil nuts produced in Mato Grosso State. Floresta e Ambiente, 26(2), 1-8. https://doi.org/10.1590/2179-8087.066017

Brasil. (2022). Agência Nacional de Vigilância Sanitária-ANVISA. Instrução Normativa (IN) nº 160 de 01 de julho de 2022. Diário oficial. Ed.: 126; Seção:1. Página: 227. https://www.in.gov.br/en/web/dou/-/instrucao-normativa-in-n-160-de-1-de-julho-de-2022-413367081

Carbas, B., Machado, N., Oppolzer, D., Ferreira, L., Brites, C., Rosa, E. A., & Barros, A. I. (2020). Comparison of near-infrared (NIR) and mid-infrared (MIR) spectroscopy for the determination of nutritional and antinutritional parameters in common beans. Food Chemistry, 306, 125509. https://doi.org/10.1016/j.foodchem.2019.125509

Cortés, V., Talens, P., Barat, J. M., & Lerma-García, M. J. (2019). Discrimination of intact almonds according to their bitterness and prediction of amygdalin concentration by Fourier transform infrared spectroscopy. Postharvest Biology and Technology, 148, 236-241. https://doi.org/10.1016/j.postharvbio.2018.05.006

Cardoso, B. R., Duarte, G. B. S., Reis, B. Z., & Cozzolino, S. M. F. (2017). Brazil nuts: Nutritional composition, health benefits, and safety aspects. Food Research International, 100(Part 2), 9-18. https://doi.org/10.1016/j.foodres.2017.08.036

Casagrande, J., Santos, C. B., & Nicoletto, B. B. (2019). Análise da rancidez oxidativa em castanhas do Brasil em diferentes condições de armazenamento. Revista Brasileira de Obesidade, Nutrição e Emagrecimento. São Paulo. v. 13. n. 81. p.812-820. ISSN 1981-9919. https://www.rbone.com.br/index.php/rbone/article/view/1069/881

Chiang, K. Y., Matsumura, F., Yu, C. C., Qi, D., Nagata, Y., Bonn, M., & Meister, K. (2023). True Origin of Amide I Shifts Observed in Protein Spectra Obtained with Sum Frequency Generation Spectroscopy. The Journal of Physical Chemistry Letters, 14(21), 4949-4954. https://doi.org/10.1021/acs.jpclett.3c00391

Constantino, A. F., Lacerda, Jr. V., Santos, R. B. D., Greco, S. J., Silva, R. C., Neto, Á. C., & Freitas, J. C. (2014). Analysis of oil content and oil quality in oilseeds by low-field NMR. Química Nova, 37(1), 10-17. https://doi.org/10.1590/S0100-40422014000100003

Dufour, E´. (2009). Infrared Spectroscopy for Food Quality Analysis and Control; Sun, D. W. (Ed.). Academic Press: New York. ISBN: 978- 0- 12- 374136- 3. https://books.google.com.br/books?id=bOWUDeiYshsC&printsec=frontcover&hl=pt-BR

Danielski, R., Polmann, G., & Block, J. M. (2021). Brazil and Their Coproducts: Innovation in the Food Sector Through the Valorization of Food and Agro-Food By-Products,139-152. http://dx.doi.org/10.5772/intechopen.91078

Derksen, G. C., Blommaert, L., Bastiaens, L., Hasşerbetçi, C., Fremouw, R., Van Groenigen, J., & Timmermans, K. R., (2023). ATR-FTIR spectroscopy combined with multivariate analysis as a rapid tool to infer the biochemical composition of Ulva laetevirens (Chlorophyta). Frontiers in Marine Science. 10, 1-14. https://doi.org/10.3389/fmars.2023.1154461.

Escobar, G. A. C., Barrios-Rodriguez, Y. F., Bahamón-Monje, A. F., & Gutiérrez-Guzmán, N. (2023). Uses of mid-infrared spectroscopy and chemometric models for differentiating between dried cocoa bean varieties. Revista Brasileira de Engenharia Agrícola e Ambiental, 27, 803-810. https://doi.org/10.1590/1807-1929/agriambi.v27n10p803-810

EU (European Union). (2010). Commission Regulation No. 165/2010 of Feb 2010 amending Regulation No. Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Official Journal of the European Communities, 50, 8- 12. http://data.europa.eu/eli/reg/2010/165/oj

FAO (Food and Agriculture Organization). (2006). Codex alimentarius. Code of practice for the prevention and reduction of aflatoxina contamination in tree nuts. Cac/Rcp, 59, 1-9. Disponible: https://www.fao.org/input/download/standards/10221/CXP_059e.pdf.

FIB (Food Ingredients Brasil). (2009). As Micotoxinas. Food Ingredients Brasil, 7: 32- 40. https://www.foodconnection.com.br/artigos/ozonizao- como- tecnologia- sustentvel- para-conservao-de-cereais/.

Gianferri, R., Sciubba, F., Durazzo, A., Gabrielli, P., Lombardi-Boccia, G., Giorgi, F., & Lucarini, M. (2023). Time Domain NMR Approach in the Chemical and Physical Characterization of Hazelnuts (Corylus avellana L.). Foods, 12(10). https://doi.org/10.3390/foods12101950

Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis (Version 4.03) [Computer software]. University of Oslo. https://www.nhm.uio.no/english/research/resources/past/

Instituto Adolfo Lutz, I. A. L. (2008). Métodos físico-químicos para análise de alimentos. IV Edição, 1° Edição digital. 1020p. https://wp.ufpel.edu.br/nutricaobromatologia/files/2013/07/Normasadolfolutz.pdf

Joshi, R., Sathasivam, R., Park, S. U., Lee, H., Kim, M. S., Baek, I., & Cho, B. K. (2021). Application of fourier transform infrared spectroscopy and multivariate analysis methods for the non-destructive evaluation of phenolics compounds in moringa powder. Agriculture, 12(1), 10. https://doi.org/10.3390/agriculture12010010

Karoui, R., Downey, G., & Blecker, C. (2010). Mid-infrared spectroscopy coupled with chemometrics: A tool for the analysis of intact food systems and the exploration of their molecular structure-quality relationships. A review. Chemical Reviews, 110(10), 6144-6168. https://doi.org/10.1021/cr100090k

Kluczkovski, A. M & Scussel, V. M. (2015). Brazil nut allergy: A review. Journal of Pharmacy and Pharmacology, 9(26), 633-644. https://doi.org/10.5897/AJPP2014. 4206

Kluczkovski, A. M., Barros, H., Barroncas, J., Viana, C., & Lima, E. S., (2023). Aflatoxins in raw Brazil nut (Bertholletia excelsa H. B. K.). Journal of Agricultural Studies, 11(2), 14. https://doi.org/10.5296/jas.v11i2.20741

Kluczkovski, A. M., Silva, A. C., Barroncas, J., Lima, J., Pereira, H., Mariosa, P., & Vinhote, M. L. (2020). Drying in Brazil nut processing as a tool for prevention of contamination by aflatoxins. Journal of Agricultural Studies, 8(4), 70. https://doi.org/10.5296/jas.v8i4.17387

Lamas, S., Rodrigues, N., Santamaria-Echart, A., Palu, I., Manhique, J. R., Herrero, B., & Peres, A. M. (2025). Potential use of the ATR-FTIR spectroscopy as an almond cultivar recognition tool: impact of sample and spectral pre-treatments. Journal of Food Measurement and Characterization, 1-16. https://doi.org/10.1007/s11694-025-03133-9

Li, X., Qu, L., Su, X., Zhang, S., Gao, Y., & Yu, X. (2025). Utilizing low-field NMR for comprehensive quality evaluation of edible oil and oil product. Grain & Oil Science and Technology, 8(1), 43-54.https://doi.org/10.1016/j.gaost.2024.12.002

Mendes, E., & Duarte, N. (2021). Mid-infrared spectroscopy as a valuable tool to tackle food analysis: A literature review on coffee, dairies, honey, olive oil and wine. Foods, 10(2), 477. https://doi.org/10.3390/foods10020477

Maestri, D., Cittadini, M. C., Bodoira, R., & Martínez, M., (2020). Tree nut oils: Chemical profiles, extraction, stability and quality concerns. European Journal of Lipid Science and Technology, 122(6), 1900450. https://doi.org/10.1002/ejlt.201900450

Maldonado, S. A. S., Alemã, R. S., Fuentes, J. A. M., & Conceiã, M. (2020). Determination of total phenolic compounds, antioxidant activity and nutrients in Brazil nuts (Bertholletia excelsa H. B. K.). Journal of Medicinal Plants Research, 14(8), 373– 376. https://doi.org/10.5897/JMPR2020.6953

Mandrile, L., Barbosa-Pereira, L., Sorensen, K. M., Giovannozzi, A. M., Zeppa, G., Engelsen, S. B., & Rossi, A. M. (2019). Authentication of cocoa bean shells by near- and mid-infrared spectroscopy and inductively coupled plasma-optical emission spectroscopy. Food Chemistry, 292, 47–57. https://doi.org/10.1016/j.foodchem.2019.04.008

Meutter, J. & Goormaghtigh, E. (2021). Evaluation of protein secondary structure from FTIR spectra improved after partial deuteration. European Biophysics Journal, 50, 613-628. https://doi.org/10.1007/s00249-021-01502-y

OriginLab Corporation. (2024). OriginPro®: Graphing & Analysis Software (Version 10.1) [Computer software]. https://www.originlab.com

Parker, T., Limer, E., Watson, A. D., Defernez, M., Williamson, D., & Kemsley, E. K. (2014). 60 MHz 1H NMR spectroscopy for the analysis of edible oils. Trac Trends in Analytical Chemistry, 57, 147-158. https://doi.org/10.1016/j.trac.2014.02.006

Parlak, Y., & Güzeler, N. (2016). Nuclear magnetic resonance spectroscopy applications in foods. Current Reseatch in Nutrition and Food Science, 4,161- 168. https://dx.doi.org/10.12944/CRNFSJ.4.Special-Issue-October.22

Prestes, R. A., Colnago, L. A., Forato, L. A., Vizzotto, V., Novotny, E. H., & Carrilho, E. (2007). A rapid and automated low-resolution NMR method to select intact oilseeds with a modified fatty acid profile. Analytica Chimica Acta, 596(2), 325- 329. https://doi.org/10.1016/j.aca.2007.06.022

Reis, T. A. D., Oliveira, T. D., Baquião, A. C., Gonçalves, S. S., Zorzete, P., & Corrêa, B. (2012). Mycobiota and mycotoxins in Brazil nut samples from different states of the Brazilian Amazon region. International journal of food microbiology. 159(2), 61- 68. https://doi.org/10.1016/j.ijfoodmicro.2012.08.005

Rosenthal, A. J. (1999). Food texture: Measurement and perception. Aspen Publishers. 326p. ISBN-10 : 0834212382.

SAS Institute Inc. (2013). SAS® 9.4 [Computer software]. SAS Institute Inc. https://www.sas.com

Sobolev, A. P., Ingallina, C., Spano, M., Di Matteo, G., & Mannina, L. (2022). NMR-based approaches in the study of foods. Molecules, 27(22), 7906. https://doi.org/10.3390/molecules27227906

Scappaticci, C., Foschi, M., Plaku, A., Biancolillo, A., & D'Archivio, A. A. (2023). Enhancing Traceability of Italian Almonds through IR Spectroscopy and Chemometric Classifiers. Applied Sciences, 13(23), 12765. https://doi.org/10.3390/app132312765

Santos, V.R., Goncalves, V., Deng, P., Ribeiro, A. C., Teigao, M. M., Dias, B., Pinto, I. M., Gallo, J., & Peng, W. K. (2022). Novel time-domain NMR-based traits for rapid, label-free Olive oils profiling. npj Sci Food 6, 59. https://doi.org/10.1038/s41538-022-00173-z

Silva, B. S. F., Ferreira, N. R., Alamar, P. D., Melo Silva, T., Pinheiro, W. B. D. S., Santos, L. N., & Alves, C. N. (2023). FT-MIR-ATR associated with chemometrics methods: A preliminary analysis of deterioration state of Brazil nut oil. Molecules, 28(19), 6878. https://doi.org/10.3390/molecules28196878

Woolf, P. J., Fu, L. L., & Basu, A. (2011). Protein: identifying optimal amino acid complements from plant based foods. PLoS One, 6(4), e18836. https://doi.org/10.1371/journal.pone.0018836

Authorship contributions

1 – Tiago Bratilieri dos Santos

Food and Dairy Laboratory Technician at the Universidade Federal de Rondônia (UNIR).
https://orcid.org/0000-0003-3315-0577 • tbratilierisantos@gmail.com
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodoloy, Validation, Visualization, Writing – original draft, Writing – review & editing.

2 – Ladyslene Chrísthyns de Paula

Adjunct Professor and Research Coordinator at the Universidade Federal de Rondônia (UNIR), Department of Food Engineering.
https://orcid.org/0000-0001-6265-1237 • ladyslene.paula@unir.br
Contribution: Investigation, Methodoloy, Validation, Writing – review & editing.

3 – Luiz Alberto Colnago

Full Professor and Researcher at Embrapa Instrumentação, Specialist in NMR and Biocomplexity.
https://orcid.org/0000-0002-9516-9022 • luiz.colnago@embrapa.br
Contribution: Investigation, Formal Analysis, Software, Writing – review & editing.

4 – Lúcia Helena de Oliveira Wadt

IResearcher at the Brazilian Agricultural Research Corporation (Embrapa) and General Manager of Embrapa Rondônia.
https://orcid.org/0000-0002-5018-7550 • lucia.wadt@embrapa.br
Contribution: Conceptualization, Investigation, Methodoloy, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing.

5 – Samir Carvalho Buzaglo Pinto:

Master in Pharmaceutical Sciences (2019) from the Universidade Federal do Amazonas.
https://orcid.org/0000-0001-6055-1347 • sam.carvbp@hotmail.com
Contribution: Formal Analysis, Investigation, Software, Writing – review & editing.

6 – Ariane Mendonça Kluczkovski:

Post-doctorate in food science from the Universidade Federal de Santa Catarina (UFSC) in the area of biomarkers (mycotoxins) and product development.
https://orcid.org/0000-0003-1785-3766 • ariane@ufam.edu.br
Contribution: Formal Analysis, Investigation, Writing – review & editing.

7 – Anthonioni Peron Dal Sasso:

Technician in Dairy and Food Technology from the Instituto Federal de Rondônia, Brazil.
https://orcid.org/0009-0000-9946-649X • anthonioni.sasso@ifro.edu.br
Contribution: Formal Analysis, Investigation, Writing – review & editing.

8 – Gabrieli Oliveira Folador:

PhD in Food Engineering from the Universidade Federal do Paraná (UFPR).
https://orcid.org/0000-0001-9077-8737 • gabrieli.oliveira@unir.br
Contribution: Conceptualization, Data curation, Formal Analysis, Investigation, Methodoloy, Validation, Visualization, Writing – original draft, Writing – review & editing.

How to quote this article

Santos, T. B., de Paula, L. C., Colnago, L. A., Wadt, L. H. de O., Pinto, S. C. B., Kluczkovski, A. M., Dal Sasso, A. P. & Folador, G. O. Application of mid-infrared spectroscopy and nuclear magnetic resonance to assess the quality of processed Amazon nuts. *Ciencia e Natura*, Santa Maria, v. 48, e92511, 2026. DOI: https://doi.org/10.5902/2179460X92511.