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Enhancing the extraction efficiency of beneficial compounds from *Tapirira guianensis* fruit using ultrasound-assisted extraction and choline chloride deep eutectic solvent

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Abstract

In recent decades, a new class of solvents known as natural deep eutectic solvents has emerged. In this study, the efficacy of different natural deep eutectic solvents, prepared by ultrasound-assisted synthesis, was evaluated for extracting bioactive compounds from *Tapirira guianensis* fruits. A total of seven natural deep eutectic solvents systems, combining choline chloride with various hydrogen bond donors (carboxylic acids, alcohols, and sugars), were analyzed for total phenolic content and antioxidant activity. The results showed that carboxylic acid-based natural deep eutectic solvents were the most efficient, with the best results obtained using choline chloride/citric acid/glycerol (Stap7) > choline chloride/citric acid (Stap1) > choline chloride/lactic acid (Stap2), yielding total phenolic contents of 715.7 ± 1.1, 648.5 ± 1.2, and 630.7 ± 0.7 mg GAE/g, respectively. Ultrasonic extraction with 40% (v/v) eutectic mixtures showed that combinations with citric acid were more effective, producing extracts with high concentrations of polyphenols and antioxidant potential. The antioxidant activity, measured by 2,2-Diphenyl-1-picrylhydrazyl, 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, and ferric-reducing antioxidant power assays, showed a similar trend. Spectroscopic analysis (nuclear magnetic resonance) identified gallic acid and 5-hydroxyvanillin, providing structural details of the extracts. Macro- and micronutrient analysis revealed some elements below detection limits. Therefore, these extracts can be used directly for application development without additional isolation.

Keywords: ultrasound; NADES; antioxidant compounds; eutectic solvents.

Practical Application: Utilization of natural deep eutectic solvents (NADES) and ultrasound to efficiently extract bioactive compounds from *Tapirira guianensis* fruits.

1 INTRODUCTION

The species *Tapirira guianensis* is widely distributed in all Brazilian biomes. In its composition, this species presents a high antioxidant and nutraceutical potential, containing polyphenols, specifically galloylquinic acids and flavonols (Mar et al., 2023).

Foods and beverages supply essential macro- and micronutrients—such as carbohydrates, proteins, lipids, vitamins, and minerals—which support key biological functions including energy metabolism, oxygen transport, bone health, blood clotting, and fluid balance (Fiorentini et al., 2022).

Conventional phytochemical extraction methods are time- and energy-consuming, often using non-generally recognized as safe (GRAS) solvents, making them costly and environmentally unfriendly. Even with GRAS solvents like ethanol or water, the extracts typically need further processing, limiting their efficiency and sustainability (Ferreira et al., 2020). In recent decades, a new class of solvents known as natural deep eutectic solvents (NADES) has emerged. These solvents are composed of primary plant metabolites, including sugars (such as glucose, fructose, sucrose, trehalose, and maltose), sugar alcohols (such as sorbitol, glycerol, 1,2-propanediol, and xylitol), organic acids (such as lactic, citric, maleic, malic, oxalic, and ascorbic acids), amino acids (such as proline, glycine, and alanine), and amines (such as betaine and choline) (Fuad & Nadzir, 2021).

Some NADES, though natural, may exhibit cytotoxic effects. Several preparation methods exist, including stirring with heating, lyophilization, evaporation, and microwave-assisted

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synthesis. Recently, ultrasound-assisted synthesis has emerged as a more sustainable, faster, and more efficient alternative (Gamela et al., 2020). Ultrasound-assisted extraction (UAE) shows promise, where ultrasonic energy is employed to facilitate the analyte extraction process from the matrix (Santana et al., 2020).

This study developed an UAE method using NADES to recover micro- and macronutrients from *T. guianensis* fruits. It identified the most effective system for extracting phenolic compounds and antioxidant activity and characterized key compounds via ^{1}H NMR and multivariate analysis. The results support eco-friendly applications in food and pharmaceutical industries.

1.1 Relevance of the work

This study highlights the relevance of natural deep eutectic solvents (NADES), combined with ultrasound assistance, for the efficient extraction of bioactive compounds from *Tapirira guianensis* fruits. Among the seven systems tested, those based on citric acid (especially STap7) showed higher total phenolic content and antioxidant activity. The presence of phenolic acids, such as gallic acid, was confirmed by nuclear magnetic resonance (NMR), and the extracts revealed potential for direct applications. These results reinforce the effectiveness of NADES in the extraction and bioaccessibility of beneficial compounds, highlighting their promising use in sustainable and functional formulations.

2 MATERIALS AND METHODS

2.1 Preparation of natural deep eutectic solvents

Choline chloride (ChCl), used as the hydrogen bond acceptor (HBA), was dried at 40°C for 30 min, and then mixed with one or more hydrogen bond donors (HBDs) in specific molar ratios. The mixture was heated at ~80°C with stirring for 30 min, and then diluted with distilled water (30% v/v) to form a colorless, uniform, non-crystallizing solution. The NADES names and molar ratios are shown in Table 1 (Lin et al., 2022).

2.2 Ultrasound-assisted natural deep eutectic solvents extraction

The fruits of *T. guianensis* were collected from Manaus, state of Amazonas (3°6′26″S, 60°1′34″W, SISGEN: A2FF2EEss), carefully washed with distilled water, freeze-dried, and ground for later use. The lyophilized fruits of *T. guianensis* (0.5 g) were mixed with each NADES at a solid–liquid ratio of 1:15 (w/v) as described previously (Silva, Pauletto et al., 2020).

Table 1. Natural deep eutectic solvents abbreviations and molar ratios.

Serial number	Natural deep eutectic solvents	Molar ratio
STap1	Choline chloride: citric acid	1:1
STap2	Choline chloride: lactic acid	1:1
STap3	Choline chloride: malic acid	1:1
STap4	Choline chloride: glucose	1:1
STap5	Choline chloride: glycerol	1:2
STap6	Choline chloride: citric acid:glucose	1:1:1
STap7	Choline chloride:citric acid: glycerol	1:1:1

Natural deep eutectic solvents (30%, v/v).

All NADES were prepared by sonication and heating at 50°C using power amplitudes equivalent to 40% (ultrasonic energy density level of 1.8 kJ·cm^{-3}). The treatment duration was 10 min, and it was performed in an ice bath to prevent sample overheating (Santos-Martín et al., 2023). During the ultrasonic homogenization process, the NADES system was treated using a 25-mm diameter probe, operating at a frequency of 20 kHz and with a nominal power of 750 W (VibraCell VCX 750 da Sonics, Shawnee, OK, EUA) (Mar et al., 2023). Each system was followed by filtration, and the solution was centrifuged to remove small sample particles. The resulting concentrate was collected and stored at 18°C (Lin et al., 2022).

2.3 Nuclear magnetic resonance chemical profile

An aliquot of 20 mg of each extract from the fruits of *T. guianensis* was dissolved in 520 µL of DMSO-d_6 with tetramethysilane (TMS) (0.05% v/v), sonicated in an ultrasonic bath for 10 min, and transferred to a 5-mm NMR tube. The nuclear magnetic resonance (NMR) spectra were acquired at the Nuclear Magnetic Resonance Laboratory (NMRLab) of the Federal University of Amazonas (UFAM) using a Bruker Avance IIIHD NMR spectrometer (Bruker, Billerica, Massachusetts, USA), operating at 11.7 T, equipped with a 5-mm BBFO Plus SmartProbe™ with Z-axis gradient. The pulse sequence used was zgpr with the following acquisition parameters: time domain (TD) data points of 32k, spectral width (SW) of 10 kHz, 90° pulse (P1) of 10.0 µs, relaxation delay (D1) of 2.0 s, acquisition time (AQ) of 1.64 s, receiver gain (RG) value of 64, number of scans (NS) equal to 32, free induction decay (FID) resolution of 0.60 Hz, center frequency (O1) set at 1,562.04 Hz, and suppression power (PLW9) of 8.1292 e^{-005}. Phase and baseline corrections of the spectra were performed manually using TopSpin 3.6.3 software. The chemical shift (in ppm) of the ^{1}H NMR spectra was referenced to the methyl signal of TMS at δ 0.0 and coupling constants (*J*) were recorded in Hz. correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) NMR experiments were conducted to verify the ^{1}H–^{1}H and ^{1}H–^{13}C correlations of the main compounds observed in the ^{1}H NMR spectra.

2.4 Antioxidant capacity assessment

The antioxidant activity based on DPPH$^{\bullet}$ radical and ABTS$^{\bullet+}$ cation radical and ferric-reducing antioxidant power (FRAP) were performed based on a previous report with modifications on an Epoch 2 Biotek microplate reader (Mar, Silva, Moreira et al., 2021).

2.5 Determination of total phenolic content of extracts

Total phenolic compounds were quantified using the Folin–Ciocalteu method. After a 5-min reaction with the reagent, 6% sodium bicarbonate was added, and the mixture was incubated for 90 min. Absorbance was measured at 750 nm using a microplate reader. Gallic acid was used as the standard ($y = 0.0029x + 0.0208$; $R^2 = 0.9915$), and results were expressed as milligrams

of gallic acid equivalents per milliliter of sample (mg GAE/mL), presented as mean ± standard deviation (Mar et al., 2023).

2.6 Digestibility assay in vitro

The concentration of bioactive compounds after digestion was assessed using the international in vitro digestibility protocol. In the gastric phase, tea infusions were mixed with simulated gastric fluid (SGF), pepsin, $CaCl_2$, HCl, and ultrapure water, adjusted to pH 3.0, and stirred at 200 rpm, 37°C for 2 h. In the intestinal phase, the gastric product was combined with simulated intestinal fluid (SIF), $CaCl_2$, lipase, pancreatin, bile extract, and water, adjusted to pH 7.0, and stirred under the same conditions. After digestion, the samples were centrifuged and stored for antioxidant analysis. All enzymes used were high-purity reagents from Sigma-Aldrich. Results were expressed as mean±standard deviation (Mar, Silva, Rabello et al., 2021).

2.7 Determination of macro- and microminerals by inductively coupled plasma optical emission spectroscopy

Acid digestion of 0.5 g of lyophilized *T. guianensis* fruit powder and extracts was performed using a MultiWave 5000 microwave digestion system with 10 mL of ultrapure HNO_3. The digestion program ramped to 220°C in 5 min, held for 15 min, and cooled to 70°C. Digested samples were diluted to 25 mL and stored at 4°C for inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis. Macro- and microminerals were quantified using an Agilent 5800 ICP-OES, with argon as the nebulizer gas. Calibration curves used certified reference materials, showed $R^2 > 0.999$, and %RSE (relative standard error) within ± 10%, confirming the high accuracy, sensitivity, and reproducibility of the method (Rodrigues et al., 2025).

The limit of detection (LOD) and limit of quantification (LOQ) were determined using calibration curves, with LOD ranging from 0.003 mg/100 g (Cr) to 0.18 mg/100 g (K, Ca, and Mg) and LOQ from 0.01 mg/100 g (Cr) to 0.6 mg/100 g (K, Ca, and Mg), indicating high sensitivity. The precision and accuracy of metal analysis via ICP-OES were confirmed using blanks and fortified samples prepared with standard solutions. The %coefficient of variation (%CV) was ≤ 10% for all analytes, showing good repeatability. Recovery rates ranged from 85 to 115% for blanks and 82 to 114% for fortified samples, all within the accepted range (80 to 120%), confirming the method's reliability (Instituto Nacional de Metrologia, Qualidade e Tecnologia [Inmetro], 2020).

2.8 Fourier transform infrared

To determine the chemical profile of the samples, the Fourier-transform infrared spectrometry with attenuated total reflection (FTIR-ATR) module from Agilent Cary (630 FTIR-ATR) was employed, using a ZnSe ATR crystal. The analysis of the STAP1 to STAP7 samples were conducted in the range of 680 to 4000 cm^{-1}.

2.9 Statistical analysis

The analysis of variance (ANOVA) was performed using the R software (version 3.5.1). Differences between the mean values obtained for each treatment were evaluated at a 5% significance level ($p \leq .05$) using Tukey's test.

3 RESULTS AND DISCUSSION

3.1 Extraction of bioactive compounds from natural deep eutectic solvents of T. guianensis *fruits based on spectrophotometric determination*

This study compared the performance of seven NADES combinations—comprising choline chloride (HBA) and various HBDs (carboxylic acids, alcohols, and sugars)—prepared via ultrasound-assisted synthesis for extracting bioactive compounds from *T. guianensis* fruits. The goal was to identify synergistic effects that enhance biological activity. Extraction efficiency was assessed based on total phenolic content (TPC) and antioxidant activity, with ANOVA used to compare the performance of each system.

The NADES based on carboxylic acids as the HBA component provided higher extraction efficiency for both parameters. As summarized in Table 2, the best results were obtained with the use of the systems choline chloride/citric acid/glycerol (STap7) > choline chloride/citric acid (Stap1) > choline chloride/lactic acid (Stap2), yielding TPCs of 715.7 ± 1.1 mg GAE/g, 648.5 ± 1.2 mg GAE/g, and 630.7 ± 0.7 mg GAE/g, respectively.

The extractions conducted using ultrasound-assisted methods with eutectic mixtures tested as aqueous solutions at 40% (v/v) showed that the combination with citric acid exhibited high efficiency. The results demonstrated extracts with high concentrations of polyphenols and antioxidant potential for NADES systems containing choline chloride/citric acid (Bakirtzi et al., 2016). According to Soukaina et al. (2024), the phenolic compound values ranged from 20.34 to 90.15 mg GAE/g of dry

Table 2. Results are expressed as mean ± standard deviation ($n = 3$).

Samples	DPPH (μM TE/mL)	ABTS (μM TE/mL)	TPC (mg GAE/g)	FRAP (μM Fe (II)/mL)
Stap1	1520.2 ± 7.6^{b}	1899.0 ± 8.8^{c}	648.5 ± 1.2^{c}	1575.4 ± 3.9^{c}
Stap2	1503.5 ± 9.0^{a}	1880.8 ± 8.4^{a}	630.7 ± 0.7^{a}	1522.0 ± 2.8^{a}
Stap3	1477.7 ± 6.3^{c}	1830.8 ± 6.9^{d}	607.5 ± 1.1^{d}	1506.9 ± 2.2^{d}
Stap4	1392.7 ± 5.2^{e}	1731.9 ± 6.9^{f}	447.9 ± 0.9^{f}	1266.2 ± 2.8^{f}
Stap5	1427.7 ± 8.0^{d}	1763.0 ± 8.8^{e}	503.3 ± 1.0^{e}	1375.0 ± 3.4^{e}
Stap6	1098.5 ± 6.6^{f}	1466.3 ± 3.3^{g}	409.5 ± 1.0^{g}	1209.8 ± 3.6^{g}
Stap7	1526.0 ± 6.6^{b}	1928.6 ± 7.7^{b}	715.7 ± 1.1^{b}	1607.2 ± 2.8^{b}
Sdtap1	567.7 ± 7.6^{h}	461.9 ± 8.4^{l}	148.2 ± 1.4^{k}	788.0 ± 2.9^{l}
Sdtap2	582.7 ± 3.8^{g}	367.4 ± 8.4^{n}	118.9 ± 0.9^{m}	709.1 ± 2.9^{n}
Sdtap3	553.5 ± 5.0^{i}	600.8 ± 8.4^{k}	168.8 ± 0.9^{j}	831.3 ± 3.3^{k}
Sdtap4	354.3 ± 7.6^{l}	861.9 ± 6.9^{h}	227.3 ± 1.1^{h}	976.1 ± 3.3^{h}
Sdtap5	421.0 ± 9.0^{j}	701.9 ± 8.4^{j}	168.8 ± 1.1^{j}	886.8 ± 4.0^{j}
Sdtap6	402.7 ± 7.6^{k}	806.3 ± 8.8^{i}	196.5 ± 1.1^{i}	942.0 ± 2.6^{i}
Sdtap7	557.7 ± 5.2^{hi}	403.0 ± 8.8^{m}	129.4 ± 0.9^{l}	763.5 ± 2.9^{m}

Results are expressed as mean ± standard deviation ($n = 3$). Different letters in the same column are significant ($p < .05$). DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; TPC: total phenolic content; FRAP: ferric-reducing antioxidant power; μM TE/mL: micromoles of Trolox equivalent per milliliter of sample; μM Fe(II)/mL: micromolar of ferrous sulfate per milliliter of sample; mg GAE/mL: milligram of gallic acid equivalent per milliliter of sample.

weight (DW), indicating significant variation in the efficiency of NADES, with a particular emphasis on systems containing citric acid. According to Barbieri et al. (2020), choline chloride-based eutectic solvents have the ability to stabilize phenolic compounds, primarily due to the formation of hydrogen bonds between phenolic acids.

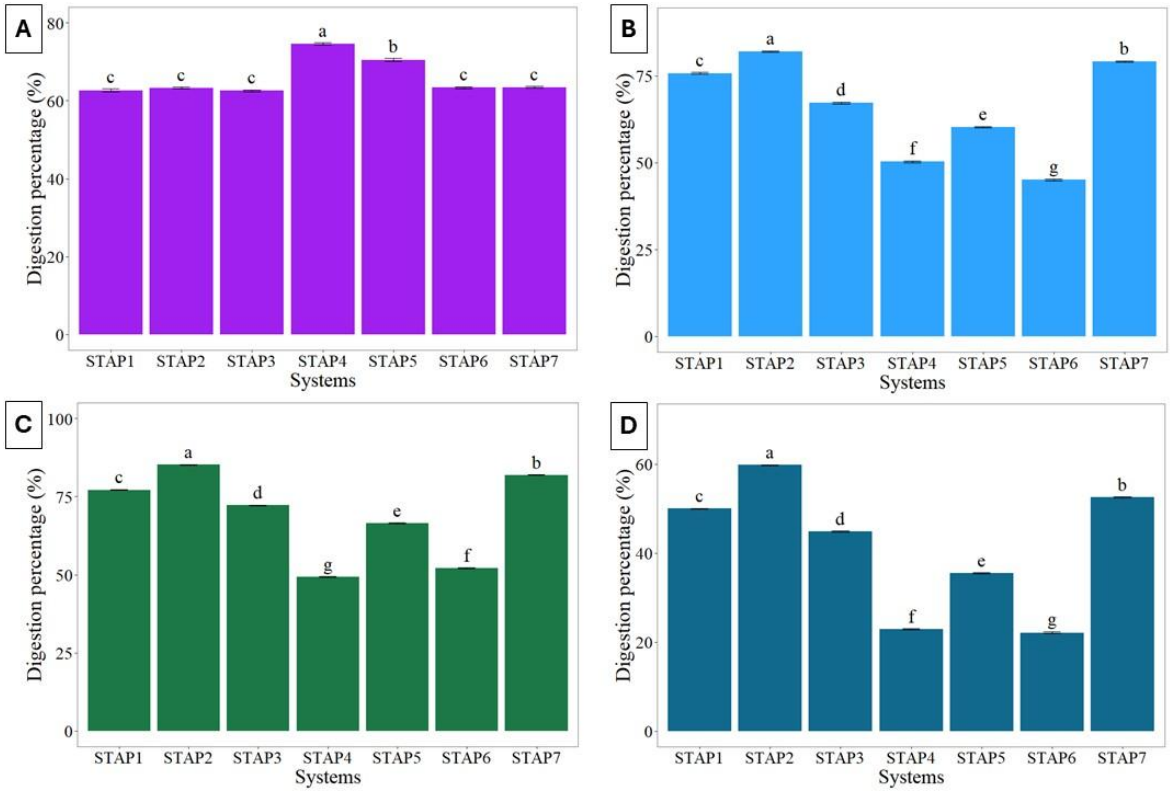
Antioxidant activity, measured by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and FRAP assays, showed an increasing trend among all *T. guianensis* extracts in the following order: STap7 (1526.0 ± 6.6; 1607.2 ± 2.8) > STap1 (1520.2 ± 7.6; 1575.4 ± 3.9) > STap2 (1503.5 ± 9.0; 1575.4 ± 3.9) (µM Trolox/mL; µM Fe (II)/mL), respectively. Similarly, the DPPH values showed significant differences among the various NADES combinations ($p \leq .05$), indicating a clear influence of the solvents used. These results indicate a similar pattern with the bioactive compounds (TPC) extracted using citric acid-NADES. Previous studies have also reported a strong correlation between polyphenolic content and antioxidant activity (Hsieh et al., 2020).

The in vitro antioxidant capacity values of the *T. guianensis* samples analyzed in this study were assessed using 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt $(ABTS)^{+\bullet}$ assays. As shown in Table 2, it is observed that the antioxidant activity of the extracts obtained with NADES significantly ($p \leq .05$) exceeded that of the aqueous extract (1274.3 ± 6.7 µM Trolox/mL), according to the studies by Mar et al. (2023). These findings are consistent with the trends observed in phenolic compounds, showing greater recovery of TPC in extracts using ChCl. The potential against the $ABTS^{+\bullet}$ radical showed the same trend as the potential against DPPH and FRAP radicals (STap7 > STap1 > STap2).

Bioaccessibility is defined as the fraction of a food/component that is released from the food matrix in the gastrointestinal tract and becomes available for absorption (Paterson et al., 2024). *In vitro* digestion reduced the TPC of STap7 from 715.7 to 129.4 mg GAE/100 g, indicating that 81.9% of the TPC was bioaccessible, while for STap1, the reduced TPC value was 77.1% (Figure 1).

During the gastric and fermentative phases, the release of polyphenols from cashew seeds was more efficient. According to Lima et al. (2014), the TPC in undigested cashew residue was 566.10 mg GAE/100 g DW, which decreased to 105.03 mg GAE/100 g DW after digestion, indicating a bioaccessibility level of only 18.6%. For cashew juice, the phenolic contents were 338.60 mg GAE/100 g DW in undigested samples and 130.60 mg GAE/100 g DW after digestion, resulting in a bioaccessibility of 39%.

The eutectic systems of *T. guianensis* exhibited a reduction in antioxidant activity after the *in vitro* digestion process for the ABTS, DPPH, and FRAP assays, with bioaccessibility ranging from 79.1, 63.4, and 52.5%, respectively, for the sample with the highest antioxidant potential. Cashew showed a decrease in antioxidant activity after *in vitro* digestion and fermentation in the ABTS, DPPH, and FRAP assays, with bioaccessibility ranging from 3.18 ± 0.93 to 17.69 ± 1.58% (Andrade et al., 2022).



Means that do not share the same letters on each graph (A-D) are significantly different.

Figure 1. Bioaccessibility reduction (%) of eutectic solvents. 2,2-Diphenyl-1-picrylhydrazyl (A), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (B), total phenolic content (C), *and* Fe(II) (D) *reduction assays.*

The low antioxidant activity observed at the end of digestion suggests the poor stability of compounds under pH and enzymatic changes. Antioxidants are more reactive in acidic (gastric) conditions and less so in neutral (intestinal) pH. During digestion, compounds may transform into less active forms or new antioxidant metabolites (Scrob et al., 2019).

3.2 Macro- and micronutrient content of natural deep eutectic solvents from T. guianensis *fruits*

The extraction capacity of eutectic solvents is largely influenced by their ability to form hydrogen bonds with analytes (Espino et al., 2016). Reports indicate that NADES act as Lewis acids and, in the presence of electron-donating groups, are capable of extracting inorganic elements from a solution (Huang et al., 2018). After the synthesis of NADES, the performance of seven different systems was evaluated in the extraction of macro- and micronutrients for the determination of K, Ca, Mg, P, Fe, Na, Mn, Cu, Zn, Ba, B, V, and Cr by ICP-OES.

The determination of metal content by ICP-OES revealed that the crude extract exhibited substantially higher concentrations of macroelements such as calcium (Ca), potassium (K), and magnesium (Mg) compared to the extracts obtained using different NADES systems (Table 3). This difference can be attributed to the non-selective nature of conventional extraction, which solubilizes a broader range of compounds from the plant matrix.

In contrast, the NADES systems showed greater selectivity in the extraction of these minerals, with the Stap2 (chloride:lactic acid) system standing out by presenting the highest concentrations among the eutectic solvents tested (Table 2). Although the values obtained with NADES were lower than those of the crude extract, the results indicate that this specific system is particularly efficient in mineral recovery, combining extraction performance with environmental viability and potential for sustainable applications.

It is worth noting that the elements Na, Mn, Cu, Zn, Ba, B, V, and Cr were present below the detection limit (0.05–0.25). Table 3 presents the concentration of different elements (Ca, Fe, K, Mg, and P) in various samples (Stap1 to Stap7), expressed in mg/100 g. Potassium (K) is the predominant element in all samples, with the highest values observed for Stap2 (~20 mg/100 g) and Stap3 (~17 mg/100 g), followed by Stap1, Stap4, and Stap5. Calcium (Ca), iron (Fe), magnesium (Mg), and phosphorus (P) are present in lower concentrations, with values significantly lower than potassium. Statistical differences are indicated by the letters above the bars, where samples with different letters exhibit statistically significant differences.

A comparison with the lyophilized (Table 3) fruit extract reveals significant differences in mineral concentrations. In the extract, potassium (K) is the most abundant element (~950 mg/100 g), followed by calcium (Ca) (~200 mg/100 g), both at much higher levels than in the raw samples. In contrast, iron (Fe) exhibits very low concentrations in the extract, suggesting lower extraction efficiency. Statistical analysis indicates that the extraction process influences the retention and concentration of minerals, particularly K and Ca, which may impact nutrient bioavailability.

Horse chestnut seed flour from the wild pure species contains significantly higher metal levels than the hybrid, with iron at 80.05 mg/100 g versus 1.42 mg/100 g. Potassium, calcium, manganese, nickel, and copper are also more abundant in the pure species (Durante et al., 2021). In pepper seeds, mineral concentrations varied as follows: calcium (1,639–4,861 mg/kg), iron (51–196 mg/kg), potassium (558–13,050 mg/kg), magnesium (553–1,662 mg/kg), manganese (7–46 mg/kg), phosphorus (2,306–3,793 mg/kg), and zinc (8–21 mg/kg) (Gamela et al., 2020).

Potassium is essential for regulating cellular osmotic pressure, membrane transport, and enzyme activation, with a recommended daily intake of 782 mg. Calcium, crucial for bones, muscles, and teeth, has a recommended intake ranging from 400 to 1500 mg/day, depending on age (Agostini-Costa et al., 2017). Magnesium is an essential element for the body due to its role in metabolism and as a constituent and activator of many enzymes, particularly those associated with the conversion of high-energy phosphate compounds. The daily requirement is 300–500 mg (Bezerra et al., 2019). Phosphorus is essential for metabolism, with a daily requirement of 800–1,200 mg, found mainly as phosphate in free or bound forms. Iron, needed at 1.5–2.2 mg/day, is present in hemoglobin and myoglobin, playing a key role in oxygen transport (Gamela et al., 2019). The elemental content of various eutectic solvents used to extract compounds from dried licorice roots (*Glycyrrhiza glabra L.*) was measured using ICP-OES. In the sucrose/lactic acid NADES system, high concentrations were found for Ca (12,570 ± 364 mg/kg), Fe (146 ± 2 mg/kg), K (2,470 ± 64 mg/kg), and Mg (2,552 ± 63 mg/kg), while Cu and Li showed the lowest levels (Shikov et al., 2022).

Table 3. Concentration values of Ca, Fe, K, Mg, and P obtained from samples and raw extract of *T. guianensis* fruits. Results are expressed as mean ± standard deviation.

	Elements				
Samples	**Ca (mg/100 g)**	**Fe (mg/100 g)**	**K (mg/100 g)**	**Mg (mg/100 g)**	**P (mg/100 g)**
Raw extract	186.3 ± 6.2	2.7 ± 2.7	1092.6 ± 43.0	119.92 ± 5.2	27.6 ± 1.2
Stap1	2.8 ± 0.2	-	14.0 ± 0.3	1.6 ± 0.0	-
Stap2	3.6 ± 0.3	1.0±0.1	19.6 ± 0.1	2.4 ± 0.1	0.4 ± 0.1
Stap3	3.3 ± 0.1	-	16.7 ± 0.5	1.5 ± 0.1	0.3 ± 0.0
Stap4	1.6 ± 0.1	1.4 ± 0.1	11.7 ± 0.2	1.2 ± 0.0	0.2 ± 0.0
Stap5	1.4 ± 0.1	-	10.8 ± 0.1	1.0 ± 0.0	0.2 ± 0.0
Stap6	1.0 ± 0.0	-	7.8 ± 0.1	0.2 ± 0.0	-
Stap7	1.4 ± 0.2	-	8.3 ± 0.2	0.7 ± 0.2	-

3.3 Fourier-transform infrared spectroscopy

In this study, the individual components of carboxylic acid-based natural deep eutectic solvents (CA-NADES) and CA-NADES synthesized with the molar ratios showing the best extraction potential were investigated. The objective was to determine the intermolecular interactions between the functional groups of the HBA and HBD.

Pure choline chloride has several functional groups, but few of them coexist after the formation of deep eutectic solvents (DESs). Vibrational bands at 3,200 cm^{-1} and 1,200–880 cm^{-1} are associated with hydroxyl or amino groups (the former corresponds to N—H stretching and the latter to symmetric C—N^+ stretching). Vibrational bands at 1,485–1,420 cm^{-1} indicate the presence of alkyl groups, with the CH_2 bending at 1485 cm^{-1} being the most prominent group detected in all choline chloride-based DESs (Hayyan et al., 2015).

The spectra black, red, and blue represent the pure HBDs: citric acid, lactic acid, and malic acid, respectively. The 3,280–2,500 cm^{-1} range indicates OH stretching, while 1,750–1,660 cm^{-1} corresponds to C=O groups in carboxylic acids. Peaks from 1,450 to 800 cm^{-1} reflect C–O and C–H stretching and O–H bending. Multiple C=O peaks in citric and malic acids (black and blue, respectively) result from their multiple carboxylic groups (Airouyuwa et al., 2023).

According to Figure 2, which concerns the choline chloride/glucose system, vibrational bands in the region of 3,600–3,000 cm^{-1} correspond to the vibrational stretching of O—H. Bands at 2,920 cm^{-1} refer to C—H stretching, 1,470 cm^{-1} to a combination of C—H and C—O—H bands, 1,485 cm^{-1} to the bending of CH_2 of an alkyl group, and 1,665 cm^{-1} to the stretching of C=O. The intense bands near the 1,000 cm^{-1} region are due to the stretching vibrations of C—O and C—C, while bands at 917 cm^{-1} are attributed to the stretching of C=C (Hayyan et al., 2015).

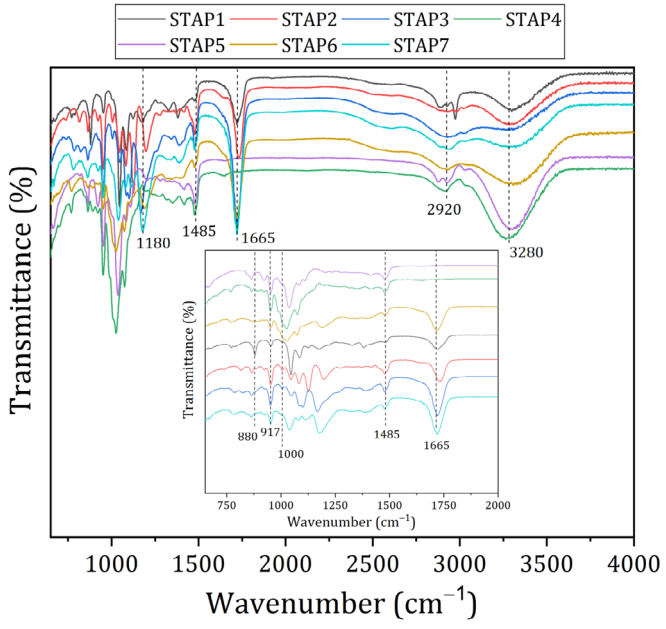


Figure 2. Normalized FTIR-ATR spectra for different DESs, according to their respective natural deep eutectic solvents.

Figure 2 shows the spectrum for the choline chloride/glycerol DES. Vibrational bands at 1,485 cm^{-1} correspond to the CH_2 bending of an alkyl group, and bands near 1,110 cm^{-1}, 1,035 cm^{-1}, and 862 cm^{-1} correspond to functional groups, namely C—O stretching, asymmetric C—C—O stretching, and symmetric C—C—O stretching, respectively (Ibrahim et al., 2006).

The results confirmed the suggested adjustment in the molecular interaction between the synthesized NADES and their respective pure components. These findings are consistent with previous literature (Naseem et al., 2021) and confirm the presence of new hydrogen bonds in the synthesized NADES.

3.4 Chemical profile analysis by nuclear magnetic resonance

In the ^{1}H NMR spectra of the seven extracts from the fruits of *T. guianensis*, a signal at δ 6.93 (s) was identified, characteristic of the hydrogens of gallic acid (Figures 3 and 4), which has already been identified in the leaves of this species (Silva, David et al., 2020).

The structure was confirmed through analysis of the HSQC and HMBC spectra of the extracts, which allowed for the assignment of positions C-2 and C-6 (δ 108.6), C-5 (δ 137.3), and COOH (δ 164.4) (Figure 4). In addition to the signal of gallic acid, the Stap1, Stap2, Stap3, Stap6, and Stap7 samples exhibited signals at δ 7.50 (H-6, *d*, *J* = 3.5 Hz), δ 6.60 (H-2, *d*, *J* = 3.5 Hz), δ 9.54 (H-1′, s), and δ 4.50 (H-1″, s), respectively. The HSQC spectrum analysis allowed for the identification of the C-1' and C-1" positions at δ 177.9 and δ 55.7, respectively (Figure 5).

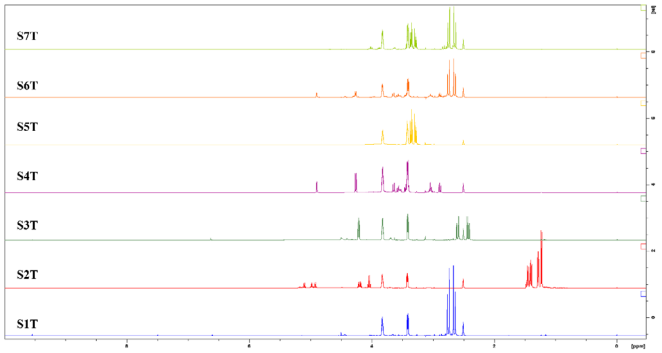
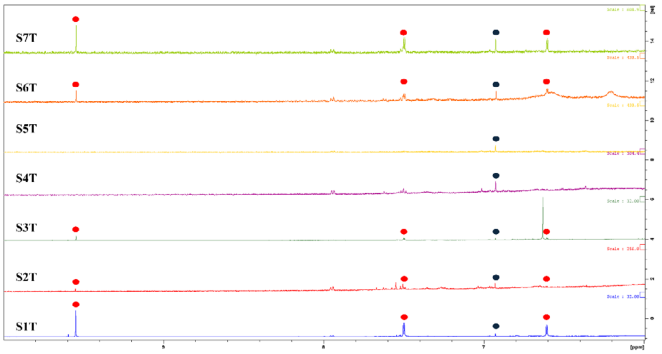


Figure 3. ^{1}H NMR spectra of the seven extracts of *T. guianensis* fruits (500 MHz, DMSO-*d*6).



The red and black circles correspond to the signals of the compounds 5-hydroxyvanillin and gallic acid, respectively.

Figure 4. Expansion of the aromatic region (6.0–10.0 ppm) of the ^{1}H NMR spectra of the seven extracts of *T. guianensis* fruits (500 MHz, DMSO-*d*6).

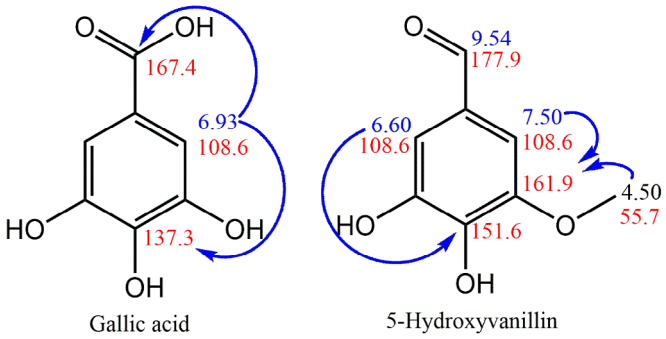


Figure 5. Aromatic compounds identified in the different extracts of *T. guianensis* fruits.

The HMBC correlation map revealed interactions of H-6 with δ 109.6 (C-2), δ 151.6 (C-4), and δ 161.9 (C-5), while H-1″ exhibited a correlation with δ 161.9 (C-5). Therefore, the combined analysis of the one-dimensional (1D) and two-dimensional (2D) NMR spectra allowed the structure of this phenolic compound to be proposed as 5-hydroxyvanillin.

FTIR analysis confirmed key intermolecular interactions in NADES formation, showing vibrational bands linked to hydrogen bonds between HBD and HBA, which stabilize the DESs. New bands in NADES spectra, compared to pure components, indicated additional hydrogen bonds. These molecular changes enhanced the extraction of phenolic compounds, such as gallic acid and 5-hydroxyvanillin, as confirmed by NMR.

4 CONCLUSION

This study evaluated seven ultrasound-assisted NADES for extracting bioactive compounds from *T. guianensis* fruits. Systems using carboxylic acids as HBAs—especially choline chloride/citric acid/glycerol (STap7), choline chloride/citric acid (STap1), and choline chloride/lactic acid (STap2)—achieved the highest TPC and antioxidant activity (DPPH and FRAP assays). STap7 showed the greatest overall efficacy and TPC bioaccessibility, confirming the efficiency of citric acid-based NADES. Mineral analysis indicated effective extraction of Ca, K, and Mg. FTIR and NMR confirmed key phenolic compounds, including gallic acid and 5-hydroxyvanillin. These findings underscore NADES potential for optimizing phenolic extraction and bioaccessibility.

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