

An eco-friendly high-pressure biorefinery approach for the recovery of valuable compounds from cashew nut testa shell (*Anacardium occidentale* L.)

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Abstract

Abstract The cashew agroindustry generates substantial by-products that are often improperly used. Cashew nut testa shell (CNTS) has attracted interest due to its elevated fatty acid contents and phenolic compounds, raising the relevance of environmentally friendly extraction techniques for its recovery. CNTS was submitted to high-pressure (Supercritical Fluid Extraction – SFE; Pressurized Liquid Extraction – PLE; and Subcritical Water Extraction – SWE) methods compared to traditional low-pressure (Soxhlet and maceration) techniques. Supercritical fluid extraction with CO₂ as solvent was selective to recover fatty acids, such as palmitate (12.63 mg g⁻¹), stearic (26.65 mg g⁻¹), and oleic (25.61 mg g⁻¹) acids, as well as behenic (46.42 mg g⁻¹) and erucic (28.00 mg g⁻¹), quantified by GC-MS. In contrast, the ethanolic and aqueous extracts, by pressurized liquid and subcritical water extraction, presented polyphenols like catechin, epicatechin, and procyanidin identified by UPLC-PDA-ESI-QDa, known for their high antioxidant potential and biological activities. In addition, fractions of proteins and sugars were also recovered. Considering the different compounds in the raw material, sequential extraction routes were conducted to fractionate the CNTS and provide different products from an underestimated raw material, a novelty that increased the value of the cashew processing chain.

1. Introduction

The cashew nut industry generates significant amounts of by-products that are usually discarded as waste, leading to environmental concerns and economic losses. Cashew nut testa shell (CNTS) is a by-product (3% of the weight of cashew nuts) of the cashew nut processing industry (Sharma et al. 2020), and recent studies have demonstrated that CNTS contains valuable fatty acids and phenolic compounds, with potential uses across different industrial sectors, such as food, cosmetics, and pharmaceuticals. (J. da Silva et al. 2022).

Fatty acids, especially stearic, palmitic, oleic, linoleic and linolenic acids, represent vegetable oils' most prevalent constituents (Kamatou and Viljoen 2017). Cashew nuts with testa shell are also abundant in fatty acids, mainly oleic, linoleic, and palmitic acids (Trox et al. 2010). The fatty acids from natural products are of particular interest because of their diverse biological activities and nutritional importance to human health (Jamil et al. 2020). They play roles in numerous physiological processes, such as energy metabolism, cell signaling, and membrane structure (Chandrasekara and Shahidi 2011a).

Phenolic compounds, found abundantly in plant tissues, are secondary metabolites with widespread distribution. CNTS exhibit significant antioxidant activity due to the presence of radical-scavenging polyphenols (Chandrasekara and Shahidi 2011b). Furthermore, CNTS has been reported to be a good source of hydrolysable tannins (Rajini 2011) and abundant reservoir of phenolic compounds, such as catechins, epicatechins, and procyanidins (Kannan et al. 2009; Sruthi et al. 2023; Trox et al. 2011). The bioactive compounds and antioxidative properties associated with them have been reported to offer various health advantages, including reducing the likelihood of chronic ailments like cancer, diabetes, and cardiovascular disease (Kamath et al. 2008; Lee et al. 2020).

However, extracting these compounds from CNTS is challenging due to their complex structure and chemical composition. Traditional extraction methods for these compounds involve the use of organic solvents, which are often toxic and present health and environmental risks. Moreover, these traditional solvent extractions also result in the coextraction of unwanted compounds, leading to decreased purity and increased processing costs.

Supercritical fluid (SFE), pressurized liquid (PLE), and subcritical water (SWE) extraction are modern extraction methods widely used for the recovery of bioactive compounds from various plant materials (Rebelatto et al. 2020). SFE using CO₂ is an attractive alternative due to its ability to selectively extract nonpolar and moderately polar compounds. Supercritical solvents, such as CO₂ in the supercritical state, exhibit gas-like properties and solvent-like behavior (Ray et al. 2023), which facilitates their penetration in the sample matrix and dissolution of the target compounds.

The PLE process, in which ethanol and ethanol/water mixtures are used as solvents, maintains the solvent in its liquid state by operating below the critical point. To improve the mass transfer rate, pressure and temperature conditions are carefully selected to reduce the solvent's surface tension and viscosity while increasing the solubility of the components. This helps the solvent permeate into the solid matrix, thereby facilitating extraction (Krümmel et al. 2022; Mazzutti et al. 2018).

SWE uses water as a solvent in its subcritical region. Water is heated to high temperatures but maintains its liquid state due to pressures higher than its vapor pressure (Essien et al. 2020). In this state, fluid properties such as the dielectric constant and viscosity are changed to improve mass transfer and water solubility (Gan and Baroutian 2022).

These high-pressure (SFE, PLE and SWE) extraction methods have advantages over conventional extraction methods, such as higher efficiency, shorter processing time, and lower solvent and energy consumption (Del Castillo-Llamas et al. 2021). Moreover, these methods are considered environmentally friendly because they use nontoxic solvents and result in less waste (Gil-Martín et al. 2022).

Recently, the simultaneous extraction of fatty acids and phenolic compounds from plant materials using CO₂ and ethanol and water has attracted increased interest. This is because these compounds are often found in plant tissues and have been reported to have synergistic health effects. Moreover, using SFE, PLE, and SWE allows for the extraction of a broader range of polyphenols. These methods are effective and selective at extracting specific classes of compounds from plant materials and are important for adding value to byproducts such as CNTS.

Therefore, this study aimed to investigate sequential extractions of fatty acids and polyphenols by SFE, PLE and SWE, using solvents with different polarities in a biorefinery strategy for recovering valuable fractions from the underestimated CNTS. The extracted compounds were characterized using various analytical techniques, which includes gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography coupled to diode array and mass spectrometry detectors (UPLC-PDA-

ESI-QDa). The study also evaluated the antioxidant activity of the extracted compounds using in vitro assays. This innovative sequential approach highlights the relevance of recovery different bioactive fractions from CNTS, increasing the cashew chain value.

2. Materials and methods

2.1. Raw material

Cashew nut testa shells (CNTS) samples were obtained from Embrapa Tropical Agroindustry (Fortaleza, CE, Brazil). This byproduct was spread on trays for drying at 45°C in an oven with circulation air (MS Metalúrgica e Comércio, Canoinhas, SC, Brazil) for 24h. After drying, the CNTS were ground in a stainless-steel knife mill and were kept in a freezer at -18°C until analysis.

2.2. Sample characterization

The sample's moisture content was assessed through the gravimetric method involved using a vacuum oven at 70°C (method 926.12) (TECNAL, model TE-395, Brazil) (AOAC 1996). The Kjeldahl procedure was used to evaluate the total protein content (TPC) (method 928.08) with a nitrogen-protein correspondence factor of 6.25 (AOAC 2000). The ash content was determined employing a muffle furnace (QUIMIS, Q318M24, São Paulo) at 550°C for 6 h (method 900.02) (AOAC, 2000). The crude fiber content was estimated using an automatic fiber extraction system, “Fibra Plus” (FES 2 R TS, Pelican Equipment, Chennai, India) (Prosky et al. 1984). The total fat content was estimated with the help of a Soxhlet apparatus using n-hexane as a solvent at a boiling temperature of 60°C (method 920.39C) (AOAC 2005). Carbohydrate content was quantified by difference.

2.3. Extraction procedure

Various extraction methods and techniques have been developed to obtain desired compounds from raw materials. This section explores high-pressures equipment types (supercritical fluid (SFE), pressurized liquid (PLE), and subcritical fluid (SWE) extraction), to compare with low-pressure methods (Soxhlet-SOX and Maceration-MC). Each method uses different conditions and solvents to achieve the best results to recover specific fractions from the raw material (CNTS).

Previous kinetic evaluation was performed to establish the extraction time for SFE, PLE, and SWE, where samples were collected at pre-established time intervals. Based on the kinetics, the extraction time was fixed for each method, representing the period of diffusion controlled by extraction. A piecewise empirical model with three segments (Eq. 1) was fitted to the kinetic curves, and its performance was evaluated applying the coefficient of determination (R^2) and the root mean square error (RMSE).

$$y = \begin{cases} a_1 + k_1 x & (x < x_{i1}) \\ y_{i1} + k_2 (x - x_{i1}) & (x_{i1} \leq x < x_{i2}) \\ y_{i2} + k_3 (x - x_{i2}) & (x \geq x_{i2}) \end{cases}$$

1

This results in two equations (Eqs. 2 and 3):

$$y_{i1} = a_1 + k_1 x_{i1}$$

2

$$y_{i2} = y_{i1} + k_2 (x_{i2} - x_{i1})$$

3

where a_1 = intercept, k_1 = slope, x_{i1} = intersection, k_2 = slope, x_{i2} = intersection, and k_3 = slope.

2.3.1. High-pressure extractions

The schematic design of the high-pressure units used are shown in Fig. 1.

The SFE was performed in a unit adapted by Michielin et al. (2005) and Mazzutti et al. (2018) (Fig. 1a). The extraction conditions were chosen according to studies carried out by Andrade et al. (2012) and Mazzutti et al. (2018). The parameters for SFE conditions evaluated were 40 °C, a flow of 1.2 kg h⁻¹, and two pressure conditions (15 and 25 MPa), and the assays were coded as SFE1 and SFE2, respectively. The extraction procedure involved placing 20 g of CNTS into a stainless-steel extraction vessel (329 mm long, 20.42 mm internal diameter), which was completed with glass beads. The extracts were collected in pre-weighed amber glass vials and stored at -18°C in a freezer. The solid residue obtained after processing was collected and stored for later use.

The PLE and SWE assays were performed on a self-assembled apparatus (Fig. 1b) described by Gonçalves Rodrigues et al. (2019) using ethanol and water as extraction solvents, respectively. Briefly, 5 g of CNTS powder and glass beads were placed in a stainless-steel extraction cell (25 mm ID and 180 mm height). An HPLC pump (Waters, model 515, USA) was used to pump the solvent, with the flow rate set at 4 mL min⁻¹. The cell pressure was controlled (10 MPa) by a needle valve (HiP, model 20–11LF4, NFA, USA) for both methods, PLE, with ethanol as the solvent, and SWE, with water as the solvent. The temperature for PLE was controlled at 40, 60, 80, 100, or 120°C, with the assays codifying as PLE1, PLE2, PLE3, PLE4, or PLE5, respectively. For the SWE assays, the extraction temperatures were 80, 100, 120, 140, and 160°C, and the assays were codified as SWE1, SWE2, SWE3, SWE4, and SWE5, respectively. The recovered PLE and SWE extracts were dried by rotary evaporation or lyophilized to remove the solvent and stored at -18°C in the dark.

2.3.2. Low-pressure extractions

SOX extraction was performed according to the 920.39C method using hexane (Synth, São Paulo, Brazil) (AOAC 2005). Briefly, 150 mL of solvent was added to a Soxhlet extractor with 5 g of dry CNTS sample for 6 h. Maceration extraction (MC) was performed using ethanol (MCE) and water (MCW) as solvents following the method of Reyes et al. (2014). Briefly, one gram of CNTS was mixed with 40 mL of the

solvent, containing 0.1% (w v⁻¹) butylated hydroxytoluene (BHT), and stirred for 24 h at 250 rpm and 37°C on an orbital shaker (DOS-20 L, Elmi Ltd., Riga, Latvia) (Kamath et al. 2008). The extracts obtained by MCE or MCW were vacuum filtered, after which the CNTS residues were retained. The SOX and MCE recovered extracts were rotary evaporated (Fisatom, model 801, São Paulo, Brazil). Extracts recovered by MCW were lyophilized to remove the solvent and stored at -18°C in the dark.

2.3.3. Sequential extractions

The combination of high-pressure or low-pressure sequential extractions was conducted following six routes (3 high-pressure and 3 low-pressure), as presented at Fig. S1. Initially, the CNTS underwent to the removal of the nonpolar fraction (oily fraction) by SFE with CO₂ or by SOX with hexane to recover the fatty acids, representing the high-pressure, and the low-pressure routes, respectively. Then, the defatted CNTS (after SFE or after SOX) was conducted to 3 routes each: (I) the phenolics recovery routes using ethanol as solvent, represented by SFE-PLE or by SOX-MCE; (II) the water extraction routes, represented by SFE-SWE or SOX-MCW; and finally, the tree steps routes (III), represented by SFE-PLE-SWE or by SOX-MCE-MCW.

The primary goal of the sequential extractions was the CNTS compounds fractionation, as a function of solvent and process used, providing different products: non-polar rich fractions (mostly fatty acids), polyphenols (phenolic acids and flavonoids) rich fractions (sugar, polysaccharide, and protein components), mainly the ethanolic extracts; and high-molecular weight rich fractions, the water extracts.

2.4. Extraction yield

The total extraction yield (E_y) was calculated as the percentage of the ratio between the extracted mass (m_e) and the dry mass (m_d) of the CNTS (Eq. 4). The experiments were performed in duplicate.

$$E_y = \frac{m_e}{m_d} \times 100$$

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2.5. Profiling of the extracted compounds

2.5.1. GC–MS analysis and fatty acid profile

GC–MS analysis was performed for the oily samples recovered by SFE with CO₂ and by SOX with hexane and was conducted on an Agilent GC 7890A instrument coupled with an Agilent 5975C MS detector. The HP-5MS (Agilent) fused silica capillary column (30 m length × 250 µm i.d. × 0.25 µm thick film composed of 5% phenyl-95% methylpolysiloxane) was connected to an EI source (electron impact ionization) operating at 70 eV with a quadrupole mass analyzer. The mass scan ranged from 30 to 550 m/z. Helium 5.0 (purity of 99.999%) was used as the carrier gas at a flow rate of 1.2 mL min⁻¹. The injector (split ratio of 1:50) and transfer line temperatures were 300 °C. The injection volume was 1.0 µL with an Agilent GC

Sampler 80 autosampler equipped with a 10 μL syringe. The oven temperature program consisted of ramping up from 60°C for 3 min, followed by a ramp from 4°C min^{-1} to 270°C for 2 min and then from 30°C min^{-1} to 300°C for 5 min with a 63.5 min runtime. The fatty acid (FA) composition was used to evaluate the profile of the CNTS oil. The samples were prepared using the protocol reported by O'Fallon et al. (2007). The fatty acid composition of the CNTS oil was expressed as g/g extract (F.A.M.E.). C4-C24 Supelco® (18919-1AMP) was mixed.

2.5.2. Total phenolic and flavonoid contents

The total phenolic content (TPC) and total flavonoid content (TFC) were evaluated for the extract samples recovered by high-pressure (PLE and SWE) and by low-pressure methods (MCE and MCW), and also from the sequential extractions (section 2.3.3). TPC was assessed using the Folin–Ciocalteu assay (Singleton et al. 1999). The CNTS extracts were diluted to concentrations up to 10 mg mL^{-1} and mixed with a reaction mixture consisting of 10 μL of extract or solvent, 50 μL of Folin–Ciocalteu reagent (Sigma Aldrich), 150 μL of 20% sodium carbonate (Lafan), and 790 μL of distilled water. The reaction took place at room temperature in the absence of light. After 2 hours, the absorbance was assessed at 760 nm using a spectrophotometer (Epoch, BioTek, São Paulo). The experiments were carried out in triplicate, and the findings are presented in milligrams of gallic acid equivalent (GAE) per gram of dried CNTS extract (mg GAE g^{-1}).

TFC was evaluated according to the methods of Zhishen et al. (1999) with modifications. A solution of NaNO_2 (0.5 mol L^{-1}), AlCl_3 (0.3 mol L^{-1}) and NaNO (1 mol L^{-1}) was prepared. Briefly, the extract was added along with water and NaNO_2 . After the 5 min reaction at 37°C, AlCl_3 was introduced, and the mixture was permitted to undergo a reaction in the dark. After 6 min, NaOH was added, the solution was mixed well again, and the absorbance was measured at 510 nm with a spectrophotometer (Epoch, BioTek, São Paulo). The experiments were performed in triplicate. The results are expressed in milligrams of catechin equivalents (CAE) per gram of dried CNTS extract (mg CAE g^{-1}).

2.5.3. Chemical profile by UPLC-PDA-ESI-QDa analysis

Ultra-performance liquid chromatography (UPLC) analysis was used to determine the chemical profile of the polar extracts recovered from CNTS. The evaluated extracts were selected as the processing extraction conditions that provided higher performance in the recovery of polyphenols and antioxidant components, according to results from section 3.4. The samples represented the high-pressure (PLE and SWE) and the low-pressure (MCE and MCW) extraction routes. Briefly, approximately 4 mg of extract was dissolved in 1 mL of a 50/50% methanol/water mixture. The material was filtered through a 0.22 μm PTFE filter, placed in vials, and stored at -80°C for analysis. The analyses were conducted employing an Acquity UPLC (Waters, USA) system coupled to a PDA (210–600 nm) and a QDa mass spectrometer (Quadrupole, Waters). A Waters Acquity BEH C18 column was used for separation (150 mm $\times$ 2.1 mm, 1.7 μm) at 40°C. An aliquot of each 5 μL extract was subjected to an exploratory gradient with a mobile phase composed of deionized water (A) and acetonitrile (B), both of which contained formic acid (0.1% v/v). The extracts were subjected to an exploratory gradient as follows: 2–100% B (22.0 min), 100% B

(22.1–25.0 min), and 2% B (26.0–30 min) with a flow rate of 0.3 mL min^{-1} . Ionization was performed employing an electrospray ionization source in negative mode in SIR mode using the following ions: procyanidin B dimer, m/z 577; catechin/epi-catechin, m/z 289; catechin gallate, m/z 441; and the procyanidin B trimer, m/z 865. The adjusted instrumental parameters were as follows: capillary voltage, 0.8 kV; cone voltage, 15 V; source temperature, 120°C ; desolvation temperature, 350°C ; and desolvation gas flow, 500 L h^{-1} (G. S. da Silva et al. 2017; Rockenbach et al. 2012). The system was controlled using Empower 3 software (Waters Corporation). The areas of each substance were exported to Morpheus software and converted into a heatmap, and the samples were grouped using Euclidean distance and mean linking.

2.5.4. Antioxidant capacity

The antioxidant potential of the CNTS extracts was investigated for the extracts obtained by high-pressure methods (PLE and SWE), by low-pressure methods (MCE and MCW), and from the sequential extractions (section 2.3.3). The antioxidant potential was analyzed by different methods, described below.

FRAP assay

To measure the antioxidant capacity of the samples using ferric reducing antioxidant power (FRAP), a protocol was described by Benzie & Strain (1996). The selected CNTS extracts were diluted in the extraction solvent. The FRAP reagent was formulated by combining a 0.3 M sodium acetate buffer solution (pH 3.6), a 20 mM ferric chloride solution ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and a diluted TPTZ solution (2,4,6-tripidylstriazine) in a 40 mM hydrochloric acid solution. In preparing the reagent, 60 mL of sodium acetate buffer solution was combined with 6 mL of TPTZ solution and 6 mL of ferric chloride solution. Subsequently, the solution was diluted and incubated for 30 min at room temperature in darkness. The absorbance at 593 nm was measured utilizing a spectrophotometer (Epoch, BioTek, São Paulo). The results are expressed as millimoles of Trolox equivalent (mmol TE) per gram of dried CNTS extract (mmol TE g^{-1}).

DPPH assay

To assess antioxidant capacity, the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) was used following the protocol established by Mensor et al. (2001). The selected CNTS extracts were diluted in the extraction solvent. Subsequently, the DPPH radical solution was introduced, and the mixture was homogenized. The samples were left to react for 30 min at room temperature without light. A spectrophotometer (Epoch, BioTek, São Paulo) was used to measure the samples at a wavelength of 517 nm. The results are presented in millimoles of Trolox equivalent (mmol TE) per gram of dried CNTS extract (mmol TE g^{-1}).

ABTS assay

The antioxidant capacity of the selected extracts of CNTS was analyzed via 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid according to the protocol described by Re et al. (1999). The ABTS radical was generated by mixing 2.45 mM potassium persulfate with 7 mM ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) in the dark at room temperature for 16 h prior to the execution of the method. Subsequently, the generated ABTS radical was diluted in 5 mM PBS (phosphate-buffered saline) at pH 7.4 to achieve an absorbance of 0.7 (± 0.2) at 734 nm. The diluted CNTS extracts were mixed with the ABTS solution and allowed to react for 30 min in the dark. After the reaction, the absorbance was read at 734 nm in a spectrophotometer (Epoch, BioTek, São Paulo). The results are presented in millimoles of Trolox equivalent (mmol TE) per gram of dried CNTS extract (mmol TE g^{-1}).

2.5.5. Reducing and total sugars

The quantification of reducing (RS) and total (TS) sugars present in the polar extracts was investigated for the samples obtained by high-pressure methods (PLE and SWE), by low-pressure methods (MCE and MCW), and from the sequential extractions (section 2.3.3) performed based on the methods of Miller (1959), with modifications. Briefly, 2.5 g of 3,5-dinitrosalicylic acid (DNS) was added to 50 mL of sodium hydroxide (NaOH: 2 mol/L) and 125 mL of distilled water to prepare the solution. The solution was stirred until completely dissolved. Subsequently, 75 g of tetrahydrate potassium sodium tartrate ($\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$) was added, and the mixture was adjusted to a volume of 250 mL. For the determination of TS, hydrolysis was performed on the sample. In summary, a reaction was performed with the sample and hydrochloric acid (HCl: 2 mol/L) for 10 minutes at 100°C, following sodium hydroxide (NaOH – 2 mol/L) was added at a 1:1:1 (v/v/v). The DNS reaction occurred at a 1:1 (sample:DNS solution) ratio, then the mixture was subject to heating in a water bath at 100°C for 5 minutes. The solution was chilled using an ice bath, after which the absorbance was read on a spectrophotometer (Epoch, BioTek, São Paulo) at 540 nm. The contents of total and reducing sugars were expressed as glucose equivalents (GLU) per gram of dried CNTS extract (mg GLU g^{-1}).

2.5.6. Protein content

The protein content of the CNTS extract samples was determined for the extracts obtained by high-pressure methods (PLE and SWE), by low-pressure methods (MCE and MCW), and from the sequential extractions (section 2.3.3) performed based on the methods of Miller according to method 928.08 (AOAC, 2005) for the polar fractions (ethanolic and water extracts) recovered by different methods. Briefly, the procedure consisted of digesting the extracts together with a catalyst (copper sulfate (CuSO_4): sodium sulfate (K_2SO_4) – 1:10 n m⁻¹) using sulfuric acid (98% P.A.) in tubes through a digester block (TECNAL, model N1040, Brazil). After digestion, the mixture was distilled through a nitrogen distiller (TECNAL, model TE-0364, Brazil) using a 40% m v⁻¹ sodium hydroxide solution. The distillate was gathered in a boric acid solution (4%) and titrated with a standardized hydrochloric acid solution (0.1 mol L⁻¹). The protein percentage was calculated according to the nitrogen-protein correspondence factor of 6.25. Analyses were performed in duplicate.

2.6. Statistical analysis

The results were statistically analyzed using Statistica 13.5 software (TibCo, USA) through analysis of variance (ANOVA). Multiple mean comparisons were performed utilizing the Tukey test, with a confidence level of 95%.

3. Results and discussion

3.1. Centesimal composition content

The centesimal composition of the CNTS is shown in Table 1. The values detected in the present work were compared with those from da Costa (2019) for the essential oil from CNTS and from Oliveira (2016), who studied the isolation of CNTS constituents and evaluated its potential as a natural antioxidant, which results are also presented at Table 1. The values observed for the centesimal composition of the CNTS were similar to those in the literature, with variation possibly due to the analysis method used, seasonality, soil, cultivation and variety (Schiassi et al. 2018).

Table 1
Centesimal composition of CNTS

Centesimal composition	Present work (%)	Literature data (%)	
		Oliveira (2016)	da Costa (2019)
Moisture	4.86 ± 0.16	9.50	7.05
Fat	21.00 ± 1.88	20.10	15.82
Ash	1.71 ± 0.01	2.02	1.72
Fiber	9.67 ± 0.55	10.30	14.89
Protein	11.71 ± 0.17	19.00	10.47
Carbohydrate	51.05 ± 2.77	39.08	50.03

CNTS has high lipid content (21.00%), which a valuable fatty acid profile can benefit to human health (see section 3.4.1, profile rich in unsaturated fatty acids). The high percentage of carbohydrates suggested a relevant content of natural sugars (Nagaraja 2000). The ash and protein contents of CNTS and shelled nuts, in general, are analogous, showing that the family groups (nuts) have similar chemical compositions in some aspects. Tunçil (2020) evaluated the dietary fiber profiles of hazelnuts and obtained a composition of 2.66% for ash and 7.5% for protein present in hazelnut skin. Muñoz-Arrieta et al. (2021) evaluated the nutritional and bioactive composition of peanut skin (*Virginia*) and obtained values of ash (2.13%) and protein (12.7%). Although present in smaller amounts than other protein sources, they are important for several metabolic functions. CNTS contains compounds that contribute to its nutritional characteristics (Sruthi and Naidu 2023). Although this composition is variable due to seasonality, this by-product has the potential to be used for the recovery of value-added compounds.

3.2. Kinetics extraction

The kinetic curves for the SFE, PLE, and SWE techniques are presented in Fig. S2. The mass transfer mechanisms were evaluated from kinetic curves consisting of three periods: constant extraction rate (CER), falling extraction rate (FER), and diffusion-controlled rate (DCR), as presented by Weinhold et al. (2008).

The kinetics behavior was adjusted and described by the piecewise model. Mathematical modeling is important for interpreting extraction behavior and mass transfer mechanisms to obtain parameters that help improve the economic viability of these processes on an industrial scale (Muruges et al. 2018). Table S1 shows that the parameters of the piecewise model fit the experimental data well, with R^2 values above 0.99 and RMSEs below 0.06. Kinetic modeling makes it possible to precisely determine the total extraction time required for each process. It is possible to observe the three mass transfer periods via piecewise adjustment. The CER period was conducted at rates of 10.0327 (SFE1), 0.0651 (SFE2), 0.5133 (PLE3), and 0.1018 (g min^{-1}) (SWE3), and the end of the CER period was detected at 73.68, 36.30, 2.67, and 7.86 min, respectively. This period (CER) is represented by the first fitted line, in which the extraction of soluble material available on the surface of the plant matrix occurs (Mezzomo et al. 2009).

The FER periods occurring at rates of 0.0062 (SFE1), 0.0125 (SFE2), 0.0727 (PLE3), and 0.0169 g min^{-1} (SWE3) were identified between 73.68 and 154.27, 36.30 and 71.44, 2.67 and 7.54, and 7.86 and 46.93 min, respectively. This period is characterized by convection and diffusion mechanisms due to the partial exhaustion of solute from the surface of the particles, which causes a decrease in the extraction rate (Sovová 1994). DCRs occurring at rates of 0.011 (SFE1), 0.0016 (SFE2), 0.086 (PLE3), and 0.043 (SWE3) (g min^{-1}) are presented for times (154.27), (71.44), (7.54), and (46.93 min), respectively. During the DCR period, the diffusion mechanism controls the mass transfer of the process. Additionally, it is possible to observe that the extraction rate decreases due to solute exhaustion from the particle surface (Ferreira and Meireles 2002). The extraction time is defined by achieving DCR period, characterized by low (almost negligible) extraction rate, where the process yields are above 90%. This analysis result in extraction times defined as 180, 100, 10, and 50 min for SFE1, SFE2, PLE3, and SWE3, respectively.

3.3. Extraction yield

Figure 2 shows the extraction yield (E_y) for the extraction assays conducted at high-pressure and low-pressure methods applied to the raw material (CNTS) at different process conditions.

The behavior of the solvents with different polarities (CO_2 , hexane, ethanol, and water) suggested a wide variety of compounds present in the CNTS. The results indicate high contents of non-polar components (rich in fatty acids – the oily fraction) and polar components (polyphenols, antioxidants, and sugars).

The oily fraction yields recovered by SFE with CO_2 were 12.31% (SFE1) and 14.46% (SFE2), increasing with the pressure increase at constant temperature. These results were lower than obtained by SOX with n-hexane (20.38%), as compared at Fig. 2a. However, despite its widespread use and various applications,

the SOX technique has notable drawbacks compared to SFE. SOX extraction is time-consuming (6 h), leading to significant energy consumption, while the SFE method was conducted up to 180 min of extraction (section 3.2). Moreover, SOX method uses considerable amount of solvent (hexane), which requires further evaporation or concentration steps afterward (Torres et al. 2022). Additionally, thermal degradation of compounds occurs due to the extended exposure to high temperature at the solvent boiling point. As a result, SFE has emerged as a more efficient alternative with improved performance and reduced environmental impact.

The polar fraction recovery by PLE2 and SWE1, with ethanol and water respectively, showed yield performance with no significant difference in E_y compared to MCE and MCW respectively ($p < 0.05$), as presented at Fig. 2b. From the ethanolic fractions, the increase in PLE temperature (from 40 to 120°C) significantly increased the E_y compared to MCE ($p < 0.05$). The same behavior was observed for SWE2, SWE3, SWE4, and SWE5 compared to that of MCW. The best yield value was from SWE5 (E_y of 48.99%), a water extraction at 160°C, and significantly different than other assays ($p < 0.05$) (Fig. 2b). An increase in temperature decreases the density, viscosity, and surface tension of water in the subcritical state, increasing the mass transfer and favoring the extraction yield (Plaza and Marina 2019). The yield of the assays PLE3, PLE4, PLE5, SWE2, SWE3, and SWE4 were statistically similar, with values higher than that obtained by MCE and MCW ($p < 0.05$) (Fig. 2b). Similar to SWE, the temperature influence on PLE assays also contribute to an increase in the solubility of the compounds, enhancing the extraction yield due to higher solvent diffusivity, and reduced viscosity, which increases solvent penetration in the solid matrix enhancing the mass transfer (Gonçalves Rodrigues et al. 2019). Notably, PLE (10 min) and SWE (50 min) methods expend lower time and energy compared to MCE or MCW (24 h), respectively, contributing to indicate the high-pressure methods as viable alternatives to improve the value of the cashew nut processing chain.

3.4. Profiling of the extracted compounds

This section will discuss the findings concerning the profile of the extracts, both polar and non-polar, acquired through high and low-pressure extraction methods.

3.4.1. GC-MS analysis and fatty acid profile

Fatty acids are the main elements of cashew nut oils and are predominantly composed of stearic, palmitic, oleic, linoleic, and linolenic acids (Leal et al. 2023). Figure 3 shows the profile of fatty acids extracted from CNTS by SFE with CO₂ (SFE1 and SFE2) and by Soxhlet with n-hexane (SOX).

The samples recovered by SFE1, SFE2, and SOX assays revealed, respectively, the presence of: saturated fatty acids (SFAs) of 53.88, 65.36, and 60.36%; monounsaturated fatty acids (MUFAs) of 32.69, 38.41, and 26.14%; and polyunsaturated fatty acids (PUFAs) of 13.43, 17.07, and 13.80%. High SFA content is crucial for the stability of CNTS oil, and it has been stated that SFA plays an important role in the antioxidant stability of lipids (Shahidi and Zhong 2010).

SFE2 was more selective to the acids palmitic (12.63 mg g^{-1}), oleic (29.65 mg g^{-1}), linolenic (25.61 mg g^{-1}), behenic (46.42 mg g^{-1}), and erucic (28.00 mg g^{-1}), compared to SFE1 and SOX ($p < 0.05$). CNTS oil also contained considerable concentrations of stearic acid (39.09 mg g^{-1}). An increase in pressure improved the selectivity of this compound; however, no significant difference was detected compared to SOX sample ($p < 0.05$). The increase in pressure contributes to fatty acids recovery, where CO_2 is a viable solvent compared to hexane (Soxhlet), which is undesirable to health and environment (Arturo-Perdomo et al. 2021).

High concentration of SFA on the oily fraction has cardioprotective effects, mainly attributed to oleic acid (Nishi et al. 2014). Then, the CNTS oily fraction presented good lipid quality, with high content of MUFAs, especially oleic acid with high oxidative stability (Reis Ribeiro et al. 2020). Frigolet & Gutiérrez-Aguilar (2017) reported that methyl palmitate and oleic acid are beneficial for treating diabetes, obesity, autoinflammatory diseases, cancer, and cardiovascular disease.

In the recovered fractions, variations in the fatty acid profile may be associated with the polarity of the solvent. Long-chain fatty acids, especially unsaturated fatty acids, can have low polarity compared to saturated fatty acids, which justifies the higher contents of SFAs from the SFE and SOX samples, obtained by the nonpolar solvents CO_2 and n-hexane, respectively (Cuco et al. 2019).

Leal et al. (2023b) evaluated the impact of different kernel sizes on the cashew nut oil profile and identified the main fatty acids as saturated fatty acids (SFAs), palmitic (C16:0), and stearic (C18:0) acids, and the monounsaturated fatty acid (MUFAs) oleic acid (C18:1). Polyunsaturated fatty acids (PUFAs) and linoleic acid (C18:2) were obtained. From this study, it can be observed that CNTS has a profile with components associated with nuts. Muñoz-Arrieta et al. (2021) evaluated the fatty acid composition of peanut skin (*Valencia*) using petroleum ether (Soxhlet) as a source of extraction and reported the concentrations of palmitic (8.05 mg g^{-1}), stearic (2.07 mg g^{-1}), and oleic (37.2 mg g^{-1}) acids. As a nut family film, these values are close to the concentrations presented in the CNTS. However, the recovery method can influence the concentration of these acids.

Fatty acid recovery from CNTS positively impacts environmental sustainability, the economy, and renewable energy and contributes to more conscious practices in the use of natural resources. This approach aligns with the search for more sustainable solutions and can contribute to the transition to a greener, low-carbon society.

3.4.2. Total phenolic and flavonoid contents

Polyphenols represent the predominant class of phytochemicals in CNTS. TPC and TFC values from the extract samples obtained by high-pressure methods (PLE and SWE) and by low-pressure assays (MCE and MCW) are shown in Fig. 4.

The TPC and TFC values ranged from 196.69 to $302.74 \text{ (mg GAE g}^{-1}\text{)}$ and 212.74 to $354.27 \text{ (mg CAE g}^{-1}\text{)}$ respectively. PLE2 and SWE2 were the best assays for TPC recovery, with values of 302.74 and 296.35

(mg GAE g⁻¹), respectively (Fig. 4a). PLE2 was efficient in phenolics recovery due to the use of ethanol at high pressure and moderate temperature, which increases the desorption and solubilization speed of the components from the solid matrix, improving the mass transfer (Plaza and Turner 2015).

High TPC values suggest the presence of the relevant content of polyphenols from CNTS. Trox et al. (2011) reported that the main phenolic acids in CNTS are syringic, gallic, and p-coumaric acids. Chaves et al. (2010) evaluated the chemical constituents of CNTS obtained from ethanolic extracts, and the TPC was approximately 185.44 (mg GAE g⁻¹). Kamath & Rajini (2007), through a stirring process in a shaker at 37°C using ethanol to obtain the TPC of CNTS, obtained 243 (mg GAE g⁻¹). In the recovery of phenolic compounds from peanut skin (*Arachis hypogaea* L.) Sorita et al. (2022) used PLE (ethanol, 10 MPa, 80°C) and SWE (water, 10 MPa, 160°C) and obtained TPC values of 24.61 and 84.47 (mg GAE g⁻¹), respectively; these concentrations are lower than obtained from CNTS extracts, corroborating with the relevance of this underestimated raw material, valued by using alternative high-pressure methods.

The SWE2 and SWE3 samples presented TFC values of 354.27 and 351.59 (mg CAE g⁻¹), respectively (Fig. 4b). These values were significantly greater than those recovered by MCE and MCW ($p < 0.05$). The presented data suggest that flavonoids are significant polyphenols in CNTS (Sruthi and Naidu 2023). (+)-Catechin, (-)-epicatechin, epigallocatechin, and catechin gallate are the main flavonoids present in CNTS (Sruthi et al. 2023). Using water as the solvent, SWE2 and SWE3 recovered significant amounts of TPC and TFC. This can be explained by the change in polarity of the dielectric constant to water, which increases the solubility of some less polar compounds (Plaza and Marina 2019). In addition, it decreases viscosity and surface tension, reduces intermolecular interactions (solute-matrix), and weakens hydrogen bonds, resulting in a higher mass transfer rate and high solubility of polyphenols (Zhang et al. 2020). An increase in the temperature of PLE and SWE reduced the TPC and TFC, which can be attributed to the degradation of thermolabile compounds (Mariatti et al. 2021). High temperatures can affect the plant matrix (Maillard reaction) under severe temperature conditions, favoring the formation of unwanted products (Pagano et al. 2021; Plaza and Marina 2019). This shows that high-pressure techniques are more efficient at recovering TPCs and TFCs than traditional techniques.

3.4.3. Antioxidant capacity

The antioxidant capacity of the CNTS extracts was investigated using FRAP, DPPH, and ABTS assays. Figure 5 shows the values obtained from the CNTS extracts recovered by different methods.

FRAP results from the CNTS extracts ranged from 1.10 to 2.73 (mmol TE g⁻¹), where SWE2 and SWE3 provided higher values compared to other assays (Fig. 5a). The DPPH radical scavenging capacity of CNTS extracts obtained by PLE, SWE, MCE, and MCW ranged from 1.15 to 5.51 mmol TE g⁻¹ (Fig. 5b). SWE1, SWE2, and SWE3 provided statistically similar values (5.51, 5.46, and 5.23 mmol TE g⁻¹, respectively), which were different and higher than obtained by PLE2, MCE, and MCW (Fig. 5b).

The capture of ABTS radicals through electron transfer reaction showed that CNTS extracts obtained by SWE had high antioxidant potential, with values from 5.01 to 6.92 (mmol TE g⁻¹) (Fig. 5c), where the SWE3 sample exhibited the best antioxidant capacity. These values were significantly higher than obtained by PLE2 (5.72), MCE (5.43), and MCW (6.34 mmol TE g⁻¹) ($p < 0.05$). Nevertheless, ABTS results (Fig. 5c) show the importance of CNTS in terms of the antioxidant potential. Sorita et al. (2022) recovered phenolic compounds from the peanut (skin) and observed that SWE (water, 10 MPa, 160°C) provided higher ABTS value (0.36 mmol TE g⁻¹) than PLE (ethanol, 10 MPa, 80°C), SOX (ethanol), and SOX (water). These results showed that CNTS has higher antioxidant capacity than peanut skin, although they are from the same family of raw material.

In general, SWE samples showed higher antioxidant potential compared to PLE samples. This may be related to the water polarity, high temperature, and composition of the CNTS. In addition, high antioxidant capacity of SWE samples can be attributed to the polysaccharides recovery (Ballesteros et al. 2017). Differences in the data obtained are due to the extraction technique and origin of the raw material, seasonality, and interference with the characteristics of the compounds in the raw material (Schiassi et al. 2018). These results show that high-pressure techniques are viable alternatives for recovering compounds with antioxidant capacity from the CNTS.

3.4.4. Reducing and total sugars

Reducing (RS) and total (TS) sugars were evaluated for the extracts obtained by high-pressure (PLE and SWE) and low-pressure (MCE and MCW) methods (Fig. 6). The RS and TS concentrations ranged from 114.80 to 327.37 and 222.28 to 421.38 (mg GLU g⁻¹), respectively.

Higher RS value was provided by MCW (327.27 mg GLU g⁻¹), with significant difference to PLE, SWE, and MCE ($p < 0.05$) (Fig. 6a). It has been reported that higher temperatures result in longer reaction times, favoring hydrolysis at aqueous medium (Nanda et al. 2016). An increase in H⁺ concentration of in subcritical water facilitates the breaking of glycosidic bonds leading to the hydrolysis (Zhu et al. 2011), with breaking of cellulose, hemicelluloses, and lignin, converting in simple sugars (monosaccharides) (Heidari et al. 2019). The decrease in the RS (SWE5 conducted at 160°C) could be attributed to monosaccharides degradation at high temperatures, resulting in Maillard reaction (Chamika et al. 2021). In addition, SWE at high temperatures increases the solubility of polysaccharides such as sugar in water (polar solvent), compared to ethanol (solvent with moderate polarity).

SWE4, SWE5, and MCW showed high TS values (411.10, 421.38, and 413.30 mg GLU g⁻¹, respectively), which were significantly different from PLE and MCE ($p < 0.05$) (Fig. 6b). The TS values increased with SWE temperature due to polysaccharides depolymerization by selective hydrolysis of glycosidic bonds, increasing the soluble sugars content (Zhang et al. 2019). Overall, the sugars present in CNTS still need to be explored. No studies in the literature have quantified these components in CNTS. Therefore, the RS and TS values provide important data on sugar recovery from CNTS by high and low-pressure extraction.

3.4.5. Protein content

The total protein content was evaluated from the samples recovered by SWE, PLE, MCE, and MCW, with values from 3.05 to 5.60 (g 100 g⁻¹), as shown in Fig. 7.

The best protein recovery was by PLE2, SWE3, and SWE4 (values of 5.19, 5.47, and 5.60 g 100 g⁻¹ proteins, respectively), with significantly equal values ($p < 0.05$) (Fig. 7). Moderate extraction conditions prevented protein degradation. Increasing the extraction temperature can cause protein denaturation, reducing its recovery (Mannu et al. 2021). This is elucidated by the increase in protein hydrophobic interactions with temperature, although, at very high temperatures, the hydrophobic interaction between the surface of the protein molecules and the amino acids is weakened (Iqbal et al. 2016).

3.5 Sequential extraction process integration

The effect of CNTS defatting pretreatment by SFE or SOX was evaluated for the extracts recovered as a second step by high-pressure (PLE and SWE) and low-pressure (MCE and MCW) methods. The analysis was conducted in terms of the recovery of polyphenols (TPC and TFC), antioxidant capacities (FRAP, DPPH, and ABTS), sugar contents (RS and TS), and proteins. The high-pressure sequential extractions were conducted as: SFE2-PLE2; SFE2-SWE3 and SFE2-PLE2-SWE3, based on the individual extraction conditions that recovered higher concentrations of the non-polar (fatty acids) and polar (polyphenols, antioxidants, and sugars) fractions. The low-pressure sequential methods were conducted as: SOX-MCE; SOX-MCW and SOX-MCE-MCW, with results presented at Fig. 8.

The last steps of each sequential assays were compared with the isolated assays (conducted at the conditions selected for the integration process), i.e., the individual extractions of PLE2, SWE3, MCE, and MCW.

The extraction yield of the polar fraction was not affected by solid defatting (at high- or low- pressure) ($p < 0.05$), showing that defatted material has no significant influence on the yield of SFE-PLE2 (compared to only PLE2), and of SOX-MCE (compared to only MCE), respectively. The yield of the last step of route SFE2-PLE2-SWE3 decreased, compared to SWE3 and to SFE2-SWE3. It means that previous extractions in combined routes already solubilized components from the raw material, which was also detected for the low-pressure route (SOX-MCE-MCW), as presented at Fig. 8a.

TPC and TFC of the polar samples were favored at two-step routes SFE2-PLE2, SFE2-SWE3, SOX-MCE, and SOX-MCW (Fig. 8b and 8c), where defatted samples were used, compared to the individual assays (PLE2, SWE3, MCE, and MCW, respectively). This behavior suggests that the CNTS lipid fraction may hindered the desorption of phenolic compounds (Viganó et al. 2016). Chandrasekara & Shahidi (2011a) evaluated the antioxidant potential of cashew phenolics in foods and biological model systems affected by roasting and observed TPC values of 656.2, 701.2, and 790.9 (mg GAE g⁻¹) for raw and roasted defatted CNTS at low and high temperatures, respectively. The values were close to those obtained by degreasing with SFE and SOX.

The behavior of antioxidant capacity (by FRAP, DPPH, and ABTS) was similar to the TPC and TFC values, suggesting that polyphenols are the main antioxidant components from CNTS (Fig. 8d, 8e, and 8f). Also, defatted samples affected positively the antioxidant capacity, compared to non-defatted samples. Correlation analysis between polyphenols (TPC and TFC) and antioxidant capacity (FRAP, DPPH, and ABTS) revealed positive and strong correlations between these compounds (Table S2). Sruthi et al. (2023) analyzed the profile of phenolic compounds in CNTS and evaluated their antioxidant properties; the antioxidant capacity of the phenolic fractions of CNTS was positively correlated with its polyphenol content. Therefore, polyphenols may be the dominant antioxidant component in CNTS. The high concentrations attributed to the antioxidant capacity of CNTS can be attributed to the high content of polyphenols (phenolic acids and flavonoids) (Trox et al. 2011). The antioxidant potential of phenolic compounds is attributed to the presence of one or more hydroxyl groups (-OH) within their aromatic ring structure (Moazzen et al. 2022). Though the antioxidant function of flavonoids is commonly tied to the existence of phenolic hydroxyl groups (-OH), the C-H bonds contribute to their antioxidant capacity. Furthermore, it has been shown that the preferred mechanism for radical scavenging by flavonoids is through hydrogen atom transfer (Vo et al. 2019). This difference may be related to the concentrations of phenolic acids (gallic, syringic, and p-coumaric) present in the CNTS (Trox et al. 2011). Additionally, (+)-catechin and (-)-epicatechin are two important flavonoids in CNTS (Chandrasekara and Shahidi 2011b). They are responsible for various biological activities, including antioxidant effects (Bernatova 2018). This correlation can be attributed to the large amounts of flavonoids in the CNTS. Several authors have identified a strong correlation between polyphenols and antioxidant capacity (Sorita et al. 2022; Sruthi et al. 2023).

No significant differences in the RS or TS contents were detected with sequential processes (SFE2-PLE2, SFE 2-SWE3, SOX-MCE, and SOX-MCE), compared to individual assays (PLE2, SWE3, MCE, and MCW) (Fig. 8g and 8h). Removing the lipid fraction does not affect the sugar content in the CNTS extract since the sugars have low solubility in nonpolar solvents. In the sequential steps SFE2-PLE2-SWE3 and SOX-MCE-MCW, the recoveries of these compounds decreased since some of these compounds were previously solubilized in two steps. Regarding the protein content, removing the nonpolar fraction prior to sequential extraction with water (SFE2-SWE3) provided greater protein recovery (Fig. 8i). In addition, it was still possible to recover the amounts of compounds retained in the solid material in sequential SFE2-PLE2-SWE3.

3.6. Chemical profile by UPLC-PDA-ESI-QDa analysis

The extracts obtained by high- and low-pressure assays (individual extractions), and by the sequential processes were evaluated by liquid chromatography using negative ionization mode, as it is adequate for phenolic substances. The major compounds detected at the samples were catechin and epicatechin, or their condensed units, attributed to procyanidins. Figure 9 shows the UV–Vis chromatograms at 280 nm of the analyzed extracts, with the mass spectrum data of the identified compounds shown in Fig. S3.

Peaks 2 (t_R - 5.58) and 4 (t_R - 6.22), with molecular ion $[M-H]^-$ at 289 m/z , were dominant in the CNTS extracts (Fig. S3b) and identified as catechin ($C_{15}H_{14}O_6$) and epicatechin ($C_{15}H_{14}O_6$), respectively. Detailed fragmentation patterns are shown in Figure S3a and S3d. Sandhu & Gu (2010) reported that catechin and epicatechin $[M-H]^-$ at 289 m/z exhibited fragmentation characteristics at 245, 205 and 203 m/z (cleavage of the A-flavan-3-ol ring). Sruthi et al. (2023) observed predominant peaks from CNTS extracts, with main ion $[M-H]^-$ at 289 m/z , were catechin and epicatechin monomers with molecular formula $C_{15}H_{14}O_6$. Trox et al. (2011) and Chandrasekara & Shahidi (2011b) also reported catechin and epicatechin as the main compounds from CNTS. Catechin has attributes such as potent antioxidant, anti-inflammatory potential Higdon & Frei (2003), and also antibacterial properties and contributes to cardiovascular protection (Kalender et al. 2002).

Peaks 1 (t_R -5.14), 3 (t_R -5.89), 5 (t_R -6.40), and 8 (t_R -7.8) (Fig. 9) of the CNTS spectrum showed molecular ion $[M-H]^-$ at m/z 577, 579, and 865. Detailed fragmentation patterns are shown in Fig. S3a, S3c, S3e, and S3h. The monomeric flavonoid unit at m/z 289 is the result of cleavage of the C-C bond between two catechin units or cleavage of the quinone methide (Sun et al. 2020). After comparing retention times and m/z values, peaks 1 and 3 were tentatively identified as procyanidin B2, while peaks 5 and 8 were tentatively identified as procyanidin B3 (G. S. da Silva et al. 2017; Rockenbach et al. 2012). Procyanidins have been receiving recognition for their diverse therapeutic properties, which include antitumor, antioxidant, anti-inflammatory, immune-modulatory, and hypoglycemic effects (Zineb et al. 2023). Peaks 6 (t_R -7.02) and 9 (t_R -10.63) had molecular ions at 579 m/z $[M-H]^-$ (Fig. S3f and S3i), and were reported as derived from procyanidin, according to fragmentation pattern reported by Sruthi et al. (2023). Peak 7 (t_R - 7.41) presented molecular ion at 441 m/z $[M-H]^-$ (fragmentation pattern at Fig. S3g), as reported by Sandhu & Gu (2010), and was identified as catechin gallate.

This analysis corroborates with Sruthi et al. (2023), which reported the main polyphenols from CNTS. Then, catechin, epicatechin, catechin gallate, and procyanidin dimers in the procyanidin trimer, identified by the presence of a three-ring structure, are the significant polyphenols from CNTS extract. Based on structural variations, flavonoids are subdivided in flavanols, flavanols, flavones, isoflavones, anthocyanidins, flavanones, and chalcones (Shen et al. 2022).

A heatmap was constructed for further analysis of phenolic compounds identified by UPLC-PDA-ESI-QDa in the CNTS extracts (Fig. 10).

We observed that darker tones indicate higher concentrations of phenolic compounds from the CNTS extracts. Defatted samples presented higher concentration in phenolic compounds, i.e., the sequential methods SFE2-PLE2 and SOX-MCE presented higher polyphenols concentration, compared to one step samples. Furthermore, the polyphenols were also detected, in small concentrations, from the water extracts at the third extraction steps (SFE2-PLE2-SWE3 and SOX-MCE-MCW). These results contribute to upcycling the cashew agroindustry because the identified polyphenols are valuable compounds for further applications in food, pharmaceutical and chemical industries.

4. Conclusions

The results obtained in this study show that high-pressure techniques provided satisfactory recovery of bioactive compounds compared to conventional techniques. SFE was more effective than Soxhlet method in fatty acids recovery. Also, PLE and SWE were comparable to MCE and MCW, respectively, for phenolics and antioxidant recovery. Furthermore, high-pressure processes are less solvent-, time-, and energy-consuming, compared to traditional methods. Sequential extractions contributed to the yield and the fractionation of the extractable components from CNTS, concentrating the phenolics in the polar fraction. Epicatechin, catechin and procyanidins were the major compounds from CNTS polar extracts, which contribute to value the recovered extracts. This novel study provided an in-depth recovery of bioactive compounds from CNTS, following a biorefinery approach with viable alternative high-pressure methods.

Declarations

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Author's contribution

Jonas da Silva: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Validation; Visualization; Writing - original draft; Writing - review & editing. **Talyta Mayara Silva Torres:** Data curation; Formal analysis; Software; Visualization; Writing - original draft; Writing - review & editing. **Paulo Riceli Vasconcelos Ribeiro:** Data curation; Formal analysis; Software; Visualization; Writing - original draft; Writing - review & editing. **Edy Sousa de Brito:** Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing. **Sandra Regina Salvador Ferreira:** Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

Declaration of competing interests

The authors declare no competing interests.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

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Figures

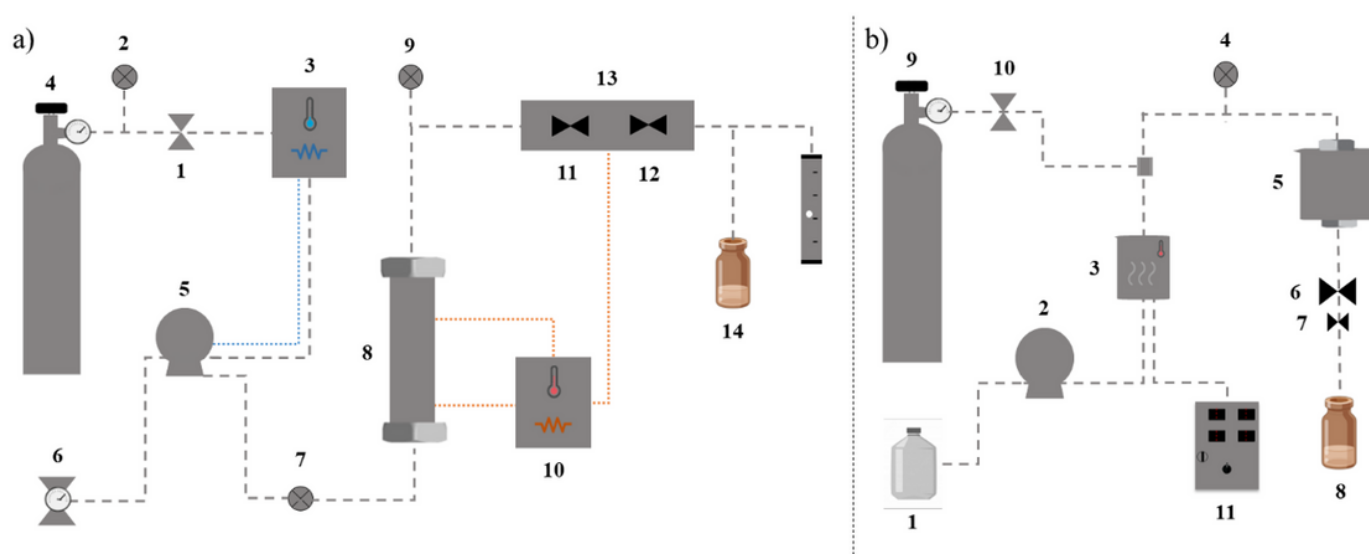


Figure 1

High pressure extraction scheme: a) SFE, b) PLE and/or SWE. **Source:** Adapted of Sorita et al. (2022)

*a) SFE - 1: CO₂ regulator valve; 2, 7, 9: Manometer; 3: Cooling bath; 4: CO₂ cylinder; 5: CO₂ pump; 6: Compressed air regulator; 8: Stainless steel extraction cell; 10, 13: Heating bath; 11, 12: Regulator needle valve; 14: Extract Reservoir; 15: Rotameter

*b) PLE and SWE - 1: Solvent reservoir; 2: Pump; 3: Heat exchanger; 4: Manometer; 5: Stainless steel extraction cell; 6, 10: Regulator valve; 7: Regulator needle valve; 8: Extract reservoir; 9: N2 cylinder,11: PID controller.

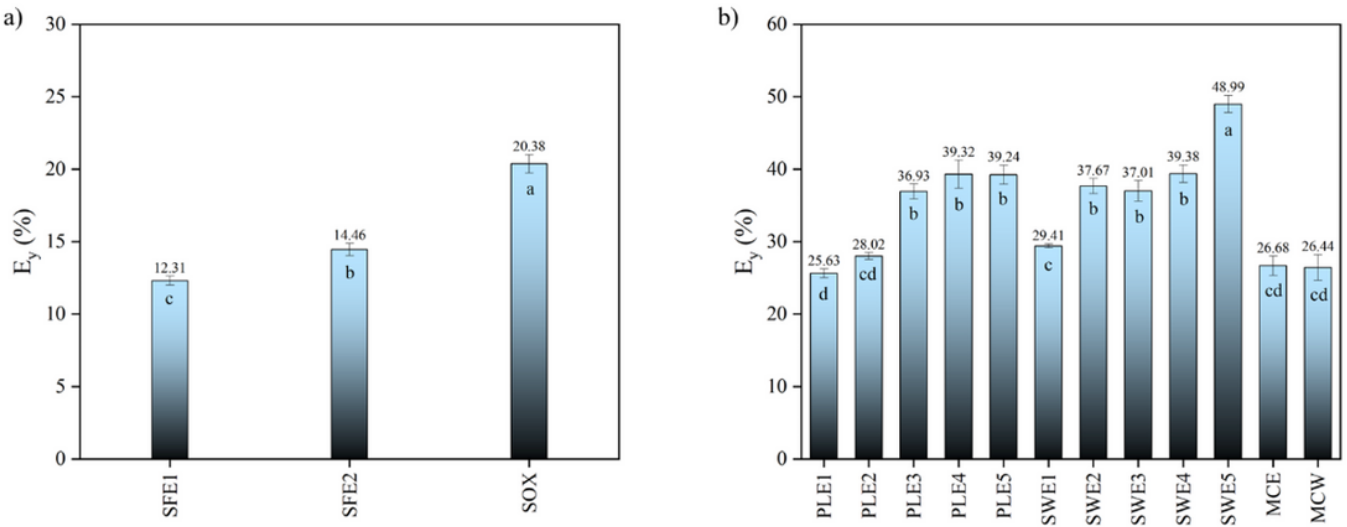


Figure 2

Extraction yield (E_y) obtained from CNTS using a) SFE and SOX (the oily fraction), and b) PLE, SWE, MCE, and MCW (the polar fractions).

*Samples identified with the same letter in the column did not present a significant difference ($p < 0.05$) in the Tukey test.

**High pressure: PLE-ethanol (PLE1: 40, PLE2: 60, PLE3: 80, PLE4: 100, and PLE5: 120 °C), and SWE-water (SWE1: 80, SWE2: 100, SWE3: 120, SWE4: 140 and SWE5: 160 °C); Low pressure: MCE-ethanol (MCE: 37°C), and MCW-water (MCW: 37°C).

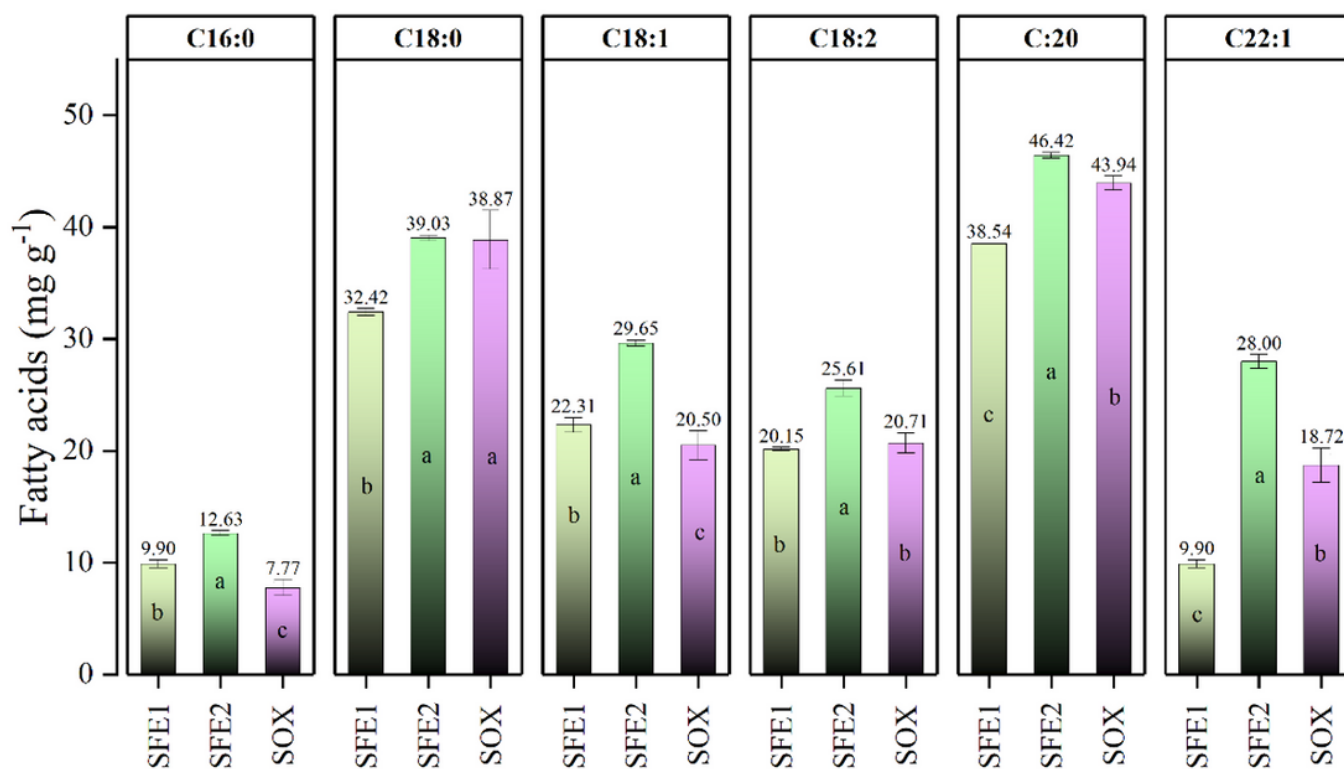


Figure 3

Fatty acids profile of CNTS oil extracted by SFE with CO₂ and SOX with n-hexane.

*Samples identified with the same letter in the group did not present a significant difference ($p < 0.05$) in the Tukey test.

**C16:0 - Palmitic acid, C18:0 Stearic acid, C18:1 - Oleic acid, C18:2 - Linolenic acid, C22:1 Behenic acid, C22:1 - Erucic acid.

***High pressure: SFE-CO₂ (SFE1: 15 and SFE2: 25 MPa), Low pressure: SOX-hexane.

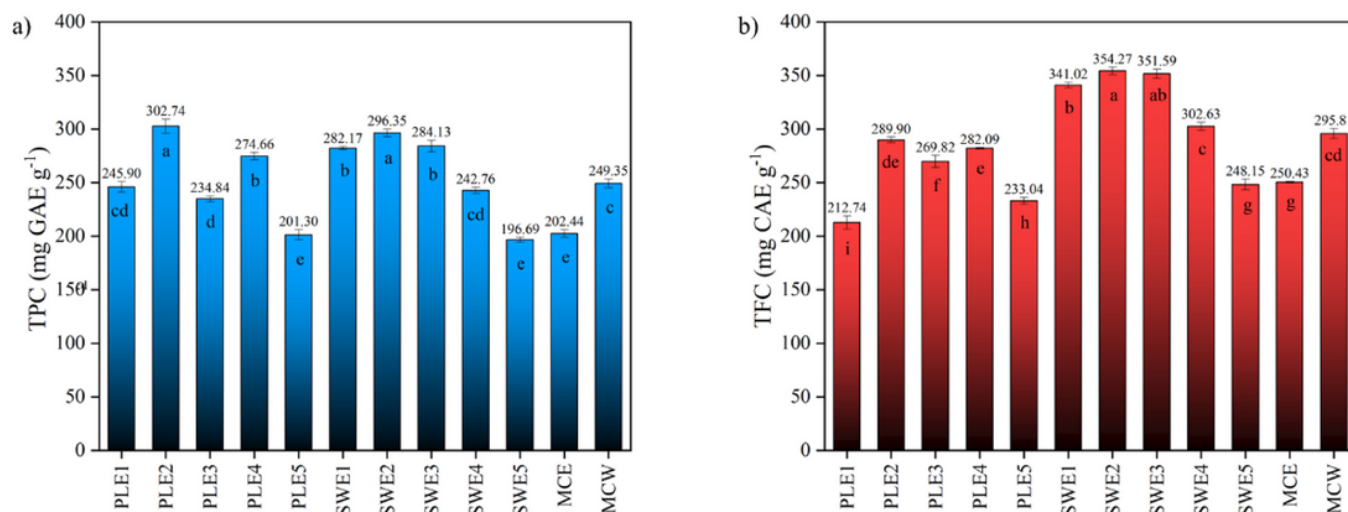


Figure 4

TPC (a) and TFC (b) of CNTS extracts obtained by PLE and MCE with ethanol, and SWE and MCW with water.

*Samples identified with the same letter in the column did not present a significant difference ($p < 0.05$) in the Tukey test.

**High pressure: PLE-ethanol (PLE1: 40, PLE2: 60, PLE3: 80, PLE4: 100, and PLE5: 120 °C), and SWE-water (SWE1: 80, SWE2: 100, SWE3: 120, SWE4: 140 and SWE5: 160 °C); Low pressure: MCE-ethanol (MCE: 37°C), and MCW-water (MCW: 37°C).

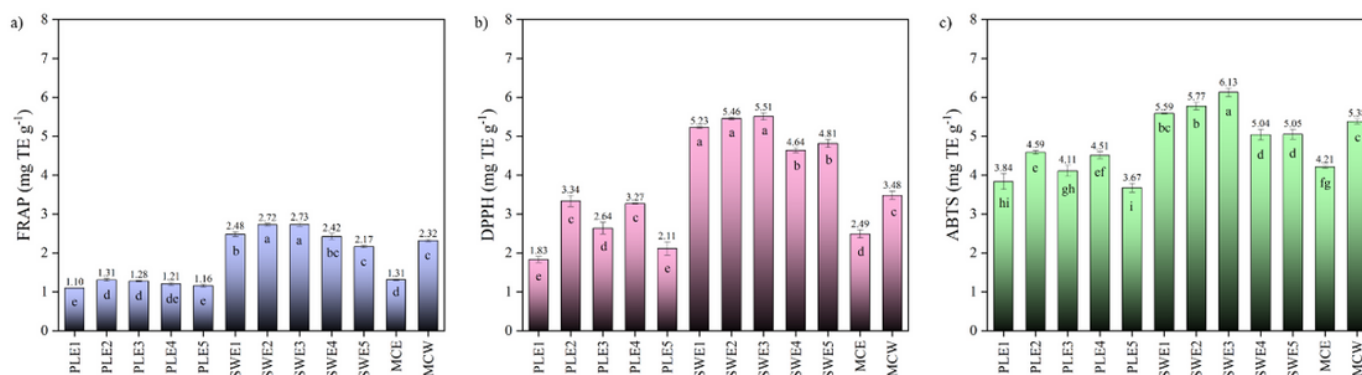


Figure 5

Antioxidant capacity by FRAP, DPPH, and ABTS assays for CNTS extracts obtained by PLE, SWE, MCE, and MCW.

*Samples identified with the same letter in the column did not present a significant difference ($p < 0.05$) in the Tukey test.

**High pressure: PLE-ethanol (PLE1: 40, PLE2: 60, PLE3: 80, PLE4: 100, and PLE5: 120 °C), and SWE-water (SWE1: 80, SWE2: 100, SWE3: 120, SWE4: 140 and SWE5: 160 °C); Low pressure: MCE-ethanol (MCE: 37°C), and MCW-water (MCW: 37°C).

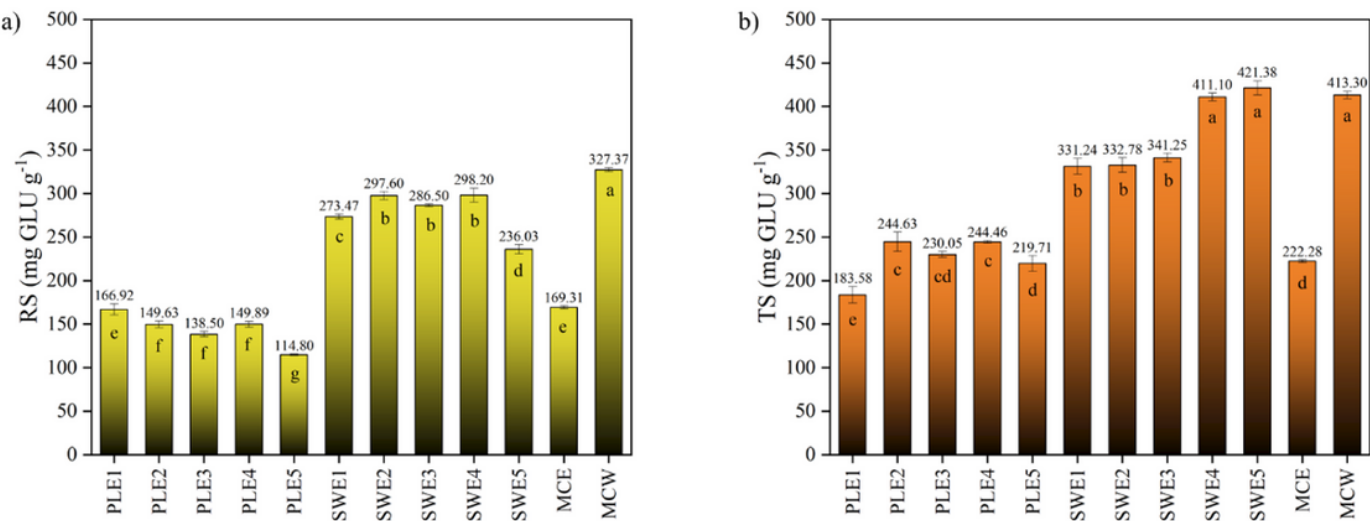


Figure 6

RS (a) and TS (b) sugars for CNTS extracts obtained by PLE, SWE, MCE, and MCW.

*Samples identified with the same letter in the column did not present a significant difference ($p < 0.05$) in the Tukey test.

**High pressure: PLE-ethanol (PLE1: 40, PLE2: 60, PLE3: 80, PLE4: 100, and PLE5: 120 °C), and SWE-water (SWE1: 80, SWE2: 100, SWE3: 120, SWE4: 140 and SWE5: 160 °C); Low pressure: MCE-ethanol (MCE: 37°C), and MCW-water (MCW: 37°C).

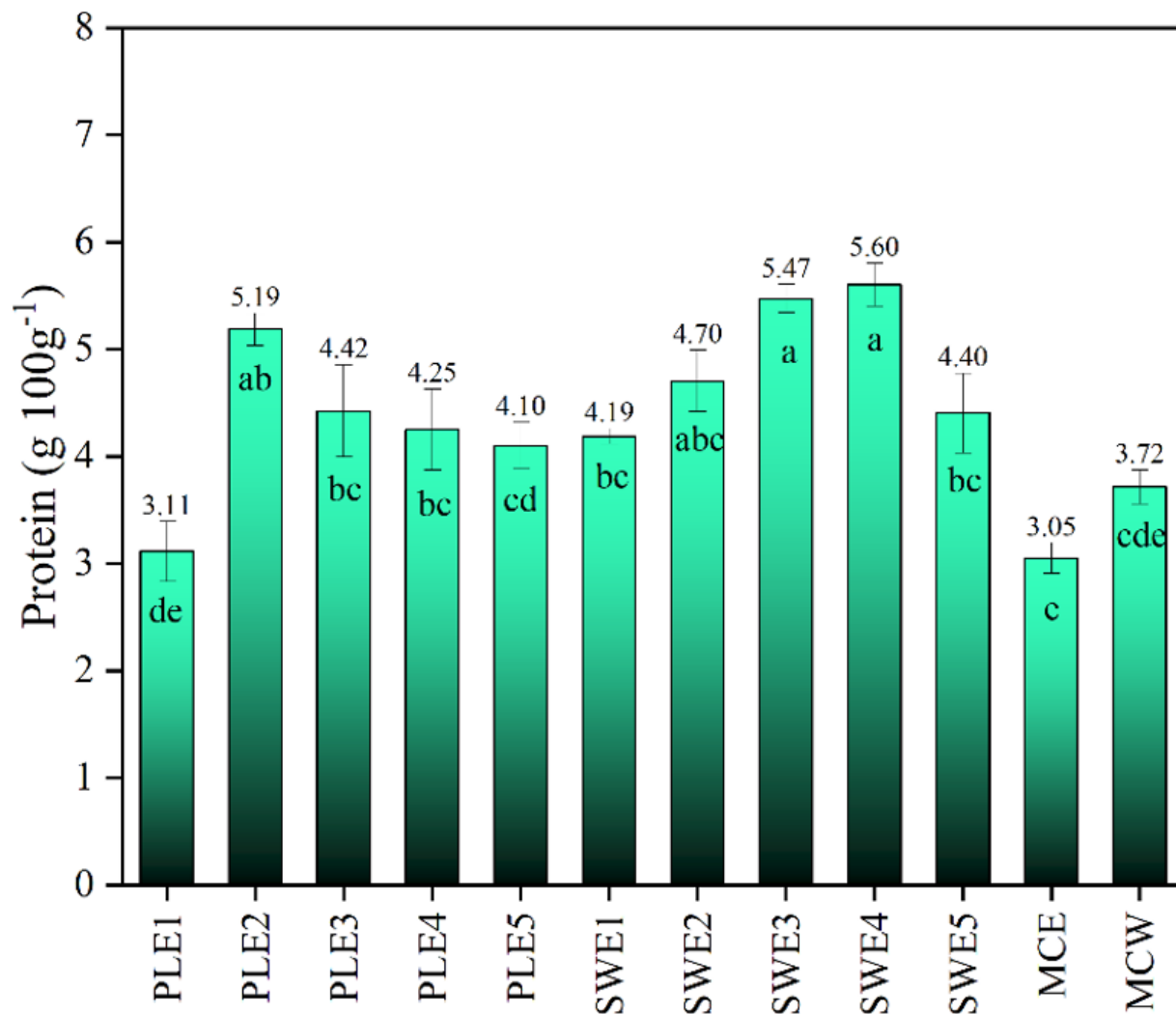


Figure 7

Protein recovery for CNTS extracts obtained by PLE, SWE, MCE, and MCW.

*Samples identified with the same letter in the column did not present a significant difference ($p < 0.05$) in the Tukey test.

**High pressure: PLE-ethanol (PLE1: 40, PLE2: 60, PLE3: 80, PLE4: 100, and PLE5: 120 °C), and SWE-water (SWE1: 80, SWE2: 100, SWE3: 120, SWE4: 140 and SWE5: 160 °C); Low pressure: MCE-ethanol (MCE: 37°C), and MCW-water (MCW: 37°C).

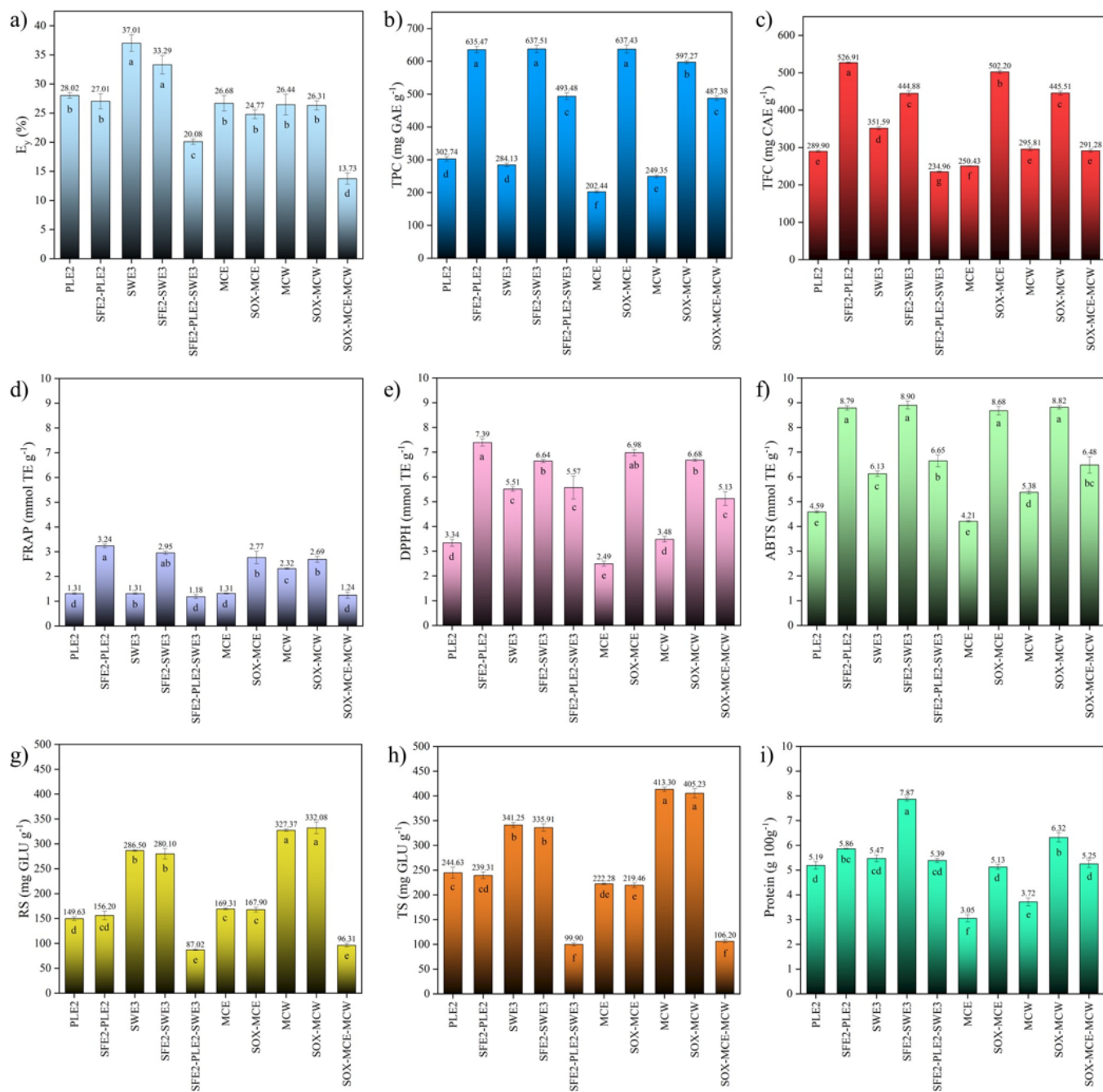


Figure 8

Effect of sequential integration of high-pressure and low-pressure extraction methods on: a) yield (E_y), polyphenols b) TPC and c) TFC, antioxidant capacity d) FRAP, e) DPPH, and f) ABTS, sugars g) RS and h) TS, and i) protein.

*Samples identified with the same letter in the column did not present a significant difference ($p < 0.05$) in the Tukey test.

**High pressure: SFE2 (CO_2 , 25MPa), PLE2 (ethanol, 60 °C), and SWE3 (water, 120 °C). Low pressure: SOX (hexane, 60 °C), MCE (ethanol, 37°C), and MCW (water, 37°C).

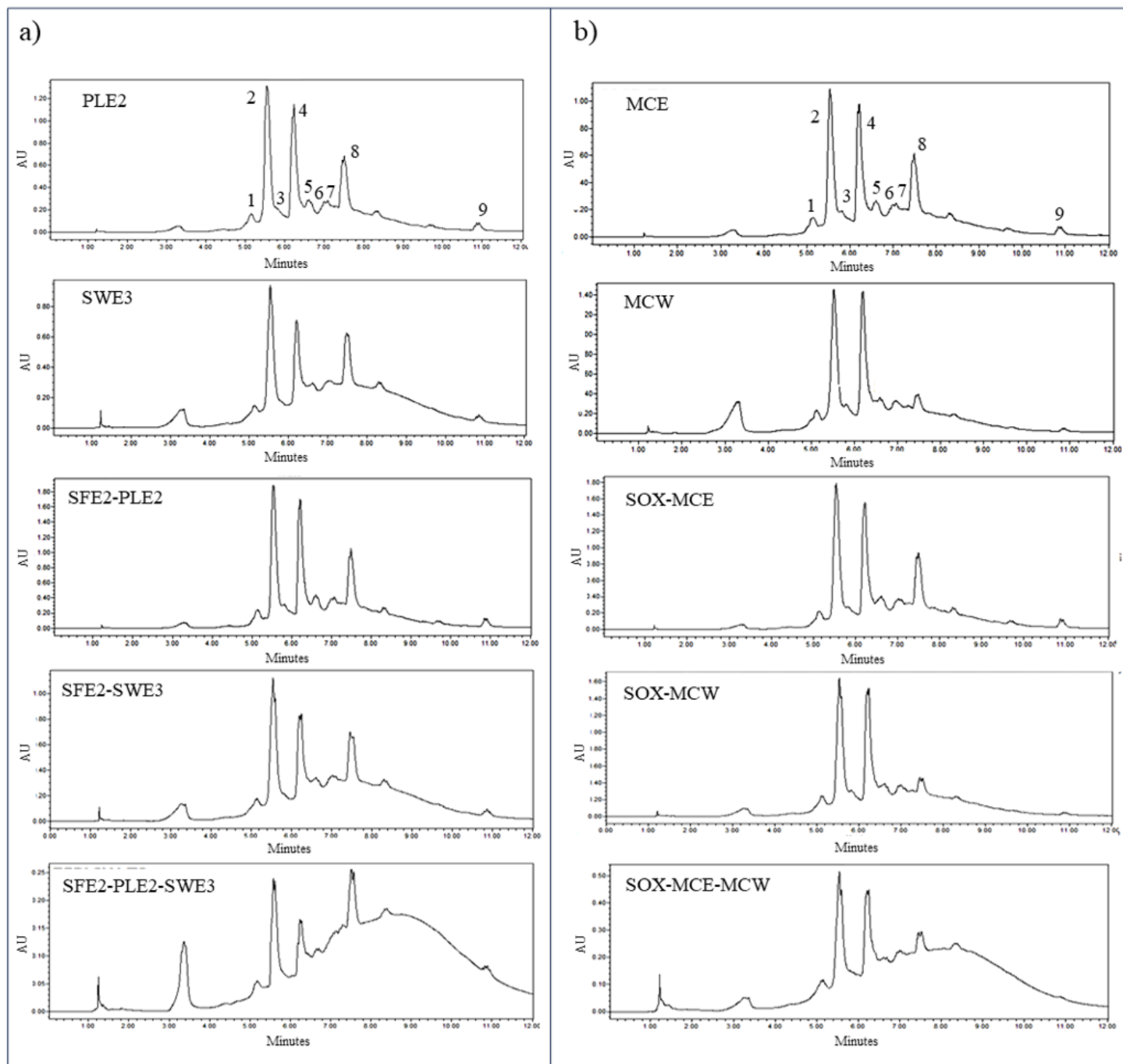


Figure 9

Uv-Vis chromatogram at 280 nm of extracts obtained by optimal conditions and integration of the sequential process by a) high-pressure and b) low-pressure analyzed by UPLC-PDA-ESI-QDa.

*High pressure: SFE2 (CO₂, 25MPa), PLE2 (ethanol, 60 °C), and SWE3 (water, 120 °C). Low pressure: SOX (hexane, 60 °C), MCE (ethanol, 37°C), and MCW (water, 37°C).

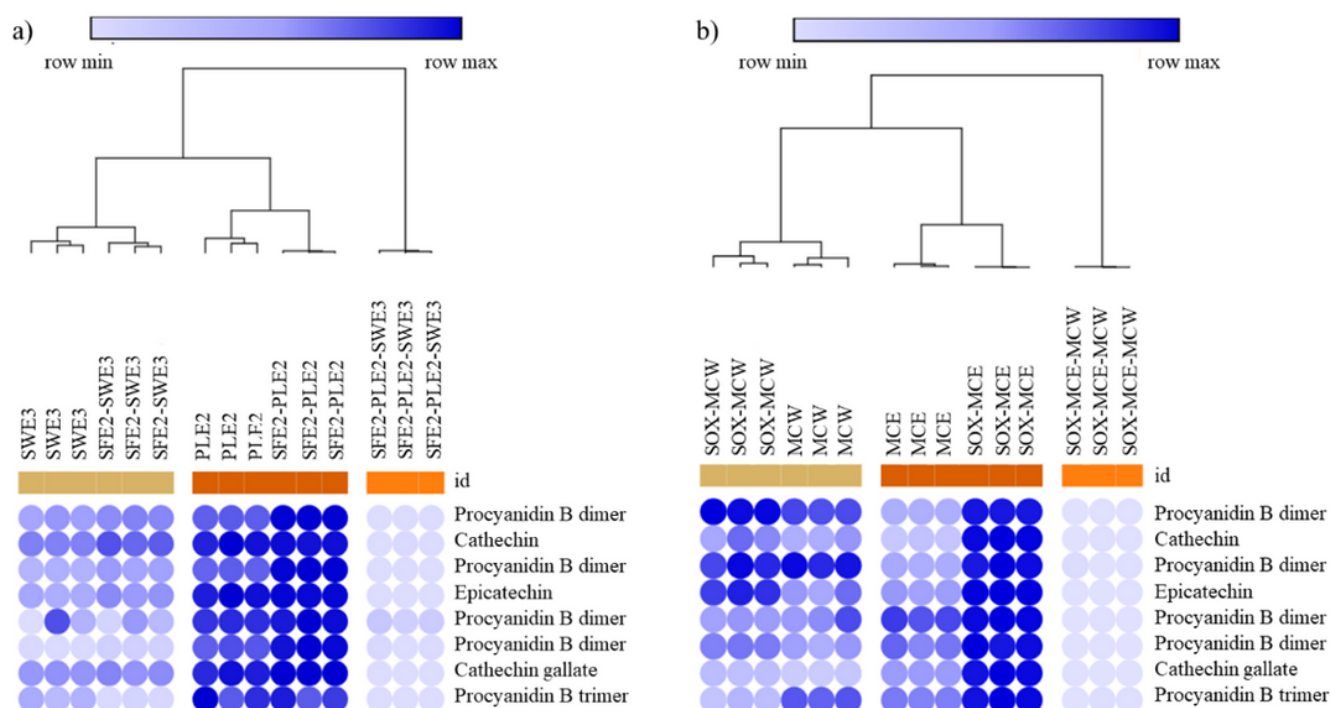


Figure 10

Heatmap of the extracts obtained by extraction and sequential integration by a) high-pressure and b) low-pressure.

*High pressure: SFE2 (CO₂, 25MPa), PLE2 (ethanol, 60 °C), and SWE3 (water, 120 °C). Low pressure: SOX (hexane, 60 °C), MCE (ethanol, 37°C), and MCW (water, 37°C).

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