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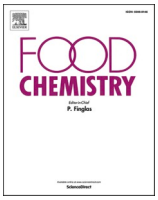

Characterization of a novel natural protein-polysaccharide complex from cashew apple bagasse and its functional implications

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ABSTRACT

Cashew apple bagasse (CAB) constituting about 20 % of the cashew apple's (CA) weight, is often overlooked and considered a waste product. This study aims to valorize CAB by extracting and studying a nutritional and functional compounds from CAB, particularly proteins. Response surface methodology (RSM) design and ultrasound-assisted extraction (UAE) are employed to optimize a protein-enriched fraction extraction process. Analysis of CAB-Protein-Pellet composition reveals that its main constituents are sugars (42.49 %) and proteins (22.10 %). HPSEC analysis confirmed the existence of a new natural protein-polysaccharide complex (PPC), an high level of Ara (11.85 g/100 g) and Gal (17.45 g/100 g) indicating the presence of polysaccharides rich in arabinose and galactose (PRAG) with the main class of polymers in the CAB-PPC being AGPs. MIR-FTIR and ^{1}H NMR spectra allowed new insights into the structural features of the PPC derived from CA. The effects of protein-polysaccharide interactions within CAB-PPC on structure and functionality were investigated, revealing interesting functional properties and their correlation relationship. The findings highlight some similarities between CAB-PPC and gum Arabic with minor differences. The interfacial tension of CAB-PPC (21.32 mN/m) was lower than that of gum Arabic (23.71 mN/m). Therefore, CAB-PPC could be suitable for a range of food applications including thickening, stabilization, gelling, water retention, emulsification, and foaming.

1. Introduction

The cashew tree (*Anacardium occidentale* L.) is an important tropical tree that yields a fruit with two edible components: the cashew nut and the cashew apple (CA), an enlarged peduncle. Presently, the cultivation of this tree primarily focuses on the cashew nut. CA is approximately 9–10 times the weight of the nut. Unfortunately, the CA is often overlooked and considered a waste product in many cases (Tamiello-Rosa, Cantu-Jungles, Iacomini, & Cordeiro, 2019).

The primary product derived from the CA is its juice (Pinho, Afonso, Carioca, da Costa, & Ramos, 2011). The by-product generated during the juice extraction process, referred to as CA bagasse (CAB) is typically undervalued. It constitutes about 20 % of the CA's weight, presenting a significant volume of material, nearly 9.2 million tons of CAB according to Zié, Alabi, Karamoko, and Blecker (2023), that could have substantial potential for valorization. CAB has been identified as a source of ascorbic acid, antioxidants; carbohydrate (Fonteles et al., 2016; Patra, Abdullah, & Pradhan, 2021; Silva, Mendes, Correia, Rocha, & Micoli, 2018) along with proteins ranging from 9 % to 16 %, offering potential for valorization (Patra, Abdullah, & Pradhan, 2022).

In a recent review article by Zié et al. (2023), the focus is on the valorization of CAB, particularly exploring the extraction and utilization of nutritional or bioactive compounds. Considering the current trend in the protein industry, which actively seeks alternative and underutilized plant sources (Hadnadjev et al., 2017), exploring the potential of vegetable and fruit wastes like CAB could become economically interesting for obtaining vegetable protein fractions (Wani et al., 2006; Firatligil-Durmus & Evranuz, 2010; Horax, Hettiarachchy, Kannan, & Chen, 2011). Indeed, CA is one of the major solid wastes generated by cashew nut producers and CA juice industries in several countries. The

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valorization of CA waste into valuable products, such as protein-enriched materials, offers considerable potential for application. Proteins are appreciated in the food industry for their functional properties (emulsification, gelation, foaming, etc.) and nutritional values (Akasha, Campbell, & Euston, 2012; Zhao et al., 2021). Therefore, protein extract can be used as a functional additive and flavor enhancer in various food formulations such as candies, sauces, bakeries, etc. This approach would not only create more income for farmers and food processors but also contribute to sustainable waste management practices (Zié et al., 2023).

Therefore, the extraction of a protein fraction from CAB and the subsequent study of its physicochemical properties are highly relevant to reduce food losses and obtain new compounds and products. Actually, few studies related to the extraction of protein from CAB are present in literature (Abas Wani, Sogi, Grover, & Saxena, 2006; Patra et al., 2022; Zié et al., 2023). Moreover, no study based on the physicochemical characterization of CAB protein extract has been investigated until now. The findings from this study could provide scientific fundaments on the extraction process of CAB proteins, on their composition and on their characteristics to evaluate a potential suitability for future use in food applications.

Conventional extraction methods pose challenges for fruits like CA, as their bioactive compounds are deeply embedded within the innermost layers of their cell walls (Da Rocha & Noreña, 2020). Consequently, researchers are now focusing on employing innovative technologies, such as ultrasound and microwaves, to extract bioactive compounds from CAB. These methods offer higher yields while requiring less solvent, energy, and time (Zié et al., 2023). Furthermore, these emerging technologies are regarded as alternatives to conventional extraction methods due to their cost-effectiveness and high extraction efficiency, while preserving thermolabile bioactive compounds from degradation (Fonteles et al., 2017). The utilization of novel technologies for plant-based protein extraction are found to increase the protein yields by preserving their functional as well as nutritional values. Ultrasound-Assisted Extraction (UAE) is a versatile, non-thermal, and cost-effective method, known for its ability to recover maximum compounds in a shorter time while preserving the quality of the final product. In UAE, the rapid formation and collapse of gas bubbles generated by ultrasonic waves on the cell surface induce microstreaming and shockwaves, which exert intense shear and mechanical forces. This leads to the disruption of cell membranes and walls, allowing the surrounding solvent to effectively penetrate the cells through the resulting fissures, releasing intracellular proteins into the solvent (Kumar et al., 2021). Moreover, using green technologies such as UAE to extract proteins from cashew apple by-products (CAB) will benefit industries by enabling the development of value-added food products (Zié et al., 2023).

However, optimizing the extraction process through the traditional "one factor at a time" approach is time-consuming and labor-intensive. To address this, various empirical methods based on statistical techniques or artificial intelligence are employed (Patra et al., 2022). Response Surface Methodology (RSM) is a statistical toolset used to design experiments, develop predictive models, assess factor interactions, and determine optimal conditions for the extraction process (Desai, Survase, Saudagar, Lele, & Singhal, 2008).

In this study, response surface methodology (RSM) design and ultrasound-assisted extraction (UAE) were employed to obtain a protein-enriched CAB extract. The chemical composition, structural analysis (FT-IR and ^{1}H NMR), surface/interfacial properties, zeta potential, and thermal behavior of this fraction were subsequently investigated.

2. Materials and methods

2.1. Materials

Yellow (50 %) and red (50 %) cashew apples (*Anacardium occidentale* L.) harvested at the commercial maturity stage in a cultivated field in the north of Côte d'Ivoire (Korhogo) were used as the raw material. After manually removing the nuts, CA were washed with distilled water at room temperature, and the juice was extracted by pressing (using a hydraulic screw press). The obtained bagasse was then packaged and stored at −20 °C. All reagents used in this study were of analytical grade and were purchased from Merck and VWR.

2.2. Sample preparation

Bagasse samples were freeze-dried (Christ Gamma 2–16 LSC plus, Martin Christ, Germany) for 72 h. The dried samples were then ground (Fritsch, ROHS, Oberstein, Germany) and sieved through a mesh with a diameter less than 750 μm. The obtained powder was stored at −20 °C for further analysis.

2.3. CAB characterization

CAB analysis included determination of moisture, protein, lipids, ash, starch, soluble and insoluble dietary fibers, cellulose, hemicellulose, lignin, free sugar, and pectin contents. All experiments were carried out in triplicate.

The moisture (by gravimetry) and ash content of the CAB was determined according to Cruz Reina et al. (2022). Crude protein was determined using the Dumas combustion method (rapid-N cube Elementar analyzer system, Villeurbanne, France) with a conversion factor of 6.25. The Soxtherm method (Type S306 AK/S306 A, Gerhardt, Konigswinter, Germany) was employed to determine total lipid content using petroleum ether as the extraction solvent. The determination of native starch content was conducted using the Ewers polarimetric method as per the standard "ISO 10520 – First edition: 1997-09-01". Insoluble and soluble dietary fibers were determined according to AOAC 991.43 method (AACC 32–07). The contents of lignin, hemicellulose, and cellulose were determined using Fibertec (FT 121 Fibertec, FOSS) according to Van Soest and Robertson (1979) method. Free sugar was determined by dissolving 500 mg of the sample in 10 mL of water and stirring for 30 min at 50 °C. Derivatization to alditol acetate was performed before sample injection on an Agilent GC-FID. The average monosaccharide percentage is expressed relative to the initial dried sample. Pectin analysis was conducted by quantifying the major constituent sugars, namely rhamnose and galacturonic acid. 150 mg of CAB powder was hydrolyzed with 7 mL of 2 mol.L^{-1} trifluoroacetic acid at 105 °C for 6 h. The solution was neutralized with NaOH and injected in a Dionex HPAED-PAD, ICS5000.

2.4. Experimental design

Many main factors, often not independent, influence the solubilization of proteins that are the subject of an experimental approach based on the establishment of experimental designs. Among the most important, we can mention the solid/solvent ratio, the particle size, the stirring mode, the temperature, the time, the pH, the ionic strength, etc. Alkali extraction is the most commonly used conventional method for plant-based protein extraction. Based on this, RSM (design expert software version 10) was used to determine the influence of four independent variables and optimum conditions for CAB protein extraction. A range of independent variable was chosen depending on the combined effects of temperature, extraction pH, solvent/CAB ratio and extraction time on the yield and techno-functional properties of plant protein isolates studied in the literature (Sajib, Forghani, Kumar Vate, & Abdollahi, 2023) as well as preliminary tests carried out on CAB protein extraction.

Independent variable used for performing the extraction process were temperature (x_1) (25–45 °C), extraction pH (x_2) (8–10), solvent/CAB ratio (x_3) (10:1 to 30:1 *v/w*) and extraction time (x_4) (1–5 h). Each variable was coded at five levels = −2, −1, 0, 1, 2 (Table 1) according to Eq. 1.

Table 1
Variables and levels for central composite design.

Variable	Symbol	Coded variables levels				
		−2	−1	0	1	2
Temperature (°C)	x_1	25	30	35	40	45
Extraction pH	x_2	8	9	10	11	12
Extraction Time (h)	x_3	1	2	3	4	5
Ratio solvent/CAB (*v/w*)	x_4	10:1	15:1	20:1	25:1	30:1

$$Xi = (xi - \overline{xi})/\Delta xi \quad (1)$$

where X_i is the dimensionless value of an independent variable, x_i is the real value of an independent variable, $\bar{x}_i$ is the real value of an independent variable at the center point, Δx_i is the step change. The levels and values of independent variables are represented in Table 1 with their coded forms. The central composite design using design expert software *v*ersion 10 was used to find the experimental runs number and the combination of independent variables. The experiment contained 27 runs with three center points which are presented in Table 2. The investigated responses function was Y_1 = g of soluble protein from extract/100 g CAB and Y_2 = Protein purity percentage. The influence of independent variables on the responses was mathematically modelled using RSM. All experiments were carried out in a randomized order to minimize any effect of extraneous factors on the observed responses. Each experiment was conducted in triplicate, and the average value of responses was used for analysis after every experimental run.

2.5. Protein extraction process

According to the experimental design (Table 2), the solvent was mixed in different ratios with 3 g of CAB powder to investigate its effect on the extraction process. The mixture was then placed in a water bath with stirring (GFL 1083) at the desired temperature. The pH was adjusted using 6 mol.L^{-1} and 1 mol.L^{-1} HCl solutions or 6 mol.L^{-1} and 1 mol.L^{-1} NaOH solutions. The resulting slurry was centrifuged (Beckman Coulter Avanti J-E Centrifuge) at 11000*g* for 20 min. After centrifugation, the supernatant was collected and freeze-dried under the same conditions as the raw material. Soluble protein content was determined by the Dumas combustion method using a conversion factor of 6.25. All experiments were conducted in triplicate.

Table 2
Central composite design arrangement.

Run	Coded variables*				Uncoded variables			
	X_1	X_2	X_3	X_4	x_1	x_2	x_3	x_4
1	0	0	0	0	35	10	3	20:1
2	0	0	0	0	35	10	3	20:1
3	−1	−1	−1	1	30	9	2	25:1
4	2	0	0	0	45	10	3	20:1
5	0	0	0	0	35	10	3	20:1
6	0	0	0	2	35	10	3	30:1
7	1	1	1	1	40	11	4	25:1
8	1	−1	1	1	40	9	4	25:1
9	−1	1	1	1	30	11	4	25:1
10	1	−1	1	−1	40	9	4	15:1
11	0	0	0	−2	35	10	3	10:1
12	1	1	−1	1	40	11	2	25:1
13	−1	1	−1	−1	30	11	2	15:1
14	1	−1	−1	1	40	9	2	25:1
15	−1	−1	1	1	30	9	4	25:1
16	1	−1	−1	−1	40	9	2	15:1
17	1	1	−1	−1	40	11	2	15:1
18	−1	−1	−1	−1	30	9	2	15:1
19	−1	1	−1	1	30	11	2	25:1
20	−1	1	1	−1	30	11	4	15:1
21	−1	−1	1	−1	30	9	4	15:1
22	0	2	0	0	35	12	3	20:1
23	1	1	1	−1	40	11	4	15:1
24	0	−2	0	0	35	8	3	20:1
25	0	0	−2	0	35	10	1	20:1
26	0	0	2	0	35	10	5	20:1
27	−2	0	0	0	25	10	3	20:1

X_1: temperature; X_2: extraction pH; X_3: Extraction time; X_4: Solvent/CAB ratio.

2.6. Ultrasound-assisted extraction

2.6.1. Solubilization step

High-intensity ultrasonication (20 kHz, CPX 750, Cole 115 PA Parmer Instruments, Illinois, USA) was employed to enhance CAB protein extraction under optimal conditions obtained from the experimental design (temperature, pH, solvent ratio) by adjusting 39.26 °C to 40 °C and pH 10.99 to 11. The ultrasonic conditions were applied at a frequency of 20 kHz, a power of 700 W according to (Patra et al., 2022), an amplitude of 40 %, stirring speed of 250 rpm, and an extraction time of 30 min. The ultrasonicated whole sample was then centrifuged (Beckman Coulter Avanti J-E Centrifuge) at 11000 *g* for 20 min. After centrifugation, the supernatant was collected, freeze-dried and the soluble protein content was determined by the Dumas combustion method using a same method at 2.5.

2.6.2. Precipitation step

For the precipitation step, we only used the ultrasonic method since the soluble protein content was high. The objective was the optimization of the protein content; however, we followed the same extraction process (see Fig. 1). To summarize, for the optimization of the second step of the extraction, the pH of the supernatant obtained after centrifugation was adjusted to precipitate the protein, with optimization conducted at four pH levels (1.5, 2.5, 3, 3.5, and 4). The protein precipitate was washed twice using de-ionized water at the precipitation pH, followed by centrifugation. The protein was re-solubilized by adjusting the pH to 7.0 using 1 mol.L^{-1} NaOH, freeze-dried, and stored at −20 °C until used for analysis (Fig. 1). All experiments were carried out in triplicate.

The obtained protein yield was calculated using the following formula:

Protein yield = Extracted protein (g)/ Total protein in the initial sample used (g) *100.

The purity (%) of protein isolates was determined as the percentage of protein in the extracted protein isolates.

2.7. CAB protein pellet (CAB-PP) analysis

2.7.1. Structural characterization

2.7.1.1. HPSEC analysis. Structural characterization was performed using HPSEC analysis. CAB-PP was dissolved in distilled water (3.5 mg/mL). After passing through a 0.45 μm filter, 100 μL of the samples were injected into an HPSEC (Shimadzu Nexera XR) coupled with refractive index (RI) and ultraviolet (UV) detectors. Separation was achieved using a TSK Gel 2500 PWXL column (300*7.8 mm; 6 μm) at 30 °C, with a mobile phase of $NaNO_3$ (0.05 mol.L^{-1}) at a flow rate of 0.7 mL/min. Molecular weight (MW) distribution of the fractions was estimated using dextran standards (12,000, 25,000, 50,000 and 150,000 Da).

2.7.1.2. Mid-infrared (MIR) Fourier transform infrared spectroscopy (FT-IR) analysis. CAB-PP structural changes were monitored using Mid-Infrared (MIR) Fourier Transform Infrared Spectroscopy (FTIR) on an IRTracer-100 spectrometer (Shimadzu, Duisburg, Germany) equipped with an attenuated total reflection (ATR) accessory. The wavenumber of the analysis ranged from 700 to 4000 cm^{-1} using a resolution of 4 cm^{-1} with an accumulation of 40 scans at 20 °C.

2.7.1.3. Nuclear magnetic resonance (NMR) spectroscopy. NMR spectra of CAB-PP for ^{1}H was obtained using a magritek Spinsolve Benchtop 43

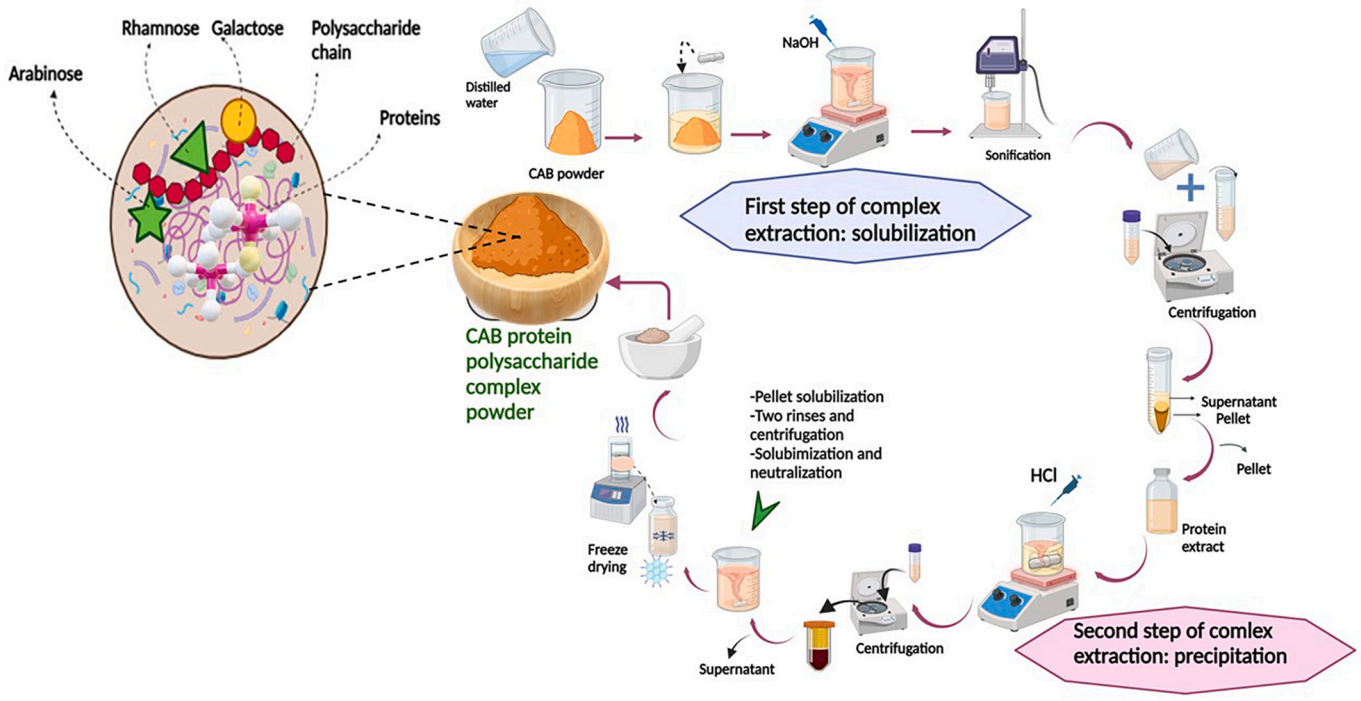

Fig. 1. Cashew apple bagasse protein-polysaccharide complex extraction process, using ultrasound.

MHz spectrometer (Magritek, Wellington, New Zealand). Dried CAB-PP (15 mg) were dissolved in deuterium oxide (1 mL *w/v*) at room temperature and allowed to stand for 3 h before NMR analysis.

2.7.2. Physicochemical and macromolecular characteristics of CAB-PP

Pectin, protein, moisture and ash were determined as described above with the raw material (Cruz Reina et al., 2022).

2.7.2.1. Polysaccharide quantification using gas chromatography. Quantification of the polysaccharide was carried out by acid hydrolysis of the obtained pellet. 50 mg of CAB-PP powder was mixed with 3 mL of H_2SO_4 (1 mol.L^{-1}) at 100 °C for 3 h. The released monosaccharides content after degradation was determined by GC analysis (Agilent GC FID) preceded by neutralization and derivatization in alditol acetate.

For the determination of galacturonic acid, the Megazyme K-uronic kit was used. 50 mg of PP was hydrolyzed with 2.5 mL of H_2SO_4 (2 mol. L^{-1}) at 100 °C for 6 h, after neutralization a reading at 340 nm was performed using a UV spectroscope.

2.7.2.2. Differential scanning calorimetry (DSC). Thermal behavior was observed by DSC on a Q 1000 (TA Instruments, USA). Samples weighing 1 mg were mixed with distilled 9 μL water in aluminum pans. They were hermetically sealed and stored at 4 °C for hydration during 1 h. The samples were then heated from 25 °C to 150 °C at 5 °C/min under a nitrogen flow rate of 50 mL.

2.7.2.3. Surface/interfacial tension measurements. Surface tension is the property of the liquid (in our case either milli-Q water or our samples dissolved in milli-Q water) in contact with the air phase. Interfacial tension, on the other hand, is the property between two substances in our case sunflower oil with milli-Q water or sunflower oil in contact with our samples dissolved in milli-Q water.

A 0.1 wt% aqueous solution of CAB-PPC or gum Arabic (GA) was prepared for the determination of air-water (not for GA) and sunflower oil-water interfaces (commercial oil). Adsorption kinetics were studied under adsorption times ranging from 0.05 to 30 min for air-water tension and to 24 min for sunflower oil-water interface using a drop volume tensiometer (DVT50, Krüss, Germany) equipped with a 0.25 mL syringe and a 2.016 mm diameter metal capillary. Both analyses were performed at 25 °C.

2.7.2.4. Average particle size, polydispersity index (PDI) and zeta potential of complex particles. The average particle size and PDI (at neutral pH) and Zeta potential (from neutral pH to pH 1.8) of 1 % CAB-PPC suspension were measured by a particle size and ζ-potential analyzer Delsa Nano C (Beckman Coulter, USA). The determination of the zeta potential on a wide range of pH was to determine the isoelectric point of the proteins and also to make a correlation of the electrostatic properties with the result of the precipitation and the complex obtained. The refractive index and absorbance were set to 1.3328 and 0.001, respectively. All tests were performed at 25 °C.

3. Results and discussion

3.1. CAB characterization

Table 3 presents the composition of the CAB sample. The water content is 3.42 ± 0.50, consistent with a freeze-dried sample. Carbohydrates are the major constituents of CAB. The level of starch (22.27 %)

Table 3
Characterization of CAB.

Compounds	
% fresh basis	
Moisture	3.42 ± 0.50
% dry basis	
Crude fat	5.17 ± 0.24
Crude protein	10.02 ± 0.30
Ash	0.91 ± 0.00
Starch	22.27 ± 0.20
Cellulose	19.79 ± 4.34
Hemicellulosic	23.73 ± 3.27
Lignin	13.56 ± 3.93
Free sugar	0.46 ± 0.13
Pectin contents	0.31 ± 0.02

is higher than those reported by (Cruz Reina et al., 2022); cellulose (19.79 %), hemicellulose (23.73 %), and lignin (13.56 %) are close to those reported in the literature (Cruz Reina et al., 2022; da Correia, Júnior, Gonçalves, & Rocha, 2013; Kouassi, Soro, Vaca-Garcia, & Yao, 2018). However, the pectin and free sugar contents are much lower than previously published values (Zié et al., 2023). The protein content is around 10 %, falling within the expected range (between 9 and 16 %). The fat content (5.17 %) is slightly lower than that reported by Kouassi et al. (2018).

3.2. Optimization using RSM and extraction of CAB protein

Generally, protein concentrates or isolates are extracted in an alkaline solution, which is then precipitated at an isoelectric pH (Wani et al., 2006). We attempted to follow the same protocols to obtain a CAB extract concentrated in protein.

Indeed, the first step involved extraction at higher pH values, away from the isoelectric point, promoting the maximum solubilization of proteins, where the proteins gain a net negative or positive surface charge (Vogelsang-O'Dwyer, Zannini, & Arendt, 2021).

A factorial design was implemented to optimize this initial extraction step. The choice of parameters was based on the understanding that various factors, such as pH, temperature, extraction time, and solvent-to-powder ratio, affect protein solubility (Wani et al., 2006).

RSM was employed to study the optimal conditions for maximizing the yield and purity of CAB protein extraction. As given in Table 1, four factors were selected: solvent/CAB ratio (from 10:1 to 30:1), temperature (from 25 to 45), pH (from 8 to 12), and extraction time (from 1 h to 5 h). Protein yields ranged from 5.24 % to 31.74 %, and purity varied from 7.73 % to 11.23 % under the tested conditions. The results are higher than those obtained (46.95 mg/g) by Patra et al. (2021, 2022) after protein extraction from CAB using a conventional method without optimization. Very little data exists on protein extraction from CAB; there are only two at the time of writing (mentioned above).

The predicted protein yields were calculated using the regression model and compared with experimental values. The total determination coefficient (R^2) was 0.9845, indicating a reasonable fit of the model to the experimental data. Additionally, the predicted R^2 of 0.9595 is in reasonable agreement with the adjusted R^2 because the difference is less than 0.2.

The significance of each coefficient was determined using the Student *t*-test and *p*-value (p). Temperature, pH, and solvent/CAB ratio were identified as the most significant factors ($p < 0.0001$). Extraction time was not a significant factor ($p > 0.05$).

Analysis of variance (ANOVA) of independent variables was performed. The quadratic model was used to interpret the results of the composite central plan. The statistical analysis data revealed that the model for protein extractability was highly significant ($p < 0.0001$).

The failure of the model to represent the data in the experimental domain at points that are not included in the regression is measured by the lack of fit test. The lack-of-fit analysis was not significant ($p > 0.05$), revealing the adequacy of the fitted model.

The ratio of the standard error of the estimate to the mean value of the observed response is called the coefficient of variation (CV). This value is expressed as a percentage and measures the reproducibility of the models. When CV is not greater than 10 %, the model can be considered reasonably reproducible (Firatligil-Durmus & Evranuz, 2010). The CV of this model was calculated at 5.74 %.

The prediction profile showed that the optimum conditions for CAB protein extraction were 39.26 °C, 10.99 pH and a ratio of 25:1 for 3.82 h, resulting in a predicted protein yield of 25.20 % and a purity of 10.41 as the maximum, with a desirability of 0.97.

In the extract from the first step, only 25.20 % of soluble proteins were obtained. The optimal conditions determined by RSM show that a large quantity of proteins were not solubilized (74.8 %). Furthermore, other compounds are solubilized even with a wide range of pH values, resulting in a low extract protein purity (10.41 %) barely higher than the raw material protein purity (10.02 %). Similar extraction yield was obtained by Firatligil-Durmus and Evranuz (2010) when RSM was used to determine optimum conditions for protein extraction from red pepper seed meal.

Subsequently, the second step, which involves precipitation at a pH close to the isoelectric point, was carried out to purify the extract by eliminating non-protein components solubilized in the first step. Under these conditions, proteins are less soluble where they have zero net charge and can more easily approach each other with minimal charge repulsion. Various pH values were tested (1.5, 2.5, 3, 3.5, and 4), and pH 3.5 was found to be optimum (results not shown). Precipitation at this pH resulted in an extract with a protein purity of 12.62 % and a yield of 25.61 %. However, the proteins were not concentrated enough, with a low yield (25.61 %), indicating that 74.39 % of the extract comprises other compounds than proteins. The purity of 12.62 % also remained low after parameter optimization.

3.3. Use of ultrasound-assisted extraction (UAE)

In the pursuit of higher yield and greater purity, UAE was employed, following a two-step procedure similar to the extraction of plant proteins. This involved solubilization using the optimum conditions obtained with the RSM method followed by precipitation at a pH close to the isoelectric point (pH 3.5). The steps of the final extraction procedure are schematized in Fig. 1. The principle of UAE is cavitation, relying on the formation, growth, and implosion of microbubbles generated by consecutive compression and rarefaction (Patra et al., 2022). Cavitation during UAE of CAB disrupted the CAB cell wall, leading to internal structures modification. Ultrasound also increased solvent penetration into CAB cells, enhancing the protein transfer rate into the supernatant. Thus, 55.12 g/100 g of proteins were solubilized. In similar studies on CAB, the protein content yield obtained under optimal conditions of microwave-treated CAB by Patra et al. (2021) was 166.25 mg/g (16.62 g/100 g), while the protein content found by Patra et al. (2022) after optimization of ultrasound-assisted protein extraction from CAB, using artificial neural network-genetic algorithm and response surface methodology, were 354.92 mg/g (35.49 g/100 g) and 325.73 mg/g (32.57 g/100 g), respectively. These observed differences can be explained by the varying extraction methods, optimization processes, the variety of cashew apples used, and the treatments performed on the cashew apples to obtain CAB. Moreover, it appears that these authors did not determine the purity of the extracted proteins nor purify the extract, as was done in this study using precipitation. Similarly, Sitthiya, Devkota, Sadiq, and Anal (2018) also used ultrasonic assisted alkaline extraction of protein from banana flower that was optimized using the response surface methodology. The maximum protein yield of 252.25 mg/g (25,25 g/100 g) was obtained under optimized extraction conditions. This result represents half of the protein yield obtained in our study (55.12 g/100 g).

The purity was 14.67 % which meant that other compounds were still solubilized along with the proteins.

The second step involved precipitation at a pH close to the isoelectric point to purify the extract by eliminating non-protein components solubilized in the first step. Precipitation at this pH (3.5) resulted in an extract twice as pure in protein content as the first step. Proteins were concentrated with an enrichment factor of 2, achieving a purity of 22.10 %, which remained relatively low indicating that 77.9 % of the extract still comprised other compounds than proteins. The yield was also doubled to 55.12 %. Ultrasound facilitated protein release, raising both the yield (25.61 % to 55.12 %) and the purity (12.62 % to 22.10 %) of extracted proteins after precipitation. A similar trend for protein yield from *Dolichos lablab L* and CAB using ultrasound was observed by Zhao et al. (2021) and Patra et al. (2022).

Other studies on the effects of ultrasound have shown that increased mass transfer due to ultrasound treatment is attributed to the reduction

of the boundary layer thickness produced by pressure variations, oscillation velocities, and microflux (Fonteles et al., 2016). Unlike extraction without sonication, UAE not only increased protein purity (from 12.62 % to 22.10 %) but also reduced extraction time as described by Suchintita Das, Tiwari, Chemat, and Garcia-Vaquero (2022). Other authors also found that ultrasound assistance both shortened the extraction time and increased the rate of carotenoid (Ye, Feng, Xiong, & Xiong, 2011) and of ascorbic acid, protein and total antioxidants (Patra et al., 2022).

Despite these treatments, the purity remains low. This suggests that some compounds were not only co-extracted but also associated with proteins; otherwise, they should not precipitate with the proteins after isoelectric precipitation. Indeed, it is possible that proteins form a complex, as the presence of binding components affects protein solubility (Wani et al., 2006). A similar complex has previously been identified in cashew sap (cashew gum). So it could be that the CAB proteins form a complex with the polysaccharides. To explain why the yields were relatively low and the purity was not significantly improved, further analyses were conducted to address the hypothesis and identify compounds associated with proteins.

3.4. Monosaccharide composition of the CAB protein pellet (PP)

Sugar composition of the protein precipitate was determined to gain a better understanding of its content. The results obtained are presented in Table 4.

The precipitate primarily contains 22.10 % proteins and 42.29 % non-cellulosic sugars, confirming the hypothesis that CAB proteins form a complex with polysaccharides. Such a complex had already been identified in cashew sap which is similar to gum Arabic and for the first time with this study, in CAB. Indeed, the major monosaccharides are galactose (17.45 %), arabinose (11.85 %) which are also predominant in cashew gum and gum Arabic (de Paula & Rodrigues, 1995) and glucose (9.79 %). Therefore, CAB-PP are similar to gum Arabic, being complex polysaccharides containing a certain amount of proteins that cannot be removed by purification (Williams & Phillips, 2009). Several authors such as (Kang, Guo, & Shi, 2019; Kang, He, & Shi, 2024; Rumpagaporn et al., 2015; Zhang, Smith, & Li, 2014) have used alkaline extraction followed by precipitation to obtain other natural polysaccharide-protein complexes. These natural hydrocolloids are rich in hydrophilic groups, which exhibit good water retention capacity (Liu & Xu, 2019) and they have garnered attention from academia and industries due to their functional properties such as thickening, suspension, emulsification, stabilizing, gelling, film formation (Li et al., 2020), and adhesive properties in the food industry (Li et al., 2021). The high protein percentage in CAB Protein Polysaccharide Complex (CAB-PPC) is crucial for its enhanced emulsion stabilizing capacity (Yadav, Fishman, Chau, Johnston, & Hicks, 2007; Yadav, Moreau, Hotchkiss, & Hicks, 2012) thickening properties, and film-forming capacity (Cai, Wei, Zhang, Rao, & Wang, 2021).

Table 4
Monosaccharide composition of the protein pellet.

Monosaccharide	Composition (g/100 g dry solids)
Rhamnose (Rha)	1.24 ± 0.16
Arabinose (Ara)	11.85 ± 1.98
Xylose (Xyl)	0.69 ± 0.19
Mannose (Man)	0.77 ± 0.15
Glucose (Glc)	9.79 ± 2.55
Galactose (Gal)	17.45 ± 3.73
Galacturonic Acid (GalA)	0.70 ± 0.06
Ratio	
Ara/Gal	0.68
Rha/Gal	0.07
GalA/Gal	0.04
Gal/Ara	1.47
Ara + Gal/Rha	23.6
Rha/GalA	1.77

Different characteristic ratios were calculated to elucidate the polysaccharide and oligosaccharide sugar structure of the protein polysaccharide complex: Arabinose to Galactose (Ara/Gal), Rhamnose to Galacturonic acid (Rha/GalA), Galactose to Rhamnose (Gal/Rha) and Arabinose + Galactose to Rhamnose (Ara + Gal)/Rha (Table 4). Polysaccharides rich in arabinose and galactose (PRAG) are a large family that contain structurally well-defined polysaccharides such as arabinans, arabinogalactans (AG), and arabinogalactan proteins (AGP) (Canalejo et al., 2022).

The Ara/Gal ratio is characteristic of PRAG-like structures, and higher values of this ratio indicate higher contents of arabinose and galactose or structures rich in arabinose and galactose (Canalejo et al., 2022). The Ara/Gal, Rha/Gal, GalA/Gal, Gal/Ara, Rha/GalA and Ara + Gal/Rha Ratio were 0.68, 0.07, 0.04, 1.47, 1,77 and 23.6 respectively, indicating a major presence of structures rich in galactose and arabinose (AG, AGP) and a low content of homogalacturonan (HG)-like structures. The results are similar to those reported by several authors who studied monosaccharides from different natural protein-polysaccharide complexes, such as Paulsen, Craik, Dunstan, Stone, and Bacic (2014) when they worked on gum Arabic arabinogalactan protein (Ara/Gal 0.75 to 0.12 and Rha/Gal 0.27 to 0.04), Redgwell, Curti, Fischer, Nicolas, and Fay (2002) on the arabinogalactan Proteins content of green coffee beans (Gal/Ara: 3.3 to 1.5), Canalejo et al. (2022) on polysaccharide extracts recovered from different grapes and winemaking products (Ara/Gal and Rha/GalA: 1,1 to below 0.5) but our Rha/GalA was higher (1.77). The Ara + Gal/Rha ratios (23) of CAB-PPC showed the relative importance of the neutral side-chains to the rhamnogalacturonan type I (RG-I) backbone (Apolinar-Valiente, Romero-Cascales, Gómez-Plaza, López-Roca, & Ros-García, 2015). L. Tan et al. (2013) suggested RG-I covalent links with the rhamnosyl residues of the arabinogalactan protein (AGP) and with arabinoxylan attached to either a rhamnosyl residue in the RG I domain or directly to an arabinosyl residue in the AGP, supporting the presence of protein polysaccharide complex in CAB-PPC. The analysis of CAB-PPC monosaccharide composition data which indicated the presence of Rha, Gal and Ara suggests the presence of RG-I side chains and AGP similar to the findings of Gao, Fangel, Willats, Vivier, and Moore (2015). An abundance of Ara (11.85 g/100 g) and Gal (17.45 g/100 g) showed PRAG presence in CAB-PPC and the main class of polymers in the CAB protein polysaccharide complex is AGPs.

3.5. HPSEC analysis

Fig. 2 shows the HPSEC chromatograms of CAB-PP using UV (at 280 nm) and RI. UV absorbance is sensitive to the chemical nature of the eluting species, especially the proteinaceous component, whereas RI is a sensitive measure of concentration (Mahendran, Williams, Phillips, Al-

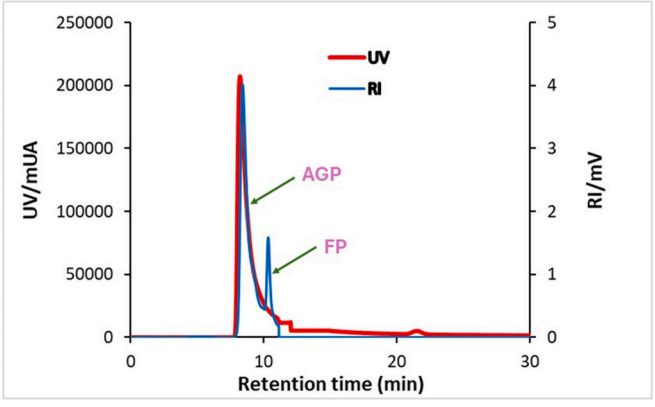

Fig. 2. Size exclusion chromatogram of CAB-PP using UV (280 nm) and RI detectors.

Assaf, & Baldwin, 2008).

The HPSEC profile of CAB-PP shows two main fractions. The first elutes from 8 to 10 min, and the second elutes from 10 to 11 min with average molecular weights of 12.155 kDa and < 12 kDa, respectively, determined using a dextran calibration curve. Furthermore, UV (280 nm) signals were found in both fraction 1 and fraction 2, attributed to the presence of aromatic amino acids (tryptophan, tyrosine, and phenylalanine) in the proteinaceous moiety (Lin, Wang, Meng, & Guo, 2021), indicating a polysaccharide-protein complex (PPC). This CAB-PP behaves as a heterogeneous system similar to gum Arabic and cashew gum (de Paula & Rodrigues, 1995).

The elution profiles obtained by UV (8–12 min) show a significant peak covering both fractions shown by RI. The intensity of each fraction differs due to variations in protein content, as reported by Idris, Williams, and Phillips (1998) in characterization of *Acacia senegal* trees gum. The major fraction (peak 1) and a higher molecular mass peak might correspond to the arabinogalactan-protein (AGP) fraction, which is the main molecular fraction of our extract. The second fraction, with a smaller molecular weight, should be the free protein (FP) population. These findings align with the composition analysis results, which showed that the major monosaccharides are galactose and arabinose with AGPs as the dominant class of polymers in the CAB-PPC. Our results are consistent with those of (Redgwell, Schmitt, Beaulieu, & Curti, 2005) in their study on coffee hydrocolloids, which showed the physicochemical and functional properties of an arabinogalactan-protein (AGP) fraction. Using the Yariv test, they demonstrated that some of the proteins (up to 20 %) were covalently bound to arabinogalactan, while the remainder were likely intracellular proteins that co-precipitated with AGP during alcohol precipitation.

Therefore, the HPSEC results, in agreement with the monosaccharide composition, confirm the hypothesis that the CAB-PPC extract contains a protein-polysaccharide complex.

Similar to studies on gum Arabic (Idris et al., 1998; Lin et al., 2021; Mahendran et al., 2008; Rhazi et al., 2020), (Kang et al., 2024) also used HPSEC to demonstrate the conformational properties describing the heterogeneous structure of the natural complex (arabinoxylan protein) from corn bran (Kang et al., 2019, 2024). Moreover, our raw material CAB has a composition almost similar to corn bran, a by-product from the corn milling process.

Gum Arabic considered the gold standard is an amphiphilic polysaccharide comprising major galactose chains and highly branched galactose/arabinose side chains (Dai et al., 2018; Mariod, 2018; Tang, Gao, Zhang, & Tang, 2024). Gum Arabic, primarily consisting of arabinogalactan (AG) and Arabinogalactan protein (AGP) with a highly branched structure, is a popular plant-based emulsifier in the food industry and finds applications in various other industrial sectors. The AGP fraction in gum Arabic plays a significant role in its emulsifying properties, primarily due to its covalent linkage between protein and polysaccharide (Cai et al., 2021; Castellani et al., 2010; Kang et al., 2024).

Like gum Arabic, CAB Protein-Polysaccharide (CAB-PPC) is a natural protein-polysaccharide complex composed mainly of proteins, galactose, and arabinose, which might have wide applications in various fields. We know that individual biopolymers in the food industry often do not provide the desired properties and viscosity of edible gels, including the ability to retain the predominant solvent and the desired rheological properties (Miao et al., 2023), which limits their wide application. To address this issue, many scientists have shown that the most effective approach is to add an additive, i.e., adding protein as an additive to polysaccharide-based edible gels (Lin et al., 2023; Miao et al., 2023). However, the discovery of new sources of a natural hydrocolloid like CAB-PPC could limit these artificial modifications.

3.6. Fourier transform infrared (FTIR)

Fourier Transform Infrared (FTIR) analysis provides valuable insights into the nature of interactions between molecules (Dai et al., 2018), offering insights into the characteristic vibration patterns of covalent bonds within molecules (Etzion, Linker, Cogan, & Shmulevich, 2004). This analysis presents quantitative data about all constituents that absorb IR radiation, including proteins and polysaccharides (Dai et al., 2018; Etzion et al., 2004). Moreover, Mid-Infrared (MIR) spectroscopy directly provides information about specific constituents in the sample, their characteristic molecular structure, and chemical bonds (Etzion et al., 2004; Nandiyanto, Oktiani, & Ragadhita, 2019). IR spectroscopy analyzes absorption peaks at specific wave numbers (expressed in cm^{-1}) and is crucial for identifying polysaccharides and proteins structure (Dai et al., 2018; Li, Bai, Yang, & Li, 2023).

In this context, FTIR spectroscopy was utilized to elucidate the structural characteristics of CAB-PPC a natural protein-polysaccharide complex sample. The results revealed numerous peaks, indicating the complex structure of the novel hydrocolloid (as depicted in Fig. 3A). The infrared spectra of CAB-PPC exhibited similarities to those observed in gum Arabic, particularly within the peak at 3330 cm^{-1}, which is associated with hydrogen bonds (Hay, Kontogiorgos, Thompson, Nastasi, & Fitzgerald, 2024; Nandiyanto et al., 2019) confirming the presence of hydroxyl (O—H), and the two characteristic bands at 1600–1630 and 1420–1450 cm^{-1} (Hay et al., 2024; Nandiyanto et al., 2019). In the literature (Türker-Kaya & Huck, 2017), the bands from 3500 to 3200 cm^{-1} are assigned to CH_3, N—H and O—H stretch: carbohydrates, proteins, alcohols and phenolic compounds. According to Dong, Sørensen, He, and Engelsen (2017) signals between 2100 and 2490 cm^{-1}correspond to the structure of arabinogalactan, with vibration modes including O—H deformation + C—O stretching, CH stretch + deformation of CH2 and C—H stretch, and C—C stretch. Moreover, according to Li et al. (2024), amides are considered the most reliable indicator for determining the secondary structure of a protein, more essentially the amide I band (Türker-Kaya & Huck, 2017). The peaks around 1630 cm^{-1} are ascribed to amide I and are associated with C = O stretching (Li et al., 2021; Nandiyanto et al., 2019). The vibrational signal at 1540 cm^{-1} (amide II) originated from N—H bending and C—N stretching (Feng et al., 2024; Li, Zheng, et al., 2021). Additionally, the vibrational peak at 1450 cm^{-1} may be attributed to the structure of glucuronic acid (C═O third overtone). The peak observed at the wavelength of 1360 cm^{-1} corresponds to the structure of rhamnose (C—H stretch first overtone + CH deformation of CH3). The same peaks have been identified in gum Arabic by Dong et al. (2017). The amide III (1150 cm^{-1}) peaks arisen due to the interplay between N—H angular vibration and CN stretching vibration (Li, Zheng, et al., 2021).

It appears that the model information in the region from 1630 to 1150 cm^{-1} primarily are from the proteinaceous moieties in CAB-PPC, with some contribution from the carbohydrate moieties (aliphatic CH stretching) (Dong et al., 2017).

3.7. Nuclear magnetic resonance (^1H NMR) spectroscopy spectra

The chemical structure of the CAB-PPC particles was further elucidated using ^1H NMR spectroscopy. The ^1H NMR spectrum displayed characteristic chemical shifts and peaks, as illustrated in Fig. 3B. The scanning region of CAB-PPC sample 4.4–5.4 contained significant cross peaks indicating the high structural complexity of CAB-PPPC (Grein et al., 2013), demonstrated by FTIR analysis, suggested the presence of sugar constituents within the polysaccharide chains, particularly in the anomeric region according to (Hay et al., 2024). The signal from 5.01 to 4.69 ppm could be assigned to Ara, Rha and β Gal (Hay et al., 2024). An additional peak at 3.76 ppm was observed for 1,3-linked Gal (Capek, Matulová, Navarini, & Suggi-Liverani, 2010). These peaks in the CAB-PPC sample, along with the presence of β-Gal, confirmed that the polysaccharide composition of CAB-PPC closely resembles that of gum Arabic. Gum Arabic polysaccharide typically comprises a β-(1 → 3) galactose backbone with linked branches, predominantly from C-6, but also C-4 and C-2 of Galp and Arap, terminating in Rha and GlupA (Rhazi

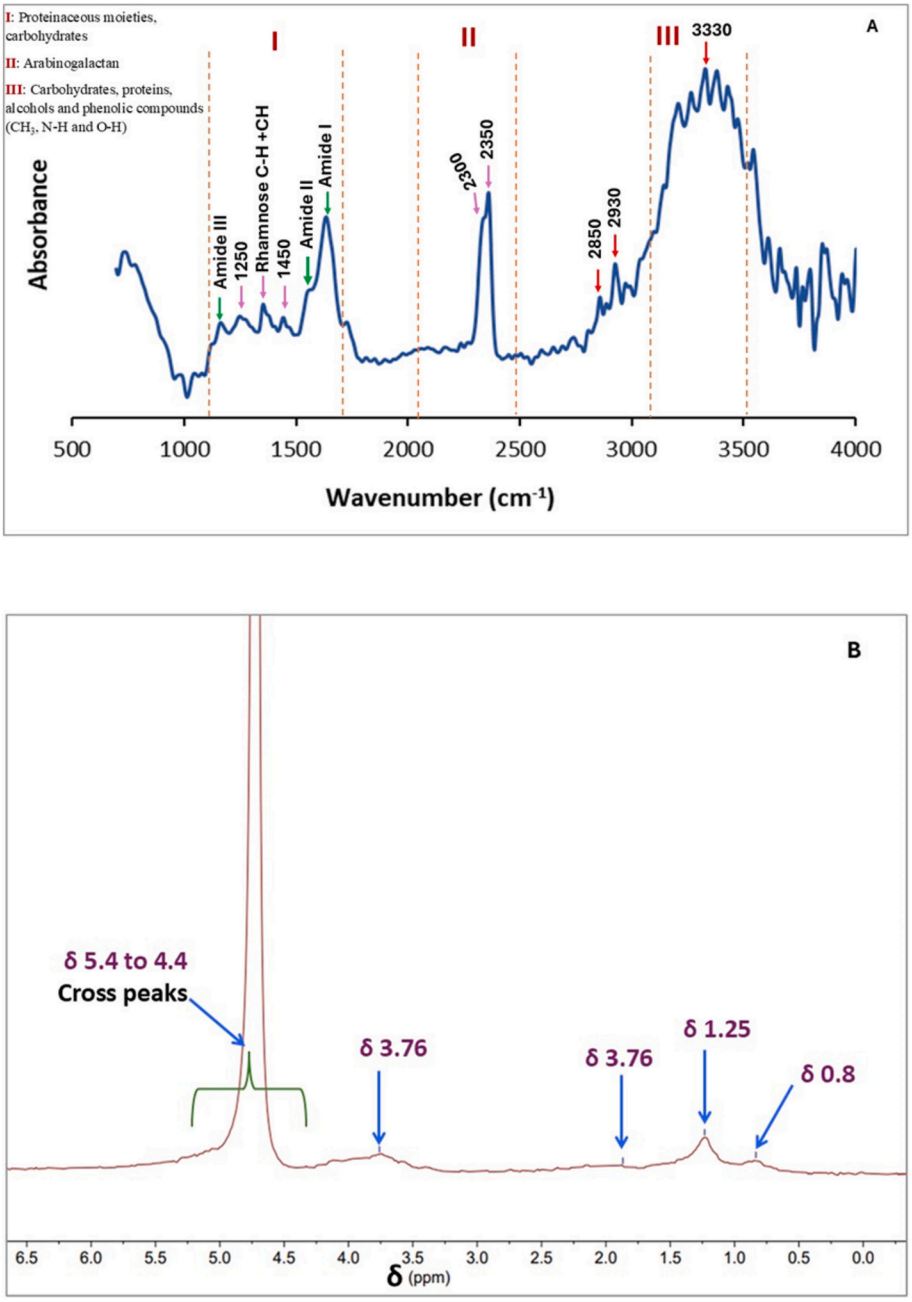

Fig. 3. Mid-infrared spectra of CAB-PPC (A) and Nuclear magnetic resonance (^{1}H NMR) spectra of CAB-PPC (B).

et al., 2020).

In the ^{1}H NMR spectrum of CAB-PPC, in addition to signals attributable to carbohydrates, signals corresponding to the protein component of the molecule were also observed in the region δ 3.1–0.8 (Capek et al., 2010). The NMR results confirmed those obtained with the MIR analysis.

3.8. Physicochemical properties characterization of CAB-PPC

The complex obtained was therefore characterized by three main physicochemical properties including thermal behavior, surface/ interfacial tension and zeta potential (Fig. 4). Particle size and polydispersity were also determined to elucidate the relationship between their results and the properties of the composite.

3.8.1. Differential scanning calorimetry (DSC)

The DSC profile of CAB-PP powder revealed a melting point (Tm) at 77.66 °C, attributed to the denaturation of the protein fraction (Fig. 4A).

The presence of a weak endothermic peak at 77.66 °C corresponding to free protein denaturation containing in CAB-PPC. The enthalpies of protein in PP were low compared to those of known protein with high purity (Jennings et al., 2024). Since the enthalpy changes represent the ratio of the content of the ordered structure (Qin et al., 2013), it could be inferred that the ordered structure of protein in CAB-PPC was weak. This could be due to the deployment of some proteins (Dehnad, Emadzadeh, Ghorani, Rajabzadeh, & Jafari, 2023) and the fixation of a large portion of the proteins to the polysaccharides contained in CAB-PPC. This confirms that not all proteins are free in CAB-PPC, as demonstrated by HPSEC.

An exothermic peak is then observed which corresponds to the decomposition of the CAB-PPC powder compounds. The occurrence of an exothermic reaction, characterized by the release of energy due to the liberation of heat, implies the potential breakdown of protein-

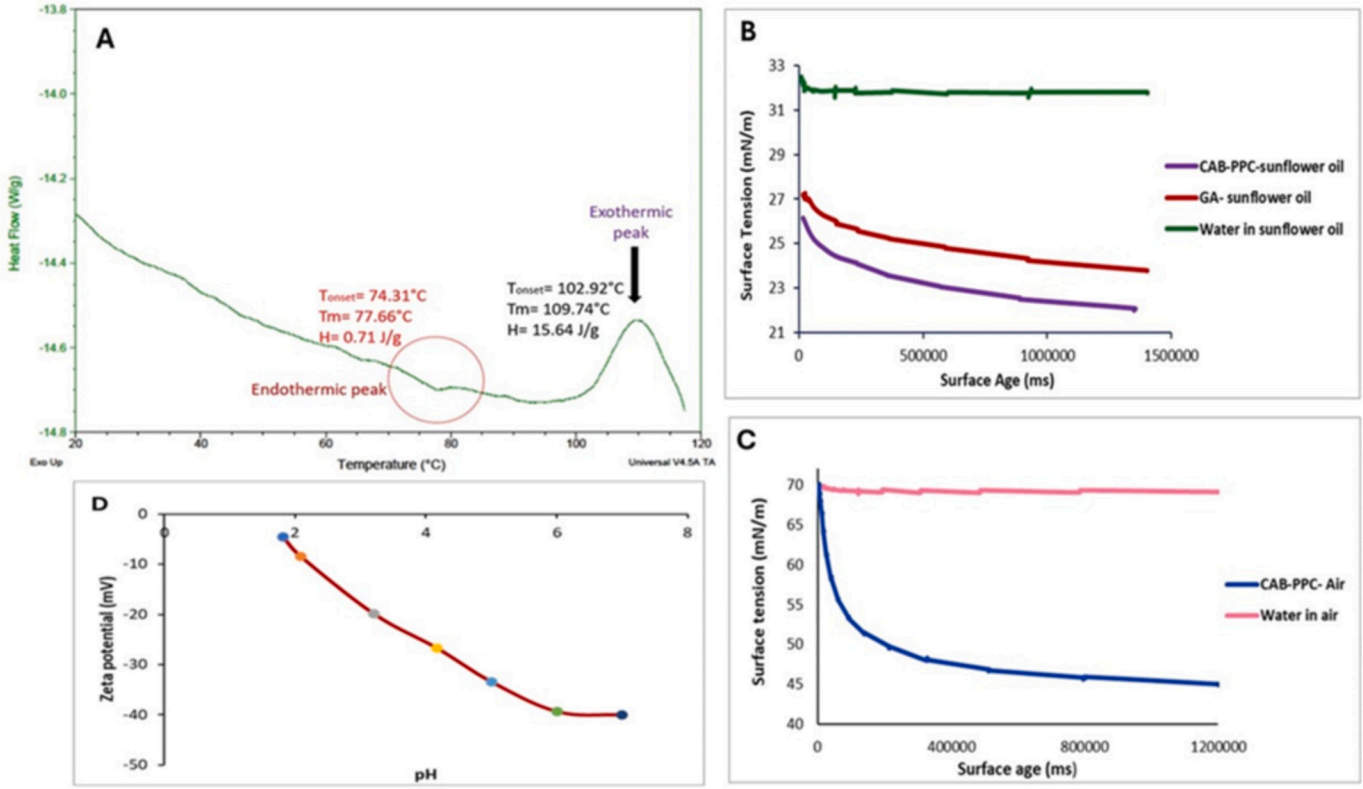

Fig. 4. DSC thermograms of CAB-PPC (A); Dynamic surface(B) and interfacial (C) tension of CAB-PPC solutions as a function of surface Age and Influence of pH on the zeta potential of the CAB-PPC solution (D).

polysaccharide bonds. The Tm of CAB-PPC powder (109.74 °C) indicate its thermal stability. This trend can be linked to the interactions between the hydroxyl groups of polysaccharides and the amino and carboxyl groups of the protein as reported by Aguilar-Vázquez, Loarca-Piña, Figueroa-Cárdenas, and Mendoza (2018), Tan, Qiu, Lamport, and Kieliszewski (2004) and Tang et al. (2024). The DSC result is also consistent with the HPSEC results.

3.8.2. *Interfacial tension*

To assess the colloidal behavior of the CAB-PPC, adsorption kinetics were evaluated at the air-water (Fig. 4B) and sunflower oil-water interfaces (Fig. 4C). The study of the adsorption kinetics on a longer time scale and the extrapolation of the equilibrium SFT value were carried out using the drop volume tensiometer. The surface tension dropped significantly, indicating the rapid diffusion and adsorption capacity of molecules at the interface. Subsequently, the molecules were gradually adsorbed at the interface from 71 mN/m to a stable SFT value of approximately 45 mN/m (Fig. 4B). Hay et al. (2024) and Castellani et al. (2010) showed that the surface tension of GA decreased from 72.4 to 60.1 mN/m and from 71 mN/m to 57.4 mN/m respectively, which is higher than our result. This value further demonstrates the favorable interaction between the protein and polysaccharide fractions of CAB-PPC. The hydrophilic nature combined with the more hydrophobic nature of the PPC constituents gives them a low surface tension.

The adsorption of particles at the oil-water interface primarily involves two phases. Initially, the particles move toward the oil-water interface and infiltrate; subsequently, the particles gather and rearrange at the interface to create a viscoelastic interfacial layer. This behavior is essential for the emulsifying properties of particles (Tang et al., 2024). The interfacial tension of CAB-PPC and GA was measured under identical analytical conditions to examine the effect of the high protein content in CAB-PPC on the interfacial tension compared to GA. As depicted in Fig. 4C, the interfacial tension decreased swiftly within the first 350 s due to the complex particles adsorbing at the oil-water interface for both GA and CAB-PPC. This indicates that both samples exhibited the fundamental traits of efficient emulsifiers. Afterward, a gradual decrease in interfacial tension was noted, attributed to the extensive stacking and rearrangement of the protein-polysaccharide complex particles at the oil-water interface (Tang et al., 2024). The rapid reduction in interfacial tension could also be because of CAB-PPC's small particle size, making the particles more prone to adsorb at the oil-water interface (Han, Chen, Gao, Zhang, & Tang, 2020; Tang et al., 2024). This decrease in interfacial tension indicated that an appropriate enhancement of the electrostatic interaction occurred and facilitated the adsorption of protein-polysaccharide complexes at the interface. This indicates that CAB-PPC might be excellent candidates as Pickering stabilizers (Xu et al., 2021). The interfacial tension of CAB-PPC (21.32 mN/m) was lower than that of gum Arabic (23.71 mN/m), probably due to the high protein content of CAB-PPC.

3.8.3. *Zeta potential, polydispersity index and particle size data*

The particle size of the natural complex particles was 365.1 nm showing strong intermolecular interactions. The polydispersity index (PDI) of the particle solutions were 0.308, indicating that all particles had good dispersibility as found by Tang et al. (2024).

The zeta potential serves as a measure of the electric charge that develops on the surface of a molecule or suspended particle (Luo et al., 2024). This measurement provided an insight into the electrical properties of CAB-PPC. In the studied pH range, the zeta potential value remains negative even as the pH drops to 2 (Fig. 4D). This phenomenon can be attributed to the electrostatic interactions within the CAB-PPC complex. This electrostatic interaction between functional groups with opposite charges could effectively contribute to their stability (Doublier, Garniera, & Renarda, 2000; Xu et al., 2021). Highly negatively charged polysaccharides expose more negative charges, and the negative zeta potential value in the pH range as shown in Fig. 4D was found in a

similar study by Tan et al. (2021). This result showed that polysaccharide has a major impact on the zeta potential.

Moreover, below a pH of 5.5, the protein can either aggregate with other protein molecules due to self-association near the isoelectric point or interact with oppositely charged groups on the anionic polysaccharides in the surrounding solution (Jones & McClements, 2011). In the CAB extract, most protein molecules would be bound to polysaccharide molecules, and few free proteins would self-associate. The formed complexes result in a net negative charge even below pH 2, which would explain the 22.10 % of protein purity after precipitation. Jones and McClements (2011) also reported similar results, where the measurement of negative ζ-potentials in protein-polysaccharide systems within the same pH range demonstrated protein-polysaccharide complexation. This complex could result from thermodynamic compatibility induced by associative electrostatic interactions between proteins and polysaccharides due to the net opposite electrical charges carried by the two biopolymers (Le, Rioux, & Turgeon, 2017).

These discussions focus on the process behind a novel finding and its implications. Although significant optimizations have been made to achieve these results, many other factors must be considered in future research. Variables such as species, climate, annual variations in harvest, region, soil conditions, extraction methods, and sourcing bagasse from a cashew apple juice production industry will likely impact extraction outcomes, purity, and the properties of the resulting complex similar to other plant-based products. Additionally, for effective waste valorization, it may be more beneficial to conduct the extraction on bagasse produced at an industrial scale, as storage and handling at this level could influence the quality of both the bagasse and the extracted complex.

3.9. Potential applications of CAB-PPC in the food industry

CAB-PPC has potential applications as an emulsifier and stabilizer in the food industry, particularly for oil-in-water emulsions. Its surface-active properties stem from the protein-rich fraction, which adsorbs onto the surface of oil droplets, while the polysaccharide chain prevents coalescence and flocculation through electrostatic and steric repulsion forces. This makes CAB-PPC ideal for use in the beverage and confectionery sectors, not only as an emulsifier and stabilizer but also for flavor encapsulation. For instance, it could be utilized as an emulsifier in the production of concentrated juices and cola-flavored soft drinks. Moreover, its solubility and relatively low viscosity allow it to be incorporated into processed foods without altering their original taste or texture.

Spray drying aromatic oils with CAB-PPC solutions can be employed to create microencapsulated powders, ideal for use in convenient dry food products like soups and dessert mixes. Additionally, CAB-PPC could be incorporated into candy manufacturing to prevent sugar crystallization and emulsify fatty ingredients in products such as pastilles, caramel, and toffee. Moreover, CAB-PPC has potential as a thickening agent in the production of chewing gum, enhancing its texture and stability.

Despite its potential, CAB-PPC faces technical challenges stemming from factors such as species variation, climate, year-to-year collection, region, soil environment, extraction conditions, and sourcing of bagasse from the cashew apple juice industry. These variables can affect the consistency, purity, and functional properties of the complex. Addressing these issues in future research will be crucial to ensure CAB-PPC meets the necessary standards for food applications.

To advance toward industrial applications, further studies are needed to ensure consistency, product standardization, and variability control, similar to the research conducted on gum Arabic. However, before CAB-PPC can be marketed and used in consumer products, in vivo analyses must be performed to validate its bioactivity, stability, safety, and bioavailability. It would be particularly important to incorporate technologies ensuring the safety of formulated food products to avoid toxic or allergic reactions in consumers.

4. Conclusion

This study has provided new insights into the potential valorization of cashew apple bagasse (CAB), specifically focusing on the extraction and characterization of nutritional, techno-functional and bioactive compounds, with an emphasis on protein and polysaccharide fractions. The analysis of CAB-PPC composition allowed us to hypothesize the presence of a protein-polysaccharide complex (PPC). Detailed characterization using HPSEC, coupled with RI and UV detectors, confirmed that CAB-PPC contains two fractions including proteins which form a complex with polysaccharides, particularly arabinogalactan-proteins.

Further molecular insights were gained through MIR FTIR spectroscopy and ^{1}H NMR techniques, elucidating the characteristic molecular structure of the CAB-PPC. The effects of protein-polysaccharide interactions within CAB-PPC on structure and functionality showed good functional properties as well as their correlation relationship. These interactions influence the structural integrity and enhance the functional capabilities of CAB-PPC, particularly in applications such as emulsification and stabilization.

The emulsifying properties of CAB-PPC were particularly attributed to the arabinogalactan-protein fraction, which plays a critical role in its functional properties. Comparisons with gum Arabic (Table 5), known for its wide range of industrial applications, suggest that CAB-PPC could act similarly due to its structural and functional similarities. Given its technological characteristics, CAB-PPC shows promise as a stabilizer, emulsifier and thickener in various industries.

Ultimately, this study highlights the identification of a novel natural protein-polysaccharide fraction within CAB, providing valuable knowledge for future applications of this hydrocolloid complex. The findings open new avenues for the utilization of CAB, contributing to the development of sustainable and value-added products from agricultural by-products.

As a perspective, an approach will be carried out to deepen our understanding of the technological properties of the CAB protein-polysaccharide complex by including other physicochemical parameters and other functional properties such as emulsification properties. The characterization of other samples from other extraction conditions such as a comparative study with gum Arabic would be relevant. The influence of the source, the age of the trees from which CA was obtained, climatic conditions, soil environment, species, and extraction conditions on the properties of the obtained fractions could be studied in future research. An advanced study could also be investigated to better understand the detailed structure of this matrix in order to increase the added value of cashew apple bagasse.

CRediT authorship contribution statement

Madinatou Zie: Writing – original draft, Methodology,

Table 5
Comparison of some physicochemical properties of CAB-PPC with GA.

Properties	PPC	GA	references
Galactose	17.45 g/100 g	39–42 g/100 g	(Idris et al., 1998)
Arabinose	11.85 g/100 g	24–27 g/100 g	
Rhamnose	1.24 g/100 g	12–16 g/100 g	
Ara/Gal	0.68	0.57–0.68	
Proteins	22.10 g/100 g	1.5–2.6 g/100 g	
Molecular mass components	2 (AGP, FP)	3 (AGP, AG, GP)	
Molecular weight	12×10^3 g/mol^{-1}	25×10^3 g/mol^{-1}	(Hay et al., 2024)
Surface tension	45 mN/m	57 mN/m	(Castellani et al., 2010)
Interfacial tension	21.32 mN/m	23.71 mN/m	Current study
polydispersity	0.308	1.3–1.8	(Idris et al., 1998)
Viscosity	<1	<1	(Data not shown)

Investigation, Formal analysis, Data curation, Conceptualization. **Nicolas Jacquet:** Writing – review & editing, Methodology, Conceptualization. **Gaoussou Karamoko:** Methodology. **Taofic Alabi:** Supervision, Funding acquisition. **Aurore Richel:** Resources. **Romdhane Karoui:** Resources. **Christophe Blecker:** Writing – review & editing, Validation, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Data availability

Data will be made available on request.

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