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Production of pectin from cashew apple waste (*Anacardium occidentale*) using ultrasound-assisted extraction: Process optimization, physicochemical and functional characterization

Bio Sigui Bruno Bamba[1]*, Say Jean Baptiste Anouhé[1], Koffi Nicaise Oussou[1], Doudjo Soro[2], Lamin Kassama[3]*

[1]Department of Biochemistry-Genetics, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire.
[2]Food, Chemical and Environmental Process Sciences Unit, National Polytechnic Institute Félix Houphouët Boigny, Yamoussoukro, Côte d'Ivoire.
[3]Department of Food and Animal Sciences, Alabama Agricultural and Mechanical University, Normal, Alabama 35719, United States of America.

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ABSTRACT

Ivory Coast is the world's largest producer of cashew nuts, generating significant amounts of waste that can be used as a source of beneficial natural compounds such as pectin. However, its properties depend on extraction conditions and methods. The aim of this study was to recover pectin from cashew apple pomace (*Anacardium occidentale* L.) using ultrasound-assisted extraction (UAE). A three-factor, three-level Box–Behnken design coupled with a desirability function was employed to optimize the process. The physicochemical and functional properties of this pectin were analyzed and compared to those of conventional extraction (CE). The results showed that the optimal conditions were 10:1 mL/g (solvent–solid ratio), 40 min (sonication time), and 1.5 (pH), resulting in a maximum yield of 9.54 ± 0.35%, higher than that of CE (7.63 ± 0.53%). The pectin characterization showed that ultrasound-assisted extraction increased the equivalent weight, methoxyl content, anhydrouronic acid content, and degree of esterification. However, the conventionally extracted pectin exhibited the best water holding capacity, emulsion activity, and foaming capacity, as well as emulsion and foaming stability. Moreover, cashew apple pectin obtained through both extraction techniques was categorized as highly methyl-esterified and showed similarity in structure (Fourier-transform infrared spectroscopy [FT-IR]). Thus, cashew apple pomace could be a valuable source of pectin with interesting functional properties, thereby relieving pressure on currently commercial sources of pectin.

1. INTRODUCTION

The growing demand for natural ingredients for food and pharmaceutical applications has placed the valorization of agro-wastes at the center of the circular bioeconomy. The contribution of the cashew sector (*Anacardium occidentale* L.) to waste production is approximately 40.34 million tons, of which more than 90.73% relates to cashew apple processing [1,2]. In 2023, Côte d'Ivoire, the world's largest producer of cashew nuts with more than 1.20 million tons, contributed 11.4 million tons of cashew apples considered waste [3]. Moreover, processing this volume into juice would generate about 1.5 million metric tons of waste, out of other cash crop waste. This leads to environmental problems, including the generation of greenhouse gas emissions during anaerobic decomposition [4]. Therefore, its valorization into bioproducts, mainly pectin, remains a promising strategy due to the growing demand for pectin, for new sources of pectin, and for pectin with desired properties [5-7].

Pectin is a hydrocolloidal heteropolysaccharide of α-D polygalacturonic acid, partially acetylated or esterified by methyl groups, found in the cell wall and middle lamellae of dicotyledonous plants [8]. Many beneficial food and non-food properties are attributed to it due to its non-toxicity and ecological nature. Traditionally, it is used as a gelling, emulsifying, and thickening agent in food. Recently, pectin has been increasingly utilized as a fat replacer, as a health-promoting functional ingredient, and as a prebiotic [9-12]. For medicine and pharmaceutical purposes, it has been used as a carrier to control drugs or bioactive release [13,14]. However, these properties are related to raw material origin, extraction conditions, and method [8,15].

Recently, pectin has been recovered from many sources, such as peach pomace [15], *Spondias purpurea* L. peel [16], durian rind [15], and cocoa byproducts [17]. However, processing conditions affect production yield and physicochemical and functional properties [18,19]. For instance, longer extraction time at a higher temperature increases

*Corresponding Author
Bio Sigui Bruno Bamba,
Department of Biochemistry-Genetics, Peleforo Gon Coulibaly University, Korhogo, Côte d'Ivoire. E-mail: bamba_bio @ upgc.edu.ci
Lamin Kassama,
Department of Food and Animal Sciences, Alabama Agricultural and Mechanical University, A-101 Carver Complex Thomas Wing 4900 Meridian Street, Normal, AL 35719, United States of America.
E-mail: lamin.kassama @ aamu.edu

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pectin yield but decreases its molecular weight, resulting in a decrease in pectin solution viscosity [18]. Besides, at a low extraction solvent pH, the pectin degree of methylation is high, but its water dissolution capacity is reduced [20]. Moreover, the conventional method, commonly based on maceration in acidified hot water, consumes time, energy, and solvents, leading to low extraction yield, pectin property modification, and an enormous amount of wastewater generation that affects the environment [7,21]. As a result, several innovative extraction methods, such as microwaves, supercritical CO_2, subcritical water, high-voltage pulsed electric fields, high hydrostatic pressure, and ultrasound-assisted extraction (UAE), have been developed [8,22]. UAE has emerged as an advanced, cost-efficient, eco-friendly, adjustable, quick, sustainable, and most utilized technique for extracting bioactive compounds from plant-based agro-industrial foods [23-25]. It utilizes acoustic cavitation to disrupt cell walls, facilitating pectin release from the plant matrix, which enhances extraction yield, shortens extraction time, and preserves native physicochemical and functional properties [16,26]. Moreover, combining acidified hydrochloric acid (HCl) water with UAE (UAE-HCl) yields higher pectin extraction compared to UAE-organic acids, microwave-assisted extraction-HCl (MAE-HCl), and conventional extraction-HCl (CE-HCl) [27]. In addition, due to the significant reduction of extraction solvent volume, HCl consumption in the UAE is low [24], leading to sustainable production of pectin [16]. Furthermore, Khedmat *et al*. [28] showed that UAE effectively preserves technological, biofunctional, and nutritional properties of pectin. Therefore, it has been successfully employed to recover pectin from many agro-wastes [4,6,16,17,29,30].

The UAE efficiency is affected by both operating conditions and extraction processes that impact the molecular structures of targeted molecules, resulting in variation in their biological properties [25]. Therefore, optimization is necessary to maximize yield and obtain a standardized quality pectin. In this context, response surface methodology (RSM) is a mathematical model-based tool commonly used to optimize the extraction conditions by evaluating the influence of multiple operational parameters [22].

To our knowledge, no previous studies have reported the optimization of UAE conditions for pectin extraction from cashew apple pomace despite the previous work of Yapo and Koffi [5] and Tamiello-Rosa *et al*. [6], which used conventional Soxhlet extraction. Therefore, this study investigates the optimization of the UAE pectin extraction process yield using the Box–Behnken design (BBD) coupled with the desirability function. The pectin was extracted by UAE and CE under optimized conditions and compared in terms of physicochemical and functional properties.

2. MATERIALS AND METHODS

2.1. Materials and Reagents

Ripe cashew apples were purchased at the local market in Korhogo, Côte d'Ivoire. Ethylene diamine tetraacetic acid, sodium azide, HCl, sodium hydroxide (NaOH), sodium chloride, phenolphthalein reagent, and ethanol were purchased from Merck, Germany. All chemicals used were of analytical grade.

2.2. Sample Preparation

After soaking in 2 ppm chlorinated water for 5 min, the fresh cashew apples were washed with distilled water, sliced into small pieces, and then crushed in a laboratory fruit blender. The pomace obtained, after mixture filtration on nylon cloth, was immediately spread in thin layers on stainless steel trays and oven-dried (model number: UM500, Memmert GmbH, Schwabach, Germany) at 50°C until constant weight and then powdered. The powder was sieved through a 200 µm mesh and stored in a tightly closed bottle at 4°C until further analysis.

2.3. Production of Pectin by UAE and CEs.

The UAE of cashew apple pomace pectin (CAPP) was carried out according to the method described by Dranca and Oroian [7] with slight modifications [Figure 1]. Briefly, 20 g of CAPP were mixed with an adequate volume of a 1 M hydrochloric acid solution diluted in distilled water until the desired pH was achieved [Table 2]. The

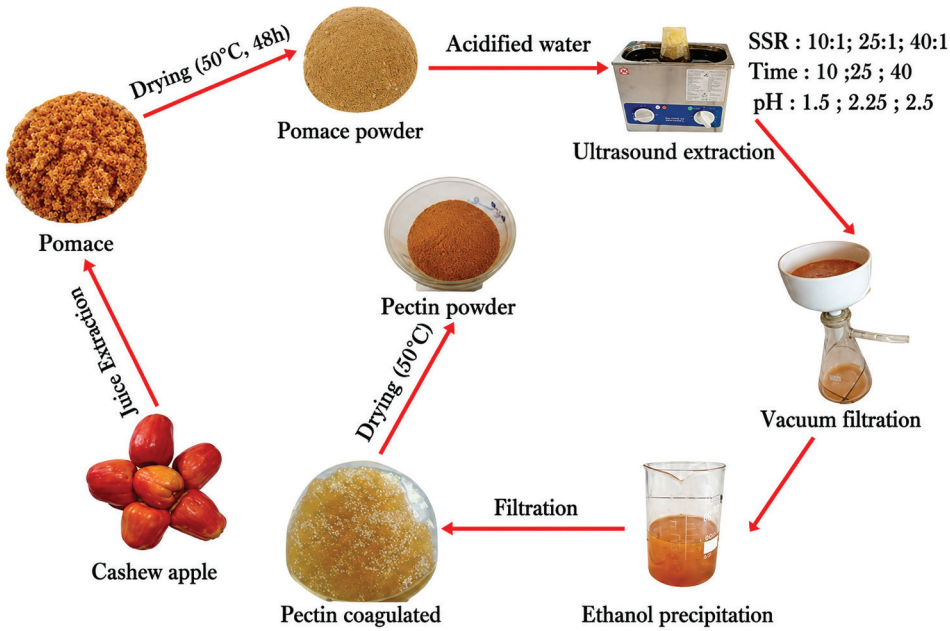

Figure 1: Diagram of the pectin extraction process from cashew apple pomace using ultrasound-assisted extraction.

Table 1: Analysis of variance of the fitted quadratic model for cashew apple pectin yield.

Source	Sum of squares	df	Mean square	F-value	*P*-value
Model	58.96	9	6.55	186.51	<0.0001***
SSR	0.4753	1	0.4753	13.53	0.0143*
Time	19.22	1	19.22	547.19	<0.0001***
pH	33.42	1	33.42	951.33	<0.0001***
SSR×Time	0.4225	1	0.4225	12.03	0.0179*
SSR×pH	1.95	1	1.95	55.4	0.0007***
Time×pH	0.81	1	0.81	23.06	0.0049**
SSR^2	0.252	1	0.252	7.17	0.0439*
$Time^2$	2.15	1	2.15	61.32	0.0005***
pH^2	0.5509	1	0.5509	15.68	0.0107*
Residual	0.1756	5	0.0351		
Lack of Fit	0.0956	3	0.0319	0.7969	0.5982 ns
Pure Error	0.08	2	0.04		
R^2	0.997				
Adjusted R^2	0.992				
Predicted R^2	0.991				
C.V.	3.57%				

SSR: Solvent to solid ratio; df: Degree of freedom; C.V.: Coefficient of variation; R^2: Coefficient of determination; ns: Non-significant; *: Significant; **: Highly significant; ***: Extremely significant.

mixture was magnetically stirred (150 rpm) for 2 min and placed in an ultrasonic bath (Bandelin SONOREX Super RK 100 H, 35 KHz) at maximal power (320 W) for the required process time. The resulting mixture was vacuum filtered on a 0.45 µm Whatman paper to obtain clarified pectin extract, immediately cooled to room temperature using an ice bath. Pectin was precipitated by adding 96% ethanol (1:1) while gently stirring at 50 rpm and incubating overnight at 4°C. The precipitated pectin was washed 3 times with 100 mL of 96% ethanol and then dried in an air oven at 50°C until a constant weight. For the conventional method, maceration was carried out at 80°C using a heating plate with magnetic agitation at 150 rpm, following the same procedure under the optimal conditions determined by UAE optimization.

2.4. Experiment Design and Optimization

To evaluate the factor effects and optimize pectin extraction yield, a BBD was conducted using Design-Expert version 13 software (Stat-Ease, MN, USA). Fifteen experiments, including three repetitions at central points, were randomly performed [Table 2]. The quadratic polynomial model was developed from experimental data, and its reliability was evaluated based on lack of fit, regression coefficient, and analysis of variance (ANOVA), *P*-value allowing to determine the significant factors of the model.

$$Y = \beta_0 + \sum_{j=1}^{K} \beta_j X_j + \sum_{j=1}^{K} \beta_{jj} X_j^2 + \sum_i \sum_{<j=2}^{K} \beta_{ij} X_i X_j \quad (1)$$

where Y is the predicted response; β_0 is a constant, β_j, β_{jj}, and β_{ij} are regression coefficients for linear, quadratic, and linear interactive effect terms, respectively; k is the number of factors (k = 3), and X_i and X_j are factors (independent variables) in coded values.

Table 2: Box–Behnken design matrix of independent variables with actual and coded values along with the experimental and predicted responses of ultrasound-assisted extraction of pectin from cashew apple pomace.

Run	Independent variables			Pectin yield (%)	
	SSR (mL/g)	Time (min)	pH	Experimental	Predicted
15*	25:1 (0)	25 (0)	2.25 (0)	6.00	6.00
6	40:1 (+1)	25 (0)	1.5 (−1)	6.98	6.94
9	25:1 (0)	10 (−1)	1.5 (−1)	5.00	4.89
1	10:1 (−1)	10 (−1)	2.25 (0)	2.80	2.86
14*	25:1 (0)	25 (0)	2.25 (0)	5.80	6.00
12	25:1 (0)	40 (+1)	3.0 (+1)	3.80	3.91
11	25:1 (0)	10 (−1)	3.0 (+1)	1.80	1.71
7	10:1 (−1)	25 (0)	3.0 (+1)	2.33	2.37
5	10:1 (−1)	25 (0)	1.5 (−1)	7.80	7.85
13*	25:1 (0)	25 (0)	2.25 (0)	6.20	6.00
8	40:1 (+1)	25 (0)	3.0 (+1)	4.30	4.25
3	10:1 (−1)	40 (+1)	2.25 (0)	6.75	6.61
10	25:1 (0)	40 (+1)	1.5 (−1)	8.80	8.89
4	40:1 (+1)	40 (+1)	2.25 (0)	6.50	6.44
2	40:1 (+1)	10 (−1)	2.25 (0)	3.85	3.99

*: Center point run, SSR: Solvent to solid ratio.

The pectin extraction process was optimized using Derringer's desirability function numerical methodology as described by Kamal *et al*. [22]. The optimal conditions obtained were then validated by statistically comparing the predicted model response value with the mean value of three experimental trials conducted under these optimal conditions.

2.5. Determination of Physicochemical Properties of CAPP

2.5.1. Extraction yield

Pectin extraction yield was calculated using the equation proposed by Kamal *et al*. [22].

$$Y_p(\%) = \left(\frac{W_d}{W_{cp}}\right) \times 100 \quad (2)$$

where Y_p is the yield of extracted pectin (%), W_d is the weight of obtained dried pectin (g), and W_{cp} is the amount of dried cashew pomace powder used for extraction (g).

2.5.2. Moisture content (MC) and ash content (AC)

MC was determined using the Association of Official Analytical Chemists method [31] by drying 1.0 g of pectin in an oven (Memmert U 15, Germany) at 105°C until a constant weight. The MC was then calculated using the equation.

$$MC\ (\%) = \frac{W_1 - W_2}{W_1 - W_0} \times 100 \quad (3)$$

where W_0, W_1, and W_2 are the weight of the empty crucible, the weight of the crucible + pectin sample before drying, and the weight of the crucible + pectin sample after drying, respectively.

AC was determined using the AOAC [31] method by ashing 1 g of pectin sample in a crucible at 550°C for 4 h using a muffle furnace

(Heraeus electronic, France). After cooling in a desiccator at room temperature, the AC was calculated using the equation.

$$AC\ (\%) = \frac{W_3 - W_0}{W_1 - W_0} \times 100 \quad (4)$$

where W_0, W_1, and W_3 are the weight of the empty crucible, the weight of the crucible + pectin sample before ashing, and the weight of the crucible + pectin sample after ashing, respectively.

2.5.3. pH and titratable acidity (TA)

The AOAC [31] method, with slight modifications, was used to determine the pH of a 1 g solution of pectin powder in 50 mL of distilled water using a pH meter electrode (Hanna HI5222-01).

For TA, 10 mL of pectin vacuum filtrate (1 g in 50 mL) under magnetic stirring was titrated with 0.1 M NaOH using phenolphthalein as an indicator [32]. TA was calculated as follows:

$$TA = \frac{0.1 \times V_{NaOH}}{W} \times 1000 \quad (5)$$

where W is the pectin sample weight (g), V_{NaOH} is the volume of NaOH poured (mL), and 0.1 is the NaOH normality.

2.5.4. Degree of esterification (DE)

The DE of pectin was determined using the titrimetric method of Wang *et al*. [33]. After complete dissolution of 50 mg of pectin in 10 mL of boiling distilled water under magnetic stirring, the resulting solution was titrated with 0.1 M NaOH volume (V_1) using phenolphthalein (4 drops) as an indicator. Thereafter, this solution was saponified by adding 20 mL of NaOH under continuous stirring at 400 rpm for 30 min and then neutralized with 20 mL of HCl 0.5 M prior to a titration with 0.1 M NaOH (V_2). The DE was then calculated using the equation.

$$DE\ (\%) = \frac{V_2}{V_1 + V_2} \times 100 \quad (6)$$

2.5.5. Methoxyl content (MeOC)

The MeOC was determined following the procedure described by Fakayode and Abobi [34]. This involved adding 25 mL of 0.25 N NaOH to the final solution obtained from the EW procedure and thoroughly mixing it. After incubating the mixture for 30 min at room temperature, 25 mL of 0.25 N HCl was added before titration with 0.1 N NaOH. The MeOC (%) was then calculated using Equation 7.

$$MeOC = \frac{V_{NaOH} \times N_{NaOH} \times 31}{W_p} \quad (7)$$

where Wp is the weight of the sample (g), N_{NaOH} and V_{NaOH} are the normality and the volume (mL) of NaOH, respectively, and 31 is the molecular weight of the methoxy group.

2.5.6. Equivalent weight (EW)

EW was determined using the method reported by Fakayode and Abobi [34]. Initially, 0.5 g of pectin powder was placed in a 250 mL conical flask, followed by the sequential addition of 5 mL of ethanol, 1 g of NaCl to sharpen the endpoint, and 100 mL of distilled water, while stirring at 300 rpm. After 1 h, the mixture was slowly titrated with 0.1 N NaOH until the solution was pink using a phenolphthalein indicator. The equation was then used to calculate the EW.

$$EW\ (\%) = \frac{W_p \times 1000}{N_{NaOH} \times V_{NaOH}} \quad (8)$$

where Wp is the weight of the sample (g), N_{NaOH} and V_{NaOH} are the normality and the volume (mL) of NaOH, respectively.

2.5.7. Anhydrouronic acid content (AUAC)

AUAC, which indicates the purity of the extracted pectin, was estimated according to the following equation of Nguyen and Pirak [35].

$$AUAC\ (\%) = \frac{176 \times 0.1\ z \times 100}{W_p \times 1000} + \frac{176 \times 0.1y \times 100}{W_p \times 1000} \quad (9)$$

where 176 g/mol is the molecular weight of AUA; *z* and y are the volume (mL) of NaOH in EW and MeOC determination, respectively, and Wp is the sample weight (g).

2.6. Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy was performed to gain insight into the chemical functionality of CAPP. Before the measurement, samples were mixed with potassium bromide (1:100), pressed into pellets, and then the FTIR spectra were determined using a Nicolet 5700 FTIR spectrometer (Thermo Fisher Scientific, USA) in the wavenumber range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.7. Determination of Functional Properties of CAPP

2.7.1. Water solubility

The method described by Bamba *et al*. [32], with a slight modification, was used to determine pectin water solubility (PWS) by dissolving 1 g of powder in 100 mL of distilled water under magnetic agitation for 20 min. After centrifugation of the resulting mixture at 4000 rpm for 5 min (HETTICH model EBA III centrifuge), 25 mL of supernatant was oven-dried at 105°C until a constant weight. The PWS was then calculated as follows:

$$\text{Water solubility}\,(\%) = \frac{W_{dw}}{0.25 \times W_S} \quad (10)$$

where W_{dw} denotes the dry weight of 25 mL (g), and W_S is the weight of the supernatant (g)

2.7.2. Water holding capacity (WHC) and oil holding capacity (OHC)

WHC and OHC of pectin were determined using the method described by Kazemi *et al*. [21] with a few modifications. Pectin powder (2 g) was added to 20 mL of distilled water or palm oil in a pre-weighed 50 mL centrifuge tube and then vortexed for 1 min. After standing for 10 min for complete wetting, the tube was centrifuged at 3500 rpm for 30 min, the supernatant was discarded, and the swollen pectin slurry was weighed. WHC and OHC, expressed as grams of water or oil retained per gram of pectin sampled, were calculated as follows:

$$\text{WHC or OHC}\ (\%) = \frac{W_2 - W_1}{W_1 - W_0} \quad (11)$$

where W_0 is the weight of the empty centrifuge tube, W_1 is the weight of the centrifuge tube containing the sampled pectin powder, and W_2 is the weight of the centrifuge tube containing the swollen pectin slurry.

2.7.3. Emulsifying activity (EA) and emulsion stability (ES)

Pectin EA and ES were evaluated following the method described by Kazemi *et al*. [21] with some modifications. For this purpose, 5 mL

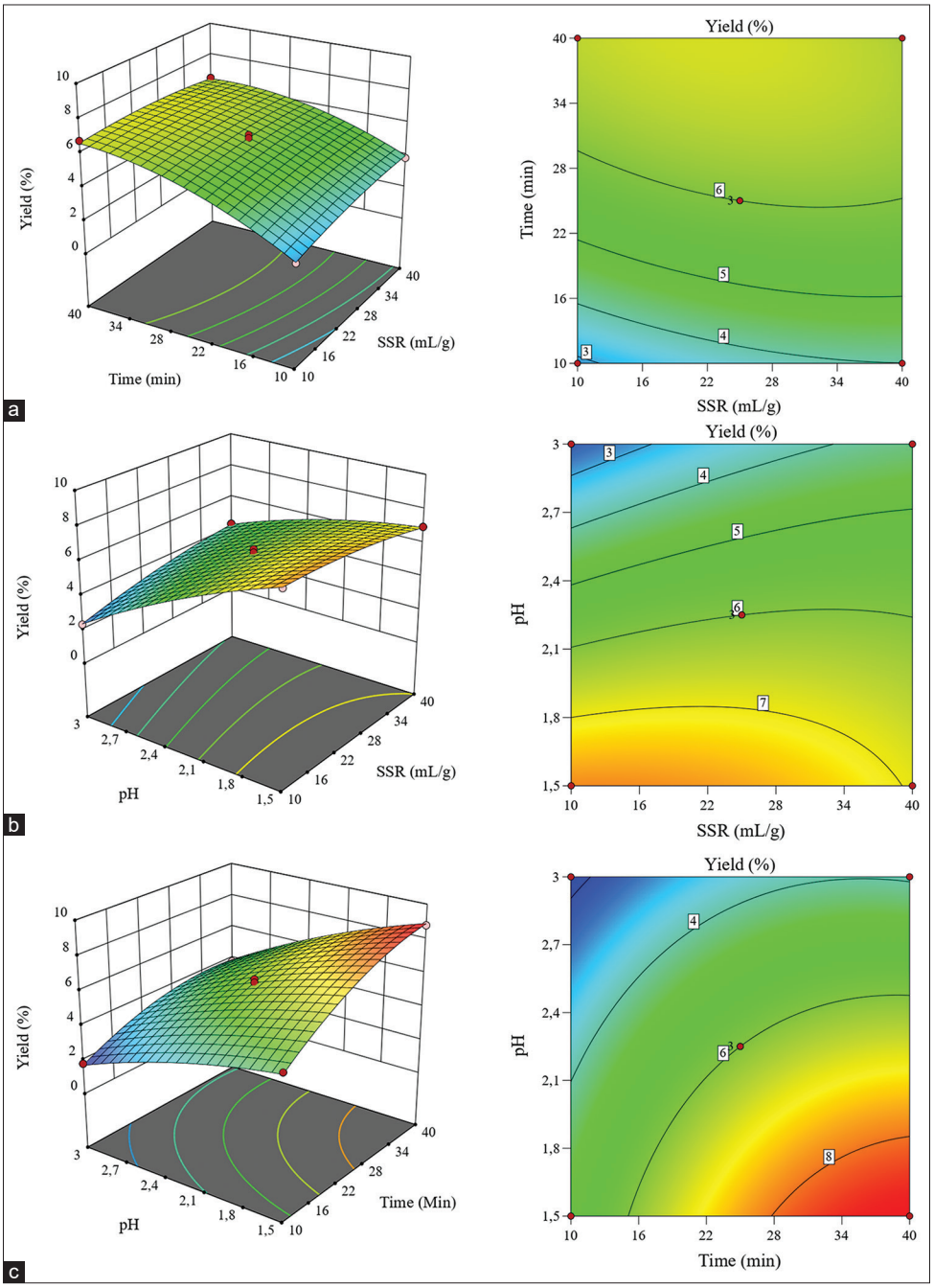

Figure 2: 3D response surface and contour plots of significant interaction effects between independent variables on pectin yield: Sonication time and solvent-to-solid ratio at pH 2.25 (a), solvent pH and solvent-to-solid ratio at 25 min (b), solvent pH and sonication time at 25 mL/g (c).

of palm oil was added to a 5 mL pectin aqueous solution (0.5% w/v) containing 0.02% sodium azide in a 15 mL centrifuge tube. The mixture was then subjected to 10 min of ultrasonic bath treatment and subsequently centrifuged at 4500 rpm for 10 min. Thereafter, the resulting emulsion was stored at 4°C for 1 and 30 days. The EA and ES were calculated using equations.

$$EA(\%) = \frac{V_E}{V_T} \times 100 \quad (12)$$

$$ES(\%) = \frac{V_R}{V_E} \times 100 \quad (13)$$

where V_E is the volume of the fresh emulsifying layer (t = 0 day), V_T is the total volume of the system, and V_R is the volume of the remaining emulsifying layer after storage (t = 1 and 30 days).

2.7.4. Foaming capacity (FC) and foam stability (FS)

For pectin FC and FS, two pectin solutions at 2% and 4% were prepared by gently dissolving 0.5 and 1 g of pectin into 25 mL beakers at 100 rpm at room temperature, respectively. Subsequently, 10 mL of each prepared solution was poured into a 15 mL centrifuge tube and vortexed for 3 min [35]. FC and FS were expressed as the percentage of volume increase and the remaining volume after vortexing and after storage for 30 min, respectively. They were calculated using equations.

$$FC(\%) = \frac{V_0 - V_I}{V_I} \times 100 \quad (14)$$

$$FS(\%) = \frac{V_{30} - V_I}{V_I} \times 100 \quad (15)$$

where V_I, V_0, and V_{30} are the initial volume before vortex, just after vortex, and after storage for 30 min, respectively.

2.8. Statistical Analysis

The experimental design and statistical analysis of the BBD were carried out using Design-Expert version 13 (Stat-Ease, MN, USA), whereas the characterization analysis results were examined using Statistica 13.5. All characterization experiments were performed in triplicate, and the results were presented as mean values with their standard deviations using Excel 2019. The Student's t-test and one-way ANOVA were performed to compare the data as appropriate, and significance was determined based on a $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Modeling of the Ultrasound Pectin Extraction Process

The pectin production yield using a three-level BBD is presented in Table 2. The results showed that pectin yield ranged from 1.8% to 8.8%. The lowest value was obtained in run number 11 (solvent/solid ratio = 25:1 mL/g, extraction time = 10 min, and pH = 3.0), whereas the highest was in run number 10 (solvent/solid ratio = 25:1 mL/g, extraction time = 40 min, pH = 1.5). In addition, the multiple regression analysis applied to experimental data generated a second-order polynomial equation (Equation 16) as a model for the extraction process.

$$\text{Yield (\%)} = 6.000 - 0.244 \times \text{solvent–solid ratio} + 1.550 \times \text{Time} - 2.044 \times \text{pH} - 0.325 \times \text{solvent–solid ratio} \times \text{Time} + 0.698 \times \text{solvent–solid ratio} \times \text{pH} - 0.450 \times \text{Time} \times \text{pH} - 0.261 \times \text{solvent–solid ratio}^2 - 0.763 \times \text{Time}^2 - 0.386 \times \text{pH}^2 \quad (16)$$

As observed from the ANOVA results in Table 1, the *P*-value of the model was significant ($P = 0.000096 < 0.05$), suggesting that the generated model is significant. In addition, the *P*-value of the lack-of-fit was insignificant ($P > 0.05$), indicating the adequacy of the model. Furthermore, the good fitness of the model is demonstrated by the high values of the determination coefficients ($R^2 = 0.997$, adjusted $R^2 = 0.992$, and predicted $R^2 = 0.991$). This result implies that more than 99.7% of the yield variation could be explained by the proposed model [21]. Besides, the very low coefficient of variation (3.7 < 10%) suggests the conducted experiments' reliability. These values are consistent with those reported in recent studies on optimizing pectin extraction from several agricultural waste matrices. Shivamathi *et al*. [37] reported a R^2 of 0.9996 and a CV of 0.32% for cinnamon apple skin, indicating excellent correlation and minimal variability. Similarly, Hosseini *et al*. [38] observed a R^2 of 0.9961 for bitter orange peel. For guava pomace, Kamal *et al*. [22] reported a R^2 of 0.9865 and a CV of 1.96%, whereas Mada *et al*. [39] found a R^2 of 0.9926 and a CV of 3.01% for mixed banana and papaya peelings. The high reliability could be attributed to the combined action of drying, grinding, and sieving applied to the sample before extraction, which stabilizes its MC, particle size, and analyte distribution [40-42], leading to the reduction of sample heterogeneity, the improvement of reproducibility, and the predictive performance enhancement of statistical models. Furthermore, the lack-of-fit test, which measures the model's inability to represent the data, was insignificant [Table 1], providing further evidence that the obtained model was significant and the quadratic polynomial model was well fitted with the data [22,33]. Based on the above results, the second-order polynomial model developed by RSM-BBD is suitable to describe pectin maximization extraction from cashew apple pomace.

3.2. Effects of Extraction Factors on Pectin Yield

The ANOVA illustrates the impact of each variable on pectin yield [Table 1]. All independent variables (solvent-to-solid ratio, extraction time, and pH) significantly affected pectin yield. The linear terms of pH and time, the interaction term of solvent–solid ratio×pH and $Time^2$ were the most significant factors ($P < 0.001$) in pectin extraction, followed by the interaction term of Time×pH ($P < 0.01$), then the linear term of solvent–solid ratio, its interaction term with Time, and its square term and pH^2 ($P < 0.05$). Moreover, all terms negatively affected the pectin yield, except the linear term of time and the interaction term solvent–solid ratio×pH, which had a positive effect.

Three-dimensional response surfaces and contour plots [Figure 2] generated from the model made it possible to visualize the first-order individual and interaction effects of factors on the pectin extraction while placing the third at its central level. Thus, [Figure 2b and c], presenting the effect of pH on extraction yield, shows that a decrease in pH from 3 to 1.5 results in a significant increase in pectin yield. This finding is in line with the results of Dranca and Oroian [7] and Hosseini *et al*. [38] on apple pomace and sour orange peel, respectively. However, it contradicts the findings of Zamboi *et al*. [8] and Kamal *et al*. [22], who showed an increase in pectin yield from guava pomace and *Opuntia ficus-indica* cladodes, respectively, with an increase in pH up to the optimal value, followed by a decrease. Low

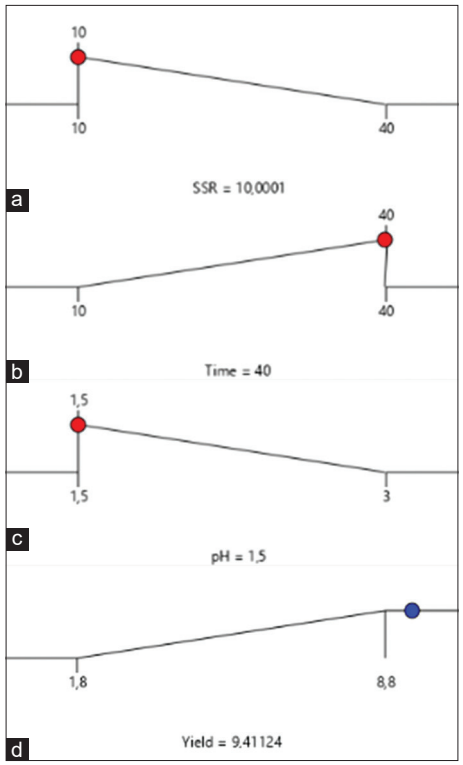

Figure 3: Desirability functions of maximizing pectin extraction yield from cashew pomace showing optimized extraction conditions for each independent variable: Solvent-to-solid ratio (a), Sonication time (b), Solvent pH (c), and the associated predicted yield value (d).

pH conditions result in a high concentration of hydrogen ions that activate the hydrolysis of protopectin to soluble pectin, increasing the mass transfer of pectin from the plant matrix into the solvent [7,34], which results in an enhancement of yield. Furthermore, the interactive combination of pH with time and pH with solvent–solid ratio showed a negative and positive effect on the pectin extraction process, respectively. Dranca and Oroian [7] reported a negative effect with the pH-time interaction, while the pH-solvent–solid ratio interaction had no significant effect. This could be attributed to pectin degradation by ultrasonic cavitation throughout the sonication time [8] while a high solvent–solid ratio increased the exchange area between solvent and plant matrix, thus increasing mass transfer and facilitating pectin extraction.

Sonication time also highly affects the pectin extraction from cashew apple pomace, Equation (16). By varying sonication time from 10 to 40 min, the pectin extraction yield increased, as shown in [Figure 2a and c]. Regarding the impact of sonication time on pectin extraction yield, the results are diverse and varied. Some researchers [34,43,44] reported similar results, others indicated an initial increase followed by a decrease over time [7,45,46], whereas Hosseini *et al*. [38] found opposite results. The increase in yield observed in this study could be attributed to the enlargement of plant cell wall pores by ultrasonic cavitation, facilitating diffusion of solvent into the plant matrix and release of pectin, which increases the pectin yield [7,45,46]. Conversely, the decrease in extraction yield could be due to pectin fragmentation resulting from overexposure to ultrasound [38], the gradual decrease in concentration gradient, and the increase in viscosity of the extraction medium [34]. In addition, this study showed that the interaction between sonication time and solvent/solid ratio had a negative effect on pectin extraction yield, whereas Dranca and Oroian [7] reported that this had no effect on pectin yield.

The solvent–solid ratio showed a significant linear effect on the pectin extraction from cashew apple pomace. As shown in Figure 2a and b, the increase in solvent–solid ratio from 10:1 to 40:1 leads to an enhancement of pectin yield. However, literature showed contradictory results regarding this factor's effect. The authors observed an increase in pectin yield [43,44], an increase followed by a decrease in pectin yield [37,45], a decrease in pectin yield [8], and a lack of effect [7] with the increase in solvent–solid ratio. The pectin yield increases with the increase in solvent–solid ratio, which could be due to the higher exchange surface area between the plant matrix and the solvent, resulting in a higher mass transfer induced by ultrasonic waves [37]. However, the pectin yield decreased in this study, despite the increase in ratio, which could probably be related to the difficulties of ultrasonic waves to propagate in a large volume of work [8] and/or due to the incorrect distribution of ultrasonic power within the system [47].

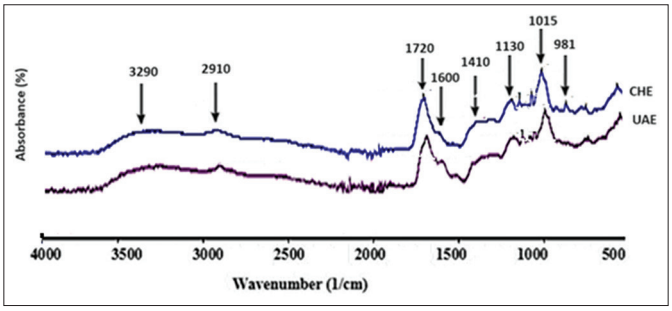

Figure 4: Fourier transform infrared spectrum of cashew apple pomace peel pectin using ultrasound-assisted extraction and conventional extraction.

3.3. Numerical Optimization and Experimental Validation of Pectin Extraction Conditions

The conditions maximizing CAPP extraction were obtained from the developed quadratic model using the Derringer desirability function approach [22]. As shown in Figure 3, the optimal conditions were a sonication time of 40 min, a solid–solvent ratio of 10:1 mL/g, and a solvent pH of 1.5, resulting in a predicted pectin yield of 9.41% with a desirability value of 1.00. Experimental validation of these conditions yielded 9.54 ± 0.35%, which was not significantly different from the predicted value, as the coefficient of variation (CV) was below 10% [Table 3]. These results demonstrated the reliability of the developed model for optimizing pectin extraction from cashew apple pomace.

Under these optimal conditions, the UAE showed a significantly higher pectin yield (24.9 % higher) than that of conventional maceration extraction (CME) ($P < 0.05$). These results are in line with those for grapefruit peel using HCl (16.34% [32]), finger citron pectin using HCl (46.15% [27]), and pomelo pectin using citric acid (28.14% [48]). This could be explained by the fact that ultrasound improves the solvent's access to the internal structure of the particles by breaking down the cell wall caused by the bursting of cavitation bubbles on contact with the tissue, thereby releasing more pectin [33,35,48]. Furthermore, this action is reinforced by the ability of ultrasound to rapidly improve tissue hydration and reduce swelling during soaking, thereby increasing extraction yield in less time [35].

However, the yields obtained in this study were lower than those reported by Yapo and Koffi [5] (10.7–25.3%) but higher than the 6.5% reported by Tamiello-Rosa *et al*. [6] for CAPP extracted using the conventional Soxhlet method. These differences among studies indicate that CAPP yield is strongly influenced by the experimental conditions and the extraction technique applied. These authors have demonstrated that conventional methods require a lot of time and high temperatures to obtain a high yield.

Furthermore, the pectin yield obtained through UAE in this study was comparable to that reported for *Malus domestica* Fălticeni pectin extracted with citric acid at pH 1.8 (9.18% [7]), chayote pectin extracted with distilled water at pH 1.9 (6.19% [49]) and *Helianthus annuus* heads pectin with citric acid at pH 3.2 (8.89% [45]). Nevertheless, it remained lower than the yields obtained from pummelo peel pectin with HCl at pH 2 (8.89% [24]) and *Spondias tuberosa* L. pectin with citric acid at pH 1.5 (22% [26]). These findings suggest that pectin extraction yields greatly depend on the vegetal source, operating conditions, and extraction process applied.

Table 3: Cashew pomace pectin extraction yield using ultrasound-assisted (UAE) and conventional (CE) extraction under optimal extraction conditions.

Prediction vs Experiment	Pectin yield (%)	
	UAE	CE
Predicted value	9.41[a]	-
Experimental value	9.54±0.35[a]	7.64±0.53[b]
Error (%)	1.38	-

Values in the same line and same column with different superscript symbols (a, b) mean significant difference ($P<0.05$).

Table 4: Comparative physicochemical and functional properties of pectin extracted under optimal conditions using ultrasound-assisted (UAE) and conventional (CE) extractions.

Properties	Characteristics	CE	UAE
Physicochemical	Moisture content (%)	6.23±0.17^{a}	5.36±0.13^{b}
	Ash content (%)	3.57±0.24^{a}	2.79±0.25^{b}
	pH	3.23±0.08^{a}	2.91±0.15^{b}
	Titratable acidity (%)	2.49±0.10^{a}	2.18±0.03^{b}
	Degree of esterification (%)	70.90±1.35^{b}	75.20±1.04^{a}
	Equivalent weight (g/mol)	680.63±4.04^{b}	900.11±6.54^{a}
	Methoxyl content (%)	5.03±0.19^{b}	6.23±0.33^{a}
	Anhydrouronic acid content (%)	68.81±0.91^{b}	71.16±0.16^{a}
Functional	Solubility (%)	31.33±0.57^{a}	28.67±1.52^{b}
	Water holding capacity (g water/g)	3.30±0.39^{a}	2.18±0.13^{b}
	Oil holding capacity (g oil/g)	1.85±0.16^{b}	2.19±0.07^{a}
	Emulsifying activity (%)	39.5±0.86^{a}	36.67±1.52^{b}
	Emulsifying stability (%)		
	After 1 day	43.01±1.77^{a}*	40.88±1.09^{a}*
	After 30 days	41.34±0.55^{a}*	38.79±2.87^{a}*
	Foam capacity (%)		
	2	13.66±0.57^{a}*	12.33±0.57^{b}*
	4	14.66±0.57^{a}*	13.33±0.58^{b}*
	Foam stability (%)		
	2	2.66±0.58^{c}	1.67±0.58^{c}
	4	4.66±0.57^{a}	3.33±0.57^{b}

Values in the same line with different superscript symbols (a, b, c) mean significant difference (P<0.05), and values in the same column with the same star symbols (*) mean significant difference (P<0.05).

3.4. Physicochemical Properties of Pectin Obtained Under Optimal Conditions

3.4.1. Moisture and AC

Table 4 shows that pectin MC obtained by UAE (5.36 ± 0.13%) was significantly lower (P < 0.05) than that of CME (6.23 ± 0.17%). These results contrast with the findings of Nguyen and Pirak [35], who reported similar pectin MCs for CME and UAE for dragon fruit peel, but are consistent with those reported for grapefruit peel using UAE (5.19%, [24]) and for seed-free grape marc using ultrasound-assisted microwave extraction (UAME) (5.85%, [50]). Although higher than the 4.61% reported for *S. purpurea* L. bark using UAE [16], these results were below the permissible limit of 12% recommended by the International Pectin Producers Association [51]. Indeed, low MC ensures better stability of pectin during storage by preventing or mitigating the potential of enzymatic and microbial degradation and also facilitates powder flow properties [15,35].

Pectin AC indicates the purity of pectin [15]. As shown in Table 4, the AC for pectin extracted by UAE (2.79 ± 0.25%) was significantly lower (P < 0.05) than that of CME (3.57 ± 0.24%). This is in agreement with the results reported by Hossain *et al*. [48] for pomelo peel pectin extracted by UAE (3.22%) and CHE (5.34%). However, these values were below the acceptable limit (10%) indicated in the International Pectin Producers Association guidelines [39], suggesting that CAPP extracted by UAE and CE are of high purity. In fact, lower AC demonstrates the good gel-forming capacity of pectin [35].

3.4.2. pH and TA

CAPP using UAE had a pH of 2.91 ± 0.15 and TA of 2.18 ± 0.03%, significantly lower (P < 0.01) than those obtained using CME, which were 3.23 ± 0.08 and 2.49 ± 0.10%, respectively [Table 4]. The findings for UAE and CME in this study contradicted those of Hossain *et al*. [48], who reported statistically similar pH values for UAE (3.03) and CE (2.98). However, pH values of both methods were lower than 3.15 and 3.0 reported for onion peel pectin using MAE with natural deep eutectic solvents and CE [47], respectively, and higher than 2.81 for *Averrhoa bilimbi* pectin using a shaking deep eutectic solvent [52]. The pH values in this study indicate that pectin was acidic and suitable for preservation during storage [32]. Furthermore, the low titratable acidity values obtained in this study suggest that the use of CAPP in food preparations would not be harmful to consumers and would make them taste less acidic/sour because fewer total acid molecules are present to react during consumption.

3.4.3. DE and MeOC

The DE describes the ratio of methanol-esterified carboxylic groups to free carboxyl groups [34]. It allows us to classify pectin. DE >50% indicates a highly methoxylated pectin (HMP), which forms gels in the presence of sugars; otherwise, it is classified as a low-methoxylated pectin requiring the presence of divalent ions to form gels [35]. The obtained pectin in this study had a DE > 50% regardless of the extraction process [Table 4], which suggests that it was HMP. These results contrast with those of Yapo and Koffi [5], who obtained low methylated pectin (DE 28–46%) using Soxhlet extraction with HNO_3, but coincide with those of Tamiello-Rosa *et al*. [6], who obtained highly methylated pectin (DE = 76%) using CE with water reflux for cashew apple pomace. The difference suggests that the use of different extraction methods and solvents led to different DE values, suggesting a strong dependence of DE on extraction conditions.

Moreover, UAE (75.20 ± 1.04%) resulted in significantly higher DE (P < 0.05) than CME (70.90 ± 1.35%). These results were not consistent with those reported by Wang *et al*. [33], who obtained a similar DE (~66.56%) for grapefruit HMP using CE and UAE with HCl, and Hossain *et al*. [48], who obtained a lower DE for UAE (63.95%) compared to CE (68.94%) for pomelo skin HMP using citric acid. In addition, lower DE values were reported by Nguyen and Pirak [35] for dragon fruit peel (26.57–49.87%) and by Vathsala *et al*. [24] for sweet lime and grapefruit (27.53% and 22.14%, respectively) using UAE with HCl. From the above results, it can be concluded that the DE value greatly depends on the extraction method, acid type, and concentration, and organ origin [24,48]. Furthermore, HMPs, such as those obtained in this study, exhibit rapid gelation properties at high temperatures with a more effective action on lipid profiles [34], and are generally used for canning applications [21].

The DE is always evaluated with the MeOC, which represents the actual amount of methyl esterification in a sample [34], i.e., the number of moles of methyl alcohol/100 moles of galacturonic acid [35]. From Table 4, the extraction method significantly affected the MeOC of CAPP. UAE leads to higher MeOC (6.23 ± 0.33%) than CME (5.03 ± 0.19%). These values were lower and not consistent with those reported by Hossain *et al*. [48] for pomelo peel, where CE (10.34%) showed higher MeOC than UAE (8.37%) using citric acid. Higher MeOC (8.36 ± 0.28%) was also reported by Bhat *et al*. [15] for immature apple pectin using CHE with citric acid, while a similar MeOC value (6.23%) was reported by Fakayode and Abobi

[34] using CHE with HCl. Therefore, the difference observed seems to be related to the type of acid, extraction method, and organs [24,35]. However, the MeOC values in this study are within the standard pectin tolerable values (2–7%) reported by the International Pectin Producers Association [50]. MeOC controls gel-forming ability, setting time, sensitivity to metal ions, gel strength, and overall structural and functional properties of pectin gels [24,35].

3.4.4. EW

EW indicates the presence of a free, non-esterified galacturonic acid group within the pectin molecular chain [24] and determines pectin gel-forming and emulsion capacities and stabilities [15]. Table 4 shows that UAE (900.11 ± 6.54 g/mol) presents a significantly higher EW value ($P < 0.05$) than CE (680.62 ± 44.04 g/mol). These results are in line with EW values of 685.01 ± 9.39 and 717.86 ± 12.92 g/mol reported for CE and UAE, respectively, by Hossain *et al*. [48]. Besides, Filho *et al*. [4] found a lower EW value (478.12 g/mol) for *S. purpurea* L. peel pectin using UAE. Thus, the lower EW value could be attributed to the molecular chain degradation effect of CE due to high temperature [53,54]. However, the high EW values obtained in this study indicate that the CAPP can retain large amounts of water to form a gel [15,24] and to stabilize food systems [43].

3.4.5. AUAC

AUAC indicates the purity of pectin, which should be at least 63% [34,48]. In the present study, the AUAC of the obtained pectin was significantly different, with the highest value found with UAE (71.16 ± 0.16%) compared to CE (68.81 ± 0.91%) [Table 4]. These findings are in contrast with those reported by Hossain *et al*. [48], who found AUAC using UAE (72.04%) lower than CE (82.72%) with citric acid. However, Nguyen and Pirak [35] reported similar AUAC values using UAE (87.19%) and CE (83.30%) at 75°C for 30 min for dragon fruit peel. Moreover, they showed lower AUAC values (66.55%) with UAE than CE (78.45%) using citric acid at 75°C for 60 min. As a result, AUAC depends on the extraction conditions and pectin origins. However, CAPP with a high AUAC value demonstrates its high purity and low protein, starch, and sugar levels [35,48].

3.5. FTIR

The infrared Fourier transform spectra of CE and UAE are illustrated in Figure 4. A general comparison of the spectra reveals that the pectin obtained from both extraction methods has the same FTIR peak profile across all regions (4000–500 cm^{-1}), suggesting that the extraction method did not influence the primary structural conformation. Similar results were reported for pectin extracted from durian rind, pomelo peel, and dragon fruit peel using UAE and CE [48,55,56]. The broad peak within 3500–3000 cm^{-1} corresponds to O–H stretching vibrations [8,24,48,55,56], which were related to the inter- and intramolecular hydrogen bonding of galacturonic acid backbone [15,57]. Moreover, a smaller broad shoulder observed around 2900 cm^{-1} can be attributed to C–H stretching vibration of –CH, –CH2, and –CH3 [51,57] and the methyl ester of the galacturonic acid [8,56]. The absorption band at around 1720 cm^{-1} and a smaller broad shoulder band at 1610 cm^{-1} correspond to C=O of esterified carboxylic groups (-$COOCH_3$) and free carboxylic groups (-COOH), respectively [15,24,33,55]. In addition, the increased peak intensity at 1720 cm^{-1}indicates that the extracted pectin belongs to the HM group, regardless of the extraction method, which aligns with the analysis of DE [7,46]. Furthermore, the peak at 1410 cm^{-1} observed in the 1400–1450 cm^{-1} region is often interpreted differently. Some authors attributed it to the C–H bending vibration, [24,30] whereas others related it to the symmetric carboxylate ion (COO−) stretching [8,49]. Moreover, spectra ranging from 1300 to 800 cm^{-1} are generally referred to as the «fingerprint» zone for carbohydrates [23,47,57]. In this region, only the peaks at 1130, 1015, and 981 cm^{-1} were observed for both pectin types. The peaks at 1130 and 1015 cm^{-1} correspond to C–OH, C–C, and C–O–C glycosidic bond stretching vibrations, suggesting the presence of pyranose in the pectin structure as previously reported by Yu *et al*. [27] for cucurbitaceae pectin and Vathsala *et al*. [24] for pummelo peel pectin. Similarly, the presence of pyranose was observed at 1015 cm^{-1} [6] and 1010 cm^{-1} [33]. In addition, the absorption band at 981 cm^{-1} is associated with the presence of D-glucopyranosyl groups [58].

3.6. Functional Properties of Pectin Obtained Under Optimal Conditions

3.6.1. Solubility of pectin

Solubility serves as an indicator of how an ingredient will behave in the preparation process and its potential impact on the final product's appearance, texture, structure, and taste [32]. Table 4 shows the solubility values of CAPP extracted by UAE and CE. The solubility of pectin in UAE (28.67 ± 1.52%) was significantly lower than in CE (31.33 ± 0.57%). This trend is similar to the solubility values reported by Bamba *et al*. [32] for lemon peel pectin (29.22%), but much lower than those of sunflower by-product (81.36%) reported by Ezzati *et al*. [46]. Indeed, water solubility is greatly influenced by the analytical conditions (hot or cool water) and the pectin drying process [32]. Hossain *et al*. [48] demonstrated that pomelo peel pectin extracted by UAE and CE was insoluble in cool water but soluble in hot water. In general, a lower solubility indicates a higher gel formation capacity [59]. Moreover, a higher DE and molecular weight lead to lower solubility [56], which is consistent with the average solubility observed in this study due to the average DE and MeOC.

3.6.2. WHC and OHC

Table 4 presents the WHC and OHC of CAPP. The results indicate that WHC and OHC were significantly different ($P < 0.05$) between UAE and CE. UAE exhibited the lowest WHC value (2.18 ± 0.13 g/g) and the highest OHC value (2.19 ± 0.07 g/g) compared to those of CE (3.30 ± 0.39 and 1.85 ± 0.16 g/g, respectively). These WHC values were lower than those of *O. ficus-indica* cladodes using CE (5.63 g/g [36]) and eggplant peel using UAE (6.22 g/g [21]), but higher than those of water-soluble polysaccharide of pistachio using CE (1.46 g/g [56]) and similar to those of chayote using UAE (3.14 g/g [49]) and watermelon rind using CE (2 g/g [59]). Various factors, including chemical composition, structure, and porosity of pectin, pH, temperature, and ionic strength [60], plant species and tissues, as well as extraction methods [13], affect pectin WHC. Regarding OHC, the values recorded for CAPP were higher than those reported for *O. ficus-indica* cladodes using CE (1.24 g/g [36]), lower than those indicated for chayote using UAE (3.73 g/g [49]) and watermelon rind using CE (4 g/g [59]), but similar to those reported for eggplant peel using UAE (2.12 g/g [21]). Nevertheless, the WHC and OHC values in this study were high, suggesting that CAPP may be used as a stabilizer or emulsifier in high-fat foods such as some meat products and as a syneresis-reducing agent in yogurt, dairy desserts, etc. [13,61].

3.6.3. Emulsifying properties

Emulsifying properties of pectin, including emulsion activity (EA) and ES, are shown in Table 4. The pectin obtained through UAE exhibited a significantly lower EA (36.67 ± 1.53%) than that of CE (39.47 ± 0.87%). In both methods, EA values were lower than those

reported for eggplant peel using UAE (56.16% [22]) and higher than reported data for *O. ficus-indica* cladodes using CE (26.9–30.77% [36]). They were similar to the EA of chayote using UAE (35% [49]) and *O. ficus-indica* cladodes using CE (35% [36]). This suggests that the emulsifying properties of pectin depend on both the extraction process [60] and the plant species. In addition, EA increases with the rise in pectin concentration [36,56]. Moreover, ES values were about 41.01% after storing the pectin emulsions at 4°C for 1 and 30 days, regardless of the pectin extraction method. These values were lower than those reported by Kazemi *et al*. [21] for eggplant pectin (96.36% after 1 day and 91.66% after 30 days) and lower than those reported by Ke *et al*. [49] for chayote pectin (88.36% after 1 day and 85.33% after 30 days), all obtained using UAE and stored at 4°C. Indeed, the decrease in ES values with the extension of storage time is due to the significant change in the pectin emulsion droplets' size distribution and spatial arrangement [13]. In this study, the ES can be attributed to the non-coalescence of emulsion droplets promoted by the formation of a solid network related to the high pectin molecular weight, the presence of methyl and acetyl groups in the main chain, and contaminants (ferulic acid, proteins, and minerals) in the structure of pectin [21,49,62].

3.6.4. Foaming properties

The FC and FS of the CAPP fractions obtained by UAE and CE at 2% and 4% concentrations are described in Table 4. As shown, these properties depended on both the extraction method and pectin concentration. The increase in pectin concentration from 2% to 4% increased foam FC and FS regardless of the extraction method. The highest FC values were reported for CE pectin (14.17 ± 0.75%) compared to UAE pectin (12.83 ± 0.74%) and were similar irrespective of pectin concentration for each method. For these pectin concentrations, Kazemi *et al*. [21] found an increase in FC value for eggplant peel pectin (FC: 14.33 and 32.67% at 2 and 4%, respectively) obtained by UAE, whereas Ke *et al*. [49] observed a decrease for chayote pectin obtained by UAE (2% (90.58 and 49.67% at 2% and 4%, respectively). Similarly, Ezzati *et al*. [45] showed a decrease in FC at low concentrations (FC: 12.87% and 11.97% at 0.3 and 1%, respectively) for sunflower byproduct pectin obtained by UAE. Moreover, a higher FC value (52%) than that of this study was obtained by Bayar *et al*. [62] for *O. ficus-indica* cladodes pectin obtained by CE at 2%. From these data, it can be stated that several factors, such as the technique of extraction and pectin origin, affect FC. On the other hand, an increase in CAPP concentration demonstrated a positive effect on FS values, and the highest value (4.66 ± 0.57%) was observed at 4% pectin obtained by CE, followed by UAE (3.33 ± 0.57%). This observation was in line with the reported data of Kazemi *et al*. [21] and Ezzati *et al*. [39] for sunflower byproduct pectin and eggplant peel pectin obtained by UAE, but contradicts the findings of Ke *et al*. [49] for chayote pectin obtained by UAE. The increase in FS at high pectin concentration could be due to the increase in the viscosity of the continuous phase, leading to the prevention of the coalescence of air bubbles [45].

4. CONCLUSION

Traditionally discarded cashew apple pomace was valorized into pectin. The effects of various factors investigated on the yield of pectin using BBD showed that UAE results in a higher yield than CE under optimal conditions of 40 min (sonication time), 10:1 mL/g (solvent-to-solid ratio), at a pH of 1.5 with ultrasonic bath power set at 320 W. Pectin from both methods had high purity due to its high AUAC (>68.81%) and low MeOC. Moreover, they were highly methylated (DE > 70.90%) with an EW higher than 680.62 g/mol. FTIR analysis pointed out a similar molecular structure for UAE and CE pectins. In addition, UAE pectin exhibited lower solubility, WHC, and EA, FC and FS, higher OHC, and similar ES and FC compared with that of CE. Moreover, the foaming properties increased with increasing pectin concentration regardless of the extraction method. Thus, cashew apple pomace can be a valuable source of pectin with interesting functional properties. However, rheological, release, and biofunctional studies of the extracted pectin are needed to determine its potential use and application in food product formulations.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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9. ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY STATEMENT

The study data are available from the authors upon request.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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