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PRODUCTION AND CHARACTERIZATION OF PECTIN FROM PRICKLY PEAR (*OPUNTIA FICUS-INDICA*) PEEL BY ORGANIC ACID EXTRACTION USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

The improper disposal of agro-industrial waste has emerged as a crucial environmental issue. Therefore, the extraction of valuable compounds from these residues presents a promising strategy to mitigate their environmental impact while promoting resource efficiency. Prickly pear (*Opuntia ficus-indica*) peels, which constitute about 45% of the whole fruit, represent an abundant and significant by-product of food processing. These peels have garnered scientific interest due to their potential as a sustainable source of pectin, a polysaccharide widely used in the food and pharmaceutical industries. Therefore, the aim of this research was to extract and characterize pectin obtained from prickly pear peels using citric acid as an environmentally friendly solvent. The effects of temperature and pH on pectin yield were evaluated using a central composite design, which allowed the identification of significant interactions and effects of these factors. The results revealed that pH significantly influences ($p = 0.001$) pectin yield, while temperature is an important factor to take into account during experiments ($p = 0.089$). The optimal conditions were found to be a temperature of 94.1°C and a pH of 1.8, under which a predicted pectin yield of 11.1% was obtained. Physicochemical characterization of the extracted pectin showed that its equivalent weight ranged from 224.2 to 897.8 g/mol. Methoxyl content varied between 3.6% and 5.0%, while degrees of esterification and anhydrouronic acid content ranged from 20.5% to 59.2% and from 48.0% to 98.6%, respectively. The degree of esterification of 20.5% at the optimal pH of 1.8 classified this pectin as low-ester pectin, which has distinct gelling and functional properties suitable for various applications. These results suggest that pH significantly affects both pectin yield and its physicochemical properties. In conclusion, prickly pear peels, typically considered waste, have the potential to be a viable and sustainable source of pectin. The extracted pectin exhibits physicochemical characteristics comparable to those of commercial pectins, highlighting its potential for industrial utilization in food formulations.

Key words: prickly pear peel, pectin, citric acid, acid hydrolysis, green process



INTRODUCTION

Pectin is the most demanded natural hydrocolloid in the food industry due to its functional properties, which include gelling, thickening, stabilizing, and emulsifying and is one of the main polysaccharides in plant cell walls, along with cellulose and hemicellulose [1, 2]. Currently, waste biomass, particularly from the processing of fruits and vegetables, is emerging as an attractive alternative for pectin extraction, contributing to reduce the impact of agro-industrial activities on the environment [3,4]. Pectin is characterized by its high galacturonic acid (GA) content and has various degrees of esterification (DE), which determine its functional properties and largely depend on the extraction method, processing conditions, and raw material [5]. Pectins are classified as high methoxyl when the degree of esterification exceeds 50%, and low methoxyl when it is lower, which confers them with different properties and gelling capacities [6].

The production of pectin through efficient and economical methods has generated interest due to its use as a gelling agent, thickener, texture enhancer and stabilizer in foods, as well as a soluble dietary fiber, drug carrier, film-forming polymer and in cosmetic applications [5,7]. In the food industry, polysaccharides from pectin provide increased viscosity and act as stabilizers in food and beverages [8].

Pectin can be obtained from waste generated by the food industry, such as biomass derived from seeds and peels, which are of great interest due to their lower moisture content compared to the original fruit [5]. Citrus, mango, guava, sunflower, plum, and prickly pear have been reported as rich sources of pectin [1,9]. Such raw material supports a circular economy and allows pectin to be used both for local consumption and export to countries where large amounts of plant waste are produced.

Prickly pear (*Opuntia ficus-indica*) belongs to the cactus family. Its fruit is oval-shaped, with a thick, waxy and spiny peel, available in a variety of colors ranging from green, yellow and purple, to red and orange [10]. The peels of prickly pear account represent a large proportion of the whole fruit (40% to 50%) and are a source of bioactive compounds, particularly phenolics, flavonoids, and betalains [11]. Due to the hardness and characteristics of its peel, this species has been reported as sources of pectin [12].

Currently, pectin is industrially obtained through a conventional method based on its extraction with mineral acids for long periods of time and at low pH, which can cause corrosion in the system [13]. Banerjee [5] reported that the conventional method of extracting pectin from mango peel with HCl (25.2%) yielded a higher extraction rate compared to the sonication method (14.9%). On the other hand, the use of phosphoric acid to extract pectin from orange peel resulted in a yield of



29.4% at 95°C during 2 h [14]. However, the main disadvantage of using mineral acids is the need to remove toxic compounds when pectin is intended for applications in the food industry [8].

Pectin obtained from prickly pear peel can be extracted by acidic methods, although its yield and quality depend on the variety of prickly pear, the pH level, temperature, and extraction time [15]. According to previous studies, pectin extracted from prickly pear peel is characterized as a low-methoxyl acetylated pectin, which means that it has a low degree of esterification and a high content of acetyl groups. This type of pectin is capable of forming gels in the presence of calcium ions [10].

Pectin extraction without the use of mineral acids is an attractive and environmentally friendly process. Food-grade organic acids have been approved as materials recognized as safe without limitations by the Food and Drug Administration (FDA) in the USA [16].

MATERIALS AND METHODS

Raw materials

The prickly pear (*Opuntia ficus-indica*) were purchased at a local market in Lima (Peru), where the study was conducted, and were selected based on their ripeness.

Sample preparation

The prickly pears were washed using potable water, 5% sodium hypochlorite and distilled water. Subsequently, two transverse and one longitudinal cut were made to separate the pulp from the peel, and the peels were cut into 1 cm² pieces. During the blanching process, the cut peels were added to osmotized water, reaching a temperature between 95 and 98°C for 5 min. This was followed by two rinses with osmotized water to remove the residues. Finally, the peels were dried in an oven (Ecocell 55, Standard Oven) at 55°C for 48 h until a constant weight was obtained, and then ground to a powder. This procedure was conducted in an area of the Physicochemical Laboratory designated for the Grupo de Investigación en alimentos GIA-FIQT-UNI (Lima, Perú).

Pectin extraction

In this study, the extraction of pectin from prickly pear peel was investigated using an eco-friendly and safe extraction solvent such as citric acid. The methodology used consisted on drying the biomass in an oven at 55°C for 48 h until a constant weight was achieved, hydrolysis of the biomass with food grade citric acid following a statistical design with varying pH levels (between 1.8 and 3.0) and temperature range (66 to 90°C) for 55 min at a raw material: acid ratio 1:19. The hydrolyzed pectin was separated by centrifugation (3000 rpm, 25 min), followed by



precipitation for 1 h by adding 96 % ethanol at a hydrolyzed pectin -to-alcohol ratio of 1:1.2. The pectin was then filtered by manually pressing it using a cotton cheesecloth which is soft, nearly lint-free, tightly woven, and highly absorbent, as described by Tulashie [17] and purified through successive washes with 70 % ethanol to remove water-soluble residues, including trace metals and ash. After that, pectin was dried at 40°C for 20 h until constant weight was obtained. Finally, the dried pectin obtained was weighed to determine its yield, and this was correlated with the variables involved in the experimental design applied in this study.

The pectin yield was calculated using the mass of pectin obtained from the raw material (dry basis) by equation (1):

$$Yield (\%) = \frac{M_{pectin}}{M_{prickly\ pear\ peel}} \times 100\% \quad (1)$$

Where M_{pectin} is the mass of dry pectin (g) and $M_{prickly\ pear\ peel}$ is the precursor material used in pectin extraction (g).

This procedure was conducted in an area of the Physicochemical Laboratory designated for the Grupo de Investigación en alimentos GIA-FIQT-UNI (Lima, Perú).

Characterization of pectin

The moisture content of the pectin samples was determined using the method described by Wathoni *et al.* [18]. This process consisted of placing a 0.5 g sample in an oven at 100°C for 4 h until a constant weight was reached. Then, the sample was cooled and its final weight was determined. The ash content was obtained by placing 0.5 g of the pectin by sample in a porcelain crucible and subjecting it to a muffle at 600°C for 4 h.

The equivalent weight (EW) represents the number of free carboxylic that react with the added base (NaOH) in such a way that the functional group (-COOH), which gives the acidic characteristics to the molecule, suffers the separation of the proton due to the action of NaOH. The equivalent weight was measured by weighing 0.5 g of pectin in a flask, to which 5 mL of ethanol, 1 g of NaCl, 100 mL of distilled water and six drops of phenolphthalein were added. Subsequently, the mixture was stirred and slowly titrated with 0.1 N NaOH until the indicator turned pink (pH 7.5). The neutralized solution was subsequently used to measure the methoxyl content. The equation for calculating the EW is presented in equation (2).



$$EW \left(\frac{eq}{g} \right) = \frac{\text{Sample weight} \times 100}{\text{Vol. alkali} \times N. \text{ alkali}} \quad (2)$$

The determination of methoxyl content was carried out by adding 25 mL of 0.25 N NaOH to the neutralized solution. This mixture was continuously stirred and allowed to stand at room temperature for 30 min in a closed flask. Subsequently, 25 mL of 0.25 N HCl, along with the phenolphthalein indicator, was added, and the solution was titrated with 0.1 N NaOH until the solution turned a pinkish shade [9]. The equation to calculate the DM is presented in equation (3).

$$DM (\%) = \frac{\text{Vol. used of NaOH} \times 3.1}{\text{Sample weight}} \quad (3)$$

Anhydrouronic acid (AUA) was calculated using the NaOH consumption values obtained from the measurements of EW and DM [9]. The corresponding equation for AUA is shown in equation (4).

$$AUA (\%) = \frac{176 \times 0.1(z) \times 100}{W \times 100} + \frac{176 \times 0.1(y) \times 100}{W \times 100} \quad (4)$$

Where molecular weight AUA is 176 mg/meq; z: represents the mL of alkali (NaOH) resulting from equivalent weight; y: represents the mL of alkali (NaOH) from methoxyl content and W: represents the weight of sample used.

The degree of esterification was determined by considering the percentage of methoxyl (methyl esterified carboxylic acid units) and the percentage of AUA [9].

The SHIMADZU Prestige-21 IR spectrometer was used to analyze the presence of functional groups in the pectin obtained in the three optimum conditions, within a wavelength range of 400-4000 cm⁻¹. The IR analysis was conducted in the Center for Development of Advanced Materials and Nanotechnology, Faculty of Sciences, while the physicochemical characterization was conducted in the Organic Chemistry Laboratory at the Faculty of Chemical and Textile engineering, both of Universidad Nacional de Ingeniería

Evaluation of the influence of pH and temperature on the pectin extraction

The effects of processing variables, such as extraction temperature and pH, on pectin yield were studied. Response surface methodology, with experimental data fitting to a second-order mathematical model, was employed to determine the optimal extraction conditions and maximize yield according to a central composite design (Table 1). A total of 15 experiments were conducted to determine the relationship between these two factors.

The results of the tests obtained through the central composite design were subjected to an analysis of variance (ANOVA) using Minitab software, version 17,



in order to determine the significance of the coefficients in the model at a 95% confidence level ($p < 0.05$). The model fit was verified using the R^2 value.

RESULTS AND DISCUSSION

Physicochemical characterization of pectin

The physicochemical characterization was carried out for the three pectin that obtained the highest yield under the temperature and pH conditions of the experimental design and are shown in Figure 1. Figures 1d, 1e and 1f show the pectin obtained at different pH values after the alcohol washing operation and Figures 1a, 1b and 1c show the pectin after dehydration in the oven.

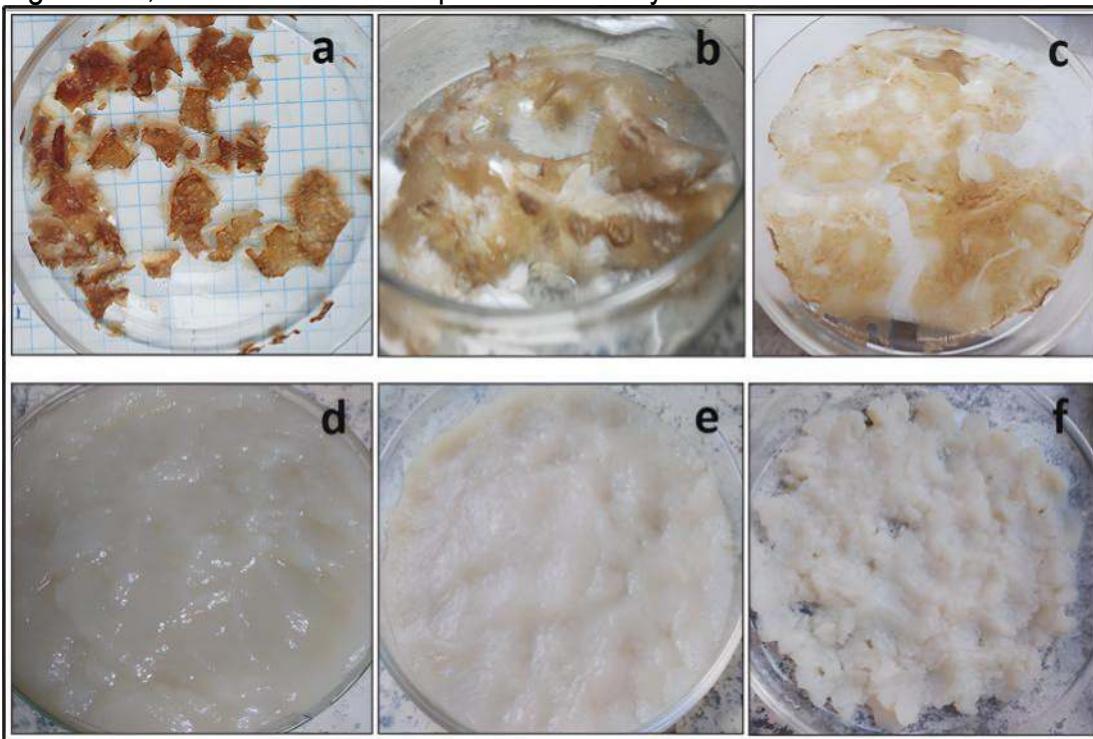


Figure 1: Pectin obtained at different pH conditions: 1.8 (a and d), 2.0 (b and e), 2.5 (c and f)

The chemical composition of pectin is shown in Table 1. Moisture control is a factor that influences the safe storage of pectin, as it is closely related to the stability of agents that inhibit the growth of microorganisms [14]. According to IPPA [16], a moisture content higher than 12% can promote the growth of microorganisms, which may potentially affect the quality of pectin due to the production of pectinase enzymes. The moisture content ranged from 5.6 to 8.4%, thus meeting the specifications for high quality pectin. These results agree with those reported by Lekhuleni [8], who worked on the extraction of pectin from prickly pear peel, obtaining moisture values between 7.6% and 8.9%, and by Weldearegay [13], with a range of 6.1 to 9.1%.

The ash content of pectin is indicative of the presence of minerals such as potassium, sodium, magnesium, and iron, and reflects the level of purity of the pectin, where higher purity corresponding to lower ash content [20]. The pectin obtained in this study exhibited an ash percentage of 6.6%, which did not vary with change in pH, placing it below the established maximum limit of 10% for a good quality pectin [19]. Weldearegay [13] reported an ash value of 10.87% for pectin from prickly pear peel, whereas Lekhuleni [8] reported a higher range, between 25% and 34%, for pectin extracted from different varieties of prickly pear peel. These variations can be attributed to different extraction and purification conditions. The use of acidified alcohol washing can help to reduce the ash content by decreasing the presence of minerals in the pectin [20].

The content of free (non-esterified) galacturonic acid in pectin molecular chains is measured by the equivalent weight EW of pectin [15]. The EW values were influenced by pH, with an increase in EW being observed as pH increased. This behavior is congruent with that reported by Mada [17]. Previous studies have demonstrated that a higher equivalent weight could favor the formation of gels, while a lower EW tends to increase the fractional degradation of pectin Kamal [18]. The pectin obtained in this work falls within the standard range of 600 to 800 [15] for pH 2.5 and below for the pH ranges of 1.8 and 2. Low PE values were also reported by Lekhuleni [8] with values between 119.7 and 155.0 for pectins from different varieties of prickly pear peels.

The degree of methoxylation (DM) of pectin is an important parameter for determining their application. The methoxyl content of pectin is the measure of its gelling capacity, water dispersibility, and is an important factor for controlling sensitivity to polyvalent cations, as well as its usefulness in the preparation of gels, films and low-solid content fibers [22]. Pectin with a methoxyl content higher than 7% is called high methoxyl (HM) pectin, which is characterized by its greater solubility [15]. On the other hand, low methoxyl (LM) pectin, containing less than 7% methoxyl, forms gels with lower sugar concentrations, but requires the presence of divalent cations, such as calcium. These ions are necessary for establishing bonds between adjacent pectin polymers and stabilizing the gel [9]. The results from this study indicate that the pectin has a low methoxyl content, with values ranging between 3.5% to 5%, consistent with the findings of Kamal [18] who reported values between 5.4 and 5.6 % as pH increased. Additionally, Lekhuleni [8] reported that pectin extracted from prickly pear peel using sulfuric acid, assisted by microwave, presented values in the range of 2.3% to 3.9%. However, a higher value of 8.33% was reported for pectin obtained through sequential ultrasound and microwave-assisted extraction (UMAE) [15].



The total anhydrouronic acid content (AUA%) in pectin serves as a parameter for assessing its purity and degree of esterification [9]. In this study, the AUA content ranged from 47.9% to 98.6%, depending on the pH at which the pectin was extracted. The highest AUA content was observed in pectin extracted at pH 1.8. According to, Food Chemicals Codex [20] pectin containing less than 65% AUA is considered contaminated with proteins, starch and sugars. The results obtained in this study confirm the purity of pectin extracted at pH 1.8 and 2. Kamal [18] also highlighted that pH and temperature during the extraction process can significantly affect the AUA content, with lower pH and higher temperatures resulting in increased AUA levels. For food additive and pharmaceutical applications, pectin with an AUA content of at least 65% is recommended [24].

The degree of esterification (%DE) is an important factor characterizing the pectin chains of the carboxyl groups and was determined to evaluate the type of pectin. The percent esterification is related to the methoxyl content (%MeO), and the results show an increase in both DE and %MeO as pH increases. Based on the degree of esterification, low methoxyl pectin is classified as having a DE \leq 50%, whereas high methoxyl pectin has a DE $>$ 50%. The values obtained in this study fall below 50%, indicating that the pectin has a low degree of esterification, which is consistent with the low methoxyl content. These values may be attributed to the low pH conditions, which resulted in a lower degree of esterification due to a higher deesterification of the polygalacturonic chains [25]. The lowest DE, obtained at pH 1.8, coincides with that reported by Mota [10], who reported a DE of 18.51% for pectin extracted from *Opuntia robusta* fruit peel using EDTA. However, this DE is lower than those reported by Biratu [24] who extracted pectin from the pulp of four varieties of coffee using different inorganic acids (53% to 68.5%), and Mamiru and Gonfa [25] who extracted pectin from watermelon rind using acetic acid (57.3%).

Infrared spectroscopy was performed to identify the main functional groups of the three pectin samples with the highest extraction yields. Figure 2 shows the infrared spectra of pectin at different pH levels and temperature. Common to all three spectra is a broad band between 3200 and 3650 cm^{-1} , corresponding to OH stretching. The hydroxyl and/or carboxylic acid groups in the pectin monomer known as D-galacturonic acid are the source of the -OH group in pectin [15].

The methyl ester of D-galacturonic acid⁻¹, produced by methyl esterification of the monomers, was identified by an absorbance peak in the range of 2923 and 2934 cm^{-1} , in agreement with the pH. The signals between 3000 and 2840 cm^{-1} correspond to the stretching vibrations of CH and CH₂ groups. Two bands at (1604-1630 cm^{-1}) and 1417 cm^{-1} , correspond to the asymmetric and symmetric vibration of the carboxylate (COO⁻) group respectively [28]. A strong absorbance at 1015 cm^{-1} is characteristic of the glycosidic bonds between the sugar units. The



bands in the range of 1713 - 1728 cm⁻¹ are attributable to the stretching vibration of the C=O group of the carboxylic acid methyl ester (or protonated carboxylic acids).

The functional group of C-O-C, around 1210 and 1226 cm⁻¹, results from the stretching of C-O bond in alkyl aryl ether (R-O-R') or from the asymmetric tensile vibration of C-O-C at the methoxy group (-O-CH₃) [15]. The FTIR spectra of pectin extracted at different pHs were almost the same, although slight variations were observed in certain regions, due to differences in the equivalent weight of the pectin [26].

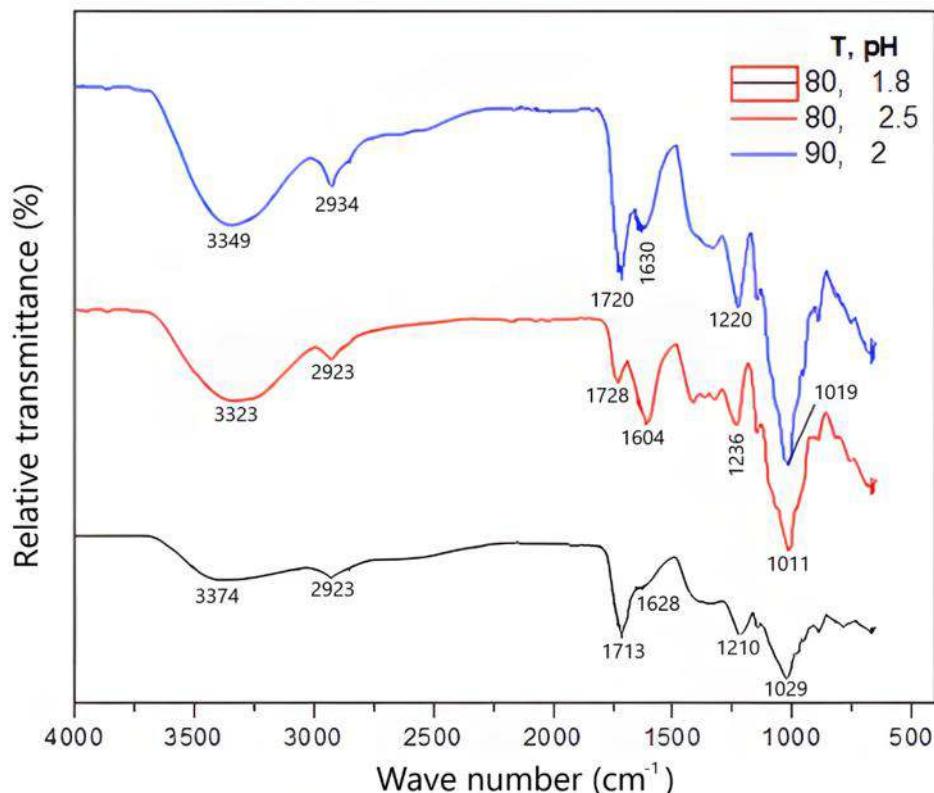


Figure 2: FT-IR spectra of pectin extracted from prickly pear peel at different pH and temperature conditions

The total extraction yield reflects the pectin extraction efficiency. The highest pectin yield was obtained when prickly pear peels were extracted at the lowest pH level of 1.8 (Table 2). This result is consistent with the studies of Khan and Nandkishor [7], who reported that an increase in the ionic strength of acids enhances their affinity for catins, which stabilizes the pectin molecule and, therefore, allows better precipitation of pectin [9].

The analysis of variance (ANOVA) for the significance test of the model coefficients is shown in Table 3. The factors and their combinations were: factor X₁: Temperature (T (°C)) and factor X₂: pH. The analysis indicates that the linear effect

of pH is the factor with the highest significance ($p < 0.05$), while temperature and the combined factors did not significantly affect yield.

Regression analysis was performed to fit the experimental data to statistical models, aiming to identify the optimal conditions for maximizing the response variable (yield). The significance of each coefficient was determined using the F-test and the p-value in Table 2. The mathematical model can be described by the following equation in terms of coded values:

$$\text{Yield \%} = -16,7 + 0,779 X_1 - 3,29 X_2 - 0,00319 X_1 * X_1 + 0,92 X_2 * X_2 - 0,0843 X_1 * X_2$$

The R^2 adjusted value of 0.9074 indicates that the model adequately explains the variability of the data. An increase in yield is observed as pH decreases, which is consistent with the negative coefficient of X_2 . On the other hand, the positive influence of temperature is congruent with the positive coefficient of X_1 . The negative interaction $X_1 X_2$ indicates the absence of synergy between temperature and pH.

Response surface methodology (RSM) combines experimental design, regression analysis, and optimization methods [8]. Figure 3a and 3b shows the development of the response surface, where the optimal combinations of factors were identified, both individually and in their interactions. The objective was to maximize desirability function, a score parameter that evaluates whether the combination of factors satisfies the criteria established for the response. In this context, the desirability function approaches a value of 1, indicating that the predicted combination of factors yields the optimal outcome. The model suggests that the highest score is obtained at a temperature of 94.1 °C and pH of 1.8, achieving a pectin yield of 11.1%, within the confidence interval of 9.6% to 12.6%. Furthermore, the contour plot indicates a pectin yield of over 10%, with higher yields observed at lower pH levels and higher temperatures.

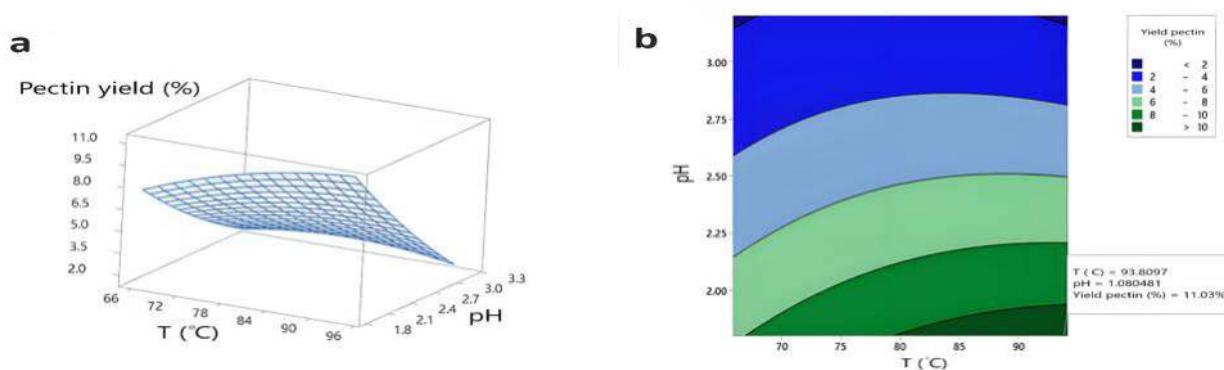


Figure 3: (a) Response surface plot and (b) Contour plot with respect to pectin yield as a function of pH and temperature (T (°C))

The increase in temperature improved the yield, possibly due to the enhanced solubility and diffusivity of pectin and other pectic substances in the solvent [21]. The results regarding the effects of pH and temperature are consistent with those of Kamal [18] who extracted pectin from orange peel using hydrochloric acid, with their highest yield at temperature of 95 °C (21.5%) and a lower pH of 1.5 (21.3%). In addition, acidic conditions promote the hydrolysis of insoluble pectin components into soluble forms, thus increasing pectin recovery. This agrees with Qui *et al.* [29], who with a response surface analysis, demonstrated improved pectin extraction from banana peel under low pH conditions.

Compared to other non-commercial sources of pectin, the yield obtained from prickly pear peel was higher than that from apple peels treated with various organic acids, such as citric acid (5.3-6.4%) [16], and from cocoa (*Theobroma cacao L.*) cobs peels treated with ascorbic acid (2.3-4.9%) [30]. However, the yield was lower than that obtained from watermelon peel using acetic acid [27] and from the mixture of papaya and banana peels extracted by a microwave-assisted method [20].

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The use of the experimental design and response surface analysis allowed the optimization of pH, temperature and their interactions on the yield of pectin extracted from prickly pear peel. The pH had a more significant effect on the response value. The results showed that increasing the temperature and decreasing the pH led to a higher extraction yield. The optimization study predicted that the optimum extraction conditions corresponded to a temperature of 94.1°C and pH of 1.8, which produced a pectin yield of 11.1% pectin. Various quality parameters, such as moisture (6.3-8.4%), equivalent weight (224.2-987.8 g), methoxyl content (3.6-5.0%), anhydrouronic acid (AUA) content (48.0~98.6%) and degree of esterification (20.5-59.2%), were also influenced by the extraction conditions. The extracted pectin was classified as low methoxyl pectin (DE 29.5%, methoxyl content 3.6%) and exhibited high quality (AUA >65%). All quality parameters were within acceptable range and a high AUA content together with a low ash percentage indicated high pectin purity. Notably, the highest AUA value and lowest ash percentage were observed in pectin extracted at pH 1.8, although the equivalent weight was lower at this pH. Overall, the extracted pectin meets the established criteria for commercial applications, suggesting it is a viable alternative for industrial use. Moreover, the use of citric acid in the extraction process presents an environmentally friendly and sustainable method, offering potential advantages for industrial-scale pectin production. These findings demonstrate that citric acid



extraction from prickly pear peel yields pectin of good quality and reasonable quantity.

On the other hand, for future studies and further application of pectin extracted from prickly pear peels in food industry, it is recommended to complement the characterization using advanced instrumental techniques, such as thermogravimetric analysis and differential scanning calorimetry, which can provide valuable insights for food application under different temperatures of processing. Additionally, scanning electron microscopy analysis can be used to determine its surface morphology of pectin fibers to evaluate its functionality. Finally, to validate the properties resulting from the previous analysis, it is crucial to evaluate the use of pectin in different food formulations.

Conflict of interest

The authors declare no conflict of interest.

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Table 1: Physicochemical properties of the pectin extracted from prickly pear peel

Characteristics	pH: 1.8	pH: 2.0	pH: 2.5
Moisture (%)	8.4	5.6	6.3
Ash (%)	6.5	6.5	6.7
Equivalent weight	224.2	306.0	897.8
Methoxyl content (%)	3.6	4.5	5.0
Anhydrouronic acid (%)	98.6	83.3	48.0
Degree of esterification (%)	20.5	31.0	59.2

Table 2: Performance of pectin extract from prickly pear peels using design of experiments

Nº Assay	Factors		MO: Mass obtained	Yield (%)
	T (°C)	pH		
1	80	2.5	1.14	5.7
2	90	3.0	0.50	2.5
3	70	2.0	1.27	6.3
4	80	2.5	1.19	5.9
5	80	2.5	1.18	5.9
6	65.9	2.5	1.10	5.5
7	94.1	2.5	1.32	6.6
8	80	3.2	0.65	3.2
9	70	3.0	0.36	1.8
10	90	2.0	1.68	8.4
11	80	1.8	2.21	11.0
12	80	2.5	1.17	5.8
13	80	2.5	1.15	5.7
14	90	1.8	2.19	10.9
15	94	1.85	2.17	10.8



Table 3: ANOVA for pectin yield

Factors	DF	SS	AS	F	p
Model	5	107.793	21.5586	27.19	0.000
X ₁ : temperature	1	2.871	2.8710	3.62	0.089
X ₂ : Ph	1	67.100	67.1001	84.62	0.000
X ₁ *X ₁	1	0.795	0.7950	1.00	0.343
X ₂ *X ₂	1	0.410	0.4100	0.52	0.490
X ₁ *X ₂	1	1.057	1.0567	1.33	0.278
Error	9	7.136	0.7929		
Total	14	114.929			
S		R-quad.	R-quad. (fit)	Coefficient of Variation	
0.890461		93.8%	90.3%	14%	

Note. DF: Degrees of freedom, SS: Sum of squares, AS: average of squares, F: F distribution, p: p value

REFERENCES

1. **Ciriminna R, Fidalgo A, Avellone G, Carnaroglio D, Danzì C, Timpanaro G, Meneguzzo F, Ilharco LM and M Pagliaro** Economic and technical feasibility of betanin and pectin extraction from *opuntia ficus-indica* peel via microwave-assisted hydrodiffusion. *ACS Omega*. 2019; **4**(7): 12121–12124. <https://doi.org/10.1021/acsomega.9b00960>.
2. **Bedzo OKK, Gottumukkala LD, Sasso G Lo, Kaminski K, Schrage W, Goffman F, Ivanov N, Hoeng J and DJ Hayes** Process development for efficient pectin extraction from tobacco residues and its characterisation. *Biomass Conversion and Biorefinery*. 2023; **1**:1–21. <https://doi.org/10.1007/s13399-023-04750-4>
3. **Roman-Benn A, Contador CA, Li MW, Lam HM, Ah-Hen K, Ulloa PE and MC Ravanal** Pectin: An overview of sources, extraction and applications in food products, biomedical, pharmaceutical and environmental issues. *Food Chemistry Advances*. 2023; **2**:100192. <https://doi.org/10.1016/J.FOCHA.2023.100192>
4. **Kumar S, Konwar J, Purkayastha M Das, Kalita S, Mukherjee A and J Dutta** Current progress in valorization of food processing waste and by-products for pectin extraction. *International Journal of Biological Macromolecules*. 2023; **239**: 124332. <https://doi.org/10.1016/J.IJBIOMAC.2023.124332>
5. **Guízar-Amezcua MG, Pineda-Santana A, González-Domínguez MI, Cajero-Zul LR, Guerrero-Ramírez LG, López-Miranda A, Nambo A and J López-Mercado** Evaluation of pectin extractions and their application in the alkaline Maillard reaction. *Scientific Reports*. 2022 12:1 2022; **12**(1): 1–12. <https://doi.org/10.1038/s41598-022-22002-9>
6. **Xie F, Gu BJ, Saunders SR and GM Ganjyal** High methoxyl pectin enhances the expansion characteristics of the cornstarch relative to the low methoxyl pectin. *Food Hydrocolloids*. 2021; **110**: 106131. <https://doi.org/10.1016/J.FOODHYD.2020.106131>
7. **Banerjee J, Vijayaraghavan R, Arora A, MacFarlane DR and AF Patti** Lemon juice-based extraction of pectin from mango peels: Waste to wealth by sustainable approaches. *ACS Sustainable Chemistry and Engineering*. 2016; **4**(11): 5915–5920. <https://doi.org/10.1021/acssuschemeng.6b01342>

8. **Santos JDG, Espeleta AF, Branco A and SA De Assis** Aqueous extraction of pectin from sisal waste. *Carbohydrate Polymers*. 2013; **92**(2): 1997–2001. <https://doi.org/10.1016/J.CARBPOL.2012.11.089>
9. **Khan M and Nandkishor** Optimization of Extraction Condition and Characterization of Low Methoxy Pectin from Wild Plum. *Journal of Packaging Technology and Research*. 2019; **3**(3): 215–221. <https://doi.org/10.1007/S41783-019-00070-Z>
10. **Lekhuleni ILG, Kgatla TE, Mashau ME and AIO Jideani** Physicochemical properties of South African prickly pear fruit and peel: Extraction and characterisation of pectin from the peel. *Open Agriculture*. 2021; **6**(1):178–191. <https://doi.org/10.1515/opag-2021-0216>
11. **Chougui N, Djerroud N, Naraoui F, Hadjal S, Aliane K, Zeroual B and R Larbat** Physicochemical properties and storage stability of margarine containing *Opuntia ficus-indica* peel extract as antioxidant. *Food Chemistry*. 2015; **173**:382–390. <https://doi.org/10.1016/J.FOODCHEM.2014.10.025>
12. **Mota J, Muro C, Illescas J, Hernández OA, Tecante A and E Rivera** Extraction and Characterization of Pectin from the Fruit Peel of *Opuntia robusta*. *Chemistry Select*. 2020; **5**(37):11446–11452. <https://doi.org/10.1002/SLCT.202002181>
13. **Pereira DTV, Méndez-Albiñana P, Mendiola JA, Villamiel M, Cifuentes A, Martínez J and E Ibáñez** An eco-friendly extraction method to obtain pectin from passion fruit rinds (*Passiflora edulis* sp.) using subcritical water and pressurized natural deep eutectic solvents. *Carbohydrate Polymers*. 2024; **326**:121578. <https://doi.org/10.1016/J.CARBPOL.2023.121578>
14. **Tovar AK, Godínez LA, Espejel F, Ramírez-Zamora R-M and I Robles** Optimization of the integral valorization process for orange peel waste using a design of experiments approach: Production of high-quality pectin and activated carbon. *WaMan* 2019; **85**: 202–213. <https://doi.org/10.1016/J.WASMAN.2018.12.029>
15. **Weldearegay SG, Gaddala B, Fentie EG, Sundramurthy VP and LS Priya** Valorization of waste prickly pear peels: optimization for pectin extraction, characterization, and development of edible film. *Biomass Conversion and Biorefinery*. 2024:1–23. <https://doi.org/10.1007/s13399-024-05833-6>

16. **Cho EH, Jung HT, Lee BH, Kim HS, Rhee JK and SH Yoo** Green process development for apple-peel pectin production by organic acid extraction. *Carbohydrate Polymers*. 2019; **204**: 97–103.
<https://doi.org/10.1016/J.CARBPOL.2018.09.086>
17. **Tulashie SK, Amenakpor J, Atisey S, Odai R and EEA Akpari** Production of coconut milk: A sustainable alternative plant-based milk. *Case Studies in Chemical and Environmental Engineering*. 2022; **6**: 100206.
<https://doi.org/10.1016/J.CSCEE.2022.100206>
18. **Wathoni N, Yuan Shan C, Yi Shan W, Rostinawati T, Indradi RB, Pratiwi R and M Muchtaridi** Characterization and antioxidant activity of pectin from Indonesian mangosteen (*Garcinia mangostana* L.) rind. *Helijon* 2019; **5(8)**: e02299. <https://doi.org/10.1016/J.HELION.2019.E02299>.
19. **IPPA**. *Pectin commercial production and pectin in organic food products*. (International Pectin Producers Association, ed.); 2014.
20. **Mada T, Duraisamy R and F Guesh** Optimization and characterization of pectin extracted from banana and papaya mixed peels using response surface methodology. *Food Science & Nutrition* 2022; **10(4)**:1222–1238.
<https://doi.org/10.1002/FSN3.2754>
21. **Kamal MM, Kumar J, Mamun MAH, Ahmed MNU, Shishir MRI and SC Mondal** Extraction and Characterization of Pectin from *Citrus sinensis* Peel. *Journal of Biosystems Engineering* 2021; **46(1)**: 16–25.
<https://doi.org/10.1007/s42853-021-00084-z>
22. **Roy S, Priyadarshi R, Łopusiewicz L, Biswas D, Chandel V and JW Rhim** Recent progress in pectin extraction, characterization, and pectin-based films for active food packaging applications: A review. *International Journal of Biological Macromolecules*. 2023; **239**: 124248.
<https://doi.org/10.1016/J.IJBIOMAC.2023.124248>
23. **Committee on Food Chemicals Codex**. *Food Chemicals Codex*. Washington, DC: National Academy Press; 1996.
24. **Khamsucharit P, Laohaphatanaert K, Gavnlertvatana P, Sriroth K and K Sangseethong** Characterization of pectin extracted from banana peels of different varieties. *Food Science and Biotechnology*. 2018; **27(3)**: 623–629.
<https://doi.org/10.1007/s10068-017-0302-0>

25. **Hosseini SS, Khodaiyan F and MS Yarmand** Optimization of microwave assisted extraction of pectin from sour orange peel and its physicochemical properties. *Carbohydrate Polymers*. 2016; **140**: 59–65.
<https://doi.org/10.1016/J.CARBPOL.2015.12.051>
26. **Biratu G, Gonfa G, Bekele M and HW Woldemariam** Extraction and characterization of pectin from coffee (*Coffea arabica* L.) pulp obtained from four different coffee producing regions. *International Journal of Biological Macromolecules*. 2024; **274**: 133321.
<https://doi.org/10.1016/J.IJBIOMAC.2024.133321>
27. **Mamiru D and G Gonfa** Extraction and characterization of pectin from watermelon rind using acetic acid. *Helijon* 2023; **9(2)**: e13525.
<https://doi.org/10.1016/J.HELION.2023.E13525>.
28. **Manrique GD and FM Lajolo** FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biology and Technology* 2002; **25(1)**: 99–107.
[https://doi.org/10.1016/S0925-5214\(01\)00160-0](https://doi.org/10.1016/S0925-5214(01)00160-0)
29. **Qiu LP, Zhao GL, Wu H, Jiang L, Li XF and JJ Liu** Investigation of combined effects of independent variables on extraction of pectin from banana peel using response surface methodology. *Carbohydrate Polymers* 2010; **80(2)**: 326–331. <https://doi.org/10.1016/J.CARBPOL.2010.01.018>
30. **Priyangini F, Walde SG and R Chidambaram** Extraction optimization of pectin from cocoa pod husks (*Theobroma cacao* L.) with ascorbic acid using response surface methodology. *Carbohydrate Polymers* 2018; **202**: 497–503. <https://doi.org/10.1016/J.CARBPOL.2018.08.103>