

Optimization and Physicochemical Characterization of Pectin Extraction from Watermelon Rind (*Citrullus lanatus*) with Citric Acid

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ABSTRACT

Watermelon rind was used for the pectin extraction with citric acid as the extractant solvent. The effects of pH (2.0-3.0), extraction time (45-75 min) and liquid-solid ratio (1:10-1:40 g/ml) on the pectin yield, degree of esterification, methoxyl content and anhydrouronic acid content were investigated using Box-Behnken surface response experimental design. The pH was the most significant variable for the pectin yield and properties. The responses optimized separately showed different optimal conditions for each of the variables studied in this work. Therefore, the desirability function was used to determine the sole theoretical optimum for the highest pectin yield and highest anhydrouronic acid content, which was found to be: pH of 2.0, extraction time of 62.31 min and liquid-solid ratio of 35.07 g/mL. Under this optimal condition, the pectin yield, degree of esterification, methoxyl content and anhydrouronic acid content were 24.30%, 73.30%, 10.45% and 81.33%, respectively. At optimal conditions, watermelon rind pectin can be classified as high methoxyl and rapid-set pectin with high quality and high-purity.

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a Cucurbitacea, creeping herbal plant or climbing plant characterized as a large and juicy fruits. Watermelon fruit is composed of flesh (edible part), seeds distributed throughout the flesh and the rind representing 30-40% of the total weight^{1,2}. Health benefits such as prevention against cardiovascular diseases are attributed to the fruit^{3,4}. The literature have determined that the watermelon seeds⁵ and watermelon rinds⁶ contain antioxidant properties. Watermelon is mainly used for local production of juices, which generate large amounts of waste without a proper disposal treatment. These watermelon residues have great potential to produce pectin and other value-added products.

Pectin is a linear polysaccharide mainly composed of anhydro galacturonic acid units with $\alpha(1\rightarrow4)$ bonds, and the carboxyl acid groups are esterified with methyl groups partially⁷. Its structure includes other components such as neutral sugars: arabinose, galactose, rhamnose and xylose^{7,8}, which can react with methanol to form methyl esters or neutralized by a base⁹. Pectin has gelling properties, that are used for the aqueous gels formation in the food industry^{10,11}. Some of the pharmaceutical applications of pectin are to diminish lipid digestion¹², to improve lipid hepatic accumulation¹³, glucose tolerance for anti-diabetic effects¹⁴ and its anti-inflammatory activity in high methoxyl pectins¹⁵.

The variables that affect the extraction yield are pH, temperature, time and liquid-solid ratio (LSR). The quality parameters of pectin include extraction yield and properties such as ash content¹⁶ free acidity¹⁷, methoxyl content¹⁸, degree of esterification¹⁹, anhydro galacturonic acid content^{20,21} and Fourier transform infrared spectroscopy (FTIR) analysis^{22,23}. Pectin is classified through its degree of esterification (DE) due to define its gelling properties. High methoxyl pectins, characterized by forming gels in high soluble solids and acidic systems, which have galacturonic acid units that are more than 50% (DE > 50%) esterified with methyl groups. In low methoxyl pectins (DE < 50%), the gelation occurs on a widespread pH range than high methoxyl pectins, and require the presence of divalent cations^{7,8,24}.

The primary sources of industrial pectin extraction are apple pomace and citrus peels^{24,25}. However, pectin from agro-industrial processes wastes is generating a great interest because their use can reduce environmental impacts provoked by themselves and give added value to the agro-industrial production chain. Many biological residues such as banana peels²⁶, mango peels^{27,28} and melon peels¹¹ have been utilized to obtain pectin. Hence, watermelon rind can be used to produce pectin, while adding value to the watermelon's agribusiness.

Several works have reported the extraction of pectin from watermelon wastes by using different methods such as acid hydrolysis^{29,30} and assisted microwave extraction^{31,32}. However, the acid hydrolysis is the most employed method for the pectin extraction from food waste. Therefore, the objectives of this study are to optimize and characterize the pectin extraction from watermelon rind using citric acid as solvent for extraction through the response surface methodology (RSM) and determine optimal conditions for the highest pectin yield and anhydrouronic acid content (AUA) simultaneously.

RESULTS AND DISCUSSION

Experimental results and quality parameters

Table 1 presents the experimental values obtained for pectin yield, methoxyl content, degree of esterification, and anhydrouronic acid content at each Box Behnken design (BBD) point. The pectin yield range (4.19-27.86%) was within the typical values reported of 15-20% and 30-35% for dried apple pomace and citric peels, respectively²⁴. The results indicate that the watermelon rind pectin can be classified as high methoxyl pectin²⁵ due to its DE and methoxyl content (MeO) were higher than the reference values of 50% and 6.7%³³, respectively. Furthermore, according to the Food Agriculture Organization (FAO), and the World Health Organization (WHO), the AUA suggests that a high-quality pectin (AUA > 65% FAO/WHO) was obtained³⁴, except for runs carried out at pH 3.0.

Run	Independent variables			Experimental responses			
	X1 ^a	X2 ^b (min)	X3 ^c (g/mL)	Yield (%)	DE (%)	MeO (%)	AUA (%)
1	2.0	45	25	17.02	71.13	9.91	79.11
2	3.0	45	25	5.31	67.71	7.45	62.49
3	2.0	75	25	21.23	66.83	8.36	71.02
4	3.0	75	25	5.95	66.67	6.75	57.52
5	2.0	60	10	14.59	71.42	9.39	74.68
6	3.0	60	10	4.19	71.53	6.82	54.11
7	2.0	60	40	27.16	73.33	10.66	82.50
8	3.0	60	40	5.99	75.44	7.21	54.23
9	2.5	45	10	6.77	73.43	9.40	72.69
10	2.5	75	10	8.80	68.22	7.89	65.69
11	2.5	45	40	6.12	68.42	7.71	64.00
12	2.5	75	40	4.47	79.17	10.16	72.83
13	2.5	60	25	11.48	73.47	10.51	81.24
14	2.5	60	25	11.50	74.61	10.44	79.46
15	2.5	60	25	13.00	72.93	10.03	78.08

Table 1. Experimental conditions of pectin extraction with citric acid, and responses for the Box Behnken experimental design. ^a X1 (pH with three levels: 2.0, 2.5 and 3.0), ^b X2 (extraction time with three levels: 45, 60 and 75 minutes) and ^c X3 (LSR with three levels: 10, 25 and 40 g/mL).

Statistical analysis

The analysis of variance (ANOVA) of the results was used to determine the effects of pH (X_1), extraction time (X_2) and liquid-solid ratio (X_3) on each of the responses of watermelon rind pectin (Table 2). ANOVA revealed that the linear effect (X_1) was significant ($p < 0.05$) for pectin yield, MeO and AUA. Interactions ($X_2:X_3$) had significant for DE and MeO. Furthermore, square effects X_1^2 and X_2^2 ; X_1^2 and X_3^2 and X_2^2 were also significant for MeO, AUA and DE, respectively.

		Pectin yield (%)					DE (%)				
Source	Df	Sum sq	Mean sq	F value	Pr (>F)	Df	Sum sq	Mean sq	F value	Pr (>F)	
Model	9	584.37	64.93	6.08	0.0306*	9	156.22	17.36	4.24	0.0631	
Linear	3	443.10	147.70	13.82	0.0074**	3	17.52	5.84	1.43	0.3911	
Interaction	3	35.57	11.86	1.11	0.4274	3	67.34	22.45	5.48	0.0488*	
Square	3	105.70	35.23	3.30	0.1159	3	71.36	23.79	5.81	0.0438*	
Residuals	5	53.44	10.69			5	20.48	4.10			
Lack of fit	3	51.92	17.31	22.77	0.0424	3	19.00	6.33	8.61	0.1058	
Pure error	2	1.52	0.76			2	1.47	0.74			
Total	28	1275.62				28	353.39				

		MeO (%)					AUA (%)				
Source	Df	Sum sq	Mean sq	F value	Pr (>F)	Df	Sum sq	Mean sq	F value	Pr (>F)	
Model	9	27.00	3.00	7.40	0.0201*	9	1238.65	137.63	7.84	0.0177*	
Linear	3	13.57	4.52	11.15	0.0118*	3	800.20	266.73	15.19	0.0060**	
Interaction	3	4.29	1.43	3.53	0.1041	3	79.90	26.63	1.52	0.3184	
Square	3	9.14	3.05	7.51	0.0267*	3	358.55	119.52	6.81	0.0323*	
Residuals	5	2.03	0.41			5	87.80	17.56			
Lack of fit	3	1.89	0.63	9.39	0.0978	3	82.78	27.59	10.99	0.0845	
Pure error	2	0.13	0.07			2	5.02	2.51			
Total	28	58.06				28	2652.90				

(Significant codes: 0 ‘***’, 0.01 ‘**’, 0.05 ‘*’)

Table 2. Results of ANOVA for pectin yield, DE, MeO and AUA.

By using multiple regression analysis on the experimental responses, the second-order model was utilized to fit experimental data and predict the effects of dependent variables. The validity of the model could be confirmed because the lack-of-fit tests were not significant, and the following determination coefficients (R^2) 0.9162, 0.8841, 0.9338 and 0.9301 were obtained for pectin yield, DE, MeO and AUA, respectively. These results show that the calculated responses by the model were reliable and adequate. The second-order equations (coded factors) for pectin yield (Eq. 1), DE (Eq. 2), MeO (Eq. 3) and AUA (Eq. 4) were given as follows

$$\begin{aligned} \text{Pectin yield}(\%) = & 11.99 - 7.32 X_1 + 0.65 X_2 + 1.17 X_3 - 0.89 X_1 X_2 \\ & - 2.69 X_1 X_3 - 0.92 X_2 X_3 + 3.41 X_1^2 - 3.03 X_2^2 - 2.42 X_3^2 \end{aligned} \quad (1)$$

$$\begin{aligned} \text{DE}(\%) = & 73.67 - 0.17 X_1 + 0.025 X_2 + 1.47 X_3 + 0.82 X_1 X_2 \\ & + 0.50 X_1 X_3 + 3.99 X_2 X_3 - 2.48 X_1^2 - 3.10 X_2^2 + 1.74 X_3^2 \end{aligned} \quad (2)$$

$$MeO(\%) = 10.33 - 1.26 X_1 - 0.16 X_2 + 0.28 X_3 + 0.21 X_1 X_2 - 0.22 X_1 X_3 + 0.99 X_2 X_3 - 1.24 X_1^2 - 0.97 X_2^2 - 0.57 X_3^2 \quad (3)$$

$$AUA(\%) = 79.59 - 9.87 X_1 - 1.40 X_2 + 0.80 X_3 + 0.78 X_1 X_2 - 1.93 X_1 X_3 + 3.96 X_2 X_3 - 7.24 X_1^2 - 4.81 X_2^2 - 5.97 X_3^2 \quad (4)$$

Effect of independent variables on pectin yield

ANOVA showed that pH (linear effect) was the only variable that affected pectin yield significantly³⁵. The determination coefficient indicates that 91.62% of the total variation can be explained by the quadratic model (Eq. 1), which suggest that the model is suitable to predict the yield pectin from watermelon rind under the experimental conditions evaluated in this work. Fig. 1a shows that the pectin yield was favored at lower pH (negative regression term) and, the higher pectin yield was obtained at the lowest pH of 2.0. The carboxyl groups present in pectin are hydrated due to the acidified extraction solvent at lower pH; the loss of charges in carboxyl groups tends to reduce the repulsive forces, promoting the pectin precipitation¹⁰. Indeed, more pectin dissolution by hydrolysis of non-soluble pectin and increasing of pectin mass transfer from the plant source at lowering pH have shown by the literature^{19,20,35-37}. These conditions were shown to have led to increased the pectin release and recovery.

These results were similar to other reported pectins, which showed a significant rise of pectin yield at lower pH for extracted pectin from mango peels²⁷ and watermelon rind³¹. Fig. 1a illustrates that at high points of extraction time (X_2) and low values of LSR (X_3) the pectin yield decreased; this behavior is in agreement with results reported in the pectin extraction from seed watermelon peel³⁸ and pectin extraction from carrot pomace¹⁹. This is likely because of extended extraction time; the citric acid causes the breakdown of glycoside bonds and ester bonds of pectin³⁹. On the other hand, excessive or insufficient amounts of LSR influenced the pectin mass transfer due to the solution saturation or low dissolution capacity. Therefore, the pectin yields were smaller at extreme LSR conditions³⁸.

Effect of independent variables on DE

As can be seen, in Table 2 and Fig. 1b, the extracted pectin is considered like a high methoxyl pectin with DE ranged from 66.67% to 75.44%. According to ANOVA, linear effects were not significant, and the interaction between time extraction and LSR ($X_2:X_3$) and the quadratic effect of the time extraction (X_2^2) affected the DE (Table 2). High methoxyl pectin (DE > 50%) has been obtained from pomelo peel³⁶, pomelo albedo¹⁷ and watermelon rind²⁹. Indicating that our findings are in agreement with these previous works. It was noticed that the DE increased for more extensive extraction time. These results are in accordance with the reported values given in pectin from cocoa husks¹⁶. Under the conditions tested in this study, the DE showed that the obtained watermelon rind pectin was characterized with a low degree of de-esterification of polygalacturonic chains.

Effect of independent variables on MeO

The methoxyl content listed in Table 2, varied from 6.75 to 10.66%. Based on the Regression analysis (Eq. 3), it can be suggested that pH (linear and quadratic coefficients) negatively influenced the MeO (Fig. 1c). In the other word, decreasing pH led to get higher the methoxyl content. Other significant factors were interaction coefficients terms ($X_2:X_3$, Fig. 1c) and quadratic coefficient of the time extraction (X_2^2). In addition, the R squared pointed out that 6.62% of data variability could not be explained by the Eq. (3). The acidic extraction (citric acid) was favorable towards the methoxylation of side acid groups of polygalacturonic chains. This is probably due to the methoxylation was catalyzed by acidity medium (low pH), which increases the reactivity of the acid groups by shifting the

equilibrium forward to methyl ester formation. The methoxyl content of citrus peel pectin and apple pectin⁴⁰ were comparable with obtained results.

Effect of independent variables on AUA

Similar to the pectin yield, pH was the most significant variable for the anhydrouronic acid content. Unlike the yield, the quadratic coefficient term of LSR (X_3^2) was also significant. In Fig. 1d observes that AUA (purity pectin) increased at lower levels of pH and low-intermediate levels of LSR. However, the highest value of AUA (82.50%) was obtained at the lowest pH (2.0), greatest LSR (40 g/mL) and time extraction of 60 minutes. Several works from different plant sources like orange peels³⁷, ponkan peels²⁰ and banana peels²⁶ have shown that low pH and LSR between 20 and 25 g/mL increased the AUA^{16,20,37}. By contrast, it has been previously shown that longer extraction time improved pectin purity^{16,21,26}, which did not agree with the results in the present study.

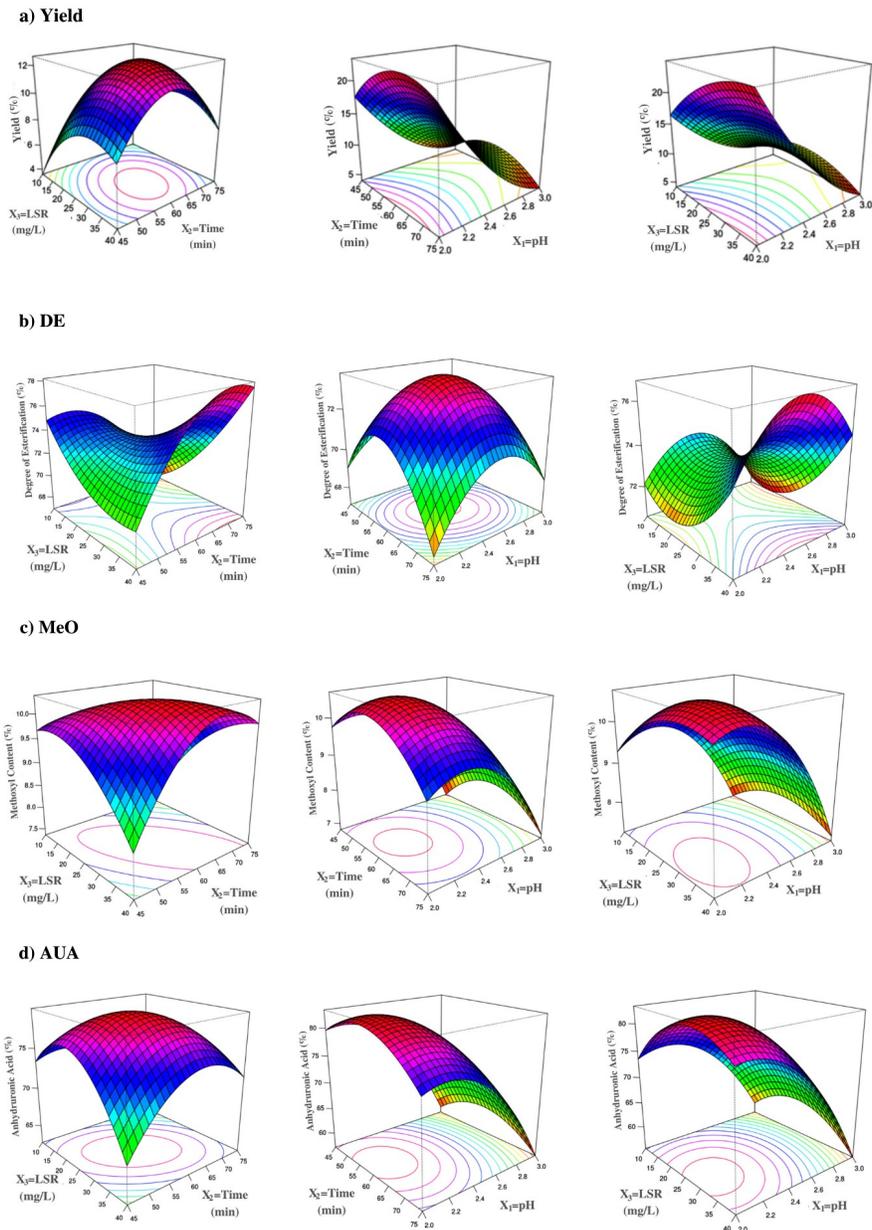


Figure 1. Response surface showing the effect of dependent variables (pH, time extraction and LSR) on the pectin yield (a), DE (b), MeO (c) and AUA (d).

Pectin optimization

Based on the polynomial models fitted for each of the responses, a separate optimization process was carried out to find out the optimal conditions that maximized pectin yield, DE, MeO and AUA independently. Different optimal conditions were obtained (Table 3). These optimal predictions confirmed the strong influence of pH (low values) on the pectin yield, MeO content and AUA content as well as previously shown. It is noteworthy that for all responses except DE, the optimal conditions were at low pH, intermediate values of time extraction (nearly 60 min) and midpoints to high levels of LSR for the study regions tested in this work.

In order to unify to one optimal condition (theoretical optimum), an analysis of the multiple responses through the desirability discontinuous functions⁴¹ using R-programming software⁴² was performed. The conditions obtained for the highest pectin yield and highest AUA simultaneously were found at pH of 2.0, extraction time of 62.31 min and LSR of 35.07 g/mL (Table 3). Theoretical optimum was quite similar to the optimal conditions predicted for pectin yield model in Eq. (1) as is shown in Table 3. In fact, the theoretical optimum pectin yield (24.30%) can be considered equivalent to highest predicted by the Eq. (1), which is slightly lower than the experimental value, 27.16%. In addition, regarding the other responses, the theoretical predictions are very good, similar to the experimental values (Table 1). According to these findings, the models were well fitted to predict the effect of independent variables and their responses.

Dependent variable	X ₁	X ₂ (min)	X ₃ (g/mL)	Optimal value†	Theoretical predicted‡
Pectin yield (%)	2.00	62.07	36.57	24.32	24.30
DE (%)	2.59	70.05	40.00	78.26	73.00
MeO (%)	2.23	60.97	31.12	10.72	10.45
AUA (%)	2.15	57.77	26.97	83.24	81.33

Table 3. Optimized responses and theoretical optimum. † Optimum calculated by each model separately ‡ Responses at pH = 2.0, extraction time = 62.31 min and LSR = 35.07 g/mL.

The optimal pectin yield (24.30%) was comparable with other studies that also used the desirability function to optimize simultaneous responses. The pectin extraction from melon peels¹¹, mango peels²⁸ and ponkan peels²⁰ reported maximum pectin yields of 29.48%, 30.0% and 25.6%, respectively. Previous works from watermelon rind have reported pectin yields of approximately 19%³¹ and 25%^{30,32}. It has shown that citric acid was a better solvent extractant than hydrochloric acid in the pectin extraction from watermelon rind²⁹. Nevertheless, Their maximum pectin yield (8.38%) obtained was very low compared to the reported values by this study and previous works, despite their optimal conditions (pH of 2.0, extraction time of 180 min, LSR of 25 and temperature of 80 °C) were pretty similar to ours. This might be due to the Colombian variety of watermelon and using methanol as a precipitating agent, which has shown to be a better precipitating agent than ethanol in the extraction of pectin from pomelo albedo¹⁷. For this reason, the highest properties of pectin were obtained from watermelon rind.

Characterization of pectin

The characteristics of extracted pectin from watermelon rind using citric acid under the highest yield experimental conditions (pH of 2.0, time of 60 min and LSR of 40 g/mL) were comparable with commercial pectin (Merck). Table 4 shows the remarkable properties of the pectin obtained. The ash content and alkalinity of ash were 1.24% and 0.39%, respectively, which were lower than the accepted levels for the standard pectin. The ash content is a measure of the degree of purity⁴⁰ and quality⁴³ of

pectin. Based on these results, the extracted pectin presented low amounts of soluble solids and high purity. Free acid registered a high value compared to standard pectin; this might be due to the chemical nature of carboxyl groups, which in harsh pH conditions are hydrolyzed, increasing pectin acidity.

High methoxyl and rapid-set pectin was obtained from watermelon rind because of the MeO (10.66%), and DE (73.33%) values are ranged for this pectin type (Table 4). Rapid-Set pectin is used in high sugar jams, jellies, and marmalades^{7,8}, indicating that pectin from watermelon rind could be employed in food applications. High methoxyl pectin from watermelon rind has been previously reported²⁹⁻³², and these findings are in agreement with obtained results. From the results of AUA, the extracted pectin had a value of 82.5%, which was higher than those reported by similar works with watermelon rind²⁹⁻³¹ and as well as the percent requirements for commercial food or pharmaceutical purpose^{33,34}. It should be pointed out that the AUA is a determining quality parameter and gelling properties of pectin^{19,33}. Therefore, the results related to AUA also indicated that high-quality pectin was extracted from watermelon rind by using citric acid.

Properties	Standard pectin	Extracted pectin
Ash (%)	3.77 ± 3.39	1.24
Alkalinity of ash (Calcium carbonate, %)	2.34 ± 2.90	0.39
Free acid (meq/g)	0.78 ± 0.46	1.25
MeO (%)	≥ 6.70 (USP)	10.66
DE (%)	≥ 50 high methoxyl pectin 71-74 rapid set	73.33
AUA (%)	≥ 74 (USP) ≥ 65 (FAO/WHO)	82.50

Table 4. Chemical properties pectin obtained from watermelon rind compared with standard pectin.

FTIR analysis

The information on the functional groups presents in the extracted pectin from watermelon rind was evaluated by FTIR analysis at highest yield experimental conditions (pH of 2.0, time extraction of 60 min and LSR of 40 g/mL). The FTIR spectra of watermelon rind pectin is illustrated in Figure 2. The peaks related to O-H and C-H stretching vibrations were between 3500-3250 cm⁻¹ and 3000-2700 cm⁻¹, respectively. Stretching vibrations (=CO) of esterified carboxyl groups and free carboxyl groups (1800-1600 cm⁻¹) were observed in the fingerprint regions of the pectin spectrum^{10,44-46}. The peak of CH bending vibrations for pyranose ring (approximately 1338 cm⁻¹) and the peak of COO⁻ stretching vibration for ester groups (1330-1210 cm⁻¹) can be assigned in Fig. 2⁴⁴. Furthermore, the characteristic overlapped peaks of glycoside bonds and pyranose cycles around 1000 cm⁻¹ and weak peaks (830-500 cm⁻¹) associated with α- and β-configurations were identified, indicating that the pectin is the principal component^{44,47}. The bands of FTIR spectra of the obtained pectin were similar to reported with analogous work of pectin extraction from watermelon rind²⁹.

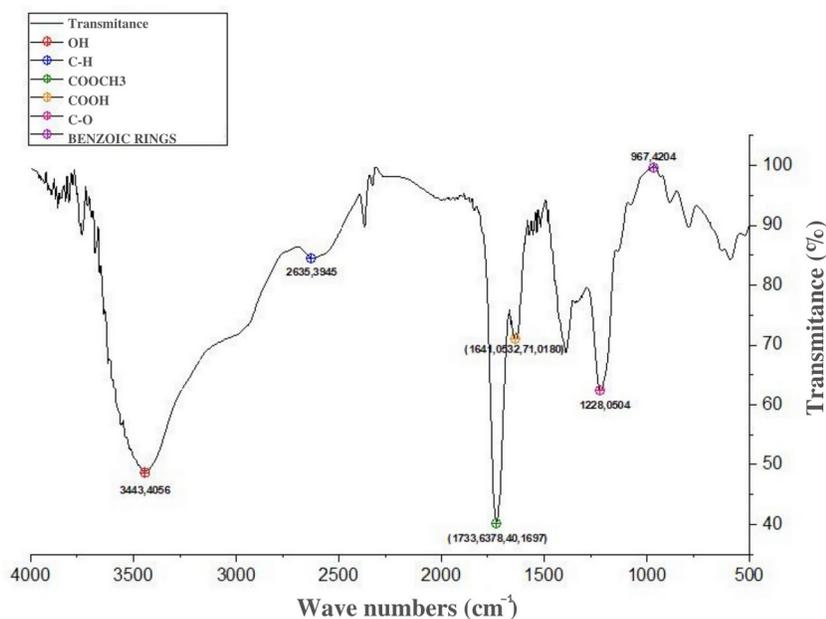


Figure 2. FTIR spectra of extracted pectin from watermelon rind.

CONCLUSIONS

In this study, the pectin extracted from watermelon rind with citric acid is considered as a high methoxyl pectin (DE > 50% and MeO > 6.7%) and has high quality (AUA > 65%, FAO/WHO, except for runs at pH of 3.0). Based on the results of BBD, pH was the most significant variable on the pectin yield and its properties. The simultaneous optimization to obtain the highest pectin yield and highest AUA showed that the optimal conditions were found at pH of 2.0 (lowest level), extraction time of 62.31 min (intermediate points) and 35.07 g/mL (high- midpoints), which were close to optimal experimental conditions (pH of 2.0, time of 60 min and LSR of 40 g/mL) for the highest yield pectin. Under this optimum the yield pectin, DE, MeO and AUA were 24.32%, 73.33% (rapid-set pectin), 10.66 and 82.50% (AUA > 74% USP, high quality), respectively. In addition, ash content and alkalinity of ash revealed high purity of pectin at the optimal condition.

MATERIALS AND METHODS

Samples preparation

Watermelon rinds were obtained from local market of watermelon juices located in the Barranquilla center and around the Universidad del Atlántico, Colombia. They were washed, manually cut, and heated in distilled water until boiling to denature enzymes and inactive microorganisms.⁴⁸ The collected material was milled for homogenization and was dried in a convection oven at a temperature no higher than 80 °C for 1440 minutes. Finally, the treated watermelon rind was packed and stored in a desiccator for later use.

Chemicals and solvents

All chemicals and solvents such as citric acid, ethanol and methanol used were of analytical grade.

Pectin extraction

The pectin extraction was carried out through acid hydrolysis with citric acid, according to the methodology with some modifications.⁴⁹ The dried watermelon rind was stirred in citric acid solutions with the following defined conditions for all runs of the Box-Behnken design: pH (2.0, 2.5 and 3.0);

extraction time (45, 60, 90 min) and liquid-solid ratio (1:10, 1:25 and 1:40 g/mL) at a constant temperature of 80 °C. The resultant slurry was vacuum filtered with a micro cloth using vacuum pressure. The residual liquid was precipitated with methanol in a 60% volume solution. The obtained precipitate was washed three times with 70% ethanol and subsequently was rewashed with 96% ethanol. The collected precipitate was dried in a convection oven until constant weight at 50 °C for 12 hours.

Pectin yield

The pectin yield of watermelon rind on dry weight basis was determined as follow (Eq. 5)^{10,43}

$$Pectin\ yield(\%) = \frac{weight\ of\ dried\ pectin(g)}{weight\ of\ dried\ watermelon\ rind(g)} \quad (5)$$

Where the dried pectin was obtained after the treatment of filtration, precipitation and drying; the dried watermelon rind was collected as result of sample preparation.

Pectin characterization

Determination of degree of esterification and anhydrouronic acid content

The degree of esterification and the anhydrouronic acid content were determined by titration relating the methoxyl content with the equivalent weight. 0.50g of watermelon rind pectin was dissolved into ethanol/water solution (1:20 v/v); 5 drops of phenol red indicator were added, and the sample was titrated with 0.1N sodium hydroxide (V₁, mL) until the indicator changed. Then, 25 ml of 0.25N NaOH was added, and the sample was heated and stirred vigorously. Five drops of phenol red and 25 mL of 0.25N HCl were added again and, it was titrated with 0.1N NaOH (V₂, mL) until the color change from yellow to faint pink endpoint.⁵⁰ The MeO and the DE were then calculated according to Eq. (6)^{18,51} and Eq. (7)^{11,19,52}, respectively.

$$MeO(\%) = \frac{V_2(mL)0.1(N)31}{weight\ of\ sample(mg)} \times 100 \quad (6)$$

$$DE(\%) = \frac{V_2(mL)}{V_1(mL) + V_2(mL)} \times 100 \quad (7)$$

The Eq. (8) was used to calculate the anhydrouronic acid content¹⁸

$$AUA(\%) = \frac{0.1(N)(V_1(mL) + V_2(mL))176}{Weight\ of\ sample(mg)} \times 100 \quad (8)$$

Where 176 is the molecular weight of anhydrouronic acid expressed as mg/meq; V₁ and V₂ were the volumes used for first and second titration, respectively.

Fourier transform infrared spectroscopy

Fourier transform infrared (FTIR) spectra of the extracted pectin was used to evaluate its structural chemical properties. FTIR analysis was performed in the SHIMADZU (IRAffinity-1) spectrometer with a resolution of 4cm⁻¹ and 130 scans of wavelengths ranging from 7000 to 400 cm⁻¹.

Experimental design

The Box-Behnken design response surface design was used to evaluate the effect of three independent variables such as pH (X_1 : 2.0-3.0), the extraction time (X_2 : 45-75 min) and the liquid-solid ratio, LSR (X_3 : 10-40 g/ml) on simultaneous responses (pectin yield, the MeO, the DE and AUA). Table 5 summarizes the levels and code of three independent variables.

Dependent variables	Real values of coded levels		
	-1	0	1
X_1 : pH	2.0	2.5	3.0
X_2 : Time (min)	45	60	75
X_3 : Liquid-solid ratio, LSR (g/mL)	1:10	1:25	1:40

Table 5. Levels and code of variables chosen for the Box-Behnken design.

The experimental design and variance analysis (ANOVA) were performed using R-programming software 4.0.2. The responses variables were fitted to the second-order polynomial model as given for the following Eq. (9).

$$Y = B_0 + \sum_{i=1}^3 B_i X_i + \sum_{i=1}^3 B_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 B_{ij} X_i X_j \quad (9)$$

Where: Y is the response variable; B_0 , B_i , B_{ii} and B_{ij} are the coefficients terms for the regression model; X_i and X_j are the levels of independent variables. The responses were initially optimized separately to predict the 3D surface and the maximum values. Latterly, an optimization of simultaneous responses using the desirability function⁴¹ was performed.

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Author contributions

J. P. designed and supervised the work and wrote-edited the manuscript. K. G. and L. V. carried out the experiments and chemical analysis. K. G. edited the main manuscript. All authors did the statistical and data analysis.

Competing interests

The authors declare no competing interests.

Additional information

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