Research Article

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Journal of Nutraceuticals and Food Science

2020
Vol.5 No.6:1

Extraction and Physicochemical Pectin from Extraction Watermelon

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Received Date: December 12, 2020; **Accepted Date:** December 15, 2020; **Published Date:** December 25, 2020

Citation: Lee KY, Choo WS (2020)Extraction and Physicochemical Pectin Extraction. from Watermelon J Nutraceuticals Food Sci Vol.5 No.6:1.

Abstract

Extraction of pectin fr om watermelon (*Citrullus lanatus*) rind using citric acid, an organic acid was optimized using Response Surface Methodology (RSM) and compared to that using hydrochloric acid, a mineral acid. A five-level, four-factor central composite design was employed to optimize the conditions for maximum yield of pectin. The effects of pH, reaction temperature, reaction duration and substrate to extractant ratio were investigated. The optimized conditions using citric acid as an extractant were at pH 2, 80˚C, 3h and substrate to extractant ratio of 1:25. The optimized conditions using hydrochloric acid as an extractant were similar except for a shorter extraction time of 2h. At these extraction conditions, the yield of pectin extracted using citric acid (8.38 ± 0.43) was significantly higher than that using hydrochloric acid (6.52 ± 0.15) but showed similar uronic acid content (50.2-58.8%), degree of methylation (66.3-71.5%) and degree of acetylation (106.0-106.8%).

Citric or hydrochloric acid-extracted watermelon rind pectin showed near Newtonian like flow behavior at 1% concentration and this behavior were similar to citrus peel pectin. This study demonstrated the use of citric acid as the more effective acid than hydrochloric acid to extract pectin and also the transformation of fruit waste to use material from watermelon rind.

Keywords: Central composite design; Fruit waste; Response surface methodology; RSM; Watermelon peel

Introduction

Citrullus lanatus or watermelon is a tropical fruit that is widely cultivated around the world. Watermelon possesses a thirst-quenching effect as it has greater than 90% water [1]. The outer part of the fruit is known as the rind or peel which is green with dark green stripes and the inner part of this rind is white in color [2]. Watermelon rind contains mainly carbohydrates [3]. As watermelon rind represents about one-third of the total fruit mass, researchers have been trying to discover new ways to utilize it. Watermelon rind could be turned into a sorbent for the removal of heavy metals such as nickel and cobalt from aqueous solution [4].

Recent reports proposed the use of watermelon rind as a pectin source [5-8]. Pectin is a polysaccharide that contains mainly galacturonic acid joined by α-(1,4) linkages. Pectin is also a multifunctional component found in the intercellular space and middle lamella of higher plants [9]. In the food and pharmaceutical industries, pectin is known as a biopolymer that is widely used due to its hydrocolloid properties. By-products of the apple and citrus fruit industries are the major pectin sources [10]. In the food industry, pectin is widely used as a food additive. It can function as a stabilizer in beverages such as yogurt drinks and milk, a thickener in bread, frozen dough and yogurt, an emulsifier for cream, milk and ice cream and a gelling agent for jam and jellies [11]. The applications of pectin are influenced by a number of factors such as the degree of methylation (DM) and degree of acetylation (DA). High methoxyl pectin (DM>50%) is used in frozen dough, breads and pre-proofed dough to improve the volume of these products. Low methoxyl pectin (DM<50%) is used as a thickener in yogurt and a gelling agent in dairy products and desserts [11]. The physicochemical properties of pectin determine its application. The physicochemical properties of pectin varied according to the plant source and the extraction conditions used [12].

On the industrial scale, mineral acids such as hydrochloric acid, nitric acid, and sulphuric acid are commonly used to extract pectin [13]. Previous studies on the pectin extraction from watermelon rind used hydrochloric acid, nitric acid or sulphuric acid as an extractant [5-8]. Citric acid, an organic acid had been previously shown to increase the yield of pectin extraction from other various sources [12-15]. Therefore, the objective of this study was to use Response Surface Methodology (RSM) to optimize the extraction conditions to

obtain pectin from watermelon rind using citric acid and compare to those using hydrochloric acid as an extractant. Furthermore, the properties of the pectin produced using the optimized conditions were determined.

Methodology

Materials

Watermelons were purchased from a local supermarket in Selangor, Malaysia. All chemicals and solvents used in this study were of analytical or HPLC grade.

Sample preparation

Watermelon rind was cut into 3 cm × 3 cm pieces and dried at 60˚C for 24h. The dried watermelon rind was ground using a blade mill (Polymix Kinematica, Littau, Switzerland).

Experimental design and extraction of pectin

RSM was used for pectin extraction optimization. In this study, four factors were extraction time, pH, temperature, and substrate to extractant ratio and the response was the yield of pectin. The levels of the factors or independent variables used in the CCD are shown in **(Table 1)**. The levels selected for extraction time differed between the two acids due to hydrochloric acid being a stronger acid and thus require a shorter time. A central composite design (CCD) consisting of 30 experimental runs was used **(Tables 2 and 3)**. The software used in this optimization study was Design Expert 7.0 (Stat-Ease, USA).

Table 1: Levels of independent variables used in the central composite design for pectin extraction.

Independent variable	Symbol	Coded levels				
		-α	-1	0	1	+α
Extraction pH	X1	1.5	2	2.5	3	3.5
Extraction time (hours) for citric acid	X2	1	1.75	2.5	3.25	4
Extraction time (hours) for hydrochloric acid	X2	0.5	1	1.5	2	2.5
Extraction temperature (°C)	X3	50	60	70	80	90
Substrate to extractant ratio	X4	10	15	20	25	30

Table 2: Central composite design for pectin extraction using citric acid with experimental values.

Variables					
	X1	X2	X3	X4	Yield (%)
Run no.	pH	Time (h)	Temperature (˚C)	Substrate to extractant ratio (w/v)	
1	2.5	2.5	70	20	4.4
2	2.5	2.5	50	20	4
3	3	3.25	60	15	0.7
4	2.5	4	70	20	4.9
5	3.5	2.5	70	20	1.5
6	3	3.25	80	15	3.2
7	2	1.75	80	25	10.6
8	2.5	2.5	70	30	4.2
9	2.5	2.5	70	20	2.7
10	3	1.75	80	25	3.7
11	1.5	2.5	70	20	12.8
12	3	3.25	80	25	4.4
13	2.5	2.5	70	10	3.2

14	2.5	2.5	70	20	5
15	2	1.75	60	25	6.6
16	2.5	2.5	70	20	4.6
17	2.5	1	70	20	3.7
18	2.5	2.5	90	20	6.3
19	3	1.75	80	15	1.6
20	2	3.25	80	15	10.6
21	2	3.25	60	25	3.1
22	2	1.75	60	15	5.7
23	3	1.75	60	25	2.5
24	2	3.25	60	15	3.5
25	3	3.25	60	25	0.9
26	2	1.75	80	15	6.9
27	2.5	2.5	70	20	4
28	2	3.25	80	25	9.7
29	3	1.75	60	15	1.4
30	2.5	2.5	70	20	5.4

Table 3: Central composite design for pectin extraction using hydrochloric acid with experimental values.

Variables					
	X1	X2	X3	X4	
Run No.	pH	Time (h)	Temperature (˚C)	Substrate to extractant ratio (w/v)	Yield (%)
1	2	1	60	15	3.2
2	3	1	60	15	1.8
3	2	2	60	15	3.6
4	3	2	60	15	1.8
5	2	1	80	15	5
6	3	1	80	15	2
7	2	2	80	15	6.2
8	3	2	80	15	2.6
9	2	1	60	25	3.9
10	3	1	60	25	1.7
11	2	2	60	25	4.6
12	3	2	60	25	1.9
13	2	1	80	25	5.1
14	3	1	80	25	2.2
15	2	2	80	25	6.3
16	3	2	80	25	2.9
17	1.5	1.5	70	20	5.5
18	3.5	1.5	70	20	1.7
19	2.5	0.5	70	20	2.6
20	2.5	2.5	70	20	3.3
21	2.5	1.5	50	20	1.7
22	2.5	1.5	90	20	5.1
23	2.5	1.5	70	10	1.7
24	2.5	1.5	70	30	2.6
25	2.5	1.5	70	20	3.5
26	2.5	1.5	70	20	3.3
27	2.5	1.5	70	20	3.1
28	2.5	1.5	70	20	2.8
29	2.5	1.5	70	20	2.9
30	2.5	1.5	70	20	2.5

Pectin extraction was carried out using the dried watermelon rind prepared as above [13]. The dried watermelon rind was treated with 85% ethanol at 70˚C for 20 min in a shaking water bath and then filtered with a micro cloth (60 µm). These steps were carried out another three times followed by extraction of the residue using citric or hydrochloric acid. After extraction, the residue was treated with five times the volume of 95% ethanol and centrifuged at 14,500 xg for 10 min. The pellet was washed with 70% ethanol followed by 95% ethanol. Lastly, the washed pellet was dried

overnight at 50˚C and this was the dried watermelon rind pectin.

Pectin yield

The pectin yield was calculated using the equation stated below.

$$Yield\ (\%) = \frac{Weight\ of\ dried\ watermelon\ rind\ pectic\ (g)}{Weight\ of\ dried\ watermelon\ rind\ (g)} \times 100$$

Fourier transform infrared spectrophotometry (FTIR)

A Nicolet iS10 FTIR spectrometer 2 (Thermo Scientific, Waltham, USA) was used to record FTIR spectra of hydrochloric acid-extracted pectin, citric acid-extracted pectin and citrus peel pectin (Sigma-Aldrich, St. Louis, USA) in the range of 400 to 4000 cm^{-1}. Scans were collected at a resolution of 4 cm^{-1}.

Determination of uronic acid content

Spectrophotometric determination of uronic acid content was carried out according to Filisetti-Cozzi and Carpita [16]. Distilled water containing 0.4 mL of 0.1% pectin was mixed with 40 µL of 4 M sulfamic acid-potassium sulfamate (pH 1.6). This mixture was then added to 2.5 mL of 0.0125 M sodium tetraborate in sulfuric acid and mixed before cooling in an ice bath. Next, the mixture was brought to the boil for 20 min followed by cooling in an ice bath. After cooling, 80 µL of meta-hydroxy diphenyl reagent was added and incubated for 20 min. The absorbance was read at 520 nm with D-galacturonic acid as a standard.

Determination of degree of methylation (DM) and degree of acetylation (DA)

High-Performance Liquid Chromatography (HPLC) was used to determine the DM and DA of the pectin according to Levigne et al. [17]. Pectin (5 mg) was added to 10 mM copper sulfate and 0.5 mL of 25 mM isopropanol as an internal standard. Next, 0.5 mL of 1 M NaOH was mixed with the mixture and left to stand at 4˚C for 1.5h for saponification. Then, the mixture was centrifuged at 8000 xg for 10 min and the supernatant was neutralized with a syringe equipped with a Maxi-clean IC-H filter membrane (Alltech, Deerfield, U.S.A.). Afterward, the sample was analyzed using an HPLC system (Agilent Technologies, Santa Clara, U.S.A.) equipped with a Superspher 100 RP-18 endcapped LiChroCART® 250-4 column (Merck, Darmstadt, Germany) under refractometric detection. The mobile phase used was 4 mM sulfuric acid and the flow rate was 0.7 mL/min at 25˚C.

Flow analysis

Pectin extracted from watermelon rind using citric or hydrochloric acid (1%, w/v) was dissolved in Milli-Q water. The flow behavior was measured using a rheometer (Programmable DV-III+Rheometer, Brookfield, U.S.A.) with CPE-42 spindle at 25°C. The shear rate was set from 0 to 1000 s^{-1} using a Rheocalc software version 3.

Statistical analysis

All experiments were performed in independent triplicate. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 21 (IBM Corporation, New York, USA). One-way ANOVA and Tukey's posthoc test was carried out and the statistical significance was evaluated at $p<0.05$.

Results and Discussion

Fitting the RSM model

In this study, a second-order model was used to explain the relationships between extraction conditions and the yield of pectin. The second-order model is shown below:

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \left(\sum_{i=1}^{n} b_{ii} X_i\right)^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X$$

where Y is the predicted response, b_o is the constant coefficient, b_i is the linear coefficient, b_{ii} is the quadratic coefficient, b_{ij} is the interaction coefficient, and X_i and X_j are the coded values for the extraction variables. The equations for extraction using citric acid are listed below after eliminating the insignificant terms **(Table 4)**. The significance level was set at $p<0.05$.

Yield (%)=25.16272-9.76379 X_1-0.092084 X_3-0.14388 X_1X_3+0.10889 X_2X_3+2.55188 X^2_1

The equations for extraction using hydrochloric acid are listed below after eliminating the insignificant terms **Table 4:**

Yield (%)=25.16272-9.76379 X_1-5.68607 X_2-0.092084 X_3-0.14388 X_1X_3+2.55188 X^2_1

The lack of fit for these two prediction models was not significant. This indicates that the prediction models are suitable for predicting the optimum conditions to extract pectin from watermelon rind using citric or hydrochloric acid as an extractant. In addition, the R-squared values for the model to predict the yield of pectin extracted using citric acid and hydrochloric acid were 0.9321 and 0.9507, respectively. These indicate that these models can explain 93.2% and 95.1% of the variation in the yield of pectin, respectively.

The optimized conditions using citric acid as an extractant were determined to be at pH 2, 80˚C, 3h, and substrate to extractant ratio of 1:25 whereas the optimized conditions

using hydrochloric acid as an extractant were at pH 2, 80˚C, 2h and substrate to extractant ratio of 1:25.

Table 4: Galacturonic acid (GalA); Degree of Methylation (DM); Degree of Acetylation (DA); Degree of Esterification (DE); and yield of pectin extracted using citric acid or hydrochloric acid under optimum conditions.

Extractant	Yield (%)	GalA (%)	DM (%)	DA (%)
Citric acid	8.38 ± 0.43a	50.2 ± 11.8 a	66.3 ± 20.1 a	106.8 ± 30.1 a
Hydrochloric acid	6.52 ± 0.15b	58.8 ± 20.3 a	71.5 ± 22.8 a	106.0 ± 23.6 a

Effect of extraction conditions on the yield of pectin

The interactions between time and temperature as well as pH and temperature showed significant effects ($p<0.05$) on the yield of pectin extracted using citric acid. Lower pH and higher temperatures increased the yield of pectin extracted from watermelon rind using citric **(Figure 1)** or hydrochloric acid. Longer extraction time and higher temperature also increased the yield of pectin extracted from watermelon rind using citric acid. The effects of time, temperature or pH on the yield of pectin had been demonstrated in pectin extraction from other sources [18-21].

The substrate to extractant ratio did not show a significant effect on the yield of pectin from watermelon rind. However, another study showed that substrate to extractant ratio has a significant effect on the yield of pectin from the orange peel with an increase in the substrate to extractant ratio resulted in an increase in the pectin yield to a certain extent followed by a decrease in the pectin yield when excessive extractant was added. Saturation of extractants can inhibit mass transfer [22].

The yield of pectin extracted using optimized conditions

The yield of watermelon rind pectin extracted using optimized conditions in this study is in accordance with one reported study by Hartati and Subekti [7] but lower than others [5,6,8]. The discrepancy is most likely due to the use of different extraction conditions and calculation of yield. The yield of pectin extracted using citric acid in this study was significantly higher ($p<0.05$) than that using hydrochloric acid. This result is in accordance with the other studies on pectin extraction from cocoa husks by Chan and Choo [12], pomelo by Chan et al. [13], buttercup squash flesh by Methacanon et al. [15] and apple pomace by O'Donoghue and Somerfield [14]. The amount of pectin extracted using strong acid is generally lower than using weak acid [23]. This is due to strong acid that could produce highly soluble and smaller pectin molecules as a result of partial hydrolysis and be easily lost during the filtration process [24]. In addition, smaller pectin molecules may not be precipitated during alcohol precipitation and some might be eluted out with the alcohol and reduce the yield of pectin [23].

Properties of pectin extracted at optimum conditions

There was no significant difference in the uronic acid content of pectin extracted using citric or hydrochloric acid. This result is not in accordance with a study on yellow passion fruit peel pectin [18]. The discrepancy is most likely due to the source of pectin used. In addition, the uronic acid content of watermelon rind pectin in this study is lower than those reported by other studies on watermelon rind pectin [5,8]. Protein or starch in the extracted pectin can be removed to obtain a higher uronic acid content [25,26].

Based on the DM, the pectin extracted from watermelon rind using citric or hydrochloric acid was high methoxyl (HM) pectin. Moreover, there was no significant difference in the DM of pectin extracted using citric or hydrochloric acid. The DM of watermelon rind pectin in this study is in accordance with a reported study on watermelon rind pectin [8]. There was no significant difference in the DA of pectin extracted using citric or hydrochloric acid under the optimum conditions. The DA of pectin extracted from watermelon rind using citric or hydrochloric acid was more than 100%. The maximum DA could be as high as 200%, as each uronic acid is esterified with two or three acetyl groups [27]. Additionally, other factors such as the presence of sugars in the pectin molecules could also contribute to a high DA value [28].

Fourier transform infrared spectroscopy (FTIR) analysis

Validation of the pectin from watermelon rind was done using FTIR. Previous studies by Jiang et al. [5], Maran et al. [6], Hartati et al. [7], Petkowicz, et al. [8] on pectin extraction from watermelon rind using hydrochloric acid, nitric acid or sulphuric acid as an extractant did not conduct FTIR analysis of the watermelon rind pectin. Pectin from watermelon rind in this study was compared with citrus peel pectin with a DE of more than 85%. The fingerprint regions of pectin are from 950-1200 cm^{-1} which indicate the presence of pyranose cycle vibration and the bands ranging from 12000-1800 cm^{-1} indicate the presence of carboxylic groups in pectin [29].

The similar bands between pectin from watermelon rind and citrus peel pectin include the pyranose cycle region at 1016-1019 cm^{-1}, 1052 cm^{-1}, 1076 cm^{-1}, 1104 cm^{-1}, and 1149 cm^{-1}. All these bands are important characteristic bands found in pectin from different plants [30]. Furthermore, the band for

non-carboxylic groups of pectin which is located at 1600-1650 cm^{-1} is also shown in the pectin from watermelon rind. Additionally, the C=O bond at 1750 cm^{-1} is also shown in the pectin **(Figure 2)** as a result of the esterification of galacturonic acid [30].

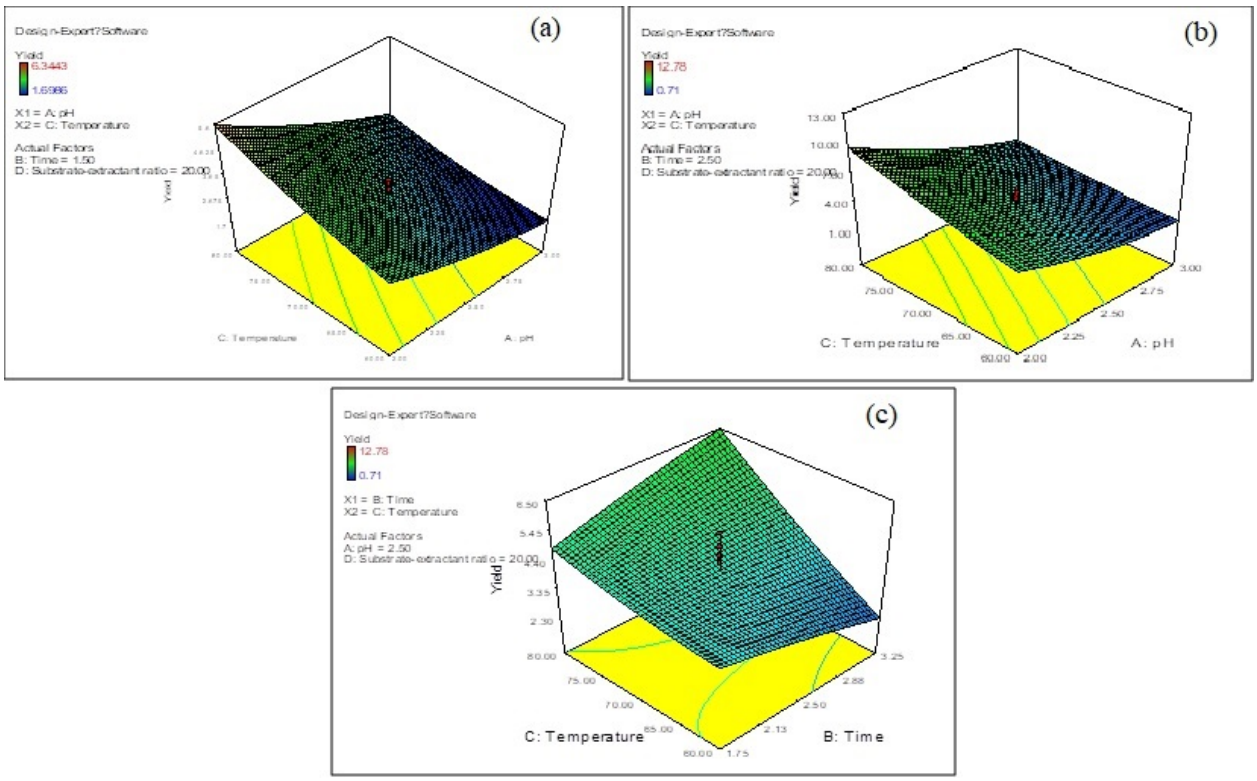

Figure 1: 3D surface plots for the yield of pectin extracted from watermelon rind using hydrochloric acid. A): Effect of pH and temperature; B): Effect of pH and temperature; C): Effect of time (h) and temperature.

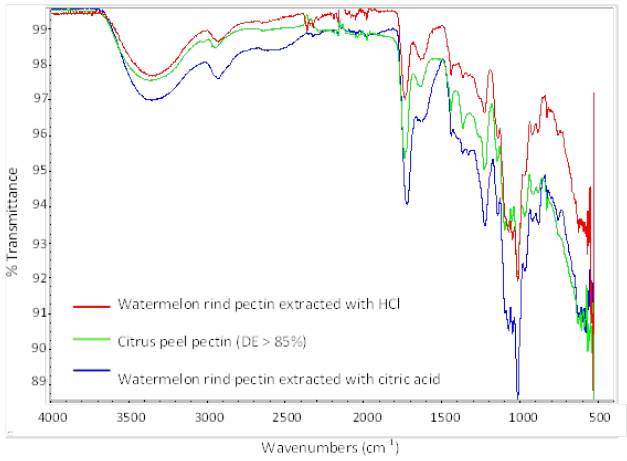

Figure 2: FTIR spectrum of pectin extracted from watermelon rind using hydrochloric acid or citric acid and citrus peel pectin (>85% esterified).

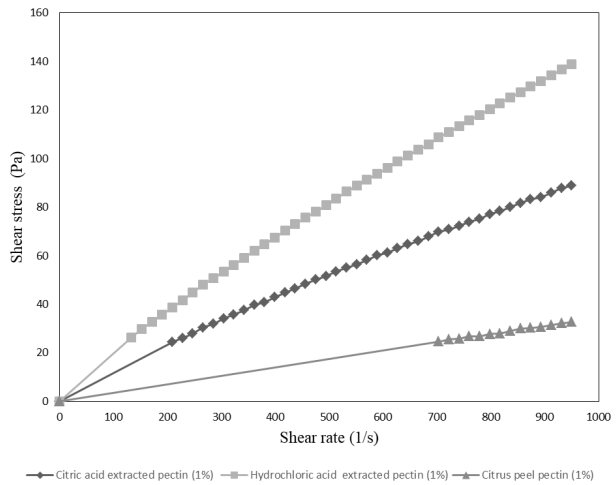

Figure 3: Flow behavior of the pectin extracted using hydrochloric acid or citric acid and a citrus peel pectin at 1% concentration.

Flow behavior of pectin from watermelon rind

The flow behavior of pectin from watermelon rind using citric (flow behavior index=0.9955) or hydrochloric acid (flow behavior index=0.996) and a citrus peel pectin (flow behavior index=0.9988) at 1% concentration showed a linear relationship between the shear stress and share rate **(Figure 3)** This near Newtonian like flow behaviour has also been [31]. reported on pectin extracted from [31] citrus peel and on dragon fruit peel at 1% concentration [32].

Conclusion

The potential of using citric acid as an extractant in pectin extraction from watermelon rind was shown in this study as the yield of pectin extracted using citric acid was higher than using hydrochloric acid with similar properties in terms of uronic acid content, DM, DA and flow behavior. Pectin from watermelon rind was determined as HM pectin with potential applications in food products containing a high amount of sugar or acidic pH conditions.

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