EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology 2024, 25(8), 308–315
https://doi.org/10.12912/27197050/190081
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Received: 2024.06.01
Accepted: 2024.06.18
Published: 2024.07.01

Optimization and Characterization Pectin Extraction from Rosy-Pulped Pomelo Using Ethanol Precipitation Method

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ABSTRACT

The goal of this study is to use a simple approach to synthesize pectin from grapefruit peel, an agricultural waste common in tropical regions. Additionally, the study employed optimization techniques to identify the ideal conditions for the maximum extraction efficiency while also determining the importance of studying factors. Besides, extracted pectin characteristics are also presented in this research. Under several analysis, some chemical groups for pectin were presented in extracted pectin by Fourier transform infrared spectroscopy analysis (FTIR). For instance, C-H methyl groups at the peak 1442 cm^{-1}; methyl ester groups C=O appears at the wavelength 1747 cm^{-1} and aliphatic C-H groups and hydroxyl groups. Furthermore, under three major conditions acid (%); temperature and time extraction, an optimization study was conducted to figure out the optimal conditions for pectin extraction efficiency. Under optimal conditions acid 10%; 77.5 °C and 80 mins is the favorable condition for obtaining pectin extraction efficiency. Basically, this study is the first one extracting pectin from rosy-pulp pomelo peel by sulfuric acid-ethanol precipitation method. This is a preliminary that lays the foundation for purifying extracted pectin from rosy-pulp pomelo, also solving this agricultural waste bring higher economic values.

Keywords: green source, rosy-pulp pomelo, pectin, extraction, efficiency.

INTRODUCTION

Pectin can be utilized as a stabilizer in acidic protein beverages as well as to enhance the mouth feel and pulp stability in drinks with juice as the foundation. Pectin also increases the gel strength of low-calorie jams and decreases syneresis in jams and marmalades. In confectionary jellies, pectin is employed to provide a solid gel structure and a clean bite. Pectin is typically used in food applications at a dosage of 0.5% to 1%. Additionally, the food sector employs it. Pharmaceutical and cosmetic products both use pectin (Analese et al. 2023). As an emulsifier, gelling agent, glazing agent, stabilizer, and/or thickening in industrial applications, pectin is a naturally occurring, biocompatible, biodegradable, and renewable polysaccharide. These functions are all subsets of the phrase "rheology modifier." When the molecule chains cross-link, a three-dimensional network is created that retains water and co-solutes, creating pectin gels (Ana et al. 2019; Didem et al. 2021).

Pectin is a naturally occurring substance that is found in the cell walls of many plants. It has long been employed for its ability to make gels, thicken liquids, and stabilize substances in a variety of fields, including the food, pharmaceutical, and cosmetic sectors. Pectin consumption has been demonstrated to lower blood cholesterol levels (Analese et al. 2023). Microorganisms break down pectin in the large intestine and colon, releasing short-chain fatty acids that are beneficial to health. Fruit and vegetable leftovers, primarily citrus or apple peel, can be used to make pectin (Didem et al. 2021). The main benefits of processing these by-products are their high reliability and economic value in the case of pectin derived from citrus fruits, particularly from oranges, grapefruits, etc. Almost 60% of the weight of oranges is thought to be processed, according

to estimates (Elnaz et al. 2017; Ana et al. 2019; Licelander et al. 2021).

According to this perspective, various methods are available to extract pectin from food waste. However, most conventional procedures rely on acidic hot extraction and use chemicals like hydrochloric acid, nitric acid, or sulfuric acid that have substantial environmental implications (Licelander et al. 2021; Luiz et al. 2022). They also result in low extraction yields, high extraction times, and heating requirements. Compounds could volatilize or high-quality chemicals could degrade. Recent proposals for green extraction techniques show promise for increasing the sustainability of pectin extraction, especially given the reduction in operation time and the restriction of high-impact compounds (Luiz et al. 2022; Nasrul et al. 2019).

These are based on the use of Deep Eutectic Solvents (DESs), thanks to their insignificant volatility at room temperature and their capability to form homogenous solutions with water, organic acid and/or pure water as extracting solvents (Muhamamd et al. 2019). Additionally, innovative extraction choices are based on the exploitation of ultrasound or microwave-assisted technique, due to their low energy and reagent consumption, shorter treatment time and greater safety of the operators, in comparison to the conventional extraction techniques (Seyed et al; Ricardo et al. 2019). However, an efficient, reliable, economic, reproducible, and environmentally safe extraction method is still sought after. Due to their negligible volatility at room temperature and capacity to generate homogeneous solutions with water, organic acid, and/or pure water as extracting solvents, these are based on the usage of Deep Eutectic Solvents (DESs) (Muhammad et al. 2019; Sayed et al. 2016). Additionally, due to their lower energy and reagent usage, faster treatment times, and increased operator safety over conventional extraction procedures, cutting-edge extraction choices are based on the use of ultrasonic or microwave-assisted approaches. An effective, dependable, affordable, reproducible, and ecologically secure extraction process is still desired.

The purpose of this research is to develop an optimization method for extracting pectin from rosy-pulp pomelo peel by using strong acid such sulfuric acid combined with ethanol precipitation method. The study seeks to fill the gap, namely using strong acid to optimal pectin extraction from fruit wastes such rosy-pulp pomelo, which we did not find in the existing scientific literature. Because, existing research mostly use weak acid to extract pectin from organic materials. In this study we select rosy-pulp pomelo peel as a material for extracting pectin.

Because the rosy-pulp pomelo is a widely consumed fruit in many tropical nations due to its abundant growth and high yield. Because of this, its peel is likewise quite large. Using their peels to produce new economic values is constantly promoted. Besides, the assessment of optimal conditions has not been applied much to pectin extraction studies using this material. Therefore, our research focuses on finding optimal conditions for pectin extraction from this material to help reduce costs and time for the experimental process. At the same time, choose suitable conditions for optimal extraction efficiency. This study is also supplemented with some basic analyzes to compare extracted pectin and commercial pectin to show the difference between the researched pectin and the finished commercial pectin. Fourier Transform Infrared (FTIR) and Scanning Electron Microscope (SEM) were applied to examine extracted pectin characteristics.

MATERIALS AND METHODS

Chemicals

Sulfuric acid 98% and ethanol 95% were bought from Xilong – China.

Rosy-pulped pomelo

Rosy-pulped pomelo peels were collected from a local market in Hanoi city. The fresh peel was sorted and thoroughly washed to remove impurities. Fresh, rosy-pulped peels were processed: cut into coarse pieces (size > 1 cm).

Pectin extraction preparation

Rosy-pulped pomelo peels weighing 25 gram and were cooked under studied temperatures and investigated contact times (detailed information for temperature and contact time were performed in optimization study section). After that, add sulfuric acid with studied concentrations, stirring often, until the substance changes shape. Ethanol (6 mL 95%) was added to the center of the solution after it had naturally cooled down, and it was physically agitated until the gel had formed. Using a paper filter, the gel

and water from the solution were separated. The gel was dried in a low-temperature oven (under 70 °C) until all the water was gone. The gel was ground and stored in a plastic bag after being dried into a solid shape (Sayed et al. 2016). Pectin recovery efficiency was computed by Equation 1 below:

$$H_{pectin}(\%) = (P/25) \times 100 \quad (1)$$

where: P – the mass of pectin extraction (gram).

Optimization study – response surface methodology - central composite design

Central composite design (CCD) is a commonly used statistical method based on a multivariate nonlinear model for optimizing biosorption process factors. It is also used to construct regression model equations and operating conditions from appropriate experiments (Elnaz et al. 2017). Additionally, it is helpful in analyzing how different process factors interact with one another. In the present study, the CCD was used to identify the ideal process factors for pectin extraction from rosy-pulped pomelo peels. A second-order model that requires the fewest possible experiments for fitting was fitted using the CCD. The CCD consists of 2n factorial runs (coded using the standard notation), 2n axial runs [with six duplicates of each (±α, 0, 0, …, 0), (0, ±α, 0, 0, …, 0), …, (0, 0, …, ±α)] and 2n accentor runs (Ana et al. 2019; Elnaz at el. 2017; Luiz et al. 2022). The more components there are, the more runs are required to fully recreate the design described in Equation 2:

$$N = 2^n + 2n + n_c \quad (2)$$

Essential, the optimization process consists of three basic steps: (1) ducting statistically planned tests; (2) estimating the coefficients in mathematical model, and (3) predicting the response and assessing the model's suitability (Ana et al. 2019). To analyze the influence of parameter interactions, an empirical model was built to link the response to the pectin recovery efficiency. It is based on a second order quadratic model for pectin extraction using rosy-pulped pomelo peels as provided by equation (3) (Ricardo et al. 2019). In this study, response surface methodology – central composite design (RSM-CCD) was used to determine the optimal conditions for pectin extraction through three driving factors: acid (%); temperature (°C); and contact time (min). The pectin extraction efficiency was measured under equation below:

$$H_{pectin} = a_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_1^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} b_{ij} X_i X_j \quad (3)$$

where: H_{pectin} – pectin extraction efficiency (%), a_0 – zero-order regression coefficient, X_i – ith independent factor affects pectin extraction efficiency, b_i – first-order regression coefficient describes the influence of factor X_i to objective function H_{pectin}, b_{ii} – the interaction regression coefficient describing the influence of factors X_i to H_{pectin}, b_{ij} – interaction regression coefficient describing the influence simultaniety of X_i to X_j to H_{pectin}.

Fisher's determination (= 0.05) and lack of fit (Lack of fit) tests are used to determine how closely the model and regression equation match the experiment. To determine the coefficients b of the regression equation regulation, use the square technique minimal. The significance of the regression coefficients is examined using the Student's test (t-test) (P = 0.05). The significance level of the model is assessed using an analysis of variance. Optimizing the function target using the expectation function approach using the regulatory software experimental plan Design-Expert 11 (StaS-Ease, Minneapolis, MN, US). The next step was to conduct three repetitions of experiments on the article's ideal condition to assess the compatibility of the properties model.

RESULTS AND DISCUSSIONS

Pectin characterization

FTIR results

The chemical groups in the pectin that was extracted from the rosy-pulped pomelo were

Table 1. Range and level of the independent variables

Factor		Unit	Range and level				
			-α	-1	0	+1	+ α
Acid	A	%	6.64	8	10	12	13.4
Temperature	B	°C	64.88	70	77.5	85	90.11
Contact time	C	min	46.4	60	80	100	113.64

identified using FTIR analysis, and the results were compared to those of commercial pectin. (Figure 1). There are parallels between the extracted and commercial pectin spectra. For both extracted and commercial pectin, the C-H methyl group was visible at the peak at 1442 cm^{-1} (Muhammad et al. 2019; Narsul et al. 2019). The methyl ester group's C=O is responsible for the wavelength of 1747 cm^{-1} ($COOCH_3$) (Licelander et al. 2021; Muhammad et al. 2019). The aliphatic C-H groups are described by a wavelength of 2927.9 cm^{-1} (Nasrul et al. 2019; Ricardo et al. 2019). The two main hydroxyl group peaks were seen at 3402 and 3367 cm^{-1} (Elnaz et al. 2017; Didem et al. 2021; Luiz et al. 2022).

SEM results

The surface of extracted pectin is like large fragments interwoven and overlapping with each other. Sometimes the surface forms dense clusters with dissimilar morphology. For commercial pectin, the surface resembles blocks stacked in layers. Besides, the surface has clearer roughness than extracted pectin. The size of commercial pectin is much larger than extracted pectin (Figure 2).

Optimization study results

The present investigation employed response surface methods in conjunction with an experimental design comprising three indicators to optimize the pectin extraction procedure. Twenty experimental runs at various extraction settings were conducted in total, and Table 2 tabulates the anticipated and experimental data about the catch run. Using Stat-Ease Design-Expert version 11.0 software. Regression and ANOVA analysis were performed on the experimental outcome. The model was then fitted to the experimental responses to explain the relationship between the independent variables (acid extraction, temperature, and extraction time) and the response of pectin extraction efficiency (%). Regression analysis was used to create a quadratic order polynomial

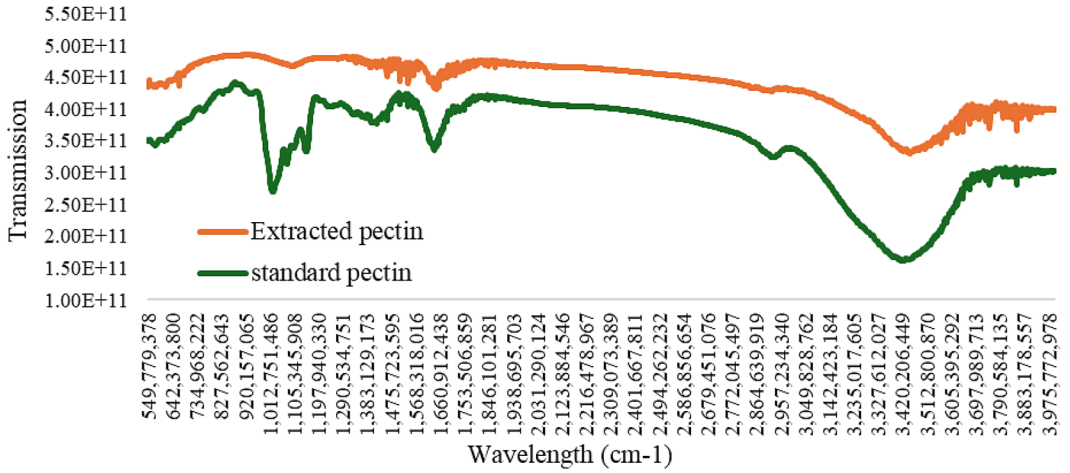

Figure 1. FTIR spectra of extracted pectin from rosy-pulped pomelo and commercial pectin

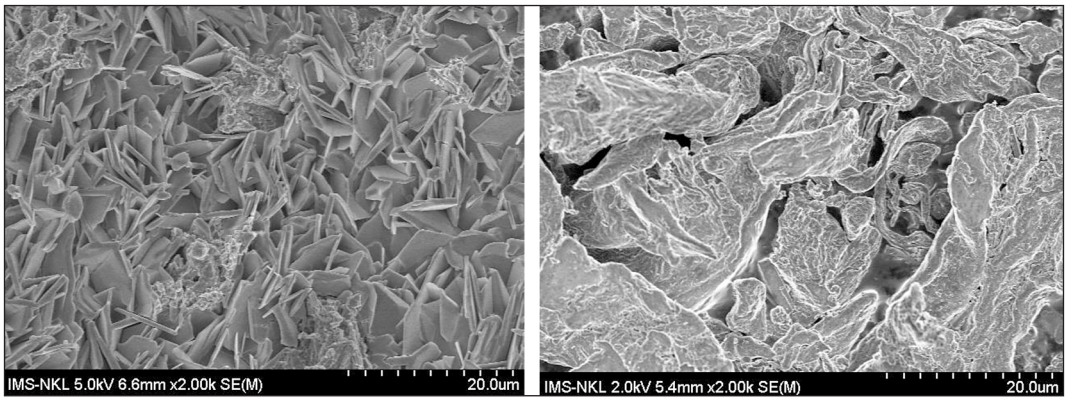

Figure 2. (a) SEM result of extracted pectin and (b) commercial pectin

Table 2. Central composite design (CCD) matrix with the experimental and predicted values for pectin extraction efficiency

No.	Acid (%)	Temperature (Degree)	Contact time (min)	Actual result pectin (%)	Predicted result pectin (%)
1	8	70	100	16.78	16.46
2	8	70	60	12.72	12.94
3	10	77.5	80	29.98	29.96
4	8	85	60	19.41	19.23
5	12	85	60	23.98	24.47
6	12	70	60	22.74	22.65
7	6.64	77.5	80	15.75	15.84
8	10	64.88	80	13.59	13.57
9	10	77.5	80	30.03	29.96
10	12	70	100	21.19	21.54
11	10	77.5	80	29.83	29.96
12	10	77.5	46.4	24.7	24.52
13	12	85	100	25.03	24.98
14	8	85	100	24.12	24.38
15	10	77.5	80	30.01	29.96
16	10	77.5	80	29.99	29.96
17	10	90.11	80	21.98	21.76
18	13.4	77.5	80	24.84	24.51
19	10	77.5	80	29.88	29.96
20	10	77.5	113.64	27.97	27.91

model. Equation 4 is the quadratic order polynomial model for pectin extraction efficiency (%).

$$\textit{Pectin extraction efficiency}\ (\%) = 29.96 + 2.58A + 2.43B + 1.01C - 1.12AB - 1.16AC + 0.4062BC - 3.46A^2 - 4.35B^2 - 1.32C^2 \quad (4)$$

The R^2, adjusted R^2, projected R^2, and CV values in the ANOVA analysis were 0.9986, 0.9974, 0.9887, and 1.23, respectively. It shows that the model is applicable. Table 3 displayed the characteristics of the chosen model for extracted pectin.

Table 3. ANOVA for aquadratic model

Model	618.80	9	68.76	808.87	< 0.0001	Significant
A-Acid	90.71	1	90.71	1067.21	< 0.0001	
B-Temperature	80.81	1	80.81	950.67	< 0.0001	
C-Time	13.88	1	13.88	163.33	< 0.0001	
AB	10.01	1	10.01	117.80	< 0.0001	
AC	10.74	1	10.74	126.37	< 0.0001	
BC	1.32	1	1.32	15.53	0.0028	
A^2	172.45	1	172.45	2028.81	< 0.0001	
B^2	272.28	1	272.28	3203.26	< 0.0001	
C^2	25.25	1	25.25	297.11	< 0.0001	
Residual	0.8500	10	0.0850			
Lack of fit	0.8183	5	0.1637	25.79	0.0014	Significant
Pure error	0.0317	5	0.0063			
Cor total	619.65	19				
Std. dev.	0.2915	Adjusted R^2: 0.9974				
Mean	23.73	Predicted R^2: 0.9887				
C.V.% (Coefficient of variation)	1.23	Adeq precision: 82.5537				
R^2	0.9986					

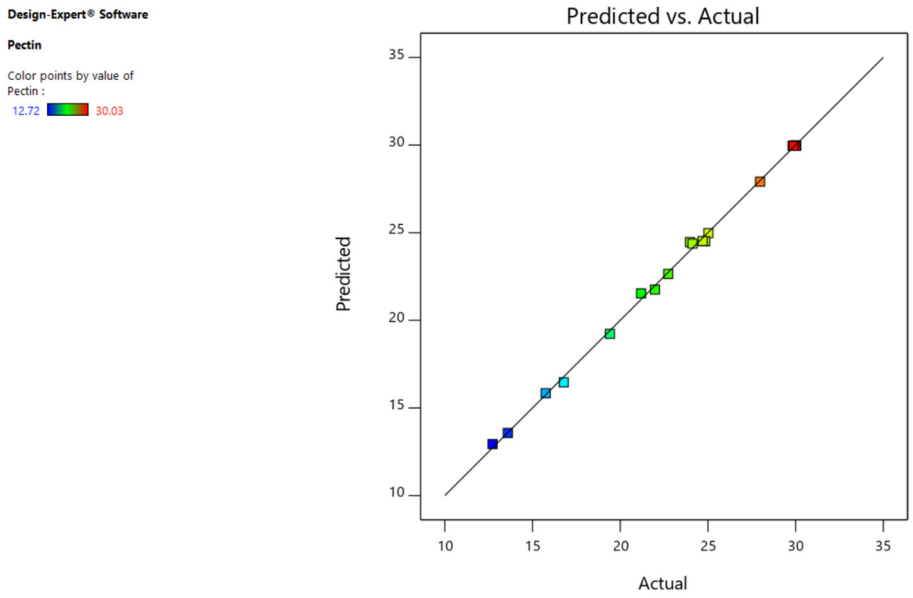

Figure 3. Correlation between predicted and actual values

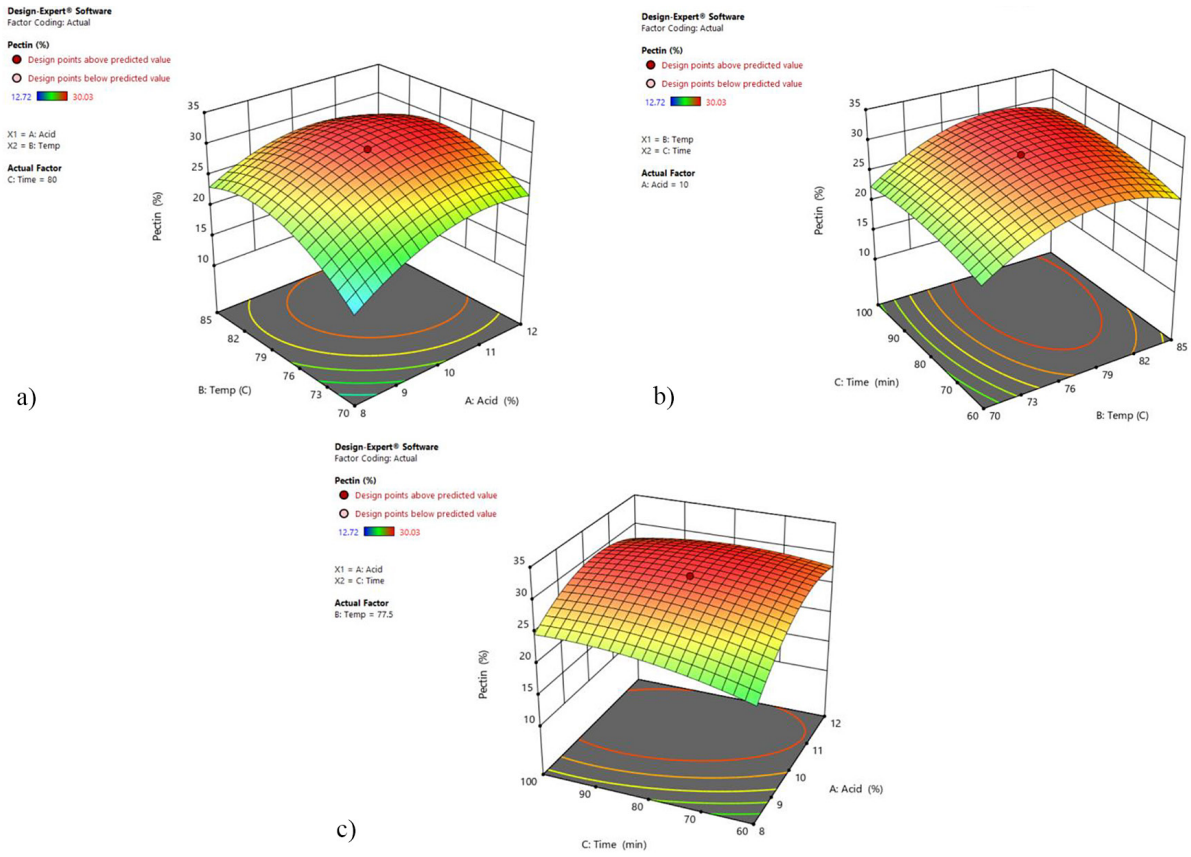

Figure 4. Response surface plots showing the effect of the interaction: (a) between temperature and acid extraction (%); (b) between time extraction and temperature; (c) between time extraction and acid extraction (%)

According to the R^2 (0.9986), a sizable portion of the variation in the observed data may be explained by this model. CCD (Table 3) revealed a substantial lack of fit (p-value = 0.8183), indicating a relatively well-fitted recommended model. Furthermore, a significant degree of correlation between the anticipated and experimental results was found (Figure 3).

Response surface graphs illustrating the impact of the temperature and acid (%) interaction

are displayed in Figure 4a. This chart illustrates how increasing the extraction temperature led to a considerable improvement in pectin extraction efficiency. One possible explanation for this might be that pectin dissolves more readily in water and acid due to their lower dielectric constants. The contour plot's ellipse-shaped area represented the relationship between acid and temperature (Figure 4a). The center of the ellipse represented the ideal pectin extraction, which was correlated with greater temperature and higher acid extraction. The optimal pectin extraction declined from the center to the border, corresponding to lower temperature and acid extraction (%).

Figure 4b depicts the effects of temperature and time. Whereas Figure 4c shows the effects of acid and time on the extraction efficiency of pectin. The trend in these figures is similar to each other. As can be observed, the ideal circumstances for pectin extraction are represented by the center point of the ellipse in Figures 4b and 4c. At the ellipse's edge, this tendency somewhat declines. In order to extract pectin as well as possible, the temperature should be about 77.5 °C and the acid (%) should be around 10 and time extraction 80 minutes. These are the ideal conditions.

Pectin has been effectively extracted from one of grapefruit peels (rosy-pulp pomelo) in this study using an easy-to-implement, straightforward procedure that calls for readily available materials and chemicals. Due to the chemical functional groups in the FTIR study, the extracted pectin's findings are also mostly compatible with those of commercial pectin. To generate purer finished pectin that may be utilized for commercial reasons, more study is necessary as evidenced by the existence of certain considerably distinct peaks. However, this is generally regarded as preliminary research that has to be overcome and improved upon in order to produce purer pectin products in the future. Furthermore, the study supports the use of auxiliary software to reduce the number of trials, time, and effort required to identify the best.

CONCLUSIONS

This is one of the pioneering studies using a type of grapefruit (rosy-pulpe pomelo) to extract pectin. In addition, the study combined with the optimization study to significantly support the determination of optimal conditions that bring the highest extraction efficiency. Most previous studies often chose relatively complex methods and unpopular chemicals. Therefore, the above factors prevent the applicability and popularization of the pectin extraction model from fruit peels.

However, the use of agricultural and food waste is a trend to develop a green and sustainable economy. Therefore, it can be said that this research is a potential study using green materials and simple methods with common chemicals to create great economic value for agricultural waste product as rosy-pulp pomelo.

Acknowledgement

This work was greatly supported by HaUI Institute of Technology – HIT, University of Industry, Hanoi city, Vietnam.

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