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Original Article

Electromagnetic Pre-Treatment for Extraction of Bitter Orange Peel Pectin: Response Surface Methodology Yield/Degree of Esterification

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ABSTRACT

Pectin was extracted from bitter orange peels by subjecting them to electromagnetic field treatment and utilizing citric acid as an acidic catalyst. Fourier-Transform Infrared Spectroscopy (FTIR) analysis verified the presence of distinct functional groups in the pectin samples extracted using acidic catalysts, which exhibited spectra similar to commercially available pectin mentioned in previous studies. Scanning electron micrographs demonstrated that subjecting bitter orange peel to microwave heating led to the destruction of parenchymal cells. Furthermore, pectin samples were examined using scanning electron microscopy (SEM), revealing their structural changes. The extraction procedure was optimized using a multi-objective strategy based on yield-response surface techniques and the esterification degree. The experimental design employed a central composite design with five levels. The key variables under consideration were microwave power (ranging from 150 to 450 W) and solvent pH (ranging from 1.5 to 3.00). Optimal conditions were determined at a microwave power of 450 W and a solvent pH of 1.5, resulting in the highest yield (36.02%) and esterification degree (45.4) of pectin. The applied extraction model and its correlation were significant, as shown by the validation of the optimization model, which exhibited less than a 10% discrepancy between experimental and anticipated outcomes. To improve extraction efficiency, it is recommended to microwave fresh pectin raw materials before drying.

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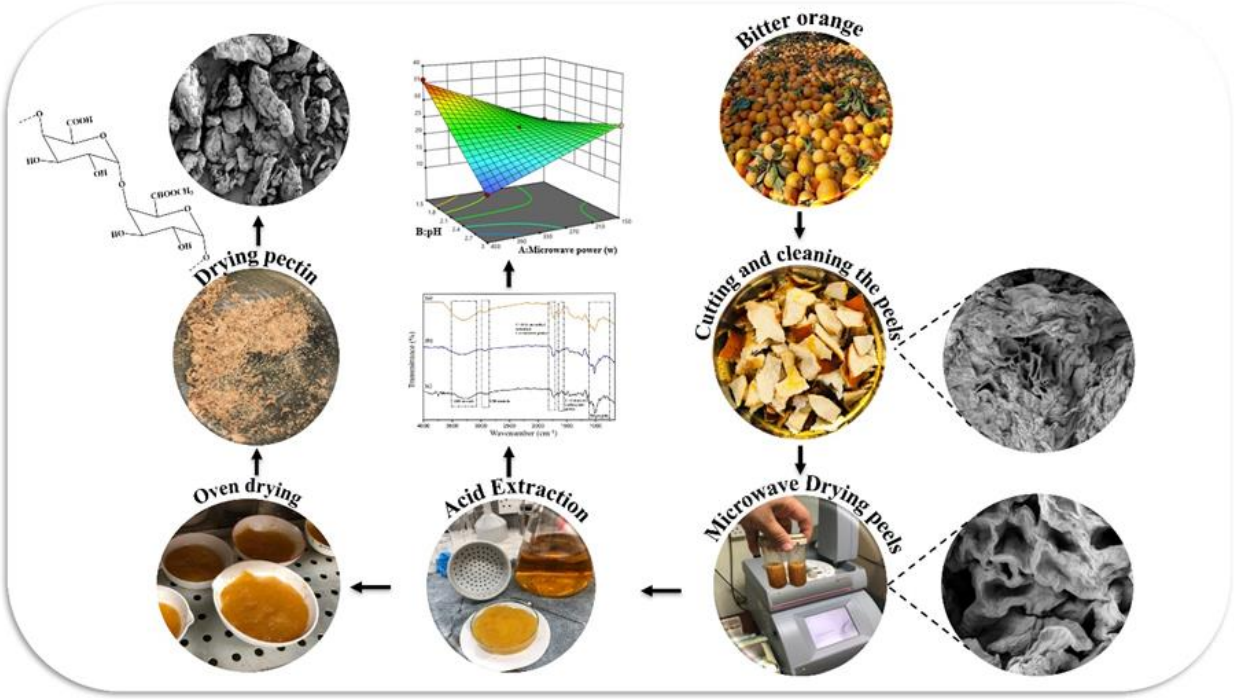
GRAPHICAL ABSTRACT



Introduction

Pectin, a complex mixture of the polysaccharide group, is predominantly found in higher plants' primary cell walls and intercellular layer. Its structure comprises a backbone of polygalacturonic acid linked by α-(1-4') bonds, adorned with rhamnose residues and neutral sugar side chains. Bitter orange, scientifically known as *Citrus aurantium* L., is an interspecific hybrid resulting from the crossbreeding of *Citrus reticulata* and *Citrus maxima* [1]. According to data from the Food and Agriculture Organization of the United Nations, the global production of citrus fruit in 2020 amounted to approximately 143.755 tons [2, 3]. The global fruit processing industry produces one hundred ten tonnes of citrus fruit waste yearly. Citrus peels account for nearly 40 to 60% of processed fruit [4]. The improper disposal of citrus waste, specifically peels and seeds, presents environmental obstacles. Current initiatives are focused on transforming these waste materials, which tonnes contain significant bioactive constituents. Citrus fruit waste, commonly discarded by the fruit processing industry, encompasses a range of components such as sugars, carbohydrates, proteins, fats, organic acids, flavonoids, oils, and pigments. These components are the subject of interest for their conversion into high-value products as a means to address the environmental consequences [5,6]. The process of esterification occurs in the galacturonic acid chain, resulting in the formation of methyl and acetyl ester groups [7,8]. The degree of esterification (DE) is used to quantify the percentage of galacturonic acid units that have undergone esterification within the chain. This measurement is important for distinguishing and modifying the gelling characteristics of different pectin types [9]. Pectin that possesses a Degree of Esterification (DE) value exceeding 50% is referred to as high methoxyl (HM) pectin, whereas pectin with a DE value below 50% falls under the category of low methoxyl (LM) [10,11]. Due to its gelling abilities, Pectin finds diverse applications in hydrogels and the food industry, especially jam and jelly production [12]. The appropriateness of pectin for specific applications is significantly influenced by the degree of esterification (DE). LM pectin is frequently utilized in the production of low-sugar products, while HM pectin is the preferred option for generating gelling effects in high-sugar

products [13]. Pectin also has pharmaceutical uses, such as cholesterol reduction and as a drug carrier for colon treatment [14,15].

In addition, pectin potential as a biodegradable alternative in food packaging due to its preservation properties that are comparable to those of synthetic plastics [16,17]. In agriculture, pectin-based hydrogels offer slow nutrient release and favorable biodegradability, promoting a pollution-free environment [18,19]. Currently, pectin is extracted from citrus peel, "lemon and mango peel" for commercial purposes [10]. However, researchers and manufacturers are actively seeking alternative sources such as cocoa husks [20], mulberry [21], apple pomace [22], sunflower head, mango peel [23,24], melon peel [25], sisal waste [26], pomegranate peel [27], banana peel, and papaya peel [28]. Bitter orange, scientifically known as *Citrus aurantium* L., belongs to the citrus family and is less often eaten because of its harsh and bitter flavor. However, many people use its fruit for culinary and medical purposes [29,30]. In traditional Iraqi medicine, the flowers of this plant were utilized to treat neurological disorders and other conditions. The increasing demand for pectin has led to the exploration of alternative sources, such as citrus peels, particularly those from bitter oranges. This approach offers a sustainable and appealing option by utilizing waste materials that are typically discarded by fruit juice companies. By doing so, not only can valuable products be obtained, but also environmental concerns can be addressed. Therefore, it is crucial to optimize pectin production to fully capitalize on these benefits.

This study investigates the fundamental parameters required to achieve the optimized extraction of pectin from bitter orange peels through the utilization of the response surface methodology, specifically employing the central composite design (CCD) approach. The primary objectives of this investigation are to maximize the yield of pectin and to determine the degree of esterification as key response variables.

Experimental

Materials and Methods

We obtained the peels of bitter oranges (*Citrus aurantium*) from a fruit orchard located in Baqubah, Diyala, Iraq. The cut fragments were placed in standard borosilicate glass vials and dried using a microwave reactor. The exposure duration was 170 s, with varying microwave power levels. The drying procedure was carried out until a consistent weight was attained. Afterwards, the peels were pulverized and filtered using a 40-mesh sieve. Prior to conduct the experimental analysis, the powder was dried and stored in conditions that prevented exposure to light. We procured the sodium hydroxide, phenolphthalein, hydrochloric acid, and citric acid reagents from Sigma Aldrich in the Netherlands.

Pectin Extraction from Bitter Orange Peels

The process started by placing 20 g of bitter orange peels into standard borosilicate glass vials. Then, the peels underwent treatment in a microwave reactor (Mono Wave 450) before extraction. The treatment involved different levels of microwave power, ranging from 150 to 450 W, for 170 s. After drying, the peels were extracted by adding 250 mL of water to 5 g of mass. The pH of the distilled water was adjusted to 2 using either citric or hydrochloric acids, both at a concentration of 0.5 M. The mixture was heated to a temperature between 80 and 82 °C, and extraction was carried out for one hour with continuous stirring. After heating, the mass was filtered using a cloth, and the resulting filtrate was solidified by adding an equal volume of 96% ethanol and allowing it to stand for an hour. The coagulated pectin was separated by filtration and washed three times: once with 70% acidic ethanol (0.5% acidity), twice with 70% ethanol to achieve a neutral pH, and once with 96% ethanol. Finally, the pectin was dried in a laboratory dryer at 60 °C. The pectin yield was calculated using Equation 1 [31].

$$\text{Yield (\%)} = \frac{\text{Mass of Extracted Pectin (g)}}{\text{Mass of Dried Bitter Orange Peels(g)}} \times 100(\%) \quad (1)$$

Acid Solvent Extraction Comparison

To compare the efficacy of various acid solvents for pectin extraction, as mentioned previously, we replicated the extraction procedures using two types of acid solvents: organic (citric acid) and inorganic (hydrochloric acid). Each solvent was prepared at a concentration of 0.5 M and utilized for extracting pectin from orange peel powder. The resulting pectin yields and DE (Degree of Esterification) values were meticulously compared to determine the most efficient solvent. The objective of this study is to further optimize the solvent that yields the highest pectin content and achieves the desired DE value.

Functional Group Characterization

Fourier Transform Infrared Spectroscopy (FTIR) was used to determine the structural characteristics of pectin recovered from orange peel powder using different acid solvents (hydrochloric acid and citric acid). FTIR Spectrometer (PerkinElmer Spectrum Two) collected the spectra from 4000 to 650 cm^{-1} wavelength.

Surface Morphology Analysis

To investigate the surface structure of both the tissue from Bitter Orange Peels and the extracted pectin, we utilized the Scanning Electron Microscopy (SEM) Zeiss Supra 55VP instrument. The dried samples were sectioned into approximately 1 mm in depth and up to 5 mm in length. To prepare the samples for analysis, they were affixed to a circular specimen stub using double-sided adhesive tape.

The Degree of Esterification (DE)

The degree of esterification (DE) in bitter orange peels was measured using a method that was slightly modified from the one described by Bahare *et al.* [32]. The measurement process involved combining 30 mg of bitter orange peels with 20 ml of deionized water and 3 ml of a modified solution. To dissolve the pectin, ethanol was used. The samples underwent vigorous agitation until complete dissolution of the pectin occurred. The titration process involved adding five drops of phenolphthalein solution and 0.1 M sodium hydroxide (V_1) to the mixture. The process of hydrolysis was facilitated by combining the samples with 10 ml of 0.1 M sodium hydroxide and vigorously shaking the mixture for 15 minutes. To neutralize the solution, 10 ml of 0.1 M hydrochloric acid was introduced and stirred until the pink color vanished. The solution was then titrated with 0.1 M sodium hydroxide until the pink color (V_2) returned. Ultimately, the DE was computed using the provided formula:

$$\text{DE (\%)} = \frac{V_2}{V_1+V_2} X_{100} \quad (2)$$

Optimizing Bitter Orange Peel Pectin Extraction

In this study, the Central Composite Design (CCD), an experimental design with five levels, was utilized to enhance the pectin extraction procedure. The Design-Expert 13.0 software was employed for this purpose. The independent variables investigated were microwave power (A) and solvent pH (B), while the response variables observed were pectin yield (%) and DE value (%). Table 1 lists the possible values for the process variables under investigation. 13 tests were conducted in which the experiments were randomly performed. These experiments were designed to consider all possible combinations of component levels within the CCD design. The main objective was to assess the influence of extraction process variables.

Table 1: The experimental variables and coded levels of the Central Composite Response Surface Design (CCRD)

Variable	Unit	Coded variable levels				
		-2	-1	0	+1	+2
Microwave power	W	87.87	150	300	450	5.12
PH	-	3.31	1.5	2.25	3.00	3.31

Analysis of Statistics and Verification of Ideal Circumstances

Analysis of statistics and verification of optimal conditions Results of the experiment were subjected to statistical analysis, specifically using analysis of variance (ANOVA) with the assistance of Design Expert software 13. This analysis involved conducting an F-test, determining the associated probability (p-value), and calculating the coefficient of determination (R^2). In addition, the pectin extraction process was conducted in triplicate to verify the reliability of the generated models. Under the predicted optimized conditions, we obtained average values for the experimental pectin yield and DE and compared them to the expected results.

Results and discussion

Various acid Solvents Influence the Extraction Process

Prior to optimizing the extraction conditions, a comparative assessment was conducted in order to evaluate the effectiveness of two acid solvents, specifically citric acid and hydrochloric acid, for extracting pectin from the layers of bitter oranges. Table 2 indicates the results of the pectin extraction method conducted on bitter orange peels using the aforementioned acid solvents, indicating the pectin yield and degree of esterification (DE). The pectin yield obtained using citric acid was significantly higher compared to hydrochloric acid in this study. The results indicated that the choice of acid solvent and the utilization of Microwave-Assisted extraction influenced the quantity of extracted pectin. These findings align with previous studies on pectin extraction from sweet orange [33] , bitter orange [34], cocoa husk [35], pomelo peels (*Citrus maxima*) [36], buttercup squash [37], and watermelon rind [38]. Generally, the use of strong acids tends to result in lower pectin extraction compared to weak acids [39]. The observed phenomenon can be explained by the formation of smaller pectin molecules as a result of partial hydrolysis using strong acids. These smaller molecules exhibit increased solubility but are also more prone to being lost during the filtration process [38]. Furthermore, smaller pectin molecules may not precipitate effectively during alcohol precipitation, and some may elute with the alcohol, thereby reducing the overall pectin yield [40, 41].

Table 2: Pectin yield and DE (Degree of Esterification) of bitter orange peel pectin extracted using various acid solvents

Acid type	Acid solvent	Yield (%)	DE (%)
Organic	Citric acid	8.39	20.76
Inorganic	Hydrochloric acid	4.07	19.05

According to Table 2, the organic acid solvent was superior to the inorganic acid solvent in extracting pectin from bitter orange peels. The variability in the acid solvents used in the experiments could be responsible for the differences observed in the yield. In addition, the data presented in Table 2 showed no significant distinction in the degree of esterification of pectin extracted using citric acid and hydrochloric acid. However, in the study conducted by Yan Du *et al.* [42], researchers observed that pectin extracted from Akebia trifoliata var. australis peel using citric acid showed a higher DE value (76.64%) in

comparison to pectin extracted with hydrochloric acid (59.46%). These findings suggest that citric acid exerts a reduced impact on the de-esterification of pectin. Furthermore, Caian *et al.* [43]. Furtehrmore, citric acid exhibited the least significant de-esterification effect when extracting pectin from the yellow passion fruit rind, in contrast to mineral acids. Consequently, pectin extracted with citric acid demonstrated the most remarkable DE compared to hydrochloric acid-extracted pectin. The discrepancy between the findings of this study and the existing literature may be attributed to variations in pectin sources and extraction conditions.

This study examined the results of using various acid solvents in the extraction process and determined that citric acid is the most appropriate solvent to be used in future optimization studies. Citric acid was chosen as the preferred option for extracting pectin from bitter orange peel. This choice was based on its ability to produce the largest amount of pectin and achieve the desired DE value, which falls within the acceptable range of HM. Additionally, citric acid is considered to be more environmentally friendly compared to mineral acids [44-45].

FT-IR Analysis

Pectin was extracted from bitter orange peels using different acid solvents and confirmed its presence through FTIR analysis to identify the most critical functional groups. The FTIR spectra in Figure 1 depict the extracted pectin samples obtained using citric acid and hydrochloric acid solvents. Intriguingly, a consistent pattern was noticed in all pectin samples, regardless of the solvents used. This pattern indicates the presence of hydrogen bonding within the galacturonic acid structure at both intra- and intermolecular levels, as supported by [11] and [46]. Another 2800-3000 cm^{-1} peak corresponded to the CH functional group.

The absorption peak between 1680 and 1810 cm^{-1} represented the stretching vibration of carbonyl functional groups (C=O) in the methyl-esterified carboxylic groups. Furthermore, the 1490-1700 cm^{-1} range exhibited the stretching vibration of carboxylate functional groups (COO-) in pectin. Interpretation of the "fingerprint" area (800-1200 cm^{-1}) was challenging due to multiple active groups. However, the presence of the pectin molecule was evident [45,47].

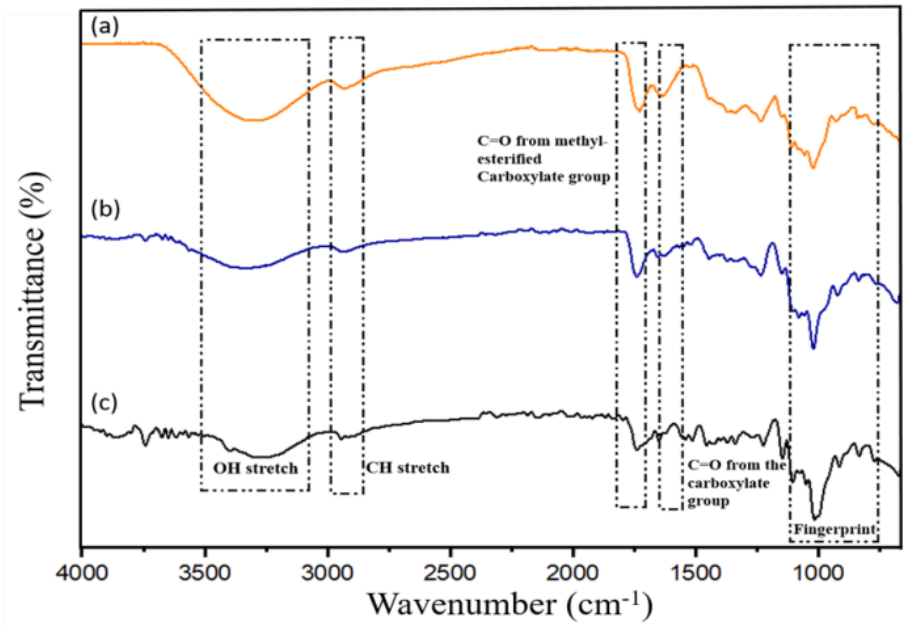


Figure 1: FTIR spectra of pectin from bitter orange peels extracted with (a) citric acid, (b) hydrochloric acid, and (c) commercial pectin

The vibration of the pyranose ring structure in the pectin fingerprint area (950-1200 cm^{-1}) was identifiable [38]. The functional groups of the extracted pectin were compared to those of commercial pectin using FTIR wavenumbers specified by [48]. Table 3 revealed close correspondence between the pectin from bitter orange peels and commercial pectin. The findings depicted in Figure 3 indicate that the pectin extracted from bitter orange peels possesses comparable attributes to commercially available pectin.

Table 3: Selected FTIR peaks and comparison of peaks from commercial pectin (A) and the pectin extracted with citric acid (B) and HCl (C) from bitter orange peels

Wavenumber for FTIR (cm^{-1})			Functional Group
A	**B**	**C**	
3310	3332.45	3287	OH group
2929	2921	2940	CH group
1746	1742	1753	C=O stretching vibrations of the methyl-esterified carboxylate groups
1437 and 1605	1440 and 1614	1441and 1631	C=O from the carboxylate group
800-1200	800-1200	800-1200	Fingerprint

Surface Morphology Analysis

The importance of treating bitter orange peels lies in the fact that the energy of the electromagnetic field is primarily converted into heat in substances composed of polar molecules. Intensive vapor formation within the capillary-porous structure of plant material leads to increased pressure, thereby altering its physical characteristics [49]. Utilizing microwave energy can aid in breaking down cell walls and liberating pectin molecules, ultimately leading to higher extraction yields [50]. This treatment enhances the efficiency of extraction, preserves the properties of pectin, and reduces the extraction time. In this study, Microwave pre-treatment of fresh bitter orange peels led to destructive changes in the plant tissue. The changes resulted in an increase in the capillary-porous characteristics and the water absorption capacity of the plant material. The heating inactivated the pectin esterase activity in the bitter orange peels. Figure 2 displays the scanning electron microscopy images illustrating alterations in the tissue's structure and integrity, a phenomenon similar to what was observed in the previous study [51]. SEM was performed to characterize Pectin extracted from bitter orange peels (Figure 3 a, b, and c) and commercial [51] pectin (Figure 3d) samples by visualizing their structures and morphology. The images demonstrate that the pectin-extracted particles are of distinct shapes, illustrating bulky and rough particles, which differs significantly from the shape of the commercial Pectin, which has a comparatively smooth surface. Nevertheless, the particles obtained from bitter orange peels subjected to 150 watts of processing exhibit a somewhat crumbly form and possess a character with limited pores. In comparison, the pectin particles isolated from peels processed at 450 watts have a more porous surface and seem even more crumbly. In addition, the pictures of the extracted Pectin indicate (Figure 3b) a rough, broken, and wrinkled texture. It might be because the temperature rose during the processing of the peels.

Similarly, Liew *et al.* [52] suggested that the rapid temperature rise may cause the coarse surface of the Pectin extracted by microwave heating. The sources of the raw materials and the extraction methods may significantly impact the final Pectin's shape. Regarding the form of commercial

Pectin, the surface had laminate structures and was fluffy with a smooth surface [53], which was very different from the surface of the extracted Pectin. These changes in the plant tissue after a microwave pre-treatment allowed or instead caused a considerable increase in the yield of extractable pectin.

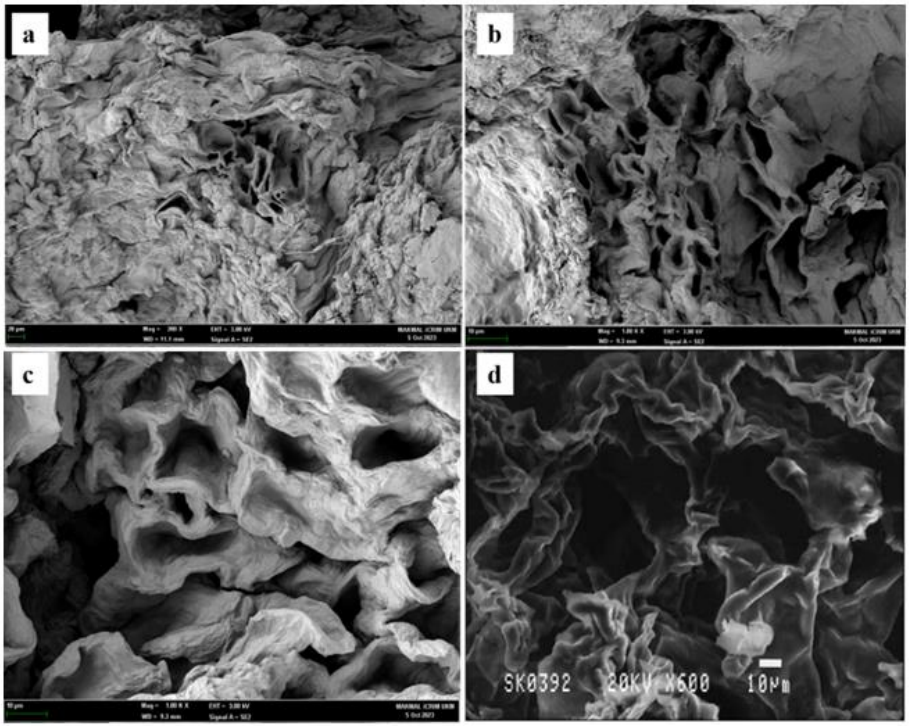


Figure 2: Scanning electron micrographs of Bitter orange peel tissue. (a) the control sample, (b) MW-Heating 150 W, (c) MW-Heating 450 W, and (d) MW-Heating for an orange peel at 450 w [51]

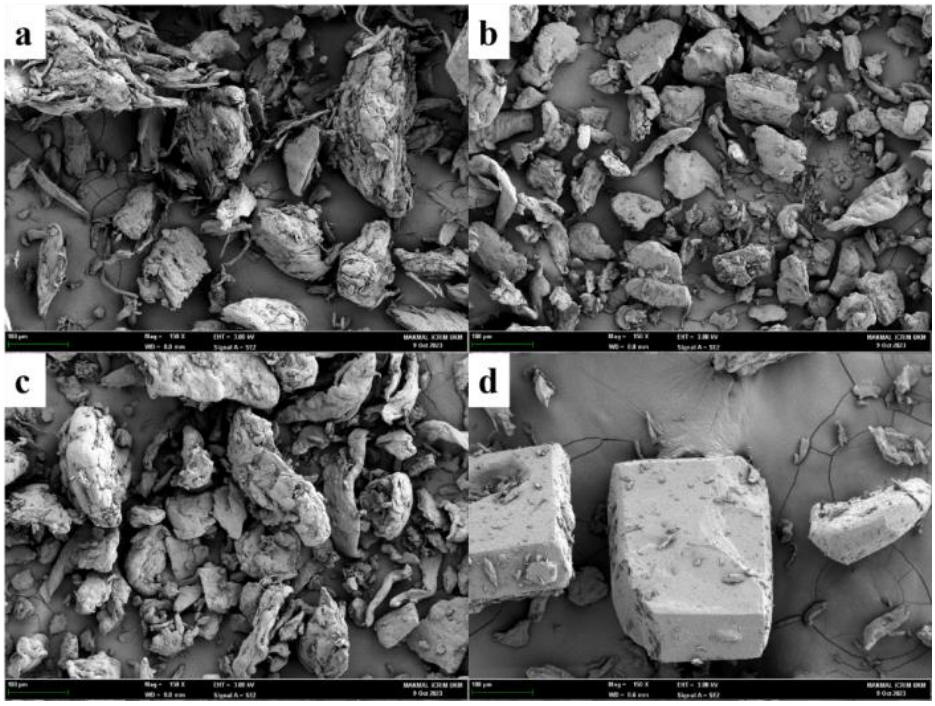


Figure 3: SEM images of Bitter Orange Peel pectin extracted (a) air-dry sample, (b) MW-Heating 150 W, pH 1.5 M, (c) MW-Heating 450 W, pH 1.5 M, and (d) commercial pectin

Optimizing the Process for Extracting Pectin Using Multi-Objective

This study used a central composite design (CCD) trial with a five-level approach to optimize the extraction of pectin from sour orange peels using citric acid. Thirteen experiments were conducted under varying extraction conditions, and the expected and actual results for each stage were documented in Table 4. Regression and analysis of variance (ANOVA) The trial data were analyzed using Stat-Ease Design-Expert version 13.0. A quadratic polynomial model was developed through regression analysis, which described the relationship between the independent factors (microwave power, fluid pH) and the responses (pectin yield and DE). The quadratic polynomial Equations 1 and 2 represent the pectin yield and DE models, respectively. These models elucidated the connections between the independent and the response variables, with the terms A and B representing the coded values for microwave power and liquid pH, respectively.

By examining the sign of the regression coefficients in the models, it was possible to determine the impact of the independent factors on the responses. A positive sign indicated a positive effect, while a negative sign indicated the opposite [54, 55]. For instance, in Equation 1, a positive sign meant that an increase in the linear term A would increase pectin production. In contrast, a negative sign indicated that an increase in the linear term B would result in a drop in pectin output.

Table 4: Measured and expected values of the pectin yield and Degree of Esterification (DE) extracted from bitter orange peels

	Independent variables		Experimental responses		Predicted responses	
Run	**Microwave power (w)**	**pH**	**Yield (%)**	**DE (%)**	**Yield (%)**	**DE (%)**
1	300	2.25	27.66	16.12	27.50	14.98
2	300	1.18	29.08	1.76	29.62	1.77
3	300	2.25	27.39	13.71	27.50	14.98
4	300	2.25	26.58	15.67	27.50	14.98
5	87.86	2.25	23.18	1.69	23.07	1.99
6	150	3	26.09	1.58	26.38	1.46
7	450	3	12.63	25.53	12.53	25.95
8	150	1.5	20.45	6.55	20.21	6.30
9	300	2.25	28.33	14.75	27.50	14.98
10	300	2.25	27.52	14.65	27.50	14.98
11	300	3.31	18.02	15.74	17.81	15.56
12	512.13	2.25	23.57	16.45	24.01	15.99
13	450	1.5	36.02	1.32	35.39	1.61

Table 5 summarizes the results of ANOVA and regression analysis. According to the results, the F-value for the yield model was 486.72, and for the DE model, it was 951.86. Both models had p-values that were less than 0.05, indicating their significance. The statistical analysis revealed the importance of the models, as evidenced by the high F-value and low p-value (< 0.05). There is a 32.32% chance for the lack of fit F-value of this magnitude to occur due to noise in the case of yield. Similarly, there is a 45.84% chance for the lack of fit F-value of this magnitude due to noise in the case of DE.

Table 5: ANOVA results of the response surface quadratic model, specifically for (a) yield and (b) DE percentage variables

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	*P*-value
Pectin yield					
Model	396.96	3	116.98	486.72	< 0.0001
A-Microwave power	0.8855	1	0.8855	3.68	0.0871
B-pH	139.37	1	139.37	579.88	< 0.0001
AB	210.69	1	210.69	876.59	< 0.0001
A^2	27.18				
B^2	24.83				
Residual	2.66	9	0.2403		
Lack of Fit	1.08	5	0.2916	1.65	0.3232
Pure Error	1.57	4	0.1763		
Corrected Total	399.62	12			
R^2	0.9934				
Adjusted R^2	0.9886				
DE					
Model	715.63	5	143.13	237.42	< 0.0001
A-Microwave power	195.96	1	195.96	325.06	< 0.0001
B-pH	190.23	1	190.23	315.55	< 0.0001
AB	212.87	1	212.87	353.11	< 0.0001
A^2	62.45	1	62.45	103.60	
B^2	69.30	1	69.30	114.96	
Residual	1.89	7	0.6028		
Lack of Fit	0.6695	3	0.2232	0.2514	0.8571
Pure Error	3.55	4	0.8876		
Corrected Total	719.85	12			
R^2	0.9941				
Adjusted R^2	0.9900				

Furthermore, the high values of the correlation coefficients, R^2 (99.34% and 99.41%, respectively), and the corresponding adjusted R^2 values (98.86% and 99.00%, respectively) for both the yield and DE models, indicate a strong correlation between the models and the experimental results. The models used in this study showed excellent accuracy in predicting how the independent variables and responses interacted [47,48]. The R^2 values of the yield and DE models indicated that the models accounted for 0.66 and 0.59 of the variance in the respective responses, leaving only 0.66 and 0.59 unexplained. The significance of each model term was assessed using the p-value, where a value below (0.05) indicated a significant influence of the corresponding variable on the response. This investigation was significantly influenced by the practical linear terms (A and B) and quadratic term (AB) of the yield and DE models.

The Influence of Response Variables on the Variables Involved in the Extraction Process

Figure 4 utilized 3D response surface graphs and contour plots to analyze the relationship between the independent factors and the yield and DE of pectin. Each visual presentation illustrated the impact of two independent factors on the pectin reaction while maintaining a constant level for the third independent variable within the studied range.

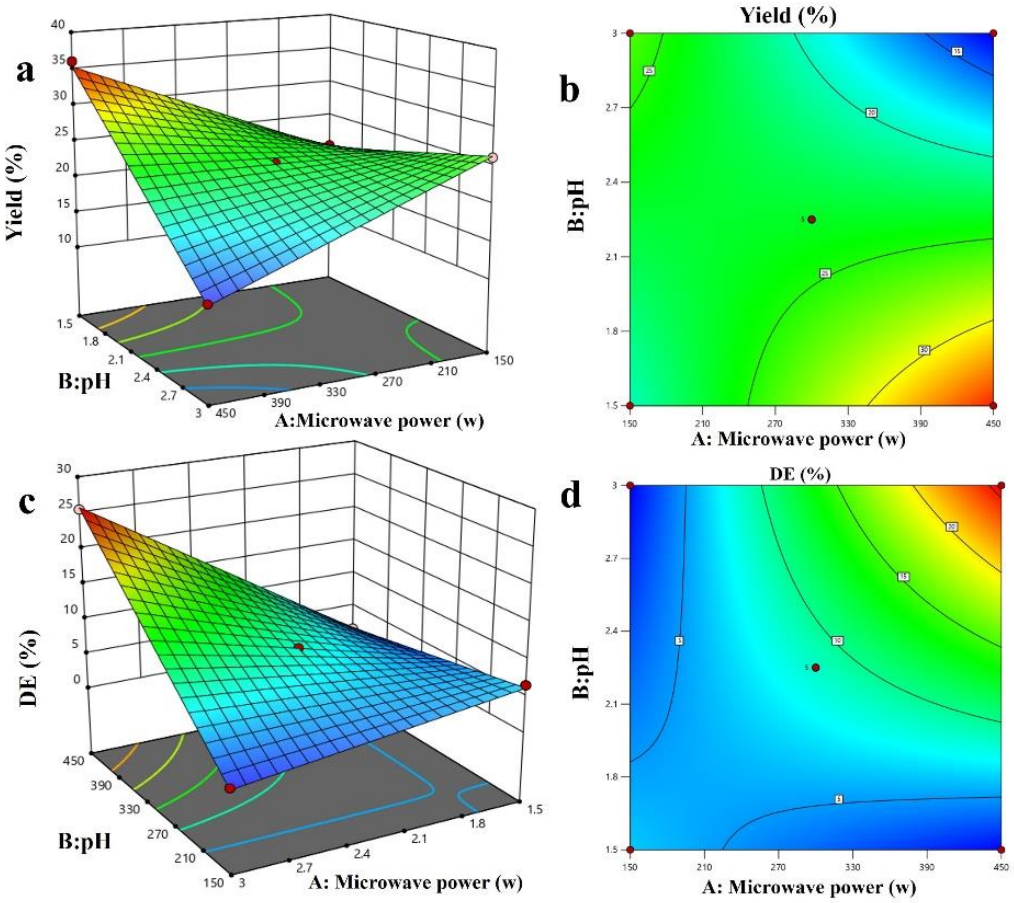


Figure 4: The 3D response surface and contour plot shows how two independent factors (a and b) pectin yield (c and d) DE interact

Influence of extraction factors on response variables

Table 4 indicates the impact of extraction method factors on the quantity of extracted pectin. It is evident that different extraction conditions led to varying pectin yields, which ranged from 12.63 % (run no. 7) to 36.03 % (run no. 13). The extraction efficiency depended on microwave power and pH, as depicted in Figure 2. Extraction efficiency was observed to be dependent on microwave power to heat the peels and pH, as illustrated in Figure 2.

The extraction efficiency demonstrated a significant dependence on microwave heating power. Furthermore, the results demonstrated that the extraction of pectin increased as the microwave power to heat the peels was raised from 300 to 450 W. The presented results confirm previous findings by M. Panchev *et al.* [51, 56-57].

In terms of extracting pectin, microwave heating has positively affected the quantity and quality of the extracted pectin. Moreover, the significant impact of solvent pH on both pectin production and DE was observed in this study. Pectin has been extracted from various fruits and vegetables, and this finding agrees with prior studies [32,33,58]. Pectin yield increased significantly from pH 4 to 2. The response surface graphs depicted in Figure 4 (a and b) illustrate

the findings. Prior research has indicated that decreasing the pH of the solvent leads to increased pectin production [45,58-59]. The hydrolysis process of protopectin was accelerated by the presence of a greater number of hydrogen ions in the solvent. A decrease in pH resulted in an acceleration of the hydrolysis process of insoluble protopectin, leading to an increase in soluble pectin [58,60].

Influence of extraction process variables on the degree of esterification

The optimal extraction conditions for pectin from bitter orange peels were determined using the software Stat-Ease Design-Expert version 13.0. The predicted optimal conditions included extraction, a solvent pH of 1.5, and a microwave power of 450 W, as demonstrated in Figure 4. Under these optimized conditions, the extracted pectin's predicted yield and degree esterification (DE) was 36.02% and 1.32%, respectively. Three independent extractions of pectin were conducted to ensure the reliability and consistency of the results. The obtained results are presented in Table 6. At the experimentally determined optimal conditions, the pectin yield was (36.02), whereas the DE was (1.32). The models' reliability was evaluated by implementing the optimal conditions and comparing the resulting behavior to the predictions made by the models. The results closely matched the predictions, with a less than 10% percentage error, demonstrating the models' accuracy in predicting pectin yield and DE from orange peels. Notably, the optimal yield of pectin in this study surpassed some previous research [41,54, 64-65], and the yield achieved using microwave pretreatment of bitter orange peels for pectin extraction was comparable to or higher than values reported in the literature. Additionally, the degree of esterification (DE) of the pectin which was extracted using the optimized conditions was found to be less than 50%.

This indicates that these conditions were appropriate for obtaining pectin with a low DE, which is advantageous for hydrogel preparation. Pectin with a low DE has the ability to form gels and improve the stability of hydrogels. The reduced esterification level in the pectin also enables better control over gel properties such as strength and porosity, which are crucial for various applications [66-68]. The variations in pectin production, DE, and type of pectin obtained from bitter orange peels or fruit residues can be explained by the differences in extraction conditions used in various studies. This highlights the importance of optimizing the extraction parameters to achieve the desired characteristics of the final product [69-71].

Table 6: Comparison of predicted and actual results on pectin yield and DE at optimized parameters

Experimental trial	Yield pectin (%)	DE (%)
1. 1	2. 36.22	3. 1.39
4. 2	5. 37.67	6. 1.45
7. 3	8. 38.45	9. 1.34
10. Average	11. 37.44	12. 1.37
13. Predicted	14. 35.39	15. 1.61
16. Error (%)	17. 5.79	18. 5.5

Conclusion

This study investigated the impact of electromagnetic pre-treatment and various acid solvents on the yield and DE of pectin produced from bitter orange peels, namely citric acid and hydrochloric acid. The results revealed that citric acid emerged as the most efficient solvent for pectin extraction among the acids examined. It exhibited the highest pectin yield and attained optimal DE levels within the tested range, performing hydrochloric acid in extraction efficiency. A comparison was made between citric

acid and hydrochloric acid. The experimental results confirmed the accuracy of generated models for pectin extraction from bitter orange peels was multi-objective optimized with a 10% lower error rate. Likewise, the study revealed that the extraction process factors conflicted with pectin yield and DE. Consequently, optimized conditions proved more suitable for extracting low-esterified (DE < 50%) pectin from bitter orange peels. These results validate that the extraction conditions impact the DE value of pectin.

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