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ORIGINAL ARTICLE

Green extraction of pectin from *Citrus limetta* peels using organic acid and its characterization

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Abstract

The approach of converting waste to wealth has become a crucial topic and intense research has been ongoing globally for their proper management and utilization. With an aim to follow up this concept, this study is aimed to valorize *Citrus limetta* peels by extracting pectin using green organic acid. A Box-Behnken experimental design was used to optimize the extraction process and study the effect of process parameters, i.e., temperature (70–100 °C), extraction time (30–120 min), pH (1–2.5), and liquid:solid ratio (10–40 v/w) on the yield of pectin. The maximum pectin yield of 22.03 ± 0.13% was obtained at optimum extraction conditions of 90 °C temperature, 95-min time, 1.8 pH, and 30 v/w LSR which was observed to be in close agreement to the predicted value of 22.7%. Furthermore, the pectin extracted under optimal conditions was low methoxy pectin with a degree of esterification of 45.62 ± 0.64% and methoxyl content of 5.45 ± 0.04%. The structural properties of pectin were characterized using analytical techniques including X-ray diffraction and Fourier transform infrared spectroscopy. The morphological analysis of peel powder before and after pectin extraction examined using scanning electron microscope indicated that the peel structure after extraction treatment was damaged and disrupted. Besides, the emulsifying activity, emulsifying stability, water holding, and oil holding capacities of extracted pectin were evaluated to determine its potential to be considered as a natural food additive. The results suggested that *C. limetta* peels have a great potential to be used as a novel low-cost source for the pectin extraction having good properties.

Keywords *Citrus limetta* peels · Pectin · Organic acid · Optimization · Box-Behnken design · Characterization

1 Introduction

Citrus limetta, also known as sweet lime, is one of the well-known indigenous citrus fruits which is mainly cultivated in Southern and Southern East Asia. The world's leading producers of *C. limetta* include India, China, Pakistan, southern Japan, Egypt, Vietnam, Malaysia, Thailand, Indonesia, tropical America, and Mediterranean countries [1]. In India, annual production of approximately 3.26 million tons of *C. limetta* was observed in 2018 (http://nhb.gov.in/statistics/Publication/Horticulture%20Statistics%20at%20a%20Glance-2018.pdf). Furthermore, considering numerous promising health benefits due to the presence of a wide array of nutrients and bioactive compounds along with their exceptional sensorial properties, consumption, and processing of this fruit have increased exponentially. Processing of *C. limetta* generates an enormous quantity of peels accounting for 8–10% of fresh fruit which is arbitrarily discarded into the landfills without any prior treatment,

Highlights
- Pectin was extracted from *C. limetta* peels using the organic acid-mediated heating extraction method.
- A maximum pectin yield of 22.03 ± 0.13% was achieved at optimal conditions of 90 °C temperature, 95-min time, 1.8 pH, and 30 v/w LSR.
- The extracted pectin exhibited a low degree of esterification with good emulsifying and water/oil binding properties.
- *C. limetta* peel can be potentially used as a new source of pectin production which can reduce and valorize citrus waste.

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causing environmental pollution [2]. However, ironically *C. limetta* peels (CLP) are being recognized as an alluring repository of high-value compounds, especially pectin, which makes it preferable to be used in functional foods and nutraceuticals. Therefore, the extraction of pectin from this by-product can not only help in reducing the volume of citrus waste followed by its subsequent ecological effects but can also increase the financial profits obtained from conversion plants.

Pectin is a mixture of complex heteropolysaccharides distributed extensively in the cell wall and middle lamella of plants. Chemically, pectin is composed of α-(1-4)-D-galacturonic acid units which are partially esterified with acetic acid or methanol at some carboxylic acid groups [3]. It is a popular natural food additive (INS 440) used in food industries as a fat replacer as well as thickening, emulsifying, stabilizing, and gelling agents [4]. Furthermore, it is also used in pharmaceutical industries for drug encapsulation and tablet formation [5]. In addition, previous studies have reported numerous pharmacological benefits of pectin, such as lowering of cholesterol levels [6], antioxidant, immunity modulation [7], anti-cancer [8], and reduction in the postprandial concentration of glucose and insulin [9]. Therefore, considering the increasing demand for natural pectin owing to the wide array of health and technological applications, the global market size of pectin is expected to reach USD 1.5 billion by 2025, at a CAGR of 6.5% from the year 2019 (https://www.marketsandmarkets.com/Market-Reports/pectin-market-139129149.html#:~:text=%5B181%20Pages%20Report%5D%20The%20pectin,USD%201.5%20billion%20by%202025).

Commercially, pectin is commonly extracted from citrus peel and apple pomace. Despite the fact that pectin obtained from by-products is chemically equivalent, citrus fruits are generally preferred for pectin extraction as it contains almost twice pectin content than apple, i.e., 20–30% and 10–15%, respectively. Moreover, due to the presence of more phenolic compounds, apple pectin is darker in color than citrus pectin and is therefore not preferred for application in clear food products [10]. Previous studies have reported pectin extraction from most common citrus species, such as *C. sinensis*, *C. aurantifolia*, *C. reticulata*, *C. paradisi*, and *C. limon* using different techniques [11]. However, to meet the increasing demand of consumers for pectin, researchers are currently exploring other citrus sources for pectin extraction.

The traditional method of pectin extraction involves the use of strong mineral acids, i.e., hydrochloric, sulfuric, or nitric acid [12]. However, due to increasing concerns of mineral acids due to their corrosive nature and production of toxic compounds, green organic acids, such as citric acid have recently gained attention for pectin extraction as they are environment friendly [13, 14]. Besides, because of their low dissociation constant, organic acids have a low hydrolyzing capacity which will reduce the risk of pectin depolymerization which could lead to pectin with high yield, molecular weight, and viscosity as compared to mineral acids [15].

During the acid extraction method, several factors such as temperature, pH, time, and liquid:solid ratio (LSR) affects the extraction yield and quality of pectin [16]. So, in order to achieve the maximum extraction efficiency, optimization of these conditions is needed. For this purpose, response surface methodology (RSM) is applied extensively to determine the relationship among independent and response factors using a group of statistical and mathematical procedures [17].

Studies on the optimization of pectin extraction using organic acids from CLP are meager. Therefore, this study is aimed to optimize the different process variables for efficient extraction of pectin from CLP using citric acid using Box-Behnken RSM and further study the physicochemical and structural properties of extracted citrus pectin.

2 Materials and methods

2.1 Plant material and chemicals

C. limetta peels (CLP) were procured from the local juice vendors in Longowal, Punjab, India. The peels were first washed and dried in a tray drier (SICO House, Patiala, India) at 45 °C until a constant weight was achieved. The dried peels were then grounded using an electric grinder (Philips, India), and it was passed through a mesh sieve (BSS 44) to obtain fine powder. Finally, the samples were packed into air-tight containers and were stored in a dry environment (24–24 °C) for further use.

Citric acid, hydrochloric acid (37%), sodium hydroxide, phenolphthalein reagent, and ethanol (99%) were purchased from Merck (Germany) and Sigma chemicals (USA). Ultrapure water for analysis was collected from the Milli-Q purification system (Millipore, France). All the other chemicals used for further analysis were of analytical grade.

2.2 Design of experiment for optimization

RSM was utilized to evaluate the optimal conditions for maximum extraction of pectin from CLP powder. A Box-Behnken design (BBD) with three levels was used to evaluate the effect of four extraction variables, i.e., temperature (°C, X_1), time (min, X_2), pH (X_3), and LSR (v/w, X_4) on the extracted pectin yield, selected as a response. The range of

four independent variables, i.e., temperature, time, pH, and LSR was selected as 70–100 °C, 30–120 min, 1–2.5, and 10–40 v/w, respectively. The complete RSM design consisted of 27 experiments including 3 replicates at the center point to determine any possible pure error. All the experiments were performed randomly in triplicates, and the data obtained was fitted to the second-order polynomial equation (expressed as Eq. 1) to demonstrate the relationship among independent variables and response.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1X_2 + \beta_{13} X_1X_3 + \beta_{14} X_1X_4 + \beta_{23} X_2X_3 + \beta_{24} X_2X_4 + \beta_{34} X_3X_4 \quad (1)$$

where Y is the dependent variable (pectin yield), β_0 is model constant, β_1, β_2, β_3, and β_4 are the linear coefficients, β_{11}, β_{22}, β_{33}, and β_{44} are quadratic coefficients, and β_{12}, β_{13}, β_{14}, β_{23}, β_{24}, and β_{34} are interaction coefficients of the independent variables (X_1, X_2, X_3, and X_4). The outcomes obtained were analyzed statistically using analysis of variance (ANOVA), and any difference was considered to be significant at a level of $p < 0.05$ [18]. Furthermore, the competency of the model was determined valuating the coefficient of determination (R^2), adjusted coefficient of determination (adj. R^2), lack of fit test, and F test value generated from ANOVA. In addition, the model was verified using the observed vs. predicted values, and regression coefficients were used to obtain the response surface. It must be noted that all the calculations and graphical analysis were done using the Design-expert software (Stat-Ease, Version 11, USA).

2.3 Preparation of alcohol-insoluble reside from CLP powder

The process for pectin extraction is supervened by preparing alcohol insoluble residues, with the purpose to remove the compounds with low molecular weight, such as traces of galacturonic acid, partly polar compounds, and proteins. The alcohol insoluble residue was prepared by following the method of Oliveira et al. [19]. CLP (100 g) powder was washed twice, firstly using boiling 70% v/v ethanol solution (600 mL) for 10 min and then by absolute ethanol (600 mL) for 10 min at room temperature. Finally, the residue was washed using acetone (200 mL). Amidst all the washings, the residue was filtered via 10-µm nylon mesh and was allowed to dry at room temperature for 24 h.

2.4 Citric acid-assisted pectin extraction

Pectin was extracted from alcohol insoluble residue using citric acid solution according to different extraction conditions using a water bath under stirring (150 rpm). The mixture was allowed to cool at room temperature, and it was then centrifuged (3000 rpm) for 20 min at 10 °C. Thereafter, the supernatant was filtered through cheesecloth, followed by precipitation by the addition of ethanol in a ratio of 1:2 v/v, and was left overnight at 4 °C. The precipitated pectin was then collected, washed twice with ethanol, and oven-dried at 50 °C until a constant weight was achieved. After complete drying of pectin, it was made into powder using a pestle mortar and was stored in a container for further analysis. The complete process of pectin extraction from CLP has been depicted in Fig. 1 [20].

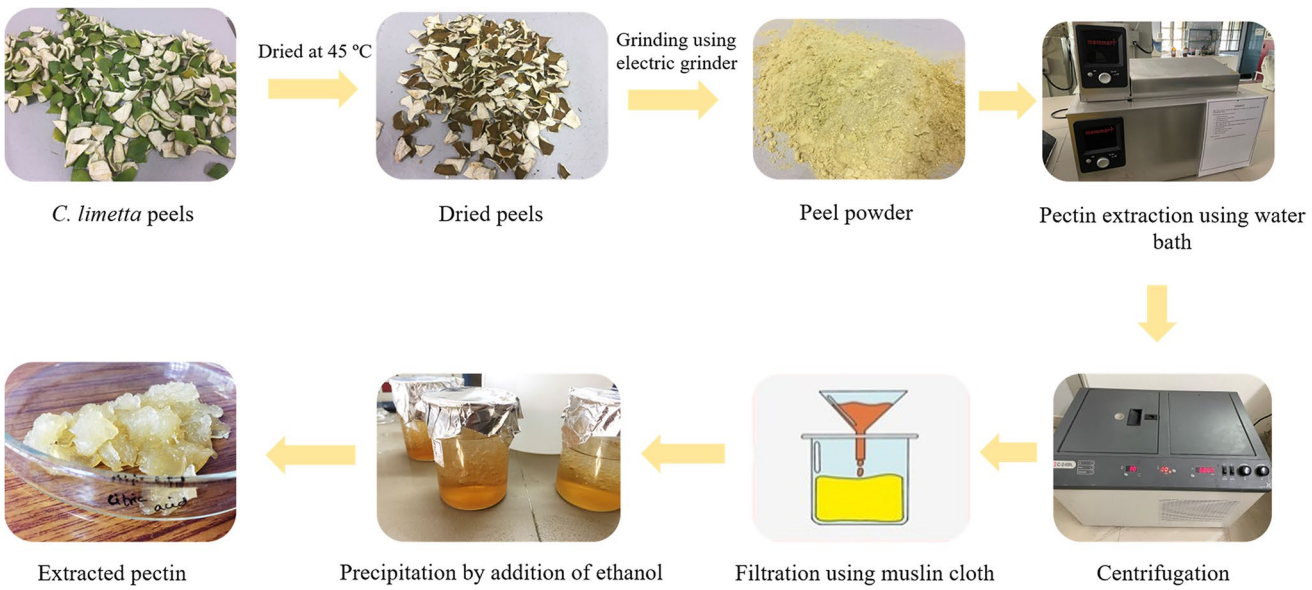


Fig. 1 Flow diagram for the pectin extraction process from *C. limetta* peels

The pectin yield was determined using the formula (Eq. 2) below:

$$\text{Yield}\% = \frac{\text{Weight of dry pectin (g)}}{\text{Weight of AIR (g)}} \times 100 \quad (2)$$

2.5 Physicochemical characterization

2.5.1 Degree of esterification

Degree of esterification (DE) of extracted pectin was demonstrated using the titration method, as reported by Wai et al. [21] with minor modifications, and the result was calculated using the following formula (Eq. 3):

$$\text{DE}(\%) = \frac{V_2(\text{mL})}{V_1(\text{mL}) + V_2(\text{mL})} \times 100 \quad (3)$$

2.5.2 Determination of equivalent weight, methoxyl content, and total anhydrouronic acid content

The equivalent weight (Eq. W.), methoxyl content (MeO), and total anhydrouronic acid content (AUA) were determined using the method as reported by Khan et al. [22]. The Eq. W. and MeO were then calculated using the following formulas (Eqs. 4 and 5, respectively):

$$\text{Equivalent weight} = \frac{\text{Weight of dried pectin (g)}}{\text{Volume of alkali (mL)} \times \text{Normality of alkali}} \times 1000 \quad (4)$$

$$\text{Methoxylcontent}(\%) = \frac{\text{Volume of alkali (mL)} \times \text{Normality of alkali} \times 31 \times 100}{\text{Weight of sample (g)} \times 1000} \quad (5)$$

Finally, total AUA was calculated using the following formula (Eq. 6):

$$\text{Total AUA}\ (\%) = \frac{176 \times 0.1x \times 100}{\text{weight of sample (g)} \times 1000} + \frac{176 \times 0.1y \times 100}{\text{weight of sample (g)} \times 1000} \quad (6)$$

where: x = titer volume (ml) of NaOH obtained from equivalent weight determination, and y= titer volume (ml) of NaOH obtained from MeO content determination.

2.5.3 Color

For the color measurement of pectin, a hunter calorimeter (Model D25 optical sensor, Hunter Associates laboratory Inc., USA) was used on the basis of L^*, a^*, and b^* values. The pectin powder was placed in a glass cell kept above the source of light covered with a white plate and the color values were noted.

2.6 Technological properties

2.6.1 Emulsification properties

Emulsion activity (EA) and emulsion stability (ES) of pectin powder were assessed by the method of Jafari et al. [14] with slight modifications. For the preparation of emulsions, aqueous pectin solution (2%, 5 mL) was homogenized with olive oil (5 mL). The pre-emulsions were then centrifuged (3500 rpm) for 5 min. Finally, the EA was calculated using the formula as mentioned in Eq. 7:

$$\text{EA}(\%) = \frac{\text{Volume of emulsified layer}}{\text{Volume of total content in tube}} \times 100 \quad (7)$$

Furthermore, ES of pectin was determined by storing the prepared emulsions at 24 °C for 1 and 30 days. ES was then calculated using the formula (Eq. 8):

$$\text{ES}(\%) = \frac{\text{Volume of remaining emulsified layer}}{\text{Volume of total content in tube}} \times 100 \quad (8)$$

2.6.2 Water holding capacity and oil holding capacity

Water holding capacity (WHC) and oil holding capacity (OHC) were determined by the procedure of Shchekoldina et al. [23] with minor modifications. Dried pectin (1 g) was added in distilled water/olive oil (10 mL), followed by vigorous stirring for 5 min, and the mixture was allowed to stand at room temperature for 30 min. The solution was then centrifuged (4500 rpm) for 30 min, and the supernatant was discarded, and the tube was drained on a filter paper to remove extra water/oil. WHC and OHC were calculated using the formula (Eq. 9):

$$\text{WHC/OHC} = \frac{x - y}{z} \quad (9)$$

where

x weight of tube with sample and water/oil (g)
y weight of tube and sample (g)
z weight of sample (g)

2.7 Structural properties

2.7.1 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) of extracted CLP pectin powder was analyzed using an FT-IR spectrometer (RX-U, FTIR, USA) using the KBr disc method in the wavelength range of 500–3500 cm^{-1}.

2.7.2 X-ray diffraction analysis

The X-ray diffraction (XRD) pattern of CLP pectin powder was analyzed using an X-ray diffractometer (PAN-analytic-Xpert PRO MRD, Almedo, Netherlands) where the sample was scanned at a diffraction angle (2θ) within the range of 10 to 80° with a step size of 0.05°.

2.8 Scanning electron microscopy

The surface morphology of CLP powder before and after the pectin extraction process was observed by scanning electron microscope (SEM, JSM-7610 F plus, JEOL, Japan). The dried peel powder was dispersed uniformly on a conductive sample table and was coated using a thin gold layer under argon atmospheric conditions by an iron sputter coater. The images of both samples were obtained at a magnification of 3000 ×.

2.9 Statistical analysis

All the experiments were carried out in triplicates, and the results were demonstrated as the mean values with standard deviation.

3 Results and discussion

3.1 RSM model fitting and statistical analysis

The effect of four extraction conditions, i.e., temperature, time, pH, and LSR in the range of 70–100 °C, 30–120 min, 1–2.5, and 10–40 mL/g, respectively were studied using a 3-level BBD design in order to achieve a maximum pectin yield from CLP. A total of 27 different experiments were obtained having a different set of input for process parameters to evaluate their combined effect on the pectin yield. The experimental and predicted values of pectin yield are shown in Table 1. A multiple regression analysis was utilized on the achieved experimental data to develop a quadratic second-order polynomial equation that includes linear, interactive, and quadratic terms (Eq. 10), to establish the relationship between process parameters and response, i.e., pectin yield.

$$\begin{aligned} Y(\%) = {} & 21.56 + 3.56X_1 + 3.41\,X_2 - 1.05\,X_3 - 0.47\,X_4 - 1.45\,X_1X_2 - 1.05\,X_1X_3 \\ & + 0.50\,X_1X_4 + 2.71\,X_2X_3 + 1.49\,X_2X_4 - 1.30\,X_3X_4 \\ & - 3.85X_1^2 - 3.86\,X_2^2 - 6.46\,X_3^2 - 3.80\,X_4^2 \end{aligned} \tag{10}$$

The reliability and adequacy of the developed experimental model were analyzed using ANOVA and R^2, which were acceptable statistically at a confidence level of 95% ($p < 0.05$). The results of ANOVA are represented in Table 2. Statistical results showed that the p value of the experimental model was less than 0.0001 and lack-of-fit (0.4002) was insignificant, which indicated that the suggested model was significantly well-fitted. Furthermore, the high value of R^2 (0.9915), adjusted R^2 (0.9815), and predicted R^2 (0.9537) along with the low value of the coefficient of variation (C.V. = 4.93%) confirmed the higher accuracy and reliability of the model in determining the relationship among pectin yield and process parameters [24]. In addition, the variables in each coefficient including linear (X_1, X_2, X_3, and X_4), quadratic (X_{12}, X_{22}, X_{32}, and X_{42}), and interactive (X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, and X_3X_4) were also found to be lower than 0.05, suggesting their significant effect on the extraction pectin yield.

3.1.1 Effect of process variables on CLP pectin yield

The yield of pectin extracted from CLP ranged from 3.97 to 22%. The linear and interactive effects of all the process variables on pectin yield were visualized using three-dimensional (3D) plots (Fig. 2). It was observed that the extraction temperature had a significant effect on pectin yield (Fig. 2a, b, and f). Results showed that the pectin yield increased with an increase in temperature, which might be due to increased solvent penetration into the plant matrix, thereby increasing the solubility as well as diffusivity of pectin from the solid matrix into the solution through the disruption of hydrogen bonds and ester linkages [3, 14]. Moreover, extraction at lower temperatures might not be sufficient for complete hydrolysis of the insoluble fraction of pectin (protopectin) by solvent, therefore achieving low pectin yields [13]. However, further increase in temperature beyond the optimum limit can result in thermal depolymerization of pectin which cannot be precipitated by alcohol, thereby reducing the pectin yields [25]. These findings were similar to the results obtained by extraction of pectin from peels of orange and passion fruit, as reported by previous researchers [26, 27].

Another important parameter affecting pectin extraction yield is time, and the results showed that with an increase in the extraction time, yield of pectin increased (Fig. 2a, c, and e). This increase in pectin yield at the initial stage of the extraction process is probably due to the prolonged reaction time between plant matrix and solvent, thereby providing high mass transfer from solid material into the solution [14]. This result was in agreement with the work of Chaharbaghi et al. [28] and Colodel et al. [29] who studied the influence of extraction parameters on pectin yield from pistachio green hull and grape pomace, respectively. However, after a certain duration, the pectin yield decreased with an increase in extraction time which could be due to the hydrolysis of glycosidic bonds and methyl ester of pectin due to the effect of citric acid, thereby leading to an overall reduction in yield [30].

Table 1 Box-Behnken design matrix with experimental and predicted values

Run no.	Independent variables				Response variable (yield)	
	Temperature (°C)	Time (min)	pH	LSR (v/w)	Experimental yield (%)	Predicted yield (%)
1	100	75	1.75	10	17.15	17.44
2	70	75	2.5	25	7.39	7.68
3	70	120	1.75	25	15.75	15.16
4	85	30	1.75	10	12.35	12.46
5	85	120	1.75	40	18.02	18.34
6	70	75	1	25	7.92	7.68
7	85	30	2.5	25	3.97	4.06
8	100	75	2.5	25	12.03	12.70
9	85	75	1.75	25	21.68	21.56
10	70	30	1.75	25	5.24	5.43
11	85	75	1.75	25	22.00	21.56
12	85	75	1.75	25	21.00	21.56
13	85	75	1	10	11.69	11.51
14	85	120	1.75	10	15.82	16.29
15	85	30	1.75	40	8.57	8.52
16	100	75	1.75	40	17.89	17.50
17	85	120	1	25	12.95	12.97
18	70	75	1.75	40	9.56	9.38
19	100	30	1.75	25	15.42	15.45
20	100	120	1.75	25	20.12	19.37
21	85	75	2.5	10	13.21	12.01
22	85	120	2.5	25	15.80	16.30
23	100	75	1	25	16.76	16.89
24	85	30	1	25	11.97	11.57
25	85	75	2.5	40	8.85	8.47
26	70	75	1.75	10	10.84	11.33
27	85	75	1	40	12.52	13.16

The surface plots in Fig. 2b, c, and d depict a considerable increase in the yield of pectin with a decreasing value of pH. The acidic solvent used for the extraction process hydrolyzes the insoluble portions of pectin into the soluble form; therefore, the pectin recovery is increased at a low pH of 2. Moreover, lower pH values could reduce the molecular weight of pectin and, therefore, improve its release from plant matrix with minimum degradation [31]. Nonetheless, at high pH levels, the pectin yield was reduced considerably, which was probably due to the accumulation of pectin, thus reducing its release [32]. These results were in close accordance with the results obtained from the extraction of pectin from pomegranate peel [33], carrot pomace [14], and citron peel [17].

The effect of LSR on the pectin yield was demonstrated, and it was clearly evident from the results that it is an important parameter influencing the process yield (Fig. 2d, e, and f). As it can be seen that with an increase in LSR up to 30 mL/g, the pectin yield increased. A study on the effect of process parameters on the pectin yield extracted from ponkan peel reported that this increase in yield with increased LSR is due to the enhanced contact area between plant matrix and solvent, which further increased the driving force for the mass transfer of polysaccharides into the solution [10]. In addition to this, increased LSR might induce swelling in the cell wall of the plant matrix, leading to the disruption of cells and, therefore, promotes pectin solubilization [34]. On the other hand, an increase in LSR after an optimum value led to a reduction in the pectin yield. These results were in agreement with the work of previous researchers who extracted pectin from sugar beet pulp [35] and sour orange [31], respectively. The possible reason for this decreased pectin yield was that with an increase in LSR beyond a certain limit, the solution starts to get saturated with a solute which negatively affected the mass transfer of polysaccharides into the solution.

Table 2 Analysis of variance (ANOVA) for the fitted model of pectin yield

Source	Sum of squares	df	Mean square	*F* value	*p* value
Model	623.29	14	44.52	99.53	< 0.0001
X_1-temperature	151.73	1	151.73	339.19	< 0.0001
X_2-time	139.67	1	139.67	312.24	< 0.0001
X_3-pH	13.15	1	13.15	29.39	0.0002
X_4-LSR	2.66	1	2.66	5.95	0.0312
X_1X_2	8.44	1	8.44	18.87	0.0010
X_1X_3	4.41	1	4.41	9.86	0.0085
X_1X_4	1.02	1	1.02	2.28	0.1569
X_2X_3	29.43	1	29.43	65.79	< 0.0001
X_2X_4	8.94	1	8.94	19.99	0.0008
X_3X_4	6.73	1	6.73	15.05	0.0022
X_1^2	78.88	1	78.88	176.34	< 0.0001
X_2^2	79.34	1	79.34	177.37	< 0.0001
X_3^2	223.40	1	223.40	499.42	< 0.0001
X_4^2	76.84	1	76.84	171.79	< 0.0001
Residual	5.37	12	0.4473		
Lack of fit	4.85	10	0.4846	1.86	0.4002
Pure error	0.5216	2	0.2608		
Cor total	628.66	26			

R^2 0.99

Adjusted R^2 0.98

Predicted R^2 0.95

CV% 4.93

3.2 Validation of optimized experimental conditions

The adequacy of developed model equation in order to predict the maximum target CLP pectin yield was carried out using the selected experimental range of process parameters. The optimal conditions were computed by solving Eq. 10, and the highest yield (22.7%) was obtained at a temperature of 90 °C, pH of 1.8, LSR of 30 v/w after 95-min extraction time. Furthermore, under these optimal conditions, three verification experiments were then carried out to validate the developed experimental model, and the pectin yield was found to be 22.03 ± 0.13%, which was found to be in close proximity to the predicted values and validated the overall suitability of optimized experimental conditions.

3.3 Physicochemical characterization

Different physicochemical parameters of CLP pectin extracted under optimum conditions were determined (Table 3) and are discussed in detail below:

3.3.1 Degree of esterification

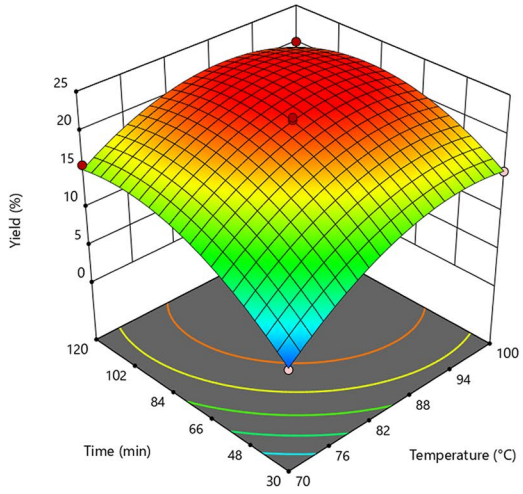
DE is a vital parameter influencing the classification of pectin having their respective applications in the food sector. It describes the limit to which the carboxyl groups are esterified in a pectin molecule [36]. On the basis of DE, pectin is being classified into two categories, namely low methoxyl pectin (DE less than 50%) and high methoxyl pectin (DE higher than 50%). The DE of pectin extracted using citric acid was found to be 45.62 ± 0.64%, indicating that the CLP pectin can be categorized as low methoxyl pectin (LMP). The reason for the low DE of extracted pectin is probably due to extraction at harsh conditions (high temperature, longer time, and low pH) which could lead to de-esterification of polygalacturonic acid chains in pectin structure [14]. This type of pectin can form gels even in the absence of sugar and therefore can be used for the development of low-calorie food products. Similar results were observed for the pectin extracted from other plant sources such as mulberry bark [37], Japanese plum [38], sour orange peel [31], and wild plum [22].

3.3.2 Equivalent weight, methoxyl content, and total anhydrouronic acid content

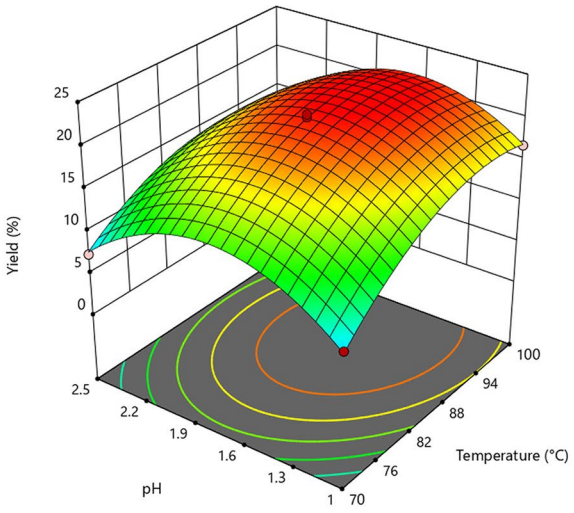
Eq. W. is one of the significant characteristics of pectin affecting its functional abilities, such as gelling properties. Pectin with high Eq. W. is known to have higher gel-forming capability, whereas, lower Eq. W. is probably due to the partial degradation of pectin which is unfavorable during gel formation. The Eq. W. of CLP pectin was observed to be 477.89 ± 9.43, and this low equivalent weight was probably due to extraction at high temperature, longer time, and low pH levels [39].

MeO is another key characteristic of pectin indicating the gel formation and its dispersibility in water. It has been reported that the methoxyl content of pectin obtained from different sources under different extraction conditions may vary from 0.2 to 12% [40]. In addition, pectin having methoxyl content less than 7% is considered as low methoxyl content, whereas, pectin with methoxyl content in the range of 8–12% lies in the category of high methoxyl pectin. According to the result obtained in this study, the methoxyl content of CLP pectin was 5.45 ± 0.04% which can be categorized as low methoxy pectin; therefore, it does not require sugar to form gels and can be used for the development of low-sugar food products. A similar result was reported by Fakayode et al. [26], where pectin extracted from orange peel was observed as low methoxyl with MeO of 6.23%.

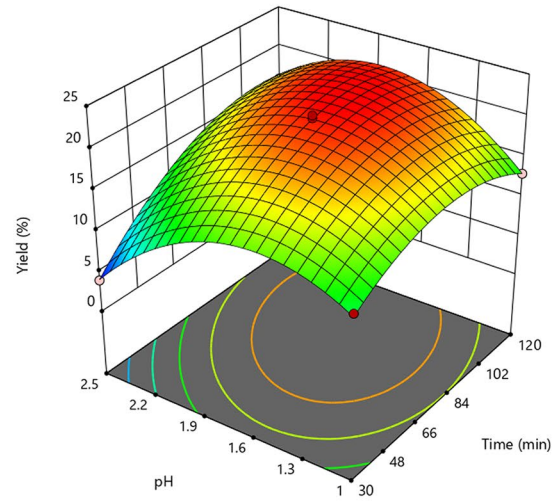
AUA content is a vital parameter that determines the purity of the pectin and affects its gelling properties. According to the standards of the Food and Agriculture Organization (FAO) and Food Chemical Codex (FCC), pectin must


Yield (%)
25
20
15
10
5
0
120
102
84
66
48
30
Time (min)
70
76
82
88
94
100
Temperature (°C)

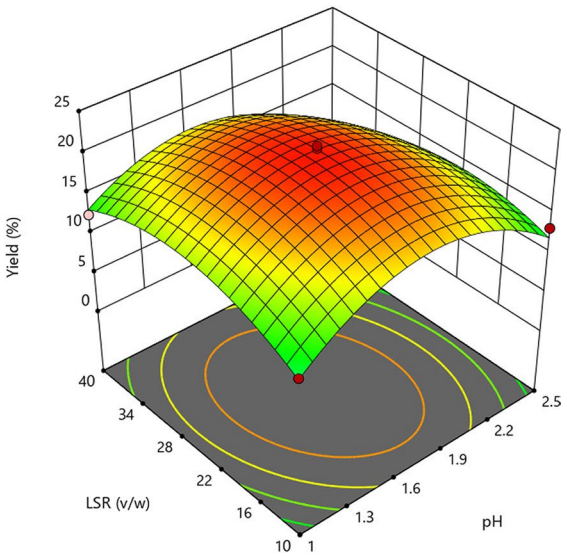
(a)


Yield (%)
25
20
15
10
5
0
2.5
2.2
1.9
1.6
1.3
1
pH
70
76
82
88
94
100
Temperature (°C)

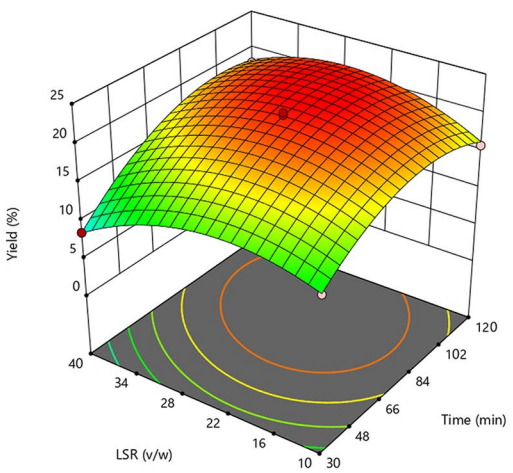
(b)


Yield (%)
25
20
15
10
5
0
2.5
2.2
1.9
1.6
1.3
1
pH
30
48
66
84
102
120
Time (min)

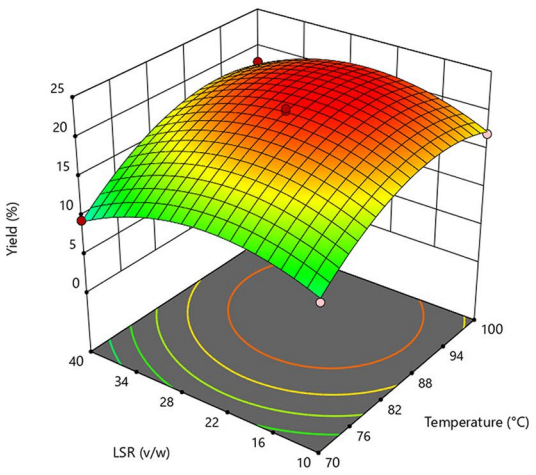
(c)


Yield (%)
25
20
15
10
5
0
40
34
28
22
16
10
LSR (v/w)
1
1.3
1.6
1.9
2.2
2.5
pH

(d)


Yield (%)
25
20
15
10
5
0
40
34
28
22
16
10
LSR (v/w)
30
48
66
84
102
120
Time (min)

(e)


Yield (%)
25
20
15
10
5
0
40
34
28
22
16
10
LSR (v/w)
70
76
82
88
94
100
Temperature (°C)

(f)

◂**Fig. 2** Response surface graphs showing the effects of independent variables. **a** Time and temperature. **b** pH and temperature. **c** pH and time. **d** LSR and pH. **e** LSR and time. **f** LSR and temperature on pectin yield

contain an AUA of at least 65% to be used in food and pharmaceutical industries [41]. AUA of CLP pectin extracted at the optimized conditions was found to be 67.81 ± 0.64% which was higher than the recommended limit and therefore confirmed the purity of extracted pectin.

3.3.3 Color

Color is a vital characteristic influencing the final appearance of food products as well as its acceptance to be used as a food additive. The color of pectin is calculated using hunter color values (*L*, a*,* and *b**), where *L** value represents the lightness in the range of 0 (dark) to +100 (light); *a** represents redness or greenness ranging from −100 (green) to +100 (red), and *b** represents yellowness or blueness ranging from −100 (blue) to +100 (yellow). The color of pectin extracted from CLP using citric acid was observed to be light brown. As correlated by visual appearance, the *L*, a*,* and *b** of CLP pectin extracted under optimum conditions were found to be 54.64 ± 0.03, 7.54 ± 0.02, and 17.45 ± 0.05, respectively. This light brown color of extracted pectin is probably due to the high temperature and long extraction times during conventional heating. A similar result was reported by Wang et al. [42], who observed the gray-brown color of pectin extracted from grapefruit using a conventional extraction process.

Table 3 Characterization of CLP pectin extracted using citric acid-assisted heating method under optimum conditions

Parameter	Value
Degree of esterification (DE %)	45.62 ± 0.64
Equivalent weight (Eq. W.)	477.89 ± 9.43
Methoxyl content (MeO %)	5.45 ± 0.04
Total anhydrouronic acid content (AUA %)	67.81 ± 0.64
Color	
L^*	54.64 ± 0.03
a^*	7.54 ± 0.02
b^*	17.45 ± 0.05
Technological properties	
Emulsifying activity (EA %)	41.31 ± 1.42
Emulsifying stability (ES %) at 24 °C	
After 1 day	70.22 ± 1.74
After 30 days	62.8 ± 1.39
Water holding capacity (WHC, g water/g pectin)	3.14 ± 0.11
Oil holding capacity (OHC, g oil/g pectin)	2.05 ± 0.17

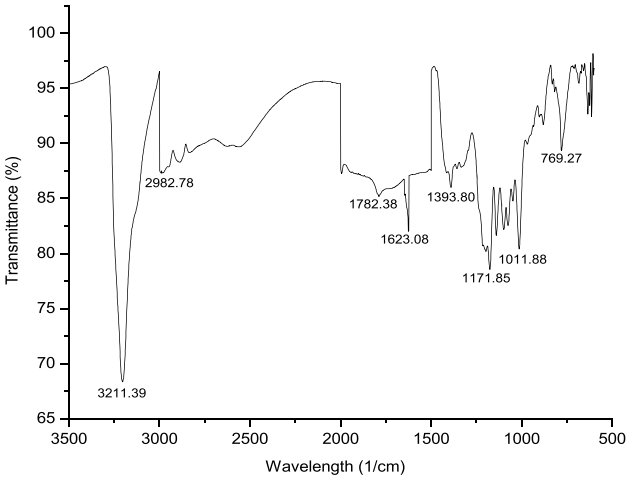


Fig. 3 Fourier transform infrared (FT-IR) spectra of *C. limetta* peel pectin extracted using citric acid under optimal conditions

3.4 Technological properties

3.4.1 Emulsification properties

In order to evaluate the technological properties of extracted CLP pectin, the EA and ES of the sample were studied. EA is the measure of the capacity to form an emulsion using a macromolecule, whereas, ES is an indicator of their capability to form a stable emulsion in a defined time period [43]. As listed in Table 3, the EA of extracted CLP pectin was 41.31 ± 1.42%, which was observed to be higher than the reported result for sour orange peel pectin (40.7%) [31] and was close to the result for pectin extracted from *C. medica* peel (46.5%) [44]. Furthermore, these emulsions were found to be 70.22 ± 1.74% and 62.8 ± 1.39% stable at

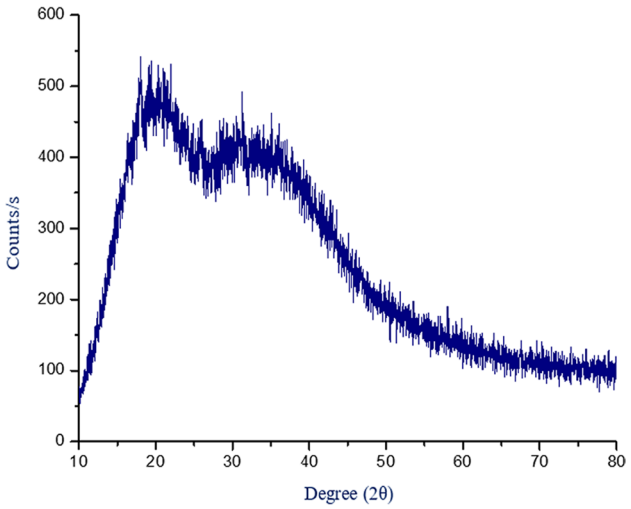


Fig. 4 X-ray diffraction (XRD) patterns for *C. limetta* peel pectin extracted using citric acid under optimal conditions

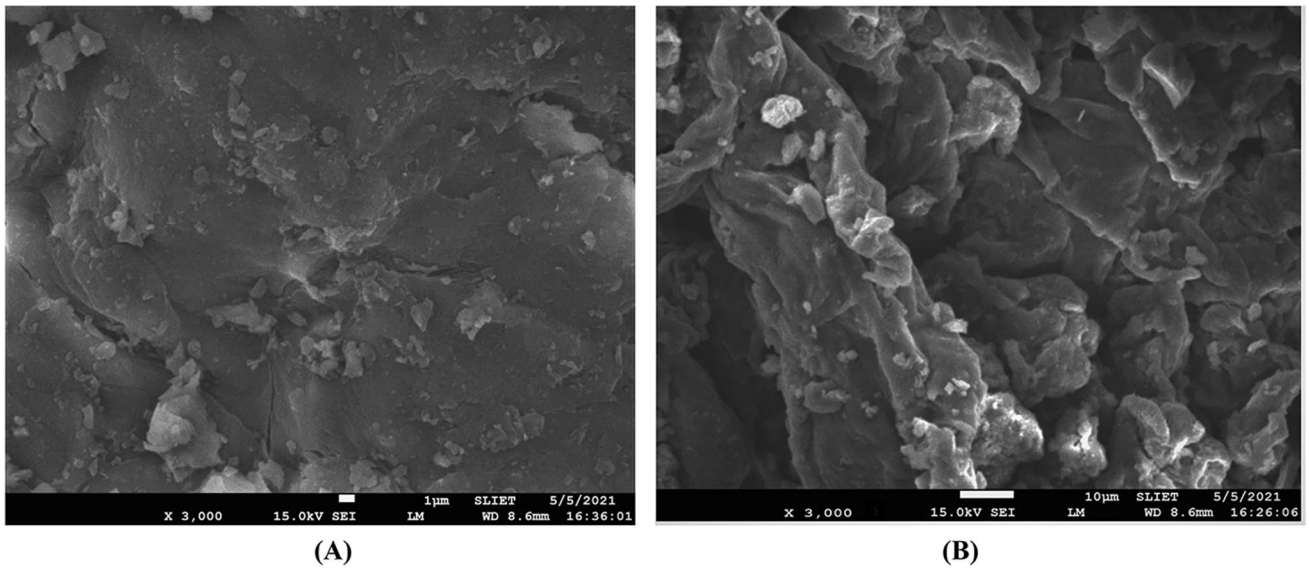


Fig. 5 SEM micrographs of *C. limetta* peel powder. **A** Before pectin extraction process. **B** After pectin extraction process at 3000×

24 °C after 1 day and 30 days of storage, respectively. It has been reported that due to the presence of reduced methyl ester groups in low-methoxy pectin, the surface activity of pectin is decreased which leads to the increase in stability of interfacial tension and particle size of emulsion, thereby positively affecting the emulsifying properties [45]. Therefore, it could be inferred from these observations that under the optimum extraction conditions, CLP pectin could be potentially used as an emulsifier and stabilizer in food industries.

3.4.2 Water holding capacity and oil holding capacity

WHC is a key technological property of many food products, and it represents the ability of 1-g pectin to retain water. Evaluation of WHC is an important parameter in food industries since pectin with high WHC is capable of holding a large quantity of water, thereby improving the textural and sensorial attributes of the product [46]. Therefore, WHC of pectin extracted from CLP under optimal conditions was evaluated, and it was observed that 1g of extracted pectin was able to hold 3.14 ± 0.11 g water (Table 3). The value was found to be higher than polysaccharides obtained from almonds (1.95 g/g) and pistachio (1.46 g/g) as well as pectin obtained from sour orange peel pectin (3.10 g/g) reported by Sila et al. [47] and Hosseini et al. [48], respectively. It has also been reported that numerous factors including the number of free hydroxyl groups present in pectin structure, pH, the porosity of dried pectin powder, and temperature could affect the WHC of the sample [49].

On the other hand, OHC is represented as the amount of oil retained by 1 g of pectin sample. Similar to WHC, OHC is also a paramount functional parameter since pectin exhibiting high OHC value can be potentially used as a stabilizer in high-fat food products [46]. The OHC of CLP pectin was observed to be 2.05 ± 0.17 g oil/g pectin (Table 3). The reported value was observed to be higher than the OHC value of commercial citrus pectin (0.93 g oil/g pectin) and *Opuntia ficus indica* pectin (1.23 g oil/g pectin) as reported by [49]. It has been reported that the high OHC value is probably due to the presence of some hydrophobic constituents in pectin structure [50].

3.5 Structural properties

The structural properties of extracted CLP pectin extracted under optimal conditions were observed using FT-IR and XRD spectroscopy which have been discussed below.

3.5.1 Fourier transform infrared spectroscopy

FT-IR is an important analytical technique used to study the primary structure of pectin by determining important functional groups in them [51]. FT-IR spectra of CLP pectin extracted under optimum conditions are depicted in Fig. 3. As observed from the spectra, broad and strong peaks were observed in the wavelength range of 3000–3500 cm^{-1} which was attributed to the stretching vibrations of inter-and intramolecular O–H groups The appeared peak at 2982.78 cm^{-1} corresponds to the bending and stretching vibrations of C–H groups (CH, CH_2, and CH_3) of galacturonic acid. Furthermore, the peaks at 1623.08 cm^{-1} and 1782.38 cm^{-1} are mainly attributed to free and esterified carboxyl groups, respectively [46]. In addition, the DE of extracted pectin was related to the peak area intensities of 1623 cm^{-1} and 1782 cm^{-1}. Therefore, as visualized by the spectra, the intensity of the peak at 1782.38 cm^{-1} was weaker than the peak of

1623.08 cm^{-1}, which confirmed that DE of CLP pectin was low. The peaks observed within the range of 1000–1250 cm^{-1} were due to glycosidic linkages (C–O–C) of galacturonic acid units [52]. It should also be noted that several peaks were observed in the range of 500–1300 cm^{-1} which is unique for each type of pectin and is therefore considered as a finger-print region [53].

3.5.2 X-ray diffraction analysis

In order to further evaluate the structure (amorphous or crystalline) of extracted CLP pectin, XRD analysis was conducted and the diffractogram is presented in Fig. 4. In XRD patterns, the amorphous structure is usually identified by a broad background peak, whereas, the crystalline structure is characterized by numerous sharp signals. As depicted by the XRD pattern, the CLP pectin exhibited an amorphous nature, while some characteristic peaks were observed at 16.28, 18.05, 20.26, 26.09, 30.35, and 34.69° (2θ) which indicated the crystalline nature in the pectin structure. Therefore, the resulted diffractogram implied that CLP pectin exhibited both crystalline and amorphous nature. Similar results have been reported for pectin extracted from pistachio by-product [54], walnut processing waste [55], sour cherry pomace [56], and black mulberry pomace [57].

3.6 Scanning electron microscopy

SEM analysis was done to examine the detailed surface morphology and the effect of extraction treatment on the raw material at a specific magnification. The SEM results of CLP powder before and after pectin extraction treatment have been depicted in Fig. 5A and B, respectively. The microstructure of CLP powder prior to extraction was observed to be uniform, dense, and compact with no disruptions and cracks in the cell wall. However, significant morphological changes in the CLP powder were evident after the conventional heating extraction method. The surface of treated peel powder was found to be disrupted with a rough, wrinkled, and irregular texture. These fractural changes in the surface of CLP powder after conventional heating extraction at harsh conditions led to the disruption in middle lamella, thereby causing complete collapse and damage of the primary cell wall of CLP which resulted in enhanced pectin yield [58].

4 Conclusions

C. limetta peels have been successfully upgraded by the production of pectin using environment friendly organic acid. The results revealed that the CLP pectin yield was in the range of 3.97 to 22% and the highest pectin yield (22.03 ± 0.13%) obtained under 90 °C temperature, 95-min time, 1.8 pH, and 30 v/w LSR, which was in close proximity to the predicted value of 22.7%, thereby validating the reliability of the developed model. The extracted pectin was classified as low-methoxyl pectin with DE of 45.62 ± 0.64% and MeO of 5.45 ± 0.04%, whereas, the equivalent weight and total AUA content of pectin were observed to be 477.89 ± 9.43 and 67.81 ± 0.64%. SEM analysis showed a significant effect of the extraction process on the raw material, and the XRD pattern demonstrated the amorphous as well as crystalline nature of extracted pectin. Besides, the FTIR characterization evidenced the presence of characteristic bands of the extracted pectin. In addition, CLP pectin exhibited promising technological and functional properties such as emulsifying properties (ES and EA), WHC, and OHC. Therefore, the pectin extracted from CLP using organic acid can be utilized as a natural additive providing a wide array of technological applications in the food sector. The proposed method for the valorization of citrus peels is in congruence with the concept of circular economy as it can help in reducing environmental pollution as well as increasing the economic benefits for industries.

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