Extraction and characterization of pectin from *Citrofortunella microcarpa* peels using conventional heating and microwave-assisted methods

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ABSTRACT

This study investigates the extraction of highly esterified pectin from *C.microcarpa* lime peels using both conventional heating extraction (CHE) and microwave-assisted extraction (MAE) methods. The highest pectin yield was obtained by applying a high concentration (0.5M) of citric acid under conventional heating at 95℃. It was also observed that an increase in microwave power could improve the yield when using a low concentration of citric acid. The quality of pectin extracted using both methods showed similar results, indicating that the application of microwave power does not necessarily alter the chemical properties of the extracted pectin. The *C.microcarpa* pectin was classified as high methoxyl pectin, which possesses an effective capability for gel formation. The FT-IR analysis confirmed the presence of functional groups characteristic of pectin structures reported in the literature. This study recommends further research to optimize extraction conditions and explore the physical and chemical properties of pectin from *C.microcarpa*, which may have potential applications in various industries, such as food, pharmaceuticals, and cosmetics.

Keywords:
C.microcarpa lime peels, pectin extraction, conventional heating extraction, microwave-assisted extraction, high methoxyl pectin

INTRODUCTION

Citrofortunella microcarpa (*C.microcarpa*), commonly known as Calamansi, is widely cultivated in Southeast Asia and used in food condiments, household cleaning, fragrances, and medicinal treatments. Citrus waste from homes and restaurants contributes to landfill accumulation, causing pollution and environmental issues.[1]Extracting pectin from fruit peels, which are typically considered waste, could create a marketable product. Researchers have been utilizing fruit peels for pectin extraction in jam and jelly production to reduce citrus waste.[2] Pectin is a complex mixture of linear hetero-polysaccharides of poly(galacturonic acid) containing methylated esters on 300-1000 galacturonic acid units[3]. Present in primary plant cell walls and middle lamella, pectin varies in structure, quantity, and chemical composition within plants over time.[2] Food and beverage manufacturers have used pectin as a thickening and gelling agent, and colloidal stabilizer for years.[4] Additionally, pectin has pharmaceutical applications, such as reducing blood cholesterol levels[5] and acting as a natural prophylactic for removing toxic cations like lead and mercury from the human gastrointestinal tract and respiratory organs.[6] It also serves as a carrier material in colon-specific drug delivery.[7]

Pectin is found in various plant tissues of different species, with varying pectin content. The extraction parameters modify the gelling abilities of diverse pectin sources, making it insufficient to consider one fruit as the primary commercial pectin source.[8] Pectin is present in many fruits, including citrus fruit peels,[9] passion fruits,[10] pomegranate,[11] melon peels,[12] pomelo peels,[13] and apple pomace.[2]

Various chemical procedures have been used for pectin extraction to investigate its structural and functional features. Common extraction methods include conventional heating, acid extraction,[14] enzymatic extraction,[15] microwave-assisted extraction (MAE),[16] and ultrasonic-assisted extraction (UAE).[17] Conventional water-based extraction involves acidified water at a pH of 2 or lower[18] and temperatures below 70℃ for two-to-four-hour periods, followed by precipitation of pectic materials using ethanol or isopropyl alcohol. The hot water extraction method is simple and efficient for pectin removal, while strong mineral acid extraction is more hazardous and environmentally concerning. The choice of acid and its concentration significantly affects the yield and properties of extracted pectin.[19,20]Citric acid has been suggested as a suitable organic acid for pectin extraction due to its minimal pectin degradation effect.[20]

Microwave-assisted extraction (MAE) is a notable alternative technique for pectin extraction that uses electromagnetic radiation within the microwave frequency range to generate thermal energy for the pectin sample.[21]MAE has been applied in various thermal processing operationsand has become a preferred method globally due to its versatile characteristics. During the MAE process, the chemical and physical properties of extracted pectin can be refined through exposure to microwaves.(Fishman et al., 2006a) The energy initiates the vibration of polar molecules, rapidly increasing the temperature and thereby expediting the extraction process.[21]Several studies have employed MAE in pectin extraction, resulting in a significant increase in pectin yield and improved pectin quality.[23–25]

MATERIALS AND METHODS

Preparation of *C.microcarpa* peels

Fresh *C.microcarpa* limes (10 kg) were sourced from the local 'Pasar Tani' market in Selayun, Brunei Darussalam. The peels were removed, and the juice was extracted and stored. The peels were then dried in a Dessini food dehydrator (Model DS-350A, China) at 50℃ until they became dry and brittle. Following this, the dried peels were ground into a fine powder using a Narutron blender (Model NBL-300, China). The *C.microcarpa* powder was further dried in an oven overnight at 60℃ and stored in a food container for future extraction.

Conventional heating extraction (CHE)

Powdered *C.microcarpa* peel samples (10.0 g) underwent extraction using a fixed solid-to-solvent ratio of 1:20 and two citric acid concentration levels (0.05M and 0.5M) as the extracting solvent. The mixtures were heated on a heating mantle at two temperature levels (60℃ and 95℃) with continuous stirring for a fixed time period of 75 minutes. After cooling, the mixtures were vacuum-filtered using Millipore miracloth. The filtrates were then treated with 95% ethanol, stirred for 2 hours, and covered overnight to allow complete pectin precipitation. Following precipitation, the solids were separated via centrifugation and washed with ethanol. Finally, the pectin isolates were dried in a Dessini food dehydrator at 50℃, and the percentage yields were calculated for all extractions.

Microwave-assisted extraction (MAE)

For microwave-assisted extraction, the same weight of peel samples, solid-to-solvent ratio, and citric acid concentrations were used. Rather than a conventional oven, the mixtures were heated in a Panasonic NN-SM33HM microwave oven at two power levels: 700W (medium) and 300W (low power) for 3 minutes. After heating, the same steps detailed for conventional heating extraction (CHE) were followed.

For the subsequent characterization of the isolated pectin, samples from extractions with the highest yield were used as a general representative for pectin isolated from *C.microcarpa* peel. All extraction experiments were conducted in triplicate.

Pectin characterisation

Moisture content. The moisture content was determined[26] by measuring 1 gram of the sample into a tared crucible, heating it in an oven at 110℃ for 5 hours, cooling, weighing, and re-drying until a constant weight was achieved. Moisture content was calculated from the weight loss before and after oven drying as follows:

$$\%\ \text{moisture} = \frac{wt\ loss\ \times\ 100}{wt\ sample}$$

Ash content. Ash content was determined[26] by measuring 1 gram of the sample into a tared crucible and igniting it in a Nabertherm muffle furnace for 3 hours at 600℃ until a constant weight was achieved. Ash content was calculated from the weight of the residue collected using the equation:

$$\% \text{ ash} = \frac{wt\ residue\ \times\ 100}{wt\ sample}$$

Equivalent weight. Equivalent weight is used to measure anhydrouronic acid content and degree of esterification. The method for determining equivalent weight[26] involved a titration procedure in which 0.5 grams of pectin sample was weighed into a 250 mL conical flask with 5 mL of ethanol added. The mixture was then titrated against 0.1N NaOH using a phenol red indicator. A faint pink color indicated the endpoint of titration. The flask containing the neutralized solution was stored for methoxyl content procedures. The equivalent weight (EW) was calculated using the equation:

$$\text{EW} = \frac{wt\ of\ sample\ (g)\ \times\ 1000}{(mL\ NaOH)(N\ NaOH)}$$

Methoxyl content. The test for methoxyl content is crucial for examining pectin's gel-forming capability. The procedures for determining methoxyl content[26] used the neutralized solutions obtained from Equivalent Weight (Titration 1), to which 25 mL of 0.25N NaOH was added while stirring. The solutions were continuously stirred under room temperature and pressure for about 30 minutes. Then, 25 mL of 0.25N HCl was added and titrated against 0.1N NaOH (Titration 2). The volume of NaOH added was recorded, and the methoxyl content (MeC) was calculated using the equation:

$$\text{MeC}\ (\%)\ = \frac{mL\ NaOH\ \times\ N\ \times\ 3.1}{wt\ sample}$$

Total Anhydrouronic Acid Content (AUA). Estimating the total anhydrouronic acid content is essential for determining the purity, degree of esterification, and assessing the physical properties of pectin.[27] The calculation of AUA content involved the values from equivalent weight and methoxyl content.

$$\text{AUA}\ (\%) = \frac{176\ \times\ 100}{z}$$

where 176 is the molecular weight of AUA, and

$$\text{z} = \frac{wt\ sample\ (mg)}{meq\ Titration\ 1 + meq\ Titration\ 2}$$

Degree of esterification (DE). DE is an important characteristic of pectin that affects its gel-forming ability. The estimation of the degree of esterification (DE) is based on the values of methoxyl content and AUA content, calculated as follows:

$$\text{DE}\ (\%) = \frac{176 \times \text{MeC}(\%) \times 100}{31 \times \text{AUA}(\%)}$$

FT-IR spectral analysis. The extracted *C.microcarpa* pectin samples were subjected to Fourier Transform Infrared (FT-IR) analysis to study and identify the functional groups present within the pectin sample. Two masses of 0.2 g of KBr and 0.002 g of pectin samples were prepared and dried in an oven for 1 hour at 100℃. The samples were ground until powdered, pelletized, and fastened on a sample holder for FT-IR analysis. The spectra were recorded with 32 scans per spectrum and set in the transmission mode within the range of 4000 cm^{-1} to 500 cm^{-1}. This analysis allowed for the identification of functional groups within the pectin sample.

RESULTS AND DISCUSSION

Pectin yield

Table 1 presents the percentage yields of extracted pectin using the CHE and MAE methods, with overall yields ranging from 0.6% to 58.4% for both extraction methods. Factors affecting pectin yield include the extraction method, acid concentration, and temperature. The highest yield (58.4%) was obtained using conventional heating at 95℃ with a high citric acid concentration (0.5M), compared to microwave heating. By keeping the solid-to-solvent ratio and extraction time constant for both methods, results showed that higher citric acid concentrations led to increased pectin yields. Low pH favors protopectin hydrolysis, causing cell rupture and greater pectin yields. Removing water molecules increases the conversion of insoluble pectic substances into soluble pectin, which then dissolves into the solvent and increases the yield.[28] Conversely, at very low citric acid concentrations, there may be insufficient pectin molecules released, resulting in lower yields.

Table 1. Percent yields from *C.microcarpa* pectin extraction using conventional heating and microwave assisted extraction methods.

Extraction method	Temperature /Power	Percentage yield (%)	
		0.05 M Citric acid	0.5 M Citric acid
Conventional heating	60℃	9.9 ± 0.084	40.8 ± 0.050
	95℃	19.0 ± 0.037	58.4 ± 0.013
Microwave-assisted	300W	0.55 ± 0.155	46.7 ± 0.976
	700W	2.7 ± 0.531	46.2 ± 4.376

Higher pectin yields were observed at higher heating temperatures (95℃) and higher microwave power (700W). Conventional heating at high temperatures induces partial hydrolysis of glycosidic ether bonds, releasing soluble pectin molecules and increasing yield.

High microwave power generates high irradiation energy from molecular interactions with the electromagnetic field, rapidly increasing temperature and causing partial cell disintegration. This triggers the efficient release of pectic substances, resulting in high pectin yield.

Table 2. Comparison of the experimental yield from *C.microcarpa* pectin extraction in this research study with the literature.

Extraction method	Extraction yield (%) (present study)	Literature		Reference
		% yield	Sample	
Conventional heating	9.9 – 58.4 %	10.7 – 40%	Sweet lime	(Yadav et al., 2017) [29]
		41.4 – 76%	Sweet lemon (*C.sinesis*)	(Devi, 2014) [27]
		36.7%	Lemon (*C.limon*)	(Kanmani, 2014)[14]
		32.4%	Sweet lime (*C.limetta*)	
Microwave-assisted	0.6 – 46.7 %	5.2 – 9.6%	Lime	(Rodsamran&Sothornvit, 2019)[21]
		5.1%	Lime *albedo*	(Fishman et al., 2006) [22]
		7.8%	Lime *bagasse*	(Thirugnanasambandham& Sivakumar, 2015) [30]
		10.1%	Citrus*lemonia*	(Zarei, 2017) [16]
		11.0%	Orange	(Kute et al., 2015) [31]

In this study, microwave-assisted extraction did not significantly improve yield compared to conventional heating at 95℃. This may be due to the short microwave heating time, which may not allow enough time for pectin molecules to be fully exposed to the irradiation energy circulating inside the microwave oven. Comparing *C.microcarpa* pectin yields with previous studies (**Table 2**) reveals that experimental yields are comparable to those from other studies. Generally, MAE yields are lower, with a maximum yield of 46.7%, compared to conventional heating at 58.4%. Yields reported by other researchers vary due to factors such as citrus fruit type, solid-to-solvent ratio, acid type and concentration, temperature, microwave power, and extraction time.

Characterisation of pectin

The moisture content of C.microcarpa pectin extracted using conventional heating and microwave-assisted extraction is 11.7% and 11.2%, respectively. These values closely align with previously reported values shown in **Table 3**. Besides citrus fruits, the moisture content found in this study is similar to those of ripe and unripe Saba banana peels, which are 10%

and 14.13%, respectively.[32] The moisture content of unripe Cardaba banana is 12.41%.[33] It is essential to maintain low pectin moisture content to prevent the growth of microorganisms that can affect pectin quality through the release of pectinase enzymes.[34] High moisture content values may result from the hygroscopic nature of pectin, necessitating storage in a closed, dry atmosphere.[29]

Table 3. The characterization results from *C.microcarpa* pectin extraction samples from using CHE and MAE methods in this research study and comparison with the literature.

Characteristic property	Experimental results		Literature reports	
	CHE	MAE	CHE	MAE
Moisture content (%)	11.7	11.2	8-10 [29]	10.0 [35]
Ash content (%)	1.9	1.6	3.3 [36]	4.4 [35]
Equivalent weight	364	358	386[14]	636 – 2219 [21]
Methoxyl content (%)	21.6	23.1	8.7-10.5 [21]	10.20 [35]
AUA content (%)	88.4	89.6	91.5 [27]	65.8 [37]
DE (%)	50.8	50.7	57.2-67.6 [29]	62.50 [35]

Ash content

The ash content of pectin extracted from conventional heating and microwave-assisted extraction (MAE) on a dry basis are 1.9% and 1.6%, respectively. Compared to literature values, the ash content in this study is lower. The variation in ash content among different sources depends on the techniques used and the range of pectin sources employed during extraction.[36] Ash content values lower than 10% are classified as low, while values above 10% indicate good quality pectin for gel formation.[38] An optimal ash content of 10% signifies superior quality pectin for gel production.[39] Pectin with low ash content, reflecting poor quality, can be refined by using a more effective acid to enhance chelation of $Ca^{2+,}$ which contributes significantly to ash content.[32] Therefore, the resulting ash content in this study demonstrates the purity of pectin. Based on this parameter, the pectin extracted using both methods in this study can be considered acceptably good quality.

Equivalent weight

The equivalent weights obtained in this study are 364 and 358 for conventional heating extraction (CHE) and microwave-assisted extraction (MAE), respectively. Equivalent weight is an indicator of pectin's gel-forming ability[40] and is one of the major physical properties determining pectin behavior. As reported in the literature, this parameter varies based on the type of citrus fruit used, its source, and the extraction method. As shown in **Table 3**, the values are comparable to those found by other researchers for pectin extraction, with equivalent weights of lemon (254),[14](sweet lemon (313),[27] and sweet lime (386).[14]In contrast to non-citrus fruits like bananas (925),[41] these citrus fruit values are considerably lower. Generally, larger equivalent weights result in stronger gel formation. However, with lower

equivalent weights, the partial degradation of pectin could be more pronounced.[29] A study by Rodsamran's group reported higher equivalent weights when using citric acid solvent under microwave heating compared to traditional acid extraction.[21]In this study, pectin extracted through conventional methods displayed a slightly higher equivalent weight than the MAE method, but the difference is small and considered insignificant.

Methoxyl content

The methoxyl content plays a crucial role in determining the type of pectin, its sensitivity to polyvalent cations, and its advantages in preparing low solid gels.[27]In this study, the methoxyl content observed from conventional heating and microwave heating are 21.6% and 23.1%, respectively. These values are considered high when compared to literature values for sweet lime (4.5%),[14]lime (8.7-10.5%),[21]and sweet orange x *P.trifoliata* (10.2%).[35] Similarly, another study found that conventional heating using citric acid as a solvent with a solid-to-solvent ratio of 1:40 resulted in a high level of methoxyl content. The literature reports a wide range of pectin methoxyl content values due to different extraction conditions.[21]

Methoxyl content characterization is essential for pectin classification, as it relates to the degree of carboxyl groups present as methyl esters in proportion to the esterified groups in the pectin molecules.[29] The larger the methoxyl content, the higher the capacity of sugar to bind and the better the quality of pectin dispersion.[29] In this study, under a pH of less than 3.5, with the presence of sufficient amounts of sugars, the high methoxyl pectin immediately produces thermally irreversible gels. Therefore, pectin's ability to disperse in water and form gels can be deduced based on the methoxyl content level. The difference in methoxyl content between the conventionally extracted and MAE samples is considered insignificant and can be classified as high methoxyl pectin.

Total Anhydrouronic acid (AUA)

Pectin is a complex polysaccharide composed of partially esterified polygalacturonide with 10% or more content of organic materials such as arabinose and galactose.[32] In this study, the total AUA content of pectin samples extracted via conventional heating extraction (CHE) and microwave-assisted extraction (MAE) are found to be 98.4% and 99.6%, respectively. These values are comparable to literature values of sweet lemon (91.5%) [27] and mangosteen rind (65.8%)[37] that produced lower AUA values.

The purpose of determining the AUA content is to measure the gelling capabilities of pectin and act as an index of pectin purity, with an expected AUA value of more than 65%.[29] AUA content values below 65% indicate the presence of impurities in the pectin, including proteins, starch, and sugars.[42] The main criteria influencing pectin purity are high galacturonic acid content and low ash content.[43]

According to the results from this study, it can be seen that the AUA content is very high. In cases of low AUA, a high quantity of protein may be present in the pectin.[29] Additionally, during the pectin precipitation process, proteins, sugars, and starch may be present, which can affect the AUA values. This shows that the presence of other impurities affects the purity and quality of pectin, which can be determined from their AUA values.

Overall, there are no significant differences in the AUA content between the two different extraction techniques used, and both can be considered as yielding good quality and pure pectin.

Degree of esterification (DE)

The degree of esterification (DE) can be calculated using the values of methoxyl content and AUA content. In this study, the DE values from conventional heating extraction (CHE) and microwave-assisted extraction (MAE) methods were 50.8% and 50.7%, respectively. These values are lower than the literature values of 57.2-67.6[29] and 62.50.[35]

DE is an essential molecular index to classify pectin and describes the degree of carboxyl groups existing as methyl ester in pectin.[29] DE is defined as the ratio of esterified galacturonic acid to total galacturonic acid groups. The structure of pectin contains three methyl esters forms at every two carboxyl groups, resulting in 60% DE, also known as DE-60 pectin.[44]

Pectin can be categorized according to its DE values, with DE > 50% considered high methoxyl (HM) pectin and DE < 50% considered low methoxyl (LM) pectin. DE depends on the type of fruit species, tissue, and maturation stage.[45] Generally, higher maturity levels result in lower DE.[29]

High methoxyl pectin, with a DE of 60-70%,[45] can form gels with sugar and acid, known as 'low water activity gels' or 'sugar-acid-pectin gels'.[44] HM pectin has qualities such as solubility in hot water, thermal reversibility, high sensitivity towards pH changes, and the frequent presence of a dispersion agent (dextrose) to prevent lumping.[45] HM pectin requires a small amount of soluble solids and an estimated pH of 3 for the gelation process.[45] In contrast, LM pectin, with a DE range of 40-50%, can form gels regardless of sugar content but requires a small amount of calcium or other divalent cations.[45]

The application of the MAE method rapidly increases the temperature of the entire sample material, efficiently deactivating the pectolitic enzyme and thus positively influencing the quality of pectic substances in the raw pectin source.[25,46] Therefore, the DE values from this study can be classified as high methoxyl pectin, as the DE is more than 50%.

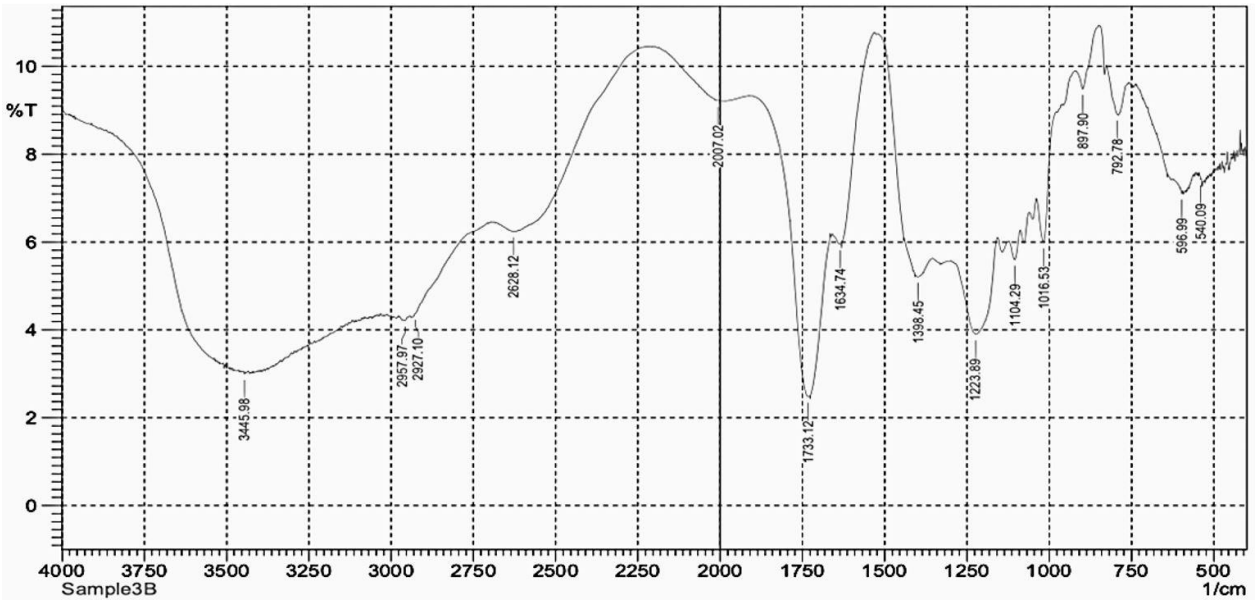

Figure 1. The FT-IR spectrum of *C.microcarpa* pectin sample extracted using conventional heating extraction (CHE).

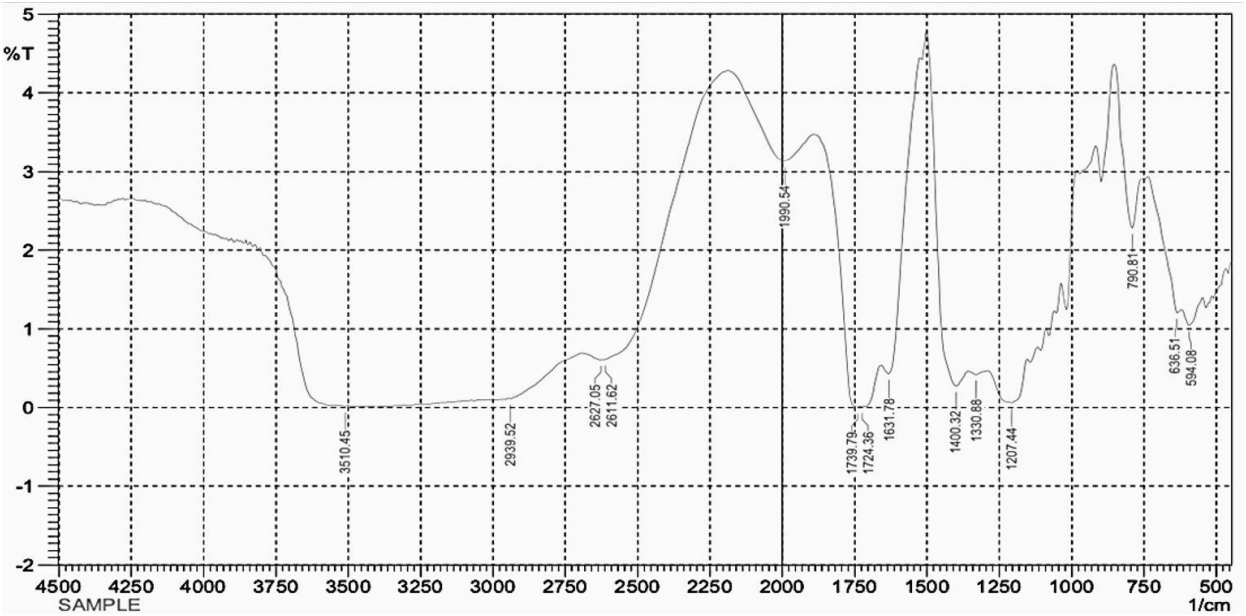

Figure 2.The FT-IR spectrum of *C.microcarpa* pectin sample extracted using microwave-assisted method (MAE).

Table 4. The FT-IR characteristic stretches and functional groups according to its wavenumber of samples from the conventional heating and MAE pectin extraction.

Characteristic stretch	Functional group	Wavenumber (cm^{-1})		
		Conventional (CHE)	Microwave-assisted (MAE)	Literature
$\nu_{(OH)}$	Alcohols	3446	3510	3440
$\nu_{(CH)}$	Alkanes	2927, 2958	2940	2930
$\nu_{(C=O)COOMe}$	α,β-unsaturated esters	1733	1725, 1740	1740
$\nu_{as(COO^-)}$	Asymmetrical esters	1635	1632	1640
$\nu_{s(COO^-)}$	Symmetrical esters	1398	1400	1435
$\nu_{s(CH)}$	Symmetric alkanes	1224	1331	1240
$\nu_{(C-C)}$	Alkanes	1104	1207	1145

Fourier-Transform Infrared (FT-IR) analysis

Figure 1 and **Figure 2** show the FT-IR spectra of pectin samples extracted via the conventional heating extraction (CHE) and microwave-assisted extraction (MAE) methods, respectively.**Table 4** lists the peak values of the FT-IR spectra for both samples and compares them to literature values. It can be observed that the experimental peak values closely match the literature values.

Pectin molecules typically produce bands in the region of 1800 to 1500 cm^{-1}, which correspond to the analysis of the degree of methylation.[11] Another region at 1730 cm^{-1} is related to the vibration of esterified carboxyl groups (C=O stretching), while the region at 1654 cm^{-1} is associated with the vibration of free carboxyl groups (C=O stretching).[47]As the intensity and the band area of esterified carboxyl groups increase, the degree of esterification is also elevated.[47,48] Visible peaks at 1725, 1733, and 1740 cm^{-1} indicate the presence of α,β-unsaturated esters, while the peak at 1224 cm^{-1} shows the presence of aliphatic amine functional groups. Regarding the 1730 cm^{-1} peak, the experimental values for the conventional and MAE pectin samples have approximately similar peak values, which correspond to their high DE values.

Another region, located at 1200 to 950 cm^{-1}, is described as the 'fingerprint' region for carbohydrates.[49]The intense experimental peaks produced are related to the characteristic stretching of pectin structural polysaccharides (polygalacturonic acid).

The characteristic stretches and functional group determination according to the IR spectra of pectin samples from both the CHE and MAE methods are in good agreement with literature values. The peaks produced from both pectin samples indicate the presence of functional groups (alcohols, alkanes, esters, α,β-unsaturated esters) corresponding to the expected pectin structures.

CONCLUSION

This study has demonstrated that *C.microcarpa* lime peels can serve as a good source of highly esterified pectin, using both conventional heating extraction (CHE) and microwave-assisted extraction (MAE) methods. The application of high concentration (0.5M) of citric acid under conventional heating at high temperature (95℃) resulted in the highest pectin yield. Additionally, it was observed that increasing the microwave power can improve the yield when using a low concentration of citric acid.

The quality of pectin extracted using both the CHE and MAE methods showed similar results, indicating that the application of microwave power does not necessarily alter the chemical properties of the extracted pectin. The *C.microcarpa* pectin was classified as high methoxyl pectin, which possesses an effective capability for gel formation. The FT-IR analysis confirmed the presence of functional groups characteristic of pectin structures reported in the literature.

This study recommends that future research should focus on further understanding the optimum extraction conditions as well as the physical and chemical properties of pectin from *C.microcarpa*. This will help in optimizing the extraction process and expanding the potential

applications of *C.microcarpa* pectin in various industries, such as food, pharmaceuticals, and cosmetics.

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