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Valorization of Sweet Lime (Mosambi) Peel and Pomace for Pectin Extraction: Process Optimization and Functional Characterization

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Abstract: *Sweet lime (Mosambi) peel and pomace, the principal by-products of juice processing, represent valuable sources of pectin widely used in the food industry for gelling, thickening and stabilizing applications. In the present study, pectin was extracted from both the peel and pomace of sweet lime using acidified water with nitric acid (HNO□) as the extraction medium. Optimization of the extraction parameters was carried out using a Box–Behnken design within the framework of response surface methodology (RSM), using three independent variables: extraction time (30–90 minutes), temperature (30–90 °C) and pH (1–2). Pectin yield, methoxyl content, anhydrouronic acid (AUA) content and degree of esterification (DE) were evaluated as response parameters. Among the variables studied, extraction pH significantly influenced pectin yield from both substrates. Optimal conditions were 78.64 °C, 59.12 min and pH 1.32 for peel and 56.87 °C, 68.45 min and pH 1.58 for pomace, yielding 20.87% and 13.5% pectin, respectively. Peel-derived pectin exhibited higher water and oil holding capacities, highlighting its superior potential for commercial pectin production and agro-industrial waste valorization*

Keywords: Pectin, Pomace, Sweet lime, peel, Response surface methodology (RSM), methoxyl content

I. INTRODUCTION

Waste valorization has emerged as a critical global strategy to address escalating environmental concerns while simultaneously generating economic and social benefits (Garcia-Garcia et al., 2019). In the agri-food sector, the effective utilization of processing by-products is particularly important, as large quantities of organic waste are generated during food manufacturing, often leading to disposal challenges, environmental pollution and resource loss. Citrus fruits represent one of the most extensively cultivated and consumed fruit groups worldwide, contributing nearly 18% of total global fruit production (Kamal et al., 2011). A substantial proportion of citrus fruits is processed into juices, resulting in the generation of considerable quantities of solid residues, primarily peels and pomace, which together account for more than 50% of the fresh fruit mass. Citrus processing waste is rich in valuable phytochemicals and functional components, including essential oils, carotenoids, polyphenols, dietary fibres, organic acids and polysaccharides (Suri et al., 2022). Among these, pectin is one of the most commercially important constituents due to its wide application in food, pharmaceutical and biodegradable material industries (Barcelos et al., 2020; Mehmood et al., 2021). Improper disposal of citrus waste not only causes environmental issues such as water pollution and microbial proliferation but also represents a loss of high-value bioresources (Suri et al., 2022). Therefore, the recovery of functional compounds from citrus by-products provides an effective route toward sustainable waste management and circular bioeconomy development. In India, sweet lime (*Citrus limetta*), commonly known as Mosambi, is widely consumed as fresh fruit and juice. The juice industry generates large amounts of peel and pomace, which are typically discarded despite their high pectin content. Sweet lime peel is considered a promising raw material for pectin extraction owing to its abundance, low cost and rich polysaccharide composition.

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Pectin is the most widely used polysaccharide in food industry, which can be extracted from peels of fruits. It is also used in pharmaceutical industries as antitumor, antioxidant, antidiabetic and anticancer drug (Hosseini et al., 2019). Pectin is used as a good additive in jams, soft drinks, milk products, as fat substitute in emulsified meat products, spreads, ice cream and salad dressing (Wang et al., 2016). Pectin is considered as an effective biomaterial film due to its biodegradability, biocompatibility and non-toxicity (Rodsamran et al., 2019). Extraction is one of the essential techniques which is used to recover pectin from various organic and inorganic wastes. Extraction technique can be carried out by both conventional and non-conventional methods. However, the conventional extraction methods have numerous limitations like long processing time, high energy requirement and high solvent consumption with high amount of waste water generation. Thus various novel approaches like microwave-assisted extraction, ultrasound-assisted extraction and enzyme-assisted extraction were developed for the extraction process (Rahmani et al., 2020). Among various extraction techniques, ultrasound-assisted extraction technique is considered more beneficial due to low energy consumption, shortened treatment time, less solvent usage, increased safety of the operators and increased yield (Chemat et al., 2017).

Sweet lime (*Citrus limetta*), commonly known as Mosambi, is a citrus species believed to be a hybrid between citron (*Citrus medica*) and bitter orange (*Citrus aurantium*). It is characterized by small to medium-sized, nearly sub-globose fruits. A significant proportion of sweet lime fruits is processed for juice production, resulting in the generation of substantial quantities of peel and pomace (approximately 50% of the fruit mass). These by-products are often discarded as waste, posing challenges related to their disposal and contributing to environmental issues, particularly water pollution. The accumulation of organic waste materials stimulates microbial activity, leading to the aerobic degradation of biodegradable matter into compounds such as nitrates, phosphates, carbon dioxide and sulphates, thereby exacerbating ecological concerns. However, this challenge can be converted into an opportunity through the recovery of value-added compounds such as pectin from citrus processing waste. The pectin extraction at an economically viable rate can enhance the commercial utility of such agro-industrial residues. In addition to potential use as animal feed or compost through biotransformation, citrus peel is known to be rich in two commercially valuable components: d-Limonene and pectin, where pectin has a significant industrial potential.

The extraction of pectin is a complex physico-chemical process influenced by several parameters, including pH, temperature, extraction time, solvent type and solid-to-liquid ratio (Patil et al., 2022). Conventional acid extraction methods, although widely used, are associated with drawbacks such as long processing times, high energy consumption, excessive solvent use and wastewater generation (Kamal et al., 2021; Kumar et al., 2023). In response to these limitations, environmentally friendly and energy-efficient techniques such as microwave-assisted extraction (MAE) (Amran et al., 2021), ultrasound-assisted extraction (UAE) (Kumar et al., 2017)have gained significant attention. Among these, UAE has emerged as a promising green technology due to its reduced extraction time, lower solvent requirement, enhanced mass transfer and improved extraction efficiency (Yang et al., 2019). Optimization of extraction conditions is crucial to maximize pectin yield while preserving its functional and physicochemical quality. Response Surface Methodology (RSM), particularly when coupled with Box–Behnken design, is a powerful statistical tool for modeling and optimizing multivariable processes with minimal experimental runs. RSM enables the evaluation of individual and interactive effects of extraction parameters, thereby facilitating process optimization in a systematic and cost-effective manner (Kamal et al. 2020). Despite extensive research on pectin extraction from various citrus species, limited information is available on the valorization of sweet lime peel and pomace, particularly using ultrasound-assisted extraction and statistical optimization approaches. Moreover, comprehensive characterization of the functional and physicochemical properties of pectin derived from mosambi processing waste remains underexplored.Therefore, the present study aims to valorize sweet lime (Mosambi) peel and pomace by optimizing pectin extraction parameters using Response Surface Methodology and to evaluate the physicochemical, structural and functional properties of the extracted pectin. The findings of this study are expected to contribute to sustainable citrus waste management and provide scientific insights into the development of high-value pectin from underutilized agro-industrial residues.

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II. MATERIAL AND METHODS

Materials

All chemicals utilized in the present study were of analytical grade. Fresh peel and pomace of sweet lime (Mosambi) were procured from local juice vendors for experimental use.

Sample Preparation

The peel and pomace were manually cut into small pieces and thoroughly washed under running tap water to remove surface impurities, followed by surface drying. The cleaned samples were then air dried at 55 °C until a constant weight was achieved. Subsequently, the dried materials were ground into a fine powder using a Waring blender. The powdered samples were stored in air-tight, moisture-proof containers under ambient conditions for further analysis.

Proximate Analysis of Peel and Pomace

The moisture content of the samples was determined by using hot air oven method, following a single-stage protocol. 5 g of the sample was placed in a hot air oven at 105°C and dried until a constant weight was achieved. Moisture content was calculated as the percentage of weight loss relative to the initial sample weight (AOAC, 2000). Ash content was estimated by incinerating 3 g of the sample. Initially, the sample was heated on a hot plate until visible fumes ceased, after which it was transferred to a pre-weighed crucible and placed in a muffle furnace at 525 °C for 5 hours (AOAC, 2000). Crude protein content was determined using the Kjeldahl method, wherein the nitrogen content of the sample was measured using a Kelplus Nitrogen Estimation system (block design and distillation system; Pelican Equipment, Chennai, India). The obtained nitrogen value was multiplied by a conversion factor of 6.25 to calculate the crude protein content (AOAC, 2000). Crude fat content was estimated using the Soxhlet extraction method (AOAC, 2020). Crude fiber content was determined by sequentially digesting the sample with sulfuric acid (H_2SO_4) followed by sodium hydroxide (NaOH), as described in AOAC (2000). Total carbohydrate content was calculated by difference, i.e., by subtracting the sum of moisture, ash, protein, fat and fiber contents from 100. The energy value of the samples was determined by using the standard calorific value of micronutrients, as illustrated below.

$$Energy\frac{kcal}{100g}sample = (Protein\%\ x\ 4) + (Carbohydrate\%\ x\ 4) + (Fat\%\ x\ 9)$$

Experimental design and Optimization of process parameters

To determine the optimal conditions for pectin extraction, an experimental design was developed incorporating three independent variables: extraction temperature (30–90 °C), extraction time (30–90 minutes) and pH (1.0–2.0). The study focused on four response variables: pectin yield, methoxyl content, anhydrouronic acid (AUA) content and degree of esterification (DE). A Box-Behnken Desing, a type of Response Surface Methodology (RSM) was employed to structure the experimental plan. This design comprised 17 experimental runs and was selected based on its suitability for optimizing the extraction parameters, as it meets the key requirements for process optimization in this context. The experimental data were analysed using Response Surface Methodology, facilitated by the Design-Expert Software (version 12.0.11.0, Stat-Ease Inc., Minneapolis, USA, trial version) This statistical tool was utilized to generate response surface plots and to conduct optimization of the process variables. The primary objective of the optimization was to identify the levels of the independent variables namely, extraction temperature, extraction time and solvent pH, that would maximize pectin yield, methoxyl content, anhydrouronic acid content and degree of esterification.

Extraction of pectin

The pectin extraction procedure was adapted from the method described by Pagan *et al.,* (2001). A 5 g portion of ground peel sample was weighed and mixed with 100 mL of acidified water with the pH adjusted according to the experimental design. Acidification was achieved using nitric acid and the pH of the solution was calibrated using a pH meter (Elico, Model No L614). The mixture was then incubated at specified temperatures and time intervals in an orbital shaking incubator set to 150 rpm. Following the incubation period, the samples were filtered through muslin cloth to remove solid residues. The resulting filtrate was treated with isopropanol to precipitate the pectin. After the

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addition of isopropanol, the mixture was allowed to stand overnight to ensure complete precipitation. The precipitated pectin was subsequently collected by filtration through muslin cloth and was washed sequentially with 70%, 80% and 90% ethanol to remove residual moisture. Finally, the purified pectin samples were dried at 50 °C overnight.

Analysis of physico-chemical properties of extracted pectin

Pectin yield

The percentage yield of pectin was determined for all samples extracted under various treatment conditions. The yield was calculated using the following formula:

$$Percentage\ yield\ of\ pectin = \frac{Pectin\ obtained\ (g)}{Total\ amou\ nt of\ peel\ or\ pomace\ powder\ (g)} \times 100$$

Methoxyl Content, Anhydrouronic Acid Content (AUA) and Degree of Esterification (DE)

These parameters were measured following the procedures outlined by Ranganna (2005). The determination was based on the saponification of pectin, followed by titration of the liberated carboxyl groups. Specifically, 25 mL of 0.25 N sodium hydroxide (NaOH) was added to the neutralized pectin solution, thoroughly mixed and allowed to stand at room temperature for 30 minutes. Subsequently, 25 mL of 0.25 N hydrochloric acid (HCl) was added and the solution was titrated with 0.1 N NaOH until the original endpoint (magenta coloration) was achieved. The values were then calculated using the following formula:

$$Methoxyl\ content\ (\%) = \frac{MI\ of\ alkali \times normality\ of\ alkali \times 3.1}{Weight\ of\ sample}$$

The estimation of anhydrouronic acid (AUA) content is critical for assessing the purity and degree of esterification of pectin, as well as for evaluating its physicochemical properties. Utilizing the values of equivalent weight, methoxyl content and ash alkalinity, the AUA content was calculated using the following expression:

$$AUA\ (\%) = \frac{176 \times 0.1z \times 100}{w} + \frac{176 \times 0.1y \times 100}{w}$$

Where the molecular unit of AUA (1 unit) = 176 g

Where,z = ml (titre) of NaOH from equivalent weight determination

y = ml (titre) of NaOH from methoxyl content determination

w = weight of sample

The degree of esterification was measured based on methoxyl (%) and AUA (%) content(Yadav *et al.,* 2017)and calculated by using the formula:

$$DE(\%) = \frac{17 \times \%Methoxyl}{31 \times \%AUA} \times 100$$

Determination of Functional Properties

Water Holding Capacity (WHC)

The water holding capacity of pectin obtained under optimized extraction conditions was determined following the method described by Lin et al. (1974). A 0.5 g sample of ground pectin was mixed with 10 mL of distilled water in a 35 mL centrifuge tube. The mixture was subjected to sonication using an ultrasonic cleaner (Atico, Haryana, India) for 1 minute. It was then shaken for 1 hour at room temperature, followed by centrifugation at 6500 × g for 25 minutes. The volume of unbound (free) water was measured and WHC was expressed as the volume of water (mL) absorbed per gram of pectin.

Oil Holding Capacity (OHC)

The oil holding capacity was also determinedaccording to the procedure outlined by Lin *et al.,* (1974). A 0.5 g pectin sample was combined with 5 mL peanut oil in a 15 mL polypropylene centrifuge tube. The contents were stirred and sonicated for 1 minute to ensure proper dispersion. The tube was then held at 25 °C and centrifuged at 4000 rpm for 25 minutes. The OHC was calculated as the amount of oil absorbed (mL/g) by measuring the volume of unabsorbed oil.

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Fourier Transform Infrared (FT-IR) Spectroscopy

The structural interactions within the film samples were analysed using Fourier Transform Infrared (FT-IR) spectroscopy (Thermo Nicolet 6700, PAU Ludhiana) to identify the functional groups and possible intermolecular interactions present in the pectin matrix. Spectral data were recorded over the wavenumber range of 400 to 4000 cm^{-1}. This range encompasses the characteristic absorption bands associated with various functional groups, including hydroxyl (-OH), carbonyl (C=O) and carboxyl (-COOH) groups, which are typically involved in hydrogen bonding and esterification reactions.

III. RESULT AND DISCUSSION

Chemical Analysis of Peel and Pomace

The chemical composition of *Citrus sinensis* (mosambi) peel and pomace is presented in Table 1. The dried peel and pomace samples exhibited moisture contents of 5.78% and 5.89%, respectively. The peel contained higher levels of fat, crude protein, crude fiber, ash and caloric energy, whereas the pomace showed a greater carbohydrate content.

Specifically, the crude protein, fat, crude fiber, carbohydrate, ash content and energy values for peel and pomace were as follows: crude protein 8.94 ± 0.3% (peel) and 3.76 ± 0.1% (pomace); fat 1.44 ± 0.1% (peel) and 1.21 ± 0.2% (pomace); crude fiber 39.40 ± 0.4% (peel) and 37.12 ± 0.3% (pomace); carbohydrates 39.6 ± 0.4% (peel) and 48.3 ± 0.3% (pomace); ash 4.77 ± 0.1% (peel) and 3.66 ± 0.1% (pomace); and caloric energy 207.12 ± 0.5 kcal (peel) and 219.13 ± 0.5 kcal (pomace). The higher carbohydrate content in pomace (48.3 ± 0.3%) suggests its potential utility as an energy-dense by-product for value-added applications. The present findings are in partial agreement with previous studies. Oikeh*et al.,* (2013) reported crude fiber contents of 13.43 ± 0.03% in the flavedo and 4.45 ± 0.06% in the albedo of *Citrus sinensis*. Similarly, Egbuonu and Osuji (2016) reported the proximate composition of dried sweet orange peel as follows: moisture 9.68 ± 0.07%, fat 6.24 ± 0.06%, fiber 13.99 ± 0.06%, ash 4.89 ± 0.06% and protein 11.00 ± 0.10%. While variations in composition may be attributed to differences in citrus variety, agro-climatic conditions, processing methods and anatomical parts analyzed, the current results confirm that mosambi peel and pomace are rich in dietary fiber and essential nutrients, thereby highlighting their potential as functional ingredients in food or feed applications.

Table 1: Chemical composition of mosambi processing waste

Processing waste	Moisture content (%)	Crude protein(%)	Fat(%)	Crude fiber(%)	Ash(%)	Carbohydrate content(%)	Energy (Kcal)
Peel	5.78±0.1	8.94±0.3	1.44±0.1	39.40±0.4	4.77±0.1	39.6±0.4	207.12±0.5
Pomace	5.89±0.2	3.76±0.1	1.21±0.2	37.12±0.3	3.66±0.1	48.3±0.3	219.13±0.5

Values are mean of three observations ± S.D

Influence of Process Variables on the Yield and Physicochemical Properties of Pectin Extracted from Mosambi Peel

The yield and physicochemical properties of pectin extracted from mosambi peel using acidified water with HNO_3 are presented in Table 2. Significant variations were observed across all four response parameters under different experimental conditions. Specifically, the yield ranged from 5.14% to 20.87%, methoxyl content from 2.79% to 9.45%, anhydrouronic acid (AUA) content from 33.44% to 68.56% and degree of esterification (DE) from 47.36% to 82.62% (Table 2). The highest pectin yield from mosambi peel was obtained when the extraction was performed at 60°C for 90 minutes at pH 1.0. In contrast, the highest DE and methoxyl content were observed at 30°C, 60 minutes and pH 1.5. The maximum AUA was achieved at 60°C, 60 minutes and pH 1.5, indicating that the extraction conditions have a significant impact on both the yield and properties of pectin.

Kanmani et al. (2014) reported a maximum pectin yield of 36.71% from *Citrus limon* under conditions of 65°C, pH 3.5 and a 67.5-minute extraction time, which was higher than the yields obtained from kinnow and apple pomace. Sharma *et al.,* (2014) extracted pectin from apple pomace waste and found a yield of 10.50% on a dry weight basis. Similarly, Sharma *et al.,* (2013) observed a maximum pectin yield of 16.1% from kinnow peel at 60°C, pH 1.75 and 70 minutes of

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extraction. Devi *et al.,* (2014) found that the yield of pectin from mosambi peel powder was higher (21.4%) when extracted with citric acid at 80°C, pH 1.5 and 60 minutes, compared to the 17.4% yield obtained using nitric acid.

Table 2: Effect of various process variables on yield and physicochemical parameters of pectin extracted from mosambi peel

		Factors			Responses			
Std	**Run**	**Temp(°C)**	**Time (min)**	**pH**	**Yield (%)**	**MC(%)**	**AUA(%)**	**DE(%)**
16	1	60	60	1.5	13.50	6.72	56.24	67.85
4	2	90	90	1.5	11.56	4.42	48.50	51.85
3	3	30	90	1.5	9.54	9.01	69.54	73.56
13	4	60	60	1.5	13.50	7.34	61.50	67.85
14	5	60	60	1.5	13.50	7.35	61.50	67.85
12	6	60	90	2.0	8.26	11.04	75.24	83.33
2	7	90	30	1.5	12.54	8.47	61.90	77.69
11	8	60	30	2.0	9.45	5.47	51.21	60.71
5	9	30	60	1.0	9.80	6.17	53.24	65.89
8	10	90	60	2.0	5.96	7.01	58.64	67.85
9	11	60	30	1.0	10.26	2.66	42.00	36.00
10	12	60	90	1.0	11.89	1.94	29.61	37.33
7	13	30	60	2.0	5.21	9.07	65.24	78.95
17	14	60	60	1.5	13.50	7.34	61.50	67.85
15	15	60	60	1.5	13.50	7.34	61.50	67.85
6	16	90	60	1.0	8.62	5.19	49.56	59.56
1	17	30	30	1.5	5.45	4.85	51.24	53.84

To further analyze the results, regression analysis was performed to fit mathematical models to the experimental data. The quadratic model derived from the regression analysis for yield, methoxyl content, AUA and DE of pectin, expressed in terms of the un-coded levels of the variables, is presented as follows:

Yield = +20.21+ 1.39 * A + 0.31* B - 4.69* C - 3.17* A * B - 0.32* A * C - 1.78 * B * C - 1.60 * A^2 - 3.34 * B^2 - 4.25 * C^2

Methoxyl content= +8.86 - 0.64 * A - 0.66 * B + 0.55 * C + 0.12 * A * B - 0.12 * A * C + 0.77 * B * C + 0.46 * A^2 - 3.28 * B^2 - 0.79 * C^2

AUA= +68.21 - 2.40 * A - 3.06 * B + 3.50 * C - 0.075 * A * B + 2.08 * A * C + 5.19 * B * C + 0.84 * A^2 - 16.75 * B^2 - 4.75 * C^2

DE=+73.80 - 3.51 * A - 4.19 * B + 2.06 * C + 0.70 * A * B -3.43 * A * C + 3.40 * B * C + 4.71 * A^2 - 13.59 * B^2 - 3.13

Effect of Process Parameters on Yield of Pectin

Figure 1 illustrates that the yield of pectin extracted from mosambi peel using HNO_3 initially increased with the duration of extraction, followed by a decline as the pH of the extraction solvent was raised from 1.5 to 2.0. Among all the process variables, the pH exhibited the lowest p-value (Table 3), indicating that it had the most significant effect on the pectin yield from mosambi peel. Additionally, the yield was significantly influenced by the interactions between temperature and time, as well as between time and pH ($p < 0.05$). These findings are consistent with those of Yadav *et al.,* (2017), who observed that the yield of pectin from sweet lime peel was notably affected by changes in pH. However, time and temperature alone did not show a significant effect on pectin yield. Similarly, Li *et al.,* (2015) reported that the yield of pectin from sugar beet increased as the pH decreased, with higher temperatures and extended extraction times further enhancing the yield.

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Effect of Variables onMethoxylContent

All process parameters exhibited a significant influence on the methoxyl content of pectin extracted from mosambi peel. Significant interaction effects were also observed for the quadratic terms of time (time × time) and pH (pH × pH). Among the individual factors, extraction time had the most pronounced impact on methoxyl content, followed by temperature and pH. These findings highlight the critical role of extraction conditions in modulating the structural characteristics of pectin.Methoxyl content is a key parameter that determines the gelling behavior and classification of pectin. It refers to the proportion of methoxyl ($–OCH_3$) groups esterified to the carboxyl groups of galacturonic acid residues in the pectin backbone. High methoxyl (HM) pectins (methoxyl content >7%) require high sugar concentrations and low pH to form gels, whereas low methoxyl (LM) pectins (<7%) can form gels in the presence of divalent cations such as calcium. The degree of methoxylation is sensitive to thermal degradation and hydrolytic reactions that can occur under prolonged heating or extreme pH conditions, which may lead to de-esterification of the pectin molecule. Hence, extraction parameters such as time, temperature and pH directly influence the retention or loss of methoxyl groups during pectin isolation.However, in contrast to these findings, Aravantinos-Zafiris and Oreopoulou (1992) reported that the methoxyl content of pectin extracted from orange peel was largely unaffected by variations in extraction conditions, suggesting that the stability of methoxyl groups may vary depending on the source and nature of the raw material.

Effect of variables on AUA

The anhydrouronic acid (AUA) content of pectin extracted from mosambi peel using HNO_3 did not exhibit statistically significant variation with respect to the individual extraction parameters—temperature, time and pHas indicated in Table 3. However, a significant effect was observed for the quadratic interaction of time (time × time), suggesting a non-linear influence of extraction duration on AUA content (Figure 1).Anhydrouronic acid content serves as an important indicator of pectin purity, as it reflects the proportion of galacturonic acid residues in the polymer backbone. High AUA values are associated with a lower presence of non-pectic impurities such as neutral sugars, proteins and phenolic compounds. The apparent insensitivity of AUA content to most process variables in this study suggests that the galacturonic acid backbone remains relatively stable under the tested extraction conditions. However, prolonged extraction times may promote partial hydrolysis or breakdown of pectic chains, which can impact the overall uronic acid content, thus explaining the significance of the time × time interaction.These results highlight the importance of optimizing extraction duration to maintain the structural integrity and purity of pectin, while minimizing degradation-related losses.

Effect of variables on DE

All three independent variables—extraction temperature, time and pHexerted a statistically significant effect on the degree of esterification (DE) of pectin extracted from mosambi peel. Among these, extraction time had the most pronounced influence on DE, followed by temperature and pH. In addition to the main effects, several interaction terms also significantly affected DE, including temperature × pH, temperature × time, time × pH, as well as the quadratic terms temperature × temperature, time × time and pH × pH ($p < 0.05$). These results underscore the complexity of DE modulation during pectin extraction and the sensitivity of esterification dynamics to multiple, interdependent process conditions.The degree of esterification refers to the percentage of methyl-esterified galacturonic acid residues in the pectin molecule and plays a critical role in determining the functional and gelling properties of pectin. Higher DE values are characteristic of high-methoxylpectins, which form gels in the presence of high sugar and low pH, whereas low-methoxylpectins (with DE < 50%) gel in the presence of divalent cations such as calcium. DE can be influenced by several physicochemical factors during extraction. Elevated temperatures and extended extraction times can accelerate the hydrolysis of ester bonds, reducing DE through de-esterification reactions. Similarly, extreme pH conditionsparticularly strongly acidic environmentscan catalyze the breakdown of ester linkages.The significant interaction effects observed suggest that the influence of one factor on DE is strongly dependent on the levels of the other variables. For example, the effect of temperature on DE may vary depending on the pH of the extraction solvent and the duration of extraction. This is consistent with findings by Wai *et al.,* (2010), who reported that the interaction

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between pH and extraction time had a strong and significant influence on the DE of pectin, reinforcing the importance of carefully optimizing extraction conditions to preserve desired functional properties.

Effect of different factors on yield and physicochemical properties of pectin extracted from mosambipomace

Pectin was extracted from mosambipomace using acidified water containing nitric acid (HNO_3) and considerable variation was observed across all response parameters under different extraction conditions as presented in Table 4.

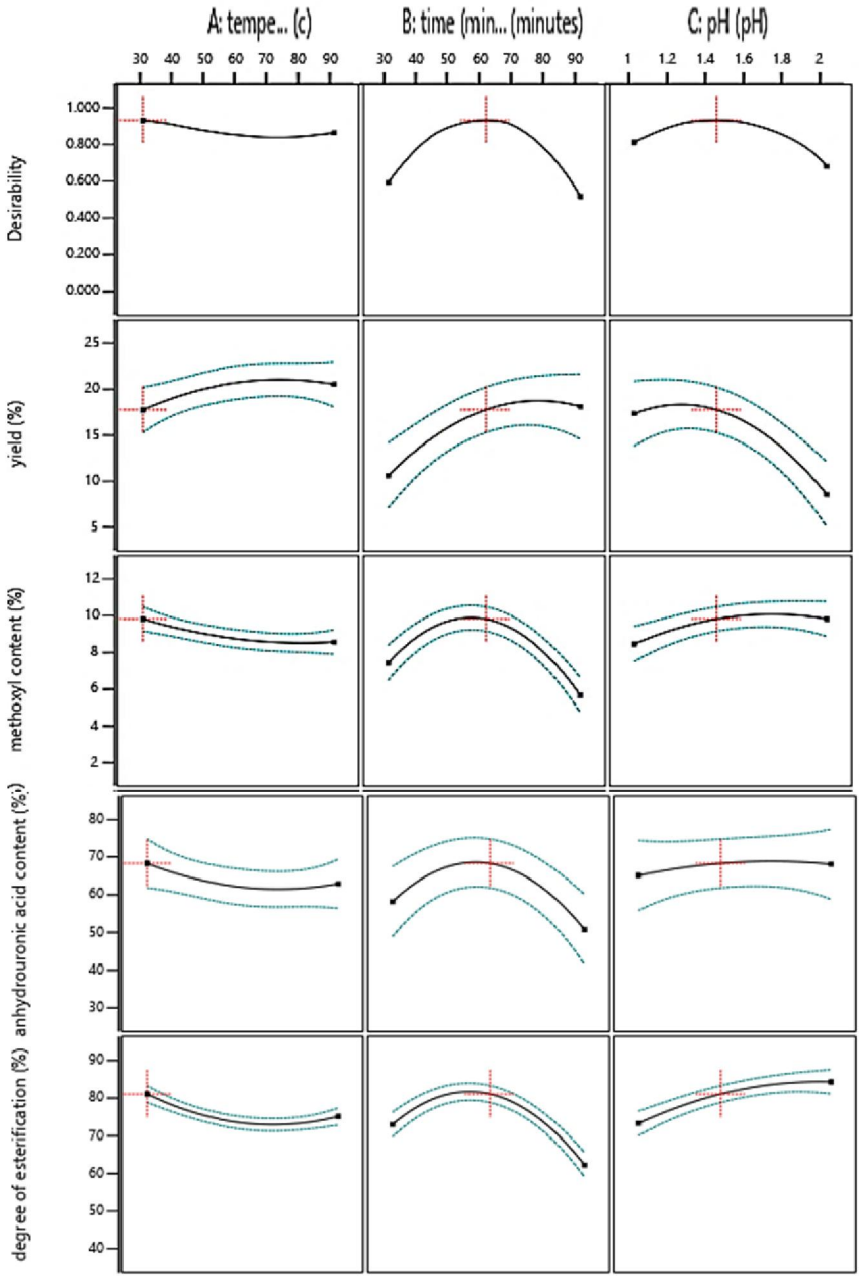

Figure 1: Effect of different factors on yield and physicochemical parameters of pectin from mosambi peel

The yield of pectin ranged from 5.21% to 13.50%, methoxyl content varied between 1.94% and 11.04%, anhydrouronic acid (AUA) content ranged from 42.00% to 75.24% and the degree of esterification (DE) spanned from 36.00% to

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83.33%.The maximum pectin yield (13.50%) was obtained at an extraction temperature of 60 °C, duration of 60 minutes and pH 1.5. In contrast, the lowest yield (5.21%) was recorded at 30 °C, 60 minutes and pH 2.0. Methoxyl content, AUA and DE reached their highest values—11.04%, 75.24% and 83.33%, respectivelyunder extraction conditions of 60 °C, 90 minutes and pH 2.0. Conversely, the lowest methoxyl content (1.94%) and AUA (29.61%) were observed at 60 °C, 90 minutes and pH 1.0, while the minimum DE (36.00%) was obtained at 60 °C, 30 minutes and pH 1.0.These findings illustrate the significant influence of extraction parameters on the yield and physicochemical characteristics of pectin derived from pomace. Lower pH and longer extraction times may contribute to acid-catalyzed hydrolysis of methyl ester and glycosidic linkages, which can reduce methoxyl content, AUA and DE. Conversely, moderate acid strength (e.g., pH 1.5–2.0) and extended extraction durations appear to favor higher recovery of structurally intact pectin with enhanced functional properties.

The marked variability in responses highlights the need for optimization of extraction conditions tailored to specific quality attributes of pectin, particularly when derived from by-products such as pomace. These results also support the hypothesis that pomace, despite being a secondary residue can serve as a viable source of functional pectin when processed under controlled conditions.

Table 3: Anova table for yield and physico-chemical parameters of pectin from mosambi peel

Parameters	Sum of squares			
	Yield	Methoxyl content	AUA	DE
Model	392.31 (0.0010)*	61.22 (0.0001)*	1226.93 (0.0105)*	1268.45 (0.0001)*
Temperature (A)	15.54 (0.0568)	3.30 (0.0058)*	45.98 (0.1792)	98.42 (0.0004)*
Time (B)	0.7875 (0.6238)	3.52 (0.0049)*	74.85 (0.0986)	140.45 (0.0001)*
pH (C)	175.59 (0.0001)*	2.39 (0.0127)*	97.79 (0.660)	33.87 (0.0079)*
AB	40.26 (0.0080)*	0.0552 (0.6285)	0.0225 (0.9746)	1.95 (0.4079)
AC	0.3969 (0.7265)	0.0600 (0.6142)	17.31 (0.3904)	46.99 (0.0035)*
BC	12.6 (0.0786)	2.40 (0.0125)*	107.64 (0.0564)	46.31 (0.0036)*
A^2	10.830 (0.0989)	0.8909 (0.0817)	55.34 (0.1456)	93.46 (0.0005)*
B^2	46.94 (0.0055)*	45.37 (0.0001)*	820.82 (0.0004)*	777.49 (0.0001)*
C^2	76.10 (0.0015)*	2.61 (0.0103)*	16.21 (0.4050)	41.22 (0.0049)*
R^2	0.8841	0.9450	0.8946	0.9688
Std. Dev.	1.73	0.4646	4.54	1.58

* Significant

Data in the parenthesis represents the p-values

Table 4: Effect of different factors on yield and physicochemical properties of pectin extracted from mosambipomace

		Factors			Responses			
Std	**Run**	**Temp(°C)**	**Time (min)**	**pH**	**Yield (%)**	**MC(%)**	**AUA(%)**	**DE(%)**
16	1	60	60	1.5	13.50	6.72	56.24	67.85
4	2	90	90	1.5	11.56	4.42	48.50	51.85

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3	3	30	90	1.5	9.54	9.01	69.54	73.56
13	4	60	60	1.5	13.50	7.34	61.50	67.85
14	5	60	60	1.5	13.50	7.35	61.50	67.85
12	6	60	90	2.0	8.26	11.04	75.24	83.33
2	7	90	30	1.5	12.54	8.47	61.90	77.69
11	8	60	30	2.0	9.45	5.47	51.21	60.71
5	9	30	60	1.0	9.80	6.17	53.24	65.89
8	10	90	60	2.0	5.96	7.01	58.64	67.85
9	11	60	30	1.0	10.26	2.66	42.00	36.00
10	12	60	90	1.0	11.89	1.94	29.61	37.33
7	13	30	60	2.0	5.21	9.07	65.24	78.95
17	14	60	60	1.5	13.50	7.34	61.50	67.85
15	15	60	60	1.5	13.50	7.34	61.50	67.85
6	16	90	60	1.0	8.62	5.19	49.56	59.56
1	17	30	30	1.5	5.45	4.85	51.24	53.84

Regression analysis was carried out to fit the mathematical models to the experimental data. The quadratic model obtained from regression analysis for yield, methoxyl content, anhydrouronic acid content and degree of esterification of pectin in terms of un-coded levels of the variables was developed as follows:

Yield = +20.21+ 1.39 * A + 0.31* B - 4.69* C - 3.17* A * B - 0.32* A * C - 1.78 * B * C - 1.60 * A^2 - 3.34 * B^2 - 4.25 * C^2

Methoxyl content= +8.86 - 0.64 * A - 0.66 * B + 0.55 * C + 0.12 * A * B - 0.12 * A * C + 0.77 * B * C + 0.46 * A^2 - 3.28 * B^2 - 0.79 * C^2

AUA = +68.21 - 2.40 * A - 3.06 * B + 3.50 * C - 0.075 * A * B + 2.08 * A * C + 5.19 * B * C + 0.84 * A^2 - 16.75 * B^2 - 4.75 * C^2

DE =+73.80 - 3.51 * A - 4.19 * B + 2.06 * C + 0.70 * A * B -3.43 * A * C + 3.40 * B * C + 4.71 * A^2 - 13.59 * B^2 - 3.13

Effect of Process Variables on the Yield of Pectin

As illustrated in Figure 2, the yield of pectin extracted from mosambipomace usingnitric acid (HNO_3) initially increased with rising pH, reaching an optimum, after which it declined. Among the independent variables, pH exhibited the most significant influence on pectin yield, followed by temperature (Table 5). Extraction time did not demonstrate a statistically significant effect on yield in isolation. However, yield was significantly affected by quadratic interactions, particularly temperature × temperature and pH × pH ($p < 0.05$). These findings suggest that the acidity of the solvent is a key factor in disrupting the plant cell wall and solubilizing pectin, but excessively low or high pH levels may lead to degradation or reduced solubility, respectively.

Effect of Process Variables on Methoxyl Content

The methoxyl content of pectin showed a decreasing trend with increasing temperature and an increasing trend with rising pH. Among all the factors, pH exerted the greatest effect, followed by temperature, while time did not have a significant linear effect on methoxyl content (Table 5). Nevertheless, methoxyl content was significantly influenced by temperature × time and pH × pH interactions ($p < 0.05$). These trends can be attributed to the thermal degradation and demethylation of galacturonic acid chains at elevated temperatures, which reduces the degree of methylation. Conversely, a slightly higher pH may preserve esterified groups better during extraction, thereby increasing methoxyl content.

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Effect of Process Variables on Anhydrouronic Acid (AUA) Content

The AUA content, an indicator of pectin purity and structural integrity, increased with higher pH levels of the extraction solvent. Among all the variables studied, only pH had a significant direct effect on AUA content (Table 5). Temperature and time did not demonstrate statistically significant effects individually. However, significant interactions were observed between temperature × time, time × pH and pH × pH ($p < 0.05$). These results imply that the preservation of anhydrouronic acid residues in the extracted pectin is enhanced under moderate acidity and optimal combinations of temperature and time, which minimize degradation.

Effect of Process Variables on Degree of Esterification (DE)

The degree of esterification (DE) of pectin increased progressively with rising pH during extraction. Of the variables studied, only pH showed a statistically significant linear effect on DE ($p < 0.05$), whereas time and temperature did not significantly influence DE individually (Table 5). However, DE was affected by the temperature × time interaction, indicating that the combination of these factors can influence the preservation or hydrolysis of methyl esters during the extraction process. A higher DE suggests the pectin extracted under these conditions retained more of its esterified carboxyl groups, which is critical for its gel-forming ability in food and pharmaceutical applications.

Table 5: Anova table for yield and physico-chemical parameters of pectin from mosambipomace

Parameters	Sum of squares			
	Yield	MC	AUA	DE
Model	123.83 (0.0080)*	64.04 (0.0001)*	1672.59 (0.0069)*	2279.41 (0.0458)*
Temperature (A)	9.42(0.0610)	2.01(0.0121)*	53.35(0.1822)	29.22 (0.5286)
Time (B)	1.58(0.3920)	0.0578(0.5868)	34.20(0.2744)	39.74(0.4648)
pH (C)	17.08(0.0198)*	15.10(0.0001)*	720.48(0.0010)*	1059.38(0.0052)*
AB	6.43(0.1080)	16.85(0.0001)*	251.22(0.0148)*	518.93(0.0268)*
AC	0.9312(0.5058)	0.2916(0.2416)	2.13(0.7758)	5.69(0.7784)
BC	1.99(0.3396)	0.1056(0.4665)	331.60(0.0077)*	113.32(0.2330)
A^2	41.71(0.0022)*	6.38(0.0006)*	21.33(0.3802)	107.49(0.2442)
B^2	1.42(0.4158)	13.06(0.0001)*	101.26(0.0807)	316.32(0.0655)
C^2	36.77(0.0031)*	10.63(0.0001)*	153.05(0.0405)*	98.63(0.2627)
R^2	0.7790	0.9563	0.7888	0.6124
Std. Dev.	1.38	0.4221	4.93	8.15

*Indicate significant effect

Data in the parenthesis represents the p-values

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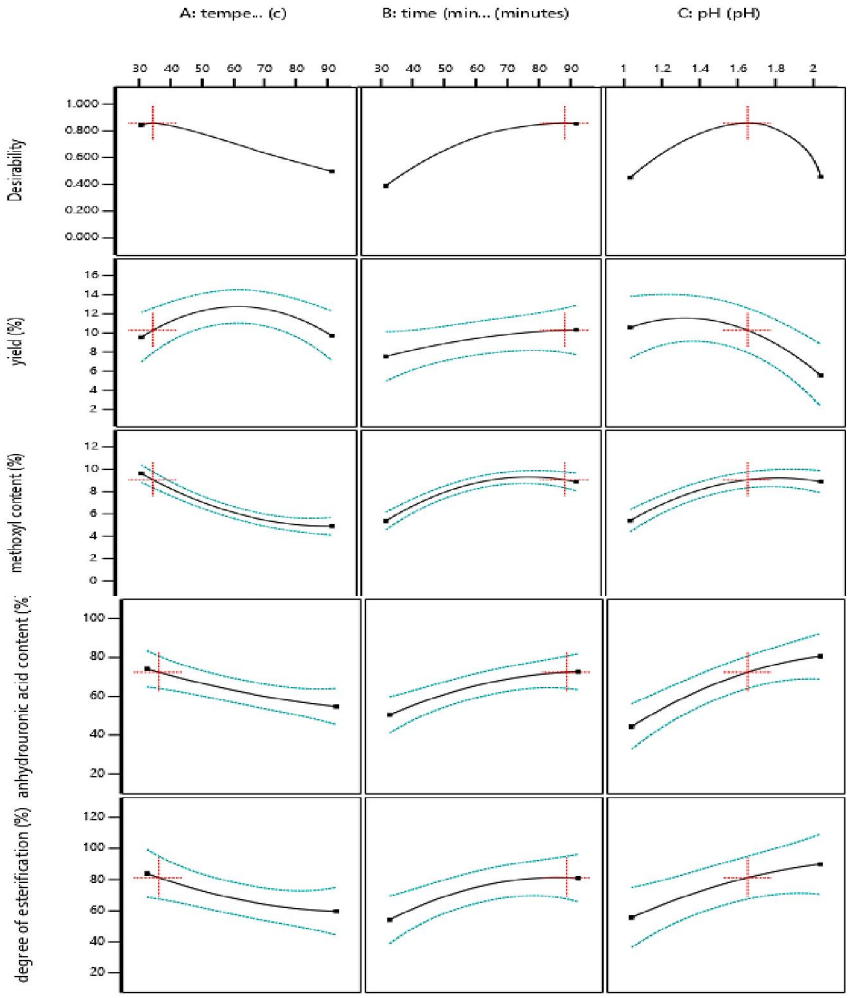

Figure 2: Effect of different factors on yield and physicochemical parameters of pectin from mosambipomace

IV. OPTIMIZATION OF OBTAINED RESULTS

Optimization of Pectin Extraction Conditions from Mosambi Peel

The optimal conditions for pectin extraction from mosambi peel using nitric acid (HNO_3) were determined through response surface methodology and canonical analysis of the response surfaces. The computer-generated optimum process parameters were 78.64 °C temperature, 59.12 minutes extraction time and pH 1.32 of the acidified solution. Under these conditions, the predicted values for the pectin characteristics were: yield of 21.70%, methoxyl content of 8.38%, anhydrouronic acid (AUA) content of 68.40% and degree of esterification (DE) of 73.18%.Experimental validation under these optimized conditions yielded actual values of 20.87% for pectin yield, 8.27% for methoxyl content, 67.47% for AUA and 74.00% for DE, which were in close agreement with the predicted values. The observed deviations were minimal, with differences of 0.83% in yield, 0.11% in methoxyl content, 0.93% in AUA and 0.82% in DE, indicating the robustness and accuracy of the optimization model (Table 6).Comparable findings were reported by Masmoudi *et al.,* (2008), who studied the influence of extraction time, temperature and pH on the yield of pectin from dried lemon peel. Their optimized conditions, extraction time of 3 hours 34 minutes, temperature of 84.34 °C and pH 2.8resulted in a pectin yield of 11.21%.

Table 6: Optimization for process variables for extraction of pectin from Peel and pomace:

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	Independent process variables			Responses			
	Temp (°C)	Time (min)	pH	Yield (%)	MC (%)	AUA(%)	DE(%)
Peel							
Optimized	78.64	59.12	1.32	21.70	8.38	68.40	73.18
Actual	78.64	59.12	1.32	20.87	8.27	67.47	74.00
Variation(%)	-	-	-	0.83	0.11	0.93	0.82
Pomace							
Optimized	56.87	68.45	1.58	13.11	7.25	62.97	70.37
Actual	56.87	68.45	1.58	13.50	7.67	61.46	71.00
Variation (%)	-	-	-	0.39	0.42	1.51	0.63

Optimization of Pectin Extraction from MosambiPomace and Functional Properties

The optimal extraction conditions for pectin from mosambipomace using nitric acid (HNO_3) were determined through response surface analysis. The optimized parameters were 56.87 °C temperature, 68.45 minutes extraction time and pH 1.58, yielding pectin with a yield of 13.11%, methoxyl content of 7.25%, anhydrouronic acid (AUA) content of 62.97% and degree of esterification (DE) of 70.37%. The experimentally obtained values were closely aligned with the predicted outcomes, with minor deviations: 0.39% in yield, 0.42% in methoxyl content, 1.51% in AUA and 0.63% in DE (Table 6), confirming the reliability and accuracy of the optimization model.Sharma *et al.,* (2013) conducted a similar study on pectin extraction from kinnow peel and pomace. Their results indicated that the maximum yield was achieved from kinnow peel (16.1%) compared to pomace (6.2%) under extraction conditions of 60 °C, pH 1.75 and 70 minutes of extraction time.

Functional Properties of Optimized Pectin Extracts

The functional properties of the pectin extracted under optimized conditions were assessed in terms of water holding capacity (WHC) and oil holding capacity (OHC) (Table 7). Pectin extracted from mosambi peel exhibited superior WHC and OHC compared to that from pomace, indicating better functional performance.Rubio-Senent*et al.,* (2015) examined similar functional properties of pectin derived from olive mill wastewater and reported low WHC, high OHC and emulsifying properties comparable to those of commercial pectin. Their findings suggest that such pectin may be suitable for use as an emulsifier in various food industry applications.

Table 7: Functional properties of pectin extracted under optimized conditions

	Peel	Pomace
WHC	5.16±0.3	3.74±0.1
OHC	2.40±0.2	1.72±0.2

Values are mean of three observations ± S.D

FT-IR Spectroscopic Analysis of Extracted Pectin

Fourier-transform infrared (FT-IR) spectroscopy was employed to characterize the chemical structure of pectin extracted from *mosambi* peel and pomace. The FT-IR spectra of the pectin powders derived from both substrates are presented in Figure 3. The spectra revealed characteristic absorption bands, indicating the presence of functional groups commonly associated with pectic substances.A broad and intense absorption band was observed at 3447 cm^{-1} (peel) and 3448 cm^{-1} (pomace), which can be attributed to the O–H stretching vibrations resulting from intramolecular and intermolecular hydrogen bonding among the hydroxyl groups of galacturonic acid residues. These interactions are indicative of the polymeric nature and hydrogen bonding capacity of pectin.Bands detected at 2966 cm^{-1} (peel) and 2925 cm^{-1} (pomace) correspond to C–H stretching vibrations, which are likely associated with methylene groups within the polymer backbone and methyl groups of methyl esters. These findings align with the results of Nisar *et al.,* (2018), who identified similar spectral features in pectin derived from citrus sources.

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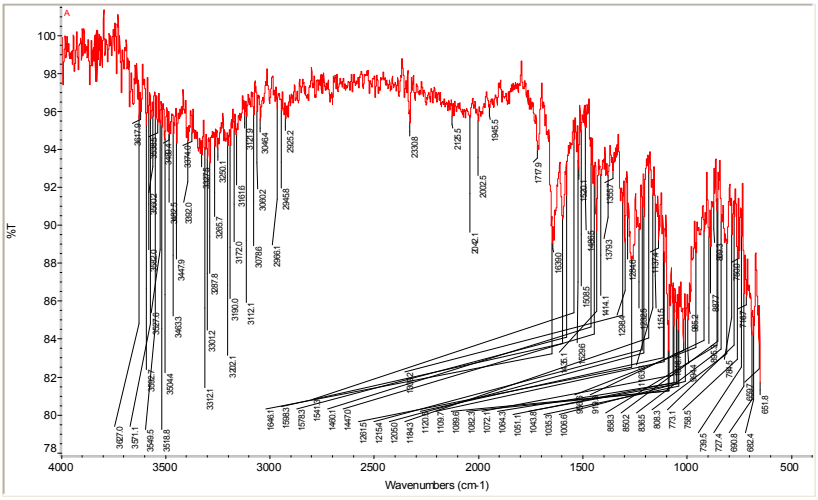
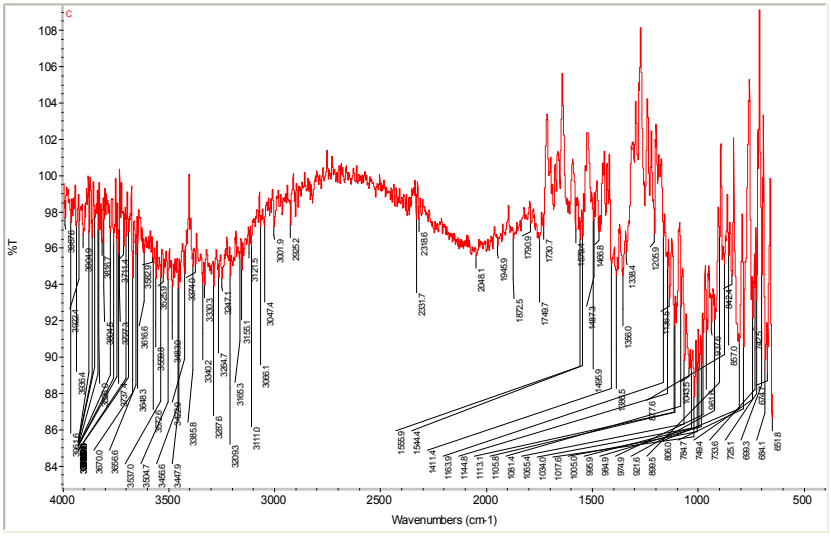
Further peaks appearing at 1638 cm^{-1} and 1525 cm^{-1}, along with 1379 cm^{-1} (peel) and 1386 cm^{-1} (pomace), represent the asymmetric and symmetric stretching vibrations of carboxylate groups, respectively, indicating the presence of free and esterified galacturonic acid units in the pectin matrix (Oliveira *et al.,* 2016). These bands are critical indicators of the degree of esterification and structural integrity of the extracted pectin.Additionally, absorption bands at 1120 cm^{-1} and 1113 cm^{-1}, along with 919 cm^{-1} and 937 cm^{-1} are assigned to C–O–C stretching vibrations within the polysaccharide backbone. These bands reflect the glycosidic linkages and confirm the presence of the polymeric pectin chain structure, further supporting the successful extraction of structurally intact pectin (Nisar *et al.,* 2018).



(a)



(b)

Figure 3: FT-IR spectra of pectin extracted from (a) peel and (b) pomace

V. CONCLUSION

The present study effectively optimized the extraction of pectin from *mosambi* peel and pomace using response surface methodology. The findings revealed that the pH of the extraction solvent significantly influenced the yield of pectin from both peel and pomace. Moreover, the pectin extracted from *mosambi* peel exhibited superior yield and enhanced physico-chemical properties compared to that from pomace. These results indicate that *mosambi* peel serves as a

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promising and sustainable source for the extraction of high-methoxyl pectin, offering potential for large-scale commercial production.

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