

EXTRACTION, CHARACTERIZATION, AND COMPARATIVE EVALUATION OF CITRUS PEEL PECTIN FOR BIOTECHNOLOGICAL APPLICATIONS

By

Amena Alam Meem

1000055691

A thesis submitted to the Department of Biotechnology in partial fulfillment
of the requirements for the degree of
Master's of Science in Biotechnology

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Declaration

It is hereby declared that

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Amena Alam Meem

1000055691

Approval

The thesis titled “**EXTRACTION, CHARACTERIZATION, AND COMPARATIVE EVALUATION OF CITRUS PEEL PECTIN FOR BIOTECHNOLOGICAL APPLICATIONS**” submitted by Amena Alam Meem (1000055691) of Summer, 2025 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Master of Science in Biotechnology on 3rd September, 2026.

Examining Committee:

Supervisor: (Member)

Dr. Munima Haque
Associate Professor,
Department of Biotechnology Brac
University

Co-supervisor:

(External)

Dr. Kanika Mitra
Principal Scientific Officer
Institute of Food Science and Technology
Bangladesh Council of Scientific and Industrial Research

External Expert Examiner:
(Member)

Dr. Md. Aminul Haque
Associate Professor
School of Pharmacy
BRAC University

Departmental Head:
(Chair)

Dr. Iftekhar Bin Naser
Associate Professor,
Department of Biotechnology
Brac University

Ethics Statement

This study did not involve human participants, animals, or clinical specimens. The research was conducted using citrus peel waste (lemon and orange peels) collected from local street vendors for laboratory analysis. All experimental procedures were carried out in accordance with the standard laboratory safety guidelines and institutional regulations. Therefore, ethical approval was not required for this study.

Abstract

Pectin is a natural polysaccharide widely used in the food industry as a gelling, thickening, and stabilizing agent. Citrus peels are abundant agro-industrial by-products and represent an economical and sustainable source of pectin. This study aimed to extract pectin from lemon and orange peels using the acid hydrolysis method and characterize the extracted pectin by comparing its physicochemical and structural properties with commercial pectin samples. Pectin extraction was carried out using hydrochloric acid (HCl) and sulfuric acid (H₂SO₄) under different extraction conditions, including pH, extraction time, and temperature. The extracted pectin was purified by ethanol precipitation, dried, and subjected to physicochemical and Fourier Transform Infrared (FT-IR) spectroscopic analyses. The evaluated physicochemical parameters included pectin yield, equivalent weight, methoxyl content, anhydrouronic acid (AUA), degree of esterification (DE), ash content, alkalinity of ash, carbonate-free ash, moisture content, and color. FT-IR spectroscopy was used to identify the characteristic functional groups of the extracted and commercial pectin samples and to assess their structural similarities. The results showed that extraction conditions significantly influenced pectin yield and quality. Sulfuric acid produced a higher pectin yield and a more desirable yellowish-brown color than hydrochloric acid, which resulted in blackish-colored pectin. The highest pectin yield obtained in this study was 25% under optimized extraction conditions. Orange peel pectin exhibited the highest methoxyl content (5.52%) and degree of esterification (73.1%), while lemon peel pectin showed the highest equivalent weight (156.09 g/mol). The moisture contents of all samples ranged from 1.46% to 4.02%, indicating good storage stability. FT-IR spectra confirmed the characteristic functional groups associated with pectin, including hydroxyl (O–H), aliphatic C–H, esterified carbonyl (C=O), carboxylate (COO[−]), and glycosidic (C–O–C) groups, supporting the presence of pectin in the extracted samples. Overall, the findings demonstrate that citrus peels are promising raw materials for pectin production and that extraction conditions strongly influence the physicochemical and structural characteristics of the extracted pectin. This study highlights the potential of citrus peel waste as a sustainable and value-added resource for producing natural pectin suitable for food science and technology applications. Further optimization of extraction and purification conditions is recommended to improve pectin quality and expand its industrial applications.

Keywords: Pectin, Citrus peel, Acid hydrolysis, Physicochemical characterization, Waste valorization.

Dedication

I dedicate this work solely to my respected mother, Asma Alam

Acknowledgement

This dissertation holds a special place in my heart, as I have carefully and diligently chosen this topic of research. It may seem like a small step, but for me, it represents a significant leap towards fulfilling my passion for research. Throughout this journey, I faced numerous challenges and setbacks, and I am immensely grateful for the support and encouragement I received during those difficult times.

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List of Acronyms

MeC = Methoxyl Content

EW = Equivalent Weight

AUA = Anhydrouronic Acid

DE = Degree of Esterification

SD = Standard Deviation

R= Replication

UK = United Kingdom

Chapter 1

Introduction

1.1 Background

Pectin is a naturally occurring, complex heteropolysaccharide found in the primary cell wall and middle lamella of higher plants. It plays an important structural role by maintaining cell wall integrity and regulating plant tissue firmness. Chemically, pectin mainly consists of α -(1→4)-linked D-galacturonic acid units, with varying proportions of neutral sugars and methyl ester groups. The degree of methyl esterification influences important functional properties of pectin, including solubility, viscosity, gel formation, and interactions with other food components (Thakur *et al.*, 1997).

Due to its unique physicochemical and functional properties, pectin is one of the most important natural hydrocolloids used in the food industry. It is widely utilized as a gelling agent, thickener, stabilizer, emulsifier, and texture modifier in products such as jams, jellies, marmalades, fruit beverages, confectionery products, dairy formulations, sauces, and edible coatings. In addition to food applications, pectin has gained increasing importance in pharmaceutical, biomedical, and cosmetic industries because of its biodegradability, biocompatibility, non-toxicity, antioxidant activity, and ability to form controlled-release matrices for bioactive compounds and drugs (May, 1990; Munarin *et al.*, 2012).

The global demand for pectin has increased due to growing consumer preference for natural, plant-derived, and clean-label food ingredients. Commercial pectin production mainly relies on citrus peels and apple pomace because these materials contain high concentrations of protopectin, which can be converted into soluble pectin through extraction processes. Citrus peel is considered one of the most valuable industrial sources of pectin because of its high pectin content, availability, and favorable functional properties (Ciriminna *et al.*, 2018). However, commercial pectin production is concentrated in a limited number of countries and imported pectin products may be expensive for developing countries. Therefore, exploring locally available agricultural waste as an alternative pectin source is important for sustainable and economical pectin production.

Bangladesh is an agriculture-based country where large quantities of fruit waste are generated from households, restaurants, juice industries, and food-processing sectors. Citrus fruits, particularly lemon (*Citrus limon*) and orange (*Citrus sinensis*), are widely consumed throughout Bangladesh. Lemon is extensively used in households, restaurants, hotels, juice shops, beverages, salad preparation, and food processing. Consequently, considerable quantities of lemon peel waste are generated and commonly discarded. Although considered waste, citrus peels contain valuable compounds such as pectin, flavonoids, carotenoids, essential oils, and phenolic compounds. Improper disposal of citrus waste contributes to environmental pollution and increases waste-management problems. Therefore, converting citrus peel waste into valuable pectin can contribute to waste reduction, environmental sustainability, and the development of value-added natural ingredients.

Citrus peels are recognized as rich sources of pectin because they contain substantial amounts of protopectin, an insoluble precursor located within plant cell walls. Acid-assisted extraction promotes the hydrolysis of protopectin into soluble pectin by disrupting plant cell-wall structures. Previous studies have successfully extracted pectin from different citrus sources, including lemon, orange, grapefruit, lime, and pomelo peels using conventional acid extraction and advanced extraction techniques (Masmoudi *et al.*, 2008; Methacanon *et al.*, 2014). However, pectin yield and quality are influenced by several extraction factors, including acid type, pH, extraction temperature, extraction time, and purification procedures.

Lemon peel was selected in this study because of its high availability, continuous consumption, and easy accessibility in Bangladesh. Unlike some agricultural wastes that are available only during specific seasons, lemon peel waste is generated throughout the year because of its extensive domestic and commercial use. Previous studies have demonstrated that lemon peel contains considerable amounts of extractable pectin with desirable functional properties (Masmoudi *et al.*, 2008).

Orange peel was selected because orange is one of the most widely consumed citrus fruits globally and is an important commercial source of citrus pectin. Comparing pectin extracted from lemon

and orange peels provides valuable information regarding differences in extraction yield, physicochemical characteristics, and structural properties between two commonly available citrus wastes. The quality and industrial application potential of pectin depend on its physicochemical and structural characteristics. Therefore, comprehensive characterization is essential for evaluating extracted pectin. Important parameters include pectin yield, equivalent weight, methoxyl content, anhydrouronic acid (AUA) content, degree of esterification (DE), ash content, alkalinity of ash, carbonate-free ash, moisture content, and color characteristics. Equivalent weight indicates the amount of free carboxyl groups present in pectin, while methoxyl content and degree of esterification influence its gel-forming behavior and classification as high-methoxyl or low-methoxyl pectin. Anhydrouronic acid content is used as an indicator of pectin purity because galacturonic acid forms the main structural backbone of pectin molecules (Ranganna, 1986). Ash-related properties provide information regarding mineral residues and extraction purity, while moisture content is important for evaluating storage stability.

In addition to physicochemical characterization, Fourier-transform infrared spectroscopy (FT-IR) will be used to identify the characteristic functional groups and confirm the structural features of the extracted pectin. FT-IR analysis can identify characteristic absorption bands associated with hydroxyl ($-OH$), carbonyl ($C=O$), esterified carboxyl, and glycosidic linkages in pectin. Comparison of FT-IR spectra between lemon peel pectin, orange peel pectin, and commercial citrus pectin will provide information on their functional-group profiles and structural similarities or differences. Thus, FT-IR analysis will complement the physicochemical characterization and help assess the structural quality of the extracted pectin.

Although numerous studies have investigated citrus pectin extraction, several research gaps remain. Most previous studies have focused on extraction optimization or evaluation of only selected physicochemical properties. Limited studies have performed comprehensive characterization of citrus peel pectin by considering multiple quality parameters together with structural analysis. Furthermore, studies comparing naturally extracted citrus pectin with commercial pectin products are limited, particularly in Bangladesh.

In this study, two commercial citrus pectin samples, namely UK Citrus pectin and Chinese pectin, will be selected as reference standards. These commercial pectin samples represent internationally available food-grade pectin products and will allow comparison between industrially produced pectin and naturally extracted citrus peel pectin. Comparing UK and Chinese commercial pectin with lemon and orange peel pectin will provide valuable information regarding extraction yield, purity, physicochemical characteristics, functional groups, structural properties, and potential industrial applications of locally sourced citrus pectin.

Therefore, the present study focuses on the extraction and comprehensive characterization of pectin from lemon and orange peels and comparison with commercial UK and Chinese citrus pectin using physicochemical and FT-IR analyses. The findings may contribute to the sustainable utilization of citrus peel waste, reduction of agro-industrial waste, and development of economical natural pectin sources with potential applications in food, pharmaceutical, cosmetic, and biotechnology industries.

1.2 Research Objective

1.2.1 General Objective

To extract pectin from lemon and orange peels and evaluate their physicochemical properties in comparison with commercially available UK citrus and Chinese pectin.

1.2.2 Specific Objectives

- ✓ To extract pectin from lemon and orange peel waste using acid-assisted extraction methods.
- ✓ To evaluate the effect of extraction parameters, including acid type, pH, extraction time, and temperature, on pectin yield.
- ✓ To compare the visual color characteristics of naturally extracted citrus pectin and commercial pectin samples.
- ✓ To determine the physicochemical properties of extracted and commercial

pectin samples, including:

- Equivalent weight
 - Methoxyl content
 - Anhydrouronic acid (AUA) content
 - Degree of esterification (DE)
 - Ash content
 - Alkalinity of ash
 - Moisture content
 - FT-IR
-
- ✓ To compare the physicochemical and quality characteristics of pectin extracted from lemon and orange peels with those of commercial UK citrus and Chinese pectin.
 - ✓ To assess the potential of pectin extracted from citrus peel waste as a low-cost, value-added food ingredient for supporting Bangladesh's circular food economy and contributing to Sustainable Development Goal 2 (Zero Hunger).

Chapter 2

Literature Review

2.1 The Plant Sources

Common name: Lemon

Scientific Name: *Citrus limon*

Common name: Sweet orange

Scientific Name: *Citrus sinensis*

2.1.1 Taxonomy

Domain: Eukaryota

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Sapindales

Family: Rutaceae

Genus: *Citrus*

Species: *Citrus limon* (L.) Osbeck

Domain: Eukaryota Kingdom: Plantae

Phylum: Tracheophyta

Class:

Magnoliopsida

Order: Sapindales

Family: Rutaceae

Genus: *Citrus*

Species: *Citrus sinensis* (L.) Osbeck

2.2 Pectin

Pectin is a naturally occurring, complex macromolecular polysaccharide that is primarily found in the primary cell wall and middle lamella of higher plants, where it contributes to cell adhesion, rigidity, and structural integrity (Chen *et al.*, 2013). Pectin was first discovered in apple juice by Vauquelin in 1790 and was later named by Braconnot in 1825 from the Greek word *pektikos*, meaning “congealed” or “solidified,” referring to its ability to form gels (Chan *et al.*, 2017). Chemically, pectin is a heterogeneous polysaccharide mainly composed of galacturonic acid residues, with different structural domains that determine its physicochemical and functional properties. Four major structural regions of pectin have been identified: homogalacturonan (HG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), and xylogalacturonan (XG) (Caffall & Mohnen, 2009; Guillon *et al.*, 1989; Ralet *et al.*, 2001; Voragen *et al.*, 2009;

Yapo et al., 2007). Homogalacturonan (HG) is the major component of pectin, accounting for approximately 65% of the total pectin structure (Wormit & Usadel, 2018). It consists of linear chains of α -(1 $\rightarrow$ 4)-linked D-galacturonic acid (GalA) residues (Caffall & Mohnen, 2009). The carboxyl groups of GalA residues in HG can undergo methyl esterification at the C-6 position and may also be acetylated at the O-2 or O-3 positions, influencing the physicochemical characteristics of pectin (O'Neill et al., 1990). Rhamnogalacturonan I (RG-I) represents approximately 20–35% of pectin and consists of repeating units of rhamnose and galacturonic acid, with side chains containing neutral sugars such as arabinose and galactose (Li et al., 2012; Peng et al., 2016). These branched structures contribute to the functional diversity of pectin (Figure 1). In contrast, xylogalacturonan (XG) and rhamnogalacturonan II (RG-II) are minor components, representing less than 10% of the total pectin structure (Mohnen, 2008; Zandleven et al., 2007). RG-II is considered one of the most structurally complex regions of pectin, consisting of a homogalacturonan backbone with four distinct side chains containing rare sugars and complex branching patterns

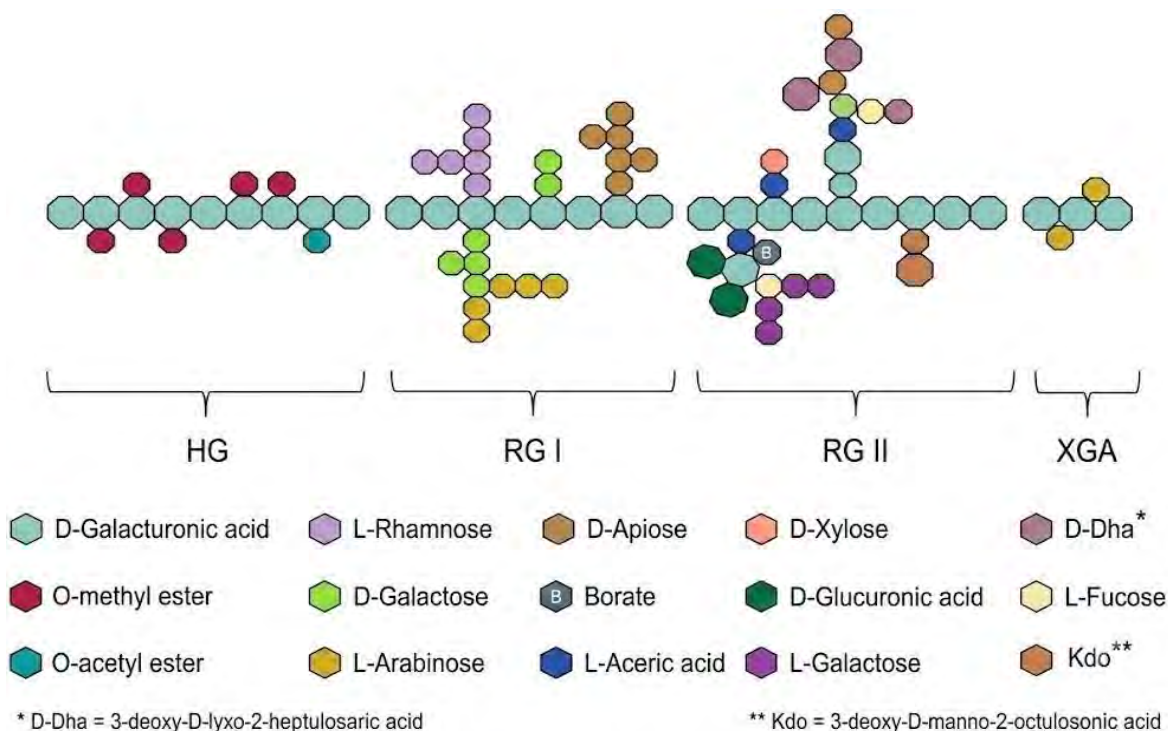


Figure 1. Chemical structure of the pectin molecule (Adapted from Freitas et al. 2021)

2.3 Characteristics of pectin

The degree of esterification (DE) is one of the most important structural characteristics of pectin. It represents the percentage of galacturonic acid residues esterified with methyl groups relative to the total galacturonic acid residues present in the polymer (Jiang *et al.*, 2005). Based on DE values, pectin is classified into two major categories: high-methoxyl pectin (HMP), with DE values greater than 50%, and low-methoxyl pectin (LMP), with DE values below 50% (Lekawska-Andrinopoulou *et al.*, 2013). The degree of esterification strongly influences pectin gel formation, molecular conformation, and rheological properties (Masmoudi *et al.*, 2012). High-methoxyl pectin forms gels under acidic conditions (pH 2.5–3.5) in the presence of high sugar concentrations (generally above 55%), where gel formation occurs through hydrophobic interactions and hydrogen bonding (Kastner *et al.*, 2014). In contrast, low-methoxyl pectin forms gels in the presence of divalent cations, particularly calcium ions, through ionic interactions between calcium and free carboxyl groups of galacturonic acid residues (Han *et al.*, 2017).

2.4 Sources of Pectin

Several extraction techniques have been developed to recover pectin from plant materials. Pectin is widely distributed in plant tissues and is particularly abundant in citrus peels, including lemon, orange, grapefruit, and pomelo peels (Table 1). Due to their high pectin content, citrus peel and apple pomace are the primary commercial sources of pectin production worldwide (Dranca & Oroian, 2018). However, several agricultural by-products have also been investigated as alternative pectin sources, including sugar beet pulp, banana peels, watermelon rinds, and pomegranate peels (Ciriminna *et al.*, 2016; Prakash Maran *et al.*, 2014; Pereira *et al.*, 2016). Among citrus-derived sources, orange, grapefruit, lemon, and lime peels have received significant attention because they are generated in large quantities as food-processing waste and contain considerable amounts of extractable pectin (Sharma *et al.*, 2017).

2.5 Extraction method of Pectin

Conventional acid extraction remains the most widely used industrial method due to its simplicity and efficiency. However, advanced extraction techniques, including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), enzyme-assisted extraction (EAE), and subcritical water extraction (SWE), have been investigated to improve extraction yield, reduce processing time, and minimize environmental impact (Dranca & Oroian, 2018).

The hot acid extraction method was selected in this study because it is a simple, cost-effective, and convenient technique that is suitable for developing countries like Bangladesh. Unlike advanced extraction methods that require sophisticated equipment and high operational costs, hot acid extraction can be performed using easily available laboratory facilities (Table 1). The table proves that citrus peels (especially lemon, orange, and pomelo) are among the richest sources of pectin and supports my research rationale for selecting *Citrus limon* and *Citrus sinensis*.

Table 1. Reported pectin yield from different plant sources using various extraction methods

Plant source	Scientific name	Pectin yield (%)	Extraction method	References
Sweet orange peel	<i>Citrus sinensis</i>	17.1	Hot acid extraction; pH 2, 0°C, 2 h	(Sarkar <i>et al.</i> , 2026)
Lemon peel	<i>Citrus limon</i>	43.0	HCl acid hydrolysis; RSM; 70°C, pH 1.25, 70 min	(Akachat <i>et al.</i> , 2025)
Lemon peel	<i>Citrus limon</i>	47.3	Intermittent microwave-assisted extraction; 900 W, 80 s, pH 1, pulse ratio 0.5	(Wani & Patidar 2025)

Banana peel	<i>Musa spp.</i>	13.06	Soxhlet extraction	(Athanasopoulou <i>et al.</i> , 2025)
Orange peel	<i>Citrus sinensis</i>	49.0–54.15	Citric acid extraction; 80°C, pH 1.0, 10 min	(Iñiguez-Moreno <i>et al.</i> , 2024)
Orange peel	<i>Citrus sinensis</i>	18.8	Citric acid extraction; Box–Behnken design; 100°C, 30 min, pH 2.48	(Iñiguez-Moreno <i>et al.</i> , 2024)
Lemon peel	<i>Citrus limon</i>	41.4	Microwave-assisted extraction; 450 W, 4 min, pH 1.0	(Pei <i>et al.</i> , 2024)
Lemon peel	<i>Citrus limon</i>	37.9	Ultrasound-assisted extraction; 60 min, pH 1.0, 55°C	(Pei <i>et al.</i> , 2024)
Colombo lemon peel	<i>Citrus limon</i>	15.20	Citric acid extraction; pH 2, 80°C, 60 min	(Rana <i>et al.</i> , 2024)
Pomelo peel	<i>Citrus grandis</i>	20.43	Microwave-assisted extraction	(Yu <i>et al.</i> , 2024)
Pomelo peel	<i>Citrus grandis</i>	11.94	Enzyme-assisted extraction	(Yu <i>et al.</i> , 2024)
Pomegranate peel	<i>Punica granatum</i>	31.89	Citric acid + ultrasound; pH 2.2, 89.87°C	(Podetti <i>et al.</i> , 2023)
Orange peel	<i>Citrus sinensis</i>	30.59	Ultrasound-assisted extraction	(Kumari <i>et al.</i> , 2023)
Jackfruit peel	<i>Artocarpus heterophyllus</i>	29.40	Radio-frequency-assisted extraction	(Naik <i>et al.</i> , 2020)
Jackfruit peel	<i>Artocarpus heterophyllus</i>	25.35 ± 0.21	Acid extraction	(Islam <i>et al.</i> , 2023)
Jackfruit core	<i>Artocarpus heterophyllus</i>	35.13 ± 0.49	Acid extraction	(Islam <i>et al.</i> , 2023)
Watermelon rind	<i>Citrullus lanatus</i>	16.01 ± 0.11	Microwave-assisted extraction; 500 W, 5 min	(Majid <i>et al.</i> , 2023)

Pineapple peel	<i>Ananas comosus</i>	2.43 ± 0.03	Microwave-assisted extraction optimization	(Zakaria <i>et al.</i> , 2023)
Papaya peel	<i>Carica papaya</i>	25.41	Microwave-assisted extraction; 512 W, pH 1.8, 140 s	(Maran & Prakash 2015)
Apple residue	<i>Malus domestica</i>	23.2–32.4	Acid extraction from fermented apple residue and apple pomace	(Muslu Can <i>et al.</i> , 2024)
Pomelo peel	<i>Citrus grandis</i>	39.72	Citric acid extraction; pH 1.8, 141 min, 88°C	(Liew <i>et al.</i> , 2018)
Pomelo peel	<i>Citrus grandis</i>	23.19	Conventional heating with HNO ₃	(Belkheiri <i>et al.</i> , 2021)
Pomelo peel	<i>Citrus grandis</i>	22.64	Microwave-supercritical CO ₂ extraction	(Yu <i>et al.</i> , 2011)
Lemon peel	<i>Citrus limon</i>	4.96–20.14	Acid hydrolysis followed by ethanol precipitation; citric acid, 80°C, pH 3, 60 min	(Belkheiri <i>et al.</i> , 2021)
Orange peel	<i>Citrus sinensis</i>	25.09	Microwave-assisted acid hydrolysis; 55°C	(Guntero & Ferretti 2022)
Orange peel	<i>Citrus sinensis</i>	9.92	Conventional acid hydrolysis	(Guntero & Ferretti, 2022)
Apple peel	<i>Malus domestica</i>	26.26	Ion-exchange chemical extraction	(Wang <i>et al.</i> 2013)
Apple pomace	<i>Malus domestica</i>	22.0	Citric acid extraction	(Mahmoud <i>et al.</i> , 2022)
Apple pomace	<i>Malus domestica</i>	14.0	Acid extraction; pH 1.5–3.0; 60–100°C	(Belkheiri <i>et al.</i> , 2021)
Apple pomace	<i>Malus domestica</i>	15.04	Conventional heating with HNO ₃	(Belkheiri <i>et al.</i> , 2021)

Jackfruit rind	<i>Artocarpus heterophyllus</i>	14.59	Conventional heating; distilled water, 90°C	(Belkheiri <i>et al.</i> , 2021)
Papaya peel	<i>Carica papaya</i>	16.0	Conventional heating with HCl	(Belkheiri <i>et al.</i> , 2021)
Papaya seed	<i>Carica papaya</i>	8.655	Hot-water extraction; 80°C, pH 1.5, 60 min	(Madhuvanthi <i>et al.</i> , 2022)
Carrot pomace	<i>Daucus carota</i>	15.6 ± 0.5	Acid extraction; pH 1.3, 90°C, 79.8 min	(Jafari <i>et al.</i> , 2017)
Carrot pomace	<i>Daucus carota</i>	15.2	Conventional heating extraction	(Belkheiri <i>et al.</i> , 2021)
Pomegranate peel	<i>Punica granatum</i>	8.5	Conventional heating with HNO ₃	Belkheiri <i>et al.</i> (2021)
Banana peel	<i>Musa spp.</i>	15.89–24.08	Citric acid extraction	(Khamsucharit <i>et al.</i> , 2018)
Pineapple peel	<i>Ananas comosus</i>	2.27	Microwave-assisted extraction	(Zakaria <i>et al.</i> , 2021)
Watermelon rind	<i>Citrullus lanatus</i>	12.0	Nitric acid extraction; 45 min, pH 1.65, S/L ratio 0.258 g/mL	(Belkheiri <i>et al.</i> , 2021)
Watermelon rind	<i>Citrullus lanatus</i>	20.0	Enzymatic extraction using Fibrilase and Multifect XL; 50°C, 15 h	(Belkheiri <i>et al.</i> , 2021)
Watermelon rind	<i>Citrullus lanatus</i>	19.1–19.6	Microwave-assisted extraction; 500 W, pH 1.5, 7 min	(Belkheiri <i>et al.</i> , 2021)
Watermelon	<i>Citrullus lanatus</i>	19.75	Microwave-assisted extraction; S/L 1:15, pH 2, 85°C, 60 min	(Belkheiri <i>et al.</i> , 2021)

2.6 Biological Activities of Pectin

Pectin possesses various biological activities, including antioxidant, antitumor, anti-inflammatory, cholesterol-lowering, blood glucose-regulating, and gastrointestinal health-promoting effects. Due to its biocompatibility, biodegradability, non-toxicity, and functional properties, purified pectin has applications in various industries, particularly food, pharmaceutical, and biomedical sectors. In the food industry, pectin is widely used as a gelling agent, thickener, stabilizer, and texturizing agent in products such as jams, jellies, marmalades, fruit preparations, beverages, desserts, and fermented dairy products (Rascon-Chu *et al.*, 2009). Beyond food applications, pectin has gained increasing attention as a biodegradable material for edible films, coatings, adhesives, paper substitutes, plasticizers, medical device coatings, biomedical implant materials, and drug delivery systems due to its excellent film-forming ability and biological compatibility (Mortensen *et al.*, 2017). Pectin and its derived oligosaccharides have demonstrated several important biological activities, including antimicrobial, anticancer, immunomodulatory, antioxidant, and anti-inflammatory effects (Figure. 2). Modified pectins, particularly those rich in rhamnogalacturonan regions and low-molecular-weight pectin fragments, have shown potential anticancer activity through mechanisms involving inhibition of tumor cell adhesion, induction of apoptosis, and modulation of immune responses (Wang *et al.*, 2017). Furthermore, pectin acts as a functional dietary fiber and prebiotic substrate because it is partially resistant to digestion in the upper gastrointestinal tract and can be fermented by beneficial intestinal microbiota, promoting the production of short-chain fatty acids and improving gut health (Liu *et al.*, 2020). Therefore, the structural complexity of pectin not only determines its physicochemical properties but also plays a crucial role in its nutritional, pharmaceutical, and biomedical applications (Figure 2).



Figure 2. Schematic diagram representing the bioactivities of pectin (Adapted from Chengaiyan *et al.* 2023)

2.5.1 Anticancer Activity

Pectin and modified pectin derivatives have demonstrated potential anticancer effects (Figure 2) through inhibition of cancer cell adhesion, suppression of tumor cell proliferation, and induction of apoptosis. Studies have shown that modified citrus pectin and pectic oligosaccharides can inhibit tumor progression by enhancing apoptosis and reducing metastatic potential. The anticancer activity of pectin is strongly associated with structural modifications, particularly reduced molecular weight and altered degree of esterification (Maxwell *et al.*, 2015; Wang *et al.*, 2017).

2.5.2 Anti-inflammatory Activity

Pectin possesses significant anti-inflammatory properties by regulating immune responses and reducing inflammatory mediator production. Low-methoxyl citrus pectin has been reported to suppress both local and systemic inflammation, while structural modifications such as de-esterification and deacetylation can enhance its ability to activate macrophages and regulate

immune functions (Popov *et al.*, 2017; Amorim *et al.*, 2019).

2.5.3 Antimicrobial Activity

Pectin and pectin-based materials have demonstrated antimicrobial activity against various pathogenic microorganisms, including *Staphylococcus aureus* and *Escherichia coli*. Modified pectin films and pectin-based nanocomposites have shown potential applications in active food packaging due to their biodegradability and ability to inhibit microbial growth (Liu *et al.*, 2019). Pectin-based biomaterials have also been explored for biomedical applications, including antimicrobial coatings.

2.5.4 Immunomodulatory Activity

The immunological activity of pectin depends on its structural composition, particularly the presence of galactan and arabinogalactan side chains in rhamnogalacturonan-I regions. Pectin-derived oligosaccharides can stimulate immune responses by enhancing macrophage activity (Figure 2), phagocytosis, and antibody production. The degree of methyl esterification and polymerization also influences its immunomodulatory effects (Yapo, 2009; Popov *et al.*, 2017).

2.5.5 Antioxidant Effect

Pectin exhibits antioxidant activity through free-radical scavenging and reducing activity, which may be influenced by its molecular weight, degree of branching, neutral sugar composition, and hydroxyl groups. Studies have demonstrated that pectin can effectively scavenge DPPH, ABTS, and hydroxyl radicals, while extraction and modification techniques such as ultrasound and enzymatic treatment may further enhance its antioxidant potential (Minzanova *et al.*, 2018; Liu *et al.*, 2022; Qiao *et al.*, 2024). Due to these properties, pectin has potential applications in functional foods, nutraceuticals, cosmetics, and biomedical products.

2.5.6 Blood Glucose- and Lipid-Lowering Effects

Pectin is a soluble dietary fiber that may contribute to the regulation of blood glucose and lipid levels. Experimental studies suggest that pectin can improve glucose tolerance, reduce blood glucose and insulin resistance, and decrease serum total cholesterol and triglycerides. Citrus pectin has also demonstrated potential antidiabetic effects through modulation of metabolic signaling pathways, including the PI3K/Akt pathway (Liu *et al.*, 2016; Guan *et al.*, 2021; Alves *et al.*, 2024). These properties support the potential use of pectin in functional foods and dietary interventions for metabolic health.

2.5.7 Wound-Healing Activity

Pectin has strong potential for wound management because of its hydrophilic, biocompatible, and gel-forming properties. It can absorb wound exudates, maintain a suitable moist environment, and serve as a carrier for bioactive compounds. Pectin-based hydrogels and composites have demonstrated enhanced cell proliferation, antioxidant activity, and accelerated wound closure, supporting their applications in wound dressings, skin protection, and tissue regeneration (Edirisinghe *et al.*, 2020; Alsakhawy *et al.*, 2022).

2.5.8 Gastrointestinal Health and Constipation

Pectin is a soluble dietary fiber that supports gastrointestinal health by forming a gel in the intestinal tract and promoting beneficial gut microbiota. Pectin-derived oligosaccharides may act as prebiotics, while experimental studies have reported improved intestinal motility and reduced constipation symptoms (Huang *et al.*, 2022; Li *et al.*, 2019). However, further clinical studies are required to establish its effectiveness in humans.

2.5.9 Cholesterol-Lowering Effect

Pectin may help reduce blood cholesterol by increasing the binding and fecal excretion of bile acids and cholesterol. Its hypocholesterolemic activity is influenced by properties such as

molecular weight, viscosity, degree of esterification, and gel-forming capacity (Naqash *et al.*, 2017; Brouns *et al.*, 2012). Therefore, pectin has potential as a functional dietary fiber for supporting cardiovascular and metabolic health.

2.6 Applications of Pectin

Pectin is increasingly used in the development of edible coatings and biodegradable packaging films due to its film-forming ability, renewability, and environmental sustainability. Pectin-based films can improve food shelf life by acting as barriers against moisture loss, oxidation, and microbial contamination (Figure 3). Incorporation of natural extracts, antioxidants, antimicrobial compounds into pectin films further enhances their protective properties and potential application in active food packaging (Eça *et al.*, 2014; Galus & Kadzińska, 2015). Also, pectin exhibits emulsifying and stabilizing properties that help maintain the stability of colloidal food systems, including beverages, protein-enriched drinks, and oil-in-water emulsions. These properties are mainly associated with the presence of charged carboxyl groups, neutral sugar side chains, and hydrophobic regions within the pectin structure, which enable interactions between pectin molecules and dispersed phases (Leroux *et al.*, 2003; Voragen *et al.*, 2009). Therefore, pectin remains an important natural hydrocolloid with broad applications in modern food processing and sustainable packaging technologies (Table 2).

2.6.1 Edible films and coatings

Pectin is widely used to develop edible films and coatings (Table 2) because of its film-forming, oxygen-barrier, and oil-barrier properties. Pectin-based films can help maintain food quality, extend shelf life, and provide antioxidant protection. Recent studies have demonstrated that pectin-based films and coatings can improve the physical and mechanical properties of packaging materials and preserve the quality of food products (Melo *et al.*, 2019; Chakravartula *et al.*, 2019; Diaz-Herrera *et al.*, 2023). Thus, pectin has considerable potential as a biodegradable and functional material for sustainable food packaging.

2.6.2 Pectin as Dietary Fiber and Prebiotic

Pectin is a soluble dietary fiber that contributes to gastrointestinal health by increasing intestinal viscosity, reducing cholesterol absorption, and promoting beneficial microbial fermentation (Figure 2). During colonic fermentation, pectin is degraded by gut microbiota to produce short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, which support intestinal health and metabolic regulation. Due to its prebiotic potential, pectin and pectin-derived oligosaccharides are increasingly used in functional foods and nutraceutical applications (Gómez *et al.*, 2016; Liu *et al.*, 2020).

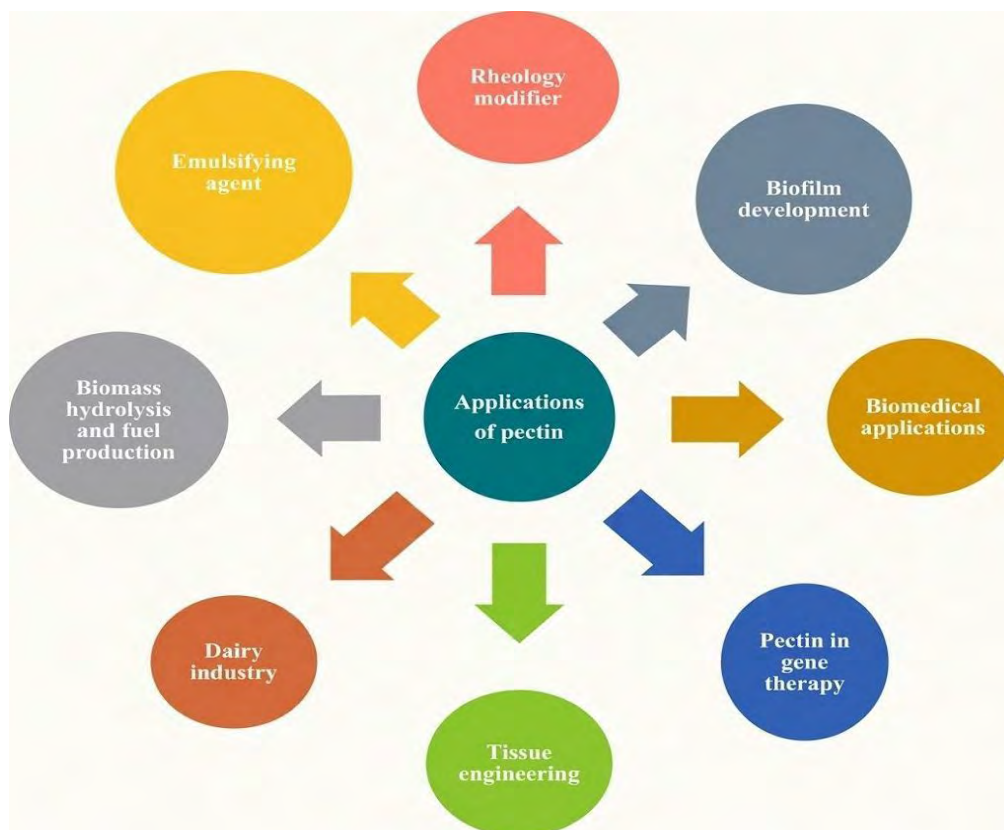


Figure 3. Applications of pectin in various fields (Adapted from Chengaiyan *et al.* 2023)

2.6.3 Application of Pectin in Biomedicine

Pectin is a promising biomaterial for biomedical and pharmaceutical applications because of its biocompatibility, biodegradability, and ability to form gels and controlled-release systems.

Pectin-based materials have been investigated for tissue engineering, wound dressing, drug and gene delivery, and targeted cancer therapy (Table 2). Its ability to encapsulate bioactive compounds and provide controlled or sustained release makes it particularly valuable for pharmaceutical delivery systems (Khotimchenko, 2020; Zaitseva *et al.*, 2020). Recent research also supports the use of pectin-based nanogels and hydrogels for improving the stability and controlled delivery of therapeutic compounds.

2.6.4 Biomass Hydrolysis and Biofuel Production

Pectin-rich agro-industrial wastes such as citrus peels, apple pomace, and sugar beet pulp can serve as renewable feedstocks for bioethanol production (figure 3). Pectin-degrading enzymes, particularly pectate lyases, facilitate the hydrolysis of pectin and improve the efficiency of subsequent saccharification and fermentation processes (Jayakody *et al.*, 2023; Wang *et al.*, 2023).

2.7.4 Drug Delivery and Cancer Therapy

Pectin is a promising pharmaceutical polymer because of its biocompatibility, mucoadhesive properties, and pH-responsive gelation. It can protect active compounds during gastrointestinal transit and facilitate controlled or colon-targeted drug release. Pectin-based nanoparticles and hydrogels have also been investigated for anticancer drug delivery and tumor targeting (Khotimchenko, 2020; Zaitseva *et al.*, 2020; Noreen *et al.*, 2017).

2.7.5 Pectin as a Rheology Modifier

Pectin can modify viscosity, yield stress, and flow behavior, making it useful in biomaterial formulation and 3D bioprinting (Figure 3). Pectin-containing hydrogels can form stable, self-supporting porous scaffolds suitable for biomedical and tissue-engineering applications (Coimbra *et al.*, 2011; Munarin *et al.*, 20).

Table 2. Application of pectin in different industries

Industry	Application	References
Food industry	Edible film	Melo et al. (2019); Shahrapour et al. (2020)
	Edible coating	Chakravartula et al. (2019); Nie et al. (2020)
	Food additive	Macku et al. (2008); Mortensen et al. (2017)
	Fat substitute	Mendez-Zamora et al. (2015); Zhang (2002); Zheng et al. (2023)
Biological medicine / Biomedical applications	Wound dressing	Sarioglu et al. (2019); Zhu et al. (2020)
	Tissue engineering	Coimbra et al. (2011); Munarin et al. (2011)
	Gene delivery vector	Bhatt et al. (2014); Katav et al. (2008)
	Drug delivery	Sabra et al. (2018); Smistad et al. (2012)
	Targeted tumor therapy	Cheewatanakornkool et al. (2017); Dutta & Sahu (2012)
Cosmetics	Emulsifier	Lupi et al. (2015); Noreen et al. (2017)
Chemical engineering	Water purification / adsorbent	Annadurai et al. (2003); Thakur et al. (2019)
Textile industry	Dye adsorbent	Kulal & Badalamoole (2020); Nsom et al. (2019)

2.7.4 Pectin as an Emulsifying Agent

Pectin can act as a natural emulsifier and stabilizer (Figure 3), with its emulsifying performance influenced by molecular weight, protein content, acetylation, ferulic acid content, and degree of esterification. It can stabilize oil–water emulsions and is therefore useful in reduced-fat mayonnaise, dairy products, ice cream, and meat products (Lupi *et al.*, 2015; Noreen *et al.*, 2017).

2.7.5 Pectin in Gene Therapy

Modified pectin can serve as a non-viral gene-delivery carrier because of its biocompatibility and ability to interact with nucleic acids. Pectin-based nanoparticles, particularly pectin–chitosan systems, have been investigated for DNA encapsulation, cellular delivery, and controlled release, offering potential for safer gene-transfer approaches (Bhatt *et al.*, 2014; Katav *et al.*, 2008).

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2.7.6 Pectin in Tissue Engineering

Pectin-based hydrogels and porous scaffolds provide a biocompatible matrix that can support cell attachment, proliferation, and tissue regeneration. Their ability to form three-dimensional structures makes pectin particularly promising for bone and other tissue-engineering applications (Coimbra *et al.*, 2011; Munarin *et al.*, 2011).

2.7.7 Pectin in the Dairy Industry

Pectin is widely used as a stabilizer and texture modifier in dairy products (Table 2). It interacts with casein and helps reduce syneresis, improve moisture retention, and stabilize acidified milk beverages, yogurt, dairy creams, and reduced-fat cheese. Low-methoxy pectin is particularly useful for improving the texture and stability of low-fat dairy products (Thakur *et al.*, 2019; Noreen *et al.*, 2017).

2.7.8 Pectin-Based Active Packaging for Food Preservation

Pectin-based films can provide antioxidant, antimicrobial, and gas-barrier functions. Their activity can be enhanced by incorporating agents such as essential oils, nisin, curcumin, or nanoparticles, improving protection against microbial contamination and oxidation (Huang *et al.*, 2020; Freitas *et al.*, 2021). Pectin coatings and films can reduce moisture loss, oxidation, enzymatic browning, and microbial spoilage, thereby maintain the quality and extend the shelf life of fresh fruits and vegetables. Pectin-based coatings containing natural extracts or essential oils have demonstrated improved preservation of products such as strawberries, apples, carrots, and other fresh-cut produce (Eça *et al.*, 2014; Huang *et al.*, 2020; Sharma *et al.*, 2021).

2.8 Importance of Citrus Peel Pectin

Citrus processing generates a considerable amount of waste, mainly in the form of peels, seeds, and pulp residues, which account for approximately 50% of the total fruit mass. Improper disposal of these wastes contributes to environmental pollution, increased waste management

costs, and loss of valuable bioresources. Citrus peel is considered one of the most important commercial sources of pectin because of its high pectin content, superior physicochemical properties, and consistent availability. Citrus-derived pectin is a complex heteropolysaccharide mainly composed of α -(1 $\rightarrow$ 4)-linked D-galacturonic acid units with varying degrees of methyl esterification and neutral sugar side chains, which determine its gelling, thickening, stabilizing, and emulsifying properties (Ciriminna *et al.*, 2016; Voragen *et al.*, 2009). Compared with pectin water-holding capacity, viscosity, and superior gel-forming ability, making it highly suitable for food and pharmaceutical applications (Sharma *et al.*, 2021).

In the food industry, citrus pectin is widely used as a natural hydrocolloid and is recognized as a safe (GRAS) food additive. Its excellent gelling and stabilizing properties make it an essential ingredient in products such as jams, jellies, marmalades, fruit beverages, yogurt, salad dressings, confectionery products, and ice cream. Citrus pectin can improve texture, prevent syneresis, enhance moisture retention, and increase the shelf life of processed foods. For example, orange peel pectin has been reported to stabilize casein networks in yogurt products, improving their texture and storage stability (Huang *et al.*, 2020).

Beyond food applications, citrus pectin possesses several biological and health-promoting properties. It functions as a soluble dietary fiber and prebiotic, promoting beneficial intestinal microbiota and increasing the production of short-chain fatty acids (SCFAs), which contribute to gastrointestinal health and immune regulation (Zhang *et al.*, 2017). Citrus pectin and its modified derivatives have also demonstrated antioxidant, anti-inflammatory, cholesterol-lowering, blood glucose-regulating, and anticancer activities, expanding its potential applications in nutraceutical and pharmaceutical industries (Sharma *et al.*, 2021; Liu *et al.*, 2020).

The recovery of pectin from citrus waste represents an important strategy for developing a circular bioeconomy by converting agricultural waste into high-value products. Valorization of citrus peel not only reduces environmental pollution associated with waste disposal but also provides an economical and sustainable source of natural pectin for industrial applications. Therefore, extraction and characterization of citrus peel pectin have significant importance for waste management, food innovation, and sustainable production of bio-based materials (Sharma

et al., 2020; Ciriminna *et al.*, 2016).

2.9 Importance of Citrus Peel in the Context of Bangladesh

Citrus fruits are among the most widely consumed fruits in Bangladesh and include lemon (*Citrus limon*), orange (*Citrus sinensis*), pomelo (*Citrus maxima*), satkora (*Citrus macroptera*), and adajamir (*Citrus assamensis*). These fruits are extensively used in households, restaurants, juice industries, beverage preparation, and traditional cuisines. However, a considerable amount of citrus peel, which constitutes approximately 25–50% of the total fruit weight, is generated as waste during domestic consumption and industrial processing (Sharma *et al.*, 2021; Rafiq *et al.*, 2018). In Bangladesh, citrus peel waste is commonly discarded with other municipal waste, leading to rapid decomposition, unpleasant odors, microbial growth, and environmental pollution due to improper waste management practices. Therefore, the conversion of citrus peel waste into valuable products represents an important approach for sustainable waste management and resource recovery. In Bangladesh, utilization of citrus peel for pectin extraction has significant economic and environmental potential. The country has a high demand for pectin in food processing industries, including jam, jelly, fruit beverage, confectionery, dairy products, and bakery industries. However, commercial pectin is mostly imported, increasing production costs for local food manufacturers. Developing a method to extract pectin from locally available citrus peel waste could reduce dependency on imported pectin and promote the production of affordable, natural food additives from indigenous resources.

Lemon (*Citrus limon*) peel is particularly promising as a pectin source in Bangladesh because lemon is consumed throughout the year and has widespread applications in households, restaurants, juice shops, hotels, and street food industries. The frequent use of lemon for beverages, salad preparation, seasoning, and culinary purposes results in continuous generation of peel waste. Unlike seasonal agricultural residues, lemon peel provides a relatively consistent and easily accessible raw material for pectin recovery. Similarly, orange (*Citrus sinensis*) peel is also generated in significant quantities due to increasing consumption of fresh oranges and processed orange beverages. Therefore, valorization of lemon and orange peel waste can provide a sustainable source of pectin while simultaneously reducing environmental burdens associated

with citrus waste disposal. Valorization of citrus peel into naturally extracted pectin can support Bangladesh's circular food economy by converting food-processing waste into a value-added food ingredient. This approach can reduce food waste, create opportunities for local agro-processing, and indirectly contribute to SDG 2 (Zero Hunger) through more sustainable and resource-efficient food systems. This is particularly relevant in Bangladesh, where an estimated 34% of food produced is lost or wasted, highlighting the need for food-waste valorization and value addition (CPD, 2025). Recent studies on Colombo lemon and BARI Malta-1 further demonstrate the feasibility of producing pectin from locally available citrus peels in Bangladesh (Rana *et al.*, 2024; Sarkar *et al.*, 2026).

Despite the abundance of citrus waste in Bangladesh, limited research has focused on the extraction, characterization, and industrial potential of citrus peel-derived pectin. Most commercial pectin available in Bangladesh is imported, while locally generated citrus peel waste remains underutilized. Therefore, investigating the extraction and physicochemical characterization of pectin from locally available lemon and orange peels may contribute to the development of sustainable bioprocessing technologies, waste valorization strategies, and cost-effective alternatives to commercial pectin.

2.10 Importance of Citrus Peel Pectin in the Asian Countries

Citrus fruits are widely cultivated and processed in Asian countries, generating substantial quantities of peel and other processing residues. Citrus peel is a valuable source of pectin, essential oils, flavonoids, carotenoids, cellulose, hemicellulose, and other bioactive compounds. The importance of citrus pectin is particularly relevant to Asian countries, where large citrus-producing and processing industries generate considerable quantities of peel residues. Countries such as China, India, Japan, Thailand, Vietnam, Indonesia, and Bangladesh have substantial citrus production and consumption, creating opportunities for recovering pectin locally rather than disposing of citrus residues. Citrus-peel valorization can provide additional economic value to agricultural and food-processing sectors while reducing the environmental problems associated with unmanaged organic waste. Recent research emphasizes that pectin recovery from food-

processing by-products can contribute to waste reduction, resource efficiency, and sustainable production systems (Sharma *et al.*, 2022; Xiang *et al.*, 2024).

Citrus pectin has important applications in the Asian food industry, particularly as a natural gelling, thickening, stabilizing, and emulsifying agent in products such as jams, jellies, beverages, dairy products, confectionery, and sauces. Citrus pectin is also being investigated for use in functional foods and nutraceutical products because of its dietary- fibre characteristics and reported biological activities. (Xiang *et al.*, 2024)

2.11 Importance of Citrus Peel in Global Context

The valorization of citrus peel waste into pectin and other value-added products has gained considerable attention due to the increasing demand for sustainable and environmentally friendly alternatives to petroleum-derived materials. Citrus peel-derived pectin possesses biodegradability, biocompatibility, non-toxicity, and antioxidant properties, making it suitable for applications in food preservation, pharmaceutical formulations, nutraceuticals, cosmetics, and biomedical materials (Canteri *et al.*, 2012). Furthermore, citrus pectin has demonstrated functional biological activities, including antioxidant, anti-inflammatory, cholesterol-lowering, immunomodulatory, and prebiotic effects, which enhance its potential in functional food and health-related applications (Zhang *et al.*, 2017; Sharma *et al.*, 2021).

The food packaging industry is one of the major areas where citrus peel-derived biopolymers have attracted increasing interest. Conventional petroleum-based plastic packaging contributes significantly to environmental pollution because of its poor biodegradability and accumulation of microplastics in ecosystems and food chains. Therefore, researchers are exploring renewable plant-based polymers, including citrus pectin, cellulose, and hemicellulose, for the development of biodegradable packaging films. Citrus peel pectin-based films exhibit good film-forming ability, mechanical strength, and potential for incorporation of antimicrobial and antioxidant compounds, making them promising alternatives for sustainable food packaging applications (Mohan *et al.*, 2020; Sharma *et al.*, 2021).

From an economic and environmental perspective, the utilization of citrus peel waste supports the principles of a circular bioeconomy by transforming agro-industrial waste into valuable products. Instead of disposal through landfilling or uncontrolled decomposition, citrus peel can be converted into high-value compounds such as pectin, essential oils, bioactive extracts, and biodegradable materials. This approach reduces environmental pollution, minimizes waste management costs, and creates additional revenue streams for the food-processing sector (Sharma *et al.*, 2021).

2.12 Research Gap

Despite extensive research on citrus peel pectin extraction worldwide, limited information is available regarding the utilization of locally generated citrus waste in Bangladesh as a potential source of high-quality pectin. This study aims to extract and characterize pectin from lemon (*Citrus limon*) and orange (*Citrus sinensis*) peels and compare their properties with commercial UK and Chinese pectin samples to evaluate their potential as sustainable alternatives for industrial applications.

2.12.1 Limited studies on locally available citrus waste resources in Bangladesh

Citrus peels are generated in large quantities in Bangladesh from household consumption, restaurants, juice shops, and food-processing industries. However, these residues are mostly discarded as waste rather than utilized as a valuable source of pectin. Although global studies have demonstrated the potential of citrus peel as a pectin source, limited research has focused on the extraction and utilization of pectin from locally available Bangladeshi citrus varieties, particularly lemon (*Citrus limon*) and orange (*Citrus sinensis*) peels.

2.12.2 Lack of comparative evaluation between locally extracted pectin and commercial pectin

Commercial pectin used in food and pharmaceutical industries is mainly imported and produced from citrus peels and apple pomace. However, limited studies have compared the quality and

functional properties of pectin extracted from locally available citrus waste with commercially available food-grade pectin. Comparative evaluation with commercial pectin from different origins (such as UK and Chinese pectin) is necessary to determine whether locally extracted pectin can meet industrial quality requirements.

2.12.3 Limited utilization of citrus peel waste within a circular bioeconomy framework

Although citrus waste contains valuable compounds such as pectin, flavonoids, phenolics, and essential oils, most citrus residues are still treated as waste globally. Further research is needed to promote the conversion of citrus peel waste into high-value products, supporting sustainable waste management, reducing environmental pollution, and contributing to circular bioeconomy approaches. Most previous studies have focused on pectin extraction from citrus peels under optimized laboratory conditions, whereas limited information is available regarding the quality and industrial suitability of pectin extracted from locally available citrus waste in Bangladesh. The novelty of this study lies in the first systematic comparative evaluation of pectin extracted from locally available lemon (*Citrus limon*) and orange (*Citrus sinensis*) peels in Bangladesh against commercial UK and Chinese pectin samples using comprehensive physicochemical characterization parameters. This comparison provides new insight into whether locally natural recovered citrus pectin from waste can achieve comparable physicochemical quality to commercially manufactured pectin.

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Raw Materials

The materials used for the study include fresh natural citrus peels of sweet orange (*Citrus sinensis*) & lemon (*Citrus limon*)

Commercial Pectin: Alfa Aesar, Thermo Fisher Scientific UK Citrus Pectin (*CAS number: 9000-69-5*) Chinese Pectin (*CAS number: 9000-69-5*)

3.1.2 Reagents

The following reagents were used during the extraction and physicochemical characterization of pectin:

- Hydrochloric acid (HCl)
- Sulfuric acid (H₂SO₄)
- Distilled water
- Ethanol
- Sodium hydroxide (NaOH)
- Phenolphthalein indicator
- Phenol red indicator
- Sodium Chloride (NaCl)

3.1.3 Apparatus

Laboratory instruments used for Pectin Extraction and Characterization:

- Digital weighing balance
- Air dryer
- Grinder
- Water bath
- pH meter
- Orbital shaker
- Hot air oven
- Magnetic stirrer
- Heating plate/heater
- Muffle furnace

- Centrifuge machine
- Filter paper
- Refrigerator
- Ice bath

3.1.4 Equipment

The following equipment was used for the extraction and physicochemical characterization of pectin:

- | | |
|----------------------|--|
| • Pestle and mortar | • Stoppered flask |
| • Polythene bags | • Conical flask (Erlenmeyer flask) |
| • Zipper bags | • Round-bottom flask with ground joint |
| • pH meter | • Measuring cylinders |
| • Centrifuge machine | • Steel tray |
| • Filter paper | • Bowl |
| • Orbital shaker | • Colored reagent bottles |
| • Hot air oven | • Airtight bottles |
| • Sieve | • Falcon tubes |
| • Forceps | • Droppers |
| • Burettes | • Beakers |
| • Muslin cloth | • Pipette |
| • Knife | |
| • Aluminum foil | |

3.2 Sampling site

A bulk amount of lemon peel was collected from street juice vendors and local coffee shops near the Dhanmondi and Shahbagh areas (Figure 4). Dhanmondi area is approximately located at 23.7461° N latitude and 90.3742° E longitude, while Shahbagh area is approximately located at 23.7389° N latitude and 90.3957° E longitude, both in Dhaka, Bangladesh. Then samples were transported to the Institute of Food Science and Technology (IFST) of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka.

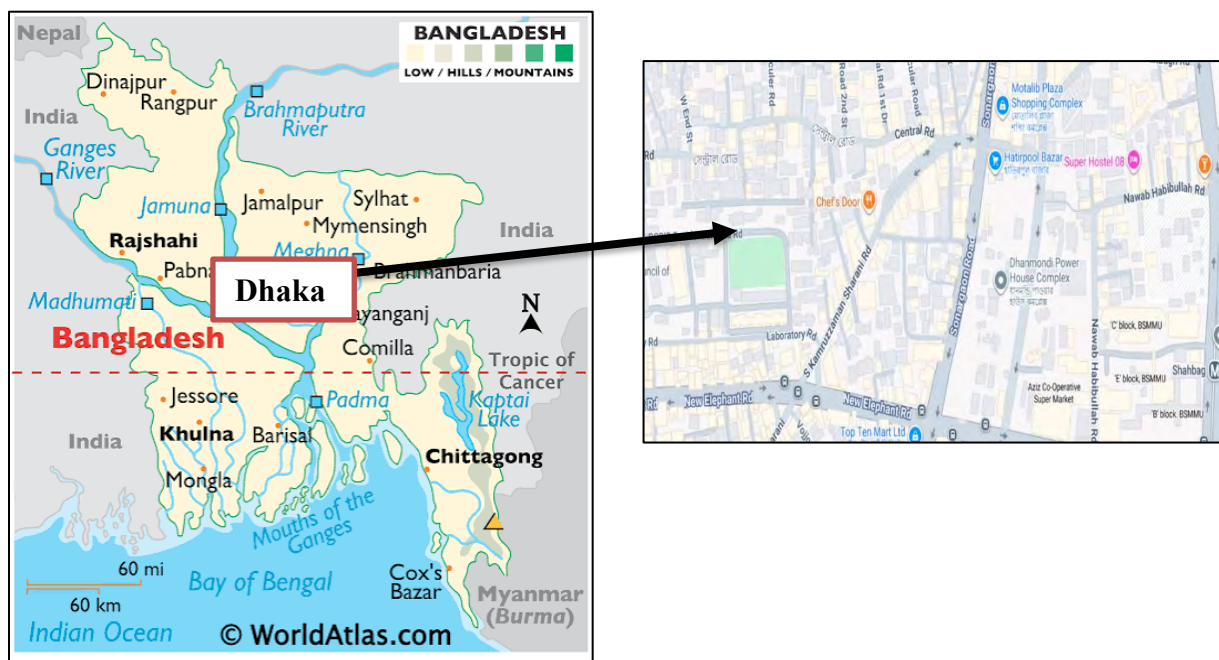


Figure 4. Map Showing Sample Collection Site

3.3 Methodology

The hot acid extraction method was selected in this study because it is a simple, cost-effective, no sophisticated machineries and convenient technique that is suitable for developing countries like Bangladesh.

3.3.1 Preparation of Raw Material

The lemon peels were washed, the albedo, which refers to the spongy white skin, was meticulously separated from the peels using forceps, and cut into pieces with a knife. Fresh citrus peels were thoroughly washed with distilled water to remove dirt and impurities. The total weight of the samples was recorded before drying. The pieces were then dried in a hot air oven at 80°C for 7 hours. After drying, they were ground into a fine powder using a grinder and sieved three times through a 400 μ m mesh to obtain a uniform fine size (Figure 5). The weight of the material lost during sieving was recorded, and the final weight of the prepared sample was measured. The sample was kept in an air-tight container for preservation before Acid Hydrolysis.

Initial Total Weight of Sample: 766.1 g

Bowl Weight: 94.7 g

Sample + Bowl weight: 860.8 g

After grinded sample weight= 87.1 g

After sieved sample weight= 77.6 g

un-sieved particle weight: 9.4 g



Figure 5. Preparation of citrus peel samples for pectin extraction: (a) collection of citrus peels from street vendors; (b) cutting the peels into small pieces; (c) air drying to remove surface moisture; (d) weighing the prepared peels; (e) drying in a hot air oven; (f) grinding and sieving through a 400 μm sieve; and (g) obtaining the citrus peel powder.

3.3.2 Extraction of pectin

A known weight (e.g., 10g) of dried citrus peel powder was mixed with distilled water in a suitable ratio (1:10 w/v). The pH of the mixture was adjusted to around 1.0–2.5 using Hydrochloric acid (HCl) and Sulfuric acid (H_2SO_4). The mixture was then heated at 70–80°C for 1–2 hours with continuous magnetic stirring to facilitate the extraction of pectin. After extraction, the hot mixture was filtered using muslin cloth followed by Whatman filter paper to remove solid residues. The filtrate containing dissolved pectin was collected (Figure 6).

3.3.3 Precipitation of Pectin

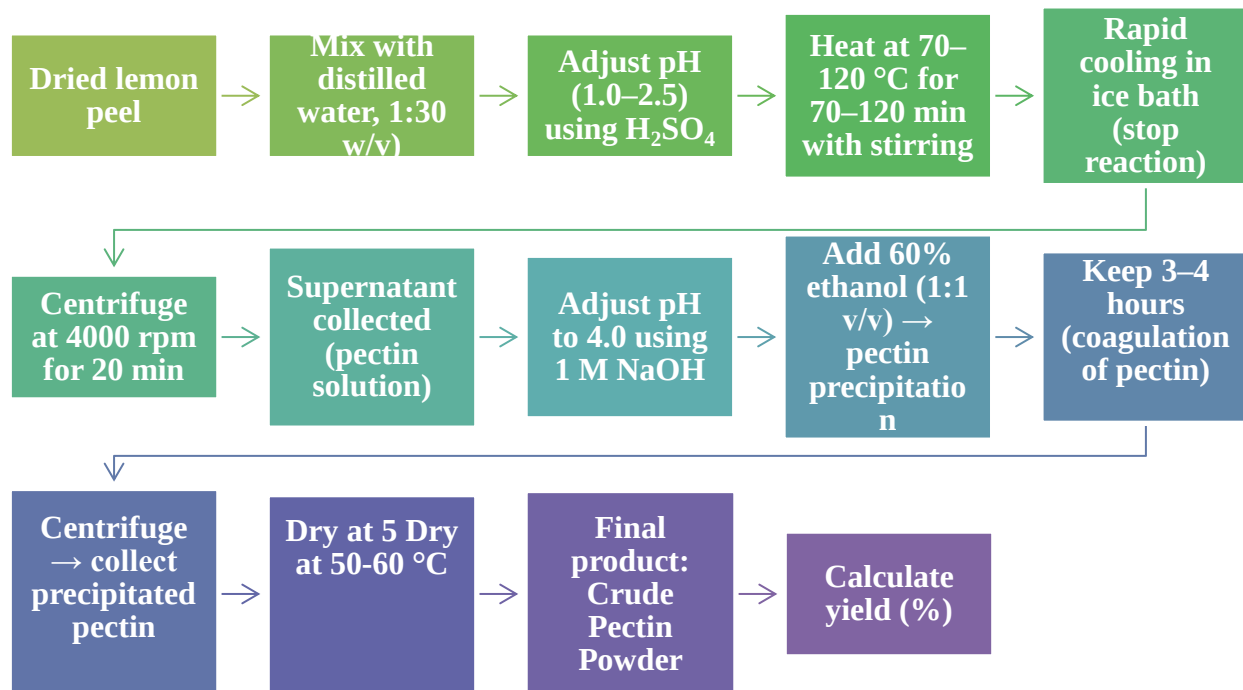
The extracted filtrate was cooled to room temperature, and an 60% volume of ethanol was added slowly with constant stirring. The mixture was left undisturbed for pectin precipitation. The gelatinous precipitate formed was separated by filtration. The collected pectin was washed several times with ethanol to remove impurities and then dried in a hot air oven at 50–60°C until a constant weight was obtained (Figure 6). The dried pectin was ground into powder and stored for analysis.

3.3.4 Determination of Yield

The yield of extracted pectin was calculated using the following formula:

$$\text{Yield (\%)} = (\text{Weight of dried pectin} / \text{Weight of dried peel sample}) \times 100$$

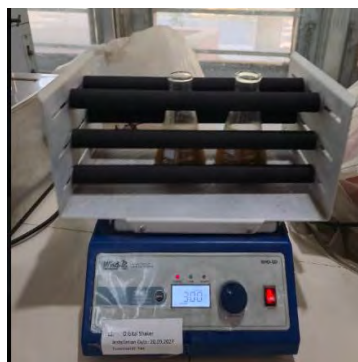
The flow diagram of pectin extraction by the acid hydrolysis method is given below-



Flowchart 1. Flow diagram of pectin extraction by the acid hydrolysis method



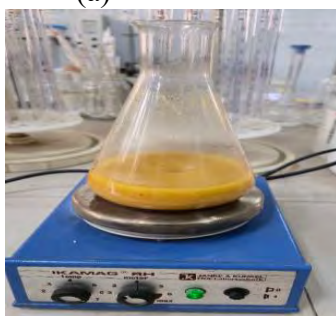
(a)



(b)



(c)



(d)



(e)



(f)



(g)



(h)



(i)



(j)



(k)



(l)

Figure 6. Procedure for extraction of pectin: (a) weighing of the citrus peel powder sample; (b) shaking of the sample solution; (c) acid hydrolysis at pH 2; (d) magnetic stirring during extraction; (e) cooling the extract using an ice-water bath; (f) filtration through muslin cloth to obtain the supernatant; (g) adjustment of pH to 4 using NaOH; (h) precipitation of pectin using alcohol; (i) centrifugation to collect the precipitated pectin; (j) pectin before drying; (k) drying of pectin in a hot air oven; and (l) grinding of dried pectin

3.4 Characterization of Pectin

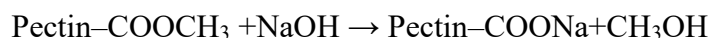
3.4.1 Determination of Methoxyl Content

Methoxyl content (MeC) is the percentage of methoxyl ($-\text{OCH}_3$) groups esterified to the galacturonic acid units in the pectin molecule. The methoxyl content (MeC) of the pectin samples was determined by the titrimetric method described by Ranganna *et al.* 1986. During the titration, the solution changes from colorless (acidic medium) to pink (alkaline medium), which indicates the endpoint of the titration.

Procedure

Approximately 0.5 g of dried pectin was accurately weighed and transferred into a stoppered conical flask. Then, 25 mL of 0.25 N sodium hydroxide (NaOH) was added to the flask. The mixture was shaken thoroughly to ensure complete mixing and allowed to stand for 30 minutes at room temperature to facilitate the saponification of the methyl ester groups present in the pectin (Figure 7).

Saponification reaction (hydrolysis of methyl ester groups):

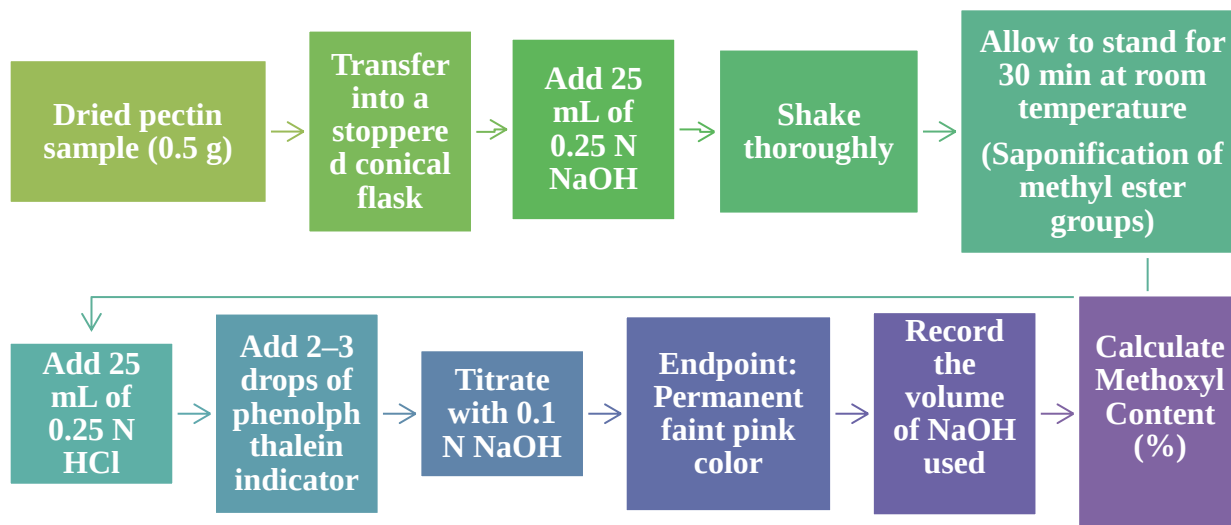


After saponification, 25 mL of 0.25 N hydrochloric acid (HCl) was added to neutralize the excess alkali. A few drops of phenolphthalein indicator were then added, and the solution was titrated with 0.1 N NaOH until a permanent pink color appeared, indicating the endpoint.

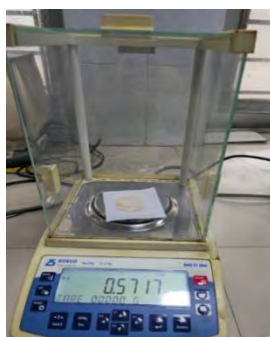
The methoxyl content was calculated using the following equation:

$$\text{Methoxyl Content (\%)} = (V \times N \times 3.1) / W$$

- V = Volume of 0.1 N NaOH used in the final titration (mL)
- N = Normality of NaOH
- W = Weight of the pectin sample (g)



Flowchart 2. Flow diagram for the determination of methoxyl content of pectin by the titrimetric method



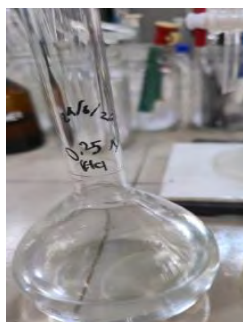
(a)



(b)



(c)



(d)



(e)



(f)

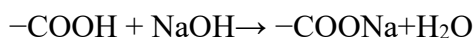
Figure 7. Procedure for determination of methoxyl content of pectin: (a) weighing of pectin sample, (b) dissolution in NaOH solution, (c) saponification of pectin ester groups, (d) addition of HCl for neutralization, (e) titration with standard NaOH solution, and (f) colour change at titration endpoint

3.4.2 Determination of Equivalent Weight

The equivalent weight (EW) of pectin is defined as the mass of pectin (in grams) that contains one equivalent (one mole) of free carboxyl (-COOH) groups. It is an important parameter used to estimate the number of free acid groups present in pectin before esterification.

Procedure

Weigh 0.5 g of pectin and transfer it into a 250 mL conical flask. Moisten the sample with 5 mL of ethanol to facilitate the dissolution of the pectin. Add 1 g of sodium chloride (NaCl) to sharpen the titration end point. Then add 100 mL of CO₂-free distilled water followed by 6 drops of phenol red indicator. Swirl the flask thoroughly to ensure that the pectin is completely dissolved and that no lumps remain adhering to the sides of the flask. Titrate the solution slowly with 0.1 N sodium hydroxide (NaOH) to minimize possible de-esterification until the indicator changes colour at approximately pH 7.5. The end point is indicated by a colour change from yellow to pink (Figure 8), and the final colour should persist for 30 seconds. During the titration, the free carboxyl groups of pectin react with sodium hydroxide according to the following reaction:



Since 1 mole of NaOH neutralizes 1 mole of free carboxyl (-COOH) groups, the amount of NaOH consumed is directly proportional to the number of free carboxyl groups present in the pectin sample. A higher equivalent weight indicates fewer free carboxyl groups, whereas a lower equivalent weight indicates more free carboxyl groups.

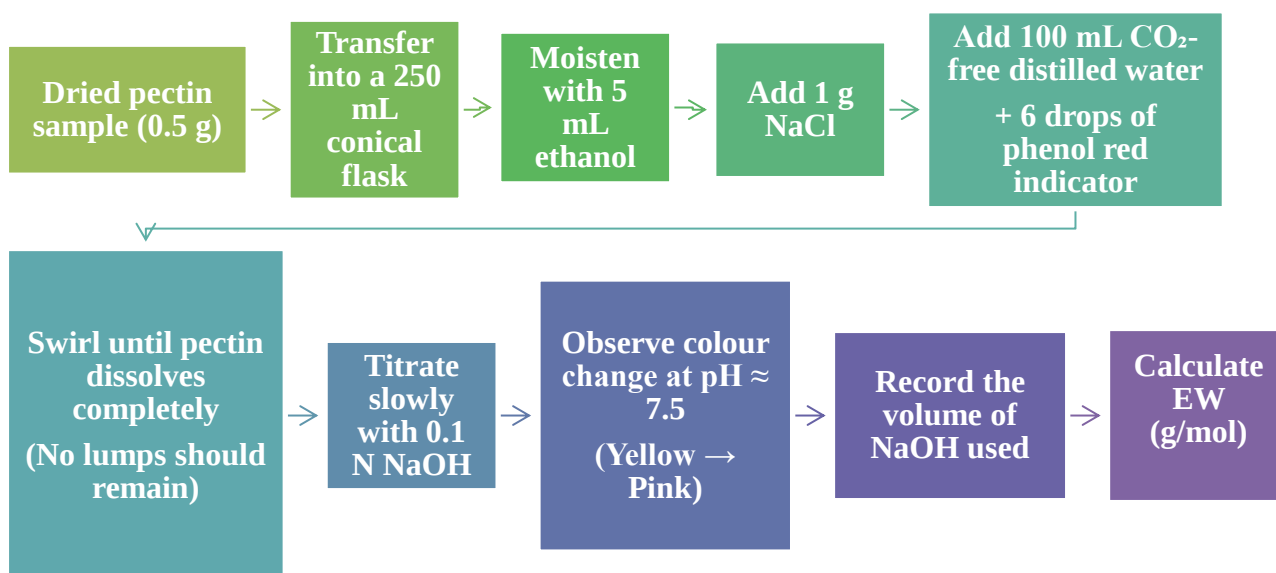
$$\text{EW (\%)} = (\text{Weight of sample (g)} \times 100) / (\text{mL of Alkali} \times \text{N of Alkali})$$

Weight of sample (g) = mass of pectin used for the analysis.

Volume of NaOH used (mL) = volume of 0.1 N sodium hydroxide required to reach the titration endpoint.

Normality of NaOH (N) = concentration of the sodium hydroxide solution.

100 = conversion factor to express the equivalent weight in grams per equivalent (g/mol)



Flowchart 3. Flow diagram for the determination of equivalent weight of pectin



(a)



(b)



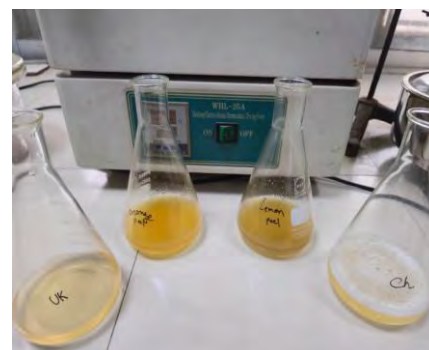
(c)



(d)



(e)

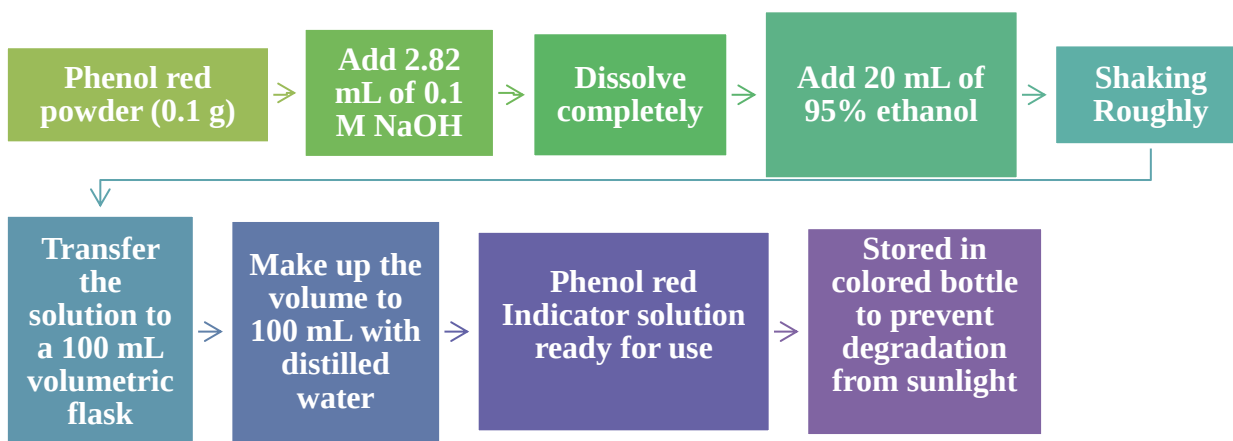


(f)

Figure 8. Procedure for determination of EW of pectin: (a) Samples are taken into conical flask, (b) moisten with ethanol, (c) add NaCl, distilled water, (d) addition of phenol red indicator (e) titration with standard NaOH solution, and (f) colour change at titration endpoint

3.4.2.i. Phenol red indicator

Phenol red is a pH indicator commonly used to detect changes in acidity or alkalinity during laboratory analyses. It is yellow under acidic conditions and changes to orange or red as the pH approaches neutral to mildly alkaline conditions, with a transition range of approximately pH 6.4–8.2. In pectin analysis, phenol red can be used as an indicator during titration to identify the endpoint, where a persistent color change indicates that the acidic components have been neutralized. The clear color transition makes phenol red useful for visual endpoint detection in acid–base titrations.



Flowchart 4. Flow diagram for the preparation of phenol red indicator solution

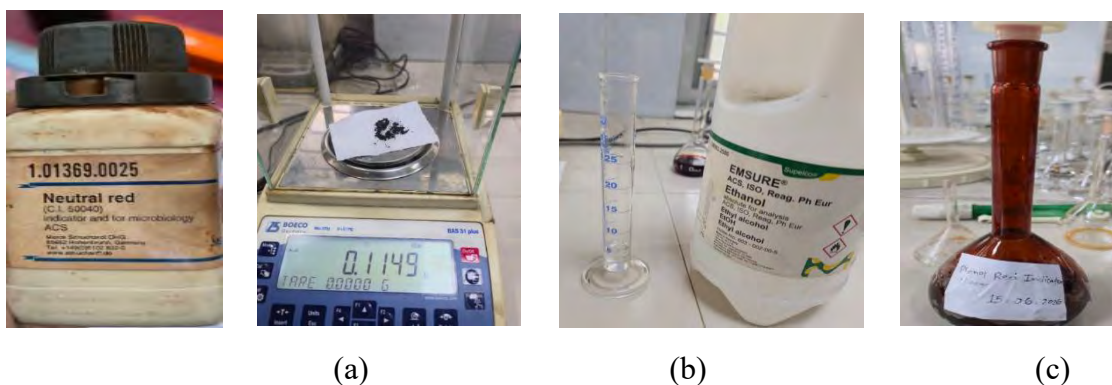


Figure 9. Procedure of Phenol red indicator, a) phenol red powder taken b) weighed c) Ethanol and Distilled water added d) phenol red indicator

3.4.3 Determination of Anhydrouronic Acid Content

Estimation of AUA content is essential to determine purity, degree of esterification (DE), and in the evaluation of pectin physical properties. Pectin, a partly esterified polygalacturonide, contains at least 10% organic material composed of galactose, arabinose, and other sugars. Making use of equivalent weight, methoxyl content, and the alkalinity of the ash data, the anhydrouronic acid was calculated using;

$$\text{AUA (\%)} = (176 \times 0.1 \text{ z} \times 100\% / \text{w} \times 1000) + (176 \times 0.1 \text{ y} \times 100\%) / (\text{w} \times 1000)$$

Where 176 is the molecular weight of anhydrouronic acid

y = ml (titer) of NaOH from methoxyl content determination.

z = ml (titer) of NaOH from equivalent weight determination.

w = weight of sample

3.4.4 Determination of Degree of esterification

The DE of pectin was determined according to the formula below (Shaha *et al.*, 2013):

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

The Degree of Esterification (DE) of pectin is the percentage of galacturonic acid carboxyl groups that are esterified with methanol ($-\text{CH}_3\text{OH}$) in the pectin molecule. It indicates the proportion of methyl ester groups present compared to the total number of carboxyl groups (esterified + free - COOH groups) in pectin.

3.4.5 Determination of Ash

Ash content is the amount of inorganic mineral residue remaining after the complete combustion of pectin at high temperature. It represents the total mineral components present in pectin, such as calcium, potassium, sodium, magnesium, and other inorganic salts. When pectin is incinerated, all organic components (carbon, hydrogen, oxygen, and nitrogen-containing compounds) are oxidized and removed as gases, leaving behind inorganic mineral residues (ash). The percentage of ash indicates the purity and mineral content of the extracted pectin (Owusu *et al.* 2025).

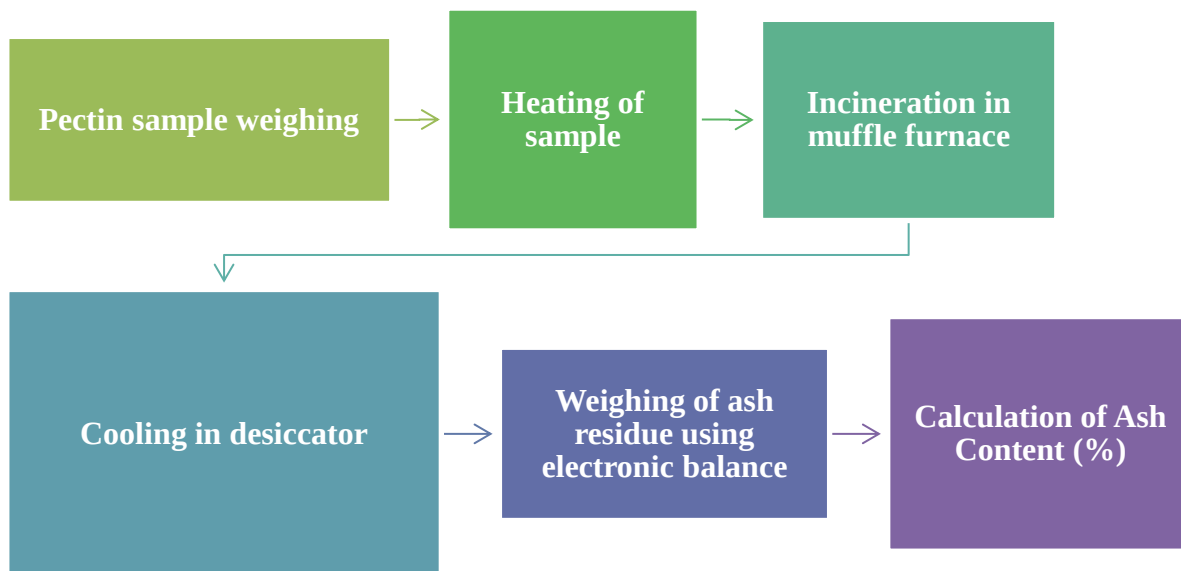
Procedure

Weigh approximately 2–5 g of the dried pectin sample into a clean, dry, and pre-weighed porcelain crucible. Record the weight of the empty crucible (W_1) and the crucible containing the sample (W_2). Place the crucible on a hot plate or over a low flame and gently char the sample until the evolution of smoke ceases to minimize sample loss during ashing. Transfer the crucible to a muffle furnace preheated to 600°C and incinerate the sample for 4–6 hours, or until a light gray or white ash is obtained, indicating complete combustion of the organic matter. Remove the crucible carefully using crucible tongs and allow it to cool in a desiccator to room temperature. Weigh the crucible containing the ash (W_3) (Figure 10).

$$\text{Ash content (\%)} = (\text{Weight of ash residue}) / (\text{Weight of dried pectin sample}) \times 100$$

The ash content is calculated using the following equation:

- W_1 = Weight of the empty crucible (g)
- W_2 = Weight of the crucible + sample before dry (g)
- W_3 = Weight of the crucible + sample after dry (g)



Flowchart 5. Flow diagram for the determination of ash of pectin

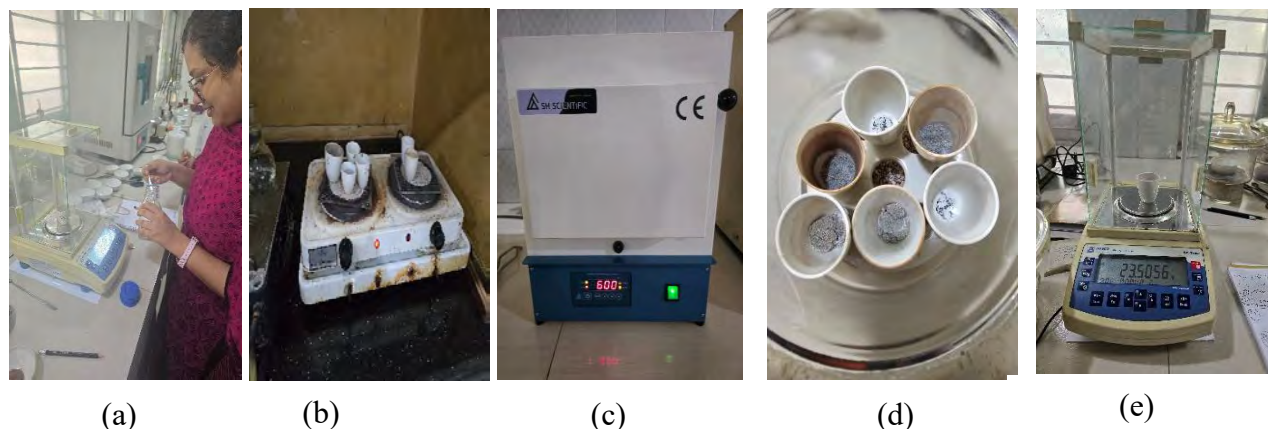


Figure 10. Procedure for determination of ash content of pectin: (a) weighing of pectin sample, (b) heating of sample, (c) incineration using a muffle furnace, (d) cooling of ash residue in a desiccator, and (e) final weight measurement using an electronic balance

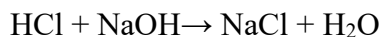
3.4.6 Determination of Alkalinity of Ash Data

The obtained ash was transferred to a flask and dissolved in 25 mL of 0.1 N hydrochloric acid (HCl). The solution was heated gently to boiling to ensure complete dissolution of the ash and subsequently allowed to cool to room temperature (Flowchart 6). The excess hydrochloric acid was then back-titrated with 0.1 N sodium hydroxide (NaOH) using phenolphthalein as the indicator until a faint pink endpoint was obtained (Figure 11). The volume of NaOH consumed was recorded and used to calculate the alkalinity of ash (expressed as carbonate). The ash alkalinity as carbonate percentage was determined using the appropriate equations.

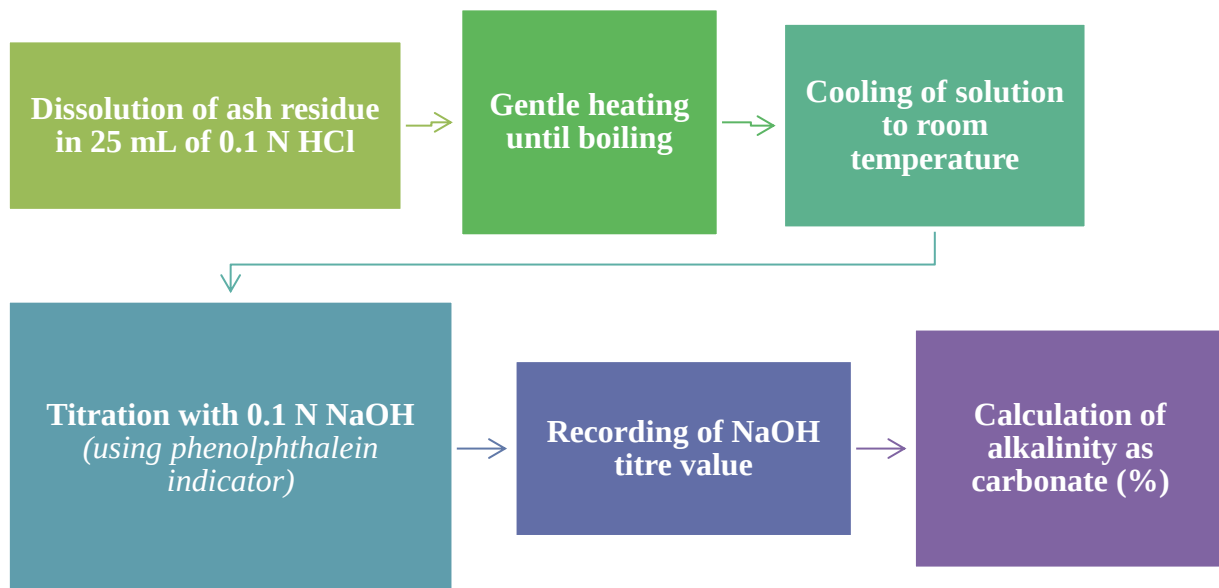
Reaction of carbonate ash with HCl:



Neutralization during titration:



$$\text{Alkalinity \% as carbonate} = (\text{Titre} \times \text{N of NaOH} \times 60) / (\text{wt of ash} \times 1000)$$



Flowchart 6. Flow diagram for the determination of alkalinity of ash of pectin



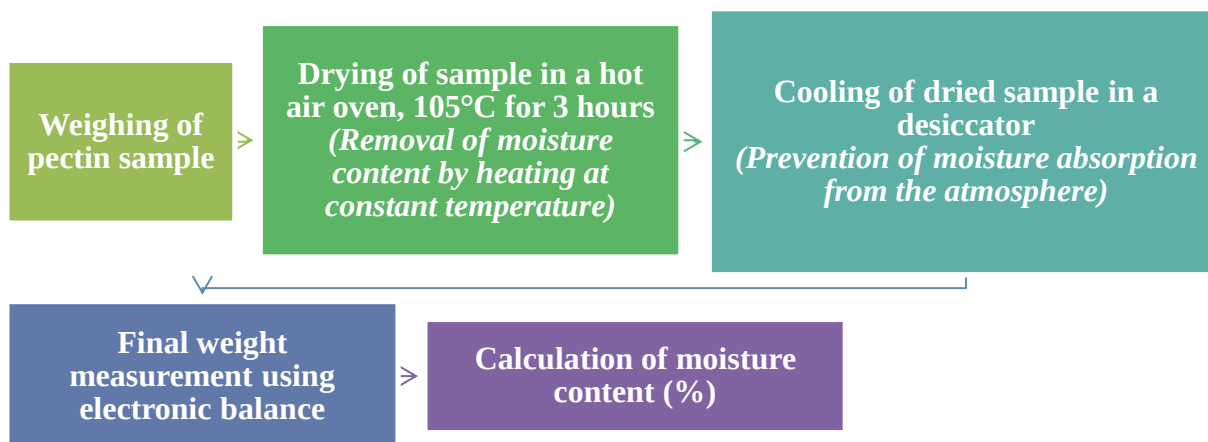
Figure 11. Procedure for determination of alkalinity (%) of ash of pectin: (a) ash sample obtained after incineration, (b) addition of 25 mL HCl solution, (c) gentle boiling of HCl solution, and (d) endpoint detection using phenolphthalein indicator with colour change from colourless to pink during titration.

3.4.7 Determination of Moisture Content

A known amount of pectin sample was accurately weighed and placed in a pre-weighed moisture dish. The sample was then dried in a hot air oven at a suitable temperature until a constant weight was obtained/ 105°C for 3 hours, allowing the removal of moisture present in the pectin. After drying, the sample was transferred to a desiccator and cooled to room temperature to prevent moisture absorption from the surrounding environment. The final weight of the dried sample was recorded using an electronic balance (Figure 12). The moisture content of pectin was calculated from the difference between the initial weight of the sample and the final dry weight, and expressed as a percentage of the original sample weight (Flowchart 7).

Calculation of moisture content (%)

Moisture content (%) = (Initial weight of sample - Final dry weight)/ Initial weight of sample×100



Flowchart 7. Flow diagram for the Procedure for Determination of Moisture Content of Pectin

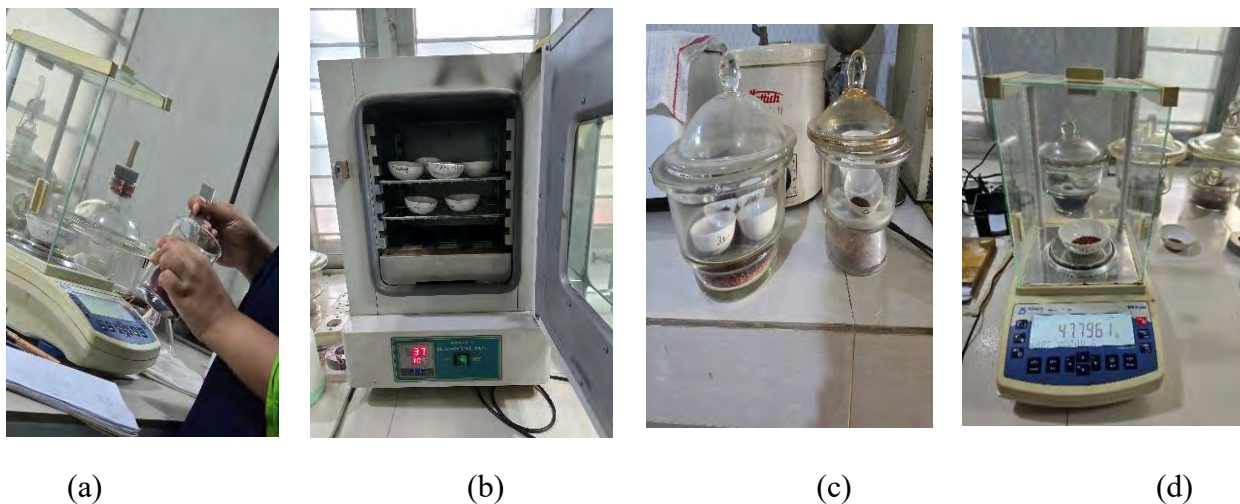


Figure 12. Procedure for determination of moisture content of pectin: (a) weighing of pectin sample, (b) drying in a hot air oven, (c) cooling of dried sample in a desiccator, and (d) final weight measurement using an electronic balance

3.4.8 FT-IR

The Fourier Transform Infrared (FT-IR) analysis of the extracted pectin samples was performed at the Analytical Service Cell, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. The analysis was conducted as a collaborative laboratory service. The dried pectin samples were finely ground and analyzed. Spectra were recorded over the wavenumber range of 4000–650 cm^{-1} at room temperature. A background spectrum was collected before each measurement to eliminate atmospheric interference. The obtained spectra were analyzed to identify the characteristic functional groups of pectin, including hydroxyl (O–H), aliphatic C–H, esterified carbonyl (C=O), carboxylate (COO^-), and glycosidic (C–O–C) groups by comparing the absorption bands with published literature.

Procedure

The dried pectin samples were finely ground into a homogeneous powder before FT-IR analysis. The FT-IR instrument equipped with an ATR accessory was first cleaned, and a background spectrum of air was recorded to minimize atmospheric interference. A small amount of the powdered pectin sample was placed directly onto the ATR crystal and pressed firmly using the

instrument's pressure arm to ensure good contact between the sample and the crystal. Similar ATR-FTIR procedures have been used for pectin characterization. The spectra were recorded at room temperature over the wavenumber range of 4000–650 cm^{-1} . Multiple scans were collected and averaged to obtain the final spectrum. The spectrum was subjected to baseline correction where necessary, and the ATR crystal was cleaned between samples to prevent cross-contamination. The resulting spectra were examined for characteristic absorption bands corresponding to O–H, C–H, esterified C=O, carboxylate (COO^-), and C–O–C/glycosidic groups. Characteristic pectin bands around 1740 cm^{-1} and 1600–1630 cm^{-1} were considered indicative of esterified and non-esterified carboxyl groups, respectively.

Chapter 4

Result and Analysis

4.1 The percent pectin yield

The pectin yield obtained from lemon and orange peel samples varied considerably depending on the sample quantity and extraction conditions. The highest pectin yield was 25.00%, obtained from lemon peel using H_2SO_4 at pH 2, 90 min, and 70 °C, whereas the lowest yield was 11.43%, obtained from orange peel. Overall, the lemon peel samples generally produced higher yields than the orange peel sample investigated in the present study. The variation in yield may be attributed to differences in extraction conditions, acid type, temperature, extraction time, and the intrinsic composition of the citrus peel. The results of pectin yield are presented in Table 3.

Table 3. Yield of Pectin

Name of Sample	Mass of Sample (g)	Mass of Pectin (g)	Pectin Yield (%)
Lemon peel pectin	5	0.73	14.6
Lemon peel pectin	5	0.79	15.8
Lemon peel pectin	10	1.4	14
Lemon Peel Pectin	10	2.5	25
Lemon peel pectin	20	3.2	16
Lemon peel pectin	20	3.5	17.5
Lemon Peel Pectin	13	1.8	13.85
Lemon peel pectin	16	2.9	18.13
Lemon Peel Pectin	30	3.8	12.67
Lemon Peel Pectin	12	2.1	17.5
Orange Peel Pectin	14	1.6	11.43

4.2 The Effect of P^H, acid, extraction time, extraction temeparure on pectin yield

The extraction parameters had a noticeable effect on pectin recovery. It was found that extraction using H₂SO₄ at pH 2, 90 min, and 70 °C produced the maximum yield of 25%.

The Effect of P^H, acid, extration time, extraction Temeparure are shown in table 4.

Table 4. The Effect of P^H, Acid, Extration Time, Extraction Temeparure

Source of Pectin	Acid	PH	Time (min)	Temperature (°C)	Yield (%)
Lemon peel	HCL	1	70	70° C	14.6
Lemon peel	HCL	2	70	70° C	15.8
Lemon peel	H2SO4	2	90	70° C	25
Lemon Peel	H2SO4	2	120	80° C	14
Lemon peel	H2SO4	2	140	80° C	16
Lemon Peel	H2SO4	2	90	70° C	13.85
Lemon peel	H2SO4	2	90	80° C	17.5
Lemon Peel	H2SO4	2	120	80° C	18.13
Lemon Peel	H2SO4	2	120	80° C	12.67
Lemon Peel	H2SO4	2	110	80° C	17.5
Orange Peel	H2SO4	2	90	80° C	11.43

Increasing the extraction temperature and extending the extraction time did not consistently increase the yield and, in some cases, resulted in a reduction in pectin recovery. This indicates that excessive temperature or prolonged acid treatment may promote degradation of pectin or other structural changes. The effects of pH, acid type, extraction time, and temperature on pectin yield are presented in Table 4, while the relationship between extraction temperature and yield is illustrated in Figure 13, and the effect of extraction time is shown in Figure 14.

The relationship between extraction temperature (°C) and pectin yield (%) is shown in the bar chart (Figure 13).

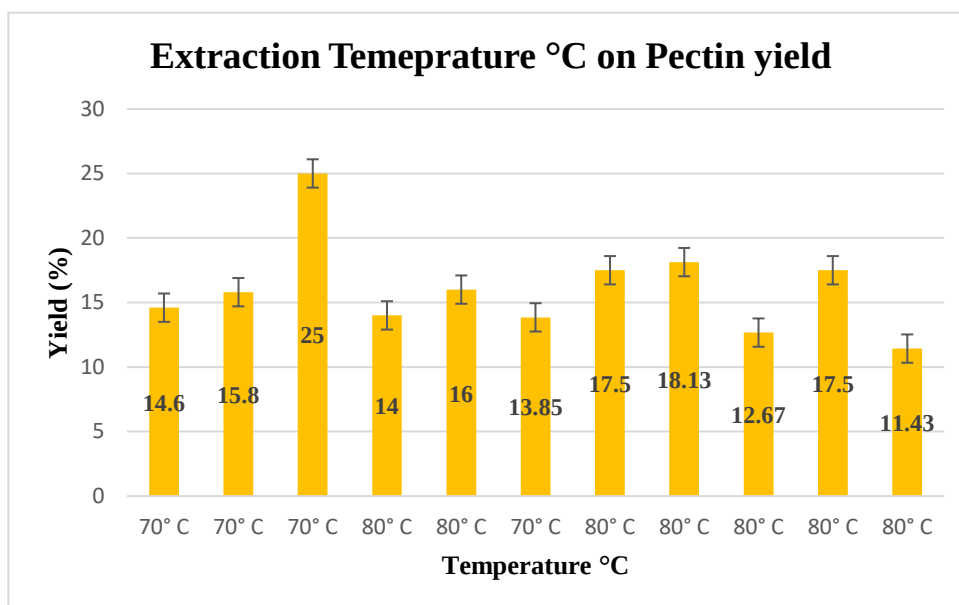


Figure 13. Illustration of the relationship between extraction temperature (°C) and pectin yield (%).
The bar chart shows the effect of different extraction temperatures on the percentage yield of pectin obtained from the samples

The relationship between extraction time (min) and pectin yield (%) is shown in the bar chart (Figure 14). The combined bar chart illustrates the interactive effects of extraction temperature and processing time on the recovery percentage of pectin. By linking both parameters directly to individual runs, the data reveals that optimizing temperature and time simultaneously is critical, rather than looking at either variable in isolation. For instance, the highest efficiency is achieved at a moderate temperature of 70°C paired with a 90-minute extraction time, reaching a peak yield of 25.0% (Figure 15). Interestingly, increasing the thermal energy to 80°C does not automatically guarantee a higher yield; prolonged heating at 80°C (up to 120 and 140 minutes) results in fluctuating or lower yields (ranging between 11.4% and 18.1%), which is consistent with the thermal degradation of pectin polymers over extended periods.

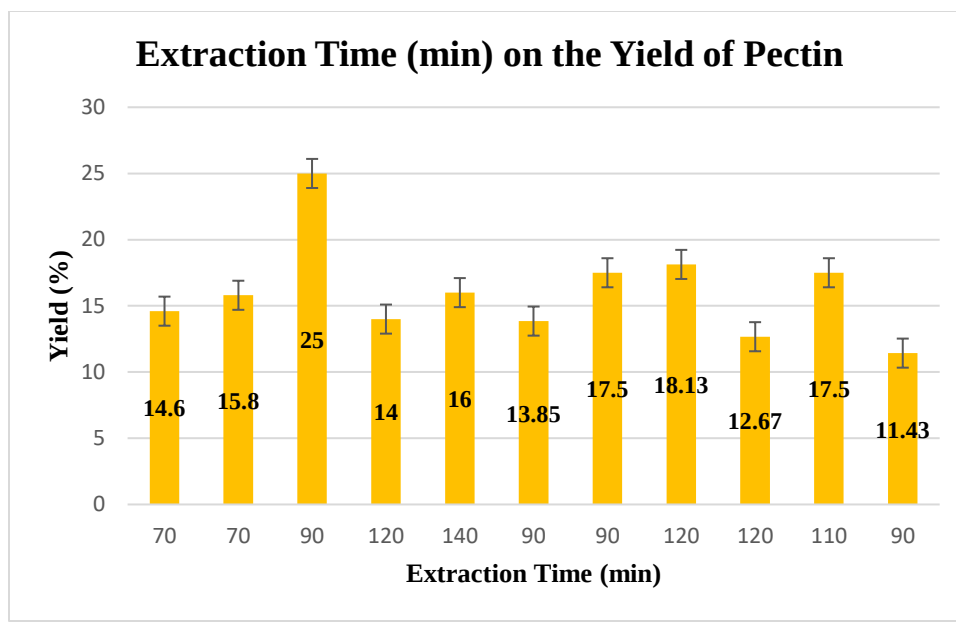


Figure 14. The effect of extraction time (min) on pectin yield (%) is illustrated in the bar chart showing the variation in pectin yield obtained at different extraction durations

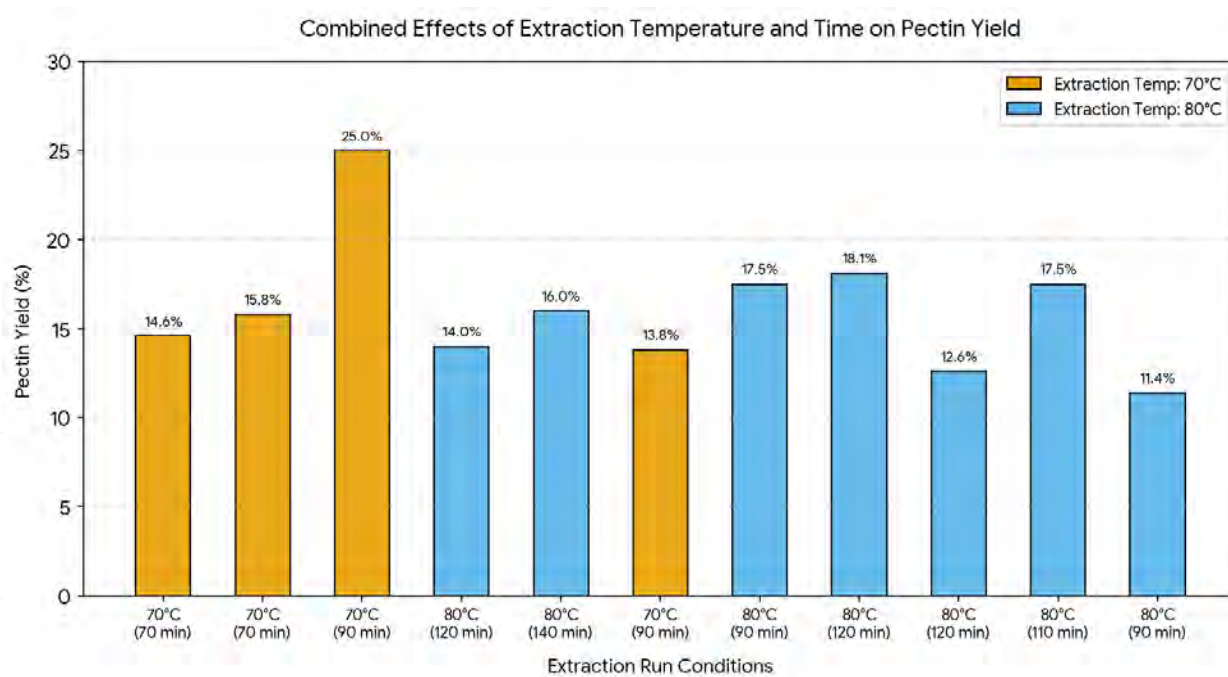


Figure 15. Combined Effects of Extraction Temperature and Time on Pectin Yield

Table 5. Visual color characteristics of commercial pectin samples and pectin extracted from lemon and orange peels

Name of Sample	Color
Chinese Pectin	white
UK Citrus Pectin	white
Lemon peel pectin	Yellowish
Orange Peel Pectin	Light Orange

4.4 The overall results of analysis

The physicochemical properties of the commercial and extracted pectin samples were evaluated based on methoxyl content (MeC), equivalent weight (EW), anhydrouronic acid (AUA) content, degree of esterification (DE), ash content, alkalinity of ash, moisture content, and colour. The results showed considerable variation among the four pectin samples. The MeC values ranged from 2.0374% to 5.5193%, while the EW ranged from 85.30 to 156.0909 mg/mL. AUA content varied between 29.216% and 48.928%, and DE ranged from 39.5919% to 78.3748%. Ash content, alkalinity of ash, and moisture also differed among the samples. The commercial samples were white, whereas the extracted lemon and orange peel pectins exhibited yellowish and light-orange colours, respectively. The average physicochemical properties of the investigated pectin samples are presented in Table 6.

Table 6. The summary of average results of Physicochemical properties of commercial and extracted pectin samples

Sample	MeC (%)	EW (mg/mL)	AUA (%)	DE (%)	Ash (%)	Alkalinity of Ash (%)	Moisture (%)	Color
UK Citrus Pectin	4.79 ± 0.08	85.30 ± 0.41	48.93 ± 0.73	55.52± 0.70	8.80± 0.41	3.50 ± 0.24	1.46± 0.24	White
Chinese Pectin	2.04 ± 0.08	100.42 ± 0.50	29.22 ± 0.52	39.59± 0.5	1.89± 0.24	16.35± 0.41	3.38± 0.23	White
Lemon Peel Pectin	3.01 ± 0.09	156.09 ± 0.50	29.22 ± 0.42	58.47± 0.5	9.80± 0.41	2.97± 0.24	3.79± 0.41	Yellowish
Orange Peel Pectin	5.52 ± 0.08	153.18 ± 0.50	43.29 ± 0.79	78.37± 0.80	5.80± 0.33	9.12± 0.48	4.02± 0.57	orange

The comparative physicochemical properties of commercial pectin samples and pectin extracted from citrus peels are illustrated in Table 6. Among the samples, orange peel pectin showed comparatively higher methoxyl content and degree of esterification, whereas Chinese pectin exhibited the lowest values for several physicochemical parameters.

4.5 Color of extracted Pectin

In Figure 17, the extracted pectin samples exhibited noticeable color differences compared with the commercial pectin samples. Both UK citrus pectin and Chinese pectin appeared white, while the pectin extracted from lemon peel showed a yellowish colour and the orange peel pectin exhibited a light-orange colour (Figure 16). The colour differences may be associated with the characteristics of the raw materials and the extraction process.



Figure 16. Precipitation of pectin after addition of alcohol



Color of orange peel pectin

Color of lemon peel pectin

Color of commercial pectin

Figure 17. Dried samples of Pectin

4.6 Equivalent Weight

The equivalent weight of the commercial and extracted pectin samples showed noticeable variation among the four samples. UK citrus pectin had the lowest equivalent weight (85.30 ± 0.33 mg/mL), with replicate values ranging from 84.95 to 85.75 mg/mL. Chinese pectin showed a higher equivalent weight of 100.42 ± 0.56 mg/mL, with replicate values of 99.70, 100.50, and 101.06 mg/mL. Among the extracted samples, lemon peel pectin exhibited the highest equivalent weight (156.09 ± 0.36 mg/mL), followed by orange peel pectin (153.18 ± 0.51 mg/mL). The higher equivalent weight observed in the extracted lemon and orange peel pectin compared with the commercial samples indicates differences in their chemical composition and the proportion of free acidic groups present in the pectin. The relatively small standard deviations observed among the replicates indicate good consistency of the measurements. Overall, the equivalent weight followed the order lemon peel pectin > orange peel pectin > Chinese pectin > UK citrus pectin. The sample weights, NaOH consumption, and calculated equivalent weights are presented in Table 7.

Table 7. Equivalent weight of the pectin

Sample	R1 (g/mol)	R2 (g/mol)	R3 (g/mol)	Equivalent weight (g/mol) (Mean $\pm$ SD)
UK Citrus Pectin	84.95	85.2	85.75	85.3 $\pm$ 0.33
Chinese Pectin	99.7	100.5	101.06	100.42 $\pm$ 0.56
Lemon peel pectin	155.7	156	156.57	156.0909 $\pm$ 0.36
Orange peel pectin	152.5	153.3	153.74	153.1818 $\pm$ 0.51

In Figure 18, the equivalent weight values showed clear differences between the commercial and extracted pectin samples. The extracted lemon and orange peel pectin exhibited considerably higher equivalent weights than the commercial UK and Chinese pectin samples.

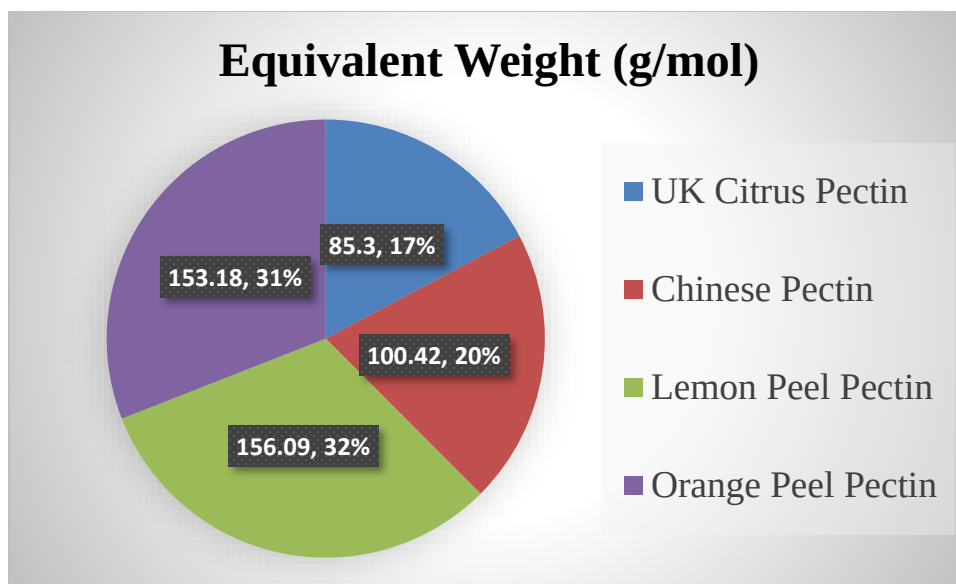


Figure 18. A pie chart of comparative distribution of equivalent weight among commercial and extracted pectin samples

4.7 Methoxyl Content

The methoxyl content (MeC) of the pectin samples was determined based on the amount of sodium hydroxide consumed during titration. The results showed that the methoxyl content varied among the four samples (Figure 19). In table 8, it is found that Chinese pectin exhibited the lowest MeC (2.0374%), followed by lemon peel pectin (3.0091%) and UK citrus pectin (4.7851%), whereas orange peel pectin showed the highest MeC (5.5193%).

Table 8. Methoxy Content of the pectin

Sample	R1 (%)	R2 (%)	R3(%)	MeC (%) (Mean±SD)
UK Citrus Pectin	4.7	4.8	4.7	4.7851± 0.06
Chinese Pectin	1.9	2.4	2.1	2.0374± 0.07
Lemon peel pectin	2.9	3.1	3.1	3.0091± 0.07
Orange peel pectin	5.4	5.5	5.6	5.5193± 0.06

The methoxyl content showed a distinct variation among the commercial and extracted pectin samples. Orange peel pectin exhibited the highest methoxyl content, while Chinese pectin showed the lowest value. The comparative distribution of methoxyl content among the investigated samples (Figure 19).

The variation in Methoxy Content of commercial and extracted pectin samples is illustrated in Figure 18.

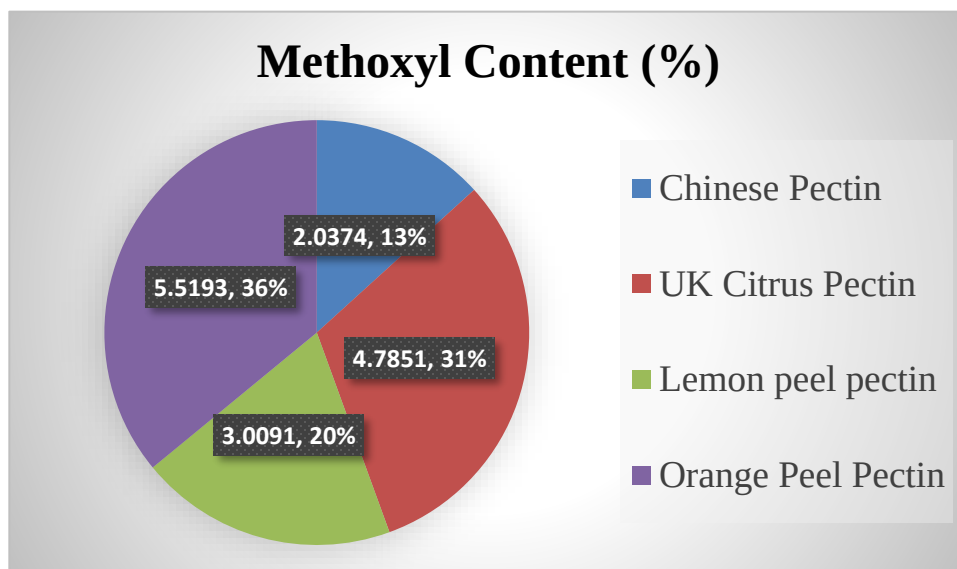


Figure 19. A pie chart of comparative distribution of MeC among commercial and extracted pectin

4.8 Anhydrouronic Acid

The anhydrouronic acid (AUA) content was determined to assess the relative purity and uronic acid composition of the pectin samples. The AUA content varied considerably among the samples, with Chinese pectin and lemon peel pectin showing the lowest value of 29.216%. UK citrus pectin exhibited the highest AUA content of 48.928%, followed by orange peel pectin with 43.296%. The AUA contents of the investigated samples are presented in Table 9.

Table 9. Anhydrouronic Acid Content of the pectin

Name of Sample	AUA (%)
UK Citrus Pectin	47.8 ± 0.73
Chinese Pectin	29.093 ± 0.515
Lemon peel pectin	28.359 ± 0.415
Orange Peel Pectin	42.8249 ± 0.795

The AUA content demonstrated considerable variation among the commercial and extracted pectin samples. UK citrus pectin showed the highest AUA content, whereas Chinese pectin and lemon peel pectin exhibited the lowest values (Figure 20).

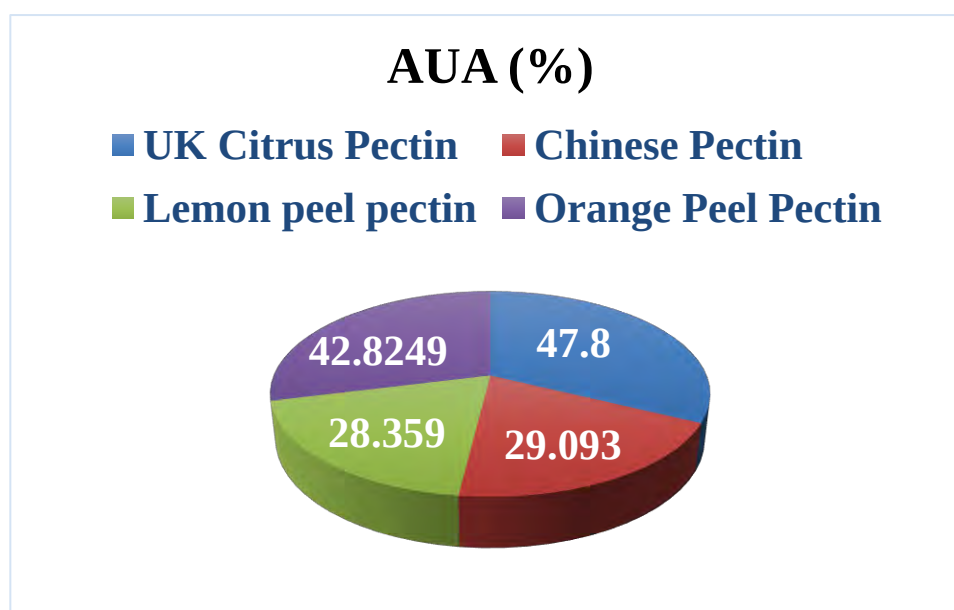


Figure 20. A pie chart of comparative distribution of Anhydrouronic Acid among commercial and extracted pectin samples

4.9 Degree of Esterification

In table 10, the degree of esterification (DE) of the pectin samples was determined to evaluate the proportion of esterified carboxyl groups present in the pectin structure. The DE values differed among the samples, ranging from 39.5919% in Chinese pectin to 78.3748% in orange peel pectin. Lemon peel pectin and UK citrus pectin showed DE values of 58.4745% and 55.5245%, respectively.

Table 10. Degree of Esterification of the pectin

Name of Sample	DE (%)
UK Citrus Pectin	56.8348 ± 0.70
Chinese Pectin	39.7593± 0.50
Lemon peel pectin	60.2416 ± 0.50
Orange Peel Pectin	73.1709 ± 0.80

It showed noticeable differences among the commercial and extracted pectin samples. Orange peel pectin exhibited the highest DE value, whereas Chinese pectin showed the lowest value (Figure 21). Lemon peel pectin and UK citrus pectin showed intermediate levels of esterification.

The variation in Degree of Esterification of commercial and extracted pectin samples is illustrated in Figure 21.

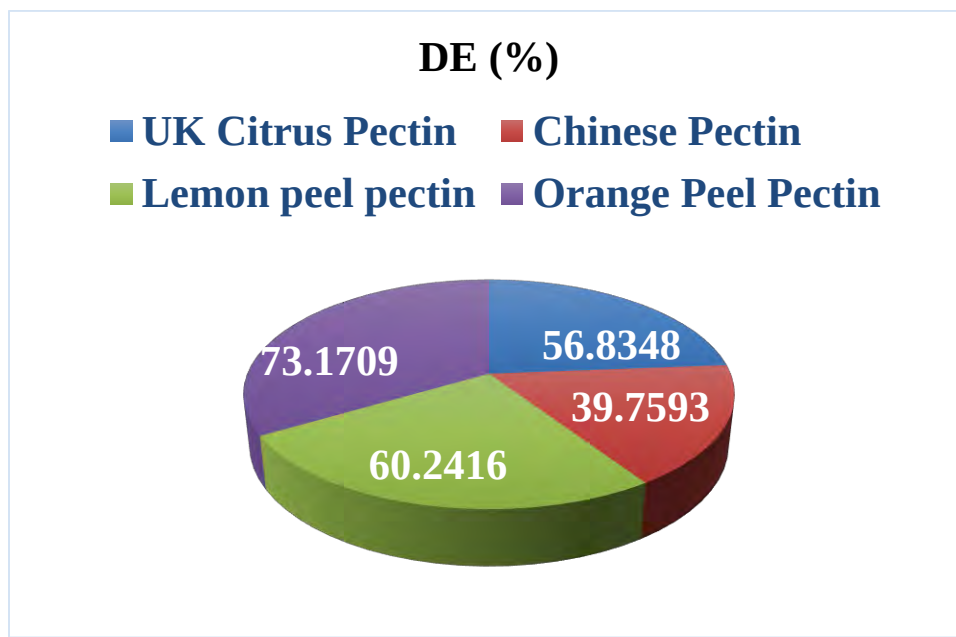


Figure 21. A pie chart of comparative distribution of DE among commercial and extracted pectin

4.10 Alkalinity of Ash Data

The alkalinity of ash showed considerable variation among the four pectin samples. Chinese pectin exhibited the highest alkalinity of ash value ($16.35 \pm 0.41\%$), with replicate values of 15.85%, 16.35%, and 16.85%. Orange peel pectin showed the second-highest value ($9.12 \pm 0.49\%$), followed by UK citrus pectin ($3.50 \pm 0.24\%$) and lemon peel pectin ($2.97 \pm 0.24\%$). The relatively high alkalinity observed in Chinese pectin indicates a greater contribution of alkaline mineral components in its ash fraction compared with the other samples (Table 11). In contrast, lemon peel pectin showed the lowest alkalinity, suggesting a comparatively lower contribution of alkaline mineral residues. The relatively small standard deviations among the three replicates indicate good consistency in the measurements. Overall, the alkalinity of ash followed the order Chinese pectin > orange peel pectin > UK citrus pectin > lemon peel pectin.

Table 11. Alkalinity of Ash Data of the pectin

Sample	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Mean	SD
UK Citrus Pectin	3.2	3.5	3.8	3.5	0.244949
Chinese Pectin	15.85	16.35	16.85	16.35	0.408248
Lemon Peel Pectin	2.67	2.97	3.27	2.97	0.244949
Orange Peel Pectin	8.52	9.12	9.72	9.12	0.489898

Chinese pectin showed the highest alkalinity of ash ($16.35 \pm 0.41\%$), which was substantially higher than the other samples. Orange peel pectin showed the second-highest value ($9.12 \pm 0.49\%$), while UK citrus pectin ($3.50 \pm 0.24\%$) and lemon peel pectin ($2.97 \pm 0.24\%$) exhibited considerably lower values. The error bars represent the standard deviation of the three replicates and are relatively small, indicating good consistency among the measurements. The graph clearly demonstrates that the alkalinity of ash differed considerably among the commercial and extracted pectin samples, with the order being Chinese pectin > orange peel pectin > UK citrus pectin > lemon peel pectin (Figure 22).

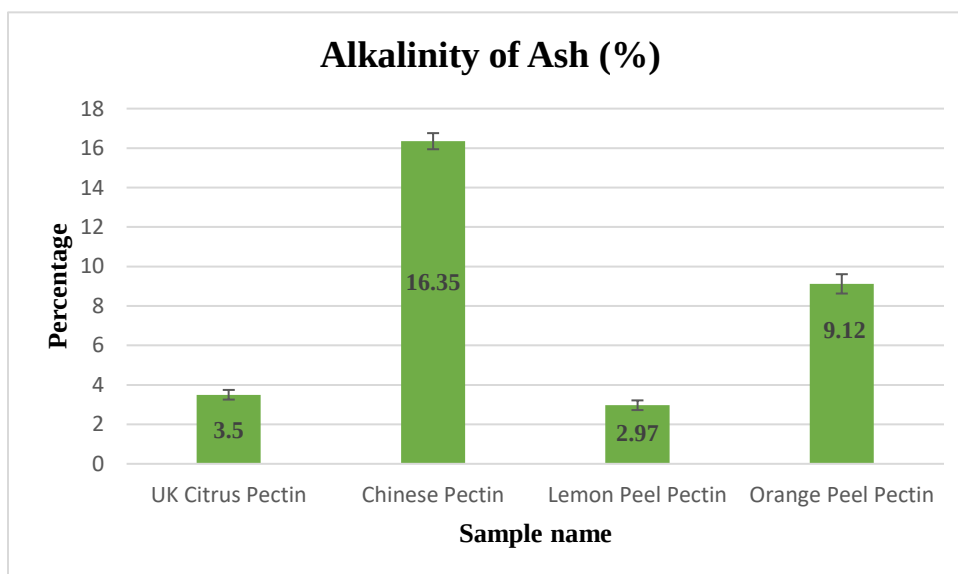


Figure 22. A bar chart of comparative distribution of Alkalinity of Ash Data among commercial and extracted pectin samples

4.11 Ash Data

The ash content of the pectin samples was determined to assess the amount of inorganic mineral residue remaining after incineration. The results demonstrated considerable variation among the samples. Chinese pectin showed the lowest ash content (1.89%), followed by orange peel pectin (5.80%). UK citrus pectin and lemon peel pectin showed higher ash contents of 8.80% and 9.80%, respectively. The ash (%) of the pectin is shown in Table 12.

Table 12. Ash of the pectin

Name of Sample	Replicate 1 (%)	Replicate 2 (%)	Replicate 3 (%)	Mean	SD
UK Citrus Pectin	8.3	8.8	9.3	8.8	0.408248
Chinese Pectin	1.59	1.89	2.19	1.89	0.244949
Lemon Peel Pectin	9.3	9.8	10.3	9.8	0.408248
Orange Peel Pectin	5.4	5.8	6.2	5.8	0.326599

The ash content showed clear differences among the commercial and extracted pectin samples. Lemon peel pectin exhibited the highest ash content, whereas Chinese pectin showed the lowest value. The ash contents of UK citrus pectin and orange peel pectin were intermediate compared with the other samples. The comparative distribution of ash content is illustrated in Figure 23.

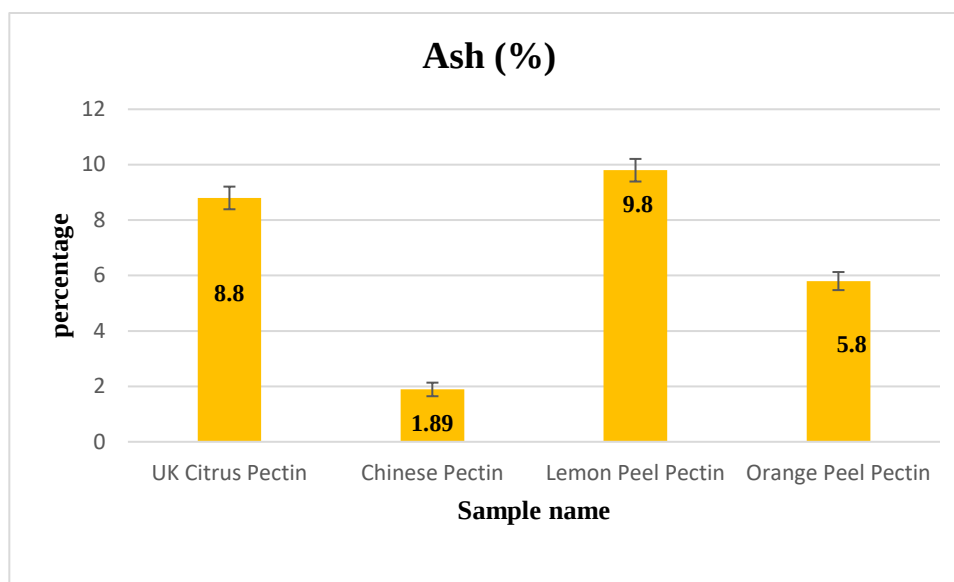


Figure 23. A bar chart of comparative distribution of Ash among commercial and extracted pectin samples

4.12 Moisture

The moisture content of the pectin samples was determined to evaluate the amount of residual water present in the dried pectin (Table 13). The results showed that moisture content varied among the samples. UK citrus pectin exhibited the lowest moisture content (1.4593%), while orange peel pectin showed the highest value (4.0228%). Chinese pectin and lemon peel pectin showed moisture contents of 3.3771% and 3.7908%, respectively.

Table 13. Moisture of the pectin

Name of Sample	Moisture (%)
UK Citrus Pectin	1.4593 ± 0.30
Chinese Pectin	3.3771 ± 0.40
Lemon peel pectin	3.7908 ± 0.50
Orange Peel Pectin	4.0228 ± 0.70

The moisture content varied among the commercial and extracted pectin samples. Orange peel pectin showed the highest moisture content, followed by lemon peel pectin and Chinese pectin, whereas UK citrus pectin exhibited the lowest value. The comparative distribution of moisture content among the samples is illustrated in Figure 24.

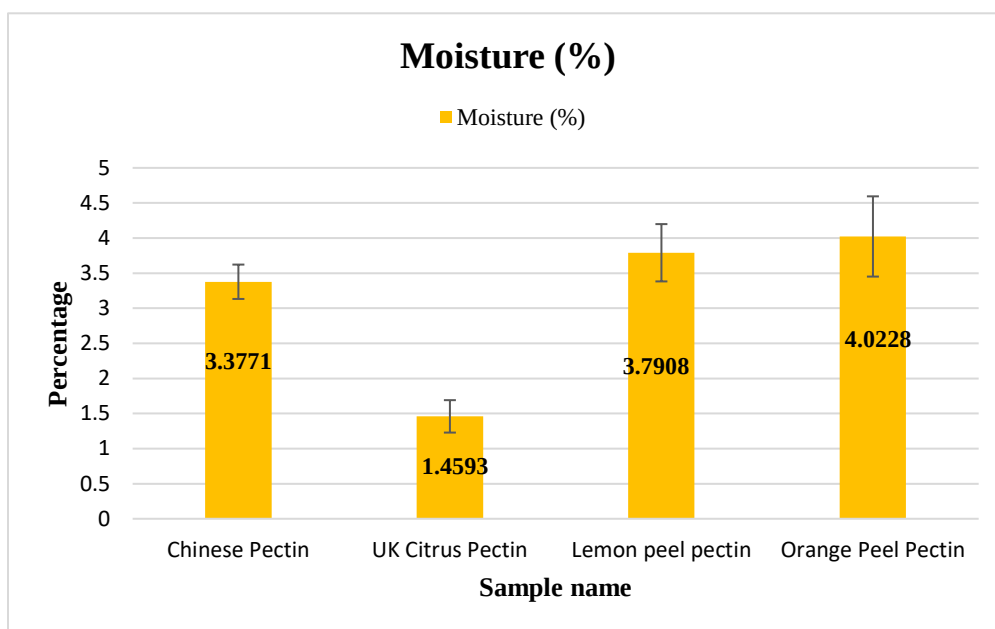
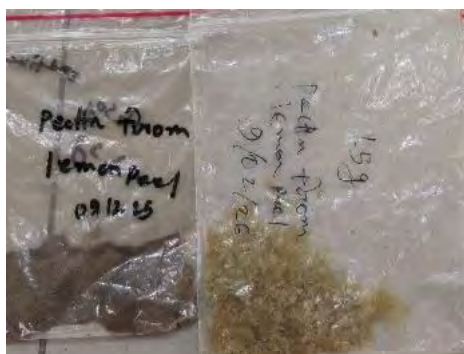


Figure 24. A bar chart of comparative distribution of Moisture among commercial and extracted pectin samples

4.13 Selection of acid for hydrolysis

Pectin extracted using HCl developed a black color (Figure 25), indicating possible degradation or oxidation during acid hydrolysis. In contrast, pectin extracted with H₂SO₄ retained a yellowish-brown color (Figure 24) and produced a higher pectin yield.



Extracted by **HCL**

Extracted by **H₂SO₄**

(A)

(B)

Figure 25. *Visual appearance of pectin extracted using different acids: (A) pectin extracted with HCl showing a blackish color and (B) pectin extracted with H₂SO₄ showing a brownish-yellow color*

4.14 FT-IR Identification with function groups

FT-IR spectroscopy was used to identify the characteristic functional groups and structural features of the extracted and commercial pectin samples. Differences in peak position and intensity among the samples may be attributed to variations in chemical composition, degree of esterification, and extraction conditions. The major absorption peaks and their corresponding functional group assignments for the extracted and commercial pectin samples are presented in Table 15.

Table 15. FT-IR Peak Identification and Functional Group Assignment of Extracted and Commercial pectin

Sample	Peak (cm ⁻¹)	Functional Group
Peel	3360	O–H stretching
	2919	C–H stretching
	1740	C=O stretching
	1625	COO ⁻ asymmetric stretching
	1222	C–O stretching
	1016	C–O–C, C–O stretching
Commercial Citrus	3346	O–H stretching
	2938	C–H stretching
	1735	C=O stretching
	1630	COO ⁻ asymmetric stretching
	1232	C–O stretching
	1011	C–O–C stretching
Chinese Commercial	911	β-Glycosidic vibration
	3332	O–H stretching
	2938	C–H stretching
	1735	C=O stretching
	1241	C–O stretching
	1002	C–O–C stretching
	911	β-Glycosidic vibration

In Figure 26, it is found that the FT-IR spectra showed characteristic absorption bands associated with hydroxyl groups, C–H stretching, esterified carbonyl groups, and glycosidic linkages, confirming the polysaccharide nature of the samples.

The FT-IR spectra of the commercial and extracted pectin samples showed characteristic absorption bands associated with the major functional groups of pectin, confirming the polysaccharide nature of the samples. A broad absorption band in the region of approximately 3200–3500 cm⁻¹ can be attributed to O–H stretching vibrations, which arise from the hydroxyl groups present in the galacturonic acid units and hydrogen bonding within the pectin structure. The absorption band around 2900 cm⁻¹ is associated with C–H stretching vibrations of aliphatic CH₂ and CH₃ groups.

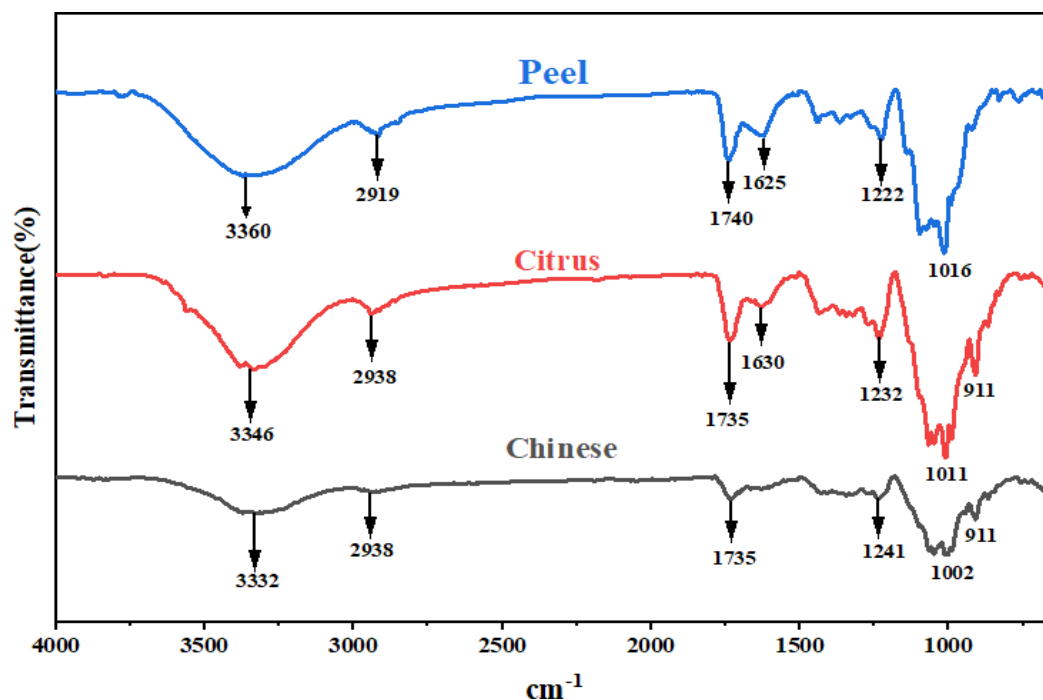


Figure 26. Fourier Transform Infrared (FT-IR) spectra of extracted lemon peel pectin, commercial UK citrus pectin, and Chinese commercial pectin

The absorption band near 1730–1750 cm^{-1} is characteristic of the C=O stretching vibration of esterified carboxyl groups ($-\text{COOCH}_3$), while the band around 1600–1650 cm^{-1} is mainly associated with the asymmetric stretching of non-esterified carboxylate (COO^-) groups. These two regions are particularly important for pectin characterization because their relative intensities are related to the degree of esterification.

Chapter 5

Discussion

The present study evaluated the yield and physicochemical properties of pectin extracted from lemon and orange peels and compared them with two commercial pectin samples. The evaluated parameters included pectin yield, equivalent weight, methoxyl content, anhydrouronic acid (AUA), degree of esterification (DE), ash content, alkalinity of ash, carbonate-free ash, moisture content, and color. These properties are important because they determine the quality, purity, and industrial applications of pectin.

Generally, the yield obtained from all citrus samples was (11.43-25%). Phaviphu *et al.* (2018) reported that the yields of pectin extracted from different sources were significantly different and varied from 10.91 to 24.08%. Among the lemon peel samples, the highest yield (25.0%) was obtained using sulfuric acid (H_2SO_4) at pH 2, an extraction time of 90 min, and a temperature of 70°C . The lowest yield (11.43%) was observed for orange peel pectin under the extraction conditions employed in this study. The increase in pectin yield under acidic conditions is attributed to the hydrolysis of protopectin into soluble pectin. However, prolonged extraction time or excessive extraction temperature may reduce yield because of degradation of the pectin polymer. Therefore, extraction time and temperature should be carefully optimized to maximize pectin recovery.

a) The best extraction condition among your tested conditions is: Temperature: 70°C

Time: 90 minutes

Acid: H_2SO_4

pH: 2

Pectin yield: 25%

The maximum pectin yield (25%) was obtained from lemon peel extraction using H_2SO_4 at pH 2, extraction temperature of 70°C , and extraction time of 90 min. Increasing the extraction temperature or prolonging the extraction time did not improve yield, possibly due to thermal degradation of pectin chains and hydrolysis of galacturonic acid polymers. Higher temperature (80°C) and longer extraction time may reduce pectin yield. The optimum extraction condition

within the experimental range was estimated at 70°C and 90 min, giving the maximum observed pectin yield of 25%. The result supports optimal conditions identified (Akachat *et al.*, 2025).

b) The use of 60% ethanol and absolute ethanol (99.5%) resulted in similar pectin yield. Therefore, 60% ethanol was selected as the preferred solvent because it is more cost-effective and reduces the overall extraction cost while maintaining comparable yield.

c) Extraction at pH 1 resulted in a lower pectin yield, whereas pH 2 produced a higher yield. This indicates that a moderately acidic condition favors pectin extraction by promoting the release of pectin from the plant cell wall.

d) Higher extraction temperatures (80–90°C) negatively affected pectin quality, resulting in degradation of pectin structure and development of a blackish color. Lower extraction temperatures produced better-quality pectin with improved color and stability.

e) The type of acid used during extraction influenced the color and quality of the extracted pectin. HCl extraction produced darker (blackish) colored pectin, whereas H₂SO₄ extraction resulted in a yellowish-colored pectin, indicating better color quality.

Pectin extracted using sulphuric acid was medium brown, while hydrochloric acid-extracted pectin was the darkest, appearing dark brown to nearly black. Citric and acetic acid-extracted pectins were lighter in colour, ranging from amber to deep orange (Mansor *et al.* 2025). The type of acid used for extraction influenced both the yield and color of the extracted pectin. Pectin extracted using HCl developed a black color, indicating possible degradation or oxidation during acid hydrolysis. In contrast, pectin extracted with H₂SO₄ retained a yellowish-brown color and produced a higher pectin yield.

Extraction conditions, including acid type, pH, extraction time, and temperature, had a noticeable influence on pectin yield. Acid-assisted extraction promotes the hydrolysis of insoluble protopectin into soluble pectin by disrupting plant cell wall structures. Sulfuric acid has been reported as an

effective acidifying agent for pectin extraction due to its ability to enhance pectin solubilization under controlled acidic conditions (Methacanon *et al.* 2014). The highest yield was achieved at pH 2, demonstrating that moderately acidic conditions promoted efficient extraction. Yield increased as extraction time increased up to the optimum extraction period (90 min) but tended to decrease with prolonged extraction, probably because of thermal degradation and depolymerization of pectin molecules. Similarly, extraction temperature influenced pectin recovery. Moderate temperatures improved pectin solubilization, whereas prolonged exposure to elevated temperatures reduced yield due to structural degradation.

Commercial Chinese and UK citrus pectin appeared white, indicating a higher degree of purification. In contrast, lemon peel pectin was yellowish and orange peel pectin exhibited a light orange color. The darker coloration of the extracted pectins is likely due to the presence of natural pigments such as carotenoids and flavonoids that were not completely removed during extraction and purification. Color is an important quality parameter because lighter-colored pectin is generally preferred for commercial food applications (Rodrigo *et al.* 2013).

Equivalent weight is an indicator of the number of free carboxyl groups present in the pectin molecule. Lemon peel pectin exhibited the highest equivalent weight (156.09), followed by orange peel pectin (153.18), whereas UK citrus pectin showed the lowest value (85.30). A higher equivalent weight generally indicates fewer free acid groups and suggests that the extracted pectin experienced less degradation during extraction. Therefore, the relatively high equivalent weights of the citrus peel pectin indicates that the extraction procedure preserved the structural integrity of the pectin molecules (Vaclavik *et al.* 2008). The high equivalent weight would have higher gel-forming effect. The lower equivalent weight may be as a result of higher partial degradation of pectin. The decreased equivalent weight could also be dependent upon the amount of free acid (Ramli and Asmawati, 2011). The equivalent weight of the pectin is total content of free galacturonic acid (unesterified) in the molecular chains of pectin. Pectin produced at a low pH has higher equivalent weight, because a low pH can cause pectin polymerization into longer chain, and in turn reduces the free acid content (Uzma *et al.*, 2015).

The methoxyl content of the pectin was found to be 3.01-5.52 %, which falls within the typical range of 0.2 % to 12 % (Aina *et al.*, 2012). Methoxyl content determines the gelling properties of pectin and is closely related to its industrial application. Orange peel pectin contained the highest methoxyl content (5.52%), followed by UK citrus pectin (4.79%), lemon peel pectin (3.01%), and Chinese pectin (2.04%). Although the extracted citrus pectins showed lower methoxyl contents than values commonly reported for highly purified commercial pectins, orange peel pectin demonstrated comparatively better methyl esterification among the samples studied. Methoxyl content is a key factor in controlling the setting time of pectins and the ability of the pectin to form gels (Constenla and Lozano, 2003). Spreading quality and sugar-binding capacity of pectin is increased with increase methoxyl content.

Anhydrouronic acid (AUA) is commonly used to evaluate pectin purity because galacturonic acid forms the principal backbone of pectin molecules. UK citrus pectin exhibited the highest AUA content (48.93%), followed by orange peel pectin (43.30%). Chinese and lemon peel pectins contained similar AUA values (29.22%). Lower AUA values generally indicate the presence of non-pectic substances such as neutral sugars, proteins, or other impurities. Additional purification steps may improve the AUA content of the extracted pectins. Pectin, which is a partly esterified polygalacturonide, contains at least 10% organic materials composed of arabinose, galactose and other sugars. AUA (%) is essential to determine the purity and the degree of esterification and to evaluate physical properties (Ranganna, 1986). The higher galacturonic acid and lower ash content are the two criteria governing its purity (Hwang *et al.*, 1992).

The degree of esterification (DE) of the pectin in this study was found to be 39.59-78.37%. Our DE value is consistent with the findings of (Koffi *et al.*, 2013), who reported a DE of 77 % for pectin extracted from lemon. However, it is notably higher than the DE values for pectin extracted from oranges (35.85 %) by Devi *et al.*, (2014) and from lemon (33.77 %) by Kanmani *et al.*, (2014). The degree of esterification determines whether pectin is classified as high-methoxyl or low-methoxyl pectin. Orange peel pectin exhibited the highest degree of esterification (78.37%), followed by lemon peel pectin (58.47%) and UK citrus pectin (55.52%). These samples are classified as high-methoxyl pectins because their DE values exceed 50%. In contrast, Chinese pectin had a DE value of 39.59%, indicating that it belongs to the low-methoxyl pectin category.

High-methoxyl pectins generally produce stronger gels in the presence of high sugar concentrations and acidic conditions, making them suitable for the manufacture of jams, jellies, and confectionery products.

The alkalinity of ash varied considerably among the four pectin samples. Chinese pectin showed the highest value ($16.35 \pm 0.41\%$), followed by orange peel pectin ($9.12 \pm 0.49\%$), while UK citrus pectin and lemon peel pectin showed lower values of $3.50 \pm 0.24\%$ and $2.97 \pm 0.24\%$, respectively. Alkalinity of ash reflects the neutralizing capacity of the inorganic components remaining after incineration and is commonly determined by acid treatment followed by titration with NaOH (Mbaku *et al.*, 2023).

The ash content varied considerably among the samples, with Chinese pectin showing the lowest value (1.89%), followed by orange peel pectin (5.80%), UK citrus pectin (8.80%), and lemon peel pectin (9.80%). Lower ash generally indicates lower inorganic/mineral residues and better purification. The variation may be related to the citrus source, extraction conditions, acid treatment, washing, and removal of residual salts. Liu *et al.* (2022) also reported that citrus cultivar and growing region significantly influence the physicochemical properties of pectin. The ash content of lemon peel pectin in the present study (9.80%) was relatively high compared with values reported for several citrus pectins. For example, a recent study reported ash contents of 1.34% for mosambi, 2.12% for kinnow, 1.02% for orange peel, and 1.78% for commercial pectin (Kaur *et al.*, 2022). The higher ash values observed in the present extracted samples may therefore indicate the presence of greater mineral residues and suggest that additional washing and purification could improve pectin purity. Overall, Chinese commercial pectin had the lowest ash content, whereas lemon peel pectin had the highest, indicating considerable differences in purity and mineral residue among the samples.

Moisture content is an important quality parameter affecting storage stability and shelf life. The moisture content of the pectin was found to be 1.46-4.02 %, which is lower than the typical moisture content range for commercial pectin (9.4 % to 11.3 %) reported in the literature (Abang Zaidel *et al.*, 2017). This value is consistent with findings from (Akhter *et al.*, 2024), for pectin extracted from lemon peels. Pectin is known to be highly hygroscopic, meaning it readily absorbs

moisture from the environment. Therefore, it is crucial to store pectin in an airtight, dry container to maintain its quality. A low moisture content is essential not only for preserving the pectin but also for preventing microbial growth, particularly the growth of microorganisms that could produce pectinase enzymes, which would degrade the pectin and reduce its quality (Halle & Ayele, 2022; Weng *et al.*, 2024; Yüksel *et al.*, 2024). UK citrus pectin exhibited the lowest moisture content (1.46%), whereas orange peel pectin contained the highest moisture content (4.02%). Nevertheless, all samples contained moisture below 5%, indicating satisfactory drying efficiency and suggesting that the pectins are suitable for storage with minimal risk of microbial deterioration.

The FT-IR spectra of the extracted lemon peel pectin, commercial citrus pectin, and Chinese commercial pectin exhibited the characteristic absorption bands of pectin, confirming that the extracted pectin retained the typical polysaccharide structure of commercial pectin. Similar FT-IR profiles have been reported for citrus peel pectin extracted using conventional acid extraction, where the principal absorption bands were observed within the regions of 3300–3400, 2900–2940, 1740–1730, 1630–1600, 1240–1200, and 1100–1000 cm^{-1} (Bosch et al.2023).

A broad absorption band at 3360 cm^{-1} (Peel), 3346 cm^{-1} (Citrus), and 3332 cm^{-1} (Chinese) was attributed to O–H stretching vibrations of hydroxyl groups involved in extensive intermolecular hydrogen bonding. This broad band is a characteristic feature of polysaccharides and reflects the hydrophilic nature of pectin molecules. The peaks at 2919 cm^{-1} for the extracted pectin and 2938 cm^{-1} for both commercial pectins correspond to C–H stretching vibrations of methyl ($-\text{CH}_3$) and methylene ($-\text{CH}_2$) groups present in the galacturonic acid backbone. These absorption bands are commonly reported for citrus pectin and indicate the presence of aliphatic carbohydrate chains (Bosch et al.2023). The strong absorption band observed at 1740 cm^{-1} in the extracted pectin and 1735 cm^{-1} in both commercial pectins is assigned to the C=O stretching vibration of esterified carboxyl groups ($-\text{COOCH}_3$). This peak confirms the presence of methyl-esterified galacturonic acid residues and is considered one of the most important characteristic bands of pectin. The occurrence of this peak is consistent with the high degree of esterification obtained in the physicochemical analysis, indicating that the extracted pectin is a high-methoxyl pectin (Santiago-Gómez et al.2025). Another characteristic absorption was observed at 1625 cm^{-1} for the extracted pectin and 1630 cm^{-1} for the commercial citrus pectin, corresponding to the asymmetric stretching

vibration of free carboxylate (COO^-) groups. The simultaneous presence of peaks near 1740 cm^{-1} and $1625\text{--}1630\text{ cm}^{-1}$ indicates that both esterified and non-esterified galacturonic acid residues coexist within the pectin structure. These two bands are widely used to evaluate the esterification degree of pectin. The absorption bands at $1222\text{--}1241\text{ cm}^{-1}$ were assigned to C–O stretching vibrations of ester and carboxylic acid groups, further confirming the presence of esterified galacturonic acid units. Similar bands have been consistently reported in citrus pectin extracted by conventional and green extraction methods (Iñiguez-Moreno, M., *et al.*, 2024). Strong peaks located at 1016 cm^{-1} (Peel), 1011 cm^{-1} (Citrus), and 1002 cm^{-1} (Chinese) correspond to C–O–C and C–O stretching vibrations associated with glycosidic linkages in the polygalacturonase acid backbone. These fingerprint peaks confirm the integrity of the polysaccharide chain. Additionally, the absorption at 911 cm^{-1} observed in the commercial pectin samples is attributed to β -glycosidic linkage and pyranose ring vibrations, further supporting the characteristic structure of pectin. The extracted lemon peel pectin exhibited FT-IR spectra highly comparable to those of commercial citrus and Chinese pectin. The presence of characteristic hydroxyl, aliphatic C–H, esterified carbonyl, free carboxyl, ester, and glycosidic functional groups confirm the successful extraction of structurally intact pectin. Minor shifts in peak positions among the samples are likely to be due to differences in extraction conditions, degree of esterification, moisture content, and molecular interactions rather than changes in the fundamental chemical structure. These findings demonstrate that lemon peel is a suitable alternative source of commercial-quality pectin for food, pharmaceutical, and biodegradable packaging applications.

Chapter 6

Limitations, Future Implications and Conclusion

6.1 Limitations

Although this study successfully extracted and characterized pectin from citrus peels, several limitations should be considered. Firstly, the study was conducted using a limited number of raw materials (lemon and orange peels), and variations in citrus variety, geographical origin, maturity stage, and seasonal changes may influence pectin yield and physicochemical properties. Therefore, the results may not represent all citrus peel sources.

Secondly, only acid-assisted extraction methods using hydrochloric acid and sulfuric acid were investigated. Other advanced and environmentally friendly extraction approaches, such as ultrasound-assisted extraction, microwave-assisted extraction, and enzyme-assisted extraction, were not evaluated. These techniques may improve pectin yield, purity, and functional properties while reducing chemical consumption.

A key limitation of this study lies in the experimental baseline used for comparing the extracted pectin. The pectin isolated in this research underwent a stringent laboratory purification process, whereas the marketable finished products used as benchmarks were tested directly in their finalized, consumer-ready states. To establish a more rigorous and scientifically valid comparison, the raw marketable samples should have been subjected to the exact same extraction and purification protocols prior to analysis. Because this uniform processing was not conducted, differences in matrix interference, additives, and purity levels between the raw finished products and the isolated laboratory sample may have influenced the comparative outcomes. Future studies should ensure that all comparative samples undergo identical processing steps to isolate the variables more accurately.

6.2 Future Implications

The present study on the extraction and characterization of pectin from citrus peels highlights the potential of citrus waste as a valuable source of functional biopolymer for food science and technology applications. The extracted pectin showed promising physicochemical properties, indicating its potential use as a natural gelling agent, thickener, stabilizer, and texturizing agent in food products.

Future studies should focus on optimizing extraction conditions, including acid concentration, pH, extraction temperature, and extraction time, to improve pectin yield, purity, and functional properties. Advanced and environmentally friendly extraction techniques, such as ultrasound-assisted extraction, microwave-assisted extraction, and enzyme-assisted extraction, may be investigated to reduce chemical usage and improve extraction efficiency. Further characterization using advanced analytical techniques such as Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and rheological analysis could provide deeper insights into the structural and functional properties of citrus peel pectin. Studies on molecular weight distribution, gel strength, and antioxidant activity would also help evaluate its suitability for specific food applications.

The utilization of citrus peel waste for pectin production can contribute to sustainable food processing by reducing agro-industrial waste and promoting the development of value-added products. Extracted citrus pectin may have potential applications in jams, jellies, confectionery products, dairy formulations, edible coatings, and functional foods. Additionally, further research could explore the use of citrus pectin as a carrier material for bioactive compounds and its applications in nutraceutical and pharmaceutical industries. Citrus peel-derived pectin represents a promising, economical, and sustainable alternative to commercial pectin sources, supporting the principles of circular economy and sustainable food technology.

6.3 Conclusion

The present study successfully extracted and characterized pectin from citrus peel and compared its physicochemical properties with commercial pectin samples. The results demonstrated that citrus peels can serve as a potential and sustainable source of pectin for food science and technological applications. Extraction conditions, including acid type, pH, extraction time, and temperature, significantly influenced pectin yield. Among the investigated conditions, sulfuric acid extraction at pH 2 produced a higher pectin yield compared with hydrochloric acid extraction. The extracted pectin obtained using sulfuric acid showed a more acceptable yellowish-brown color, whereas hydrochloric acid resulted in darker coloration, indicating possible degradation or oxidation of compounds during extraction. The physicochemical analysis revealed variations among commercial and extracted pectin samples. Orange peel pectin showed favorable characteristics, including the highest methoxyl content and degree of esterification, suggesting good gelling potential. Lemon peel pectin exhibited a higher equivalent weight, indicating comparatively better structural stability. The differences in anhydrouronic acid content, ash content, moisture content, and other parameters reflected variations in raw materials and extraction conditions. The extracted pectins showed promising functional properties and demonstrated their potential as natural hydrocolloids for food applications such as gelling agents, stabilizers, and thickeners. However, further optimization of extraction parameters and additional purification processes are recommended to improve pectin purity and functional quality.

To conclude, this study highlights the potential of citrus peel waste as an economical and sustainable raw material for pectin production, contributing to waste valorization and the development of value-added food ingredients. Further research on structural characterization, functional properties, and industrial-scale extraction could enhance the application of citrus peel-derived pectin in food science and technology.

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