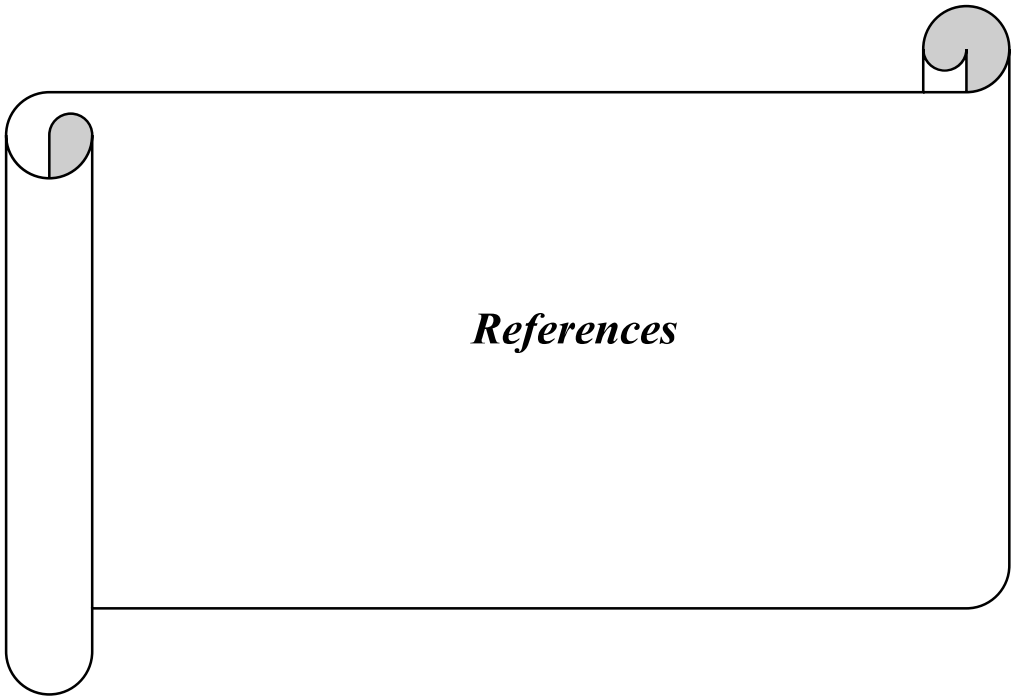
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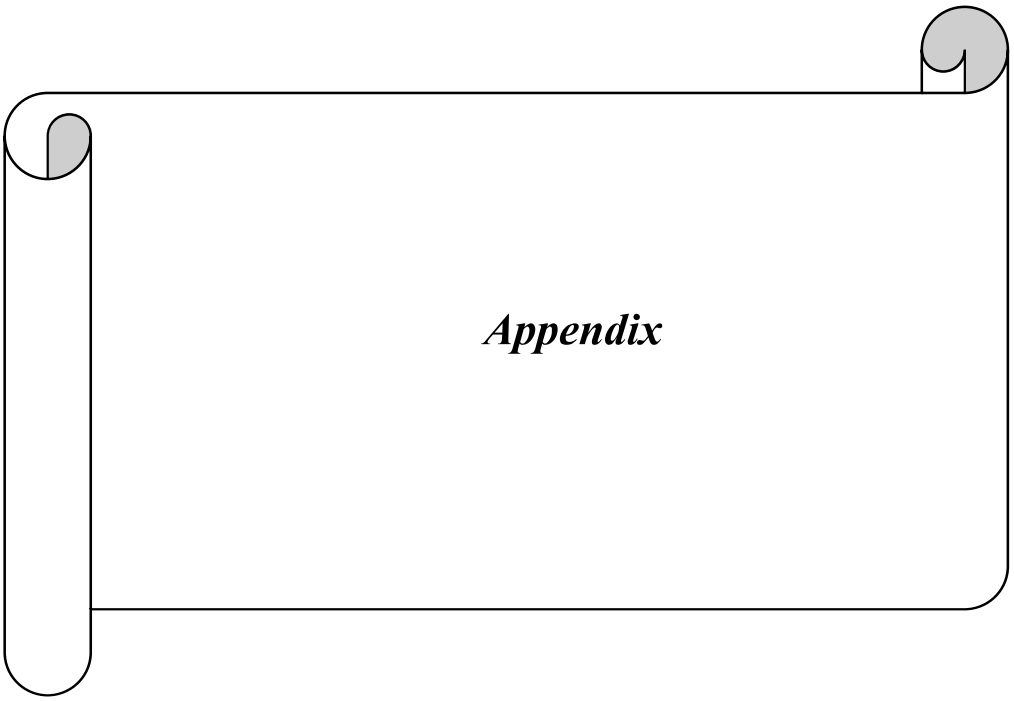
Appendix

Appendix

List of publications in peer-reviewed journals:

- Singhal, S., Hulle, N.R.S., & Koidis, A. (2024). Bioaccessibility of iron in developed pectin iron complex using *Citrus limon* peels subjected to *in-vitro* gastro-pancreatic digestion. *Food Chemistry*, *457*, 140457.
- Singhal, S., Deka, S.C., Koidis, A., & Hulle, N.R.S. (2024). Standardization of extraction of pectin from Assam lemon (*Citrus limon*) peels using novel technologies and quality characterization. *Biomass Conversion and Biorefinery*, 1-12.
- Singhal, S., & Hulle, N.R.S. (2022). Citrus pectins: Structural properties, extraction methods, modifications and applications in food systems–A review. *Applied Food Research*, *2*(2), 100215.

Participation in national/international conferences:

- Singhal, S., & Hulle, N.R.S. (2025). Development of Pineapple leather using Pectin Iron Complex (PIC) and study its quality attributes during storage Food Product and Development in Food Processing at National Conference on Emerging Technologies for Sustainable Agro-Food-Bio Systems (ET-SAFe-2025) organized by the Department of Food Engineering and Technology, Tezpur University, India (February, 2025) (**Third position**).
- Singhal, S., & Hulle, N.R.S. (2022). Extraction and characterization of pectin from Assam lemon (*Citrus limon*) peels. Waste Utilization and Management in Food Processing at International Conference on Sustainable Approaches in Food Engineering and Technology (SAFETy-2022) organized by the Department of Food Engineering and Technology, Tezpur University, India and Department of Soils, Water and Agricultural Engineering, Sultan Qaboos University, Oman (October, 2022) (**First position**).
- Singhal, S., Deka, S.C., & Hulle, N.R.S. (2021). Optimization of conventional and microwave assisted extraction of pectin from Assam lemon (*Citrus limon* Burm F.) peel. International Conference on Sustainable Approaches in Food Engineering and Technology (SAFETy-2021) organized by the Department of Food Engineering and Technology, Tezpur University, India and Department of Food Science and Technology, University of Georgia, USA (June 2021).

- Attended EFFoST International Conference (November, 2020) (Online)
- Attended 27th Indian Convention of Food Scientists and Technologists on "Raising Agro-Processing and Integrating Novel Technologies for Boosting Organic Wellness (RAINBOW)" in Tezpur University, Assam.

List of awards received:

- Received CSIR SRF-Direct Fellowship- May 2024
- Received CSIR Foreign Travel grant – October 2023
- Received Commonwealth Split-site PhD Scholarship tenable at Queen's University Belfast, United Kingdom – February 2022 -23

Table A1: Experimental parameters of Box–Behnken Design and response for conventional extraction

Run	Temperature (°C)	Time (min)	Solid-liquid ratio	Pectin yield (%)
1	75.00	60.00	0.02	13.5192
2	90.00	60.00	0.03	20.1308
3	75.00	20.00	0.01	13.1269
4	60.00	60.00	0.03	11.1654
5	90.00	100.00	0.02	20.1808
6	90.00	60.00	0.01	19.3731
7	75.00	60.00	0.02	14.6077
8	75.00	100.00	0.01	16.7654
9	75.00	100.00	0.03	18.7192
10	60.00	100.00	0.02	10.3615
11	75.00	60.00	0.02	14.6615
12	60.00	60.00	0.01	13.6923
13	60.00	20.00	0.02	8.37308
14	75.00	60.00	0.02	13.8385
15	75.00	60.00	0.02	14.8538
16	75.00	20.00	0.03	11.44
17	90.00	20.00	0.02	15.93

Table A2: Experimental parameters of Box–Behnken Design and response for microwave-assisted extraction

Run	Exposed Power (Watt)	Exposed Time (min)	Solid-liquid ratio	Pectin yield (%)
1	600.00	7.00	0.02	20.6091
2	450.00	7.00	0.03	13.5246
3	600.00	5.00	0.01	7.96753
4	450.00	5.00	0.02	7.71185
5	300.00	3.00	0.02	3.36438
6	450.00	3.00	0.01	3.3206
7	450.00	5.00	0.02	9.68663
8	300.00	5.00	0.03	5.89595
9	600.00	5.00	0.03	3.82149
10	300.00	5.00	0.01	1.99981
11	450.00	5.00	0.02	8.99826
12	300.00	7.00	0.02	10.3033
13	450.00	7.00	0.01	6.44902

14	600.00	3.00	0.02	5.18057
15	450.00	5.00	0.02	10.2827
16	450.00	5.00	0.02	11.4618
17	450.00	3.00	0.03	4.56272

Table A3: Experimental parameters of Box–Behnken Design and response for ultrasound-assisted extraction

Run	Amplitude (%)	Time (min)	Solid-liquid ratio	Pectin yield (%)
1	60.00	30.00	0.02	4.89954
2	20.00	45.00	0.02	4.38889
3	60.00	15.00	0.03	8.02083
4	100.00	15.00	0.02	11.4741
5	60.00	30.00	0.02	2.93611
6	100.00	30.00	0.03	24.1667
7	20.00	30.00	0.01	5.44028
8	100.00	45.00	0.02	31.5
9	60.00	45.00	0.03	11.1204
10	60.00	30.00	0.02	4.18287
11	100.00	30.00	0.01	11.8843
12	20.00	15.00	0.02	4.31157
13	60.00	30.00	0.02	5.76528
14	60.00	30.00	0.02	6.49213
15	60.00	15.00	0.01	4.85463
16	60.00	45.00	0.01	9.54861
17	20.00	30.00	0.03	5.27407

উদ্ভিদ বিজ্ঞান বিভাগ/ DEPARTMENT OF BOTANY
গুৱাহাটী বিশ্ববিদ্যালয়/GAUHATI UNIVERSITY
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Reference No. ***Herb./GUBH/2024/044*** **Date:** 17-03-2025

Certified that the Herbarium specimen of Somya Singhal, Department of Food Engineering and Technology, Tezpur University, Assam has been identified as appended below:

Sl. No.	Name of the Species	Family
1.	***Citrus* × *limon*** (L.) Osbeck	Rutaceae

Borah 12.3.2025
(S. Borah)
Curator, GUBH
Department of Botany
Gauhati University, Assam

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Citrus pectins: Structural properties, extraction methods, modifications and applications in food systems – A review

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ARTICLE INFO

Keywords:
Citrus pectin
extraction
Modification
Food systems, Applications

ABSTRACT

Pectin, a non-starch polysaccharide, is isolated as a byproduct from agro-processing and has numerous applications in the pharmaceutical sector, such as drug delivery, wound healing, and tissue engineering, apart from acting as a gelling, stabiliser, and thickening agent in food product formulations. The present study reviews research on pectin extraction from citrus sources and its industrial applications based on physical, chemical, functional, and rheological properties. The review also discusses studies on pectin modification using various techniques for specific applications.

1. Introduction

In 1665, Robert Hooke first identified the plant cell wall. Later, Preston and Clarke and Knox, in 1975 and 1979, respectively, characterised that the plant cell wall was present in three layers: primary wall, middle lamella, and secondary cell wall (McCann et al., 1992). The structural components of the cell wall are composed of polyuronides and polysaccharides (Ponce et al., 2010). The primary components in the cell wall include pectin, cellulose, and hemicellulose. Pectin is a complex heteropolysaccharide and is one of the crucial components determined by the multiple interaction properties within cell wall components (Dranca & Oroian, 2018). In some of the dicotyledons and monocotyledons, it contributes to one-third of the dry substance of the cell wall. Pectin in primary cells contributes to 35% in non-graminaceous monocots and dicots, 2–10% of grass, and about 5% in woody tissues (Noreen et al., 2017).

There are several sources of pectin obtained from fruits; for the past four decades, there has been a steady increase in fruit production from 338 MT to 865 MT all around the globe in which apart from banana (148 MT), citrus fruits such as oranges, soft citrus, lemons, and grapefruit (146.5 MT) contributes major role in the fruit production growth. Among the citrus fruits, the production of oranges has decreased from 67 to 55%, while grapefruit production has decreased from 8 to 7% among citrus crops. While there has been an increase in the soft citrus and lemons from 13% to 25% and 8% to 13%, respectively (World Citrus Organization, 2020).

Citrus originated in northern India and the Himalayan region of southwestern China, then spread worldwide. Citrus sources of pectin can be orange and yellow fruit types. Orange fruit types include fruits like sweet orange, mandarin, and bitter orange, while yellow fruit types include fruits such as lemon, grapefruit, and lime. From an industrial point of view, among these citrus fruits, orange is the most crucial citrus fruit, followed by grapefruit, lemons, and mandarins, respectively (Schötter et al., 2002). Citrus waste is composed of different biopolymers and requires other treatments for its application. The vast quantity of waste generated from citrus is typically low pH. It consists of organic substances such as cellulose, starch, soluble sugars, hemicelluloses, ash, fat, pectin, and water, making it more critical to analyse for further use. Due to the presence of organic matter, these may find applications for making bioplastic (Bátori et al., 2017).

Many published works report the structural properties of pectin from agricultural products depicting its utmost importance. However, limited critical studies are there for the citrus-based products especially pectin only. Thus, the review emphasises various citrus pectin extraction methods and discusses various physical or chemical functional changes take place within citrus-based pectin using different processing and extraction techniques.

2. Chemical structure of citrus pectin

Pectin refers to the glycan domains or/and family of cell wall polysaccharides. Pectin contributes approximately 70% of D-galacturonic acid remnants (GalA) associated at α-1,4 positions and several neutral sugars such as arabinose, rhamnose, and galactose. The GalA linked at α-1,4 positions can be methyl esterified or acetylated (Noreen et al., 2017; Picot-Allain et al., 2020). Polysaccharides, including pectic substances, are usually synthesised in the Golgi apparatus of the cell wall and are considered one of the complex processes.

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ORIGINAL ARTICLE

Standardization of extraction of pectin from Assam lemon (*Citrus limon* Burm f.) peels using novel technologies and quality characterization

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Abstract
The present study focuses on determining the optimal parameters of conventional (CE), microwave-assisted (MAE), and ultrasound-assisted (UAE) extraction to achieve maximum yield for pectin from Assam lemon (*Citrus limon* Burm f.) peels. In the CE, extraction time, temperature, and solid–liquid ratio were optimized. However, for MAE, exposed microwave power, time, and solid–liquid ratio were optimized while ultrasound amplitude, time, and solid–liquid ratio were optimized for UAE using Box-Behnken design. The extraction conditions had a significant effect on yield. The maximum pectin yield was 32.17% achieved by UAE while MAE and CE exhibited a pectin yield of 16.56% and 19.61%, respectively. The extract obtained under optimal conditions was characterized using Fourier transform infrared spectroscopy, X-ray diffraction, and scanning electron microscopy. The results suggest that MAE and UAE have potential to be used for sustainable extraction of pectin-rich extracts for various applications.

Keywords Assam lemon · Extraction · Pectin · Ultrasound-assisted · Box-Behnken design

1 Introduction

From the past four decades since 1980, it has been reported that there has been a steady increase in fruit production from 338 to 865 MT all around the globe, in which banana (148 MT) and citrus (146.5 MT) are the major products. Within citrus fruits, there has been a growth increase in soft citrus and lemons from 13 to 25% and 8 to 13%, respectively [1]. Citrus is one of the important components of the human diet as well as one of the major crops in the world. There are over 140 genera and 1300 species of citrus (family Rutaceae) [2]. One such species is Assam lemon (*Citrus limon* Burm. f), commonly known as acid lime, *Kaji Nemu*, or *Kagzi* lemon. It is grown mostly in the northeastern region of India. The crop is grown throughout the year, especially in the months of April–May, August–September, and November–December [2]. The fruit size varies from medium to large, contains a long elongated and oblong shape, medium-thick rind, and shining smooth surface, and has around 9–12 segments. Lemon fruit by-products encompass various components such as spent peel, seeds, compressed pulp, secondary juice (extracted from residual pulp after primary juice extraction), and leaves [3]. The peel alone constitutes nearly 50% of the fruit's wet mass post-juice extraction and is rich in pectin along with aromatic compounds, natural pigments, and polyphenols [4].

In addition to this, pectin is a natural component of plant cellular structure. It is composed of sugar molecules, primarily D-galacturonic acid, which are linked together by α-1,4 glycosidic bonds [5]. Pectin gets partially esterified with methanol or acetic acid, which gives it its unique properties [1]. It is recommended as a safe ingredient as an additive, without any limit on daily intake by the Joint FAO/WHO committee. Depending on its structural and molecular properties, including the degree of esterification (DE), galacturonic acid, and molecular weight, pectin is employed in food products. Based on the DE value, pectin is categorized into high methoxy (DE > 50%) and low methoxy pectin (DE < 50%) [6]. Due to its complex hydrophilic nature, pectin finds application as a thickener

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Bioaccessibility of iron in developed pectin iron complex using *Citrus limon* Burm F. peels subjected to *in-vitro* gastro-pancreatic digestion

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ARTICLE INFO

Keywords:
Pectin
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Caco-2 cell model
Pectin-iron complex

ABSTRACT

Pectin from the citrus peel waste has novel applications in food and biomedical industries. The present work focused on addressing iron deficiency, which is a global health concern, by developing a functional ingredient using pectin extracted from Assam lemon (*Citrus limon* Burm. F) and supplementing iron *via* the pectin-iron complex (PIC). Extracted pectin was incubated with iron chloride hexahydrate (0.90–1.80 mM) for 180 h to optimize the complexation conditions, with the optimal concentration being 1.36 mM. The iron bioavailability and its absorption in the PIC was assessed using *in-vitro* simulation digestion and Caco-2 cell monolayers. The bioaccessible form of iron in the developed PIC during the intestinal phase was 5.34 ± 0.16%, which was negligible in pectin. The absorption of bioaccessible iron in the PIC was found to be 2.93 ± 0.03%. The results demonstrated that PIC could reduce iron deficiency and increase fibre intake, leading to several health benefits.

1. Introduction

One of the major nutritional problems present globally is micronutrient malnutrition especially iron (Fe) deficiency affecting millions of people, especially in infants, children, and pregnant women. Iron deficiency is mostly prevalent in school-aged children in developing countries. The deficiency of the same induces anaemia, increased susceptibility to infection, and decreased cognitive abilities and motor activity among individuals (Kinyoki et al., 2021). The World Health Organization (WHO) has proposed three approaches to combat micronutrient malnutrition such as food diversification and education, fortification, and supplementation (Ritchie & Roser, 2024). Fe supplements, however, if taken orally could result in multiple gastrointestinal side effects due to the release of free Fe ions (Ma et al., 2021). Hence, alternative Fe supplements with limited or no side effects are highly desirable.

A study reported the effectiveness of a complex formed between a polysaccharide and Fe(III) as an oral Fe supplement. This complex demonstrated favourable characteristics such as water stability, chemical solubility, and minimal side effects (Saini et al., 2014). It was also found to be non-toxic even at high concentrations (Torino et al., 2014). However, there are certain issues linked with the utilization of polysaccharide-Fe(III) complexes such as the acidic pH in gastric fluid during the process of Fe digestion can lead to the degradation of the composition of a few polysaccharide-Fe(III) complexes, causing the release and dissolution of Fe ions (Cheng et al., 2019). Additionally, insoluble ferric compounds may develop in the intestinal juice, which may not be absorbed by the small intestine (Wang et al., 2015). The main challenge for polysaccharide-based carriers, therefore, lies in retaining Fe ions in the presence of gastric juice. Pectin, a substance with excellent gelling properties against gastric acid, is considered a promising solution. Importantly, pectin does not degrade when exposed to digestive enzymes present in the small intestine (Ma et al., 2021). Consequently, employing pectin to transport Fe ions seems to be a logical approach. The development of iron-pectin beads from the ionic gelation method has resulted in a novel system for the delivery of iron to intestinal cells (Ghibaudo et al., 2018). An innovative system for stabilizing and delivering lactic acid bacteria using pectin-iron complex beads showed that beads were stable in simulated digestive conditions and successfully released cultivable bacteria, iron, and pectin in the gut (Ghibaudo et al., 2017).

By definition, pectin is a type of anionic polysaccharide that, primarily contains of D-galacturonic acid residues linked together through α-(1 → 4)-glycosidic bonds along with different neutral sugar residues. Pectin has gained much significance globally due to its diverse range of bioactivities and effectiveness. Pectin is one of the most widely used

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Title : Extraction and characterization of pectin from Assam lemon (*Citrus limon Burm F.*) peels

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Title: Optimization of conventional and microwave assisted extraction of pectin from Assam lemon (Citrus limonBurm F.) peel

Author(s): Somya Singhal and Nishant Rachayya Swami Hulle

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