

1. Introduction

Over the past three decades, global fruit production has steadily increased, from 338 million tonnes (MT) in 1980 to 865 MT. Notably, bananas (148 MT) and citrus fruits (146.5 MT) have been major contributors to this growth. However, the growth rate of citrus fruits, encompassing oranges, soft citrus, lemons, and grapefruits, lags behind that of noncitrus fruits like mangoes, avocados, and melons by 15% (World Citrus Organization, 2019). Citrus, often hailed as the 'queen of fruits,' plays a crucial role in human diets and is a significant global crop with over 140 genera and 1300 species within the Rutaceae family (Ghosh et al., 2017). A subtle trend has emerged in the field of citrus fruits. While orange and grapefruit production has declined from 67% to 55% and 8% to 7%, respectively, soft citrus and lemons have experienced growth, increasing from 13% to 25% and 8% to 13%, respectively. This shift might be attributed to heightened competition within the citrus variety rather than with non-citrus fruits, resulting in an overall decline in the production and consumption of fresh citrus fruits in several markets (World Citrus Organization, 2019).

Assam lemon, known as Acid lime, Kaji Nemu, or Kagzi lemon, is a seedless lemon crop cultivated predominantly in the northeastern region of India. Spanning an area of 13,000 hectares in Assam, it boasts a productivity of 7.0 tonnes per hectare and is harvested throughout the year, specifically in April-May, August-September, and November-December (Ghosh et al., 2017; Kundu et al., 2022). Noteworthy for its medium to large size, elongated oblong shape, medium-thick rind, and greenish-yellow colour upon ripening, Assam lemon weighs around 81 grams with approximately 1.73 mm peel thickness (Lalramhluna and Prasad, 2016). Lemons, particularly Assam lemon (Citrus *limon*), are recognised as vital sources of natural components such as citric acid, minerals, ascorbic acid, and flavonoids. Traditionally associated with vitamin C, recent studies highlighted the crucial role of flavonoids in lemon, contributing to various biological activities, including anti-allergic, antiproliferative, anti-inflammatory, antiviral, anticarcinogenic, and antimutagenic properties (Oboh and Ademosun, 2012). Major flavanones and flavonoids in lemons, such as hesperidin, diosmin, and eriocitrin, exhibit diverse health benefits. Compared to other plant peels, Citrus fruit peels, especially from Assam lemon, contain satisfactory levels of flavonones and various polymethoxylated flavones, a rare occurrence (Roy et al., 2012).

Lemon by-products include spent peel, seeds, compressed pulp, secondary juice, and leaves. Notably, the peel alone constitutes nearly 50% of the fruit's wet mass post-juice extraction and is rich in pectin, aromatic compounds, natural pigments, and polyphenols. The physical, chemical, and biological attributes of lemons vary based on growth, development, and fruit maturity (Mukhim et al., 2015). Presently, the surge in bioplastic waste poses a significant environmental concern globally. The term "bioplastics" refers to biodegradable or renewable source-based waste and has gained popularity in recent times. Rich in various biopolymers, citrus waste presents an opportunity for eco-friendly waste utilisation. The substantial quantity of waste from citrus, often low in pH and comprising organic substances like cellulose, starch, soluble sugars, hemicelluloses, ash, fat, pectin, and water, makes it crucial to analyse for potential bioplastic applications (Batori et al., 2017).

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. The structural components of the cell wall are composed of polyuronides and polysaccharides. 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 and 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 35% in non-graminaceous monocots and dicots, 2-10% in grass, and 5% in woody tissues (Noreen et al., 2017). Pectin is a natural component of plant cellular structure. It comprises sugar molecules, primarily D-galacturonic acid, linked together by α-1,4 glycosidic bonds. Pectin is partially esterified with methanol or acetic acid, giving it unique properties (Picot-Allain et al., 2020). 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 characteristics, such as its molecular weight, galacturonic acid, and degree of esterification (DE), pectin is used in value added products. Pectin is categorised into high methoxy (DE>50%) and low methoxy pectin (DE<50%) based on the DE value (Flutto, 2003). Due to its complex hydrophilic nature, pectin finds application as a thickener (Islam et al., 2023), texture modifier (Kazemi et al., 2023), emulsifier (Firat et al., 2023), gelling, and coating agent in the food sector (Gurev et al., 2023, Zhou et al., 2023). Pectin was initially

used mainly in processing jams, jellies, and marmalades (Akusu and Chibor, 2020). Recently, pectin has gained importance in food products such as bakery fillings (Ajibade and Ijabadeniyi, 2019), prebiotics and dairy products (Gomez et al., 2016; Yuliarti et al., 2019; Khubber et al., 2021), soft drinks, fruit beverages, confectionery, conserves and glazing (Chandel et al., 2022).

Extraction of bioactive products from natural sources, such as lemon peels or pomace, involves challenges due to high moisture content, making them susceptible to fungal enzyme degradation. Fungal enzymes, including de-esterifying (pectin methylesterase) and depolymerising enzymes (pectin lyase, polygalacturonase, and pectate lyase), play a key role in breaking down pectin (Chandel et al., 2022). Various factors significantly influence pectin yield, including particle size, pH, temperature, extraction duration, solvent type (Yeoh et al., 2008), and drying methods (Monsoor, 2005). Smaller substrate particles yield more pectin as they contain more protopectin than larger particles (Ma et al., 2019). Smaller substrate particles yield more pectin as they contain more protopectin than larger particles (Ma et al., 2019). Therefore, novel extraction techniques like ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) offer cleaner, more efficient, and environmentally friendly alternatives. UAE reduces solvent usage, shortens extraction time, enhances repeatability, increases product purity, and consumes less energy than conventional methods (Colodel et al., 2019). Microwaveassisted extraction, another innovative method, offers low energy consumption, easy control, short processing time, minimal solvent requirements, affordability, high efficiency, and cleanliness (Tongkham et al., 2017).

Various physical, enzymatic, and chemical modifications are employed to enhance pectin's functionality for industrial applications. These modifications result in changes in antioxidant activity, thermal and pH stability, and biochemical properties. Complexing pectin with proteins or minerals, such as iron and zinc, has been explored to improve stability and functionality (Chua et al., 2020).

One of the significant nutritional problems globally is micronutrient malnutrition, especially iron deficiency, affecting millions of people, especially infants, children, and pregnant women (McLean et al., 2009). Iron deficiency in developing countries is prevalent in school-aged children (Best et al., 2010). The same deficiency induces anaemia, increased susceptibility to infection, decreased cognitive abilities and motor

activity among the individuals (Doom and Georgieff, 2014). The World Health Organization (WHO) has proposed three approaches to combat micronutrient malnutrition such as food diversification and education, fortification, and supplementation (World Health Organization, 2006; Sabatier et al., 2020). Iron supplements, however, if taken orally could result in multiple gastrointestinal side effects due to release of free iron ions (Lei et al., 2008; Cancelo-Hidalgo et al., 2013, Ma et al., 2021). Hence, alternative iron 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 iron supplement. This complex demonstrated favourable characteristics such as chemical stability, water solubility, and minimal side effects (Viteri et al., 2012; Saini et al., 2014). It was also non-toxic even at high concentrations (Torino et al., 2014). However, there are specific issues associated with the utilisation of polysaccharide-Fe(III) complexes, such as the acidic pH of gastric juice during the process of iron digestion can lead to the degradation of the structure of some polysaccharide-Fe(III) complexes, causing the release and dissolution of Fe ions (Cheng et al., 2019). Additionally, insoluble ferric compounds may form in the intestinal juice, which the small intestine cannot absorb (Wang et al., 2015; Lu et al., 2016). Therefore, the main challenge for polysaccharide-based carriers lies in retaining Fe ions in the presence of gastric juice. Pectin, a substance with excellent gelling capacity in gastric acid conditions, has been recognised as a promising solution (Khramova et al., 2019). Significantly, pectin does not degrade when exposed to digestive enzymes in the small intestine (Das et al., 2010; Ma et al., 2021). Consequently, employing pectin as a carrier for Fe ions seems to be a logical approach.

Several studies have been published on the other varieties of *Citrus limon*. However, the Assam variety of *Citrus limon* needs further investigation due to limited scientific information. This variety of Assam lemon is native to the state with a GI Tag and has a unique flavour and morphology, and the peel is generally discarded or pickled. This study's objective is to extract the pectin from Assam lemon peel using different extraction techniques such as conventional extraction (CE), microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) and standardisation of pectin extraction conditions and their quality characterisation using the degree of esterification, X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) analysis. Moreover, this study aims to synthesise the pectin iron

complex as a potential novel iron supplement by complexing citrus pectin with ferric ions. The obtained complex was characterised using Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), Degree of esterification (DE) and galacturonic acid analysis. Furthermore, iron absorption from citrus pectin complex was investigated by *in-vitro* assay and Caco-2 cell model. Pectin complex-based pineapple leather was formulated using a mixture design to optimise the formulation by measuring the total phenolic content, browning index, and antioxidant activity. Additionally, sensory attributes were examined to determine the product's acceptability. The produced pineapple leather was evaluated for water activity, moisture content, browning index, total phenolic content, antioxidant activity, and microbial stability during storage.

Apart from the aforementioned reasons, the novelty of the present study lies in the valorisation of regionally abundant yet underutilised Assam lemon peel through environment-friendly extraction techniques and synthesis of a novel pectin-iron complex for application in food systems. This approach promotes sustainability by minimising agro-waste and promoting circular bioeconomy, in addition to the challenges associated with the pectin utilisation and iron delivery. Furthermore, the incorporation of the pectin-iron complex into the value-added products, such as fruit leather, as well as extensive characterisation and stability investigations, adds practical value and translational significance to this work.

The whole work is divided into the following objectives to achieve the aim of the present research/thesis:

- 1) To standardise the microwave and ultrasound-assisted extraction of pectin from Assam lemon peel and its physicochemical characterisation
- 2) To synthesise pectin-iron complex from Assam lemon peel pectin, its characterisation and iron bio-accessibility
- 3) To develop a functional food product using the pectin-iron complex and study its storage stability

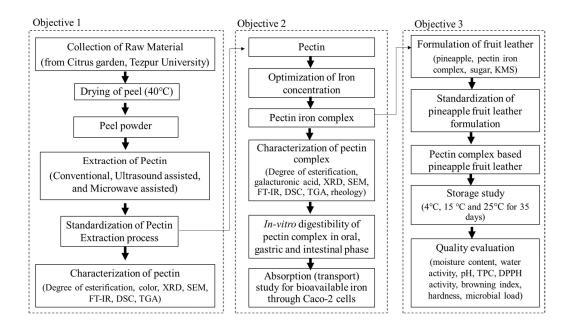


Figure 1.1. Overview of the objectives