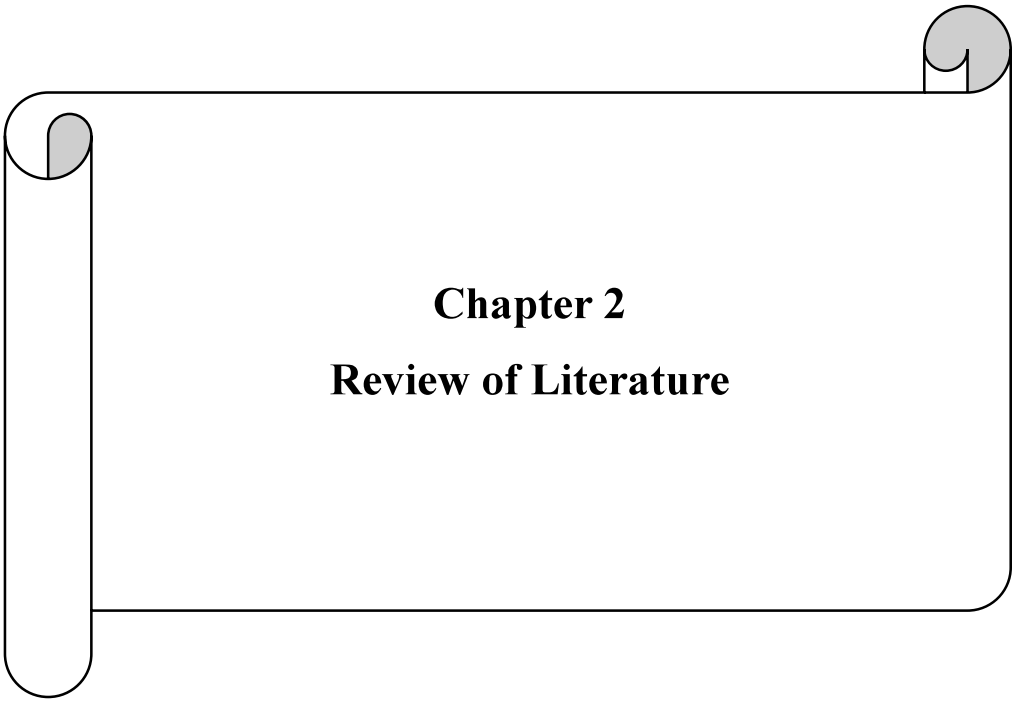

# Chapter 2

# Review of Literature

## 2.1. 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 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).

There are several sources of pectin obtained from fruits; for the past four decades, fruit production has steadily increased from 338 MT to 865 MT globally. Besides bananas (148 MT), citrus fruits such as oranges, soft citrus, lemons, and grapefruit (146.5 MT) contribute significantly to 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. At the same time, there has been an increase in 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 and then spread worldwide. Citrus sources of pectin can be orange and yellow fruit types. Orange fruit types include sweet orange, mandarin, and bitter orange, while yellow fruit types include 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 (Schöttler et al., 2002). Citrus waste comprises 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 exist for citrus-based products, especially pectin only. Thus, the review emphasises various citrus pectin extraction methods and discusses multiple physical or chemical functional changes within citrus-based pectin using different processing and extraction techniques.

### 2.2. Assam lemon

One such species is Assam lemon (*Citrus limon*), commonly known as Acid lime, *Kaji Nemu* or *Kagzi lemon*. It is a seedless *limon* crop grown mainly in the northeastern region of India. Being citrus, Assam lemon is generally used as a flavouring agent in fish, vegetarian, meat and meat products, apart from preparing beverages, including cordials, marmalades and cold drinks. The physical, chemical and biological attributes of lemon depend on numerous factors, such as the growth, development and maturity of the fruit (Mukhim et al., 2015). The crop is grown throughout the year, especially in April-May, August-September and November-December (Ghosh et al., 2017). Its size varies from medium to large, has a long elongated and oblong shape, medium-thick rind, shining smooth surface and has around 9-12 segments. It has comparatively higher juice content and is more acidic compared to lemon. As soon as the Assam lemon ripens, it turns from green to greenish-yellow in colour. Each fruit weighs around 81 g and has about 1.73 mm peel thickness (Lalramhluna and Prasad, 2016). Compared with the peels of other plants, it has been found that the peel of Citrus fruit has a satisfactory amount of flavonones and various polymethoxylated flavones, which is quite rare (Roy et al., 2012). In general, the potential of lemon has only been associated with the vitamin C levels present in them and has not been paid much consideration to the flavonoids present in them. In recent studies, it has come to know that flavonoids have an important role to play in several biological activities, including antiallergic, antiproliferative, anti-inflammatory, antiviral and anticarcinogenic antimutagenic properties. Due to such properties, lemon can be used as an active component in food as well as in the pharmacological field. Some of the major flavonones and flavonoids present in lemon are hesperidin, diosmin and eriocitrin (Del Rio et al., 2004).

Research focusing on the Assam variety of *Citrus limon* has been done to see the effect of the ripening stage, bio-fertilizers, pruning and alteration in cultivation practices on the yield, quality and storage of Assam lemon. However, limited work has been done on the

extraction of vital components and its application. In the 90s, a few studies were conducted by Chaliha et al. (1963) to study the acid extraction of pectin from Assam lemon, Misra et al. (1988) to study the effect of essential oil extracted from lemon peels against a few dermatophytes and Singh et al. (1994) to analyse the in-vitro propagation of 'Khasi mandarin' and 'Assam lemon'. To the best of our knowledge, other studies reported on Assam lemon include the following. Firstly, the genetic resources of Citrus of northeastern India and their potential use (Sharma et al., 2004). The study brings to notice that Assam lemon is resistant to scab, canker and gummosis. Secondly, a combination of drip irrigation and rainwater harvesting to increase lemon productivity (Barua, 2013). Thirdly, the effect of different stages of fruit growth and development on the physicochemical attributes of Assam lemon (Mukhim et al., 2015) It has been found in the present study that fruit when harvested at 120-130 days post-to-fruit set, exhibited the most reliable maturity indices for harvesting the Assam lemon. Fourthly, the growth of Assam lemon under Allahabad agro-climatic condition (Lalramhluna and Prasad, 2016). Fifthly, impact of various pruning severity and nutrient management on the yield and quality of Assam lemon (Ghosh et al., 2016). Sixthly, the effect of different stages of fruit growth and development on the pectin and chlorophyll content present in Assam lemon (Mukhim et al., 2016). The maximum pectin content of about 3.07% in Assam lemon was found to be at the early stage, viz. at 60 days after fruit set (DAF). With the increase in the time of maturity, the pectin content decreased. From the study, it has been found that the optimum stages for commercial extraction of pectin can be at 110 DAF (2.44%) to 130 DAF (1.56%) based on the acceptable fruit quality. Seventhly, the effect of various pruning intensities and nutrient management on the yield and quality of Assam lemon (Ghosh et al., 2017) Mrig bahar resulted in the best quality of the fruit, followed by Hasth bahar and Ambe bahar due to favourable agro-climatic conditions prevailed during fruit growth and developmental period. Lastly, analysis of bio-fertilizers, organics and bioagents along with inorganic fertilizers on yield and growth of Assam lemon (Hazarika and Aheibam, 2019).

### 2.3.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 $\alpha$-1,4 positions and several neutral sugars such as arabinose, rhamnose, and galactose. The GalA linked at $\alpha$-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.

Pectin synthesis engages multiple particular enzymes in the form of catalysts to form different glycosidic linkages and modify glycosyl residues in pectic chains. The enzymes employed in pectin synthesis include acetyltransferase, arabinosyltransferase, glycosyltransferase, galacturonosyltransferase, glucuronosyltransferase, methyltransferase, and xylosyltransferase (Dranca and Oroian, 2018).

Homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) are the three distinct domains of pectin. The linear homopolymer, or HG, makes up roughly 65% of the pectin found in plant cell structures. Because it lacks side chains, HG is regarded as a smooth area of pectin and contributes $\alpha$-(1–4) linked GalA residues. RG-I makes up a maximum of 20–35% pectin after HG. RG-I has a higher number of neutral sugars and a more complicated structure than HG. It contains roughly a hundred repetitive units of (1,2)- $\alpha$-L-Rha- (1,4)- $\alpha$-D-GalA. Compared to RG-I, RG-II has a more complicated structure and contains about 10% pectin. It is essential to the structure of plant cells even though it contributes less than pectin. Because of their non-ionic side chains, RG-I and RG-II are both regarded as the hairy regions of pectin (Dranca and Oroian, 2018; Noreen et al., 2017; Picot-Allain et al., 2020). The galacturonic acid content of pectin in citrus fruits ranges from 58.5 to 85.4%, while the degree of esterification ranges from 6.77 to 85.7%, as shown in Table 2.1 for various citrus fruits.

### 2.4. Citrus pectin and their properties

The degree of methylation (DM) and acetylation (DAc) refers to the number of methoxy and acetyl groups that can substitute the carboxylic acid groups in the GalA residues. Based on DM and DAc, pectin is classified broadly into two groups- high methoxy pectin (HMP having DM > 50%) and low methoxy pectin (LMP having DM < 50%) (Łękawska-Andrinopoulou et al., 2013). LMP is prepared from HMP using de-esterifying agents such as acids, ammonia, alkali, and pectin methylesterase in aqueous ammonia (concentrated form) or alcohol. LMP is less sensitive to acidity, while HMP is extremely sensitive. Due to hydrophobic interactions and hydrogen bonding between pectic chains, the pectin requires a higher concentration of soluble acids to form gel. LMP is commonly used in the

food industry to make reduced sugar jams since it has gelling qualities due to calcium ions, whereas HMP finds applications in the canning industry. (Noreen et al., 2017; Pérez et al., 2003).

Pectin is both soluble and insoluble in water based on its alkali metal salts. Monovalent cations in pectin are entirely water-soluble, whereas divalent and trivalent cations are partly or entirely water-insoluble. The dissolved pectin is decomposed instantly through depolymerisation or de-esterification, and the rate of decomposition is mainly due to the temperature and pH of the solution. The combination of increased temperature and low pH inflates the decomposition rate due to the hydrolysis of glycosidic association. The pectin is highly stable at a pH near 4.0 and degrades or de-esterifies rapidly at alkaline pH at room temperature (Noreen et al., 2017; Rolin, 2002).

### 2.5. Extraction of pectin

Several methods extract the pectin effectively depending on mass transfer in the solvents employed for extraction. The higher the qualitative yield obtained, the more suitable the extraction method. Based on the “green chemistry” principle, multiple novel technologies such as high-pressure processing, microwave, ultrasound, and enzyme-assisted extraction are used for pectin extraction apart from conventional extraction, as mentioned in Table 2.1. The novel technologies boosted the economy by limiting the processing time, use of strong acids, and high energy input.

#### 2.5.1. Conventional extraction

Pectin is conventionally extracted in an aqueous medium of acidic nature (pH 1.5–3) at the temperature range of 75–100 ◦C for 1–3 h (Table 2.1). The extracted pectin is then further clarified and concentrated. The yield of qualitative pectin is affected by pH, solid-solvent ratio, particle size, extraction temperature, and time. With green chemistry principles, the mineral acids can be replaced by organic acids (such as acetic acid and citric acid) using "green chemistry". Due to these factors, a considerable variation is reported in the yield of pectin obtained. Although organic acids have lower hydrolysing abilities than mineral acids, organic acids are still employed to get compounds of “clean label” (Picot-Allain et al., 2020).

**Table 2.1.** Extraction of pectin from citrus-based sources

| Fruit/Fruit by-product | Treatment | Yield of pectin (%) | Galacturonic acid | Degree of esterification (%) | References |
|---|---|---|---|---|---|
| **Conventional Extraction** | | | | | |
| Pomelo peel | Solvent: Hydrochloric acid<br>Temperature/Time: 90°C/90 min<br>pH: 2.0 | 3.11 | - | - | Chen et al., 2016 |
| Honey pomelo peel | Solvent: Hydrochloric acid<br>Temperature/Time: 85°C/80 min<br>pH: 1.24 | 17.5 | 749 g/kg | 76.6 | Guo et al., 2017 |
| Ponkan peel | Solvent: Nitric acid<br>Temperature/Time: 100°C/100 min<br>pH: 1.6 | 25.6 | 84.5% | 85.7 | Colodel et al., 2018 |
| Citron peel | Solvent: Water<br>Temperature/Time: 90°C/180 min | 21.85 | - | 77 | Pasandide et al., 2017 |
| Orange peel | Temperature/Time: 100°C/3 h<br>pH: 1.5 | 18 | - | - | Yeoh et al., 2008 |
| Orange | Solvent: Water<br>Time: 24 h | 15.25 | - | - | Sulieman et al., 2013 |

| | | | | | |
|---|---|---|---|---|---|
| Grapefruit | Temperature/Time: 90°C/90 min<br>pH: 1.5 | 19.16 | 69.9% | 75.6 | Bagherian et al., 2011 |
| Lemon | Solvent: Water<br>Time: 24 h | 20.75 | - | - | Sulieman et al., 2013 |
| **Microwave-assisted Extraction** | | | | | |
| Yellow passion fruit peel | Solvent: Tartaric acid<br>Power/Time: 628 W/9 min<br>pH: 2.0 | 30.3 | 58.5% | 50 | Seixas et al., 2014 |
| Pomelo peel | Solvent: Hydrochloric acid<br>Power/Time: 520 W/5.6 min<br>pH: 2.0 | 3.29 | - | - | Chen et al., 2016 |
| Dragon fruit peel | Solvent: Nitric acid<br>Power/Time: 450 W/5 min<br>pH: 2.0 | 21.6 | 66.16% | 57.50 | Tongkham et al., 2017 |
| Sour orange peel | Solvent: Citric acid<br>Power/Time: 700W/2 min<br>pH: 1.5 | 26.4 | 71% | 37.5 | Hosseini et al., 2016 |
| Orange peel | Solvent: Sulphuric acid<br>Power/Time: 422 W/ 2.8 min<br>pH: 1.4 | 19.24 | - | - | Maran et al., 2013 |
| Orange peel | Power/Time: 900 W/10 min | 18 | 66.5% | 74.8 | Kratchanova et al., 2004 |

| | | | | | |
|---|---|---|---|---|---|
| Grapefruit | Power/Time: 900W/6 min | 27.81 | 74.86% | 79.35 | Bagherian et al., 2011 |
| Lemon | Solvent: Hydrochloric acid<br>Power/Time: 360 W/1 min | 7.31 | - | 51.08 | Karbuz and Tugrul, 2021 |
| | Solvent: Nitric acid<br>Power/Time: 360 W/1 min | 9.71 | - | 50.65 | Karbuz and Tugrul, 2021 |
| Mandarin | Solvent: Hydrochloric acid<br>Power/Time: 360 W/1 min | 7.47 | - | 51.09 | Karbuz and Tugrul, 2021 |
| | Solvent: Nitric acid<br>Power/Time: 360 W/1 min | 7.60 | - | 51.07 | Karbuz and Tugrul, 2021 |
| **Ultrasound-assisted extraction** | | | | | |
| Yellow passion fruit peel | Solvent: Nitric acid<br>Specific conditions: 644 W/cm$^2$<br>Temperature/Time: 85°C/10 min<br>pH: 2 | 12.67 | 66.65% | 60.35 | de Oliveira et al., 2016 |
| Bitter/sour orange | Solvent: Citric acid<br>Specific conditions: 150 W<br>Time:10 min<br>pH: 1.5 | 28.07 | 65.3% | 6.77 | Hosseini et al., 2019 |
| Grapefruit | Temperature/Time: 70°C/25 min | 17.92 | 68.21% | 75.12 | Bagherian et al., 2011 |
| Lemon | Solvent: Hydrochloric acid | 8.60 | - | 51.13 | Karbuz and Tugrul, 2021 |

| | | | | | |
|---|---|---|---|---|---|
| | Temperature/Time: 75°C/45 min | | | | |
| | Solvent: Nitric acid | 10.11 | - | 50.51 | Karbuz and Tugrul, 2021 |
| | Temperature/Time: 45°C/45 min | | | | |
| Mandarin | Solvent: Hydrochloric acid | 11.29 | - | 51.63 | Karbuz and Tugrul, 2021 |
| | Temperature/Time: 75°C/45 min | | | | |
| | Solvent: Nitric acid | 10.33 | - | 51.16 | Karbuz and Tugrul, 2021 |
| | Temperature/Time: 75°C/45 min | | | | |
| **Enzyme assisted extraction** | | | | | |
| Yellow passion fruit peel | Specific conditions: 30 U/mL protopectinases | 26 | 85.4% | 67.5% | Vasco-Correa and Zapata, 2017 |
| | Temperature/Time: 37°C/45 min | | | | |
| | pH: 3.0 | | | | |
| Lime peel | Buffer: Citric acid buffer | 23 | 81.3% | 82.2% | Dominiak et al., 2014 |
| | Specific conditions: Laminex C2K (have cellulose and hemicellulose, namely, arabinoxylanse and xylanase) | | | | |
| | Temperature/Time: 50°C/4 h | | | | |

### 2.5.2. Microwave-assisted extraction (MAE)

Microwave-assisted extraction utilizes microwave energy to enhance the mass transfer rate into the solvents used. The operation of a microwave is based on the application of a microwave field to a dielectric substance. The matrix of both the sample and solvent is heated through ionic conduction and dipole rotation. Microwave energy concurrently induces the electrophoretic movement of electrons and ions, so creating an electric field. The induced electric field promotes particle movement, whereas the reconfiguration of polar molecules results in dipole rotation. These pathways jointly facilitate energy release, resulting in heat production (Hu et al., 2019). MAE demonstrates superior efficiency relative to traditional extraction for extraction duration, solvent volume, and pectin yield. From Table 2.1, it is seen that the extraction time while using MAE has reduced up to 10 times compared to that of conventional extraction. The pectin yield increased, whereas the degree of esterification decreased compared to conventional extraction (Picot-Allain et al., 2020).

In grapefruit, on the other hand, it was found that the degree of esterification did not differ considerably from that obtained from traditional extraction. Increased galacturonic acid content might be due to the relatively easy penetration of sodium hydroxide into the plant tissue during titration, which is more acceptable in food applications (Bagherian et al., 2011). While studying pectin extraction from pomelo, Liew et al. (2016a) reported that the yield of peels also increased with an increase in the microwave power from 350–650 W. If extraction time is more at low microwave power, then it might sustain the purity of the extracted sample; if done vice versa, then there could be a loss of phytonutrients due to overheating. In multiple studies, the citrus pectin extraction through MAE has been in the range of 360–900 W, while commercially, in food industries, the power employed in the microwave is 915 and 2450 MHz.

### 2.5.3. Ultrasound-assisted extraction (UAE)

Ultrasonic waves are mechanical vibrations used for extraction ranging from 20 to 100 kHz. During the pressure of acoustic waves, the varied fluctuations result in microbubbles, which then form microjets. These microjets, when collapsing, disrupt the cellular level, which helps in solvent penetration and mass transfer more efficiently. From Table 2.1, UAE exhibits a shorter extraction time, low energy requirement, less solvent, and higher

pectin yield at a lower temperature than conventional extraction. The increased ultrasound intensity increases pectin yield (Ilghami et al., 2015; Rutkowska et al., 2017). Pectin from citrus sources, including orange and passion Fruit, was extracted through UAE in 10–15 min at around 70–85°C temperature (Table 2.1). Seshadri et al. (2003) reported that ultrasonication treatment negatively affected the rheological properties of pectin. More gel formation time was identified with increased ultrasonic treatment intensity and sonication period. In agreement, Panchev et al. (1994) also concluded that ultrasonically pretreated pectin exhibited weak gelling properties with increased sonication time and power.

They observed that the yield of extracted pectin was higher; however, the degree of esterification decreased by 6%. Besides the degree of esterification value, molecular weight also decreases with increasing power and time; however, galacturonic acid exhibited an increasing trend. This might be due to the increased production of cavitation bubbles at increased temperatures. More force is required to break these bubbles, and more will be the shear stress, which will break down the pectin chain into smaller components. Thus, optimum temperature, power, and time are vital in attaining high pectin yields from various sources (Bagherian et al., 2011). Zhang et al. (2015) analysed the impact of ultrasound-assisted and ultrasound degradation on the structural properties of pectin. The study concluded that the degree of esterification decreased for both ultrasound-assisted and ultrasound-degraded pectin compared to commercial pectin, whereas pectin degraded by acid resulted in a negligible esterification value. Acid-degraded pectin was entirely devoid of neutral sugar side chains.

Xu et al. (2014) found a significant correlation between the swelling index of plant tissue and pectin yield when examining the impact of ultrasonic and thermal treatment on pectin extraction from grapefruit peel. Ultrasound aided extraction by disrupting vegetative tissue. An interaction or synergistic impact of heating and ultrasound was identified, leading to an enhanced yield (up to 27%) and reduced extraction times (up to 52 min).

### 2.5.4. High pressure assisted extraction (HPAE)

There are limited studies that analyse the efficacy of pectin extraction through high-pressure processing. The high-pressure processing method has been primarily used as a pretreatment while extracting through conventional and other novel technologies. Naghshineh et al. (2013) investigated the effect of high pressure on the enzymatic

extraction of pectin. The study reported that high-pressure exhibits polymer degradation and structural changes. Upon the removal of pressure, water molecules reassemble, resulting in a bulk water suspension that enhances the substrate's accessibility for enzymatic processes, hence resulting high-pressure treatment a viable method for pectin extraction. Guo et al. (2012) discovered that as pressure climbed from 100 to 600 MPa, pectin yield increased almost twice, primarily between 20 to 45°C. In contrast, viscosity increased with pressure up to 500 MPa but then fell dramatically at 600 MPa. A similar result was obtained at temperatures ranging from 45 to 55°C, which could be attributed to an increase in pectin solubility at rising temperatures, leading to an elevated extraction rate and yield. Another option is to increase the extraction temperature. Alternatively, greater extraction temperatures may cause degradation or denaturation of the extracted pectin (Masmoudi et al., 2008). Besides pectin, high pressure enhances the extraction of beneficial compounds. Several studies, including Prasad et al. (2009) and Xi et al. (2009), reported that ultra-high-pressure processing could be used convincingly to extract several bioactive components due to high efficiency and low time consumption.

Pectin extracted from orange peels under ultra-high pressure exhibits no significant variation in the DE or anhydrouronic acid content; nonetheless, the obtained pectin positively affects activation energy (Guo et al. 2012). The elevated activation energy indicated interactions among polysaccharide chains at the specified concentration (Wang et al., 2009). Similar to activation energy, ultra-high pressure positively influenced pectin, enhancing its texturizing and stability properties for prospective applications in pharmaceutical and food items (Guo et al., 2012).

### 2.5.5. Enzyme assisted extraction (EAE)

The efficiency of the extraction of pectin through EAE highly depends on the potential of enzymes, as they are responsible for conducting the reaction with adequate selectivity and specificity. Extraction time through EAE decreases by the action of the enzymes on the cell components, causing cell disruption and, thus, releasing the pectin (Yang et al., 2018a). The primary merits of UAE over other extraction techniques include preventing equipment from corrosion caused by strong acids, higher quality pectin due to the specificity of enzymes, and low energy input due to the processing at low temperatures (Marić et al., 2018). The commonly employed enzymes for extracting pectin are cellulases, hemicelluloses, xylanases, proteases, and protopectinases. These enzymes are costly, thus

making a significant demerit among other techniques. This technique uses buffers instead of strong acids as a solvent, as enzymes are sensitive to pH. From Table 2.1, extraction through UAE employs a lower temperature; however, it is more time-consuming (Picot-Allain et al., 2020). Liew et al. (2016b) isolated pectin from passion fruit peels with the help of a commercial enzyme, cellucast (1.67% w/w), at 61°C. Although the pectin yield from this technique is similar to that of conventional extraction (Table 2.1), the quality of pectin extracted from this method was significantly higher than that of traditional extraction. Similarly, Dominiak et al. (2014) extracted pectin from lime peel by employing laminex isolated by *Penicillium funiculosm* at pH 3.5 for 4 h maintained at 50°C. The resulting pectin exhibited about 82% methylation.

### 2.5.6. Hydro-cavitation assisted extraction

Hydro-cavitation assisted extraction is also based on green extraction technology like MAE and UAE. Hydrodynamic cavitation involves pumping the extraction solvent or liquid through the single or multiple constrictions of venturi tubes or orifice plates. Pumping liquid creates pressure, which increases the kinetic energy within the constriction, resulting in the production of microbubbles and nanobubbles. Pressure recovery causes the created microbubbles and nanobubbles to collapse, resulting in localized hot areas (Gogate and Pandit, 2001). These hot spots produce exceptional turbulence, high energy density and extremely reactive free radicals, which change a variety of physical and chemical processes (Meneguzzo et al., 2019). The primary merits of hydro-cavitation extraction over other extraction techniques include high process yields, less processing time, affordability and straightforward scalability (Albanese et al., 2019; Holkar et al., 2019).

Limited studies analyse the efficacy of pectin extraction through hydrodynamic cavitation processing. Most of these studies focus on the assessment of the antibacterial or neuroprotective effect of extracted pectin through this technology. For instance, Presentato et al. (2020) and Nuzzo et al. (2021) found that pectin extracted from grapefruit and lemon waste using this process had stronger antibacterial and neuroprotective action than commercial pectin. Meneguzzo et al. (2020) found that extraction of pectin from citrus waste using hydro cavitation leads to the migration of polyphenols, such as hesperidin, to the surface, creating a covalent conjugate with the recovered pectin, yielding hesperidin-rich pectin.

### 2.5.7. Combination of non-conventional techniques

Several studies have been done to study the interaction of various novel technologies on the extraction of vital components from the source, such as Liew et al. (2016a) studied the interaction of microwave, sonication, and irradiation on the extraction of pectin from pomelo peels. The study concluded that sonication alone consumes much more time to obtain the effect, while when combined with microwave and irradiation, time was significantly reduced and achieved higher pectin quality. In another study, Naghshineh et al. (2013) extracted pectin from lime peel powder using enzymatic extraction and a high-pressure treatment. In the study, researchers added specific enzymes to the peel and citrate buffer mixture as the solvent, then packed and treated with high pressure. The combination enhanced the pectin extraction yield as high pressure improves the rate of enzyme-catalysed reactions by changing the enzymatic structure and solid sol- vent properties such as density, viscosity, and pH (Eisenmenger and Reyes De Corcuera, 2009).

Among the different extraction technologies, such as MAE, HPAE, UAE, and ultra-high-pressure extraction (UHPE), UHPE proved to be the most efficient pectin yield and extraction time. In a study conducted by Guo et al. (2012), an increase in the extraction yield with increased pressure was reported. High pressure also has a positive effect on the extraction of phytochemicals. In the case of orange peel, UHPE resulted in a significantly higher yield than MAE, followed by conventional extraction.

## 2.6. Rheological properties of pectin

Pectin has miscellaneous applications in multiple sectors as glazing, thickening, emulsifying, stabilising, and glazing agents, contributing to the material's rheology. Rheology is the study of the flow of matter upon deformation by analysing the interaction between stress, strain, and time. In general, mostly all polymer solutions represent shear thinning behaviour exhibiting a decrease in the apparent viscosity with the increase in the shear rate that occurred due to the liberation of polymer chains. In addition, factors such as pH, temperature, sucrose concentration, and divalent ion concentration affect the rheological properties of pectin solutions (Picot-Allain et al., 2020).

The viscosity of the pectin solution positively changes with the change in pectin concentration in the solution. This is due to the decrease in the intermolecular distance

present between the pectin molecules, which results in hydrogen bonding, such as intermolecular interactions and entanglements of polymer chains. Other studies exhibited an increase in the pectin content, and the consistency index of the solution increased (Guo et al., 2012; Picot-Allain et al., 2020). In addition to pectin concentration, other intrinsic biopolymer factors like the degree of esterification, galacturonic acid, molecular mass and ionic strength, pH, and temperature of the medium also contribute to the viscosity of the solution (Picot-Allain et al., 2020).

Similarly, Gamonpilas et al. (2015) concluded that divalent ion mechanisms resulted in the gel formation from pomelo pectin. It has also been reported that the pectin gel modulus also increased with an increase in the ion size ($Mg^{2+} < Ca^{2+} < Ba^{2+}$). The viscosity of pectin gels is also affected by the degree of esterification. Another crucial factor is pectin's molecular weight, affecting pectin's rheological properties in different food systems. For instance, in low molecular weight pectin, there are comparatively fewer junction sites, resulting in reducing the cross-linking interactions and, thus, altering its gelling properties (Picot-Allain et al., 2020).

Applying heat while extracting the pectin from diverse sources significantly affects the viscosity of pectin gels. Although pectin extracted at higher temperatures results in higher pectin yield, it affects the polymeric structure and the intermolecular interactions between them (Hua et al., 2015a). This phenomenon may be attributed to enhanced pectin solubilisation, which results in increased depolymerisation, reduced chain lengths, and reduced viscosity. Pectin isolated from citron peel demonstrated pseudoplastic flow characteristics with increasing shear rate (Pasandide et al., 2017). When the polymer chain aligned in the direction of the shear flow, the viscosity of the pectin solution dropped, which led to the disentanglement of polymer branches and a reduction in intermolecular stress (Picot-Allain et al., 2020).

Guo et al. (2012) examined the impact of the extraction process on pectin derived from orange peel. The study concluded that with the increase in the extraction pressure (up to 500 MPa), extraction temperature (20–45°C), and pressure holding time (5–10 min), the viscosity of pectin also increased. It was noticed that pectin extracted through ultra-high pressure exhibited almost two times intrinsic viscosity and average molecular weight compared to conventional heating and microwave-assisted extraction. With the increase in the pectin concentration, gelling strength also increased, increasing the storage modulus

(G′). Storage modulus (G′) was significantly much higher than the loss modulus (G″) within the frequency range of 0.1 to 10 Hz (Guo et al., 2012). A further investigation on *Citrus junos* found that pectin isolated from it using a combination of enzymatic and physical methods displayed shear-thinning capabilities under regulated shear settings. Chemically modified pectin exhibited increased apparent viscosity (Lim et al., 2012). Ultrasound-treated citrus pectin exhibited enhanced thermal stability compared to untreated citrus pectin; nevertheless, its shear-thinning behavior was reduced (Chen et al., 2021). Another study on citrus pectin reflected that ultrasonic treatment at higher pH significantly reduced the pectin's weight-average molecular weight and intrinsic viscosity (Yan et al., 2021).

### 2.7. Modification of pectin

Various physical, enzymatic, and chemical variations are possible in the structure of pectin to improve its functionality; the modifications result in changes in antioxidant activity and biomedical activities. These functionalities in pectin can be achieved either by substitution (such as amidation, alkylation, sulfation, and thiolation), depolymerisation (enzymatic or acidic hydrolysis, $\beta$ elimination, and mechanical degradation), or chain elongation (crosslinking and grafting) (Chen et al., 2015a).

#### 2.7.1. Substitution

Substitution techniques involve substituting distinct groups, such as carboxylate or acetyl, through alkylation, amidation, sulfation, and thiolation to alter the properties of the desired pectin. The carboxyl group can be alkylated to COO-alkyl ester class to enhance the hydrophobicity of pectin (Miralles-Houzelle et al., 2001). Methoxylation is termed once carbon is alkylated to the carboxylate group. The conversion from carbon (C-6) carbon to carboxylic acid by eliminating methyl esters from the esterified galacturonic acid residues is termed demethoxylation. The demethoxylation reaction mainly alters the pectin's properties (Chen et al., 2015a). In general, high methoxy pectin is obtained at an industrial scale, and the same is converted to low methoxy pectin by controlled acid/alkali demethoxylation or by demethoxylating reaction of pectin methylesterase (PME) (Kim et al., 2008). The treatment of acidic or alkali demethoxylation is usually done at low temperatures to delay the pectin's depolymerisation; hence, enzymatic demethoxylation is considered more specific and a well-mild assay for the conversion of high methoxy to low

methoxy pectin. The arrangement of methoxylation, including random or blockwise arrangements in pectin demethoxylation, influences pectin properties (Ralet et al., 2001). Plant PMEs have more blockwise arrangements than fungal PMEs resulting in long stretches of unesterified HG (Willats et al., 2006). To enhance the hydrophobicity of pectin, carbons are more alkylated to the carboxylate group present in pectin by reacting the TBA salt with respective alkyl halides (Figure 2.1a).

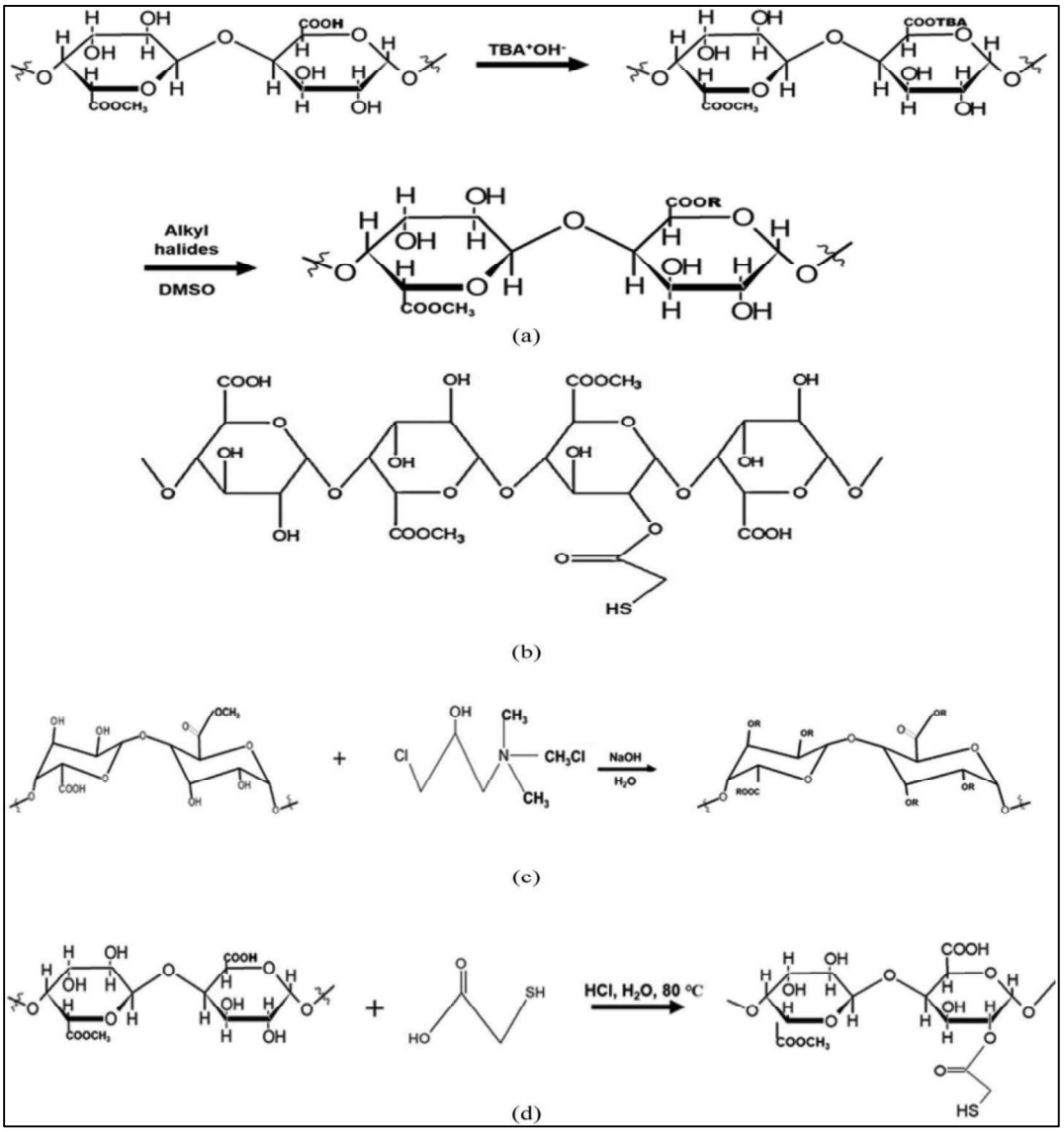


**Figure 2.1.** (a) Alkylation of pectin with alkyl halide (Einhorn-Stoll et al., 2001) (b) Amidation of Pectin (Chen et al., 2015a) (c) Quaternisation of pectin with 3-chloro- 2-hydroxypropyltrimethylammonium chloride (Fan et al., 2012) (d) Thiolated pectin synthesised by esterification of pectin with thioglycolic acid in the presence of hydrochloric acid (Sharma and Ahuja, 2011).

Similar to the alkylation of the carboxylate group, the alkylation of hydroxyl groups in pectin is also done. Acetylation is considered the most crucial alkylation of hydroxyl groups present in pectin. Pectin from plant sources can naturally be acetylated at O-2 or/and O-3 of galacturonic acid. However, this type of esterification diminishes the stability of calcium and pectin binding, which slows the gelation process. As a result, pectin treated with an acetylating agent has important applications in stabilizing or emulsifying agents (Leroux et al., 2003). Because of pectin's reduced polarity and solubility, it can be used to modulate the distribution of the weak acidic medication ibuprofen in the gastrointestinal tract (Bhatia et al., 2008). Amidated pectin is frequently utilized in food technology because of its excellent gelling capabilities and reduced sensitivity to pH and calcium ions.

Amidated pectin-based gels are thermo-reversible and can be heated, cooled, and resolidified, while conventional pectin gels are the main liquid even after heating. The gelling properties enable the amidated pectin to function as hydrogel beads. These hydrogel beads can be utilised further in delivering a colon-specific drug or entrapment of insulin for oral administration. The most widely used technique to prepare amide pectin is ammonolysis of methyl ester groups present in high methoxy pectin and ammonia in methanol (anhydrous form) (Figure 2.1b) (Chen et al., 2015a). Bae et al. (2016) modified the pectin extracted from orange peels by treating the pectin with alkaline hydroxylamine (pH 12.0, 2 M). After 4–48 h in similar conditions, pH was attained to 6.5 using hydrochloric acid followed by precipitation of pectin by 2-propanol. The resultant pectin exhibited an increased scavenging effect three-fold compared to the native one (Bae et al., 2016). Simultaneously, undesirable reactions, including demethoxylation at alkaline pH, were also carried out in addition to reduced molecular weight by depolymerisation. Another way to obtain amidated pectin, which consists of secondary amide groups, is by reacting primary amines in the place of ammonia with high methoxy pectin. The amidated pectin, which consists of aromatic/aliphatic groups, represents higher hydrophobic behaviour than conventional pectin. The reaction with amine acids also results in amidated pectin. Citrus pectin is reportedly modified using glycylglycine, glycine methyl ester, or glycine, followed by the separation of pectin by acetone. The modified citrus pectin exhibited instant solubility in water ranging from acidic to neutral pH, even with strong ionic strength (Kurita et al., 2012).

Quaternization effectively improves the functional properties of polysaccharides and converts the ionic hydrocolloids into their cationic derivative (Geresh and Dawadi, 2000). The modified pectin has potential applications in cosmetics, packaging, pharmaceuticals, and preservatives. Fan et al. (2012) studied the quaternized pectin by making a react pectin with 3-chloro-2-hydroxypropyltrimehtylammonium chloride and sodium hydroxide. The modified pectin resulted in high moisture absorption, retention, and antimicrobial properties (Chen et al., 2015a).

There are several other substitutions, including thiolation, sulfation, and oxidation, to modify the properties and structure of pectin. Sharma and Ahuja (2011) prepared thiolated pectin by esterifying pectin and thioglycolic acid to form amide or ester bonds in hydrochloric acid's presence. Another method to prepare sulfated pectin is substituting hydroxyl groups with sulfate groups resulting in antithrombotic, anticoagulant, contraceptive, antitumor, and antimicrobial properties (Martinichen-Herrero et al., 2005). In recent times, oxidation of pectin has also come to notice as oxidised pectin consists of comparatively more groups. This oxidised pectin exhibits faster degradation due to high reactivity, contributing to a crucial function in drug-controlled delivery (Takei et al., 2010).

### 2.7.2. Chain elongation

In general, a gel network in pectin is formed when HG portions cross-link, causing the entrapment of solutes and water, resulting in the development of a three-dimensional network (Willats et al., 2006). There are several other kinds of crosslinks in pectin, such as the oxidation of citrus pectin using adipic hydrazide to develop hydrogel. The hydrogel functioned to localise Doxorubcin, anti-cancer drug delivery, preventing the onset of primary cancer (Takei et al., 2010). In addition to crosslinking, pectin can be modified by synthesising pectin graft copolymers, aiding the physicochemical properties of pectin. The modification of pectin can be achieved by developing grafts or branches of polymers and adding desired properties to the pectin substrate simultaneously (Chen et al., 2015a).

### 2.7.3. Depolymerisation

The depolymerisation of pectin has always been an area of research as it is a crucial method for developing pectic oligosaccharides. Pectin degradation into smaller oligosaccharides

is essential to determine its structure; otherwise, the pectin molecule is considered a whole because of pectin's enormous size and heterogeneous nature (Coenen et al., 2008). The smaller oligosaccharides exhibit multiple applications, viz. antioxidant, a repressor of liver lipid accumulation, cancer cell inhibitor, antibacterial agent, an angiogenesis inhibitor, antimetastatic agent, and prebiotic (Chen et al., 2015a). Depolymerisation is broadly done using physical, chemical, and enzymatic depolymerisation methods. If pectin is chemically degraded through acid hydrolysis, pectin, which has less DM, hydrolyses faster (Krall and McFeeters, 1998). Alternatively, pectin degraded via hydroxyl radical-mediated scission influences the postharvest Fruit softening (Fry et al., 2002). Similarly, physical degradation significantly affects pectin degradation, including radiation, ultrasonication, high-pressure treatment, and photolysis (Chen et al., 2015a).

### 2.8. Pectin complex

Beyond the traditional uses of pectin, pectin can form complexes with various molecules such as minerals (Chirug et al., 2018), proteins (Jaramillo et al., 2011), polyphenols (Chirug et al., 2018), and lipids (Li et al., 2023), which improves its functional and bioactive qualities. Pectin-mineral complexes, particularly pectin-iron complexes, have received a lot of interest for their possible use in iron fortification. Pectin iron complex can boost iron absorption while reducing unpleasant sensory properties and gastrointestinal side effects associated with traditional iron supplements (Hu et al., 2023). Furthermore, pectin's capacity to interact with proteins and polyphenols broadens its application in food stabilisation, controlled nutrient release, and functional food design. The creation of such complexes not only improves the physicochemical stability of bioactive substances but also helps to target their delivery in food and medicinal applications (Cao et al., 2025). Pectin complexes are a viable means of enhancing the nutritional and functional qualities of food products, especially given the growing demand for sustainable and natural food fortification methods.

#### 2.8.1. Pectin-protein complexes

Pectin-protein complexes have received a lot of interest in the food and nutraceutical industries for their capacity to improve the stability, bioavailability, and controlled release of bioactive substances. These complexes are produced by electrostatic contacts, hydrogen bonding, and hydrophobic interactions (Falsafi et al., 2022) between pectin, a negatively

charged polysaccharide, and different proteins. Their applications include stabilizing emulsions and increasing the administration of nutraceuticals such polyphenols, vitamins, and probiotics. The interaction of pectin with proteins is highly influenced by pH, ionic strength, and molecular weight. Low pH values promote stable complex formation through electrostatic interactions, while higher pH levels may inhibit complexation due to charge repulsion. Furthermore, the presence of salts affects the stability of these compounds through ionic interactions (Cao et al., 2025). A recent study found that emulsions stabilized with pectin and ovalbumin fibrils have greater environmental stability, retaining structural integrity even after numerous thermal and freeze-thaw cycles (Hua et al., 2023). These findings emphasize the potential of pectin-protein complexes as stable delivery vehicles for sensitive bioactive chemicals.

Pectin-protein complexes have been successfully integrated into W/O/W (water-in-oil-in-water) emulsions to transport hydrophilic and lipophilic bioactives, including *Lactobacillus plantarum* and curcumin. These emulsions are more stable than standard emulsions, resulting in less nutrient leakage and a longer shelf life (Hua et al., 2024). Pectin-protein particles can stabilize Pickering emulsions, which rely on solid particles rather than surfactants. Tang et al. (2021a) found that pectin-bovine serum albumin complexes increased the encapsulation efficiency and bioaccessibility of hydrophobic nutraceuticals like curcumin and betanin.

A number of crucial parameters influence the stability and function of pectin-protein complexes. Complexation efficiency depends on pH, as maximum binding occurs at the protein's isoelectric point, when electrostatic attraction between oppositely charged molecules is maximal. Ionic strength affects the interaction between pectin and proteins, with specific salts boosting or inhibiting complex formation based on concentration and type. Fibrillar proteins interact more easily with pectin than globular proteins due to their increased surface area and higher binding capacity, which impacts complex stability. These characteristics all influence the functional qualities of pectin-protein complexes (Cao et al., 2025), which affects their use in food and nutraceutical formulations.

### 2.8.2. Pectin- polyphenol complex

Plant secondary metabolites called polyphenols are found in many food systems. Polyphenols are great sources of natural cross-linking agents because of their diverse

chemical structures and polarity. Multiple hydroxyls found in polyphenols can serve as binding sites for alkyl groups, sugars, or acids (Sivam et al., 2012). Polyphenols readily adsorb and compound with a wide range of chemical components due to their polyhydroxy structure (Yang et al., 2018b). Plant polyphenols can interact with macromolecules, including polysaccharides, through hydrogen bonding or hydrophobic interaction during food processing and human digestion. This can alter the nutritional properties and function of biomacromolecules (Strauss and Gibson, 2004). Because of their abundance of phenolic hydroxyl groups, polyphenols can interact with charged polysaccharides through electrostatic interaction in addition to hydrophobic or hydrogen bonding interactions. Traditional cross-linking agents like formaldehyde and glutaraldehyde can be replaced with natural, non-toxic polyphenols (Bhargavi et al., 2020). Furthermore, plant polyphenols offer a number of benefits, including antiviral, antibacterial, anticancer, anti-inflammatory, and antioxidant qualities that can give protein, polysaccharide, and other polymer systems positive functional traits (Lu et al., 2019; Lin et al., 2021).

Dietary fiber, particularly soluble fiber such as pectin, is important for human health because it can bind bioactive substances such as polyphenols (Celik and Gokmen, 2014). The interaction between pectin and polyphenols is regulated by pH, temperature, and the degree of pectin esterification (Costa et al., 2015). These complexes have important consequences for food processing, bioavailability, and functional food use. Pectin has been utilized as an adsorbent to recover and stabilize polyphenols, hence decreasing their susceptibility to degradation (Schieber et al., 2003). According to studies, pectin-polyphenol interactions increase antioxidant activity, making them useful as natural antioxidants and functional dietary additives. Pectin's capacity to form complexes with polyphenols aids in taste adjustment by masking catechin astringency (Hayashi et al., 2005). Pectin-polyphenol interactions can provide insights into their role as functional ingredients, improving stability, bioavailability, and controlled release of bioactive compounds in food systems (Liu et al., 2019a).

### 2.8.3. Pectin-lipid complex

Excessive consumption of partly hydrogenated oils has been related to an increased risk of chronic diseases, highlighting the need for better alternatives in food formulations (Huang et al., 2019). However, because of their structural and oxidative stability, replacing partially hydrogenated oils in a variety of goods, including margarine and fried meals,

presents considerable hurdles. In this regard, pectin-lipid complexes have emerged as interesting solutions for stabilising emulsions and improving the delivery of hydrophobic bioactive ingredients in functional foods. Pectin-lipid complexes stabilize emulsions by electrostatic interactions, steric hindrance, and interfacial adsorption. Pectin molecules are negatively charged and interact with the polar head groups of phospholipids and lipoproteins to form a protective barrier around lipid droplets. This barrier inhibits aggregation, flocculation, and further coalescence, resulting in long-term emulsion stability (Du et al., 2019). Furthermore, pectin increases viscosity, slowing droplet movement and preventing phase separation (Li et al., 2023).

Moreover, the food industry has experienced a rising demand for low-fat or no-fat goods in recent years. The low-fat diet may lack sufficient hydrophobic micronutrients, including fat-soluble vitamins and essential fatty acids. Consequently, there is a growing demand for non-lipid carriers to transport and safeguard oil-soluble nutraceuticals in such food products. The development of nanocarriers for hydrophobic nutraceuticals represents a viable way to meet that need (Zimet and Livney, 2009). Furthermore, because of their nanoscopic size and high density per unit mass, nano-vehicles may boost the bioavailability of these nutraceuticals (Subramanian, 2021). Another significant potential advantage is the reduction of negative impacts on sensory qualities such as the transparency of transparent food systems like certain beverages. Finally, entrapment and encapsulation may protect nutraceuticals against deterioration due to oxidation and other chemical and enzymatic processes during manufacture and shelf-life, reducing the development of undesirable tastes and aromas as well as the loss of biological value (Zimet and Livney, 2009).

Pectin-lipid complexes represent a viable technique for creating better fat replacements and lipophilic bioactive delivery systems. Their potential uses go beyond high internal phase emulsions including nano- and microencapsulation systems for vital fatty acids, fat-soluble vitamins, and plant-based emulsions.

### 2.8.4. Pectin-mineral complex

Pectin, a structurally complicated heteropolysaccharide found in plant cell walls, is well known for its dietary fiber, gelling, and stabilizing qualities in culinary applications. Aside from these functions, pectin has a high ability to bind metal ions, generating pectin-mineral

complexes that affect mineral bioavailability and bioaccessibility in the human digestive system (Kyomugasho et al., 2017). Calcium ($Ca^{2+}$), zinc ($Zn^{2+}$), iron ($Fe^{2+}$), and magnesium ($Mg^{2+}$) are essential minerals for bone formation, enzymatic activities, and oxygen transport (Cámara et al., 2005). These interactions play a crucial role in determining their absorption.

Pectin's polyanionic nature, caused mostly by its galacturonic acid residues, allows it to interact with positively charged metal ions. The degree of methylesterification (DM) in pectin has a substantial effect on its mineral-binding capacity, as lower DM results in more free carboxyl groups capable of chelating metal ions (Fraeye et al., 2010). Additionally, the presence of neutral sugars, acetyl groups, and branching structures in pectin influences its interaction with minerals (Patova et al., 2014). Citrus and sugar beet pectins differ in their mineral-binding capability, with changes in molar mass, degree of branching, and structural composition contributing to variable bioaccessibility outcomes (Kyomugasho et al., 2017).

While the development of pectin-mineral complexes can aid in regulated mineral delivery, concerns have been expressed concerning the potential loss in mineral bioavailability due to strong binding, resulting in lower absorption in the small intestine (Bosscher et al., 2003). However, new research indicates that pectin degradation in the colon may accelerate the release of bound minerals, increasing their total bioaccessibility (Dongowski et al., 2000). Understanding these interactions is critical for developing pectin-based functional foods that provide structural and nutritional benefits while maintaining mineral bioavailability.

#### 2.8.4.1. Pectin-iron complex

Iron is an important micronutrient necessary for different physiological activities such as oxygen transport, energy metabolism, and enzymatic reactions (Caetano-Silva et al., 2021). Adding iron salts, like $FeSO_4$ or $FeCl_3$, to food compositions can be challenging due to their unstable nature and interactions with other ingredients. Free iron ions are easily oxidized and hydrolyzed, resulting in insoluble ferric hydroxides that impair absorption efficiency (Hu et al., 2023). Iron supplementation can cause oxidative stress, resulting in lipid peroxidation and nutritional deterioration in food systems (Ma et al., 2021). Another key restriction is gastrointestinal irritation, since unbound iron can react with stomach acid,

causing nausea, constipation, and gastric mucosal injury (Banjare et al., 2019). To address these difficulties, pectin-iron complexes have emerged as intriguing solutions for increasing iron bioavailability and controlled release in the gastrointestinal system.

Pectin has significant advantages as an iron delivery carrier due to its chelation characteristics and biocompatibility. Pectin's carboxyl and hydroxyl groups establish strong coordination interactions with iron ions, stabilizing them and preventing undesired reactions in food systems (Chirug et al., 2018). Pectin-iron complexes are highly bioavailable because they remain soluble in water and prevent iron precipitation under physiological conditions (Ma et al., 2021). Pectin can chelate $Fe^{3+}$ ions, creating stable complexes that improve iron solubility and avoid premature release in stomach juices. Apple pectin (AP) has been found to have a high Fe(III)-binding capacity, resulting in 96.5% iron release after 4 h of intestinal digestion, ensuring adequate absorption in the small intestine (Ma et al., 2021). Furthermore, pectin-iron complexes demonstrate antioxidant characteristics that reduce the oxidative damage often associated with free iron supplementation (Chirug et al., 2018).

Several *in vitro* investigations have looked at the bioavailability, stability, and iron release patterns of pectin-iron complexes under simulated gastrointestinal circumstances. Ma et al. (2021) studied the apple pectin iron complex (AP-Fe(III)) and concluded that the AP-Fe(III) combination was very stable in stomach fluid, limiting early Fe(III) release, a significant restriction of free iron supplements like $FeSO_4$. After 4 h in the intestinal phase, iron release increased to 96.5%, showing higher solubility and bioavailability. Hu et al. (2022) used *in-vitro* tests with Caco-2 intestinal cells were employed to assess iron uptake efficiency. Pectin-iron complexes demonstrated increased iron absorption compared to free iron salts, suggesting possible advances in iron fortification. Overall, these investigations show that pectin-iron complexes outperform traditional iron supplements by increasing iron stability, lowering oxidative degradation, and improving gastrointestinal tolerability.

### 2.9. Applications of citrus pectin in food systems

Pectin is a natural component of plant materials; therefore, its use is considered safe in all countries. It is recommended as a safe ingredient as an additive without any limit on daily intake by the Joint FAO/WHO committee. Due to its complex hydrophilic nature, pectin

finds application as a thickener, texture modifier, emulsifier, gelling, and coating agent in the food sector. Pectin was initially utilized mainly in processing jellies, marmalades and jams. In recent times, pectin has gained importance in food products such as bakery fillings, soft drinks, fruit beverages, confectionery, conserves, dairy products, and glazing (Table 2.2). Pectin is utilized in food products due to its molecular and structural characteristics, including DE, galacturonic acid content, and molecular weight. Jams, jellies, and marmalades are processed using high methoxy pectin, whereas low methoxy pectin is employed in reduced-calorie jams and as a stabilizer in dairy products (Flutto 2003). When the soluble solid concentration of a conserve reaches 62%, high methoxy pectin is utilized for rapid gelling. However, if the content of soluble solids is less than 55%, then low methoxy pectin is added to attain a certain mouthfeel and texture to the product (Thakur et al., 1997).

### 2.9.1. Confectionary

Pectin (both neutral and flavoured) is used to prepare confectionery products, including artificial cherries. High methoxy pectin is incorporated to make flavoured candies. It is also employed in edible coatings of confectionery products to limit lipid migration. Pectin is added as a protective layer in food products during harsh conditions as it has the property of directly binding to the food ingredients (Ghasemi et al., 2017; Singh et al., 2018). Also, it can be used as a masking agent to eliminate unwanted compounds such as fish oil (Encina et al., 2016; Moslemi, 2020).

### 2.9.2. Dairy-based products

In gelled pudding deserts, pectin-containing fruit syrup is mixed with cold milk to maintain the consistency of the product even without refrigeration. High methoxy pectin is mainly added to stabilize certain kinds of sour milk products, while LMP is employed to maintain viscosity and even the disposition of fruit chunks in stirred or Swiss-style yoghurt. Gelatin and low methoxy pectin have been recommended in the preparation of sour cream mix to provide texture and prevent whey losses. Low-fat yoghurt is commercially manufactured with low-esterified pectin due to its interaction with milk protein (Khubber et al., 2021). This interaction led to desired yoghurt properties such as reduced syneresis and homogeneous texture (Arioui et al., 2017; Khubber et al., 2021). However, acidified dairy drinks are commercially produced using high-esterified pectin (Liang and Luo, 2020;

Moslemi, 2020). Due to the flavour release characteristics of low methoxy pectin and texture and consistency provider, it is also used in barbecue sauce (Paulionis et al., 2015).

### 2.9.3. Beverages

In beverages, pectin, especially high methoxy pectin, acts as a beverage clouding agent, texture, and mouthfeel enhancer, preventing "hard-packing" in dietetic fruit juice drinks and sugar replacers. Pectin serves as a viscosifier in soft drinks and beverages, providing a clean mouthfeel rather than the undesirable sliminess associated with gums (Flutto, 2003).

**Table 2.2** Application of pectin extracted from both citrus and non-citrus based sources

| Pectin source | Application | References |
|---|---|---|
| **Citrus based** | | |
| Lemon peel | Biodegradable starch-pectin-titanium oxide nanoparticle-based edible film | Dash et al., 2019 |
| | Prebiotic effect | Gómez et al., 2016 |
| Lime peel | Retards oxidation in soybean oil | Rodsamran and Sothornvit, 2019 |
| Grapefruit peel | Helps to remove copper (II) from water. | Zhang et al., 2020 |
| | Texture enhancer in apple jam | Khan et al., 2014 |
| Tangerine peel | Prebiotic effect | Islamova et al., 2017 |
| Orange peel waste | Prebiotic effect | Gómez et al., 2014 |
| Lemon | Jam production | Sulieman et al., 2013 |
| Orange | Jam production | Sulieman et al., 2013 |
| Citrus pectin | Antimicrobial films derived from pectin and sodium alginate | Nešić et al., 2017 |
| | Films for food packaging | Dash et al., 2019 |
| | Anti-diabetic effect | Liu et al., 2016 |
| | Antiproliferative effect | Maxwell et al., 2016 |
| | Bio-based packaging film | Kurek et al., 2021 |
| | | Roy and Rhim, 2021 |

| | | |
|---|---|---|
| | Nanoemulsion and shelf life extension of pork lion | Xiong et al., 2020 |
| | Wound-dressing | Oh et al., 2020 |
| | Post-harvest conservation of apples | Sganzerla et al., 2020 |
| Modified citrus pectin | Prostate cancer | Guess et al., 2003 |
| | Anti-Metastatic property | Glinsky and Raz, 2009, Zhang et al., 2016 |
| | Antiproliferative effect | Azémar et al., 2007 |
| | Metal removal | Eliaz et al., 2006, Zhao et al., 2008 |
| Amidated citrus pectin | Gene delivery | Katav et al., 2008 |
| Low methoxyl citrus pectin | Self-healing pectin/chitosan hydrogels | Li et al., 2021 |
| | Stabilization of low-fat set yoghurt | Khubber et al., 2021 |
| | Antimicrobial inhibition | Torpol et al., 2019 |
| **Non-citrus based** | | |
| Pumpkin | Metastasis | Zhao et al., 2017 |
| | Drug encapsulation | de Souza et al., 2013 |
| Apple | Lowers cholesterol level | Brouns et al., 2012, Krzysik et al., 2011 |
| Tomato peel | Acts as an inhibitor in tin corrosion | Grassino et al., 2016 |
| Pineapple peel | Films | Rodsamran and Sothornvit 2019 |
| Dragon fruit peel | Acts as cholesterol lowering agent | Zaid et al., 2019 |
| Apple pomace | Prebiotic effect | Islamova et al., 2017 |
| Indonesian Mangosteen rind | In biomedical field | Wathoni et al., 2019 |
| Durian rind | Increased absorption of lanthanum | Kusrini et al., 2018 |
| Gabiroba pulp | Inhibits cytotoxicity | da Costa Amaral et al., 2019 |
| Sugar beet pulp | Prebiotic effect | Chung et al., 2017, Gómez et al., 2016 |

| | | |
|---|---|---|
| *Bupleurum falcatum*roots | Prebiotic effect | Gullón et al., 2013, Matsumoto et al., 2008 |
| *F. kuhistanica*leaves (wild plant) | Prebiotic effect | Islamova et al., 2017 |
| *Passiflora glandulosa* | Hypoglycemic activity | Sousa et al., 2015 |
| Unripe apple | Anti-hyperglycaemic activity | Makarova et al., 2015 |
| Banana passion fruit | Cholesterol removal | Espinal-Ruiz et al., 2016 |
| *P. iwatensis* (seagrass) | Metal ions removal | Khozhaenko et al., 2016 |
| *L. japonica* flowers | Anti-pancreatic cancer activity | Lin et al., 2016 |
| Modified sugar beet pectin | Antiproliferative effect | Maxwell et al., 2016 |
| Fentanyl pectin | Nasal drug delivery | Ueberall et al., 2016 |
| Pectin/chitosan | Encapsulation for insulin delivery | Maciel et al., 2017 |
| Pectic polysaccharides | Breast cancer | Sathisha et al., 2007 |
| Amidated pectin | Hydrogels for drug delivery | Harris and Nasi, 2008 |
| | Wound management | Mishra et al., 2008 |
| | Wound management and skin repairing | Amirian et al., 2021 |
| | Edible food packaging with antimicrobial properties | Bayarri et al., 2014 |
| Modified pectin with oligopeptides | Tissue engineering | Jayaraman et al., 2015, Munarin et al., 2011 |
| Commercial pectin | Nasal drug delivery | Sriamornsak et al., 2010 |
| | Ocular drug delivery | Giunchedi et al., 1999 |

| | | |
|---|---|---|
| | Insoluble calcium pectinate (CaP) film | Penhasi and Meidan, 2014 |
| | Drug encapsulation | de Souza et al., 2013, Jantrawut et al., 2014, Wanasawas et al., 2013 |
| | β-carotene stability and delivery | Yi et al., 2021 |
| | Hydrogels for oral nutrient delivery | Gautam and Santhiya, 2019 |
| | Collagen-pectin hydrogels | Goel et al., 2021 |
| | Luminescent pectin-based hydrogel | Tang et al., 2021b |
| | Metal removal | Khotimchenko et al., 2012 |
| | Antiproliferative activity | Bergman et al., 2010 |
| | Modification of digestibility of potato starch | Yin et al., 2021 |
| | Functional edible films for food packaging | Melo et al., 2019 Otálora González et al., 2021 |
| | Edible film for industrial use by extrusion technology | de Oliveira et al., 2021 |
| Commercial apple pectin | Bio-based packaging material | Nešić et al., 2017 Šešlija et al., 2018 |

### 2.9.4. Food packaging

Pectin has also become crucial in food packaging by interacting with different polymers, such as alginate and cellulose, to produce edible films and coatings (Table 2.2). The reconstituted pectin-based coatings and edible films are bio-degradable and exhibit enhanced antioxidant and antimicrobial properties (Picot-Allain et al., 2020). Instead of pectin's structural and molecular properties, pectin grades are used in industry for commercial use, which also states its functionality. There are about 100–500 grades of pectin, and are depicted as the number of parts of sugar required to gel with one part of pectin such that sufficient firmness is achieved under standard conditions of 65–70% sugar, pH 3.2–3.5, and pectin limit of 1.5–2% (Thakur et al., 1997). Numerous recent studies have been done on food packaging using citrus pectin combined with other polylactic acid, polyhydroxyalkanoates, and compounds such as starch, titanium oxide

(Dash et al., 2019), chitosan (Melo et al., 2019), polyvinylpyrrolidone (Nešić et al., 2017) and nano-materials to increase the functional properties.

### 2.9.5. In the fabrication of 3D printed food

Recently, pectin has been employed in the development of 3D-printed foods. 3D food printing is formulating, designing, and producing customised foods based on nutritional and physiological needs instantly before using raw ingredients (Singhal et al., 2020). For 3D food printing, multiple food inks incorporating low methoxy pectin have been used by Vancauwenberghe et al. (2017). The authors further studied the modelling of 3D-printed honeycomb structures from pectin in terms of mechanical properties (Moslemi, 2020; Vancauwenberghe et al., 2018). Vancauwenberghe et al. (2019) successfully evaluated the 3D printing of functioning plant cells by encapsulating them in LM pectin gels at high density with precision and reproducible results.

### 2.9.6. Food additive

Milk protein is stabilised at low pH by making the electrostatic reaction of pectin bearing a negative charge and protein having a positive charge, followed by the opposition of protein using an entropy-rich loop framed by neutral areas of HMP (Yuliarti et al., 2019). Alternatively, pectin has been added to yoghurt to enhance the sensory acceptability of the product. Pectin acted as a physical barrier by unfolding the milk protein-pectin connection and trapping the volatile chemicals within the matrix. Furthermore, pectin reduced syneresis in yoghurt due to its high water-holding ability (Arioui et al., 2017). Pectin's hydrophilic nature limits lipid uptake in fried food products (Hua et al., 2015b). Additionally, decreased alkaline phosphatase activity can impair lipid metabolism (Moslemi, 2020; Sefcikova and Racek, 2016). Pectin also functions as a frozen barrier by delaying the formation of ice crystals in food, preventing syrup loss during thawing, and maintaining the shape of food and its products. The pectin interacts with $Ca^{2+}$ ions to maintain firmness during the thawing of frozen foods while reducing the losses of syrup; along with $Ca^{2+}$ ions, pectin also interacts with sucrose molecules. Low methoxy pectin is added as a coating to prevent texture and quality loss in ice creams. The addition of pectin to ice pops and lollies helps retain the flavour and colour during preparation (Thakur et al., 1997). Pectin is also used as a fat replacer in ice creams and improves the textural quality (Zhang et al., 2018).

#### 2.9.6.1. Fruit leather

Fruit leather is a value-added product made from dehydrated fruit pulp or puree that is formed into a flexible, sheet-like structure. This product has grown in popularity because of its convenience, long shelf life, and retention of important nutrients, making it a favourite snack among customers of all ages (Slavin and Lloyd, 2012). Fruit leathers are an effective way to reduce post-harvest losses, especially in nations like India, which ranks second in world fruit production but suffers large losses due to insufficient storage and preservation methods (Murthy et al., 2002). The processing of fruit into leather increases its usefulness, making it a sustainable alternative to surplus or overripe fruits that would otherwise go to waste (Habauzit et al., 2014). Fruit leathers are gaining popularity as a natural, minimally processed, and preservative-free snack due to their high fibre, vitamin, and antioxidant content. These snacks promote digestive health, immunological support, and overall well-being. They contain most of the beneficial elements found in fresh fruits, including vitamins, minerals, antioxidants, and dietary fibre (Slavin and Lloyd, 2012; Valenzuela and Aguilera, 2015). Natural sugars provide a rapid source of energy without artificial sweeteners, making fruit leather a healthier alternative to manufactured confectionery.

In fruit leather, pectin works as a gelling, thickening, and stabilising ingredient, contributing to the ideal chewy and cohesive texture of the finished product (Alam et al., 2024). One of the key advantages of pectin in fruit leather is its ability to alter texture. Apples, pears, and plums make tougher fruit leathers, while berries require additional gelling agents or combining with high-pectin fruits for maximum consistency (Bandaru and Bakshi, 2020). The use of hydrocolloids such as pectin increases the viscosity of fruit puree, which aids in the formation of a homogeneous layer during drying and lowers stickiness, increasing the handling and shelf-life of fruit leathers (Li and Nie, 2016). Pectin content in various fruit leathers is mentioned in Table 2.3.

Pectin gels in the presence of sugar and acid, making it a key ingredient in fruit leather formation. The degree of esterification determines gel formation capabilities. To produce a gel, high-methoxyl (HM) pectin requires a high sugar concentration as well as acidic conditions. Low-methoxyl (LM) pectin gels in the presence of calcium ions, making it ideal for sugar-free fruit leathers. The type of pectin employed directly impacts the firmness, elasticity, and mouthfeel of the final product (Alam et al., 2024).

Pectin helps to retain moisture in fruit leather, limiting severe dehydration and brittleness. It also affects the drying rate of fruit puree by changing the water-binding capacity, ensuring a controlled and consistent dehydration process (Diamante et al., 2013). According to Phimpharian et al. (2011), pectin can minimise syneresis (water loss) in fruit leathers, preserving the proper texture over long-term storage. Nutritional and functional benefits. Aside from its functional role in texture modification, pectin has various health benefits. The presence of pectin in fruit leather increases its nutritional content, such as improving gastrointestinal health, managing blood sugar levels, and decreasing cholesterol, making it a better snack alternative (Alam et al., 2024).

**Table 2.3** Pectin content in various fruit leathers

| S.No | Fruit leather type | Ingredients | Pectin content | References |
|---|---|---|---|---|
| 1 | Kiwi leather | Kiwi, sugar, xanthan gum, guar gum and pectin | 1% | Barman et al., 2021 |
| 2 | Mango leather | Mango pulp, guar gum, pectin, carboxymethyl cellulose, gum acacia, pectin, sodium alginate | 1-3% | Singh and Singh, 2003 |
| 3 | Pineapple leather | Pineapple, glucose syrup, pectin | 0.5-1.5% | Phimpharian et al., 2011 |
| 4 | Apricot leather | Apricot, sugar, pectin | 0.2-0.4% | Sharma et al., 2013 |
| 5 | Guava leather | Guava pulp, wheat starch, pectin and maltodextrin | - | Vijayanand et al., 2000 |
| 6 | Strawberry leather | strawberry puree, corn syrup, pectin, citric acid | - | Lee and Hsieh, 2008 |
| 7 | Pear leather | Pear puree, pectin, corn syrup, water | 16-24% | Huang and Hsieh, 2005 |
| 8 | Kiwifruit leather | Kiwifruit puree, glucose syrup, water, sugar, pectin, salt and citric acid | 1-3% | Vatthanakul et al., 2010 |
| 9 | Pineapple and mango leather | Pineapple pulp or mango pulp, sucrose, pectin and maltodextrin | 0-2% | Gujral et al., 2013 |
| 10 | *Annona muricata* L. fruit and *Avena sativa* flour leather | Soursop pulp, oats flour, pectin, citric acid, and glucose syrup | - | Ayalew and Emire, 2020 |