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Pectin: An overview of sources, extraction and applications in food products, biomedical, pharmaceutical and environmental issues

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ABSTRACT

Pectin is a complex versatile heteropolysaccharide of great importance to food, pharmaceutical and cosmetic industries. It is widely used in the food industry due to its thickening, gelling and emulsification properties and in biomedical and biomaterial applications on account of its potential anti-inflammatory and immunomodulatory effects as well as its biodegradability and biocompatibility properties. Pectin is also a soluble dietary fiber with several beneficial gastrointestinal and physiological effects. The multifunctionality of pectin is related to the nature of its molecule that has diverse chemical structures, physicochemical properties and potential functionalities depending on the sources where it is extracted and on the extraction methods. Therefore, this review focuses on the importance of pectin for today's food, pharmaceutical and cosmetic industries, compiling information on its composition and properties as determined by its origins, especially from waste biomass of the fruits and vegetables processing industry, on commercial applications and research needs. The suitability of the different extraction methods was also discussed, considering cost, energy consumption and productivity. Furthermore, the biodegradation of pectin as a complex process performed by a set of enzymes was also reviewed along with application purposes. Finally, future perspectives reveal pectin to be an astounding functional food ingredient requiring continuous research work.

1. Introduction

Pectin is a nontoxic, natural and versatile heteropolysaccharide of interest in the food, pharmaceutical and cosmetic industries. It's one of the main components of the cell walls of all higher plants and accounts for 0.5 to 4.0% of the total fresh weight of plants (Picot-Allain et al., 2022). Pectin is a structural fiber that is found in the primary cell wall and the intracellular layer of plant cells, especially in fruits such as apples, oranges and lemons (Mudgil, 2017). It is a family of galacturonic acid-rich polysaccharides that comprises homogalacturonan (HG), rhamnogalacturonan I (RG I), rhamnogalacturonan II (RG-II) and xylogalacturonan (XGA) (H.M. Chen et al., 2015). This biomolecule is a functional ingredient that has been widely used in the food and pharmaceutical industries for several decades due to its health benefits, thickening, gelling and emulsification properties (D.-Q. Li et al., 2021).

Human beings are unable to digest pectin due to the lack of pectin-degrading enzymes in our digestive system (Nasrollahzadeh et al., 2021). Pectinases are a complex heterogeneous group of enzymes that catalyze the degradation of pectic substances, either by depolymerization or de-esterification reactions (Shrestha et al., 2021). Pectinases have been widely used by the industrial sector to decompose plants' cell walls. They have now been recognized as eco-friendly biocatalysts, accounting for a 25% share of the global food and beverage enzyme market (Amin et al., 2019).

2. Why is pectin important in the field of nutrition and in the food and health industry?

The multifunctionality of pectin is due to the nature of its molecule which contain both polar and nonpolar regions, allowing it to be in-

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corporated into diverse food systems (Nasrollahzadeh et al., 2021). Mada et al. (2022) investigated the influence of pectin from mixed banana-papaya peel on the chemical composition and storage stability of Ethiopian traditional yoghurt (ergo). Their study revealed that pectin improved the sensory properties and general acceptance of the yoghurt, while the microbial load increased in response to the increase of the pectin content.

From a health and nutritional point of view, pectin is considered a soluble dietary fiber with several beneficial gastrointestinal and physiological effects, including the delay of gastrointestinal emptying and therefore decreasing the gastrointestinal transit time, the reduction of glucose absorption and an increase in fecal mass (Lara-Espinoza et al., 2018). Acetate, propionate and butyrate are produced as a result of the intestinal fermentation of pectin and other types of dietary fiber, playing a vital role in the prevention and treatment of metabolic syndrome, intestinal disorders (such as ulcerative colitis), various cancers, Crohn's disease, hypertension, diarrhea and obesity (Gullón et al., 2013). Liu et al. (2016) studied the anti-diabetic effect of citrus pectin in type 2 diabetic rats and its potential mechanism of action. They reported that the citrus pectin improved glucose tolerance, hepatic glycogen content and blood lipid levels in the rats.

According to Gunness and Gidley (2010), a diet rich in pectin results in a decrease of total cholesterol and low-density lipoprotein (LDL) in the blood without affecting the levels of high-density lipoprotein (HDL). Brouns et al. (2012) reported that 57 adults who received 15 g of pectin per day experienced up to a 7% reduction in LDL cholesterol in comparison with the control group. Additionally, pectin enhances the excretion of bile acids, a substance that assists the removal of the excess cholesterol from the body, consequently lowering the serum cholesterol levels (Zhu et al., 2017).

Pectin has also demonstrated chemoprotective properties against the metastasis of cancer and the growth of primary tumors in multiple types of cancers in human and animals (Lara-Espinoza et al., 2018). There are numerous reports that provide evidence to support the role of pectin and modified pectin in the inhibition of different types of cancers. The list includes colon cancer (Prado et al., 2019), prostate cancer (Dresler et al., 2019), pancreatic cancer (He et al., 2021), breast cancer (Delphi & Sepehri, 2016) and metastasis (Cheewatanakornkool et al., 2018).

3. Pectin composition and enzymes involved in their degradation

Pectin is one of the polysaccharide constituents of the cell walls of higher plants. Its structure is much more complex than that of other polysaccharides such as cellulose and the hemicelluloses (Scheller et al., 2007). Pectin is composed of two basic structures: a "smooth" region and a "hairy" region (Fig. 1). The "smooth" region (homogalacturonan) is a linear polymer of galacturonic acid residues with α-(1–4) linkages, substituted by methyl and acetyl residues. The "hairy" region is a complex structure, containing xylogalacturonan, rhamnogalacturonans I (RGI) and II (RGII) (Bonnin et al., 2014).

Xylogalacturonan is an α-(1→4)-linked D-galacturonic acid chain, which is highly substituted with β-D-xylose at the C-3 position. The main chain of rhamnogalacturonan I contains repeats of α-(1–4)-L-galacturonate and α-(1–2)-L-rhamnopyranoses. The rhamnose residues are highly substituted with polysaccharides such as arabinan, galactan or arabinogalactan. Rhamnogalacturonan II is the most complex glycan known containing 13 different sugars and 21 distinct glycosidic linkages. RGII has a backbone of homogalacturonan which is highly substituted by at least twelve different monosaccharides (Scheller et al., 2007).

The biodegradation of pectin is a complex process, and it is performed by a set of enzymes. Pectinase is a generic term used for a group of enzymes that catalyzes the degradation of pectin by hydrolysis, trans-elimination, as well as de-esterification reactions (Rehmana et al., 2021). Some sites of the action of enzymes are shown in Fig. 1.

3.1. Enzymes that catalyzes the degradation of "smooth" region

The enzymes involved in the degradation of the homogalacturonan include de-esterifying enzymes *i.e.* pectin methyl esterases (E.C. 3.1.1.11) and pectin acetyl esterases (E.C. 3.1.1.6) which remove methoxyl and acetyl residues, respectively, yielding polygalacturonic acid. The pectin methyl esterase preferentially acts on the methyl ester group next to nonesterified galacturonate unit end (Amin et al., 2019) and pectin acetyl esterase hydrolyses the acetyl ester of pectin forming pectic acid and acetate (Li et al., 2020).

The other subclass of homogalacturonan-degrading enzymes are broadly termed as depolymerases; they break the α−1,4-linkages of the main chain either by hydrolysis (polygalacturonases, E.C. 3.2.1.15) or via a β-elimination mechanism (pectate lyases, E.C. 4.2.2.2, and pectin lyases, E.C. 4.2.2.10) (Yadav et al., 2009). Polygalacturonase catalyzes the hydrolysis of pectin polymer into galacturonic acid monomer by addition of water molecules in α−1, 4 glycosidic linkages. Polygalacturonase is the most extensively studied pectinolytic enzyme (Satapathy et al., 2020). Pectate lyase cleaves glycosidic linkages preferentially on polygalacturonic acid forming unsaturated product (4,5-D-galacturonate) through transelimination reaction (Jayani et al., 2005). Pectin lyase acts on highly esterified homogalacturonan (Pérez et al., 2014).

3.2. Enzymes that catalyzes the degradation of "hairy" region

The enzymes involved in the degradation of xylogalacturonan are xylogalacturonan hydrolase (xylogalacturonase; EC 3.2.1.-), catalyze hydrolytic cleavage of glycosidic linkages between two galacturonate residues in xylose-substituted rhamnogalacturonan chain, producing xylose-galacturonate dimers (Vlugt-Bergmans et al., 2000).

The degradation of rhamnogalacturonans I (RG I) involves the participation of numerous enzymes. The enzymes involved in the hydrolysis of arabinan, galactan and arabinogalactan are well known (Bonnin et al., 2014), while the acetyl groups are liberated by the action of rhamnogalacturonan acetylesterases (EC 3.1.1.86) (Kaupinnen et al., 1995). Several types of enzymes have been described for the degradation of the backbone: Enzymes show endo-action: rhamnogalacturonan hydrolases (EC 3.2.1.171) and rhamnogalacturonan lyases (EC 4.2.2.23) (Silva et al., 2016), while enzymes with "exo" action are known: rhamnogalacturonan α−1,2-galacturonohydrolase (EC 3.2.1.173) (Mutter et al., 1998), rhamnogalacturonan α-L-rhamnopyranohydrolase (EC 3.2.1.174) (Mutter et al., 1994), unsaturated rhamnogalacturonyl hydrolase (EC 3.2.1.172) (Itoh et al., 2006) and exo-rhamnogalacturonan lyase (EC 4.2.2.23) (Iwai et al., 2015).

Some enzymes that catalyze the degradation of rhamnogalacturonans II (RGII), the most complex glycan known, have been described in Ndeh et al. (2017).

The biotechnological application of pectin and of its degradation products supports the interest in the study of pectinases.

4. Agro-industrial residues as sources of pectin-rich biomass

Extracting functional compounds from residues of food waste is an attractive approach to reduce the impact of agro-industrial activities on the environment. Pectin-rich plant biomass residues are therefore worthy of exploration, especially given that pectin polysaccharides are abundant in waste biomass from the processing of fruits and vegetables. Different waste streams provide pectin with diverse chemical structures, physicochemical properties and potential functionalities. However, commercially available pectin is mainly derived from three main residues: sugar beet pulp, citrus peel and apple pomace. Waste residues derived from citrus and apple processing are rich and preferred sources of pectin (Berlowska et al., 2018). Citrus used for industrial processing generates over 10 million tons of waste annually (Zema et al., 2018). Homogalacturonan domains have been isolated from citrus pectin with the

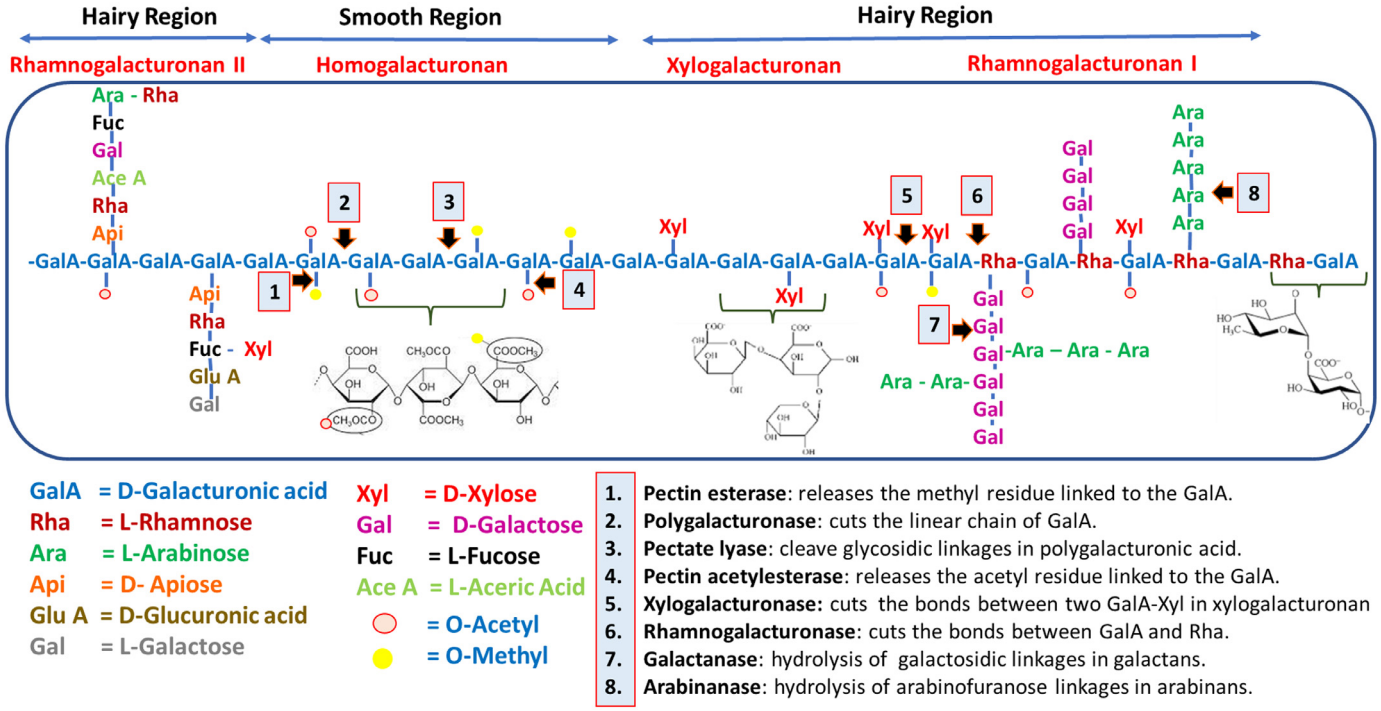

Fig. 1. Representative structure of pectin: The molecular structure of main chain of pectins and some sites of the action of enzymes involved in their degradation (adapted from Scheller et al., 2007). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

degree of polymerization ranging from 71 to 117 galacturonic acid units (Yapo et al., 2007a). Homogalacturonans with homogeneous charge density and molar mass distributions have been characterized from lime pectin samples after extraction (Hellin et al., 2005). It has been inferred that pectin from citrus peel contain rhamnogalacturonan I (RG I) and a minor rhamnogalacturonan II fraction (Yapo et al., 2007a). RG I was characterized by the presence of arabinose, galactose, galacturonic acid and rhamnose. Extraction methods have an influence on the pectin structure from citrus peel. A study by Kaya et al. (2014) showed that different extraction methods allowed the recovery of pectin from citrus peel with diverse molecular weights and variations in the RG I content. Pectin extracted from different tissues of citrus fruits may have different structural properties. Pectin isolated from lemon juice, lemon albedo, lemon core parts, seeds and carpel have shown distinct structural properties that may be also different in functionalities (Dimopoulou et al., 2019). These differences include variations in the degree of methylation in the pectin backbone and neutral sugar content. Ciriminna et al. (2017) reported low esterified pectin (< 50%) from lemon waste (40%), lemon outer skin (24%), red orange outer skin (39%), red orange waste (25%), and grapefruit peel (34%). Thus, specific sources can be selected depending on the intended applications.

The apple (*Malus* sp.) industry also generates waste, including the pomace, seeds and peels. Apple pomace and peel waste have been reported as rich and versatile pectin sources (M. Kumar et al., 2020). It is estimated that 84.7 million tons of apples are produced annually (FAOSTAT, 2020) of which, the pomace represents around 25–30% of the original fruit weight. This by-product is mainly composed of non-soluble carbohydrates, monosaccharides and disaccharides (Zacharof, 2017).

Pectin from apple pomace is predominantly protopectin, an acid soluble polysaccharide (Renard et al., 1991). Pectin recovery from apple pomace exhibits different degrees of esterification and structural properties depending on the conditions of the extraction method, similar to the situation of the citrus fruits. Pectin polysaccharides with low (43.29%) and high (65.88%) degrees of esterification (DE) have been recovered from apple pomace using citric acid solution as the solvent (Naqash et al., 2021). Other extraction conditions have reported apple pectin as highly methoxylated (54.5–67.1%) (Cho et al., 2019), and DE values between 61 and 63% (Morales-Contreras et al., 2020). While, Kumar and Chauhan (2010) reported low DE values (22.15%) from two apple varieties.

The post-processing of sugar beet (*Beta vulgaris*) generates waste in the form of pulp (insoluble beet tissue) and molasses (concentrated juice). It is estimated that approximately 20 million tons of sugar beet pulp are generated in Europe annually (Berlowska et al., 2018). It is high in pectin content (15–32%) with other main components including cellulose (22–30%) and hemicellulose (24–32%) (Hutnan et al., 2000). High-methoxyl pectin is predominant in sugar beet (Rejaii & Salehi, 2016). Pectin present in sugar beet pulp differs from pectin found in citruses and apples in the degree of acetylation linked to galacturonic acid residues, rhamnose content and presence of ferulic acid. Neutral sugar side chains carry phenolic esters (ferulic acid) attached to arabinose as well as galactose residues (Bonnin et al., 2014).

Pectin has also been recovered from sunflower (*Helianthus annuus L.*) heads. Sunflower head residues are a natural source of low-methoxyl pectin with DE values of 10–40% (J. Tan et al., 2020). Sunflower head pectin has been characterized with molecular weights ranging from 30,000–500,000 g/mol, GalA content of 70–90% and degree of acetylation of 2–4% (w/w) depending on the variety (Iglesias & Lozano, 2004). Hua et al. (2015) isolated pectin from sunflowers grown in China with 86.9% of homogalacturonans and 13.1% of RG I. Neutral sugars, rhamnose, arabinose and galactose, were found in both RG I and RG II. The GalA content reached 82.1% and trace amounts of glucose, xylose and mannose were detected in the recovered pectin. The low content of xylose was related to the low content of RG II. The degree of methyl esterification was 27% with an acetylation content of 0.38%. After structural analysis, it was suggested that sunflower pectin could follow the conventional homogalacturonan linear backbone with attached RG I and RG II.

Other less explored food wastes as pectin sources include tomato, carrot, fava beans, peas and soybeans (Müller-Maatsch et al., 2016). Many of these sources present high extraction yields. However, pectin

Table 1
Pectin content in agro-industrial residues.

Source	Type of waste	Pectin content (dry weight,%)	References
Citrus (*Rutaceae*)	Orange peel	16.70–24.80	(Kaya et al., 2014)
	Lemon peels	13.00–30.60	
	Lime peel	26.90–33.60	
	Grapefruit peel	21.60–28.00	
	Sweet orange peels	23.02	(Marín et al., 2007)
	Citrus waste	25.00	(Pourbafrani et al., 2010)
	Lemon juice waste	1.00–8.00	(Dimopoulou et al., 2019)
	Kinnow mandarin waste	22.60	(Oberoi et al., 2011)
Apple (*Malus* sp.)	Apple peel	1.21–14.50	(M. Kumar et al., 2020)
	Apple pomace	33.50	(Morales-Contreras et al., 2020)
Sugar beet (*Beta vulgaris*)	Sugar beet pulp	15.00–32.00	(Hutnan et al., 2000)
Sunflower (*Helianthus annuus L.*)	Dry sunflower heads	29.50	(Muthusamy et al., 2019)
Pea (*Pisum sativum*)	Pea pod	8.30	(Müller-Maatsch et al., 2016)
Fava bean (*Vicia faba*)	Faba bean hulls	9.57–15.75	(Korish, 2015)
Green beans (*Phaseolus vulgaris*)	Green beans cutting waste	8.10–8.30	(Christiaens et al., 2015)
Carrot (*Daucus carota*)	Rejected carrots	8.70–9.10	
	Carrot steam peels	8.90–9.10	
Wheel cactus (*Opuntia robusta*)	Fruit peel	14.64–15.71	(Mota et al., 2020)
Tomato (*Lycopersicon esculentum* Mill.)	Tomato waste	15.10–35.70	(Grassino et al., 2016)
	Tomato peel	17.00–25.00	(Casa et al., 2021)
Mango (*Mangifera indica)*	Mango peel	18.50–39.40	(Girma & Worku, 2016)

properties are the main determinant for industrial purposes such as gelling agents. The pectin contents in different agro-industrial residues are summarized in Table 1. The use of residues from peas as a source of pectin is attractive due to the amount of waste it generates per year (Müller-Maatsch et al., 2016). Pectin recovered from pea (*Pisum sativum*) pods have shown an acetylation content of 10% with a 30% degree of methylation, uronic acid between 70 and 95% and high levels of arabinose, xylose and galactose (Müller-Maatsch et al., 2016). Meanwhile, fava bean (*Vicia faba*) pods contained higher levels of pectin and pectic oligosaccharides compared to their cotyledon (Mateos-Aparicio et al., 2012). Korish (2015) found that fava bean hulls contain adequate levels of pectin for industrial purposes and that the structure of pectin varied according to the extraction method with increasing levels of galactose, arabinose and rhamnose under mild conditions while neutral sugars like glucose, mannose and xylose increased at low pH values.

5. Extraction methods of pectin

Pectin from various sources is extracted by a few commonly used techniques (Fig. 2). Usually, the published efficiencies of these methods were determined using lab setups and optimizations are essential when scaling up to industrial production. Variables included the solid-to-liquid ratio, acid strength (if used), temperature/power (depending on the method used), extraction duration and precipitation method. These variables not only affect the yield of the pectin, but they also affect the qualities of the pectin in terms of degree of esterification (DE), molecular weight, composition, purity and color.

5.1. Pre-extraction preparation

Pre-processing of the agro-industrial residues is normally needed before the actual extraction of pectin. Some of these processes, although optional, also significantly contribute to the quality and quantity of the final extracted pectin. Firstly, the agro-industrial residues are sorted and cleaned to remove undesirable materials mixed in with the agro-industrial residues since contaminants could lower the extraction efficiency. Water-washing or alcohol-washing are generally sufficient, but acid-washing could improve the yield of the pectin. Drying the raw materials is also recommended, as excess water could lower the solvent strength, hence the extraction efficiency. However, under the same extraction conditions, Salam et al. (2012) found that less pectin was extracted from dry lemon peel than from fresh lemon peel and the dry peel required extra time for rehydration.

Maceration is simply the grinding of the materials into a fine powder to increase the surface area for extraction (Farooq et al., 2022). Recently, superfine grinding has been demonstrated to be effective in improving the extraction of pectin from sunflower head by improving mass transfer (J. Tan et al., 2020).

5.2. Conventional extraction methods

The conventional extraction of pectin requires the use of acids and/or chelators. Pectin can also be extracted under alkaline conditions, although the yield is generally much lower. Heating the raw materials in an acidified buffer with agitation allows the hydrolysis of protopectin into pectin and solubilizes the pectin in the buffer. In most cases, solvents used in unconventional methods would also require a certain degree of acidification to facilitate the solubilization of the pectin. Strong mineral acids such as hydrochloric acid, nitric acid, phosphoric acid and sulfuric acids have been widely used. For organic acids, commonly used options include: acetic, citric, oxalic and tartaric acids etc. (Banerjee et al., 2016). Citric acid is both an acid and a chelating agent, with an outstanding performance in pectin extraction (Yang et al., 2018). For example, among different acids (HNO_3, HCl, H_2SO_4, acetic and citric acid) used in a comparative study, citric acid obtained the highest pectin yield (14.34%) from potato pulp, which is equivalent to over 80% of the total pectin of potato pulp (Yang et al., 2018). However, although chelating agents may be useful in this process, some could be difficult to remove after the pectin extraction. While both mineral and organic acids are effective, the choice of acid usually depends on their availability, post-processing effects, the desired properties and demands of the final products.

While Ca^{2+} is essential for the formation of the pectin matrix in the cell wall, the presence of chelating agents such as: citric acid, oxalic acid, ammonium oxalate, sodium hexametaphosphate, cyclohexanediamine tetraacetic acid or ethylenediaminetetraacetic acid, could help to capture the Ca^{2+} and release the pectin (Huang et al., 2021). Extraction solvents containing chelating agents can extract a larger amount of pectin than the acidic solutions alone (Begum et al., 2014).

5.3. Enzyme-assisted extraction

The principle of enzyme-assisted extraction is the use of enzymes to break down the cell wall matrix to release the pectin. Enzymes involved in enzyme-assisted extraction include: cellulase, hemicellulase, xylanase, pectinase, α-amylase, β-glucanase, endo-polygalacturonase

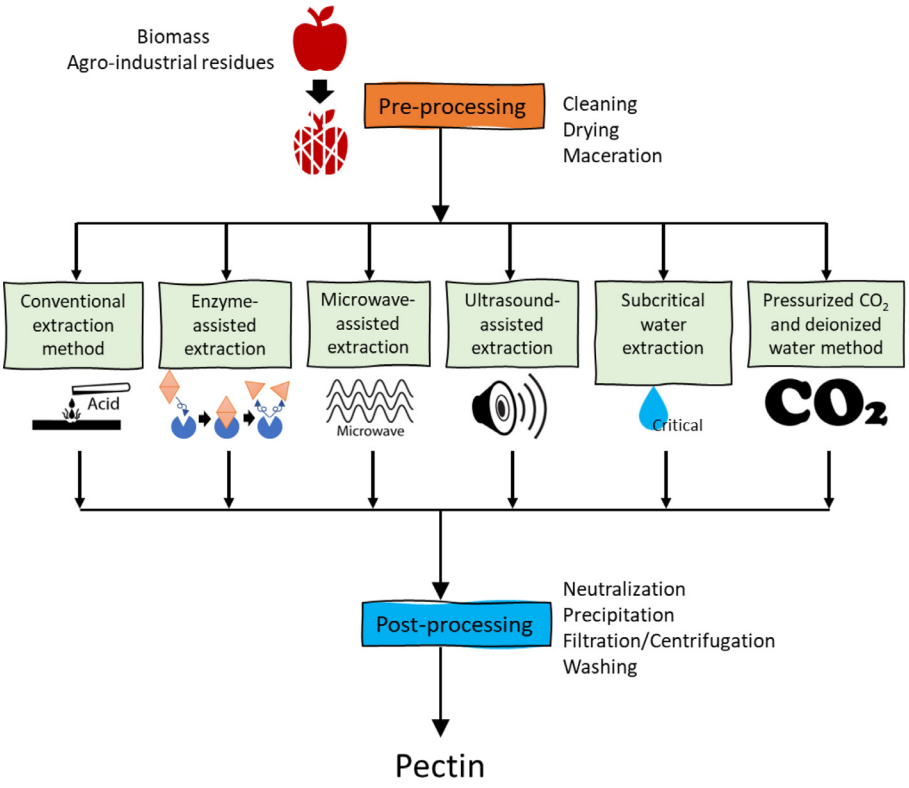

Fig. 2. A summary of the pectin extraction process.

among others (Israel et al., 2019). In some cases, crude enzymes from fungal cultures can also be used (Kumoro et al., 2020). While different enzymes serve different purposes, mixing different enzymes together can improve the rate and yield of pectin extraction. Commercial multi-enzyme blends are available for effective pectin extraction (Wikiera et al., 2015).

The quality and quantity of the pectin extracted by enzyme-assisted extraction varies a lot depending on the enzyme recipe, raw materials and extraction conditions (Zhang et al., 2020). A well optimized enzyme-assisted extraction procedure can achieve a pectin yield comparable to or even higher than that of conventional methods. Lim et al. (2012) reported that the pectin yields from Yuza pomace extracted by the chemical method and the enzymatic method were 8.0% and 7.3%, respectively. The pectin extracted by both methods falls into the category of low methoxyl pectins with similar degrees of esterification. The pectin from green tea leaves extracted by two commercial enzyme mixes: FoodPro® CBL and Viscozyme® L were 5.1% and 8.5%, respectively (Zhang et al., 2020). However, the pectin extracted by FoodPro® CBL had a much higher methoxylation degree (40.9%) than that by Viscozyme® L (22.4%) (Zhang et al., 2020). Interestingly enough, the sequence of adding the enzymes can also affect the final yield of pectin. Bayar et al. (2018) reported that the application of xylanase before cellulase in pectin extraction from *Opuntia ficus indica* Cladodes, resulted in a higher pectin yield than adding them together or adding cellulase before xylanase.

Enzyme-assisted extraction is a green solution for pectin extraction since less chemicals are needed. Another main advantage of enzyme-assisted extraction over conventional extraction is due to energy saving since the digestion and extraction are normally carried out at a lower temperature (Liew et al., 2016b). However, this method of extraction is generally more costly and time consuming, thus increasing the overall cost of extractions on an industrial scale. Another major limitation for enzyme-assisted extraction is that enzymes are sensitive to environmental conditions (temperature, pH) and chemical compositions (ion concentration, inhibitor, cofactors). Therefore, the yield and quality of the pectin extracted by enzyme-assisted extraction could vary largely from batch to batch, depending on the conditions of the raw materials and food waste.

5.4. *Microwave-assisted extraction*

Microwave-assisted extraction is more timesaving than the conventional method. Microwave-assisted extraction can significantly shorten the extraction period to a minute scale. The microwave heats up the sample and solvent through ionic conduction and dipole rotation (Llompart et al., 2019). The tremendous heat induced by microwave in the materials within a short period of time causes a disruption of the plant tissues and facilitates the dissolution of pectin into the surrounding solvent. The microwave can also rapidly denature the pectinases which cause pectin degradation. However, the quality of the pectin varies with different extraction conditions.

Dranca, Talon, Vargas, & Oroian, 2021 compared the results of conventional and microwave-assisted extraction of pectin from apple pomace. The microwave-assisted extraction was able to achieve a maximum yield of 38.06% in 90s at a microwave power of 560W, pH of 1.5 and solid-to-liquid ratio of 1:15 g/ml. The conventional extraction, however, took 120 min of extraction time at 90 °C, pH 1.5 in a citric acid solution to achieve a similar yield (38.91%).

The increase of microwave power or irradiation duration may lead to the degradation of pectin, resulting in a lower yield. Karbuz and Tugrul (2021) found that at a microwave power of 360 W, the pectin yield from Kiwi peel increased from 10.48% with a 1-min irradiation duration to 17.79%, with a 3-min irradiation duration. On the other hand, at a microwave power of 600 W, the pectin yield from kiwi peel decreased from 16.27% with a 1 min irradiation duration to 12.18% with a 3-min irradiation duration.

5.5. *Ultrasound-assisted extraction*

Ultrasound refers to sound waves with frequencies out of the human audible range, normally falling in the range of 20–40 kHz for pectin extraction. The irradiation of the raw materials with ultrasound served

multiple purposes. On one hand, the ultrasound irradiation enhances the cell disruption through cavitation, thus exposing the inner structure of the cells to the solvent. On the other hand, sonication also accelerates the rehydration of dry materials improving the penetration of the solvent (Toma et al., 2001). The heating effect of the ultrasonic vibration also facilitates mass diffusivity, improving the dissolution of the pectin into the buffer system (Yao, 2016).

When pectin was extracted from Navel orange peels in an acidic environment at 30 ˚C, sonication significantly improved the yield by 5 to 10 times depending on the pH of the solution and duty cycle of the sonication (Patience, Schieppati, & Boffito, 2021). However, the yield of pectin at this temperature range is still not comparable with the conventional heat extraction. Generally, the ultrasound-assisted method is incorporated with the heating extraction, making it an ultrasound-assisted heating extraction (UAHE) method, to improve the solubility of pectin in the extraction buffer (Xu et al., 2014). Banerjee et al. (2016) found that when extracting pectin from grape peels, UAHE achieved a slightly better yield in a shorter time at a lower temperature than the conventional heating extraction. Nevertheless, prolonged ultrasound irradiation with heat could lead to the rapid degradation of pectin, resulting in a lower yield (Xu et al., 2014). Liew et al. (2016a) found that when ultrasound-assisted and microwave-assisted extraction was used sequentially the pectin yield was better than when the conventional heating extraction method alone was used.

5.6. Subcritical water extraction

Water with a temperature higher than the boiling point (100 ˚C) under high pressure is termed as subcritical water. Under such conditions, water behaves more like a less-polar solvent due to the reduction of permittivity (Asl & Khajenoori, 2013). The high temperature also reduces the surface tension and improves the diffusivity of subcritical water (Asl & Khajenoori, 2013). Thus, subcritical water can facilitate the extraction of pectin from the agro-industrial residues. However, on the one hand, degradation of pectin occurs when the extraction temperature is over a certain temperature threshold and this threshold depends on the composition of the pectin found in the raw materials. Undesirable Maillard reactions may also take place at high temperatures leading to the browning of the pectin. However, the solubility of other cellular components (impurities) will also increase along with the improved extraction of pectin. Hence, the optimal extraction temperature for different materials needs to be determined empirically. X. Wang et al. (2014) found that citrus peel reached a maximum pectin yield, its highest molecular weight (~70 kDa) and galacturonic acid content at 120 ˚C. The maximum yield of apple pomace pectin was achieved at 150 ˚C, with a molecular weight of ~53 kDa and galacturonic acid content of ~40%, being higher than samples extracted at 130 ˚C (~65 kDa and 44%). Another study by Liew et al. (2018) also showed that the pectin yield from pomelo peel was highest at 120 ˚C and 3 MPa, through a face-centered central composite design.

Apart from the temperature, the extraction time and liquid/solid (L/S) ratio are also critical in the subcritical water extraction method. A study by Li et al. (2019) demonstrated that the yield of pectin from jackfruit peel peaked at 9.15 min with L/S ratio at 17.03 mL/g. Alterations of either of the parameters led to a drop in the yield.

Nevertheless, in some reports the molecular weight and the DE of pectin extracted by subcritical water extraction were lower than those obtained by conventional extraction, probably due to prolonged heating at high temperatures (W.J. Li et al., 2019).

Subcritical water extraction could also be coupled with other extraction techniques to combine their advantages. J. Chen et al. (2015) reported that pectin extracted from sugar beet pulp in a subcritical extraction system with ultrasonic treatment yielded 29.1%, which was comparable or even higher than results produced by the conventional method.

5.7. Pressurized carbon dioxide and deionized water method

A new extraction method was developed, given that pressurized carbon dioxide can both acidify water and generate carbonate ion (CO_3^{2-}), a natural chelate of Ca^{2+} (Tsuru et al., 2021). Tsuru et al. (2021) found that although the pressurized carbon dioxide method had a lower efficiency in extracting pectin from orange peel, when compared to hydrochloric acid (HCl) and the chelating agent (sodium hexametaphosphate) it generated pectin with a higher DE. Specifically, this method yielded about 4% pectin and over 90% DE while the HCl extraction yielded about 10% pectin and less than 80% DE. It also yielded pectin with a higher molecular weight than the HCl method done at the same time. The method is underdeveloped, but it has a lot of potential due to its low emission nature.

5.8. Post-extraction processing

After dissolving the pectin in buffer/solvent, it needs to be harvested and purified before further applications. The main goal is to remove or neutralize the chemicals mixed with the pectin and remove/replace the buffer/solvent.

The most basic and common way is to harvest the pectin through alcohol precipitation. After filtering the solid from the extract, the solubilized pectin should be precipitated by mixing the solubilized pectin with ethanol (Tsuru et al., 2021). A concentration of 60% ethanol is sufficient to precipitate the pectin. According to Tsuru et al. (2021), increasing the ethanol concentration did not increase the pectin yield. The precipitated pectin can be harvested by filtration or centrifugation. Further washing steps using different concentrations of ethanol are required to obtain pure pectin. Apart from ethanol, methanol and isopropanol can also be used for the precipitation process but considering the toxicity, ethanol would be the best choice (Salam et al., 2012). The pectin can be resolubilized in a solvent/buffer of choice for further application. Other techniques such as metal precipitation (Guo et al., 2015), ultrafiltration (Yapo et al., 2007b) have also been established for the post-processing of the extracted pectin.

5.9. Selection of extraction method for pectin production

While most of the comparisons of pectin extraction methods were done using laboratory set up and there was not a unified testing conditions even for the same method, it is difficult to cross compare the results of different studies and justify the most suitable method for industrial production. Furthermore, there are also variants of the extraction methods such as UAHE, and the sequential using of ultrasound assisted extraction and microwave assisted extraction, which make comparison more complicated. Based on literature cited above, a general comparison has been made in Table 2. Pressurized CO_2 and deionized water method is an underdeveloped method that required a lot of research and optimization before any practical application. Conventional method is basically the standard for pectin extraction. It is also used as a reference to determine the efficiency of other extraction methods. It is highly scalable with stable yield of pectin. Also, relatively low investment and technology input is required, which makes it an attractive method. Nevertheless, energy consumption and chemical pollution make it less likely to be a sustainable method. Enzyme-assisted extraction is rather a low emission method. It has a relatively low initial investment cost, but the later expense on enzymes could be a concern. Furthermore, the extraction time can be the longest among different methods, which reduces the overall output. Microwave and ultrasound assisted extraction, and subcritical water extraction required special technology. The initial investment would be high while the scalability is also limited due to the technological limitations. This could probably be compensated by the relatively short extraction time and high yield. Considering the cost, energy consumption and productivity, microwave and ultrasound assisted extraction could potentially be advantageous over the other methods.

Table 2
Comparison of different pectin extraction methods.

	Conventional method	Enzyme-assisted extraction	Microwave-assisted extraction	Ultrasound-assisted extraction	Subcritical water extraction	Pressurized CO_2 and deionized water method
Extraction temperature	+++	+	+ / ++	+ / +++	++++	+++
Yield	++ / +++	++	++ / +++	++ / +++	++	+
Extraction time	++	+++	+	+	+	++
Energy consumption	+++	++	++	++	+++	+++
Technology input	+	++	+++	+++	+++	+++
Environmental concern	+++	++	++	++	++	+

Note: "+" indicated the magnitude that increases with number of "+".

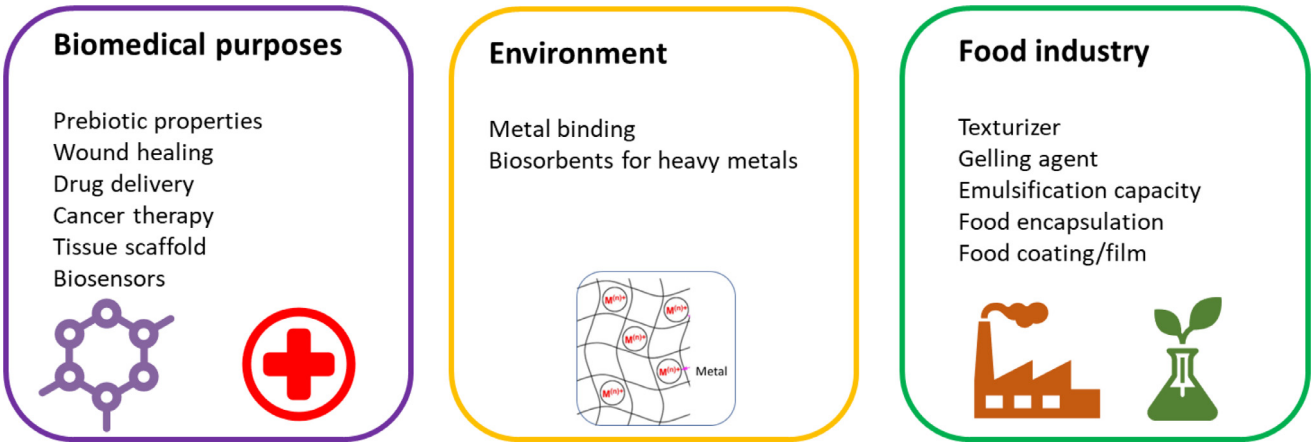

Fig. 3. Overview of applications of pectin in different areas.

6. Pectin applications

Pectin has a wide range of uses due to its biodegradability and biocompatibility properties. A summary of various applications of pectin can be seen in Fig. 3.

6.1. Biomedical and biomaterial applications

Pectin is not digested or absorbed by humans but it interacts with beneficial bacteria in the large intestine conferring prebiotic properties (Blanco-Pérez et al., 2021). The gut microbiota ferments pectin and releases secondary metabolites which promotes health benefits for the host (H. Tan & Nie, 2020). As previously mentioned, several studies have reported the positive health effects of pectin use including allergy and inflammatory diseases prevention (Blanco-Pérez et al., 2021), aiding in cancer therapy (Palko-Łabuz et al., 2021), lowering blood sugar and cholesterol levels (Yanlong Liu et al., 2016). Further, pectin applications include the controlled delivery of exogenous nutraceuticals or drugs via emulsion or hydrogel technology. This is based on pectin biocompatibility, gelling mechanism under acidic conditions, microbial degradation in the digestive tract, and its capacity to immobilize drugs, genes and proteins to prolong the retention time and improve treatment outcomes (Munarin et al., 2012). Mucoadhesive properties of pectin have been evaluated for the delivery of intranasal and ocular drugs (Sriamornsak et al., 2010). As it relates to cancer therapy, studies have examined the effects of a modified citrus pectin on galectin-3, a pleiotropic protein overexpressed in cancer cells. Results showed a protective role of the modified citrus pectin in treated mice with a reduction of galectin-3 expression and the downregulation of galectin-3 with inhibitory effects on urinary bladder cancer cell proliferation *in vitro* (Fang et al., 2018). Moreover, hydrogels have shown to be suitable for the immobilization of enzymes for the design of biosensors. Hydrogels can stabilize enzymes and provide protection against extreme operation conditions (pH, and heat) (Meyer et al., 2021). Some applications include detection of triglycerides in serum (Di Tocco et al., 2018), and determination of aldolase activity (Wang et al., 2016).

Pectin also has applications in tissue engineering since it can be manipulated to achieve 3-D matrices or scaffolds. Pectin based 3-D matrices act as a support for the delivery of bioactive compounds and promote tissue reconstruction. The use of pectin in bone tissue engineering has been the subject of numerous studies (Zhao et al., 2016) due to its biodegradability, biocompatibility and its capacity to mimic the extracellular matrix. Other biomedical applications include the use of pectin in gene therapy and polymer films (Rajabnejad keleshteri et al., 2021). Gene therapy aims to replace defective genes or silencing the expression of a certain gene that would trigger an unwanted effect. Polymer nanoparticles have the potential to offer a controlled release of genes or drugs not only at a target location but in a time-dependent manner (Vega-Vásquez et al., 2020). Drug delivery is achieved by entrapment of biomolecules/therapeutics within the interior structure of the nanoparticles or immobilized on the exterior surfaces of the nanoparticles. Polymer nanoparticles are formulations based on pectin together with other materials to avoid premature drug release because of hydrophilic functional groups present in pectin (Kedir et al., 2022). Other advantages of polymer nanoparticles include increasing delivery efficiency of drugs or genes, penetrate cells by endocytosis, and avoid clearance by phagocytes (Vega-Vásquez et al., 2020). New applications have been found beyond biomedical applications like agriculture. Potential benefits in this area include controlled delivery of pesticides, plant nutrition, and plant breeding (Vega-Vásquez et al., 2020). While polymer films, such as hydrogel films, have been employed on wounds or ulcers to prevent bacterial infection, maintain an adequate healing environment, support autolytic debridement and drug-release to promote wound healing (Fang et al., 2021). The use of hydrogels to release bioactive compounds offer great advantage to stimulate wound healing. Hydrogels can also be tailored to deliver specific antibiotics to treat infection and relieve inflammation by anti-inflammatories and antioxidants (Fan et al., 2021).

6.2. Metal binding

Several studies have shown that the adsorption properties, adsorption capacity and selectivity, of pectin for heavy metal depends on the pectin source (Wang et al., 2019). In general, pectin can bind heavy metals depending on their structure. The affinity of pectin to heavy metal ions has been evaluated with the following selectivity sequence being identified: $Pb^{2+} > Cu^{2+} > Co^{2+} > Ni^{2+} > Zn^{2+} > Cd^{2+}$ (Kartel et al., 1999). Other studies have corroborated sugar beet pectin's high affinity for Pb^{2+} and Cu^{2+} ions (Dronnet et al., 1997). However, Ni^{2+} has shown higher affinity for citrus pectin than other metal ions (Ajmal et al., 2000). Pectin derived from citrus, apples and grapes have been evaluated as biosorbents for cadmium removal (Schiewer & Patil, 2008). Schiewer and Patil (2008) reported that citrus peel has the highest adsorption capacity when compared to other pectin-rich raw materials with a metal uptake favored by increasing pH conditions. However, pectin application as adsorbent of heavy metals has some limitations since even pectin from the same source could present different adsorption capacity due to the use of different extraction methods. This fact has been observed for citrus pectin and its Pb^{+2} adsorption capacity (Balaria & Schiewer, 2008; Khotimchenko et al., 2007).

Additionally, different mechanisms may be behind the interaction between pectin and heavy metals. Plazinski (2013) proposed that formation of specific binding regions, egg-box like structures, play a key role in the proposed mechanism. In this process, free D-galacturonic acid carboxyl groups of pectin are the binding sites that allows the formation of links with heavy metals. Other proposed mechanisms suggest that the binding affinity may be cation dependent (Assifaoui et al., 2015) or associated with the formation of pectates through carboxyl groups of D-galacturonic and/or hydroxyl groups of the polysaccharide matrix (Kartel et al., 1999).

6.3. Food industry

As a natural-based polysaccharide constituents of primary cell walls of plants, pectin offers an attractive alternative to synthetic polymers owing to their renewable source (biomass waste), non-toxicity, low cost, and biocompatibility (Mellinas et al., 2020). Since its commercial production in the early 20th century, pectin has been used in numerous food processing and packaging applications, to act as a gelling agent, viscosity builder, micro and nano-encapsulating agent, as film/coating of fresh fruit and vegetables, and an emulsifier among other functionalities (Sabater et al., 2022). As a gelling agent, the degree of methylation affects its gelling properties: low methoxy pectin forms a gel in the presence of divalent cations and the pH ranges from 2 to 9.50, while high methoxy pectin gelation requires high cosolute concentrations and acidic conditions (pH 2.50–3.50) (Oakenfull, 1991). However, the choice of pectin also depends on the desired product such as jams, jellies, and fruit juices. Pectin extracted from citrus and apples is used due its gelling properties while pectin from other plant tissues like sugar beet and okra is preferred for its emulsifying capacities (Schmidt et al., 2015). Pectin's emulsification capacity has been attributed to its high acetyl content, ferulic acid moieties, covalently bound proteins and enrichment in the RG I segments (Alba & Kontogiorgos, 2017). Texture in food preparations is achieved by the use of high-ester, low-ester and low-ester amidated pectin (Flutto, 2003). The choice of pectin depends on different parameters including soluble solids and calcium content etc. Other food application includes the encapsulation of active substances such as alpha-tocopherol (Singh et al., 2018), fish oil (Encina et al., 2016), and D-limonene (Ghasemi et al., 2018). The encapsulation is used to protect biologically active material and prolong its shelf life through the application of a polymeric coating material (Singh et al., 2018). This coating film isolates the active material from the external environment, does not affect the properties of the material, and control the availability of the active materials (Singh et al., 2018). This encapsulation mechanism has been used in diverse fields like food, medicine, and textile industries.

Pectin-based coating/films provide a good barrier to preserve food quality and extend shelf life of food (Huang et al., 2021). This is a biodegradable packaging alternative to the traditional packaging which satisfies the increasing demand of consumers and organizations to reduce plastic waste and increase environment protection. Polysaccharides such as pectin have been used as a sustainable material for coating formulations. Coating is applied using liquid methods, immersion or spraying, on the food (Mohamed et al., 2020). While edible films are derived from solid sheets and then applied as a wrapping material. Pectin-based coating or films can be combined with other bio-polymers to improve physical properties like thermal stability, and mechanical and hydrophobic properties. These properties are used to evaluate the performance of the pectin-based packaging and determine the suitability for food application. Mendez et al. (2019) studied the influence of spent coffee grounds on high-methyl (HDM) pectin films and reported an increased water vapor permeability rate and thermal stability increasing their potential applications. Similarly, Lorevice at al. (2016) incorporated chitosan nanoparticles with HDM and low-methyl pectin matrices, the tensile strength increased compared with the pure pectin-based films improving the mechanical properties of the films. Other efforts have been made to improve the properties of pectin-based films. Oliveira et al. (2016) elaborated pomegranate peel pectin films with different contents of montmorillonite, the results indicated that the pectin film improved the tensile strength, and elastic modulus of films when montmorillonite was added up to 6wt%. Moreover, addition of bioactive molecules as antioxidants, and antimicrobials in the pectin-based coating/films improves functional properties. Antimicrobial properties are crucial to protect food from microbial contamination and active compounds like oregano essential oil, and nisin contribute to suppress microbial growth (S. Kumar et al., 2020). Antioxidant property can eliminate and avoid the formation of free radical (Huang et al., 2021). Plasticizers are also included in polysaccharide films to boost polymer thermoplasticity. Rodsamran and Sothornvi (2019) reported coconut water as a potential natural plasticizer for lime peel pectin films with improvements in the film flexibility compared to glycerol as plasticizer.

7. Application of enzymes involved in pectin degradation

Pectinase is one of the most important industrial enzymes that plays a significant role in the current biotechnological period (N. Sharma et al., 2013). Pectinases are used in the: fruits/vegetables processing, wine, tea/coffee and pharmaceutics industries (Mahmoodi et al., 2017). Moreover, these enzymes are used in textile processing, paper making, pectin containing waste water treatment, degumming of plants bast fibers (Oumer, 2017) (Fig. 4).

7.1. Applications in the beverage industry

7.1.1. Fruit juices and wine

Each fruit has a specific amount of pectin and its content is important regarding the enzymatic activities required to produce juices and concentrates (Grassin & Fauquembergue, 2010). Fruit juices are naturally cloudy due to presence of polysaccharides (pectin, cellulose, hemicelluloses, lignin and starch), proteins, tannins and metals (H. P. Sharma et al., 2017). The use of enzymes in fruit juice extraction and clarification processes has been widely known throughout the fruit processing industries (Barman et al., 2015). Freshly pressed fruit juices especially from fruits like apple, pear, and grape are viscous and turbid. Pectinases catalyze the degradation of the pectin, reducing the viscosity and turbidity due to cluster formation and separation through centrifugation or filtration. The juice has a higher clarity and enhanced flavor and color after pectinase treatment (Nighojkar et al., 2019). The main sources of pectinolytic enzymes are yeast, bacteria and a large variety of filamentous fungi. The *Aspergillus* strains are preferred as they

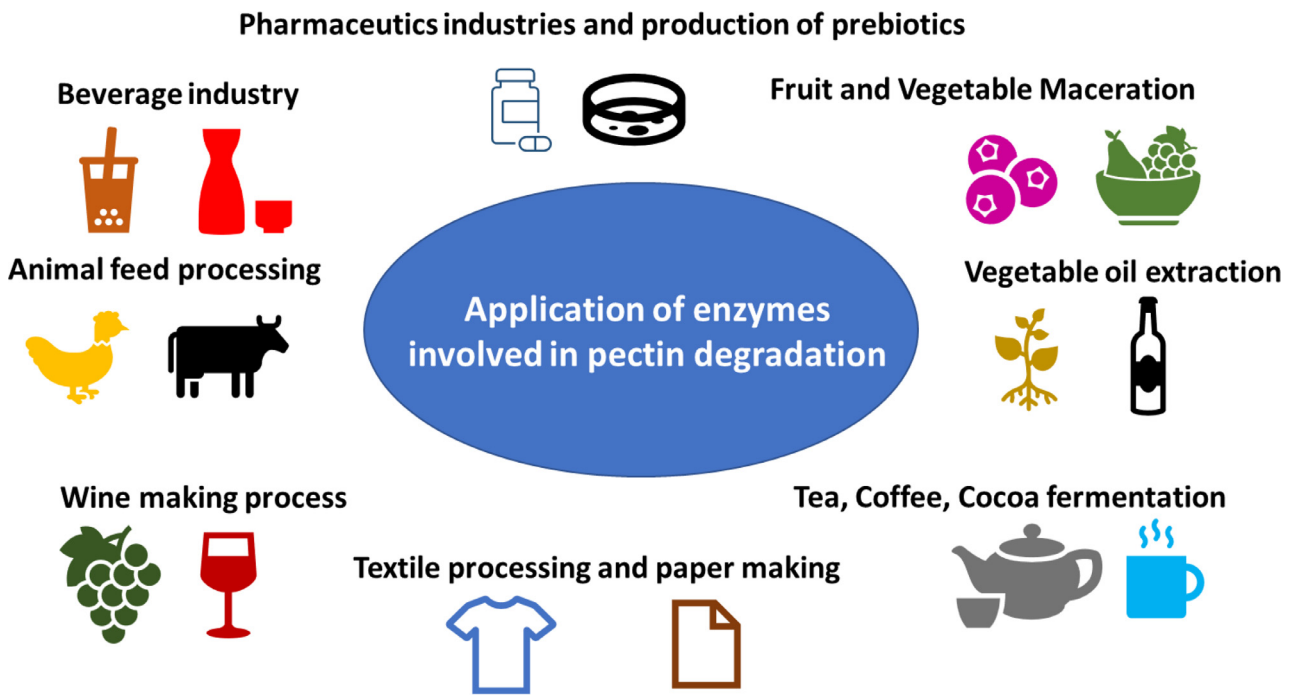

Fig. 4. Application of enzymes involved in pectin degradation.

are generally regarded as safe (GRAS) in the food industry (Eze et al., 2014). Studies have revealed that the use and combination of different pectinolytic enzymes in different fruit juices have served to clarify them. According to Gupta et al. (2015), pectin methylesterase and polygalacturonase can be used to degrade pectin in fruit juice, clarifying it. Additionally, Dixit et al. (2013) found that pectin methylesterase from *Datura stramonium* combined with polygalacturonase increased the clarity of orange, apple, pomegranate and pineapple juices by 2.9, 2.6, 2.3, and 3.6-fold, respectively. Sandri et al. (2013) using *Aspergillus niger* pectinase reported a 40% and 90% decrease of turbidity in blueberry and apple juice, respectively. While Ahmed and Sohail (2020) reported 61% clarity of orange juice after using pectinase from *Geotrichum candidum* AA15.

Pectinases are also applied in the wine making process at various stages, mainly in the crushing of fruits, before and after fermentation (Nighojkar et al., 2019). Pectinases facilitate filtration, aid in the extraction process, increase juice yield and accentuate the flavor and color. Enzymatically treated wines needed less filtration time and were more stable when compared to untreated wines. The treatment of macerated fruits with pectinases before the addition of inoculum resulted in wine with enhanced characteristics (color and turbidity), compared to untreated wines (Kalra et al., 2021). Pectinases, such as polygalacturonases, pectin esterases, pectin lyases, and rhamnogalacturonases are applied in winemaking to improve the wine processing and its final quality. These enzymes modify the polysaccharide and oligosaccharide composition of wines such as Merlot red wines (Ducasse et al., 2011).

7.1.2. Tea, coffee, cocoa fermentation

Pectinase treatment accelerates the fermentation of tea leaves by breaking down the pectin in their cell walls, improving the quality of the tea (Thakur & Gupta, 2012). The change in color of the treated, fermented leaves also results in the development of their aroma (Kumar & Vuppu, 2014). These enzymes are also used in the fermentation of coffee to remove the mucilaginous coat from the coffee beans. Degradation of mucilage improves the quality of coffee beans. Crude pectinase from *Aspergillus niger* causes about 71% degradation of the mucilaginous layer of coffee beans after 2 h of fermentation (Murthy & Naidu, 2011). Similarly, cocoa seeds are encased in a mass of white mucilaginous pulp and pectic enzymes are applied to liquefy the mucilaginous mass (Nighojkar et al., 2019).

7.2. Utilization in food processing

7.2.1. Fruit and vegetable maceration

Additionally, pectinases play a crucial role in maceration of vegetables to produce various products like puree. Several pectinolytic enzymes from *Aspergillus aculeatus* such as rhamnogalacturonase and pectin lyase were used in the treatment of carrot mash, giving it a puree-like consistency with a low degree of syneresis (Demir et al., 2001). The enzymatic maceration of fruits mostly involves enzyme preparations with only polygalacturonase or pectin lyase activities. While for vegetable maceration, bacterial endopectate lyase is preferred due to its optimum alkaline pH (Nighojkar et al., 2019).

7.2.2. Vegetable oil extraction

The use of pectolytic enzymes allows the extraction of vegetable oils such as olive, sunflower, coconut, palm or canola, in a process by degradation of cell wall components. Iconomou et al. (2010) found that the addition of enzymes to olive paste during the processing of virgin olive oil increased its phenolic antioxidant content, enhanced the overall organoleptic quality and increased the yield. While Ortiz et al. (2017) found that pectinolytic enzymes from *Aspergillus giganteus* improved the olive oil yield and rheological characteristics without affecting its chemical properties.

8. Future perspectives

Having the GRAS designation in the US and being recommended as an excellent ingredient by the European Food Safety Authority (EFSA), pectin's use as an essential food ingredient and health promoting ingredient is widespread (Muñoz-Almagro et al., 2021). The functional properties of pectin relies mainly on its heterogeneous structure, characterized by different degrees of esterification, molecular weight and neutral sugar side chains that are intrinsic to its source (C. Wang et al., 2021). This has boosted the need for continuous research on new sources of pectin as well as a valorization of fruit and vegetable agro-industrial

by-products conducive to the development of tailored food ingredients (Yahui Liu et al., 2022), while simultaneously promoting the reduction of food waste under the concept of biorefinery for environmental sustainability (Clauser et al., 2021). In the food industry pectin can be used as a low-calorie or low-fat ingredient acting as a fat replacer. Moreover, pectin plays an important role as an emulsion stabilizer. In general, the cosmetic industry uses pectin to stabilize creams, lotions or as a skin anti-aging component, while in the pharmaceutical industry it has numerous applications from drug delivery to treatment of different ailments and diseases, including blood cholesterol reduction, post-prandial glycaemic response, or the potential treatment of widespread diabetes (Muñoz-Almagro et al., 2021).

The interactions of pectin with other biopolymers like chitosan, pea protein, collagen, whey protein isolates and others generate more stable emulsions, nanoparticles, microcapsules, hydrogels or liposomes for targeted delivery systems and the encapsulation of bioactive compounds is currently a trending topic of research, favoring a new trend in food preservation. The capacity of pectin to act as a prebiotic promoting the abundance of beneficial gut probiotics and facilitating therapeutic applications still requires experimental evidence through *in vivo* studies (Yahui Liu et al., 2022). Due to its biodegradability and low toxicity, pectin is an excellent biomaterial, but further evaluation in biofilm production and tissue engineering will be important (Rajabnejad keleshteri et al., 2021). The use of pectin as a promising ingredient in edible food ink for 3D printing is another rapidly growing field of research (Cen et al., 2022). Novel industrial applications for pectin, be it in optimizing bioactive concentration in pectin-biofilms, or as an encapsulating agent to control the release kinetic of pharmaceuticals, or in determining the minimum inhibitory concentrations (MIC) values for specific microorganisms in food preservation will continue to be areas in need of research.

9. Conclusions

Pectin is and will remain an essential ingredient for food, pharmaceutical and cosmetic applications. It has a multifaceted structure that is characteristic to its origin. Extraction methods are determinant on the characteristics of the obtained pectin and must be considered at industrial level to assess cost, energy consumption and productivity. Knowledge on biodegradation processes of pectin using enzymes is essential for biomedical and food applications. Advanced research should be focused on sustainable extraction techniques from multiple raw materials in order to obtain a tailored ingredient for desired purposes. Consequently, such investigative enterprises will undoubtedly promote a circular economy through the beneficial recycling of commonly discarded waste products from the processing industries. Since its utilization on an industrial level, novel applications for pectin have been developed, which will continue as further research works disclose new potential uses of this astounding natural plant component.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Data availability

Data will be made available on request.

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