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Traditional And New Methods For Pectin Extraction: Ultrasound, Microwave, And Enzyme-Assisted Methods

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Abstract: A complex polysaccharide generated from plants, pectin is essential for food, medicine, and pharmaceutical applications because of its gelling, stabilizing, and health-promoting qualities. Conventional pectin extraction, which has historically been done from apple pomace and citrus peels using hot acid solutions, has several drawbacks, including high energy consumption, lengthy processing periods, and environmental issues related to strong mineral acids. In response, cutting-edge "green" methods that provide increased yields, shorter extraction times, and better functional quality—ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and enzyme-assisted extraction (EAE)—have surfaced. In order to improve solvent penetration and mass transfer while reducing heat degradation, UAE uses acoustic cavitation to break down plant cell walls. MAE speeds up pectin solubilization, cell rupture, and diffusion by using fast, selective heating via dielectric interactions. By using particular hydrolytic enzymes to dissolve structural barriers, EAE provides customized control over the molecular weight and esterification level. In addition to analyzing extraction mechanisms, important parameters, benefits, drawbacks, and new research advancements, this review compares standard and non-traditional approaches. Process optimization, environmental sustainability, and the possibility of combining these methods to produce pectin in an effective, environmentally responsible, and superior manner are highlighted.

Keywords: Pectin extractionnon, Conventionalextraction methods, Ultrasound-assisted extraction (UAE), Microwave-assisted extraction (MAE), Enzyme-assisted extraction (EAE), Green extraction technologies, Sustainable extraction.

1. Introduction:

A plant-based hydrocolloid, pectin is frequently used as an ingredient in countless food products, the pharmaceutical industry, and other applications. Because of its special structural and biochemical qualities, it is also used to make edible films, plasticizers, paper substitutes, and foams. Many polymers with varying neutral sugar concentration, molecular weight, and chemical structure are referred to as pectin because different plants manufacture pectin with varying functional characteristics. Numerous uses for pectin molecules are made possible by their various functional groups and specific structural alterations [1–3]. mostly because of its low production costs, non-toxic qualities, and ease of accessibility [4]. The biggest percentage of plant mass is composed of pectin, a family of complex polysaccharides, which makes up about 35% of the cells of dicotyledonous plants, compared to 2–10% in grasses [5] and 5% in woody tissues [6]. According to the American Chemical Society, there are four forms of pectic substances: protopectin, pectic acid, pectinic acid, and pectin. The parent material, protopectin, has no gel-forming qualities and is comparatively insoluble. The inner tissues of plant cell walls frequently contain protopectin [7].Pectin was deemed "generally recognized as safe" by the US Food and Drug Administration. Pectin makes up about 30% of the white rind layer of fruits like lemons and oranges [8]. This dimethyl ester is based on polygalacturonic acid and is made up of 300–1000 galacturonic acid units connected by 1α–4 linkage. With the right modifications, it has a wide range of applications due to its several functional groups that can stimulate different activities. This is mostly due to the fact that it is widely accessible, safe, nontoxic, and affordable to create [9].

Pectin is frequently extracted using enzymatic, hot water, hot acid solution, and ultrasound-assisted techniques [10]. In the industrial manufacturing of pectin isolation, the first two techniques are more traditional and frequently employed. An acidic solution with a pH of 1.5 to 3.0 and temperatures ranging from 50 to 100 degrees Celsius for 0.5 to 6 hours is used in the traditional extraction process [11]. In order to dry PMTL and deactivate enzymes, microwave treatment was employed as a pretreatment in this investigation. Additionally, the microwave-dried leaves exhibited superior pectin strength and rehydration impact [12]. Next, using a low-cost acid water-based extraction approach, the best factors were identified based on the pectin's quality and optimal extraction yield. Response surface methodology (RSM) is a statistical technology that optimizes complex processes with great efficiency [13].

The middle lamella and cell wall of higher plants contain pectin, a structurally complicated macromolecule. D-galactolacturonic acid (D-GalA) units that have been partly esterified make up the backbone of pectin. As a food additive, gelling agent, thickener, texturizer, emulsifier, stabilizing agent, and fat substitute, pectin is mostly utilized either by itself or in conjunction with other biopolymers in a variety of food formulations, including meat, dairy, jams and jellies, and bakery and confectionery goods [14].

Additionally, because pectin is a soluble dietary fiber that lowers blood glucose and cholesterol, lowers heart disease, and has anti-inflammatory properties, its application in pharmaceutical goods has increased recently [15]. As a result, the demand for this multipurpose macromolecule is rising on international markets [16]. Finding more low-cost sources, researching the extraction procedure, and characterizing the extracted pectin for appropriate uses so appear to be crucial. Commercial pectin is extracted in an industrial setting using hot water that has been acidified with powerful mineral acids, like hydrochloric and nitric, and it takes a long time to extract. The primary disadvantages of this process are the toxicity of these potent mineral acids, the caustic and environmentally unfriendly effluents produced, the lengthy processing time, and the high energy usage. According to recent research, using citric acid instead of mineral acids may increase pectin supply and quality while reducing environmental issues and yielding financial gains [17]. Pectin is primarily used as a gelling, stabilizing, and thickening agent in food products such fruit juice, confections, and jams and jellies. Pectin is also employed in the pharmaceutical business and is thought to lower gallstones and heart disease. Traditionally, a hot, diluted mineral acidic solution is used to extract pectin. Although the amount of time required varies from maker to manufacturer and depends on various aspects such as the type of pectin wanted and the raw material, this procedure is generally time intensive [18,19]. Conventional techniques are inappropriate for both the quantity and quality of the pectin extraction because of this circumstance, which causes pectin degradation. In order to obtain the optimum quality and quantity features of extracted pectin, it is crucial to use an appropriate approach. Numerous techniques for pectin extraction have already been studied, with generally positive outcomes. These solutions include employing pulsing hydrodynamic action with turbulent recirculation [20].

2. Basic structure and chemistry:

Pectin is a naturally occurring polymer found in plant cell walls that is mostly derived from foods like sunflowers, apples, and citrus peels. Long chains of α-(1,4)-D-galacturonic acid make up this complex polymer, some of which may be partly methylated, which means that some of the galacturonic acid's carboxyl groups have been replaced with methoxy groups. Polygalacturonic acid, rhamnogalacturonan-I, rhamnogalacturonan-II, and xylogalacturonan are the four constituents of pectin [21]. The covalent bonds that hold these polymers together. These four components are widely distributed, as shown by the high-resolution model of pectin (Figure 1b). The basic components of pectin are polygalacturonic acid and rhamnogalacturonan-I, while the secondary components are xylogalacturonan and rhamnogalacturonan-II. However, the amount and content of each component differs throughout pectin sources. Xylogalacturonan (XG) is distinguished by the presence of xylose at the C-3 position on some of the galacturonic acid residues in its main chain [22]. The side chains of rhamnogalacturonan-I are where variations within pectin are most noticeable. Furthermore, a rhamnogalacturonan-II dimer is created by joining two rhamnogalacturonan-II units with celery sugar residues via a boric acid ester [23]. According to the degree and kind of substitution, pectin chains are typically divided into three categories: homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II. Homogalacturonan, often known as "smooth" chains, is a simple, non-substitutable chain of polygalacturonic acid that makes up around 60% of all the pectins found in cell walls [24].

Spatial-regulated polymers make up Rhamnogalacturonan I (RG-I), which is structurally defined as a lengthy backbone sequence of alternating galacturonic acid units and D-rhamnose.Whereas rhamnogalacturonan II is made up of a homogalacturonan backbone that has been replaced with a wide range of complex glycan side chains that contain a multitude of neutral sugars, xylogalacturonan (XG) is made up of galacturonic acid in the backbone joined by xylose in branches. While rhamnogalacturonan I is connected by 1,2 glycosidic linkages (C1 on the galacturonic acid, C2 on the rhamnose) and rhamnogalacturonan II has 1,4 glycosidic linkages (C1 on the rhamnose, C4 on the galacturonic acid), 1,4 glycosidic linkages are found throughout the entire homogalacturonan pectin chains [25]

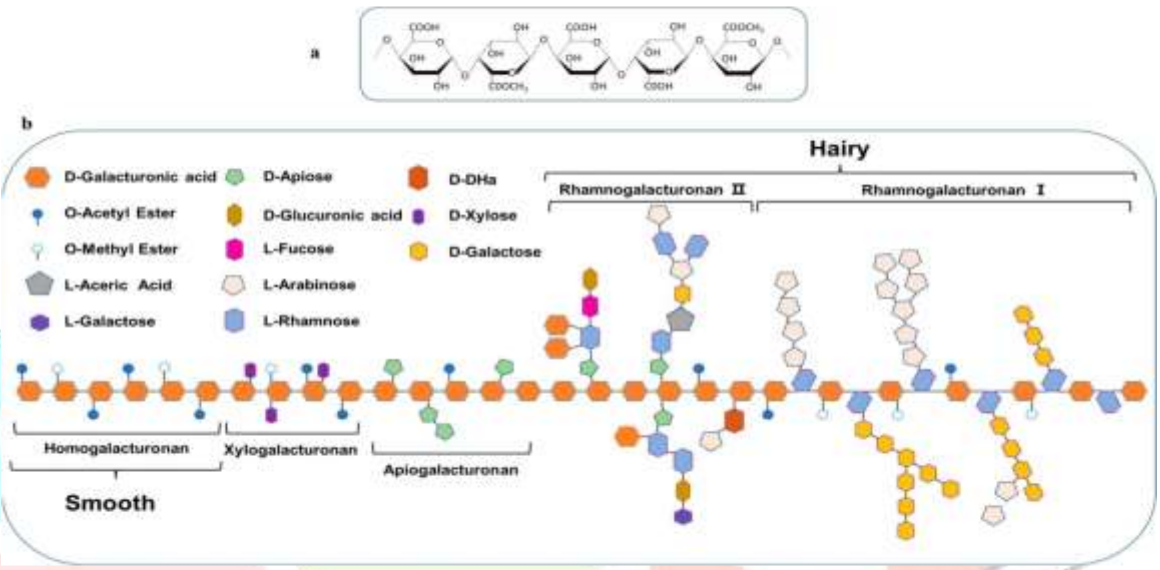

Figure 1: Schematic structure of pectin [23]. (**a**) Linear chain; (**b**) branched chain. D-DHA: 3-deoxy-Dlyxo-heptulosaric acid.

3. Extraction of pectin:

Nearly all plants contain the polysaccharide pectin, which helps to preserve the integrity of the cell structure. rotopectin is the insoluble form of pectin present in the cell walls of plants. The extraction at high temperature is initiated by the hydrolysis of the protopectin, followed by the breakage of bonds between sugars and the cell wall, resulting in the release of pectin into the extraction medium. Traditionally, acid extraction was employed to extract insoluble pectin from peels of citrus and apple pomace by heating it in an acidic medium to convert it to a soluble form. For the extraction of pectin from various agrobased industry wastes, various emerging technologies such as deep eutectic solvents, enzyme-assisted extraction, subcritical fluid extraction, ultrasound-assisted extration, and microwavebased extraction, or a combination of one or more methods, are now being used [26].

Because the raw materials needed for pectin extraction, including fruit peels or pomace, have a high moisture content and are therefore vulnerable to degradation by fungal enzymes, the process of extracting pectin from natural sources is laborious and time-consuming. The breakdown of pectin is caused by the pectic enzymes that fungi create, such as the de-esterifying (pectin methylesterase) and depolymerizing (pectin lyase, polygalacturonase, and pectate lysase) enzymes. Compared to citrus peels, pectin extraction from apple pomace is more challenging because pectolytic enzymes might cause it to decay unless it is immediately dried to lower the moisture level before being stored further for the pectin extraction procedure. In some apple varieties, enzymatic treatment of apple pulp is necessary for the efficient extraction of apple juice, thus rendering the apple pomace unsuitable for pectin extraction [27].

The extraction parameters such as particle size, pH, temperature, extraction time and type of extraction solvents greatly affects the yield of pectin [28] and drying methods [29].

The particle size of raw material affects the pectin yield, as in small particles of substrate more protopectin is available as compared to large particles [30,31].

Aqueous extraction is a typical way of extracting pectin from raw materials; the most popular techniques include microwave heating [32], direct boiling, ultrasonic [33,34], autoclaving [35], and electromagnetic induction [36].

4. Method of pectin extraction:

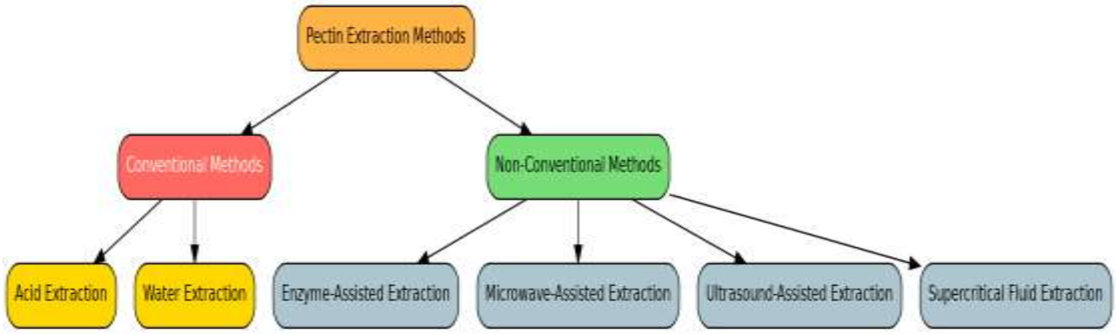

Fig.3 Methods of pectin extraction

4.1. Conventional method:

In addition to being thermally hazardous, the traditional method of pectin extraction is linked to significant time, energy, and solvent use. To get over the aforementioned restrictions and enhance pectin properties, novel extraction techniques such ultrasonic (US), microwave (MW), and enzyme have been studied [37, 38]. Long heating durations, high temperatures, equipment corrosion from the use of powerful mineral acids, a higher risk of pectin degradation, higher costs, and environmental risks are all associated with current methods. Green methods, on the other hand, produced higher-quality extracted pectin, lower prices, higher yields, environmental safety, a large reduction in extraction time, a drop in the demand for solvents, and a reduction in energy usage because of lower temperatures. Green extraction techniques, with their creative approaches and promising options for the sustainable extraction of pectin, have replaced conventional methods due to the limitations of conventional processes. As environmental protection has gained more attention, green extraction methods have become a viable alternative in recent years. To increase the quality and efficiency of pectin, a number of environmentally friendly extraction methods have now been developed, including enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE) [39–41].

5. Ultra sound assisted extractin:

Mechanical vibrations that occur above the human hearing range of 16 Hz to 20 kHz are known as ultrasonic waves [42].Ultrasound-assisted extraction frequently makes use of ultrasonic waves with frequencies between 20 and 100 kHz. It is important to note that the frequency of ultrasonic waves influences the extraction process by influencing the size of microbubbles and their resistance to mass transfer. Additionally, the generation and intensity of cavitation in liquid decreases as the ultrasonic frequency increases [43].It has been demonstrated that ultrasound-assisted extraction increases yield while decreasing extraction time. Acoustic wave pressure variations create microbubbles, which, when they collapse, create microjets, upending cellular structures, enhancing solvent penetration, and

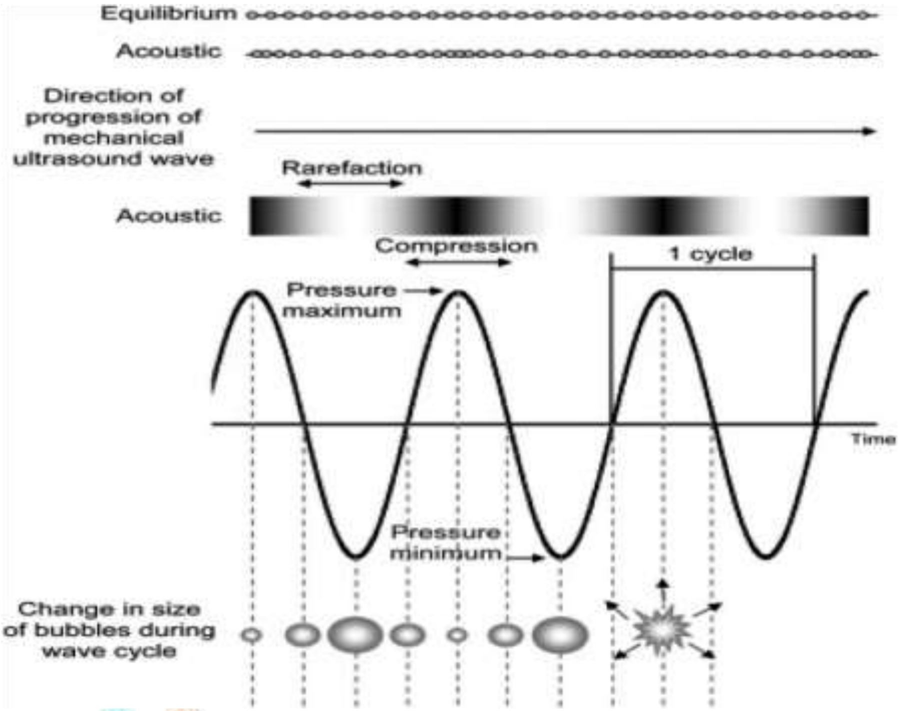

Figure 3. Schematic representation of cavitation caused by sonication. Adapted from Picó.[44].

Kinetics of mass transport (Fig. 3). Solvent penetration into the cell causes swelling and hydration, which enlarges the cell pores and enhances diffusion and mass transfer [45].The substantial advantages of ultrasound-assisted extraction—low energy consumption, reduced extraction time, reduced solvent consumption, and enhanced extraction yield—are supported by a number of studies. These all support the use of ultrasonography as a "green" extraction technique. Pectin was extracted using ultrasound from various fruit wastes and byproducts.
Ultrasound-assisted extraction has been shown to generate more pectin at lower temperatures and in less time than traditional hot extraction. Bárbara and associates [46]
examined the impact of employing ultrasonic and the traditional hot extraction method to extract pectin from mango peel. Using ultrasound for 10 minutes at 85°C resulted in an 8.1% pectin yield (Table 3), whereas the usual approach for 30 minutes at 85°C provided a 5.4% yield. These findings suggest that ultrasound-assisted extraction significantly shortened extraction times while increasing the yield of pectin extraction. In addition, ultrasonic power intensity is a crucial extraction factor. By upsetting plant cells, higher ultrasonic intensity has been shown to improve pectin output by increasing pectin extractability. The mechanism is related to the collapse of cavitation bubbles, which intensifies with increasing intensity [47].

This table provides a comparative overview of pectin extraction parameters and outcomes for different fruit

Effect of ultrasound-assisted extraction on the yield and physicochemical properties of pectin extracted from tropical and sub-tropical fruit/by-product.

Fruit/Byproduct	Extraction Conditions	Yield (%)	Temp (°C)	Time (min)	Acid	pH	Galacturonic Acid (%	Degree of Esterification (%)	Reference
Mango peel	497.4 W/cm² (Ultrasound)	8.1	85	10	Nitric acid	2.0	76.0	61.0	[48]
Yellow passion fruit peel	644 W/cm² (Ultrasound)	12.67	85	10	Nitric acid	2.0	66.65	60.36	[49]
Jackfruit peel	Not specified	14.47	60	22.5	Not specified	1.5	Not specified	Not specified	[50]
Pomegranate peel	20 kHz (Ultrasound)	23.87	62	29	Not specified	1.3	Not specified	Not specified	[51]
Sour/Bitter orange peel	150 W (Ultrasound)	28.07	Not specified	10	Citric acid	1.5	65.3	6.77	[52]

peels.

In keeping with the ideas of "green" chemistry and "green" technology, the combination of ultrasonic-assisted extraction and food-grade acids like citric and acetic acid also helps create "clean" label biopolymers. Hosseini and associates have studied the extraction of pectin from bitter oranges using ultrasonic and citric acid [53] and a traditional extraction method without acid [54], both of which are in line with the sustainable development viewpoint. According to the scientists, the pectin yield increased from 17.95% (traditional extraction: 95°C, 90 minutes, liquid–solid ratio 25 v/w, no acid) to 28.07% (ultrasound-assisted extraction: 150 W, 10 minutes, pH 1.5 using citric acid, liquid–solid ratio 20 v/w) when citric acid and ultrasounds were combined. Minjares-Fuentes along with associates [55] examined the use of ultrasonography and citric acid (140 W, 75°C, 60 minutes, pH 2.0) to extract pectin from grape pomace. According to the authors, the yield of pectin produced by ultrasonication (32.4%) was 20% higher than that produced by applying the same temperature, duration, and pH conditions without ultrasonication (25.6%). The information provided supports the hopeful application of "green" technology and "green" chemistry for sustainable development.

6. Enzyme assisted:

For better access to the cells, EAE usually requires a certain amount of size reduction of the pectin-bearing substrate. As with most other extraction methods, enzymes can be chosen for specific functions and ideal process conditions [56]. Temperature, enzyme concentration and composition, hydrolysis duration, pH and solvent ionic strength, solvent-biomass ratio, and, to a lesser extent, substrate moisture content are process parameters of special relevance in EAE processes. Numerous research have been conducted in an effort to optimize the EAE process, with varying and occasionally intriguing outcomes. Ptichkina and associates [57] examined the EAE of grey pumpkin (Cucurbita pepo) pectin. Aspergillus awamori produced the best results in a preliminary assessment of the catalytic activity (i.e., cellulase and pectinestase) of several commercial enzymes; the parameters comprised a constant at 3 hours of hydrolyzing time and an enzyme concentration of 1% (w/v). compared to the yield that other researchers got using the same substrate but a different enzyme (Bacillus polymyxa) [58]. Despite a shorter extraction time of 3 hours (as opposed to 24 hours), the authors reported poorer yields (14% vs. 22%). The presence of pectin esterase in this enzyme was thought to be helpful in the production of pectins with different levels of esterification. Jeong and associates [59].

Plant Material	Enzymes Used	Enzyme-to-Sample Ratio	Time (h)	Conditions	Key Findings	Reference
Chicory roots	Protease, Cellulase, Pectinase	1:10 (v/v)	8-48	T, 40 °C (then 100 °C post-EAE); solvent: sodium acetate	~25% higher yield increase over CHE; lower MW pectin	[60].
Mixed crops (chicory, citrus, cauliflower, endive, sugar beet)	Cellulase, Protease, Pectin methylesterase	1:10 (v/v)	4	T, 50 °C; mixing rate: 120 rpm; S/L: 1/100 and 1/50 (w/v); solvent: 0.05 M sodium acetate buffer	Healthful oligosaccharides obtainable after EAE; HM pectin; LM pectin obtainable by adding commercial pectin methylesterase	[61].
Butternut (Cucurbita moschata)	Cellulase, Hemicellulase	1:200 and 1:40 (w/w) respectively	20	T, 30 °C; S/L: 1/100 (w/v); solvent: 0.05 M sodium citrate buffer	~30% higher yield by hemicellulase; higher DE (72.6 vs 54.2); improved rheology	[62]
Lime peel	Cellulase, Hemicellulase (including xylanase, arabinoxylanase)	1:533 (v/v)	4	T, 50 °C; pH 3.5; S/L: ~1/30 (w/v); solvent: 0.05 M citric acid buffer	pH adjustment allows consistently high yield and homogeneous/repeatable quality	[63]
Lime peel	Cellulase (0–50 U/g), Xylanase	0.5 + 3.5 b	—	T, 50 °C; pH 4.5; P, 100 MPa; S/L: 1/30; solvent: 0.05 M citrate buffer	30 min high-pressure processing + 3.5 h EAE improved yield; xylanase significantly affected yield, GalA, and DE	[64]

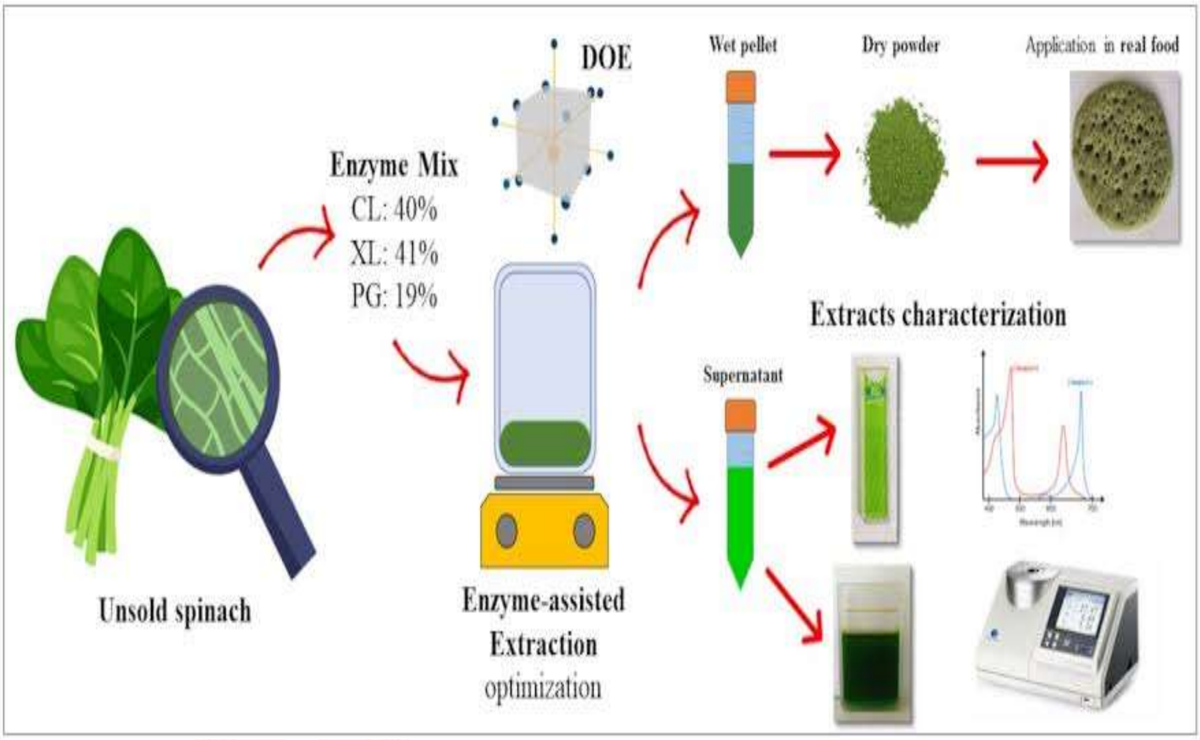

Figure 4.process of Enzyme assisted extraction of pectin

Effect of enzyme-assisted extraction on the yield and physicochemical properties of pectin extracted from fruit/by-product

Remarks:

• Ratios: w/w stands for weight in relation to biomass, and v/v for solvent.
• For Naghshineh et al., "b" in the enzyme ratio denotes a combination of high-pressure pretreatment and EAe.

Despite the fact that their galacturonic acid levels (56.56 vs. 56.44%) did not differ significantly, the scientists found that pectin produced by EAE (using Celluclast®) had a larger molecular weight (490.30 vs. 192.73 kDa) than those that were conventionally extracted. Commercial cellulases (Celluclast®) and proteases (Neutrase®) were employed in the EAE of a previous comparative study of EAE and CHE of pectin from chicory and cauliflower, and the extraction yield was once again greater (34.6%) than that of CHE (27.8%) [65].

7. Microwave assisted pectin extraction:

By means of molecular interactions with the electromagnetic field, microwave energy is directly transferred to materials [66]. Here, the material is penetrated by electromagnetic waves, which interact with the molecules. The molecules absorb the energy and transform it into heat. As a result, the microwave field's amplitude decreases as it gets farther away from the material's surface. Ionic conduction and dipolar rotation are the two main processes in dielectric materials that transform microwave energy into heat. Since most biomass materials and aqueous solvents (such in pectin extraction) include both dipolar and ionic molecules, dipolar rotation and ionic conduction take place at the same time, heating the material relatively instantly [67]. As a result, dipolar rotation takes center stage when deionized water is utilized as the extracting solvent. However, ionic conduction still has some influence since the free ions in the biomass dissolve into the solution. Ionic conduction's contribution to heating should rise with increasing ionic concentration when using solvents such as acids, alkalis, and/or salty solutions. The dielectric characteristics of a substance determine how much of the energy it absorbs and how much is transmitted through it when exposed to microwaves [68]. These are employed in the design of microwave reactors, to anticipate the rates at which materials will heat up when exposed to microwave electric fields, and to comprehend how materials react to microwaves. The dielectric loss factor (ε''), which measures the transformation of electromagnetic energy into thermal energy, and the dielectric constant (ε′), which measures a material's capacity to be polarized within an electromagnetic field, are examples of dielectric characteristics. Another definition of the dissipation factor, loss tangent, or tanδ is the ratio of the dielectric loss to constant: ε tanδ = ε′ (1) A descriptive dielectric characteristic called Tanδ can be utilized to determine a material's overall capacity to absorb microwave energy and transform it into heat [69]. A substance that warms effectively in a microwave field is referred to as "lossy." Higher dielectric loss or loss tangent components will be preferentially heated and will heat up more quickly than less lossy components when microwaves are applied to a heterogeneous material. The depth into a sample at which the microwave power has decreased to 36.8% of its transmitted value is known as the penetration depth, or dp. Materials with stronger dielectric qualities have smaller penetration depths. Food products with $\varepsilon'' < 25$ have dp in the range of 0.6 to 1.0 cm, while water, for instance, has a penetration depth of 1.3 cm at room temperature and 2.45 GHz [70]. This fluctuates according to microwave frequency and dietary composition. Eq. (2) provides an expression for the power density pd, which corresponds to a reasonable heating rate (assuming minimal heat loss) [71]. (2) ΔT^2 pd = ρCp = 2πfε0εE As a function of input power and system geometry, Δt is the material's density, Cp is its specific heat, ΔT is its temperature rise, Δt is its time increment, f is its microwave frequency, ε0 is its free-space permittivity, and E is its electric field strength [72]. It is evident from this connection that, for a given E, the components of the system with greater ε″ will heat up more quickly than those with lower ε″, leading to selective heating in heterogeneous systems. Eq. (2) makes it clear that raising E by increasing the microwave power increases power density, which in turn increases heating rate and any selective heating effects.Fig. 5 (reproduced from) shows how the non-isothermal temperature distribution brought on by microwave heating may impact various extraction stages [73] There may be a build-up of pressure inside the cellular structure, increased diffusion and hydrolysis rates, and increased solubility of pectin if the local temperature (Tlocal) at any point in the biomass is higher than the solvent temperature (Tbulk). Degradation of the extract may also be lessened by quicker extraction and lower solvent temperatures. When microwave heating is used during solvent extraction, it is widely believed that the improved processing results are due to the rapid pressure buildup that causes the interior feedstock structure to swell and break. Few research, nevertheless, have attempted to offer quantitative proof of if or how this happens. Microscopic imaging has been used in several investigations to show structural changes to the biomass structure before and after processing, such as for eggplant peel [76] and lime peel powder [75].

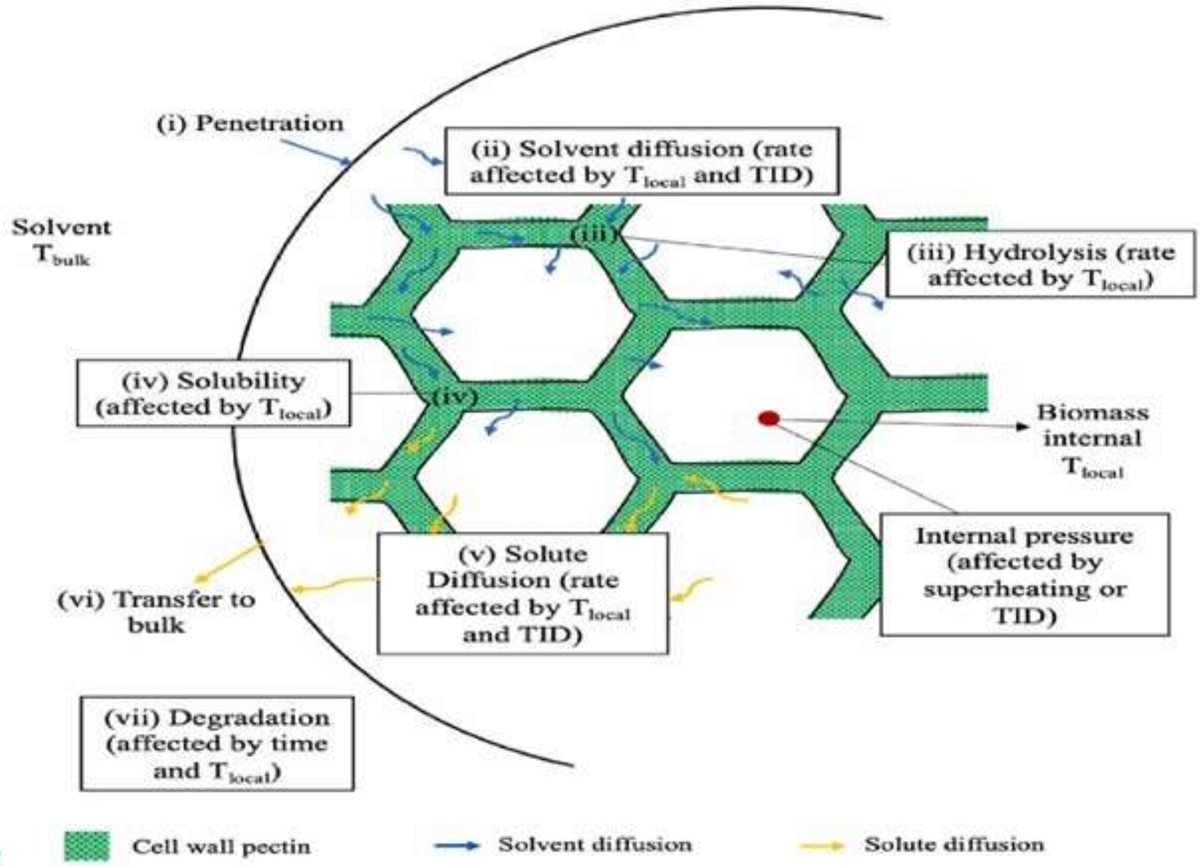

Fig.5: The steps that could improve yield or extraction rate when biomass internal temperature (Tlocal, which varies with spatial location) at steady state >solvent temperature (Tbulk) are highlighted (with boundaries) in Fig. 5. Conceptual model of pectin extraction from biomass. Temperature-Induced Diffusion, or TID. Permission to adapt from Mao et al. [74]

been Some theoretical models that describe physical occurrences during MAE were proposed in order to get around this. Until recently, the most widely accepted explanation for how microwaves improved solvent extraction was Chan et al.'s [77] model, which linked microwave heating to cell pressure because of intracellular steam creation. The model was used to predict a cell rupture time that was in agreement with experimental measurements of extraction time, marking a significant advancement towards a mechanistic description of microwave extraction. Cell rupture was unavoidable under all model input parameters, though, because there was no heat transfer from the biomass to the solvent included in the model. Lee and associates [78] suggested a novel way that microwave heating could improve mass transfer during solvent extraction: heating the biomass just a few degrees above the solvent's temperature could create chemical potential gradients that could drive solvent into the plant structure, causing swelling and possibly enough pressure to rupture cells. These gradients are comparable to the osmotic pressure that is created by concentration gradients during conventional solvent extraction [79] The MAS-II Plus Microwave Synthesis Machine Model (Sineo, China) was used to extract the pectin from pineapple peels. As seen in Figure 6, the machine was linked to the chiller distillatory to enable the condensing of the evaporated solvents, which were then retained in the flask during the extraction procedure. Strong microwave energy was absorbed as part of the extraction process mechanism, which heated the solvent and allowed the analytes in the sample matrix to partition into it [80].Sulfuric acid, with a solid-to-solvent ratio of 1:30 (w/v) and a pH of 1.83 adjusted by 0.5 N H_2SO_4, was the solvent utilized. The extraction period (1–20 minutes), temperature (70–80 °C), solvent pH (1.0–2.5), solid-to-solvent (S/S) ratio (1:10–1:30 w/v), and microwave power (400–600 W) are the parameters for microwave-assisted extraction.

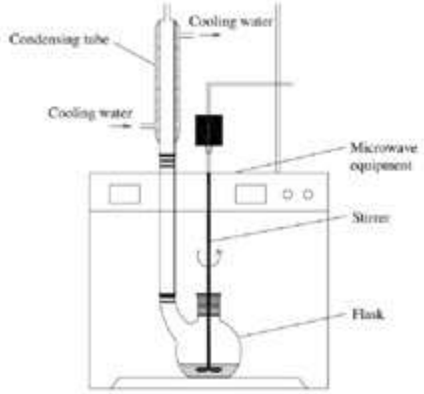

Figure 6: Schematic diagram of the reflux system-connected microwave equipment [81].

After the extraction process was finished, the hot mass extract was allowed to cool to room temperature before being filtered through fresh cheesecloth. After cooling to 4 °C, the filtrate was centrifuged for 30 minutes at 10,000 rpm. To enable the pectin to float, the collected supernatant containing pectin was coagulated with an equivalent volume of 96% ethanol and let to stand for 24 hours [82]. After centrifuging the coagulated pectin in the same manner as before, it was rinsed once with 70% acidic ethanol (0.5% HCl), then with 70% ethanol at a neutral pH, and lastly with 96% ethanol to remove the monosaccharide and disaccharide content [83]. After that, the finished product was spread out in distilled water, dried in an oven, and kept at room temperature [84].

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