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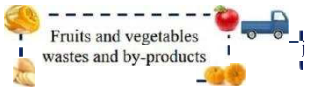
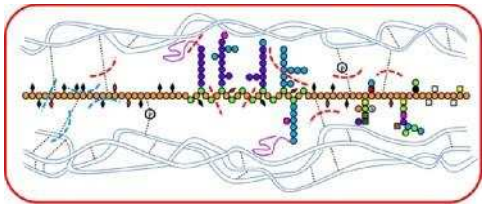
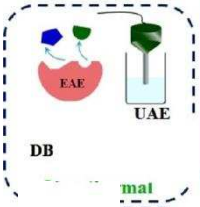


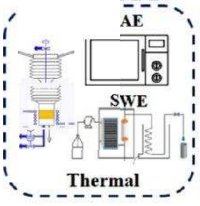
Drying and Grinding

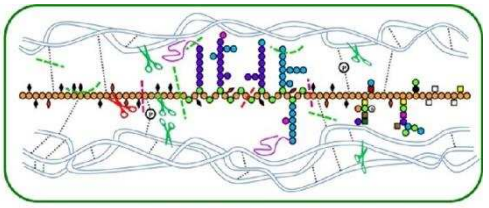
Conventional Extraction *

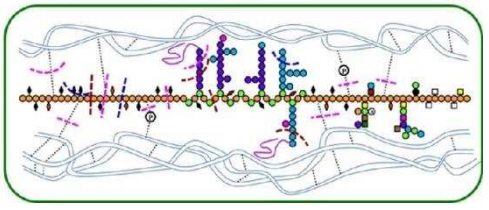
Innovative Extraction *

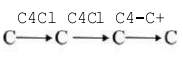


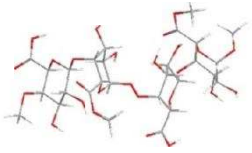




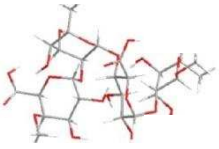


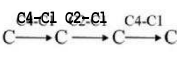






Pectin with different structure





j The combined use of the non-tberinal extraction technologies under mild alkaline condiiion is I potentially a promising method for RG-I enriched pectin extraction. *

Advantage: Relatively low cost
Disadvantage: Inconsistent pectin quality under inconstant extraction conditions (HG enriched under harsh acid conditions; RG-I enriched under mild alkaline conditions); Hard for standardization; Low extraction efficiency; Container erosion and environmental contamination

Advantage: Having a directed acting site under one specific innovative extraction method; Easy to manipulate, feasible to standardize; Relatively Higher extraction efficiency and yield; Less or even no container erosion and eco-friendly
Disadvantage: Relatively high cost

@ D-Galacturonic acid
@ L-Rhamnose
@ L-Arabinose
@ D-Galactose
@ Ferulic acid
0-methy ester
0-acetyl ester
t Borate
y
D-Kdo
{2} D-Apiose
@ D-Fucose
M Glucuronic acid
■ D-Dha
@ Aceric acid
(2 D-Xylose
@r Phosphate
Hydrogen bond
Cellulose
Protein

Action sites of heated strong acid solvents
Action sitas of mild alkaline solvents
Action sites of UAE
Action sites of different EAE
(endo-PG celluclast 1.SL)
Action sites of DBD
Action sites of SWE
Action sites of MAE
Action sites of HPE

Reconsidering conventional and innovative methods for pectin extraction from fruit and vegetable waste: Targeting Rhamnogalacturonan I

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Abstract:

Background: Rhamnogalacturonan I (RG-I) is composed of a backbone of repeating disaccharide units →2)-α-L-Rhap-(1→4)-α-D-GalpA-(1→ with neutral sugar sidechains consisting of arabinose and galactose with variable linking types and chain lengths, corresponding to the hairy regions of pectin. This polysaccharide is abundant in the primary cell walls of fruits and vegetables.

Scope and Approach: Biological functions of RG-I in immunomodulation and functional properties as a supplement and pharmaceutical expedient have increased commercial interest in RG-I extraction from fruit and vegetable waste. However, conventional extraction methods use harsh acid treatments that hydrolyze the side chains of RG-I. Innovative extraction technologies have been developed to preserve RG-I structure with better biological function. Therefore, the present review will focus on the influence of conventional and innovative methods exerts on the RG-I region of pectin from fruits and vegetables.

Key Findings and Conclusions: Non-thermal processing (ultrasound, dielectric barrier discharge plasma, and enzymatic treatment) is superior to conventional and thermal processing (relying on high pressure, microwave and subcritical water extractions) in extracting branched RG-I from fruit and vegetables waste for food and pharmaceutical applications.

Key words: RG-I, pectin, fruit and vegetable waste, innovative extraction, biomass

48 **Contents**

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1. Introduction

The fruit and vegetable processing industry produces large amounts of by-products such as peels, seeds and shells (Schieber, 2017; Pfaltzgraff, Bruyn, Cooper, Budarin, & Clark, 2013) that contain abundant bioactive components including antioxidants (polyphenols, dietary fibers), pigments, flavor compounds, proteins, essential oils, enzymes, and dietary fibers (Trigo, Alexandre, Saraiva, & Pintado, 2019). Pectin is one of the most abundant components in food processing waste and biomass by-products, thus, optimizing pectin extraction and recovery is important to fully valorize these feedstock resources (Shalini & Gupta, 2010).

Pectin is a complex, colloidal heteropolysaccharide composed of structurally distinct regions or domains which include homogalacturonan (HG), rhamnogalacturonan (RG-I), rhamnogalacturonan (RG-II) (Fig. 1). HG, accounting for approximately 65% of pectin, is a linear polymer of α-1,4 linked galacturonic acid that is partially methyl-esterified at C-6 and O-acetylated in positions 2 and 3 (Mohnen, 2008). HG has dominated pectin research due to its ability to form gels in the presence of calcium, depending on the extent and pattern of methyl esterification (Celus, Kyomugasho, Loey, Grauwet, & Hendrickx, 2018). RG-I, accounting for 20-35% of pectin, is composed of a backbone of repeating galacturonic acid and rhamnose (Rha) disaccharide with neutral side chains attached to the O-4 position and sometimes the O-3 position of α-L-Rhap backbone units. Between 20% and 80% of the Rha residues are substituted at C-4, depending on the plant source as well as the extraction conditions used (Kaya, Sousa, Crepeau, Sorensen, & Ralet, 2014). Like HG, RG-I may also be methylated and acetylated (Sun et al., 2019). RG-II, accounting for 2-10% of pectin, is composed of a HG backbone that is heavily branched with many complex side chains containing Rha, arabinose (Ara) and galactose (Gal), other minor sugars such as fucose, glucuronic acid, methyl-esterified glucuronic acid, apiose, 2-O-methylxylose, and 2-O-methylfucose. RG-II is considered the most

conserved domains among pectin molecules (Noreen, Nazli, Akram, Rasul, Mansha, Yaqoob, et al., 2017). Due to its linear structure, HG is often referred as the 'smooth region', while branched regions including RG-I, RG-II and xylogalacturonan (XG) are referred to as belonging to the 'hairy regions' (Pfaltzgraff, Bruyn, Cooper, Budarin, & Clark, 2013). Pectin is extensively used in the food industry as an emulsifier, stabilizer, gelling agent, thickening agent and color-protecting agent (Chen et al., 2015). Pectin also has promise as a bioactive, pharmaceutical ingredient for drug delivery, tissue engineering, and the formation of nanoemulsions (Chen, Guo, Zhang, Wan, Yang, & Yang, 2018). The demand for pectin is increasing approximately 4-5% annually (Raji, Khodaiyan, Rezaei, Kiani, & Hosseini, 2017), driven by demand in plant-based, clean label food ingredients and the increased functionality in pharmaceutical products.

For large and structurally complex biopolymers, extraction methods have a strong influence on the composition, structural, physicochemical and bioactive properties, and determine their application and value in the market. Traditionally, the degree of esterification (DE) and GalA content effects pectin's applications as a gelling and thickening agent because of their different influence in the gel forming mechanism of pectins (Marić, Grassino, Zhu, Barba, Brnčić M., & Brnčić R., 2018). The commercial final pectin products often require a high GalA content (65%) and a specific degree of methylation (DM) (>55% for high methylation pectins and <55% for low methylation pectins), in order to obtain the optimal gelling properties. Commercial pectins are traditionally obtained from food processing by-products including citrus peels, apple pomace, and sugar beet pulp (Putnik, Bursac Kovacevic, Rezek Jambrak, Barba, Cravotto, Binello, et al., 2017) using harsh acid extraction conditions at low pH values (1.5-3.0) and elevated temperatures (60-100 ^{o}C) over several hours (Koubala, Mbome, Kansci, Mbiapo, Crepeau, Thibault, et al., 2008). These commercial extraction conditions require high solid to liquid (S/L) ratios, large amounts of solvents, and can result in substantial adverse environmental impact including high energy and water utilization. Recently, the

food industry has expanded pectin's application from a gelling agent to an emulsifier, stabilizer, and thickening agent. In addition, pectin, and RG-I in particular, has attracted attention as a bioactive component for functional food or pharmaceutical applications. Thus, reconsideration of extraction methods is necessary to optimize pectin functionality and bioactivity.

RG-I's bioactivity is attributed to its molecular weight, composition and structure. Important criteria include the Gal, Ara, Rha and GalA contents, the degree of methylation and acetylation, and branching pattern (Ralet et al., 2005). RG-I enriched pectin putative bioactivities include prebiotic potential (Khodaei, Fernandez, Fliss, & Karboune, 2016) and potential as a pharmaceutical component due to its immunomodulatory (Zhang et al., 2012) and anti-apoptotic activities through inhibition of galectin-3 function (Zhang et al., 2016). The RG-I type pectin with abundant side chains including alpha-L-1,5-arabinan, beta-D-1,4-galactan, arabinogalactan I (AG-I) and arabinogalactan II (AG-II), exhibiting strong binding activities to galectin-3 (Cui et al., 2019). Neutral Gal side chains of RG-I region was proven to selectively bind to recombinant galectin-3 (Gunning, Pin, & Morris, 2013), through which arrested cell cycle of B16F10 cells in G2/M phase and induced apoptosis (Vayssade et al., 2010). High Gal content in RG-I region is important for pectin to inhibit cell proliferation and the induction of apoptosis (Shakhmatov, Toukach, Michailowa, & Makarova, 2014). Besides, the Gal/Ara ratio is also a critical parameter for the immunopotentiation activity of pectin oligomers (Leclere, Cutsem, & Michiels, 2013). Therefore, there is an increased interest in methods for the extraction and preparation of oligomeric pectins containing fewer HG regions and enriched in RG-I regions with branched neutral side chains specifically.

RG-I enriched pectins can either be obtained directly from various purified plant cell walls under specific mild extraction conditions or from extracted pectins using endopolygalacturonase (Endo-PG) modification in possible combination with pectin

methyl esterase and side chain degrading enzymes (Khodaei & Karboune, 2014). However, enzymatic methods are difficult and expensive to upscale at the industrial scale, studies and novel methods for the commercial production of RG-I enriched pectins need to be developed. Various innovative thermal extraction techniques have been studied to extract pectin more efficiently. These technologies rely on indirect heating by pressure, electric or magnetic field, microwaves, or light (Jérôme, Chatel, & Oliveira Vigier, 2016), rather than conventional heating (Pereira & Vicente, 2010). These methods are more effective at lower temperatures (Perez-Andres, Charoux, Cullen, & Tiwari, 2018) and enable shorter extraction times, and lower solvent requirements, and result in higher yields along with the recovery of RG-I rich pectins (Alba, Laws, & Kontogiorgos, 2015; Methacanon, Krongsin, & Gamonpilas, 2014; Wang, Chen, Wu, Wang, Liao, & Hu, 2007). However, most of the studies using these innovative technologies involve acid conditions, adversely impacting the RG-I regions, and particularly the degree and lengths of RG-I branches, within the pectin product.

Although numerous studies on pectin extraction from fruit and vegetable waste have been carried out, few considered the influence of extraction method on pectin structure, especially the recovery of RG-I enriched pectins. The aim of this review is to highlight the impact of both conventional and innovative extraction techniques on the structural changes in RG-I enriched pectin and to provide an approach for the combined application of different extraction methods for RG-I enriched pectin recovery.

2. Conventional extraction method

2.1 Thermal/non-thermal treatment in acid, alkaline or chelating agent solutions

Conventional pectin extraction is water based but relies on different chemical additives. Direct boiling is the most conventional method for industrialized pectin extraction, however, it takes several hours to obtain a good yield (Li, Jia, Wei, & Liu,

2012). During the long heating process, the pectin can undergo thermal degradation by beta-elimination of the HG backbone and significant debranching, leading to pectins of inferior quality. Thus, to reduce extraction time, heating is generally accompanied by the addition of different chemicals that facilitate pectin release from the cell wall. The influence of extraction solvent composition on pectin structure has been compared in many studies (Chan & Choo, 2013; Koubala, Kansci, Mbome, Crépeau, Thibault, & Ralet, 2008). The structural diversity of pumpkin extracted using various solvents has been demonstrated (Košťálová, Hromádková, & Ebringerová, 2014). The authors used hot water, ethylenediaminetetraacetic acid (EDTA), dilute HCl, dilute and concentrated NaOH solutions to isolate pectins. The first three solvents extract pectins with considerable polymolecularity and reduced RG-I content (1.4-28%) compared to that of alkali-extracted (39.3-49.6%) pectin, consistent with previous research (Yapo, Lerouge, Thibault, & Ralet, 2007). Because of the high xylose content in the alkali-extracted pectin, alkaline extraction is thought to promote the co-extraction of hemicelluloses such as xyloglucan and glucuronoxylan. In the study of (Kurita, Fujiwara, & Yamazaki, 2008), citrus peel pectin was extracted in water acidified with 0.05 to 1 M citric acid. Using 0.5 M citric acid under neutral pH at 65 ^{o}C, the maximum proportion of RG-I obtained was 57.5%. Pectin extracted with citric acid showed a lower DM (8.4%) and higher molecular weight distributions (50 to 2000 kDa), indicating the citric acid did not degrade pectin (Kurita, Fujiwara, & Yamazaki, 2008). Chelating agents such as oxalate, can solubilize pectin having a high DM and of high molecular weights (Kaya, Sousa, Crepeau, Sorensen, & Ralet, 2014), as previously reported (Hadfield, Rose, Yaver, Berka, & Bennett, 1998) and later verified (Koubala, Kansci, Mbome, Crépeau, Thibault, & Ralet, 2008; Lim, Yoo, Ko, & Lee, 2012). Chelating agent extractions are impacted by the number of ionic linkages in plant tissue pectin, related to the Ca^{2+} content and the distribution of free acid groups in the HG pectin domain. More pectin (yield of 15.59%) is extracted with hydrochloric acid compared with water extraction (yield of 0.95%) or sodium

196 hexametaphosphate extraction (yield of 5.17%), and the pectin yield is positively
197 associated with decreasing pH, suggesting that the pectin can bind to the
198 cellulose-hemicellulose network by hydrogen bonding (Ueno, Tanaka, Hosino, Sasaki, &
199 Goto, 2008).

200 Different stability of uronic acid residues and their linkages at different pH values
201 can determine the different structural features of pectin extracted by acid or alkaline
202 extraction. GalA-Rha or Rha-GalA linkages are less stable than GalA-GalA, besides, Ara,
203 Gal, Rha are successively acid-labile sugars, while GalA is the most resistant to acid
204 hydrolysis (Kaya, Sousa, Crepeau, Sorensen, & Ralet, 2014; Thibault, Renard, Axelos,
205 Roger, & Crépeau, 1993). Under strongly acidic conditions (pH<2) and high
206 temperatures (>65 °C), linkages between uronic acid residues are more stable than
207 linkages between uronic acid and neutral sugars (Worth, 1967). Therefore, pectin
208 extracted with alkaline solvent under low temperature has much higher RG-I content with
209 retained neutral side chains compared to that of harsh acid extraction. Citrus peel residue
210 was treated with 0.6% NaOH at 32 ℃ stirring for 10 min, the pH value was then adjusted
211 to 6-7. The pectin obtained contained 82.5% RG-I region (compared to 44% that of HCl
212 treatment at pH 3-4) with highly branched side chains according to monosaccharide
213 analysis and AFM image(Zhang et al., 2018). KOH treatment leads to less degradation of
214 Ara and Gal side chains and, the debranching of Ara side chains was more significant
215 compared to Gal side chains under harsh alkaline conditions, suggesting that Ara residues
216 are more susceptible to altered conditions than Gal residues (Khodaei & Karboune, 2014).
217 Alkaline extracted pectin also has lower molecular weight, its RG-I region content is
218 usually 2-5 times compared to pectins extracted with other conventional extraction
219 methods. (Fishman, Chau, Cooke, Yadav, & Hotchkiss, 2009). Molecular weight is
220 reduced due to β-elimination reaction, which cleaves glycosidic linkages between
221 methylated galacturonic acid units (Albersheim, Neukom, & Deuel, 1960).

In summary, RG-I content and its neutral side chains differ in different plant materials and due to the use of different extraction conditions. As shown in Table 1, potato pulp, citrus peel, sugar beet and oil-pumpkin are the best plant materials for RG-I recovery. Hot water and acid extracted pectin is usually high in HG content (GalA > 65%) and affords a high DM and DA. Low pH stimulates protopectin (water-insoluble precursor of pectin exists in plant tissues) hydrolysis (Sakamoto, 1995), promotes Ca^{2+} and Mg^{2+} removal , and increases protopectin's solubility, thus, enabling higher isolated yields of HG enriched pectin. Alkaline extracted pectin usually has high RG-I content (49.6%-82.5%, depending on temperature and pH), low DM (resulting from saponification reaction) and low yields. Alkali causes GalA instability, enriching the extracted fractions with RG-I oligomers branched with arabinan and galactose side chains. Alkaline treatment leads to pectin decomposition, therefore, the resulting product cannot be precipitated with alcohol, resulting reduced yields (Yeoh, Shi, & Langrish, 2008). Organic acid/chelating agent extracted pectin is characterized by high molecular weight and low DM. Because of their lower dissociation constant compared to mineral acids, organic acids have lower hydrolyzing capacity. The RG-I content of pectin extracted by organic acids often falls between pectins extracted by harsh mineral acids and by alkaline conditions.

3. Innovative extraction technology

3.1 Ultrasound extraction (UE)

Ultrasound refers to the sound waves with frequencies higher than 20 kHz, beyond the threshold of human auditory detection (from 16 Hz up to 16 kHz) and is mainly characterized by frequency (kHz range-MHz range) and wavelength (Koubaa, Rosello-Soto, Zlabur, Jambrak, Brncic, Grimi, et al., 2015). Its transmission depends on medium, such as solid, liquid or gas. The transmission process includes expansion (pulling molecules apart) and compression cycles (pushing molecules together). In liquid

248 medium, cavities grow and then collapse when the negative pressure exerted exceeds the
249 liquid's partial tensile strength. This process in which bubbles form, grow and collapse is
250 known as "cavitation". During phytochemical extraction, sound waves creates cavitation
251 bubbles near the tissue material, thus, breaking down the cell walls and causing enhanced
252 solvent entrance into the cells, thereby helping to release cell contents. This technique has
253 been used for pectin extraction (Bayar, Bouallegue, Achour, Kriaa, Bougatef, &
254 Kammoun, 2017). UE has been used to extract pectin from Opuntia ficusindica cladodes
255 (Bayar, Bouallegue, Achour, Kriaa, Bougatef, & Kammoun, 2017), Artocarpus
256 heterophyllus fruit peels (Moorthy, Maran, Ilakya, Anitha, Sabarima, & Priya, 2017)
257 tomato waste (Grassino, Brncic, Vikic-Topic, Roca, Dent, & Brncic, 2016), orange peels
258 (Hosseini, Khodaiyan, Kazemi, & Najari, 2019) and industrial waste of Musa balbisiana
259 (Maran, Priya, Al-Dhabi, Ponmurugan, Moorthy, & Sivarajasekar, 2017).

260 Ultrasonic treatment disrupts the cellulose network (Yang, Wang, Hu, Xiao, & Wu,
261 2018), thus, the pectin yield obtained by combined enzymatic/ultrasonic method (31.1%)
262 is about 1.5- to 3.5-times higher than those from separate enzymatic extraction (9.4%) or
263 acid extraction (5.4%). In addition to increasing yields (Liew, Ngoh, Yusoff, & Teoh,
264 2016), sonication has an effect on pectin structure and the bioactive properties of the
265 pectin (Wang, Ma, Jiang, Hu, Zhi, Chen, et al., 2016; Zheng, Zeng, Kan, & Zhang, 2018).

266 Sonochemistry severely degrades pectin microstructure, and this degradation mainly
267 occurs in the RG-I side chain and HG backbone. Pectin extracted using UE under
268 0.41W/mL, 60 ^{o}C for 28 min in water contained 41% RG-I content (Ma, Wang, Chen,
269 Ismail, Wang, Lv, et al., 2018; Wang, et al., 2016). Increased sonochemical treatment
270 leads to decreased molecular weight and a narrower molecular weight distribution for
271 extracted pectin. As the ultrasonic time increases, the decline rate in molecular weight
272 slows down, indicating the acoustic cavitation has a debranching action with less impact
273 on the main backbone structure in pectin. If ultrasonic time is relatively short, there still

will be long side chain fragments in the molecule (Ogutu & Mu, 2017; Wang, et al., 2016). After ultrasound treatment, the molar ratio of GalA/(Fuc + Rha + GlcA + Ara + Gal + Xyl) decreases demonstrating degradation of HG compared to RG-I. The proportion of RG-I in the remaining molecular fragments are higher (Wang, et al., 2017), suggesting sonication enriches the pectin extract with RG-I. Ultrasonic waves can break the covalent bond between pectin and the non-pectic polysaccharides, thereby improving pectin purity (Wang, et al., 2017). The DM of pectin is also reduced because the ester functional group is more susceptible to sonochemical effects, while the DA remains substantially unchanged. Additionally, Fenton processes are a highly efficient method for extracting RG-I enriched ultra-low molecular weight pectin. Combined treatment with ultrasound and Fenton reagent at low temperature improve the proportion of pectin RG-I from 36% to 79%, degrades pectin to 5.2 KD and accelerates the degradation process so it takes place within 35 min (Zhi, Chen, Li, Wang, Huang, Liu, et al., 2017). An ultrasound-accelerated metal-free Fenton chemistry, relying on H_2O_2/ascorbic acid, was used to develop an ultrafast approach to prepare RG-I enriched low molecular weight pectic polysaccharide (Li et al., 2019). The ultrasound was shown to enhance the efficiency of H_2O_2/ascorbic acid system for pectin degradation (from 791 kDa to 7.9 kDa within 60 min) through both chemical effects (increased the hydroxyl radicals amount and lowered activation energy of H_2O_2 decomposition) and mechanical effects (disaggregated polysaccharide clusters). More importantly, it revealed that free radicals preferentially act on the GalA backbone in the HG region while maintaining the RG-I region, the highest RG-I content of resulting fragments reached 93.7%. Ultrasound has been used to assist pectin modification (Ma, et al., 2018; Zhi, et al., 2017) decrease pectin molecular weight efficiently and highly enrich RG-I domains, inducing higher contents of galactose-containing pharmacophores in modified pectin, therefore, enhancing the bioactivity of pectin (Ma, et al., 2018).

Ultrasonic approaches have potential in processing and modification of RG-I

enriched pectin using alkaline solvent, combined with Fenton process and is promising for extracting RG-I enriched ultra-low molecular weight pectins.Pectin extracted by UE often with high purity and low DM (Table 2). UE also enables higher efficiency, lower energy consumption, reducing the use of chemical reagents, selective extraction, faster activation, and lower extraction temperatures (Chemat, Rombaut, Sicaire, Meullemiestre, Fabiano-Tixier, & Abert-Vian, 2017). However, there is poor uniformity of ultrasound waves reaching dispersed sample because the ultrasound intensity decreases with distance from the emitter, leading to poor pectin uniformity and variation between batches (Wang & Weller, 2006).

3.2 Enzyme-assisted extraction (EAE)

Pectin, cellulose, hemicellulose and protein interact with each other, resulting in the entangled network of the plant cell wall. The cellulose/xyloglucan network is embedded in a matrix of pectin along with a protein network (Panouille, Thibault, & Bonnin, 2006). Enzymes catalyzing hydrolysis have selectivity that either reduces the amount of solvent/chemical needed or increase the yield for the same amount of solvent. Enzymes work either to degrade pectin or deconstruct plant cell wall to isolate pectin, which facilitates the pectin extraction process. Through the hydrolysis of cellulose or hemicelluloses, pectin trapped within the cellulose matrix can be released. The most commonly used enzymes during pectin extraction process include cellulase, hemicellulase, protease, α-amylase, pectin methyl esterase, endopolygalacturonase, β-glucosidase (Khodaei & Karboune, 2013; Khan, Nakkeeran, & Umesh-Kumar, 2013)

Potato cell wall is potentially a rich RG-I pectin source. The effects of reaction parameters of endo-PG–catalyzed isolation of potato cell wall RG-I and their interactions by response surface methodology (RSM) have been investigated (Khodaei, Fernandez, Fliss, & Karboune, 2016; Khodaei & Karboune, 2013). The cell wall concentration and amount of enzyme are the most significant parameters affecting pectin yield, Gal and Ara

content. Under optimal conditions, 0.42 mg of cell wall material /ml buffer and 181 units of endo-PG /g cell wall material, RG-I enriched (90% RG-I proportion) pectin with high Gal content (72%) was recovered from potato cell wall. Enzymatic treatment leads to recovery of intact RG-I with higher molecular weight. The effect of combined physical/enzymatic treatments on the physical-chemical properties of pectin extracted from Yuza pomace were compared with chemically-extracted pectin (Lim, Yoo, Ko, & Lee, 2012). Pectin of low methoxyl content and reduced viscosity that contained 55% galacturonic acid was recovered with an extraction yield (7.3%) without additional chemical agents, whose yield was comparable with chemical extraction (8.0%) (Table 3). However, the RG-I region was not elevated (17.1%) because the β-glucanase used mainly focus on the cellulose hydrolysis.

Contrasts have been drawn between EAE and conventional extraction methods. Enzymatic, water, and acid extraction of pectin from kiwifruit pomace has been compared by evaluating their neutral sugar composition, pectin yield, GalA content, molar mass, viscosity and degree of branching (Munoz, Almagro, 2017). Pectin extracted with Celluclast 1.5L (including cellulases, polygalacturonase, pectin lyase and rhamnogalacturonan lyase), conducted at 25 ^{o}C (pH 3.70) for 30 min, showed the highest yield (~4.5% w/w) when compared to the yield of water-based and acidic extraction methods (~3.6-3.8% w/w). Hydrolysis of cellulose leads to the release of pectin trapped within the cellulose matrix. Enzymatically extracted pectin has lowest degree of branching (a side chain is carried by one of every 50 GalA residues) compared to pectin from acid and water extraction methods (a side chain is carried by one of every 48 and 45 GalA residues, respectively), owing to possible side chains hydrolysis caused by the rhamnogalacturonan lyase. EAE and three conventional pectin extraction methods using green tea leaf (GTL) as a model material were compared to obtain high yield leaf pectin with better viscosity and gelling properties (Zhang et al., 2020). Compared to hot water, acid, or FoodPro® CBL, Viscozyme® L and alkaline conditions can effectively extract

GLT pectin with a yield of 8.5% and 9.2%, respectively. Viscozyme® L extract had high contents of RG-I and RG-II pectin with some hydrolyzed side chains (Table 3), thus, exhibiting poor viscosity and no gelling properties. FoodPro® CBL extract had similar properties to that of hydrothermal extract, which has higher HG content. RG-I pectin is only located in primary cell wall, while HG pectin locates in both lamella layer and primary cell walls (Mualikrishna & Tharanathan, 1994). Viscozyme® L, a multi-enzyme complex containing a wide range of carbohydrases, can degrade the cell wall more thoroughly than FoodPro® CBL, therefore releasing more RG-I pectin. EAE and conventional acid extraction of apple pomace were also compared (Wikiera, Mika, & Grabacka, 2015). Celluclast 1.5L, at concentration ranging from 25-70 µL per 1 g, was used to treat apple pomace for 18 h at 50 °C pH 4.5, while acid extraction with sulfuric acid performed at 85 °C for 3 h. Even the lowest concentration of Celluclast 1.5L resulted in 15.3% recovery of pectin significantly less contaminated with glucose, however, this pectin was richer in arabinose and fucose, typical of RG-I and RG-II fractions, respectively. In an earlier report (Yoo, Mika, & Grabacka, 2015a), three different commercially available enzymatic preparations (Celluclast, Econase and Viscoferm) were used to extract pectin from apple pomace, resulting in pectins rich in HG (55.59%-61.49%). Celluclast extraction afforded higher yield (19%) than Viscoferm (18%) and Econase (12%) extractions. In addition, pectin recovered by Celluclast extraction was higher in neutral sugar content (Celluclast 17% vs Econase 13%, Viscoferm 17%). Xylanase and cellulase also promote plant cell wall degradation, enhancing extraction effectiveness.

The enzyme-assisted extracted pectin structure differs greatly based on the plant materials and enzymes that are used. RG-I enriched pectin is recovered in high purity because of the specificity of enzymatic hydrolysis, although longer reaction times (18-30 h) and low substrate concentrations (0.04-1%, w/v) are required (Khodaei, Karboune, & Orsat, 2016). EAE affords a number of advantages including oriented extraction of high

purity of extract; elimination of harsh extraction conditions with reduced equipment corrosion; some specific pre-treatments (e.g., the removal of sugars and color pigments) are eliminated. There are also some drawbacks, currently, available enzymes cannot completely hydrolyze plant cell walls, therefore limiting high yield pectin extraction. In addition, the low concentration of substrate make scale-up of the extraction process difficult (Khodaei, Karboune, & Orsat, 2016)

3.3 Subcritical water extraction (SWE)

Sub/supercritical extraction relies on distinctive states of a solvent achieved when subjected to a pressure and temperature conditions below/beyond a critical point (a pressure and temperature for which the gas and liquid phases do not exist). Subcritical water has unique properties: the hydrogen bond between water molecules weakens as the temperature increasing, and the dielectric constant can change in a great range. The ion product of water (K_w) dramatically increases as the temperature increases to 270 ^oC (Marshall & Franck, 1981). Therefore, subcritical water is effective for the extraction of both polar and non-polar compounds, including cellulose, essential oils (Carr, Mammucari, & Foster, 2011), and pectin extraction from citrus peels (Tanaka, Takamizu, Hoshino, Sasaki, & Goto, 2012; Ueno, Tanaka, Hosino, Sasaki, & Goto, 2008) (Fig. 2).

Subcritical water extraction of pectin has been applied to apple pomace and citrus peels and the effect of temperature on pectin properties has been investigated (Wang & Lu, 2014). During SWE, side sugar chains of recovered pectin increased (Table 4) while the protein content decreased with increasing temperature higher than 130 ^oC. The apple pomace pectin possibly had more proportion of hairy regions and side chains, owing to slightly higher ratio of Rha/GalA (indicating relative RG-I backbone abundance) and (Gal+Ara)/Rha (indicating neutral sugar sides chains abundance) compared to that of citrus pectin. Besides, the Gal/Ara was higher with temperature increases for both citrus and apple pomace pectin, indicating the stronger resistance to high temperature of Gal

compared to Ara (Table 4). The protein content of pectin was significantly lower than pectin extracted by conventional method owing to protein degradation caused by subcritical water, it was firstly increased from 1.01% to 2.09% when temperature increasing from 100 °C to 120 °C, then decreased to 0.24% when temperature increasing to 170 °C. Therefore, the protein was first separated and hydrolyzed from raw material while the degradation was not severe at relative lower temperature. Because protein either linked to pectin or existed in free form (Garna et al., 2007), the decrease of protein with temperature increase indicates that pectin interacts less with proteins in subcritical water. The high DE (68.9%-71.9%) of extracted pectin demonstrates probably unesterified and/or low esterified pectin was hydrolyzed during extraction. This is in contrast to previous reports (Liew, Teoh, Tan, Yusoff, & Ngoh, 2018) that pectin was recovered from pomelo peels through dynamic subcritical water extraction has low DE (38.2%). These conflicting results are mainly due to different temperatures and times used by these two researchers with the former relying on 140 °C, 5 min and the latter relying on 120 °C, 140 min. Therefore, exposure time in high temperature may be an important factor for demethylation. In another study (Ueno, Tanaka, Hosino, Sasaki, & Goto, 2008), pectin was separated from the flavedo of citrus junos using a semi-continuous flow reactor. The influence of flow rate and temperature on pectin extraction was then investigated. Pectin was rapidly extracted at 160 °C at 20 MPa with flow rates of 7.0 mL/min, during which there was no decomposition of HG. During the extraction process, potassium was eluted, reflecting the initial destruction of the cell wall and membrane by the subcritical water followed by pectin extraction. In a subsequent study (Tanaka, Takamizu, Hoshino, Sasaki, & Goto, 2012), a wider temperature range of 160-320 °C was tested and the fraction collected at 160 °C contained mostly HG enriched pectin.

The extraction process for apple pomace pectin extraction using SWE has been optimized (Wang & Lu, 2014). The physicochemical and functional properties of the resulting pectin were compared with the commercial apple pomace pectin. Under the

434 optimum conditions, an extraction temperature of 140 °C, an extraction time of 5 min,
435 and a S:W ratio of 1:14 , the resulting pectin has higher neutral sugar contents and lower
436 molecular weight, GalA content, and DM than commercial apple pectin, which is mainly
437 attributed to the hydrolysis of pectin's backbone chain. Interestingly, the amount of Ara in
438 RG-I was lower due to the hydrolysis and degradation, which can be ascribed to other
439 biomass hydrolysis in subcritical water (Lu, Yamauchi, Phaiboonsilpa, & Saka, 2009).

440 SWE can be used to extract oligosaccharides (DP>7) having HG as its main
441 component (65% of GalA) directly from the passion peels at 150 °C within 4.5 min or
442 175 °C within 5.5 min (Klinchongkon, Khuwijitjaru, Wiboonsirikul, & Adachi, 2017).
443 Under harsher conditions (hotter, longer time), subcritical water results in pectin
444 hydrolysis into oligosaccharides that can be recovered. A comprehensive investigation of
445 how temperature, water flow rate and pressure effects on pectin extraction efficiency has
446 been described (Hoshino, 2014). SWE effectively enables the separation of pectin and
447 cellulose or hemicellulose. At 120 °C, commercial pectin product with high molecular
448 weight (635 kDa) can be obtained, while at 140 °C or higher, lower molecular weight
449 (12-15 kDa) pectin is extracted having improved biological activity. At a range from
450 120-140 °C and 4-30 MPa, pectin yield and purity is the highest. Correctly controlling the
451 extraction temperature during sub-critical extraction can result in pectins of higher purity
452 with desirable properties.

453 Pectin obtained by SWE at high temperature (set value often higher than 100 °C) is
454 enriched in GalA, lacks RG-I, has a high DM, a low molecular weight and is obtained in
455 relatively lower yield among innovative extraction methods (Table 4). Pectin yields are
456 lower as pectin is decomposed into monosaccharides or small molecules under longer
457 times at higher temperatures. The most outstanding advantage of SWE, is the elimination
458 of required chemical co-solvents and, another advantage is the higher quality of extracts
459 and shorter process times (Curren & King, 2001). In addition, its GRAS status makes

460 subcritical water an ideal pectin extraction processes for pharmaceutical and nutritional
461 applications, particularly for the extensive use of pectins in drug delivery applications
462 (Nova, Nothnagel, Thurn, Travassos, Herculano, Bittencourt, et al., 2019). However,
463 improper control of process conditions leads to pectin chain hydrolysis, therefore,
464 resulting in poor quality and low yields (Khajavi, Kimura, Oomori, Matsuno, & Adachi,
465 2005).

466 **3.4 Dielectric barrier discharge plasma extraction (DBD)**

467 The past few decades have witnessed increased interests in the application of
468 non-thermal plasma extraction in food processing. Dielectric barrier discharge (DBD)
469 plasma, a kind of non-thermal plasma, has been widely used in enzyme inactivation or
470 microbiological decontamination during the food processing (Fig. 3). DBD is able to
471 break down specific bonds for the destruction of the secondary structure or to realize
472 chemical modifications of side chains through the action of the myriad of chemically
473 active species constituting the plasma (Misra, Pankaj, Segat, & Ishikawa, 2016). DBD
474 can also be used to degrade biomacromolecules including the chitosan, protein and
475 polysaccharides (Hou, Dong, Yu, Li, Ren, Zhang, et al., 2008). High-energy electron
476 produced by DBD colloids into water molecule, producing hydroxyl free radical, which
477 attacks on the pectin chains and degrade the pectin into lower molecule.

478 RSM has been used to optimize the pectin extraction conditions from pokan peel
479 using DBD (Zhang, 2014). A maximum yield of pectin (27%) can be efficiently obtained
480 under the following conditions, input voltage of 40 V, pH 2, 5.5 min and S/L 1:30 (g/mL).
481 However, longer extraction times (>5.5 min) or extreme high voltage above 40V reduce
482 recovery and pectin yield, as pectin degradation occurs during longer exposure to plasma
483 or extreme high energy throughout the system. DBD treatment was then optimized to
484 degrade pectin, and it contributes mainly to break HG region, slightly degrade side chins
485 in RG-I region. The pectin had lower linearity and contains much higher RG-I content of

71.3% compared to 36.5% of the original one, while the (GalA+Ara)/Rha ratio was slightly decreased to 1.4 compared to the original 2.4. In addition, the DE was lowered to 37.3% from 54.7%. The oxidative cleavage induced by DBD plasma selectively focuses on break down of GalA attacking the HG region but retain the RG-I domain intact. In addition, high input voltage is beneficial to RG-I enriched pectin with low molecule weight preparation because it produces enhanced electric field intensity which enables more high-energy electron colliding into water molecule to produce much more hydroxyl free radical. However, the specific mechanism of this break down still awaits further exploration.

The application of DBD plasma for pectin extraction has not attracted much attention, thus, there is limited research on this topic. The most interesting aspect of oxidative degradation by DBD plasma is its selectivity HG domains and its preservation of RG-I domains. DBD plasma degradation requires low energy consumption and can be used without additional chemical agents. Therefore, it is considered a very promising method for the recovery of RG-I enriched pectin from plant materials. However, some shortcomings restricting practical application of DBD plasma need to be addressed such as the high cost and short life time of the plasma power supply and the change of physicochemical properties in the remediation process.

3.5 Microwave-assisted extraction (MAE)

Microwaves have been used as processing tool and have played a crucial role in the food science and technology. Microwaves can be industrially used for: i) microwave-assisted extraction (MAE); ii) drying of foodstuffs; and iii) enzyme inhibition and inactivation, and microorganism inactivation (Dehghannya, Farshad, & Khakbaz Heshmati, 2018). It is used as auxiliary method combining with chemical solvent to extract bioactive compounds such as pectin, polyphenols, essential oils from food residues (Rashed, Ghaleb, Li, Nagi, Hua-wei, Wen-you, et al., 2018). MAE process is

efficient and requires small amounts of solvent. No temperature gradient results as is commonly observed in conventional heating, and the temperature distribution within the solvent is homogeneous, ensuring uniform pectin quality (Bagherian, Ashtiani, Fouladitajar, & Mohtashamy, 2011). The energy of these waves produced by irradiation of microwave leads molecules to vibrate and enhances their separation. The elaborate mechanism of microwave extraction is described in earlier reviews (Adetunji, Adekunle, Orsat, & Raghavan, 2017; Marić, Grassino, Zhu, Barba, Brnčić M., & Brnčić R., 2018)

MAE combined with acid solvent have been extensively studied. Pumpkin powder has been microwave-extracted at 120 ^{o}C for 3 min, resulting in doubling of pectin yield without loss of pectin quality (Yoo, Lee, Bae, et al., 2012), representing an advance over acid extraction discovered. The yield, GalA content, and DE of extracted pectin increases with increased microwave power and heating times (Bagherian, Ashtiani, Fouladitajar, & Mohtashamy, 2011). In addition, molecular weight is reduced as heating time or power is increased and the impact of power is dominant. Under optimum conditions microwave power of 700W; irradiation time of 165 s; pH value 1.5; a high yield (18.13%) of pistachio green hull pectin can be achieved (Kazemi, Khodaiyan, Labbafi, Hosseini, & Hojjati, 2019). The resulting pectin has low DE (12.1 ± 2.72%) and molecular weight (1.659 kg/mol), and a high percentage of HG (64%) and it was less linear than grapefruit peel pectin extracted using conventional means. Additionally, followed by irradiation time and microwave power, pH is the pivotal factor impacting pectin DE. The reduction of DE in under stringent conditions (low pH, high microwave power, and long irradiation times), is probably because of de-esterification of galacturonic acid chains (Pasandide, Khodaiyan, Mousavi, & Hosseini, 2017).

MAE extraction under mild condition is gaining increasing attention. Microwave combined with alkali has been used to extract galactan-rich RG-I enriched pectin from potato pulp (Khodaei, Karboune, & Orsat, 2016; Ueno, Tanaka, Hosino, Sasaki, & Goto,

2008). The influence of different extraction parameters on pectin yield and the structural properties of pectin were studied. A trade-off made between the multifaceted impact of high KOH concentration/solid to liquid (S/L) ratio and low power/extraction time was crucial to the efficient extraction of galactan-rich RG-I and the limitation of branching. Optimum conditions were: S/L ratio of 2.9% (w/v) with 1.5 M KOH, microwave power 36.0 W, for 2.0 min, and afforded a maximum yield of intact galactan-rich RG-I of 21.6% and productivity of 192.0 g/L. The increase of S/L and microwave power accelerated the physical rupture of cell wall increasing the concentration of arabinan released into the liquid phase, while Rha content is mainly impacted by concentration of KOH and the power applied. With increased power and KOH concentration, the RG-I backbone will be hydrolyzed. For MAE sugar beet pectin, the neutral monosaccharide recovery order was Ara > Rha > Gal > Glc > Xyl > Fuc (Fishman, Chau, & Cooke, 2009). Simultaneous extraction of citrus pectin and essential oils from waste orange and lemon peel using only water as dispersing medium and microwave as energy source was examined (Fidalgo, Ciriminna, Carnaroglio, Tamburino, Cravotto, Grillo, et al., 2016). DE and HG content depend mostly on the plant source and the extraction procedure, respectively. Fresh lemon derived pectin has a lower DE compared to fresh orange derived pectin. Pectin containing HG regions, recovered by microwave-assisted hydrodiffusion was higher in RG-I content, while this trend was reversed under hydrodistilation. HG region organizes more easily; resulting in aggregated structures, while the lateral chains of RG-I regions hinders aggregation, yielding more filamentous structures. Generally, microwave-assisted pectin extraction under alkaline conditions features higher RG-I and neutral sugar, and lower molecular weight, which is opposite to the properties of pectin extracted with HCl or water. Since some plant materials are good sources of for highly branched structures consisting of neutral sugars, the use of milder extraction solvents is promising for the recovery of RG-I enriched pectin.

3.6 High pressure processing extraction (HPE)

Ultrahigh pressure consists of pressure boost stage, pressure maintaining stage and pressure relief stage (Fig. 4) (Huang, Hsu, Yang, & Wang, 2013; Jolie, Christiaens, Roeck, Fraeye, Houben, Buggenhout, et al., 2012). In the first stage, the pressure outside rises quickly, usually in a couple of seconds, from atmospheric pressure accelerating cell wall breakage and solvent permeation. The pressure is then maintained at a certain level for some time to improve recovery yield. Finally, the pressure is returned to atmospheric pressure in the relief stage. The intracellular pressure drops sharply from ultrahigh pressure to atmospheric pressure causing the cells tends to expand, and some non-covalent bonds are broken and the 3D structure of molecules is opened, leading active ingredients to better combine with the extracting solvent. Shorter pressure relief times induce greater impact force, resulting in a higher extraction rate, reducing extracting time and improving efficiency with low energy consumption (Huang, Hsu, Yang, & Wang, 2013).

High pressure causes partial side chain degradation without destroying primary structure. The molecular weight change depends on the pressure particularly at levels of 250 Mpa to 550 Mpa (Peng, Mu, Zhang, Sun, Chen, & Yu, 2016). High hydrostatic pressure does not impact molecular weight but high pressure homogenization leads to significant molecular weight decrease, caused by the strong mechanical forces (Xie, Zhang, Lan, Gong, Wu, & Wang, 2018). Under high pressure, the size of a molecule becomes larger and the microstructure becomes looser. The filaments become slender, the blocks tend to shatter, and the overall density becomes reduced. High-pressure treatments of 200 MPa, at 25 ^{o}C for 5 min, affords pectin richer in RG-I (42%) than the untreated original pectin sample RG-I (36%), based on monosaccharide analysis, and AFM analysis showed side chains degradation of the pectin (Xie, Zhang, Lan, Gong, Wu, & Wang, 2018).

HHP shows de-esterification because the C-O ester bond is sensitive to mechanical force (Xie, Zhang, Lan, Gong, Wu, & Wang, 2018). A high-pressure enzymatic process reduced DE by half in 15 min compared to 120 min in a normal process (Zhao, Guo, Pang, Gao, Liao, & Wu, 2015). HHP has a different impact on viscosity and rheology. High pressure can change the viscoelastic characteristics of pectin with a reduction in viscosity but an increase in elasticity (Zhang, Xie, Lan, Gong, & Wang, 2018). The pectin of high-pressure enzymatic extraction performed better in viscosity and gelling ability, which is probably the result of its methoxyl content (Zhao, Guo, Pang, Gao, Liao, & Wu, 2015). Moreover, under high pressure, enzymatic hydrolysis greatly increases because pectin's structure is open under high pressure making it more accessible to enzymatic reactions (Guo, Han, Xi, Rao, Liao, Hu, et al., 2012), but this high pressure treatment does not change the molecular structure and viscosity of the pectin product (Naghshineh, Olsen, & Georgiou, 2013).

In summary, pectin recovered from HPE has a comparable content of HG and RG-I (RG-I content was a little higher than conventional acid extraction) with slightly degraded neutral side chains, and decreased molecular weight and DE. If operated at room temperature, the pectin side chains can be slightly protected since they have low thermal stability. High pressure combined with enzyme treatment is best for efficient pectin extraction. There is still no research studying the combination of proper enzyme selection or mild solvent conditions in HPE extraction of pectin. Because of the protection of RG-I by milder extraction conditions, the combined use of HPE with alkaline solvent to enrich RG-I should be feasible.

4. Hybrid extraction methods

An increasing trend has seen a synergistic use of two or more innovative technologies during the pectin extraction. For example, ultrasound-subcritical water enhancement (Chen, Fu, & Luo, 2015), microwave-ultrasound enhancement (Liew, Ngoh,

Yusoff, & Teoh, 2016), ultrasound-enzyme enhancement (Nadar & Rathod, 2017), were used for the pectin extraction. The ultrasound can enhance the mass transfer while microwave enhance heat transfer during extraction process.

Pectin-enriched material from sugar beet pulp was extracted using subcritical water combined with ultrasonic-assisted treatment (Chen, Fu, & Luo, 2015). The extract pectin (with 54.6% HG region and 35.9% RG-I) contained much more neutral side chains and Rha (4.5%) compared to pectin (Rha content of 0.4%-0.7%) extracted by merely sub-critical water. The maximum yield (24.63%) was attained under the optimum reaction conditions: L/S ratio 44.03, extraction pressure 10.70 MPa and extraction time 30.49 min. The lower Mw and higher neutral sugar (30.9%-68.2%) illustrate the ultrasonic pretreatment could attack on the backbone of pectin's HG region. It's important to optimize and standardize the combination of two or more particular innovative extraction technologies to enable the selective recovery of pectin. Pectin extracted from pomelo peel using sequential ultrasound-microwave (UMAE) assisted extraction method has the highest yield (36.3%) and lowest DE value (59.8%) compared with UAE (yield 14.3%, DE 64.4%), MAE (yield 27.7%, DE 64.1%) and microwave-ultrasound assisted extraction (yield 30.5%, DE 67.0%). Besides, pH has the most significant impact on pectin yield while microwave power for DE.(Liew, Ngoh, & Yusoff, 2016). The hemicellulase was combined with ultrasound for pectin extraction from discarded carrots. The highest yield was 27.1% compared to that of merely using cellulase (12.4%) that per se help to release the pectin from cellulase matrix. The extract pectin has low DE (24.0-49.9%) with gelling capacity (Encalada et al., 2019).

Although the hybrid extraction has been proven to enhance pectin yield, few studies have clarified their effects on the RG-I region, which need further research.

5. Comparison between conventional extraction and innovative extraction on pectin structure

The fundamentals of conventional methods differ from innovative extraction methods, leading to different pectin structure and disparate recovery yield.

Conventional extraction methods rely on various kinds of chemical additives reagent in heated higher temperature to destroy the cell wall and release the pectin, with a pectin recovery yield ranging from 0.6%-25.6%. During the extraction process, pectin structure undergoes modification because of reaction with extractants. Pectin can be degraded either by high temperature or harsh acid during acid extraction, and it undergoes a saponification reaction during alkali extraction. Besides, the totally reverse stability of GalA, GalA-GalA and Rha, Rha-GalA, GalA-Rha when facing with acid and alkali solvents, determines whether the pectin is HG or RG-I enriched to great extent. The hot water and acid extracted pectin is HG region dominant (52.9%-95.0%) with few neutral side chains and high DE (21.5%-85.7%) while the alkali-extracted pectin is RG-I region dominant (49.6%-82.5%) with neutral side chains in varying branching degrees and low DE (~10%). A comprise needed to be made between having a more uniform quality with higher RG-I content but low yield at high pH and having poor quality with higher HG content but higher yield at a low pH. Therefore, selectively combining innovative extraction methods with alkali/acid solvent for specific RG-I/HG enriched pectin extraction enables higher efficiency and quantity production.

The innovative extraction methods leads to the cell structure changes by electromagnetic, sound waves, high pressure or discharge plasma, different extraction methods produce pectin with distinctive structure features, with enhanced yield varies from 6.5% to 28.1%. UAE, DBD and EAE belong to the non-thermal relied methods, while HPE, MAE and SWE are based on thermal technologies, are promising for HG or RG-I enriched pectin efficient recovery respectively. The RG-I content of pectin obtained

by non-thermal based methods ranges from 38.3% to 90.3%, while the GalA content of pectin extracted by thermal based methods varies from 20.7% to 85.7%. The free radical polymerization and oxidative degradation respectively caused by ultrasound treatment and DBD plasma both tend to attack GalA units in HG region and protect RG-I region relatively. Among thermal based extraction methods, subcritical water extracted pectin has the lowest RG-I content (Rha content of 0.5%-0.6%), while pectin obtained by MAE and HHP has comparative HG and RG-I region content, which varies as acid or alkali solvent used. Besides, accurate extraction condition control of SWE especially temperature and time is vital for uniformity quality and good yield of pectin. Even minor change between 120 ^{o}C to 140 ^{o}C for different time exerts influence on pectin structure and DM.

6. Conclusion and perspectives

Recent research has extended our understanding of the relationship between pectin source, processes and the extraction of specific structures and functionality in recovered pectins. Acid, subcritical water or microwave treatment at high temperature are suitable for HG enriched pectin extraction while alkaline extraction under reduced temperature can be used to isolate intact RG-I domains. However, extraction of RG-I enriched pectin is enhanced by the use of multiple innovative extraction methods for efficient recovery and purity. This is particularly important for the emerging utilization of RG-I enriched pectin and oligomers as prebiotics and immunomodulators, cardiovascular disease and fibrosis treatment. The free radical inspired by ultrasound treatment and the oxidative degradation of DBD plasma both selectively attack GalA units and high-pressure treatment leads to the breakdown of C-O bonds and protect side chains of RG-I. Moreover, enzyme extraction is specific and depends on the site of action of the selected enzymes. Operating at low temperatures (25-60 ℃), these technologies can be combined with one another or with alkaline solvents, as promising methods for the targeted

692 recovery of RG-I enriched pectins.

693 However, considering the complexity of RG-I and few studies investigating the 694 influence of innovative technologies (especially ultrasound, DBD plasma) on structure, a 695 concrete mechanism of these needs further exploration. The content of Gal 696 pharmacophores, linear Ara, as well as RG-I side chains, is important for biological 697 activity. A combination of innovative technologies to control the proper ratios of Gal/Ara 698 and chain length warrants further study. There are a number of challenges and prospects.

699 (a) Improvement and standardization of analytical methods for pectin refined structure

700 Pectins from plant materials have chemically diverse structural units as well as a 701 wide distribution of molecular masses, thus, researchers face challenging 702 chromatographic separations and complicated structural characterization studies. The 703 RG-I domain (%) is often defined based on the molar content of monosaccharide residues 704 and it changes with different analytical methods. A standardization of analytical 705 approaches is required for better accurate definition of RG-I.

706 (b) Improvement of pure RG-I isolation

707 Intact pure RG-I region with specific sidechains is hard to isolate. Current studies on 708 RG-I bioactivity are normally based on HG and RG-I mixtures. In addition, certain 709 proteins in the sidechains are hard to remove. Identification and isolation of new enzymes, 710 produced by bacteria through co-culture, are needed to selectively degrade galactans, 711 branched arabinans and RG-II backbones and may represent a promising way to isolate 712 pure RG-I domains (Martens, Lowe, Chiang, Pudlo, Wu, McNulty, et al., 2011; Ndeh, 713 Rogowski, Cartmell, Luis, Basle, Gray, et al., 2017) .

(c) Targeted extraction of specific region (RG-I or HG) enriched pectins through the combined use of innovative technologies

Targeted recovery of pectins through the combined use of innovative technologies represents a new trend in isolating the structural domains of pectins. This is significant for production of pectin with specific structure considering distinct functionality of HG and RG-I domain. Plant material and extraction technology selected both need to be considered. Potatoes, ginseng, and citrus peels are all good sources of RG-I enriched pectin (Gao, Zhi, Sun, Peng, Zhang, Xue, et al., 2013; Khodaei & Karboune, 2013; Khodaei & Karboune, 2014; Zhang, Chen, Li, Yan, Ye, et al., 2018). Compared to citrus peels, pectin from sugar beets has a higher DA, a larger neutral content sugar, a lower molecular weight and less feruloyl groups (Li, Jia, Wei, & Liu, 2012). Mango peel pectin has also been reported to exhibit low GalA and high neutral sugars (Nagel, Mix, Kuebler, Bogner, Kienzle, Elstner, et al., 2015; Koubala, Kansci, Mbome, Crépeau, Thibault, & Ralet, 2008).

Future research needs to focus on the combined application of innovative non-thermal technologies (ultrasound, DBD plasma, enzyme) under mild alkaline conditions to efficiently enrich the recovery of pectins with RG-I domains. Considering difference in the resistance of Ara, Gal and Rha residues to hydrolysis, if limited Ara of RG-I were desired, a pH > 2.1 but <7.0 should be used to selectively remove the Ara while retaining Gal. For HG enriched pectin recovery, microwave or subcritical water under high temperature (above 65 °C) and acid solvent represents a promising method.

(d) Further structure-function exploration

The linear Ara of RG-I pectin from sugar beet can better enhance the immunostimulatory activity through the Syk kinase-dependent pathway better than branched Ara, due to the increased particle formation by the alignment of debranched

739 linear arabinan (Meijerink, Rosch, Taverne, Venema, Gruppen, Schols, et al., 2018). 740 RG-I-4 isolated from ginseng pectin by endo-polygalacturonase hydrolysis and 741 combination of ion exchange and gel permeation chromatography has high 742 anti-galectin-3 activity (Gao, et al., 2013; Yu, Zhang, Li, Liu, Sun, Liu, et al., 2010). 743 Future studies need to focus on the specific domain or metabolic pathways in vivo to 744 better understand the role of specific domain of RG-I on immunomodulation, 745 anti-proliferation, and anti-cancer activity.

746

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Author contribution

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Table 1 Effect of conventional water-based extraction on RG-I fraction and structure of pectin from fruit and vegetable waste

Plant material	Treatment	Extraction solvent	Extraction conditions		HG	RG-I	HG/RG-I	DM(%)	DA (%)	Yield (%)	Reference
			℃	min	(%)	(%)					
Ponkan peel	CW	water	25	900	47.8	36.4	1.30	51.2	0.0	2.9	(Colodel, Vriesmann, & Petkowicz, 2018)
	HW	water	100	120	54.6	40.7	1.30	52.6	0.4	12.4	
	CLA	0.5% ammonium oxalate	25	240	43.7	53.8	0.80	34.1	0.2	7.2	
	OA	Citric acid, pH 2.5	70	30	22.9	72.0	0.30	38.4	0.2	0.6	
Oil-pumpkin	HW	distilled water	60	120	55.5	28.0	2.00	na	na	na	(Kostalova, Hromadkova, & Ebringerova, 2013)
	CLA	0.05M EDTA, pH 4	25	120	95.0	1.4	67.9	na	na	na	
	MA	0.003M HCl	60	30	82.0	6.8	12.1	na	na	na	
	AL	0.25M NaOH	35	60	38.3	49.6	0.90	na	na	na	
	AL	1.32M NaOH	60	60	5.7	39.3	0.15	na	na	na	
Orange peel	MA	0.5% HCl, pH 1.7	82	50	nc	nc	nc	70.8	2.2	2.9	(Yordan Georgiev, 2012)
Citrus peel	MA	0.4% HCl, pH 3-4	28	40	45.6	44.0	1.04	56.0	nd	4.2	(Zhang et al., 2018)
	AL	0.6%NaOH, pH 6-7	32	10	8.6	82.5	0.10	10.0	nd	18.9	
Ponkan peel	MA	HNO3, pH 1.6	100	100	81.7	16.2	5.04	85.7	0.1	25.6	(Colodel, Vriesmann, Teofilo, & Petkowicz, 2018)
Citrus peel	OA	0.5M Citric acid, pH 7	65	120	19.9	57.5	0.35	8.4	na	7.4	(Kurita, Fujiwara, & Yamazaki, 2008)
Citrus peel	CW	water	25	30	60.6	9.8	6.18	76.5	5.5	5.8	(Yapo, Lerouge, Thibault, & Ralet, 2007)
	CLA	1% w/v Potassium oxalate, pH 4.5	25	90	69.5	8.1	8.58	73.7	2.3	14.7	
	MA	0.05M HCl	85	90	52.9	20.2	2.62	65.1	3.0	27.3	
	AL	0.05M NaOH, pH 5	40	90	43.1	16.5	2.61	10.0	na	4.8	
Yuza pomace	OA	0.25% oxalic acid/ammonium oxalate, pH 4.6	85	60	71.2	10.7	6.65	41.0	na	8.0	(Lim, Yoo, Ko, & Lee, 2012)
Orange peel	MA	Mild HNO3, pH 2.1	72	180	79.5	20.5	3.88	na	na	90.7#	(Kaya, Sousa, Crepeau, Sorensen, & Ralet, 2014)
	MA	Harsh HNO_3, pH 1.6	70	420	83.3	16.70	5.00	na	na	92.1#	
	OA	Mild citric acid, pH 4.6	85	90	79.9	20.10	3.98	na	na	85.3#	
	OA	Harsh citric acid, pH 3.5	72	150	80.4	19.60	4.10	na	na	92.9#	
Potato pulp	MA	Sulphuric acid, pH 2.04	90	60	35.1	60.77	0.58	26.68	10.51	8.38	(Yang, Mu, & Ma, 2018)
	OA	Citric acid, pH 2.04	90	60	33.4	61.49	0.54	21.51	9.21	14.34	
	OA	Acetic acid, pH 2.04	90	60	28.5	65.03	0.44	37.45	15.38	4.08	
Kiwifruit pomace	OA	1% Citric acid, pH 2.2	50	60	80.6	12.96	6.22	na	na	3.83	(Yuliarti, Goh, Matia-Merino, Mawson, & Brennan, 2015)
	CW	Water, pH 3.6	25	30	80.9	15.21	5.32	na	na	3.62	
Apple pomace	MA	Sulphuric acid, pH 2.0	85	180	55.5	11.90	4.67	56.10	7.20	8.2	(Wikiera, Mika, & Starzynska, 2015)
Grapefruit peel	MA	0.5M HCl, pH 1.5	80	90	60.9	32.11	1.87	69.03	3.65	na	(Wang et al., 2016)
Sisal waste	MA	HCl, pH 1.5	100	90	48.7	6.11	7.97	33.12	na	5.40	(Yang, Wang, Hu, Xiao, & Wu, 2018)
Grapefruit peel	MA	HCl, pH 1.5	80	90	60.6	31.54	1.92	55.31	4.00	21.10	(Wang et al., 2017)

HW:Extraction using hot water; CW: Extraction using cold water; MA: Extraction using mineral acids; OA: Extraction using organic acids; AL: Extraction using alkaline solvent; CLA: Extraction using chelating agents

DM, degree of methyl-esterification. DA, degree of acetylation

The molar percentage of homogalacturonan(HG) and rhamnogalacturonan of type I (RG-I) were calculated as the following formula:

HG (%)=GalA(mol%)-Rha (mol%)

RG-I (%)≈ 2Rha(mol%)+Ara(mol%)+Gal(mol%)

nc: nc indicates that this value can not be calculated from the data given in the article.

na: na indicates that this index was not analyzed in the corresponding article.

Table 2 Effects of ultrasound-assisted extraction or US treatment on RG-I fraction and structure of pectin from fruit and vegetable waste

Plant material /pectin material	Frequency /Power	Extraction conditions			HG(%)	RG-I(%)	HG/RG-I	Neutral sugar (%)	DM(%)	DA(%)	Yield(%)	Reference
		□	Time(min)	Solvent								
Grapefruit peel	0.41 W/mL	60	28	Water	49.16	41.09	1.20	42.64	58.78	3.98	na	(Wang et al., 2016)
Waste grapefruit peel	20 kHz	67	28	HCl, pH 1.5	54.73	38.31	1.43	39.14	65.37	3.86	23.49	(Wang et al., 2017)
Sisal waste	20 kHz	70	60	Ammonium oxalate	59.75	5.29	11.29	37.72	44.35	na	11.90	(Yang et al., 2018)
Citrus pectin	18 W/mL	20	30	Citric acid-phosphate,pH4	57.96	34.76	1.67	32.73	36.66	1.56	na	(Ma et al., 2018)
Sour orange peel	150W	30	10	Citric acid, pH 1.5	62.50	33.20	1.88	34.70	na	na	28.07	(Ma et al., 2016)
Citrus pectin	3.8 W/mL	30	5	Water, 6g/L H_2O_2;0.5mM ferrous	6.02	79.07	0.08	70.62	30.35	3.77	na	(Zhi et al., 2017)
	3.8 W/mL	30	35	Water, 6g/L H_2O_2;0.5mM ferrous	14.66	72.00	0.20	64.37	36.76	4.12	na	
Citrus pectin	11.4 W/mL	20	60	50 mM H_2O_2; 10 mM ascorbic acid	4.77	91.77	0.05	82.69	na	na	na	(Li et al., 2019)
	11.4 W/mL	30	60	50 mM H_2O_2; 10 mM ascorbic acid	2.27	92.60	0.03	84.57	na	na	na	
	11.4 W/mL	50	60	50 mM H_2O_2; 10 mM ascorbic acid	0.90	93.70	0.01	85.64	na	na	na	

Table 3 Effects of enzyme-assisted extraction on RG-I fraction and structure of pectin from fruit and vegetable waste

Plant material	Enzyme	Extraction conditions			HG(%)	RG-I(%)	HG/RG-I	Neutral sugar (%)	Gal(%)	Ara(%)	DM (%)	Yield (%)	Reference
		□	Time, h	pH									
Yuza pomace	fungal β-glucanase	40	1	nm	53.1	17.1	3.10	17.6	4.3	10.0	46.3	7.3	(Lim et al., 2012)
Potato pulp	Endo-PG	35	24	nm	25.7	73.2	0.35	61.7	55.0	11.2	na	37.9[#]	(Khodaei & Karboune, 2013)
Potato pulp	Endo-PG	35	30.4	nm	6.00	90.3	0.07	79.7	71.8	7.9	na	9.5[#]	(Khodaei & Karboune, 2014)
	Endo-PG	35	12	nm	14.00	85.2	0.16	82.8	81.2	1.6	na	63.9[#]	
Gold kiwifruit	Celluclast 1.5L	25	0.5	nm	82.91	14.15	5.86	15.27	6.86	3.87	na	4.48	(Yuliarti et al., 2015)
Apple pomace	Celluclast 1.5L	50	18	4.5	60.70	15.4	3.94	35.4	4.9	8.3	57.3	15.48	(Wikiera, Mika, & Starzynska, 2015)
Apple pomace	Celluclast	40	3	4.5	55.59	10.51	5.29	16.76	2.42	6.15	na	18.95	(Wikiera, Mika, & Grabacka, 2015)
	Econase	40	3	4.5	58.86	8.31	7.08	13.35	2.08	4.28	na	11.78	
	Viscoferm	40	3	4.5	61.49	10.06	6.11	16.64	2.78	5.56	na	17.86	
Sisal waste	Celluclast 1.5L	50	20	4	54.02	5.47	9.88	26.67	0.15	0.06	48.11	9.40	(Yang et al., 2018)
Citrus pectin	Pectinase	50	30	4	47.33	44.10	1.07	41.47	11.20	4.76	56.98	1.58	(Ma et al., 2018)
Citrus pectin(US-pre)	Pectinase	50	30	4	42.70	46.91	0.91	45.63	11.67	4.12	39.60	1.56	(Ma et al., 2018)
Green tea leaf	Viscozyme® L	30	3	4.5	nc	nc	nc	56.3*	19.14*	9.46*	22.4	8.5	(Zhang et al., 2020)
	FoodPro® CBL	30	3	4.5	nc	nc	nc	25.4*	3.45*	5.20*	40.9	5.1	

Endo-PG (Endopolygalacturonase)

Celluclast 1.5L (cellulases, polygalacturonase, pectin lyase and rhamnogalacturonan lyase); Viscozyme® L (Multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase); FoodPro® CBL (mainly contains cellulase)

[#] Yield was expressed the weight percentage of extract to the cell wall weight.

* Monosaccharides content was expressed the mass ratio instead of molar ratio

nm: nm indicates that this condition was not mentioned in the article.

Table 4 Effects of microwave, DBD plasma extraction on RG-I fraction and structure of pectin from fruit and vegetable waste

Plant material	Power (W)	Solvent	Extraction conditions		GalA(%)	Rha (%)	Gal+Ara	HG (%)	RG-I (%)	HG/RG-I	DM (%)	Yield (%)	Reference
			□	min									
microwave													
Polemo peel	1100	Water	Heating	2	70*	1.5*	24.3*	nc	nc	nc	29.7	6.5	(Wandee, Uttapap, & Mischnick, 2019)
	1100	200mM HCl, pH 1.0	Heating	2	82.2*	0.6*	13.7*	nc	nc	nc	82.5	16.1	
	1100	50mM NaOH, pH 12.1	Heating	2	85.7*	1.1*	13.8*	nc	nc	nc	na	24.2	
Sugar beet pulp	1200	50% NaOH, pH 11.5	100	10	13.4*	20.7*	64.1*	nc	nc	nc	6.4	na	(Fishman, Chau, & Cooke, 2009)
Pistachio green hull	700	16mM H_2SO_4, pH 1.5	Heating	2.75	66	2.7	29.9	63.7	35.3	1.80	12.1	18.13	(Kazemi, Khodaiyan, & Labbafi, 2019)
DBD plasma													
	Input voltage												
Fresh pokan peel	40V	HCl, pH 1.88	80	60	35.63	20.97	29.40	14.66	71.34	0.21	37.25	27.10	(Zhang,2018)

* Monosaccharides content was expressed the mass ratio instead of molar ratio

Table 5 Effect of subcritical water extraction on RG-I structure of pectin from fruit and vegetable waste

Plant material	Power(MPa)	Extraction conditions		GalA(%)	Rha(%)	Ara (%)	Gal(%)	Gal/Ara	DM (%)	Yield (%)	Reference	Remarks
		□	min									
Citrus peel	nm	100	5	60.77*	0.50*	2.38*	0.80*	0.33	71.88	19.78	(Wang, Chen, & Lü, 2014)	
	nm	120	5	68.88*	0.48*	3.10*	2.52*	0.81	74.74	21.95		
	nm	140	5	52.33*	0.62*	4.44*	4.59*	1.03	68.88	19.21		
Apple peel	nm	130	5	44.37*	0.67*	2.99*	4.23*	1.41	83.41	13.33	(Wang & Lu, 2014)	
	nm	150	5	40.13*	0.79*	2.33*	4.58*	1.96	85.99	16.68		
	nm	170	5	20.67*	0.41*	1.39*	5.40*	3.88	89.69	10.05		
Apple pomace	nm	140	5	48.20*	0.66*	2.07*	5.44*	0.38	60.23	17.55	(Wang et al., 2014)	
Sugar beet pup	10.7	120.72	30.5	59.12*	4.48*	21.66*	5.32*	0.25	55.20	24.63	(Chen, Fu, & Luo, 2015)	UAE+SWE

*The monosaccharide content was expressed as the mass ratio instead of molar ratio

Figures:

Figure. 1 Schematic representation of the structure of pectin, showing the HG, RG-I and RG-II domains. The structure of HG and RG-I backbones are highlighted.

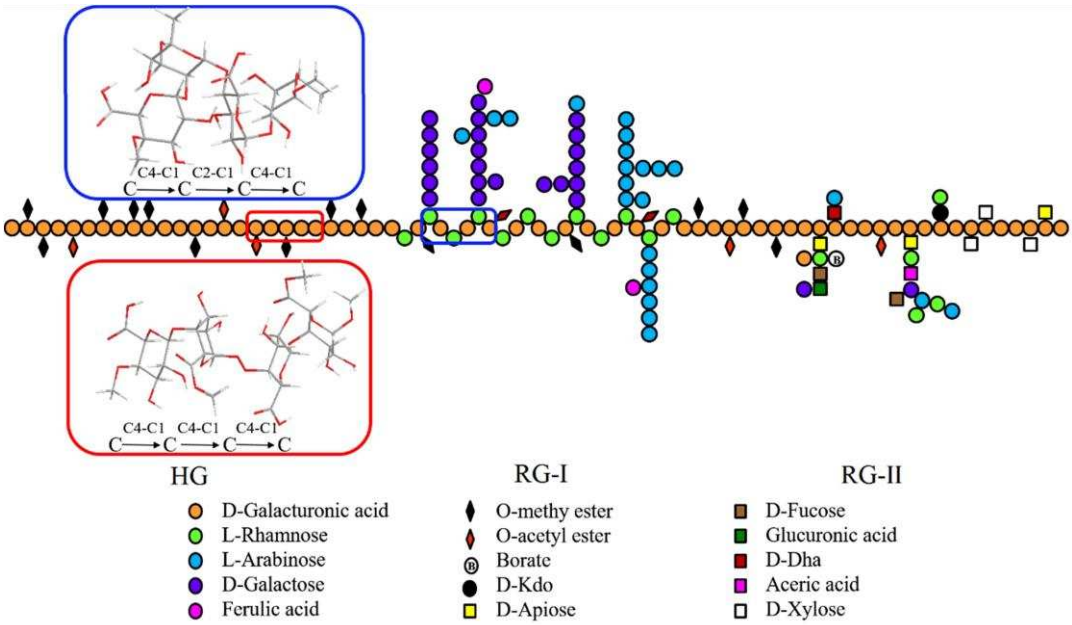

Figure. 2 Basic scheme for subcritical water extraction [adapted according to (Hoshino, Tanaka, Terada, Sasaki, & Goto, 2009) and (Ueno, Tanaka, Hosino, Sasaki, & Goto, 2008)]

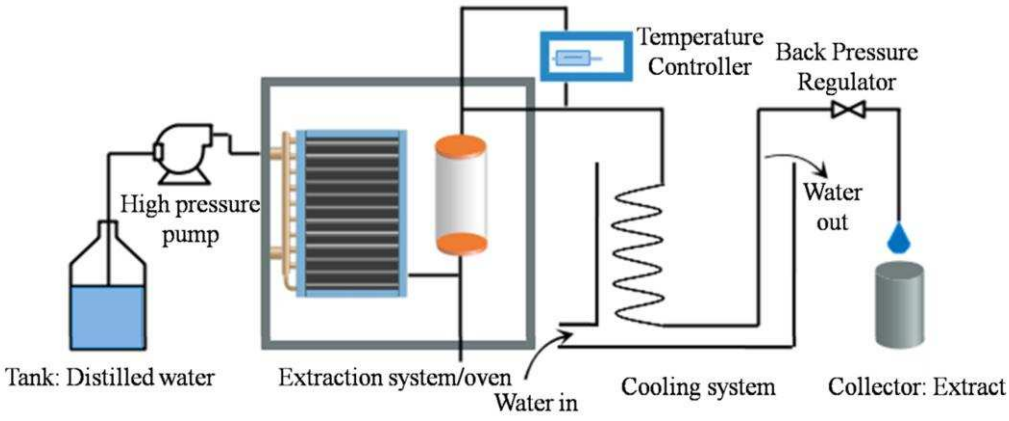

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Figure. 3 Schematic of dielectric barrier discharge [adapted according to (Misra, Pankaj, Segat, & Ishikawa, 2016)]

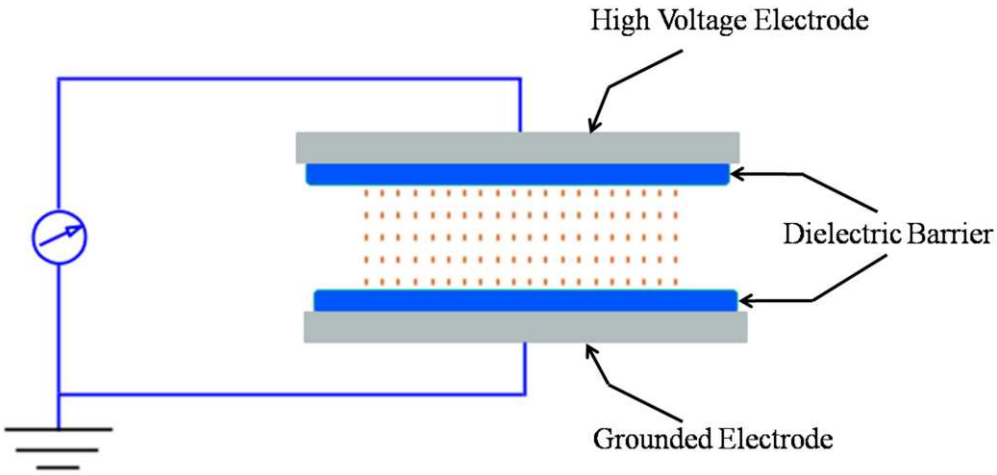
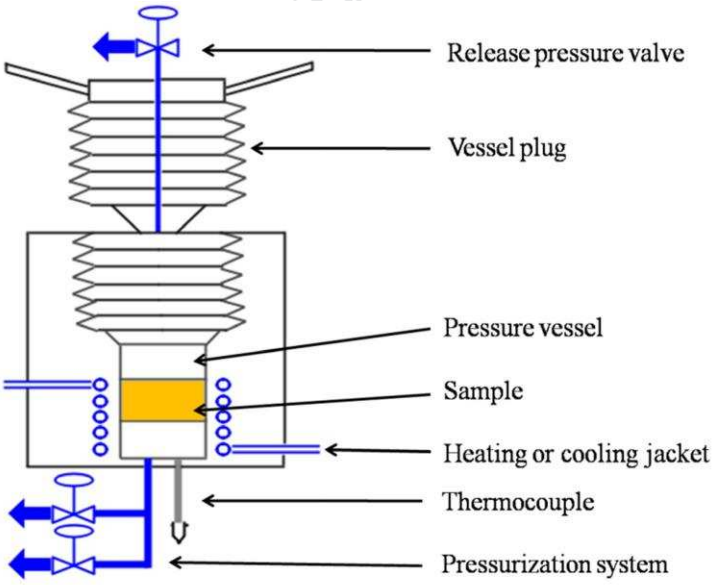


Figure. 4 Schematic diagram of ultrahigh pressure extraction device [adapted according to (Xi, Shen, Li, & Zhang, 2011)]



Highlights：

- RG-I is in the hairy region of pectin and has demonstrated biological functions
- Different extraction methods exert an influence on the final structure of pectin
- Harsh extraction conditions gives pectin rich in homogalacturonan but degrades RG-I
- Plasma/enzyme-assisted extraction or mild alkaline extraction gives RG-I pectins
- Combined non-thermal extraction gives pectins rich in neutral RG-I