

Advances in Chemical Engineering

Chapter 6

Application of High Hydrostatic Pressure for Pectin Extraction from Agro-Food Waste and By-Products

Antonela Ninčević Grassino^{1}; Damir Ježek²; Sven Karlović²; Tomislav Bosiljkov²*

¹*Department of Chemistry and Biochemistry, Faculty of Food Technology and Biotechnology, University of Zagreb.*

²*Department of Process Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb.*

**Correspondence to: Antonela Ninčević Grassino, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia*

Phone: +385-1-4605-062; Fax: +385-1-4836-083; E-mail: aninc@pbf.hr

Abstract

Food wastes and by-products produced in huge amount from a variety of sources, ranging from agricultural to food processing residues, could be valorized through the extraction and implementation of high-value components as nutritional and pharmacological functional ingredients.

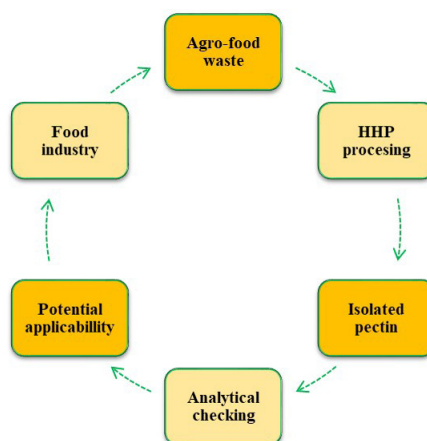
There are many well-recognized extraction techniques, such as pressurized fluid, supercritical fluid, pulsed electric field, and ultrasound-, microwave-, and enzyme-assisted, developed for conversion of agro-food wastes into various edible, food products. These technologies have gained the worldwide acknowledgment for their potential and versatile applications, which depend on type of raw materials and target biomolecules, the scale of processing (laboratory or industrial), the ratio between production costs and economic values of the compounds to be extracted.

Due to the growing consumers demand for fresh, safe and minimally processed foods has brought the great idea of food scientists

and technologists to develop many non-thermal food processing technologies which cause minimum nutritional and sensory qualities of the processed foods. Today, non-thermal technologies like high pulse electric field, high hydrostatic pressure, irradiation, ultraviolet light, ultrasound, arc discharge, oscillating magnetic fields, light pulses, plasma, chemicals (ozone, carbon dioxide, argon), and combined methods of these technologies are the major areas of researches in the process and food engineering, in order to find their suitability for processing of various food products.

In this attempt, the high hydrostatic pressure, as non-thermally processing method has been also introduced for pectin extractions from a few food waste resources. Therefore, in the present work, an attempt has been made to give an insight about the high pressure processing for valorization of agro-food wastes, its principle and process design in order to explore this novel technology in futures application for extraction of various other food waste rich in diverse bioactive and functional agents, and to develop healthier, novel food products for consumers.

Graphical Abstract



1. Introduction

The fruit and vegetables industrial processing generate a large amount of waste and by-products, such as peels, seeds and leaves. Due to the fact that these bio-organic materials usually presented an environmental problem for the industry, its potential re-utilization for recoveries of different compounds would provide economic advantages for producers. Apart from decreasing the environmental impact, the consumer would take the opportunity that some valuable compounds could be reintroduced into food. Therefore, the main focus of investigations has been placed in the re-utilization of agro-food waste for isolation of certain valuable components, such as pectin. Among various agro-food waste, the red beet [1], cocoa

husks [2], papaya peel [3], sunflower head [4], mango [5,6], banana [7], tomato [8,9] and pomelo [10] peels, hazelnut skins [11], carrots and green beans waste [12], and different other food waste streams [13] have been employed as a non-commercial resources for pectin production.

The current industrial, commercial production of pectin is based on the utilization of citrus peel and apple pomace, a residues gained from juice manufacturing. In that context, the pectin is exclusively extracted in a conventional, chemical way using nitric, hydrochloric and sulfuric acids [14]. Conventional procedure is also used for pectin isolation from a number of other food waste and by-products with mineral or organic acids [14]. In spite of the fact that conventional acid extractions of pectin is simple and efficient, this technique showed some disadvantages, such as long extraction time, degradation of target compounds, and production of contaminants that must be treated.

To overcome these limitations, new and promising extraction methods, such as ultrasound, microwave and enzyme-assisted extractions were recently reported for pectin isolation from plant food waste and by-products [14]. Besides them, another one of novel extraction technique, the high hydrostatic pressure, frequently used for preservation and processing of various foodstuffs, was recently introduced for pectin isolation from orange [15], lime [16] and potato [17] peels, and sugar beet [18].

High pressure processing (HPP), also known as ultra-high pressure (UHP) or high hydrostatic pressure (HHP) as a novel, non-thermal technology, an alternative to traditional thermal treatment, has been developed to assure the production of high quality foods. In comparison to thermal sterilization, which altered the unique sensorial and functional characteristic of foods, the HHP preserves the original properties of processed food, and day-by-day, this technology has a broad range of applications. It is widely used for processing of fruits and vegetables derived products, egg, meat and dairy products, seafood, and alcoholic beverages [19]. Meat and vegetables take the lead with a percentage of 28 and 20 %, respectively, followed by juice and beverages (18 %), and seafood and fish products (14 %). Sauces and dressings (3 %), dairy (1 %), and other food products (16 %) completed the current HHP market, with a near 20 %.

Generally, the HHP processing involves the employment of pressure in the range of 100 to 1200 MPa, with or without application of heat, causing the various physical, chemical and biological changes, due to combined effects of temperature and pressure on processed foods. The applied temperature throughout pressure could range from below 0 °C to above 100 °C, with times extending from a couple of seconds to over 20 min.

This process forces a fluid through a narrow gap valve, resulting in cavitation, turbulence and high shear stress [19]. As a consequence, the microstructure of the matrix is disrupted,

generating particles with a more uniform and smaller size, leading to better texture characteristics and improved physical stability of the isolated compound. For instance, the applied pressure increases plant cell permeability, leading to cell component diffusivity according to the phase behavior theory, i.e. the solubility of the compound is larger while the pressure increases.

Taking into account the growing consumers demand for fresh, safe and minimally processed foods, and considering the fact that there are very limited reports regarding the effect of high hydrostatic pressure on pectin isolation from agro-food waste and by products, this chapter shows the main working principles of HHP technology, which could be applied for further valorization of various agro-food waste and by-products, as a non-commercial resources for pectin production. Some of the most relevant characteristics are written below. In addition, the present chapter shows the chemistry of pectin, its applicability in relation to extraction methodology, and analytical responses, in order to have the whole idea of the pectin processing cycle.

2. Chemistry of Pectin

Pectin is a complex heterogeneous polysaccharide found in the primary cell walls of most plants. It provides mechanical strength and flexibility due to its interaction with other cell wall components. D-galacturonic acid (D-GalA), an isomer of D-glucuronic acid (D-GlcA) was discovered to be a basic constituent of all pectins (**Figure 1**). It is present in three polymeric forms, i.e. homogalacturonan (HG), a linear polymer of α -1-4 linked galacturonic acid, rhamnogalacturonan I (RG-I), a repeating disaccharide of galacturonic acid and rhamnose and rhamnogalacturonan II (RG-II), a homogalacturonic backbone with numerous complex side chains containing rhamnose and other neutral sugars [20-22]. To date, pectins are thought to be composed of at least of 17 kinds of monosaccharides of which D-GalA is the most profuse, followed by D-galactose or L-arabinose [23]. HG the most abundant of the three polymers in which D-GalA units can be partially methyl-esterified at C-6 and acetyl-esterified at O-2 and/or O-3 positions has a large impact on the functional properties of the pectin. When more of 50 % of carboxyl groups of D-GalA units in pectin are esterified with methyl (or methoxyl) groups, this category of pectin is conventionally called high methyl-esterified, with a degree of esterification (DE) > 50 %. Otherwise, the pectin is referred to low methyl-esterified, with DE < 50 %. The DE of the pectin is an important parameter for the definition of its applicability. For instance, high methoxyl pectin forms a gel when it is heated in solution with low pH (2 - 3.5) at a high concentration of sugars (55 - 75 %). On the other hand low methoxyl pectin requires presence of divalent ions, such as calcium, with or without of sugars in a broad pH range (2 - 6).

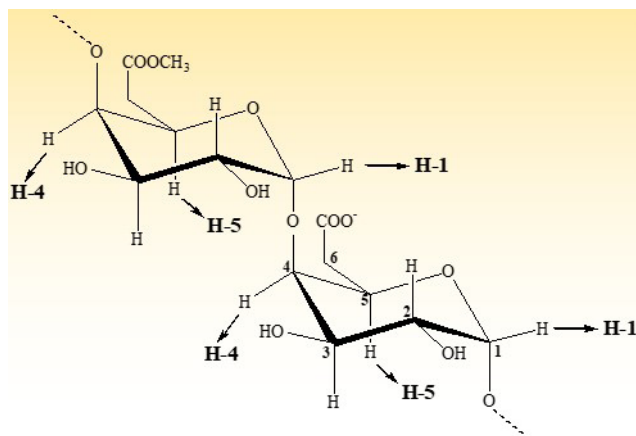


Figure 1: D-galacturonic acid as basis unit of pectin

3. Pectin Resources and Applicability

The traditional sources used for commercial production of pectin are citrus peel [24] and apple pomace [25], the residue left after juice extraction, and sugar beet pulp [26]. Due to the fact that the food processing industry generated different kinds of waste and by-products, a main focus of investigations has been placed in the utilization of their certain components, for instance pectin. In that context, the raw material for pectin production left without proper disposal mechanism could be successfully explored, offering economic advantages in decreasing disposal costs of waste. On the other hand the consumer would take the opportunity that pectin as a soluble dietary fiber could be reintroduced into food.

According to the recent literature reports, the red beet [1], cocoa husks [2], papaya peel [3], sunflower head [4], mango [5,6] banana [7], tomato [8,9] and pomelo [10] peels, hazelnut skins [11], and various other vegetable waste streams [12,13] have been studied as a naturally available resources for non-commercial production of pectin. Their evaluation as an appropriate resource for further pectin utilization is usually performed in three main steps (**Figure 2**): *i*) employment of extraction method and condition, *ii*) isolation of pectin, and *iii*) analytical checking using different tools.

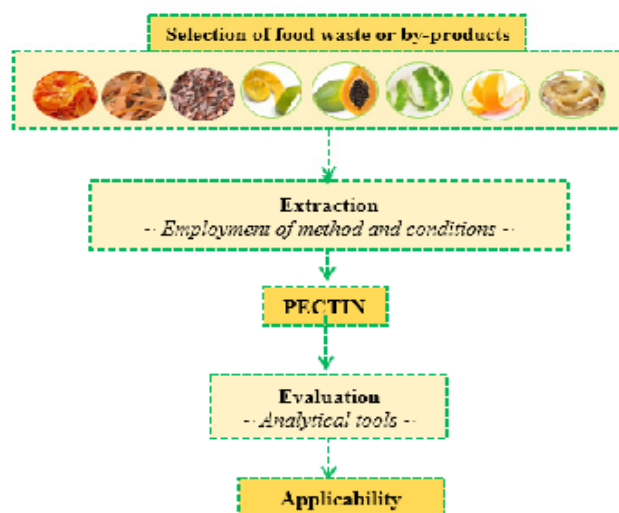


Figure 2: A schematic presentation of pectin production, from selected waste to pectin applicability

Depending on cultivation, maturation stage and storage conditions of raw material, and used extraction techniques and conditions (time, temperature and solvent), the chemical structure, composition and functional properties of pectin vary, and consequently have an influence on pectin further applicability. For instance, pectin as a water soluble carbohydrates is widely used in food processing industry as gelling agents in jams, jellies, confectionery products, as well as a stabilizer in juice and soft drinks made of milk or soy. Pectin could be used as a fat replacer in ice cream and yogurt, and a heat reversible bakery glazing. Pectin is also used in medicine and pharmaceutical industry as anti-inflammatory, anti-coagulant, anti-thrombotic, anti-microbial and anti-tumoral agents [27]. It is also utilized in hepatic regeneration, injury of atherosclerosis, preventions of endotoxemia, and in controls of calorie absorption, obesity and diabetes [27]. Pectin is used as a natural prophylactic, binding agent in tablet formulations, as an emerging prebiotic [28] and as a carrier for targeted controlled delivery of a variety of drugs.

Other applications of pectin are related to its implementation as an eco-friendly tin corrosion inhibitor [8], and carrier in bio-based edible films or coatings preparation [29], as well as an encapsulating material for the production of nano-dispersions [30].

4. Pectin Analysis

In order to responding whereby the processing (extraction) of raw material was carried out, i.e. suitable extraction time, temperature, pH, origin of extracted material, material to solvent ratio and number of extractions, and where the isolated pectin could be utilized, the choice of appropriate analytical method for detection on pectin quality parameters will have an important role. The frequently used ones as a measured of pectin purity are mentioned below.

It is well known that during the processing of bioorganic plant materials via conventional or non-conventional extraction methods can occur that change pectin solubility, polymer size, and degrees of esterification and acetylation. These changes can have important consequences on pectin quality and purity, and thus on its functionality and applicability. The particular cares have to be taken for quantification of galacturonic acid (GalA), degrees of methylation (DM) and acetylation (DA), molecular weight and intrinsic viscosity. According to the EU regulation No. 231/2012 [31], the content of galacturonic acid of food grade pectin should be $\geq 65\%$. In contrast to food use, there are no regulations regarding feed use [32].

The volumetric, acid-base titration [33] has been the preferred method for pectin quantification from commercial, as well as novel food waste resources, due to the fact that provides a simultaneous determination of methoxyl (MeO) and anhydrouronic acid (AUA) contents, and degree of esterification [34]. However, this analytical method requires that the pectin be highly purified by acid-alcohol washing in order to remove extracting solvents or salts and convert pectin into free acid form before titration. Moreover, the common problem of

titrimetric method is non-distinction of D-galacturonic acid among other uronic acids, unless the latter are primarily separated by various purification procedures.

Besides titrimetric method, the colorimetric techniques based on variants of chromogenic reagent (carbazole, *meta*-hydroxydiphenyl, 3,5-dimethylphenol or xylenol, sulfamate-*meta*-hydroxydiphenyl, copper-Folin-Ciocalteau and thioglycolic acid) were also utilized for the quantification of uronic acids in pectin isolated from food waste. The coloration obtained is proportional to the amount of uronic acids, after hydrolysis of pectic substances in concentrated sulfuric acid solutions.

Despite its expanded use, the colorimetric methods are not able to discriminate different uronic acid. Therefore, the quantitative measurement is done in terms of total uronic acid content. In addition, with the colorimetric technique another one serious problems are neutral sugars, such as hexoses and pentoses, and their degradation products formed after acid hydrolysis.

Although, colorimetric and titrimetric methods are frequently used up to now, other analytical methods have been developed to avoid the error in galacturonic acid quantification. Due to the fact that neutral sugars made an essential part of pectin, the certain amount of these compounds can be incorporated in pectin extracts gained from various plant materials during isolation, precipitation and purification procedures. In this context, the removal of neutral sugars from crude pectin extracts provides valuable information of its purity, and consequently applicability. Apart from high-pressure anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD), the composition of the neutral sugars can be examined by GC/FID, GC/MS and HPLC with refractive index and photodiode array detections.

Among chromatographic techniques, high performance size exclusion chromatography (HPSEC) is used for molecular characterization of pectin samples, such as molecular weight, molecular weight distribution, the radius of gyration and intrinsic viscosity.

Furthermore, Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) have been proposed as useful spectral techniques for characterization of pectin structure, after its isolation from various plant materials. FTIR provided functional group analysis with characteristic peaks at 3390.6, 2939.0, 1749.0 and 1052.1 cm^{-1} , which are related to -OH, -CH, C=O group of ester and acid, and -COC- stretching of the galacturonic acid. Moreover, the FTIR spectroscopy can be utilized for determination of the degree of esterification. In order to quantify the DE of pectins, a calibration curve based on pectin standard (known DE) was established from the ratio of $A_{1730}/(A_{1730} + A_{1600})$, where 1760 - 1730 cm^{-1} and 1630 - 1600 cm^{-1} bands represent ester carbonyl and free carboxylate groups, respectively.

NMR spectroscopy, i.e. proton -1 (^1H NMR) and carbon-13 (^{13}C NMR) can be utilized

for the identification of H and C atoms in extracted pectins, respectively. Therefore, the pectin purity in terms of galacturonic acid can be established on the basis of characteristic peaks (signals) found in ^1H and ^{13}C spectrums of pectin extracts.

5. Pectin Extraction

The pectin as a potential marketable component present in various foods wastes and by-products need to be separated from the matrix through selective extraction and modification, ensuring that comply the existing food regulations [31], and meet the consumer standards.

Due to the fact that certain parameters of pectin quality implicate its further applicability, the particular care should be taken to set the extraction conditions, extremely important for pectin dissolution from the sample matrix.

In order to maximize the recovery of pectin into a more suitable form for separation and detection, a simple and efficient, conventional extraction methods have been employed [14]. In the conventional procedure, such as refluxing, the pectin is extracted by treating the raw material using hydrochloric, nitric, sulfuric, and oxalic acids. The hot viscous pectin extract is separated from residues by filtration and centrifugation. Subsequently, the clarified extract is subjected to alcoholic precipitation, purification, drying and milling. The common steps of pectin production are illustrated in **Figure 3**.

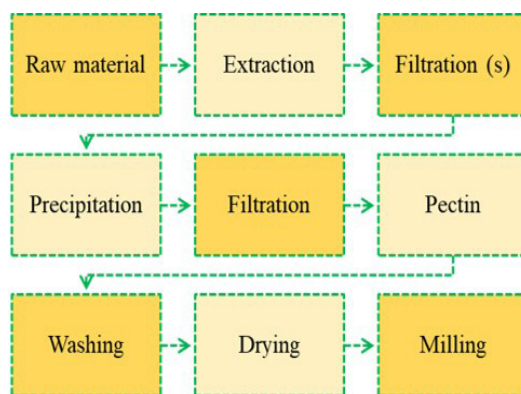


Figure 3: Flow diagram of pectin extraction procedures

Although efficient, the conventional methods showed some disadvantages, such as long extraction time, evaporation of the huge amount of solvent and creation of environmental problems producing hazardous contaminants. To overcome the limitations gained by conventional extraction, the innovative approach based on the application of ultrasound-, microwave- and enzyme- assisted extractions have been recently introduced for pectin isolation from plant food waste and by-products [14].

Besides these greener methods, another one of promising and novel extraction technique, the high hydrostatic pressure (HHP) has been recently introduced for the valorization of few food wastes, such as orange, lime and potato peels [15-17], and sugar beet [18], as non-conventional resources for pectin production. Apart from them, the high pressure homogenization was also

employment for pectin de-polymerization from apple and citrus as industrial, commercial resources, to gain a better understanding on the importance of pectin structure on the impact of high pressure homogenization [35]. The authors have pointed out that high pressure homogenization influence on rheological and textural properties of pectin, and reduce the microbial activity of liquid food system in a continuous process of pectin.

5.1. High hydrostatic pressure: work principles and process parameters

High hydrostatic pressure as a novel, non-thermal method has been developed with the aim to extend the shelf life and freshness of finished food products. As an attractive alternative method to traditional food processing, the HHP was employed for pasteurization and sterilization purposes of various foodstuffs. HHP technology has also numerous other features, listed in **Figure 4**.

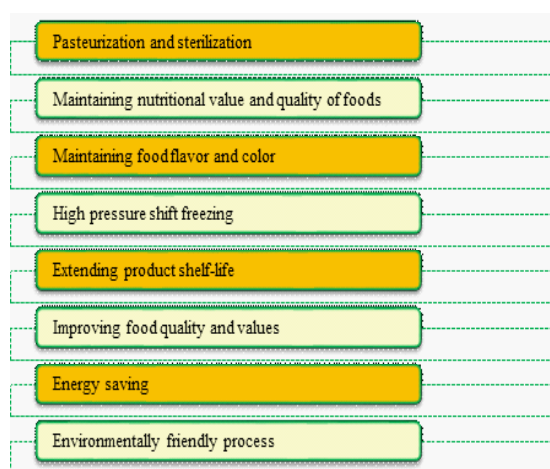


Figure 4: Characteristic of high hydrostatic pressure technology

HHP is available in the modern food industry for the last few decades and usually operate at pressures from 100 - 1200 MPa. It is applied for processing of two categories of foods: *i*) liquid and *ii*) solid and semi-solid, using batch or semi-continuous working mode.

In batch processing, the raw material is sealed in a plastic container, and placed in a pressure chamber for pressurizing, using pure water as the transmitting fluid. The chamber is then decompressed, and the cycle begins again. The cycle time depends on the type of raw material and employed temperature.

In the semi-continuous mode, the raw material is introduced periodically into a high pressure chamber, in which the filling, pressurizing, holding, decompression, and expulsion are occur.

The basic principle of high hydrostatic pressure is governed by “*Le Chatelier*” and isostatic distribution principles as a consequence of changes of three main parameters, i.e. temperature in the high hydrostatic vessel, holding time (maximum set up pressure level) and time of treatment. Therefore, when increasing the pressure at a constant temperature, at the

molecule level, the degree of arrangement of molecules in given substance increases. It results in a restriction of rotational, vibrational and translational motions of molecules [36,37].

Typical configuration of HHP equipment listed in **Figure 5** is pressure vessel (thick-wall stainless cylinder filled with water or propylene glycol), closures (to cover the cylindrical pressure vessel), system for generation and regulation of high pressure (valves, hydraulic and pneumatic pumps and intensifiers), system for process control and regulation of temperature, and handling system for loading and removing of product [36,37].

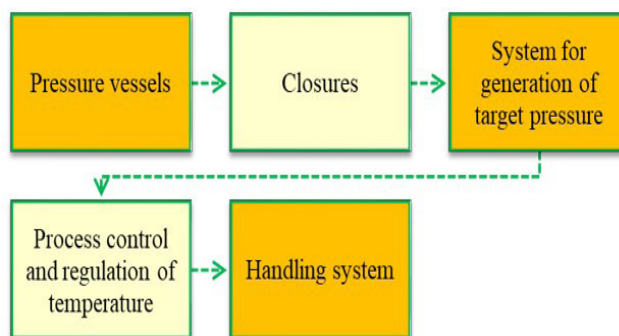


Figure 5: Typical configuration of HHP equipment

To achieve satisfactory results of HHP treatment, the three process parameters, i.e. pressure, time, and temperature must be adequately set. The lowest pressure used in the processing of raw material is around 50 MPa, while the highest pressure reaches the value of 1200 MPa.

As costly repairs and regular services of HHP equipment have shown significant contribution in the final price of processing, pressures for selected operation such as pasteurization or extraction are usually set at a minimum necessary (around 600 MPa) to achieve optimal results.

There are three possible options to choose the pressure for HHP processing of raw materials. The application of constant pressure during the full cycle is the most usual one. In the first part of the cycle, the pressure is build-up from atmospheric to target pressure value. When target pressure is reached, it is maintained during the whole time of processing. In the final part of the cycle, pressure returns to atmospheric, when the processing is finished. Decompression is usually rapid, and it can even be almost instant, depending on the valves and other equipment used.

The second mode of operation uses one or multiple stops at lower pressures (usual range is 150 - 200 MPa) for a preset amount of time. This type of processing can be beneficial for the inactivation of some bacterial spores.

The third option uses pressure pulsing, with one or more fast drops, ranging from maximal to target (lower or atmospheric) pressure values. Although efficient, particularly for

inactivation of microorganisms, this method is the most expensive, in terms of electricity needed for multiple pressurizing cycles and the largest load on the machine [38].

During HHP processes, the wide range of temperatures of pressure transmitting fluid could be employed. They vary between - 50 up to 130 °C. For instance, the temperatures from - 50 to -18 °C could be applied for freezing of various food materials. Extraction and pasteurization treatments usually worked at room temperature, due to economic reason. The higher yield of extract or larger reduction of microorganisms is gained using a slightly elevated temperature. Therefore, it is not unusual that extraction is done at temperature up to 70 °C to maintain the most relevant quality attribute, such as nutritional and antioxidant properties.

The highest temperatures are used in pressure-assisted thermal processing of various foods matrix. This emerging sterilization technique involves the preheating and processing of food materials at temperatures of 75 to 90 °C and 90 to 120 °C, respectively. Due to the fact that pressure-assisted thermal processing utilizes intensive pressure and heat, from the standpoint of engineering, the process applies significant stress of HHP equipment. On the other hand this technique enhanced the stability of valuable food ingredients, such as bioactive compounds.

Although, HHP fall into the category of non-thermal technology, one important factor which must not be disregarded is the rise of temperature during HHP processing of raw materials. The temperature usually increases from 1.5 to 3.0 °C for every 100 MPa, depending on the chemical composition of processed material and pressure fluid [38].

Besides pressure and temperature, the time is the final contributing parameter in the HHP technology. The one full HHP cycle is composed by: *i*) loading time (filling of material in pressure vessel), *ii*) compression time or alternatively pressurizing or pressure come-up time (to achieve preset process pressure), *iii*) pressure hold time (vary from seconds to hours, even days in some operations) and *iv*) unloading time (emptying of material from the pressure vessel). Therefore, the loading and unloading times are fixed, and exclusively dependent on the equipment used. From the practical, economic and commercial aspects of view, the time of 5 min or less is commonly used in HHP processing of raw materials [39].

Although, the high hydrostatic pressure was primarily focused on conservation, and transformation or processing of various products, the great challenges of HHP technology will be the extraction and preservation of bioactive and functional compounds gained from agro-food waste, with its potential application in food and biotechnological industries.

5.2. High hydrostatic pressure extraction of pectin

As it was mentioned previously, the high hydrostatic pressure exclusively used for

preservation and processing of various foodstuffs has been recently explored for pectin extraction from orange, lime and potato peels [15-17], and sugar beet [18]. Some of the most relevant and interesting characteristics of pectin isolation and detection (**Tables 1 and 2**) are described below.

Table 1: Effect of high hydrostatic pressure conditions on pectin yield extracted from few food waste and by-products

Plant material	Treatment conditions			Extraction solvent	Yield (%)	Reference
	Pressure	Time	Temperature			
	(MPa)	(min)	(°C)			
Orange peel	100-600	5-30	10-55	0.5 M HCl, pH = 1.5	8.0-15.5	[15]
Lime peel	100-200	30	50	0.05 citrate buffer, pH = 4.5, Cellulase/Xylanase	18.6-26.5	[16]
Potato peel	200	5	25	0.25 % oxalic acid/ammonium oxalate, pH = 4.6	/	[17]
Sugar beet	250-550	30	25	0.1 M HCl, pH = 1	/	[18]

Table 2: Analytical approaches used for pectin analysis after HHP extraction from few food waste and by-products (orange, lime and potato peels, and sugar beet)

Pectin quality parameters	Analytical methods/tools	Reference
DE, DA	Acid-base titration	[15], [16], [17], [18]
AUA or GalA	Spectrophotometry	[15], [16]
Monosaccharide	GC/MS	[17]
Intrinsic viscosity	Rheometer, Size Exclusion Chromatography	[16]
Viscosity-average molecular weigh	Rheometer, Size Exclusion Chromatography, Gel permeation chromatography, HPSEC	[16], [17]
Rheological properties	Rheometer	[15], [17], [18]
Activation energy	Rheometer	[15]
Gelling properties	Rheometer	[15]
Structure	FTIR, NMR, AFM, SEM	[17], [18]

Although, HHP processing of various foodstuffs operated in an ample range of pressures (100 - 1200 MPa), temperatures (- 50 - 120 °C) and times (from seconds to hours), the isolation of pectin from orange, lime and potato peels, and sugar beet are performed at pressure of 100 to 600 MPa, with temperature and time up to 550 °C and 30 min, respectively (**Table 1**).

For instance, Guo et al. [15] were applied high pressure of 100 to 600 MPa, holding time of 5 to 30 min and a temperature of 10 to 55 °C. HHP extraction efficiency was evaluated in terms of pectin yield and viscosity. At a constant temperature of 45 °C and pressure-holding time of 15 min, the pectin yield increased (8.0 - 15.5 %) with increases of pressure from 100 to 600 MPa. On the other hand, the pectin viscosity increases up to 500 MPa. Therefore, at the optimal pressure level of 500 MPa, the pectin yield and viscosity increased with the rise of

temperature and time up to 45 °C and 10 min, respectively.

On the based on these results, and with the aim to optimize the extraction, the authors [15] were used two factors, three level design, revealing that pressure of 500 MPa, the temperature of 55 °C and a holding time of 10 min are the optimal HHP conditions for extraction of pectin from orange peel.

In addition, the authors have evaluated the HHP extraction efficiency in comparison with conventional heating (80 - 82 °C, 1 h) and microwave-assisted (80 °C, 21 min) extractions. According to their reports, the HHP provided a higher yield (20.44 %), intrinsic viscosity (0.4276 L/g), and viscosity-average molecular weight (3.063×10^5 Da) than conventional and microwave-assisted extractions.

Furthermore, the Naghshineh et al. [16] were combined enzymatic extraction of the lime peel with HHP treatments employing the pressure of 100 to 200 MPa for 30 min, at 50 °C. Their results showed that the addition of individual enzymes (cellulose or xylanase) or its combination, at pressure of 200 MPa provided higher yields (18.6 - 26.5 %), galacturonic acid content (75.5 - 92.6 %) and degree of esterification (61.8 - 75.7 %) in comparison with conventional acidic (44 % HNO₃, 70 °C) and aqueous (0.05 M citrate buffer, 50 °C) treatments. On the other hand, the pressure and enzyme concentration had no effect on the molecular weight and viscosity of pectin. Thus, without reduction of molecular weight and viscosity of pectin, pressure-treated, enzymatically extracted pectin from lime as an environmentally friendly approach assured lower energy consumption due to a decrease of temperature, time and chemical in comparison to conventional extraction methods.

The high hydrostatic pressure and high pressure homogenization were applied for pectin treatment (200 MPa for 5 min cycling), after its previous isolation from potato peel waste with oxalic acid/ammonium oxalate, at 85 °C for 2 h [17]. According to Xie et al. [17] additional pectin treatments by high pressure led to increases of galacturonic acid content and degree of esterification, as well as the rise of viscosity and emulsifying properties.

In the work obtained by Peng et al. [18] is showed that pressure of 250 to 550 MPa in combinations of three different pH values (3, 7 and 8) of extracting solvent (glycine/HCl and tris/HCl) influenced on molecular weight and degree of esterification, and acetylation of sugar beet pectin. For instance, the molecular weight at pH 3, 7 and 8 significantly decreased with increases of pressure. At pH values of 3 and 7, the degree of esterification is unchanged, and the degree acylation is somewhat smaller than control with increases of pressure. According to results gained by scanning electron microscopy (SEM) and FTIR analyses, the HHP treatments provided successful modification of pectin structure, and its rheological behavior, at three different pH values.

Thus, according to few mentioned reports it is possible to conclude that high hydrostatic pressure as a green processing technique is capable to produce pectin with various physicochemical, rheological and gelling properties, depending on utilized agro-food waste and employed extraction parameters. Considering some of the main high pressure advantages, such as low temperature, short time and less uses of solvents, HHP as non-thermal technology has a great potential for the valorization of agro-food wastes for pectin production. However, further investigations in this area are required, in order to produce pectin on the industrial level, available for consumers.

6. Conclusion

Due to the fact that a number of agro-food wastes increased daily, the employment of novel, eco-friendly and quick HHP technology could have an important impact on pectin recovery. Considering the main HHP processing parameters (pressure, temperature and time), and combine them with other important extracting condition, such as type of solvents, solvent to liquid ratio, and a number of repeated extraction, may lead to modifications of structural, physicochemical and functional properties of pectin.

Therefore, the results of HHP treatment of few mentioned food waste, performed at pressure of up to 600 MPa, mild temperature (up to 55 °C) and time of 5 to 30 min, pointed out that high hydrostatic pressure could be successfully utilized for pectin production from other bioorganic materials, generated by agro-food sectors.

To assure high yields of pectin, and its excellent quality available for consumers, with simultaneous employment of green principles, nowadays, will be the great challenges for scientists.

7. References

1. E.N. Fissore, N.M.A. Ponce, L. Matkovic, C.A. Stortz, A.M. Rojas, L.N. Gerschenson, Isolation of pectin-enriched products from red beet (*Beta vulgaris* L. var. *conditiva*) wastes: composition and functional properties. *Food Sci. Technol. Int.*, 2011; 17: 517-527.
2. S.Y. Chan, W.S. Choo, Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks. *Food Chem.*, 2013; 141: 3752-3758.
3. B.B. Koubala, S. Christiaens, G. Kansci, A.M. Van Loey, M.E. Hendrickx, Isolation and structural characterisation of papaya peel pectin. *Food Res. Int.*, 2014; 55: 215-221.
4. J. Kang, X. Hua, R. Yang, Y. Chen, H. Yang, Characterization of natural low-methoxyl pectin from sunflower head extracted by sodium citrate and purified by ultrafiltration. *Food Chem.*, 2015; 180: 98-105.
5. S. Kauser, A. Saeed, M. Iqbal, Comparative studies on conventional (water-hot acid) and non-conventional (ultrasonication) procedures for extraction and chemical characterization of pectin from peel waste of mango cultivar. *Chaunsa. Pak. J. Bot.*, 2015, 47: 1527-1533.
6. C.H. Geerkens, A. Nagel, K. Meike Just, P. Miller-Rostek, D.R. Kammerer, R.M. Schweiggert, R. Carle, Mango

- pectin quality as influenced by cultivar, ripeness, peel particle size, blanching, drying, and irradiation. *Food Hydrocoll.*, 2015; 51: 241-251.
7. D. Gopi, K. Kanimozhi, N. Bhuvaneshwari, J. Indira, L. Kavitha, Novel banana peel pectin mediated green route for the synthesis of hydroxyapatite nanoparticles and their spectral characterization. *Spectrochim. Acta A.*, 2014; 118: 589-597.
 8. A. Ninčević Grassino, J. Halambek, S. Djaković, S. Rimac Brnčić, M. Dent, Z. Grabarić, Utilization of tomato peel waste from canning factory as a potential source for pectin production and application as tin corrosion inhibitor, *Food Hydrocoll.*, 2016a; 52: 265-274.
 9. A. Ninčević Grassino, M. Brnčić, D. Vikić-Topić, S. Roca, M. Dent, S. Rimac Brnčić, Ultrasound Assisted Extraction and Characterization of Pectin from Tomato Waste, *Food Chem.*, 2016b; 198: 93-100.
 10. L.P.T. Quoc, Effect of the assistance of microwave and oxalic acid on the extraction yield of pectin from pomelo (*Citrus maxima*) peel, *Bulg. J. Agric. Sci.*, 2019; 25: 191-195.
 11. Z. Kost'alova, Z. Hromadkova, Structural characterisation of polysaccharides from roasted hazelnut skins, *Food Chem.*, 2019; 286:179-184.
 12. S. Christiaens, D. Uwibambe, M. Uyttbroeck, B. Van Droogenbroeck, A.M. Van Loey, M.E. Hendrickx, Pectin characterisation in vegetable waste streams: A starting point for waste valorisation in the food industry. *LWT - Food Sci. Tech.*, 2015; 61: 275-282.
 13. J. Müller-Maatsch, M. Bencivenni, A. Caligiani, T. Tedesci, G. Bruggeman, M. Bosch, J. Petrusan, et al. Pectin component and composition from different food waste streams, *Food Chem.*, 2016; 201: 37-45.
 14. M. Marić, A. Ninčević Grassino, Z. Zhu, F.J. Barba, M. Brnčić, S. Rimac Brnčić, An overview of the traditional and innovative approaches for pectin extraction from plant food wastes and by-products: Ultrasound-, microwave-, and enzyme-assisted extraction, *Trends Food Sci. Technol.*, 2018; 76: 28-37.
 15. X. Guo, H. D. Han, H. Xi, L. Rao, X. Liao, X. Hu, J. Wu, Extraction of pectin from navel orange peel assisted by ultra-high pressure, microwave or traditional heating: A comparison, *Carbohydr. Polym.*, 2012; 88: 441-448.
 16. M. Naghshineh, K. Olsen, C.A. Georgiou, Sustainable production of pectin from lime peel by high hydrostatic pressure treatment, *Food Chem.*, 2013; 136: 472-478.
 17. F. Xie, W. Zhang, X. Lan, S. Gong, J. Wu, Z. Wang, Effects of high hydrostatic pressure and high pressure homogenization processing on characteristics of potato peel waste pectin, *Carbohydr. Polym.*, 2018; 196: 474-482.
 18. X. Peng, T. Mu, M. Zhang, H. Sun, J. Chen, M. Yu, Effects of pH and high hydrostatic pressure on the structural and rheological properties of sugar beet pectin, *Food Chem.*, (2016) 60: 161-169.
 19. C.Y. Wang, H.W. Huang, C.P. Hsu, B.B. Yang, Recent Advances in Food Processing Using High Hydrostatic Pressure Technology, *Crit. Rev. Food Sci. Nut.*, (2016) 56: 527-540.
 20. D. Mohnen, Pectin structure and biosynthesis, *Curr. Opin. Plant Biol.*, 2008; 11: 266-277.
 21. K.H. Caffall, D. Mohnen, The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carb. Res.*, 2009; 344: 1879-1900.
 22. B.M. Yapo, Pectic substances: From simple pectic polysaccharides to complex pectins-A new hypothetical model. *Carbohydr. Polym.*, 2010; 86: 373-385.
 23. B.M. Yapo, Improvement of compositional quality of monocot pectin extracts contaminated with glucuronic acid-containing components using a step-wise purification procedure, *Food Bioprod. Process.*, 2011; 88: 283-290.
 24. P. Putnik, D. Bursać Kovacević, A. Režek Jambrak, F.J. Barba, G. Cravotto, A. Binello, J. M. Lorenzo, et al.,

- Innovative “green” and novel strategies for the extraction of bioactive added value compounds from citrus wastes - a review. *Molecules*, 2017; 22: 1-25.
25. A. Kumar, G.S. Chauhan, Extraction and characterization of pectin from apple pomace and its evaluation as lipase (steapsin) inhibitor, *Carbohydr. Polym.*, 2010; 82: 454-459.
26. H.M. Chen, X. Fu, Z.G. Luo, Properties and extraction of pectin-enriched materials from sugar beet pulp by ultrasonic-assisted treatment combined with subcritical water. *Food Chem.*, 2015; 168: 302-310.
27. F.R. Buss Marena, F. Mattioda, I. Mottin Demiate, A. de Francisco, C.L. de Oliveira Petkowicz, M.H. Giovanetti Canteri, R. Dias de Mello Castanho Ambroni, Advances in Studies Using Vegetable Wastes to Obtain Pectic Substances: A Review. *J. Polym. Environ.*, 2019; 27: 549-560.
28. B.C.A. Gómez, B. Gullón, R. Yáñez, H. Schols, J.L. Alonso, Prebiotic Potential of Pectins and Pectic Oligosaccharides Derived from Lemon Peel Wastes and Sugar Beet Pulp: A comparative Evaluation. *J. Funct. Foods.*, 2016; 20: 108-121.
29. T. Nisar, X. Yang, A. Alim, M. Iqbal, Z.C. Wang, Y.R. Guo, Physicochemical responses and microbiological changes of bream (*Megalobrama ambycephala*) to pectin based coatings enriched with clove essential oil during refrigeration. *Int. J. Biol. Macromol.*, 2019; 124: 1156-1166.
30. P.Q.M. Bezerra, M.F.R. de Matos, I.G. Ramos, K.T. Magalhaes-Guedes, J.I. Druzian, J.A.V. Costa, I.L. Nunes, Innovative functional nanodispersion: Combination of carotenoid from *Spirulina* and yellow passion fruit albedo. *Food Chem.*, 2019; 285: 397-405.
31. Regulation (EU) No.231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council 1, European Commission 9 March 2012.
32. Regulation (EU) No. 68/2013 on the Catalogue of feed materials 1, European Commission 16 January 2013.
33. S. Ranganna, Handbook of analysis and quality control for fruits and vegetable, (2nd ed.), New Delhi: Mc Graw Hill Publishers, (Chapter 2), 1995.
34. A. Ninčević Grassino, F. J. Barba, M. Brnčić, J. M. Lorenzo, L. Lucini, S. Rimac Brnčić, Analytical tools used for the identification and quantification of pectin extracted from plant food matrices, wastes and by-products: A review, *Food Chem.*, 2018; 266: 47-55.
35. A. Shpigelman, C. Kyomugasho, S. Christiaens, A.M. Van Loey, M.E. Hendrickx, The effect of high pressure homogenization on pectin: Importance of pectin source and pH. *Food Hydrocoll.*, 2015; 43: 189-198.
36. S.I. Martínez-Monteaudo, V.M. Balasubramaniam, Fundamentals and Applications of High-Pressure Processing Technology. In: *High Pressure Processing of Food: Principles, Technology and Applications* (eds. V.M. Balasubramaniam, G.V. Barbosa-Cánovas, H.L.M. Lelieveld), Springer Science+Business Media LLC, New York, 2015, pp. 3-17.
37. S.I. Martinez-Monteaudo, M.D.A. Saldana, Chemical reactions in food system at high hydrostatic pressure. *Food Enf. Rev.* 2014; 6: 105-127.
38. O.P. Chauhan, *Non-thermal Processing of Foods*, New York: CRC Press, 2019.
39. T. Koutchma, *Adapting High Hydrostatic Pressure (HHP) for Food Processing Operations*, Amsterdam: Elsevier, 2014.