A Mini Review on Conventional and Alternatives Extraction Methods of Pectin from Watermelon (*Citrullus lanatus*) Rind

Wan Mazlina Md Saad[1]*, Wan Shahriman Yushdie Wan Yusoff[1] and Abdul Fatah Nuruzzaman[2]
[1]Centre of Medical Laboratory Technology, Faculty of Health Sciences, Univeristi Teknologi MARA, UiTM Puncak Alam, 43200 Bandar Puncak Alam, Selangor, Malaysia
[2]Unit Transfusi, Jabatan Patologi, Hospital Ampang, Jalan Mewah Utara, Taman Pandan Mewah, 68000 Ampang Jaya, Selangor, Malaysia
*Corresponding author (e-mail: wanmaz755@uitm.edu.my)

Pectin is a natural polysaccharide and is mainly used in food sector as gelling, thickening, and stabiliser agents and in biomedical applications including in drug delivery, tissue engineering and wound healing applications. The most common raw materials used for pectin extraction are from apple pomace or citrus peels. The conventional pectin extraction method functions through acidified water and this method mainly conducted at low pH with high temperatures for several hours. In creating awareness for a more eco-friendly experiment approach, alternative natural pectin sources and extraction methods are appropriate to be investigated. Thus, the aim of this study is to present various extraction methods used to extract pectin from watermelon rind. In this review, the conventional heating extraction (CHE) and several alternatives' methods including microwave- assisted extraction (MAE), enzyme-assisted extraction (EAE), and ultrasound-assisted extraction (UAE) had been investigated and compared. The highest result produced for pectin extraction from conventional extraction yielded 20.7% of pectin. The highest results from alternatives pectin extraction methods from MAE, EAE and UAE were 25.7%, 34.1% and 19.67%, respectively. The alternative extraction methods showed a higher pectin yield and more quality pectin. This indicates that alternative methods are able to reduce acid solvents usage and more effective experimental time. The alternative methods may also be suggested as other options to conventional method and also eco-friendlier method for pectin recovery. These alternatives pectin extraction methods can further reduce waste disposal problems and create alternatives functional food for human consumption.

Keywords: Pectin; watermelon; extraction methods; conventional; alternatives

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Pectin is one of the valuable polysaccharides compounds which is present in dicotyledonous and non-grass monocotyledonous plants in watermelon rind. Pectin is a family of complex α-d-(1-4) galacturonic acid poly-saccharides residues which are partly esterified with methyl alcohol at the carboxylic acid [1]. This compound is found within the plant cell walls [2] intercellular regions and primary cell wall of higher plants [3]. Pectin is a heteropolysaccharide, which serves as an important by-product that can be extracted from fruit and vegetable waste [4]. Pectin composition and physio-chemical properties depend mostly on the pectin source and also on the extraction conditions used. According to [5], the quantity of pectin, the structure and the chemical composition vary depending on plants types.

In recent years the pectin usage from un-conventional sources; especially from agro industrial waste such as *Citrullus lanatus* rind *(Figure 1)* began to expand. Utilization of agro industrial waste have benefitted major industries including construction, bio-fuels [6] food and pharmaceutical. It is reported that some of the agro-waste product is used as natural carbon sources that can be used in industrial enzyme production [7]. *Citrullus lanatus* or generally known as watermelon belongs to Cucurbitaceae family under the genus Citrullus. There are three major components of watermelon: the rind, flesh and seed. The water-melon rind portion represents nearly one third of the entire fruit mass [8]. The three main watermelon rind constituents include pectin, cellulose and sugars; fructose and glucose [9]. The rind is reported to contain multifunctional compounds with approximately 20% cellulose, 23% hemicelluloses, 10% of lignin and 13% of pectin [10] and 12% silica free minerals [11].

Pectin is a polysaccharide that has high-value functional food ingredient mainly used in food industries as gelling agent and emulsifier in jam and jellies [12]. From medical perspective, there are many studies showed the benefits of pectin to human health. Pectin able to reduce cholesterol level in the blood, stabilise blood pressure, remove heavy metal ions from body, and also able to restore intestine function [13].

Figure 1. *Citrullus lanatus* rind [21].

Figure 2. Pectin obtained from *Citrullus lanatus* rind using microwave assisted extraction [21].

A previous study by [14] reported that pectin can reduce cholesterol levels and low-density lipoprotein (LDL) cholesterol in blood. Furthermore, pectin is proven to exhibit an anti-tumour function due to its immunostimulatory activity [15].

Utilising pectin begins with the process of isolation from the parent plant material. Over the years, conscious of green extraction method increased awareness towards environmental protections has led to green extraction method. Therefore, pectin extraction using various methods has become a significant challenge and is yet to be fully investigated. The implementation of an effective pectin extraction method is important to optimize its extraction production and also enhance the pectin quality. Conventional extraction using acid has been the standard method used in commercial pectin extraction for years [16]. Other extraction methods that have been introduced to increase the efficiency and pectin quality include enzyme-assisted extraction (EAE), microwave-assisted extraction (MAE) [17] and ultrasound-assisted extraction (UAE). There are limited articles that investigate the different methods of pectin extraction that mainly focus on watermelon rind. Thus, the main purpose of this study is to review and compare the conventional and alternatives methods for pectin extraction from watermelon rind. The properties of the pectin produced using different extraction methods were also determined.

Composition of Pectin from Watermelon Rind

Galacturonic Acid (Ga1A) Content

Pectin is an acidic polysaccharide and a polymer of galacturonic acid [18]. According to Food and Agriculture Organization, commercial pectin must have galacturonic acid content of at least 65% [19]. As stated by [20], the content of galacturonic acid plays an important role in determining the properties of pectin solution and influences the functional properties of pectin gel formation.

The higher galacturonic acid content reflects the best quality of pectin [22]. A study [23] testified acid extraction method from watermelon rind resulted in high galacturonic acid (Ga1A) content at average value of 68.6% while enzymes-assisted extraction method for CelluPract, Fibrilase, and Multifect XL produced 47.0, 56.2, and 60.2%, respectively. Another study by [24] reported that GalA content produced by conventional extraction was between 78.3-82.8%, while pectin extracted by microwave-assisted extraction produced lower content of GalA between 74.6-76.1%. [25] and [8], also proved that extraction of pectin using conventional extraction yielded 76.84% and 74.2% of GalA content. A study by [26] showed that conventional extraction using hydrochloric acid and citric acid both produced lower yields, in the range of 50.2%-58.8% of galacturonic acid respectively. The latest studies by [27] and [28] using ultrasound-assisted extraction (UAE) showed lower GalA yield, at 44.41% and 47.41%, respectively. The difference in GalA content is probably due to the difference in methods used for pectin extraction.

Degree of Esterification

Pectin degree of esterification (DE) shows the percentage of esterified carboxyl group with methyl alcohol. The degree of pectin esterification (DE) can be categorised into two classifications which are high methoxyl pectin (HMP) (DE more than 50%) and low methoxyl pectin (LMP) (DE lower than 50) [29] that reflect different characteristics and applications. High methoxyl pectin (HMP) has DE of more than 50% and pectin with DE lower than 50% is classified as low methoxyl pectin (LMP) [29]. The previous study by [30] proved that high cosolute of sucrose that presence with pH lower than 3.5 causes HMP to form gels concentration, while in the presence of calcium ion, LMP forms gels. Table 2 concludes the differences in the degree of esterification for pectin extracted using various methods.

A study by [31] demonstrated that extracted pectin from watermelon rinds using MAE method had a degree of esterification value ranging from 56.86% to 85.76%, and this value exhibited relatively high methoxyl pectin. A study by [26] reported that pectin extracted from watermelon rind using citric and hydrochloric acid resulted in DE with 66.3% and 71.5%, respectively, were high methoxyl (HMP) pectin. However, a study by [23] showed different result where pectin extractions using acid extraction and enzyme-assisted extraction resulted in 46% and 40% which were low methoxyl pectin. The differences in DE could be caused by pectin extraction methods and the ripeness of the fruits [8].

Method of Pectin Extraction from Watermelon Rind

Various pectin extraction methods have already been applied on watermelon rind, and in most cases findings for pectin yield and properties for each method are different but satisfactory. Each method is affected by variables used during the extraction process such as temperature, time, pH, radiation, and solvent used. Novel extraction approaches, such as ultrasound assisted extraction, microwave assisted extraction and enzyme assisted extraction with different mechanisms have been investigated to enhance extraction process. It is important to use an effective method of extraction during pectin production in order to obtain the desired yield of pectin and preserve its properties. Some of the most relevant findings and comparisons of pectin yield between the methods discussed are tabulated in Table 1.

Conventional Extraction

Conventional extraction using acid is the standard method used in commercial pectin extraction for years [16]. In conventional extraction, pectin is extracted using acidified water with concentration range of 0.05-2M for various types of acid such as hydrochloric, sulphuric, phosphoric, and nitric or acetic acid at a temperature between 80 and 100 °C with continuous stirring for 1 hour [32]. A previous study by [23] reported that pectin extracted by acid extraction method using nitric acid from watermelon rind produced the highest pectin yield at 20.7%, while a study by [3] revealed 15.17% average of pectin yield by using organic acid. Studies using organic acid by [24], [25] and [8], demonstrated 17.5%, 19.75% and 19.3% yield of pectin respectively. Nevertheless, a study by [26] that compared organic acid (citric) and mineral acid (hydrochloric) only produced 8.38% and 6.52% of pectin yield. Percentage of pectin produced from the conventional extraction method depends on many elements that include acid type used, solvent concentration, temperature, pH, and solid to solvent ratio. All these variables used in the extraction process may affect the pectin yield properties. Although conventional extraction method is considered low-cost to perform, the biggest drawback of this method is the production of vast quantities of acidic effluent that need further processing before disposal, which raises environmental concerns. Besides, conventional method is considered not appropriate to obtain high-quality of pectin mainly due to effect of acid that causes degradation and depolymerisation of pectin, and also time-consuming compared to novel methods [33].

Table 1. Extraction methods and composition of pectin from watermelon rind

Extraction method	Material used	Pectin yield (%)	Galacturonic acid (%)	Degree of esterification (%)	References
Conventional method	Nitric acid	20.7	68.6	46 (LMP)	[23]
	Citric acid	15.19	-	44.37 (LMP)	[3]
	Sulphuric acid	17.4-17.6	78.3-82.8	38.2-41.2 (LMP)	[24]
	Hydrochloric acid	19.75	76.84	55.25 (HMP)	[25]
	Nitric acid	19.3	74.2±0.6	63.0±0.05 (HMP)	[8]
	Citric acid Hydrochloric acid	8.38±0.43 6.52± 0.15	50.2 ± 11.8 58.8 ± 20.3	66.3 ± 20.1 71.5 ± 22.8 (HMP)	[26]
Microwave-assisted extraction (MAE)	Sulphuric acid	19.1-19.6	74.6-76.1	44.3-48.7 (LMP)	[24]
	Distilled water with different pH	25.79	-	-	[33]
	Sulphuric acid	18	-	-	[21]
	Sulphuric acid	11.25	-	-	[11]
	Acetic acid	3.925 -5.766	-	56.86 -85.76 (HMP)	[30]

Enzymatic-assisted extraction (EAE)	CelluPract	34.1	47.0	38.3 (LMP)	[23]
	Combination of Multifect XL and Fibrilase	31.8.	52.0 - 57.3	40 (LMP)	
	Aspergillus niger	15.26	57.72	61.33(HMP)	[22]
Ultrasound-assisted extraction (UAE)	Distilled water with different pH	15.48	-	-	[34]
	Sulphuric acid	10.6	44.72	44.41 (LMP)	[27]
	Citric acid	19.67	47.41	78.13 (HMP)	[28]

Microwave-Assisted Extraction (MAE) and Solvent Used

The principle of microwave- assisted extraction is basically the employ of microwave energy penetrates molecules of solvent directly to the skin tissues of pectin origin. When penetrating the food matrix and after entering the cellular polar compounds, the micro-wave produces heat. The heat produced will interact with the molecules that cause ionic conduction and rotation of dipoles [35]. The thermal energy in the solvent may induce polar molecules vibration with rapid temperature increased may eventually increases the MAE efficiency [36].

The previous study by [24] demonstrated that pectin extractions from watermelon rind by microwave-assisted extraction (MAE) using acid solvent had yielded 19.1-19.6% of pectin. [33] investigated that using microwave-assisted extraction on optimal conditions, microwave power of 477 W, irradiation time of 128s and pH 1.52 able to produce the highest pectin yield at 25.79%. Furthermore, [11] indicated that extracted pectin from watermelon rind by MAE method, using 0.5 M sulphuric acid solution, 15 min of extraction time, and solid- liquid ratio of 1:08 had a yield of 11.25% pectin (Figure2). The research by [31] showed that MAE using acetic acid in extraction condition 279.3 W of microwave power and 12 min of irradiation time only managed to produce 5.76% of pectin. Extracted pectin from watermelon rind using acetic acid only able to recover very low amount pectin [21]. Despite lower pectin yield compared to acidic method, MAE has a greener extraction approach and a very short extraction time. It is also documented that pectin extraction using MAE is highly efficient method that can be benefitted from low energy consumption, short processing time, easy controllability, low cost and solvent required [37].

A study by [33] explained that the pectin yield increased by raising microwave power, but longer irradiation time may lead to degradation of pectin chain molecules that later may affects pectin extraction rate. From the above elaboration, we may suggest that MAE possesses many advantages and shows potential not only in producing desirable pectin yield, but also in saving time, energy and solvent consumptions as compared to conventional method.

Enzyme-Assisted Extraction (EAE)

Enzymes-assisted extraction (EAE) able to enhance the extraction process by hydrolysing the matrix of the plant cell wall. In this method, the enzyme used will generate enzymatic reactions by disintegrating the cell wall and caused increase cell permeability. Enzyme-assisted extraction method can be considered a greener method and more effective in pectin yield. A study by [38] showed that EAE method depends on the type of enzyme, enzyme concentration, reaction temperature, reaction time, pH value and particle size of plant material. The [23] studied the effectiveness of enzymes assisted extraction at optimum level by using single and combination of enzymes. CelluPract produces the highest pectin yield at 34.1% while combination of Multifect XL and Fibrilase enzymes revealed 31.8% of pectin yield. It was considered the highest pectin yield compared to all methods used in this study.

The study [22] managed to obtain 6.24% pectin yield by using *Aspergillus niger* enzyme alone. The differences in the results may be due to the specificity of the enzymes used. Based on this review, the advantages using EAE is the pectin extracted from this method does not only produce high pectin yield, but also proven to possess functional properties. EAE manifest a decrease in energy consumption, solvent used and no harmful effluent to the environment compared to conventional extraction method that applies mineral acid for extraction.

The major drawbacks in the application of enzyme-assisted extraction are the long duration process and the enzymes used, which are relatively costly to be used on commercial scale [39].

Ultrasound-Assisted Extraction (UAE)

The ultrasound-assisted extraction (UAE) is a method involves the usage of acoustic energy with an extraction solvent to extract target compounds from different plant matrices [40]. To extract the biomolecules from the sample solution, UAE uses ultrasound to penetrate solvents in contact with the solid matrix. [34] investigated that the extraction of pectin from watermelon rind using UAE able to obtain only 15.48% of pectin yield at optimum level: 20 kHz frequency, solid to liquid ratio of 45 g/mL, pH 1.1, 12 minutes of extraction time and at temperature 69°C. Furthermore, [27] reported that pectin extraction using UAE at 24000 Hz frequency and 400 watts (W), with temperature 67 °C and extraction time of 51 minutes produced 10.6% of pectin. Study by [28] also proved that extraction of pectin from watermelon rind with the power of ultrasound at 525 W, frequency at 20 kHz, with temperature 71°C and at 10 minutes produced excellent pectin yield at 19.67%.

Based on the findings from UAE method, the production of pectin from watermelon rinds would be enhanced by increasing the solid liquid ration (SLR) and temperature. However, longer extraction period and high pH would reduce the pectin output. Increase in time caused UAE reaction to reach saturation period, which may be the explanation for the reduction in the yield of pectin [34]. Over the years, UAE usage method in industry especially food processing is an economical alternative compared to conventional extraction [41], mainly due to its method simplicity, less time consuming with higher yield. However, several influencing factors including temperature, noise, and full material contact with ultrasonic transducers that may have impacted on the outputs [42] that may impact the yield production. Recovering of bioactive compounds from complex plant matrix is another challenge in UAE method following a needed constant sufficient power in enabling cavitation production [43].

CONCLUSION

This review paper has summarized the different approaches of pectin extraction from watermelon rind between conventional and alternatives methods. From this review paper, we affirm the fact that alternatives extractions are more effective and efficient in obtaining pectin from watermelon rind including extraction temperatures, extraction time and solid liquid ratio. Alternative methods that consist of MAE, UAE and EAE do not consume time in processing pectin and these methods are greener, effective and efficient compared to conventional method that requires further treatment of acid used and is more time consuming. However, although there are variability and differences from the results on pectin yield and properties from the alternatives methods, the alternative methods used are promising due to functional pectin produced and could be further improvised.

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