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Volarization of valuable compound from watermelon by-product using ultrasound-assisted extraction

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Abstract

The aim of this study was to develop an efficient, reliable, and sustainable technology for the recovery of value-added compounds from by-product, in this case, is watermelon rinds. The properties of the watermelon rinds obtained from innovative ultrasound-assisted extraction (UAE) were evaluated. In regard to this, the pectin content, degree of esterification, and galacturonic acid content of the watermelon rind extracts were determined in order to verify the efficiency of the UAE. Initially, the UAE were conducted using two types of acid: citric and hydrochloric. The highest pectin content was obtained using citric acid. Additional UAE was then performed with citric acid at 50, 60, or 70°C for 10, 20, or 30 mins. Both UAE temperature and time significantly influenced the pectin extracts and galacturonic acid. The best findings for a high galacturonic acid content (47.41%) when the watermelon rinds were extracts at 70°C for 20 mins. According to the findings, the extraction process lasted 10 or 20 mins at all temperatures was mainly high-methoxyl pectin, which can form gels under acidic conditions. This suggests that pectins derived from watermelon rinds using UAE may be especially useful as an additive in some confectionery products.

1. Introduction

Watermelon (*Citrullus lanatus*) is categorized as an industrial by-product of fresh-cut fruits usually considered under fully utilized. The re-use of by-products by transforming into more valuable raw material has garnered more attention in sustainability research. Specifically, more reliable and sustainable technologies have been investigated to reduce the impact of by-product on the environment and economy through the enhancement of the by-product's value. One-third of watermelon that called as rind (Kumar, 1985) are commonly regarded as being inedible and so discarded (Al-Sayed and Ahmed, 2013). In fact, the value of watermelon rinds has received increasing attention due to the rich well-spring of functional components such as mineral salts, fat, protein, carotenoid, carbohydrates, vitamins, phytochemicals, cellulose, and citrulline (Quek *et al*., 2007; Mort *et al.*, 2008; Al-Sayed and Ahmed, 2013; Lakshmipathy *et al.*, 2015). It has been reported, one of the major components of watermelon rind is carbohydrate, some in the form of pectin (Al-Sayed and Ahmed, 2013; Prakash Maran *et al.*, 2014). In addition, watermelon rinds can be considered as a low-cost source of materials for functional food ingredients. Particularly, the recovery of pectin from watermelon rinds might give commercial value to a by-product that is normally simply discarded.

In being sustainable of reaping the resource-rich compound value of watermelon rinds, it is very important to choose a particular extraction technique for efficient extraction of value-added compounds. Campbell (2006) compared enzymatic and acid methods to extract pectin from watermelon rind; the highest proportion of the rind's galacturonic acid content extracted was approximately 70%, achieved using the acid technique. Jiang *et al*. (2012) extracted pectin from watermelon rind by conventional heating extraction and microwave-assisted extraction and obtained pectin yields of 17.4% and 19.6%, respectively. Prakash Maran *et al*. (2014) reported that the yield of pectin extracted from watermelon rind using microwave-assisted acid extraction was approximately 26%. Hartati *et al*. (2014) recovered 11% of precipitated alcohol material (pectin) conducted using microwave-assisted extraction with 0.5 M sulfuric acid solution without further characterization of pectin. Petkowicz *et al*. (2017) obtained pectin from

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both fresh and lyophilized watermelon rinds using acid extraction. They found that the fresh watermelon rind gave higher yields (19.3%) than the lyophilized rinds (14.2%).

Strategically, the main focus for obtaining valuable compound from natural sources are green sustainability, cost- and energy-effective. The selection of the extraction technique is very important for the high recovery efficacy of bioactive compounds. It is not only obtaining highest extraction yield, but it is also necessary to use lowest non-renewable resources along with low energy consumption (Chemat *et al.*, 2017; Fierascu *et al.*, 2020). Ultrasound-assisted extraction (UAE) represents a competitive approach to the other techniques such as solvent extraction with stirring and heating, heat reflux, and hydrodistillation (Chen *et al*., 2015; Chemat *et al.*, 2017) in the production and development of nutraceuticals functional food products due to the excellent features. UAE is considered a clean method and minimized environmental impact in terms of energy and time by using a small amount of solvents and lower the quantity of carbon dioxide rejected in the atmosphere at short treatment time, respectively (Chemat *et al.*, 2017; Maric *et al.*, 2018; Zhang *et al*., 2018). Ultrasound is capable of inducing chemical and physical changes in food components. Increased emulsifying capacity, the release and diffusion of cell material, and enhanced foaming are some of the improvements to foods that can be achieved using sonication (Žlabur *et al.*, 2015). Maran and co-workers (Maran and Priya, 2015; Moorthy *et al.*, 2017) reported an efficient and economic technique for separating polyphenols and pectin using UAE.

Pectin can be categorized as low-methoxyl pectin and high-methoxyl pectin. The degree of esterification (DE) of low-methoxyl pectin is less than 50% and it can gel in the absence of sugar if calcium ions are present; it is usually used for low-sugar jellies (Fraeye *et al.*, 2010; Pasandide *et al.*, 2017). The DE of high-methoxyl pectin is over 50% can form a gel in the presence of high concentrations of sugar (55° Brix) and pH maintained lower than 3.5; it is usually used in the making of jam (Sengar *et al.*, 2020). The source of pectin, developmental stages, and extraction conditions will significantly influence the composition and structure of the pectin (Petkowicz *et al*., 2017). Therefore, the present work aims to volarize watermelon rind extracts with high-value-added using efficient UAE technique. The potential utilization of watermelon waste has been assessed by physicochemical characterization.

2. Materials and methods

2.1 Materials and chemicals

The watermelons were obtained from Klang, Selangor. Phenolphthalein, hydrochloric acid (HCl), citric acid, sodium tetraborate, and sodium hydroxide (NaOH) were purchased from Fischer Scientific. Standard galacturonic acid and 3-phenyl phenol were purchased from Sigma Aldrich. All the chemicals and reagents were of analytical grade.

2.2 Sample preparation

The watermelon rinds were prepared as described by Jafari *et al*. (2017) with slight modifications. The watermelon rinds were separated from the skin and flesh, washed with distilled water, and cut into chunks 4 cm x 2 cm x 2 cm. The rinds were then dried in an oven (ULM 500, Memmert, Germany) at 55°C for 16 hrs. The dried rinds were ground into powder using a blender (MX-GM1011, Panasonic, Malaysia) and passed through a sieve of 25 mesh size and stored in sealable bags for later analysis.

2.3 Ultrasound-assisted extraction of pectin

The ultrasonic extraction of pectin was carried out as described by Moorthy *et al*. (2017) with slight modifications. An ultrasonic probe (Q700, QSonica, USA) with a 16 mm diameter cylindrical titanium alloy tip was used with a sample/solvent ratio of 1:25 (g/mL) and pH 1.5 in a 250 mL beaker for periods of 10, 20, or 30 mins, at temperatures of 50, 60, or 70°C, and citric acid as the solvent. For hydrochloric as a solvent, the UAE of pectin was only conducted at 60°C for 20 min. The power of the ultrasound was set at 525 W and the frequency at 20 kHz.

The filtration and purification of pectin were carried out as described by Prakash Maran *et al.* (2014) with slight modifications. After extraction, the mixture was passed through filter paper and allowed to cool to room temperature. The filtrate was centrifuged using a micro refrigerated centrifuge (Model 3740, Kubota, Japan) at 1298 x *g* (5500 rpm) and 20°C for 15 mins and the supernatant was precipitated with an equal volume of 95% (v/v) ethanol. The coagulated pectin mass was washed with 95% (v/v) ethanol three times to remove the mono- and disaccharides (Minkov *et al*., 1996). The precipitate was collected and dried in an oven (ULM 500, Memmert, Germany) at 55°C until a constant weight was achieved. Pectin yield (%) was calculated using the following formula:

$$Yield\ (\%) = \frac{Mass\ of\ Pectin\ Obtained\ (g)}{Mass\ of\ Dried\ Watermelon\ Rind\ (g)} x\ 100\%$$

2.4 Degree of esterification

The DE was determined according to the method set out by Bochek *et al*. (2001). A dried sample of pectin (50 mg) was solubilized in 100 mL of water with 2 mL of ethanol until it was completely dissolved. Five drops of phenolphthalein were added using a dropper. The sample was then titrated with 0.05 mol/L sodium hydroxide and the result was recorded as an initial titer. After that, 10 mL of 0.5 mol/L sodium hydroxide was added, and the mixture was shaken vigorously and allowed to stand for 15 mins. About 10 mL of 0.5 mol/L hydrochloric acid was added and the sample was shaken until the pink colour of the solution disappeared. Then, another five drops of phenolphthalein were added and the solution was titrated with 0.5 mol/L sodium hydroxide to faint a pink colour that persisted after vigorous shaking (end-point). The volume of titration was recorded as a final titer. DE was then calculated using the following formula:

$$DE\ (\%) = \frac{Final\ Titer}{Initial\ Titer + Final\ Titer} x\ 100\%$$

2.5 Galacturonic acid content

The galacturonic acid content of the sample was determined using meta-hydroxy diphenyl reagent according to the method set out by Blumenkrantz and Asboe Hansen (1973). Sulfuric acid (6 mL) containing 0.0125 M sodium tetraborate was added to tubes containing 1 mL of sample (200 µg/mL). The tubes were cooled in an ice-water bath. The mixture was agitated with a vortex mixer, boiled for 5 mins and cooled in an ice-water bath for the second time. About 20 µL of meta-hydroxy diphenyl reagent was added and the tube was shaken for 5 mins. Finally, the absorbance of samples at 520 nm was read with D-galacturonic acid as a standard using a spectrophotometer (UVmini-1240, Shimadzu, Japan). The standard curve was obtained from standard galacturonic acid standard solutions of different concentrations (0–250 µg/mL). The concentration of each sample was obtained from the standard curve.

2.6 Statistical analysis

All experiments were performed in triplicate. All the data were subjected to two-way analysis of variance (ANOVA) using Minitab version 17 statistical package (Minitab Inc., State College, PA, USA) with a significant difference at $p < 0.05$.

3. Results and discussion

3.1 Efficiency of UAE process with different acids

Dilute mineral acids such as hydrochloric, phosphoric, and sulfuric malic acids are commonly used in pectin extraction (Wang *et al.*, 2015; Pereira *et al.*, 2016). It has been extensively used to extract pectin from crop sources due to its ability to generate pectins enriched in homogalacturonic blocks as a result of significant hydrolysis of neutral sugar-containing rhamnogalacturonic regions at low pH and high temperature (Yapo, 2007; Wang *et al.*, 2015; Hosseini *et al.*, 2019). For instance, nitric acid (HNO_3) has been used commercially to extract pectin at pH values of approximately 2 (May, 1990; Devi *et al.*, 2014). However, the main limitations for the extraction using mineral acids is requiring special treatments to remove undesirable compounds, hence, the final product can receive the GRAS (generally recognized as safe) status (Yapo, 2009). Organic acid such as citric acid has been reported to be similar to those obtained with hydrochloric acid for pectin extraction from apple pomace, cocoa husks, and passion fruit peel. The type of acid significantly affected the pectin yield (Table 1). The pectin yield extracted with citric acid was more than four times higher (at 13.31%) than the yield obtained using HCl (at 3.32%). This is in a good agreement with Yang *et al*. (2018), who found the highest pectin yield was obtained with citric acid, in a comparison of several acids, including HCl. A similar observation was confirmed for the extraction of pectin from apple pomace; Wosiacki *et al*. (2005) and Kumar and Chauhan (2010) reported higher yields of pectin when using citric acid. Chan and Choo (2013) found that the extraction of pectin from cocoa pod husks using citric acid was comparable with the HCl. In addition, Kermani *et al*. (2014) reported that citric acid extracts more pectin from mango peel than mineral acids such as HCl and sulfuric acid at the same condition. According to Kermani *et al*. (2014), the highest recovery of pectin using citric acid was related to the utilization of a chelator under acidic condition compared to the lacking chelating properties of mineral acid (HCl) due to the additional extraction activity of the chelator on the chelator-solubilised pectin fraction. Furthermore, citric acid has a low hydrolyzing capacity and the minor proton catalyzed depolymerization of polysaccharides (Maric *et al.*, 2018; Mzoughi *et al.*, 2018).

Table 1. Yield and galacturonic acid (GA) content of pectin fractions obtained from watermelon rinds at 60˚C for 20 mins of sonication time with two types of acid

Acid type	Pectin Yield (%)	Galacturonic acid (%)
HCl	3.32±0.30[a]	40.50±1.49[a]
Citric	13.31±1.51[b]	30.74±1.92[b]

Values with different letter superscript within the row are significantly different at $p < 0.05$.

Galacatutonic acid (GA) is a dominant feature of pectin in which varying proportions of the acid groups are methyl esterified (Daas *et al*., 2000). The GA content

of the pectin extracted using citric acid was slightly difference (30.74%) than that using HCl (40.50%). Kumar and Chauhan (2010) reported that GA content was obtained in the pectin extracts from apple pomace using citric acid was comparable with HCl. Again, the chelating properties of citric acid, which resulted in pectin richer in neutral sugars and other components, including proteins and phenolics make a similar tendency with the extraction of pectin using mineral acids (Kermani *et al.*, 2014). Hence, the use of citric acid will increase the pectin yield, but, conversely, lower the GA content in the pectin extracts, due to the presence of residual citric acid (Kermani *et al.*, 2014). Furthermore, citric acid is as a natural food additive and it is more attractive than commonly used mineral acids such as HCl and sulphuric acid for the extraction of pectins (Pinheiro *et al.*, 2008). As well as an economic and environmental point of view, citric acid was chosen for further analysis in order to ameliorate the yield and possibly quality characteristics of pectin extraction from watermelon rind under sustainable environment.

3.2 Extraction of pectin using UAE and citric acid under different conditions

Temperature and time need to be taken into consideration in order to achieve the target results in the extraction process assisted by ultrasound. A beneficial effect of temperature between 20 and 70°C have been reported by some authors in the case of UAE compared to non-sonicated extractions (Shirsath *et al*., 2012). The pectin yields from lyophilized watermelon rinds are tabulated in Table 2. The yield of pectin extracted from watermelon rind ranges from 2.66% (10 mins, 50°C) to 19.67% (10 mins, 70°C) (Figure 1). A similar observation reported by Petkowicz *et al*. (2017) obtained the highest yield of 19.3% for fresh watermelon rinds using a conventional acid extraction method. The findings showed the pectin yield increases with the temperature increased from 50 to 70°C at a fixed time of 10 mins. According to Xu *et al*. (2014), a higher extraction temperature may increase the solubility of pectin, which can enhance the yield. This is due to the increasing number of cavitation bubbles and a larger solid-solvent contact area, hence, improve the solvent diffusivity with consequent enhancement of desorption and solubility of the pectin (Chemat *et al.*, 2017). Overall, the increase in temperature from 50°C to 70°C resulted in an increase in yield except when the time was fixed at 30 mins. This decrease in yield may be due to the prolonged and excessive exposure of the sample to ultrasonic waves which may result in thermal degradation of the pectin. The lower yield at high temperature may be attributed to the thermal degradation of pectin at this relatively high temperature and also a decrease in ultrasonic power output under the influence of temperature (Xu *et al.*, 2014).

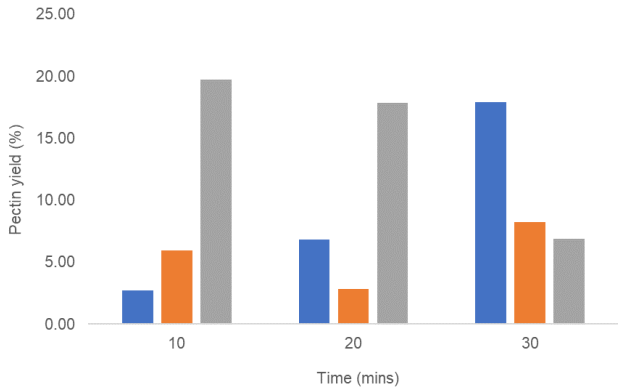

Figure 1. Evolution of the pectin yield as a function of the time and temperature (■) 50°C; (■) 60°C; (■) 70°C.

The pectin yield showed a significant increase ($p < 0.05$), from 2.66% to 17.87%, when the duration of ultrasound exposure increased from 10 mins to 30 mins at a fixed temperature of 50°C. Similarly, the pectin yield also significantly increased with exposure time at a fixed temperature of 60°C. Moorthy *et al*. (2015) explained that ultrasonic waves cause the collapse of micro-bubbles near surfaces, disrupting the cell wall,

Table 2. The pectin yield, the degree of esterification, and galacturonic acid (GA) content of pectin fractions obtained from watermelon rinds at various times and temperatures, with citric acid.

Temperature (°C)	Time (min)	Pectin yield (%)	Degree of esterification (%)	Galacturonic acid (%)
50	10	2.66±0.11^{e}	78.13±0.51^{a}	32.35±0.52^{c}
	20	6.77±0.52^{cd}	77.05±0.65^{a}	34.04±1.46^{bc}
	30	17.87±0.25^{b}	41.02±0.74^{d}	39.78±1.98^{b}
60	10	5.92±0.17^{d}	65.65±3.13^{b}	33.73±0.77^{c}
	20	2.79±0.57^{e}	73.61±2.53^{a}	36.95±0.49^{bc}
	30	8.19±1.16^{c}	51.84±1.63^{c}	47.09±1.74^{a}
70	10	19.67±0.24^{a}	66.11±0.79^{b}	34.84±1.56^{bc}
	20	17.79±0.14^{b}	42.81±1.12^{d}	47.41±2.71^{a}
	30	6.83±1.01^{cd}	45.93±3.57^{d}	34.8±2.87^{bc}

Each value is expressed as mean±standard deviation (n = 3). Different superscripts in the same row indicate significant differences ($p < 0.05$) according to the Tukey test.

which enhances the penetration of solvent into the plant matrix, and so, up to a point, a longer period of action of the ultrasound will improve the performance of the extraction process. However, the yield of pectin decreased from 19.67% to 6.83% ($p < 0.05$) as the time increased from 10 mins to 30 mins when the temperature was fixed at 70°C. This decrease in yield may be due to which may result in thermal degradation of the pectin. In principle, both viscosity and surface tension will decrease with the increase of temperature (Chemat *et al.*, 2017). According to Santos *et al*. (2009), a rise in vapour pressure causes more solvent vapours to enter the bubble cavity and numerous cavitation bubbles, hence, will collapse less violently and reduce sonication effects. In the present study, an extraction time of 10 mins combined with a temperature of 70°C gave a good yield of pectin from watermelon rind.

3.3 Characterization of pectin extracts obtained using UAE and citric acid under different conditions

As mentioned earlier, the structure and properties of the pectin will vary with the extraction conditions. Herein, the degree of esterification of pectin extracted from watermelon rind ranged from 41.02% to 78.13% (Figure 2). The highest degree of esterification was obtained at the lowest extraction time, 10 mins, and the lowest extraction temperature, 50°C. This is comparable with the report by Minjares-Fuentes *et al*. (2014). Pasandide *et al*. (2017) found that harsh conditions such as high temperatures and long extraction periods will reduce the degree of esterification significantly, due to increasing de-esterification of polygalacturonic chains. Generally, the pectin extracted from watermelon rind at less harsh conditions (10 mins at 50°C, 60°C or 70°C, or 20 mins at 50°C or 60°C) exhibited a high degree of esterification, of over 50%, and so that product can be classified as high-methoxyl pectin (HMP). HMP forms gels under acidic conditions in the presence of high sugar content (May, 1990). HMP is used as a food additive to improve the structure, bite and bake-stability of acidic jams, jellies and confectionery with high sugar content; it is also used to enhance the volume, freeze-thaw stability, moisture retention and softness of bread and frozen dough (Willats *et al*., 2006).

When the extraction time increases beyond 20 mins (with the exception of pectin extracted at 60°C for 30 mins), the degree of esterification of pectin was below 50%, and so the product would be considered low-methoxyl pectin (LMP). The significant decrease in the degree of esterification at long extraction times and high temperatures might be caused by the de-esterification of galacturonic acid chains (Masmoudi *et al.*, 2012). This is because pectin is composed of α-(1-4)-linked units of galacturonic acid or methyl ester. The glycosidic bond is a type of ether bond which readily undergoes hydrolysis. This finding is consistent with results obtained by Pasandide *et al*. (2017) on the extraction of pectin from *Citrus medica* peel. Low-methoxyl pectin (LMP) can gel in the presence of only small amounts of sugar, which makes it ideal as a food additive in low-calorie jams and jellies.

In terms of galacturonic acid content, commercial pectin must have a galacturonic acid content of at least 65%, according to the Food and Agriculture Organization (Kliemann *et al.*, 2009). The galacturonic acid content of all pectin extracted from watermelon rind was below 65% (Figure 3). However, the pectin extracted from watermelon rind by Petkowicz *et al*. (2017) had a galacturonic acid content of over 65% for both fresh and lyophilized watermelon rind processed using alcohol-insoluble residues (AIR). This difference is likely due to the difference in extraction techniques. Petkowicz *et al*. (2017) centrifuged extracts at 15,400 x *g* for 20 mins and treated the sample with 2 volumes of ethanol, kept it for 16 hrs at 4°C, centrifuged it, and then

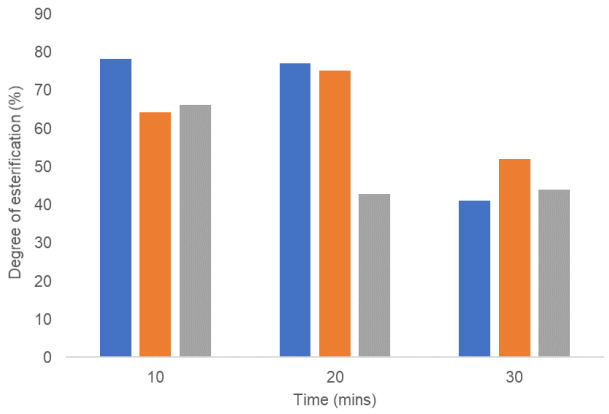

Figure 2. The degree of esterification in pectin extracts as a function of the time and temperature (■) 50°C; (■) 60°C; (■) 70°C.

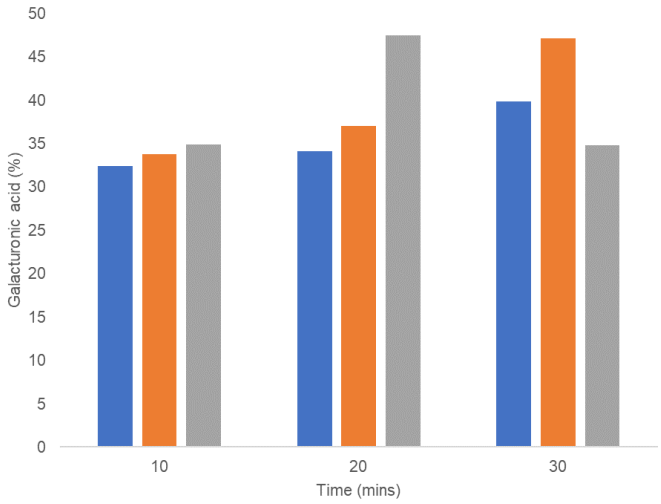

Figure 3. Effect of extraction time and temperature on the galacturonic acid content of pectin extracted using citric acid (■) 50°C; (■) 60°C; (■) 70°C.

washed it three times with ethanol before drying it under vacuum. In the present study, similar methods were employed but with less centrifugal force and less time allowed for the precipitation of pectin, which may have contributed to the pectin obtained being less pure. In general, though, there was no significant difference in galacturonic acid content under the different extraction times and temperatures used in this study.

4. Conclusion

In the present study, the use of an environmental friendly ultrasound-assisted extraction of pectin as high added value compound from watermelon rinds by-product was investigated. The pectin recovery using UAE was analyzed in terms of the degree of esterification and galacturonic acid content. The highest pectin recovery rate was 19.67%, obtained using ultrasound, citric acid, 70°C and an extraction time of 10 mins. The pectin extracted under these extraction conditions was mainly high-methoxylated homogalacturonan with a low degree of acetylation. Nonetheless, the galacturonic acid content of the pectin extracted from the watermelon rind was less than the recommended value of 65%. With regard to the findings obtained, the extraction time can be decreased remarkably using UAE and save energy.

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