Microwave-assisted extraction of pectin from pineapple peel

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Abstract
Pineapple peel has the potential to become one of the sources for pectin production due to the high content of pectin in its dietary fibre composition. Pectin is used as food thickener, emulsifier, stabiliser and gelling agent in food industry. The conventional extraction process with long operating hours at high temperature has been identified to cause thermal degradation of pectin molecules. Microwave technology application in pectin extraction has shown high potential to expedite the extraction process and produce higher yield. Therefore, this research was intended to investigate the effect of various factors (irradiation time, pH, temperature, microwave power and solid-to-solvent (S/S) ratio) of microwave-assisted extraction (MAE) on the pineapple peel pectin (PPP) yield. Pectin extracted was then analysed for its dry weight yield percentage and degree of esterification (DE). It was shown that the pectin yield was significantly affected by pH, S/S ratio and microwave power. From this study, no significant effect of irradiation time was observed from 2.5 min until 20 min to the yield of pectin, thus longer time of extraction is not necessary in MAE. The highest yield of PPP obtained was in the range of 2.27 to 2.79% w/w at pH 2.0 and S/S ratio of 1:20. The result of DE (63.93 ± 0.30%) indicated that PPP is categorized in high methoxyl pectin (HMP) pectin type because the DE values were greater than 50%. This study showed that MAE is highly potential for extraction of high yield of PPP.

Keywords: Microwave-assisted, Pineapple peel, Pectin, Extraction

Pineapple is one of the tropical fruits that can be considered as the most important in the world market and becomes the top three most preferable fruit juices after orange and apple (Upadhyay et al., 2010). Pineapple can be eaten in many ways, either raw, cooked in a meal or processed into jams or juices. The exportation of pineapple from Malaysia mainly involves fresh fruit or canned products. Based on the current statistics from Food and Agriculture Organization (FAO), Malaysia is ranked 8th in Asia after the Philipppines, Thailand, India, Indonesia, China, Vietnam and Taiwan as the largest pineapple producer (FAO, 2017). The pineapple industry shows exportation of canned pineapple increased enormously with 8,479.90 tons metric per year in 2014 to 8,853.37 tons metric per year in 2015 (LPNM, 2016). Demand of pineapple worldwide also has shown a good growth of production from 2013 to 2014 with 24.5 million tons annually to 25.4 million tons annually of production (FAO, 2017).

Activity from pineapple industry has led to the huge waste production. Wastes from pineapple usually accounted for 50% (w/w) from the whole fruit mass, by which 29-40% (w/w) comes from the peel, 9-10% (w/w) from the core, 2-5% (w/w) from the stem and 2-4% (w/w) from the crown (Ketnawa et al., 2012). According to Ketnawa et al. (2012), other than bromelain extraction, the peel could be utilized for further industrial application of its fiber. Pineapple peel has been said to be an excellent potential in food application due to its high composition in dietary fiber which is 42.2% (w/w) from the content, to produce pectin (Huang et al., 2011). The fiber-rich fractions are said to exhibit great water holding capacity, oil holding capacity, cation-exchange capacities and swelling properties that can modify the food texture, stabilize foods with high percentage of fat, and destabilize, entrap, and disintegrate the micelles formed by emulsion of lipid (Huang et al., 2011). Ukiwe and Ainnor (2011), Karim et al. (2014) and Sun et al. (2014) had earlier done several studies on pectin extraction from pineapple peel.

Pectin is a complex polysaccharides consisting of D-galacturonic acid monomers connected with glycosidic links α(1-4) (Schols and Voragen, 1996). The galacturonic acid (GalA) or anhydruoronic acid (AUA) has been suggested to be not less than 65% that is specified as pectin (FAO, 2007). Based on the source of the raw material and extraction method, there are two main groups of pectin which are high methoxyl pectin (HMP) with degree of esterification < 50% and low methoxyl pectin (LMP) with degree of esterification < 50% (Thakur et al., 1997; Wang et al., 2016). Commercialized pectin is usually produced after juice extraction of either citrus peel or apple pomace. According to May (1990), limited sources for potential food pectin is known due to its standard requirement to contain at least 65% galacturonic acid of substance. Pectin has been an important food additive in food industry and its demand in world market currently has reached approximately 45,000 tons annually with 4-5% growing per annum (Raj et al., 2017). In recent days, many studies have been done to utilize food wastes as an alternative resources for pectin production which are from mango peels (Rehman et al., 2004; Berardini et al., 2005), passion fruit peels (Pinheiro et al., 2005), dragon fruit peels (Ismail et al., 2012; Zaidel et al., 2017), sweet potato peels (Zaidel et al., 2015, Hamidon and Zaidel, 2017; Hamidon et al., 2020) and chempedak and jackfruit fruit rind (Leong et al., 2016).
Most often pectin are utilized as food thickener, emulsifier, stabiliser and gelling agent in the food industry (May, 1990). It is usually added in fruit jams, confectionary jellies, fruit juices and dairy products. A study by Thakur et al., (1997) found that the addition of pectin polysaccharide into foods and beverages helps to improve the mouthfeel and increase the satiety, thus can reduce the consumption of foods and beverages. It has also been reported in the pharmaceutical industry to lower cholesterol level in blood, treat diarrhoeal disease and duodenal ulcers extensively. Lead and mercury which are toxic metals can also be effectively eliminated from the digestive and respiratory system. The application of pectin in tablet formulations can act as a binding agent and delay the delivery of drug (Sriamornsak, 2003).

The traditional extraction method can be time consuming that takes around 6 to 10 hours in soxhlet method and 60 to 120 minutes in hot dilute acid extraction. These methods have less efficiency and produce limited yield of pectin. Pectin degradation could occur due to excessive time of extraction (Wang et al., 2016). Therefore, microwave-assisted extraction (MAE) and ultrasound-assisted extraction (UAE) as new technologies were introduced earlier in pectin extraction to increase pectin quality and yield (Maran et al., 2014; Wang et al., 2016). The MAE technique is a powerful technique and has potential to replace the existing conventional method since it has the ability to extract samples with shorter time of extraction, reduce consumption of solvent and higher rate of extraction with lower cost equipment due to its localized heat mechanism (Mandal et al., 2007; Maran et al., 2013). MAE also offers protection to thermolabile constituents and can extract the targeted bioactive compound from raw materials efficiently (Mandal et al. 2007). Extracting dragon fruit peel pectin using UAE however requires significant longer time of extraction compared to MAE and has less pectin recovery (Rahmati et al., 2015; Chua et al., 2018). One of the shortcomings of MAE is the loss of active compounds due to thermal degradation from the utilization of high microwave irradiation. However, this problem can be prevented with intermittent microwave extraction. Intermittent extractions can keep out samples from being overheated by balancing the heat and mass transfer besides enhancing the efficacy of extraction (Swamy and Muthukumarappan, 2017).

Hence, this research was intended to study the effect of different factors including irradiation time, pH, temperature, solid-to-solvent (S/S) ratio and microwave power of the microwave-assisted extraction (MAE) process on pineapple peel for pectin production. The classification of pectin produced was identified by the degree of esterification (DE).

**EXPERIMENTAL**

**Materials**
Analitical grade of hydrochloric acid (HCl) (37%), ethanol (96%), petroleum ether, sodium chloride (NaCl), sodium hydroxide (NaOH), boric acid, methyl red indictor, bromocresol green and phenol red indicator were purchased from QrëC, Malaysia and sulphuric acid (H₂SO₄) (98%) was purchased from Merck, US. Commercial pectin (M. W. 30000 – 100000, Degree of Esterification 63-66%) from R&M Chemicals. Mes-Tris buffer was purchased from Sigma-Aldrich, US. Aspergillus niger heat stable α-amylase and amyloglucosidase (30-60 units per mg protein) and protease were purchased from Sigma-Aldrich, US.

**Preparation of Samples**

Sample of pineapple (*Ananas comosus*) peels were collected from the waste product of Lee Pineapple Co. Pt located Skudai, Johor for this research purpose.

The pineapple peels were cut with sharp knife into 1 cm thick with approximately 2.5 cm² size as shown in Figure 1. The peels were washed by using potable water to remove the remaining pulp deposited and dried at 55-60 °C in conventional oven (Memmert, Germany) until constant sample weight was reached. Dried peels were finely ground and sieved to 0.60 mm size by using food blender (Waring® Commercial blender, USA) and stainless steel sieve, respectively. Samples were kept in polyethylene zipped bags and placed in airtight containers for further use.

**Proximate Composition of Pineapple Peel**

AOAC method was applied to determine the moisture content, ash content and total protein content (AOAC, 2000). Meanwhile, total dietary fibre (TDF) was determined according to the AOAC method of 991.43 (AOAC, 1995). The fat content was carried out by using soxhlet method (Nielsen, 2010). The content of carbohydrate (CHO) was calculated using the method by FAO (2003) as shown in Eq (1).

\[
CHO(\%) = 100 - (\text{ash + protein + fat + fibre}) \quad (1)
\]

**Microwave-Assisted Extraction of Pectin**

Extraction of pineapple peel pectin was done by MAS-II Plus Microwave Synthesis Machine Model (Sineo, China). The machine was connected with the chiller distillatory in order to allow the evaporated solvents to condense and kept in the flask during the extraction process as shown in Figure 2. The mechanism of extraction process involved the absorption of strong microwave energy to heat the solvent in order to permit the partitioning of analytes in the sample matrix into the solvent (Eskilsson and Björklund, 2000). The solvent used was Sulphuric acid at pH 1.83 adjusted by 0.5 N H₂SO₄, 1:30 (w/v) solid-to-solvent ratio. The parameters for microwave-assisted extraction include extraction time (1-20 minute), temperature (70-80 °C), solvent pH (pH 1.0-2.5), solid-to-solvent (S/S) ratio (1:10-1:30 w/v) and microwave power (400-600 W).

![Fig. 2 Schematic diagram of microwave apparatus connected to reflux system (Wang et al., 2007)](image)

Upon completion of extraction, hot mass extract was cooled to room temperature and filtered using clean cheesecloth. The filtrate was chilled at 4 °C and centrifuged at 10 000 rpm for 30 min. Collected supernatant containing pectin was then coagulated with an equal volume of 96% ethanol and stood for 24 h to allow the pectin floatation (Pinheiro et al., 2008). Coagulated pectin was then centrifuged similarly according to previous steps and washed with 70% acidic ethanol (0.5% HCl) once, followed by neutral pH of 70% ethanol and finally with 96% ethanol to withdraw the monosaccharides and disaccharides content (Sayah et al., 2016). The final product was then dispersed in distilled water, oven dried and stored at room temperature (Kalapathy and Proctor, 2001).

**Pineapple Peel Pectin Yield**

Pectin yield extracted from pineapple peel can be calculated by using the Eq(2) below (in the form of dry basis):

\[
Pectin\% = \frac{Pectin\; weight}{Pineapple\; peel\; weight} \times 100 \quad (2)
\]
Table 1 Proximate Composition of Pineapple Peel

| Composition | (g/100g dry peel) |
|------------|------------------|
| Protein    | 3.70 ± 0.31      |
| Fat        | 1.80 ± 0.09      |
| Carbohydrate | 52.33           |
| Ash        | 4.87 ± 0.10      |
| Total Dietary Fiber | 37.30 ± 0.33 |

*the moisture content of pineapple peel is 10.67 ± 0.57% (w/w)

Effect of Irradiation time

Figure 3 shows that the highest pectin yield obtained was 2.08% (w/w) at the time of 2.5 min followed by irradiation time 5.0 min, 20.0 min, 10.0 min and 1.0 min with their respective pectin yields of 1.94, 1.82, 1.69, and 1.50% (w/w). From 1.0 min to 2.5 min of the extraction time, the pectin yield increased rapidly but started to decrease slowly during the extraction time of 5.0 min and onwards. The findings show that irradiation time is not significant between 2.5 min until 20.0 min, thus longer time of extraction is not necessary in MAE.

Factors Influencing Pineapple Peel Pectin Yield in MAE

The protein content was about 3.70% (w/w) from the total composition. Protein is usually present in fruits and vegetables in small amount compared to legumes. Fruits also constitute high water content, containing high sugar and fibres such as pectin but low in protein and fat (Slavin and Lloyd, 2012). According to Huang et al. (2011), the preparation of pineapple peel fibre-rich fraction with removed starch and protein contains higher uronic acid than other pre-treatments. Hence, low protein content and high dietary fibre can be associated with high pectin yield.

The total dietary fibre was found to be 37.30% (w/w) which is close to the finding by Huang et al. (2011) which is 42.20% (w/w). Fibres in fruit peels was observed to be the second highest composition after the carbohydrate. Dietary fibre has been beneficial in improving human digestive system and is added as food ingredient in food products to enhance the quality. According to Huang et al. (2011), the fibre-rich fractions in the pineapple peel are capable to convert the biomass into suitable usage of functional food.

The ash content represent the total mineral content in the biomass which is used to determine total chemical composition of sample. The ash content was found to be 4.87% (w/w) higher from the findings by Huang et al. (2011), Pardo et al. (2014) and Feumba et al. (2016) which are 4.81, 1.50, and 4.39% (w/w), respectively, but lower than the finding by Morais et al. (2017) which is 5.10% (w/w). According to Nielsen (2003), most fresh foods rarely have ash content more than 5%. Fruits and its juices usually contain 0.2-0.6% (w/w) of ash while the value is higher in dried form (2.4-3.5% (w/w)). In the production of pectin, low ash content is necessary in order to obtain good gel formation of foods. The maximum ash content limit for criteria of good quality gel is 10% (w/w) (Ismail et al., 2012).

RESULT AND DISCUSSION

Proximate Composition of Pineapple Peel

The highest composition of pineapple peel was carbohydrate content which was 52.33% (w/w) whilst the least was fat which was 1.80% (w/w) as shown in Table 1. The findings are similar to Huang et al. (2011) and Morais et al. (2017) where carbohydrate was found to be the major composition with and fat showing minor composition in pineapple peel.
time of extraction is much preferred in order to reduce the energy consumption. Fishman et al. (2000) determined that 2.5 min of pectin extraction from orange albedo produced higher value of molar mass of 3.6 x 10^7 and intrinsic viscosity (n) of 10.8 dL/g than the commercial pectin with molar mass of 2.8 x 10^7 and intrinsic viscosity of 5.5 dL/g. The pectin extracted at 2.5 min was found to contain higher percentage of anhydrogalacturonic acid with 96% (w/w) compared to 3.0 min and 4.0 min with 93% (w/w) and 88% (w/w) anhydrogalacturonic acid, respectively.

**Effect of pH**

From previous studies, it has been found that pectin is usually extracted in heated acidified water in the range from pH 1 to pH 3 (Maran et al., 2013; Zaidel et al., 2015; Hamidon and Zaidel, 2017). The effect of pH values of solvent on pectin extracted from pineapple peel assisted by microwave is shown in Figure 4. pH 2.0 showed the highest pectin yield at 2.27% (w/w) followed by pH 2.5, pH 1.0 and pH 1.5 with pectin yield of 1.74, 1.48, and 1.34% (w/w), respectively. The pectin yield obtained at pH 2.0 has significant difference (p<0.05) with pectin yields obtained from pH 1.0, pH 1.5 and pH 2.0. The amount of pectin extracted tends to be decreased after higher pH (pH 2.5) has been introduced. The pectin yield between pH 1.0, pH 1.5 and pH 2.5 show no significant difference among them.

![Effect of pH values towards pineapple peel pectin yield](image)

*Values are mean ± SE of triplicate analysis; Means with different letters denote significant differences among pectin extracts (p<0.05)*

**Fig. 4** Effect of pH values towards pineapple peel pectin yield

In low pH (pH 2.0), high concentration of hydrogen ions has stimulated the insoluble pectin to become soluble pectin and reduced its molecular weight to be easily solubilized into the aqueous solvents. This phenomenon increases the proneness for pectin precipitation (Yeoh et al., 2008; Faravash and Ashtiani, 2007). However, after pH 2.5 has been introduced, the extraction of pectin was reduced to 1.74% (w/w). According to Maran et al. (2013), the release of pectin molecules has been retarded due to the pectin aggregation.

**Effect of solid to liquid ratio**

Figure 5 shows the highest pectin yield at the solid-to-solvent ratio of 1:20 (w/v) with 2.79% (w/w) pectin followed by 1:30 (w/v) and 1:10 (w/v) ratio with 2.08 and 1.16% (w/v) pectin yield, respectively. Pectin yield from all three ratios has a significant difference between them (p<0.05).

The result show similar pattern with the study by Maran et al. (2014) on pectin MAE from Citrullus lanatus fruit rinds, experimenting with a 1:10 to 1:30 (w/v) ratio and obtained highest pectin yield at a ratio of 1:20.3 g/mL. High volume of solvent is not necessary in the MAE of pectin. In MAE, the solvent volume can influence the temperature of extraction. At constant time of extraction, greater volume of solvent decreases the temperature of the extraction system thus affecting its heating rate (Spigno and De Faveri, 2009). Moreover, greater volume of solvent does not only contain greater amount of extract, but also more microwave irradiation is absorbed by the solvent (water) and causes the microwave energy to dissipate. This phenomenon may lead to excessive swelling of the material cell wall, therefore, negatively influences the mass transmission and reduces the pectin yield (Li et al., 2010).

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**Fig. 5 Effect of solid to liquid ratio (g/mL) towards pineapple peel pectin yield**

*Values are mean ± SE of triplicate analysis; Means with different letters denote significant differences among pectin extracts (p<0.05)*

**Fig. 6 Effect of different temperatures (°C) towards the pineapple peel pectin yield**

Similar result of pectin yield reduction in increasing temperature to 90 °C can be observed from both studies by Rehman et al. (2004) and Karim et al. (2014). Their study found that 80 °C of temperature was optimal and produced high pectin amount extracted from the peel of mango and pineapple. According to Karim et al. (2014), temperatures within 80 °C to 85 °C are favorable for pectin hydrolysis. In their research, when higher temperature of more than 90 °C was introduced, the pectin yield was reduced because of the degradation of pectin molecules.

**Effect of Microwave Power**

The experimental result for effect of microwave power on pectin yields extracted from the pineapple peel using MAE is shown in Figure 7. The extracted pectin was the highest at the power of 500 W followed by 600 W and 400 W with pectin yield of 1.79, 1.48 and 1.26% (w/w), respectively. The pectin yield between them all shows a significant difference (p<0.05). Hence, microwave power is one of the factors that affects MAE significantly. The pectin yield was initially 1.26% (w/w) at 400 W microwave power, and increased to 1.79% (w/w) as the...
microwave power rose to 500 W. The pectin decreased dramatically to 1.26% (w/w) after 600 W of microwave power was applied.

The irradiation of microwave energy enhances the solvent effectiveness to penetrate into the cell wall of plant. Rapid energy transfer to the solvent increases the temperature and internal pressure in sudden, causing the acceleration of cell rupture at the surface of sample and allows the hydrolysis and exudation of pectin molecules into the solvent (Yan et al., 2010; Zhang et al., 2008). However, when higher irradiation power of 600 W is introduced, the molecular interaction will be disturbed due to the superfluous energy in solvent and matrix. Thus, resulting in thermal degradation of polysaccharides and hence decreases the pectin produce (Maran et al., 2015; Sarah et al., 2018). Increasing the power of microwave between 400 W and 600 W will increase pectin yield by 17.32-29.61%.

![Image](https://example.com/image.png)

*Fig. 7 Effect of microwave power (W) towards pectin yield extracted from pineapple peel*

Microwave-assisted extraction typically uses microwave power between 300 W to 900 W. According to Tatke and Jaiswal (2011), there was no significant difference in flavonoids yield using 500 W to 1000 W microwave power. A high yield of pectin which is 25.79% (w/w) from Citrus lanatus fruit rinds with optimum condition of 477 W microwave power was achieved by Maran et al. (2014). Meanwhile, Koh et al. (2014) found that 600 W of MAE has the highest pectin yield of 17.63% (w/w) from jackfruit rinds. Considering the quality of pectin itself, lower microwave power of 450 W contains higher amount of galacturonic acid and the most effective and economic extraction.

**Degree of Esterification (DE) of Pineapple Peel Pectin**

Table 2 shows the methoxyl content, anhydouronic acid (AUA) content and DE of pineapple peel pectin. The methoxyl content and AUA content in PPP are 6.15 ± 0.07% and 54.61 ± 0.30%, respectively. The value is less than the amount of methoxyl content and AUA content found in the CP which are 6.67 ± 0.22% and 62.48 ± 1.24%, respectively. The DE in PPP otherwise shows the higher percentage found in the CP which are 6.67 ± 0.22% and 62.48 ± 1.24%, respectively. The value is less than the amount of methoxyl content and AUA content and DE of pineapple peel pectin. The methoxyl content and AUA content and DE of pineapple peel pectin.

|       | PPP          | CP           |
|-------|--------------|--------------|
| Methoxyl content (%) | 6.15 ± 0.07* | 6.67 ± 0.22* |
| AUA content (%)    | 54.61 ± 0.21*| 62.48 ± 1.24*|
| DE (%)            | 63.93 ± 0.30*| 60.41 ± 0.99*|

*Values are mean ± SE of duplicate analysis; Means with same letter denote no statistically significant difference between pectin after applying T-test (p<0.05).*

The result of DE content indicates that both PPP and CP are categorized in high methoxyl pectin (HMP) pectin type because the DE values were greater than 50% (Hamidon et al., 2020). Different types of pectin explain different mechanism in formation of the gel. High sugar concentration and low pH condition are necessary to allow the hydrophobic interaction between methoxyl groups and cross-linking homogalacturan by hydrogen bond to occur for HMP pectin to form gel (Pinheiro et al., 2008).

Meanwhile, the AUA content shows the pectin extracted purity. FAO has suggested that pectin must contain greater than 65% of AUA. However, both PPP and CP obtained less than 65% of AUA content which were 54.61 ± 0.30% and 62.48 ± 1.24%, respectively. According to Ismail et al. (2012), less AUA content might be due to the fact that pectin extracted was not sufficiently pure. This may be because the extract contains protein, sugar and starch. The AUA content of CP may be observed to be higher, but lower in DE percentage indicating that commercial pectin has experienced greater deesterification due to the higher degradation of neutral sugar (Fishman et al., 2008).

**CONCLUSION**

The results show that pectin yield in microwave-assisted extraction (MAE) from pineapple peel was significantly affected by the pH, S/S ratio and microwave power. There was no significant effect in irradiation time ranged between 2.5 min to 20 min to the pectin yield found in this study, thus longer time of extraction is not necessary in MAE. The highest yield of PPP obtained was ranged from 2.27 to 2.79% w/w at pH 2.0 and S/S ratio 1:20. The result of DE (63.93 ± 0.30%) indicates that PPP is categorized in high methoxyl pectin (HMP) pectin type because the DE values were greater than 50%. This study showed that MAE is highly potential for extraction of high yield of PPP. The extraction by using MAE has been successfully proven to provide better quantity and quality of pectin from pineapple peel. The energy released from microwave irradiation was able to extract higher pectin yield in short processing time that protects the thermolabile constituents of pectin molecules.

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