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ULTRASOUND ASSISTED EXTRACTION AND CHARACTERIZATION OF PECTIN FROM RED DRAGON FRUIT (HYLOCEREUS POLYRHIZUS) PEELS

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Abstract. *Pectin has numerous applications in the food industry because of its gelling capability. Dragon fruit peel is an ideal source of pectin since it contains pectin of around ± 10.8% of the weight. In this research, the extraction of pectin from dragon fruit peels used ultrasonic waves. This study was an experimental research with three independent variables that was a type of acid (HCl and CH_3COOH), acid concentration (0.1 N and 0.2 N), and extraction time (30, 60, and 90 minutes). The control sample was pectin extracted by conventional methods, dipped in 0.1 N acids at room temperature for 24 hours. The result showed that the ultrasound-assisted extraction was an effective method to extract pectin from peels of red dragon fruits, whereas the yield was even twice that compared with the control sample. The treatment of 90 minutes of sonication in 0.2 N HCl produced the highest yield (2.71%). The pectin has a yellowish to a white degree of color and a 2.22 g.s/cm viscosity. It has a 902.2 mg equivalent weight, 6.14% methoxyl content, 54.4% galacturonic acid content and 63.8% esterification degree. The value of esterification degree and methoxyl content indicated that pectin from this research is high ester-pectin and low-methoxyl pectin.*

Keywords: acid extraction; dragon fruit peel; pectin; ultrasound

1. Introduction

Dragon fruit is widely grown in Indonesia, especially in East Java, since it is a tropical fruit. The dragon fruit production is increasing yearly, 871,310.65 tonnes in 2017 and 906,511.61 tonnes in 2018 (Banyuwangi Center of Statistic Agency, 2019). The percentage of peel is 30-35% of the total fruit weight, where usually as solid waste from food and beverage industries (Jamilah *et al.*, 2011). The peels are still underutilized, although they are supposed to be rich in pectin.

The pharmaceutical, cosmetics, foods, and beverage industries use pectin as a gelling agent. Pectin is mainly consist of α (1,4) – D galacturonic residue, with various degrees of methyl esterification. The raw materials usually applied in the pectin industries are apple pomade or citrus peels. These fruits cannot grow in tropical countries such as Indonesia. Therefore, it is necessary to develop sources of pectin from local Indonesian fruits, one of which is dragon fruit. According to Woo *et al.* (2010), dragon fruit peels can produce pectin has 4.86 % of yield and 51.44% of esterification degree when extracted in a hot acid solution. In the research of Rahmati *et al.* (2015), dragon fruit peels using microwave-assisted extraction produced 18.35% yield.

The extraction method in pectin production usually uses immersion in an acid solution at a high temperature, and the extraction time is 20 - 360 minutes. The disadvantages of acid are the

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occurrence of pectin degradation that will influence the functional and physicochemical properties (Koubala *et al*., 2008). Besides, this method is thermally unsafe, consumes a longer time, requires a higher solvent, is energy-intensive, and offers low efficiencies (Seixas *et al.*, 2014). Acidic wastewater from the use of solid acid leads to serious environmental problems. Reducing the temperature, extraction time, and acids requirements such as HCl requires applying microwave cooking, ultrasound, and electromagnetic waves as alternative methods (Guo *et al*., 2012).

The extraction process in the food industry applied ultrasound waves as a method to use. The cavitation energy resulting from the interaction of the wave with liquid can accelerate the extraction process (Ashley *et al.*, 2001). The rupture of the cavitation bubbles can increase the temperature and pressure near the material's surface and facilitate the extraction process due to damage to the plant cell walls (Liew *et al.*, 2016). Compared with the conventional method, ultrasound-assisted extraction has benefits that are reducing solvent consumption, extraction time, and energy requirement and producing higher purity (Colodel & Petkowicz, 2019). Besides, according to Maran *et al.* (2017) ultrasound-assisted extraction is a clean, efficient, and eco-friendly technology. Because of those reasons, ultrasound-assisted extraction is the method that many researchers use to produce pectin from various fruit peels (Freitas de Oliveira *et al*., 2016; Moorthy *et al*., 2017). Furthermore, application of ultrasound in pectin extraction resulted in higher yield, shorter operation time and higher antioxidant activity (Wang *et al*., 2015)

The objective of this research was to use the combination of acid extraction and ultrasound in pectin extraction from red dragon fruit peel. The acid solutions were hydrochloride acid (strong acid) and acetic acid (weak acid) in low concentrations (0.1 N and 0.2 N). Hopefully, the ultrasound application will reduce the use of acid and extraction duration. Furthermore, the physical and chemical properties were evaluated and compared with pectin produced from a conventional method.

2. Methods

2.1. Materials

The base material in pectin production was dragon fruit peels with red flesh (Hylocereus polyrhizus). The fruit was from a local farm in Sumbersari, Jember, East Java Province, Indonesia. The peels were removed from the fruit and then cleaned with running water. Furthermore, the peels were cut into pieces (2 cm wide and 5 cm long) and then blanched in steam for 1 min. Next, they were cold at room temperature for furthermore were packed in polyethylene bags then frozen in refrigeration.

2.2. Pectin Preparation

This research was an experimental study with three independent variables. They were the type of acid (HCl and CH_3COOH), solvent concentration (0.1 N and 0.2 N), and extraction time (30 minutes, 60 minutes, and 90 minutes). The control sample was pectin produced by the conventional method using 0.2 N HCl and 0.2 N CH_3COOH at room temperature for 24 hours.

Making pectin began with crushing 750 grams of dragon fruit peel in 1500 mL of acid solution to produce a slurry. Then sonicator (20 kHz, Branson, Germany) in different time intervals (30, 60, and 90 minutes) was applied, continuing the extraction process. The slurry was then filtered, and 96% ethanol to enhance precipitation. The ratio of the filtrated to ethanol was 1: 1.5. After being left at room temperature for 17 hours, precipitated pectin had separated by centrifugation at 5000 x g for 15 min.

2.3. Measurement of yield

The following equation calculated the yield (1):

$$yeild\ of\ pectin\ (\%) = \frac{P_d}{R_m} x100 \quad (1)$$

Pd (g) is the dried weight of the final product and Rm (g) is the weight of raw material.

2.4. Measurement of whiteness

The white degree (whiteness) is important to evaluate because pectin is a powder applied as a gelling agent in the food product. The white degree was calculated by the following equation $W = 100-((100-L)^2 + (a^2 + b^2))0.5$. L, a, and b values were measured using the Minolta CR-10 Color Reader at five different points.

2.5. Determination of equivalent weight

The method for equivalent weight evaluation was according to Shaha *et al.* (2013). The procedure started by weighing 0.5 g of pectin and adding 2 ml of ethanol to perform the pectin solution. The preparation of NaCl solution by dissolving 1 g of NaCl in 40 ml distilled water and then continued with pectin solution addition. Five drops of the phenolphthalein were added to the mixture while stirred and titrated using 0.01 N NaOH until the color turned pink and persisted for approximately 30 seconds. Calculating equivalent weight by recording the volume of titration and the equal weight used the following equation (2).

$$Equivalent\ weight = \frac{pectin\ weight\ (mg)}{ml\ NaOH\ x\ N\ NaOH}\ x\ 1000 \quad (2)$$

2.6. Determination of methoxyl content

Methoxyl content was measured using the method developed by Norziah *et al.* (2000). The titrated solution from equivalent weight evaluation was added with 25 mL of 0.2 N NaOH solution and left for 30 minutes at room temperature after being shaken. Subsequently, 25 ml of 0.2 N HCl was added and continued with titration to a pink endpoint by adding five drops of phenolphthalein indicator. The following equation calculated the methoxyl content (3).

$$methoxyl\ content\ (\%) = \frac{ml\ NaOH\ x\ N\ NaOH\ x\ 31}{weight\ of\ pectin\ (gr}\ x\ 100 \qquad (3)$$

Note: 31 is the molecular weight of methoxyl

2.7. Determination of galacturonic acid content

The galacturonic acid content and molecular charge of pectin have a significant role in determining the functional properties of the pectin solution. Furthermore, the estimation of galacturonic acid used equivalent weight and methoxyl content value by the following equation (Norziah *et al.*, 2000) was used equation (4).

$$galacturonic\ acid\ content\ (\%) = \frac{(mek\ equivalent\ weight^{*} + mek\ methoxyl^{**})x176}{pectin\ weight\ (mg)} x100\% \qquad (4)$$

Information:

* = mek (milliequivalent) NaOH from equivalent weight determination

** = mek (milliequivalent) NaOH from methoxyl determination

176 is the lowest equivalent weight of pectic acid

2.8. Determination of esterification degree

Norziah *et al.* (2000) determined the degree of esterification (DE) of pectin according to the following equation (5).

$$esterification\ degree\ (\%) = \frac{176\ x\ \%\ methoxyl}{31\ x\ \%\ galacturonic\ acid} x100\% \qquad (5)$$

3. Results and Discussion

3.1. Yeild, Whiteness and Viscosity

Table 1 represents the result of extraction yields, whiteness, and viscosity measurement. According to the yield value, whiteness and viscosity, ultrasound-assisted extraction can replace conventional extraction because the method produces higher yield and viscosity. Also, the whiteness value is not too different compared to the acid method.

Yield is a parameter that shows the amount of pectin produced from the dragon fruit peel extraction. Table 1 showed that the highest yield value was obtained from ultrasonic-assisted extraction in 0.2 N HCl for 90 minutes of extraction time, and the lowest was ultrasound extraction in 0.2 N CH_3COOH and 30 minutes. The longer the ultrasonic extraction time, the higher the pectin yield. Nguyen and Pirak (2019) studied pectin extraction from white dragon fruit peels. The result showed that ultrasound-assisted extraction produced a higher yield than conventional acid extraction. The yield was 9.83% - 16.30%. Our result was lower because the yield was presented in ratio with wet dragon fruit peel without drying preparation. The yield of pectin in the research of Chua *et al.* (2020), resulted in 6.27% – 2.23% of yield.

Table 1 showed the yield had increased as the ultrasonic extraction time increased from 30 to 90 minutes. The result of Pagán *et al.* (2001) was the same as our research which increases in time concomitant with yields. Lin *et al* (2018), showed that the high yield increase obtained as the ultrasonic extraction increased from 10 to 20 minutes, but above 30 minutes, yield enhancement was slow.

Table 1. Extraction yields (% raw material), whiteness and viscosity for dragon fruit (Hylocereus polyrhizus) pectin based materials

Samples	Yeild (%)		Whiteness (W)		Viscosity (g/m.s)	
	HCl	CH_3COOH	HCl	CH_3COOH	HCl	CH_3COOH
Control	1.12±0.05	0.90±0.03	66.13±1.05	54.5±1.01	0.13±0.01	0.12±0.07
A	1.04±0.06	0.81±0.05	64.69±3.08	54.27±1.08	0.22±0.03	0.14±0.04
B	0.90±0.02	0.76±0.05	69.09±1.66	57.49±1.77	0.27±0.04	0.44±0.05
C	1.50±0.07	1.13±0.08	70.87±1.88	54.17±1.86	0.40±0.05	0.19±0.07
D	1.13±0.05	0.86±0.05	69.57±2.61	51.38±2.09	0.68±0.05	0.59±0.02
E	1.73±0.03	1.41±0.06	65.31±1.78	49.10±1.55	0.57±0.02	0.32±0.05
F	2.71±0.04	2.13±0.07	63.91±1.36	50.06±1.82	2.22±0.02	0.85±0.03

Note: A=ultrasound-assisted extraction in 0.1 N acid solution for 30 minutes; B=ultrasound-assisted extraction in 0.2 N acid solution for 30 minutes; C=ultrasound-assisted extraction in 0.1 N acid solution for 60 minutes; D= ultrasound-assisted extraction in 0.2 N acid solution for 60 minutes; E=ultrasound-assisted extraction in 0.1 N acid solution for 90 minutes; F= ultrasound-assisted extraction in 0.1 N acid solution for 90 minutes.

The yield of pectin produced by ultrasound-assisted extraction in CH_3COOH was lower than ultrasound-assisted extraction in HCl (Table 1) because CH_3COOH is a weak acid. Ismail *et al.* (2012) studied extraction in hot water (85ºC) and showed a different tendency where pectin in HCl extraction produced a lower yield than extraction in oxalic acid. These showed there was another phenomenon between ultrasound-assisted extraction and high-temperature extraction. During ultrasound-assisted extraction for 90 minutes, the increase in acid concentration increased yield. There was a different tendency at 30 minutes and 60 minutes extraction times. The acid concentration probably did not affect the pectin yield in ultrasound-assisted extraction.

Measuring the degree of whiteness was important because pectin powder will be used as an additive in food products, not as a colorant. Table 1 represents the degree of whiteness of the pectin. The whiteness degree ranged from 49.1 to 70.87. The highest degree of whiteness was pectin produced by ultrasound-assisted extraction in 0.1 N HCl and 60 minutes. The lowest was pectin extracted with ultrasonic waves in 0.1 N CH_3COOH and an extraction time of 90 minutes. The result revealed that strong acid solvents could produce pectin with a whiter color than extraction in weak acids.

Table 1 represents the results of the viscosity measurement in this study. The value was ranges from 0.12 to 2.22 g/cm.s. The highest was pectin extracted by ultrasound-assisted extraction in 0.2 N HCl and a duration of 90 minutes. Furthermore, conventional methods obtained the lowest viscosity value from extraction in 0.1 N CH_3COOH solvent. Besides that, the data showed that the higher the acid concentration and the longer the extraction time, the increased the viscosity value.

3.2. Equivalent weight, methoxyl content, galacturonic content and esterification degree

Table 2 shows the value of the equivalent weight, methoxyl content, galacturonic content, and esterification degree. The equivalent weight indicates unesterified galacturonic acid groups in the chain of pectin molecules. The value from this research was 902.2-3814.2 mg. The matter was higher than an equivalent weight of citrus pectin 445.59 ± 16.49 (Nguyen & Pirak, 2019) and did not meet the IPPA (International Pectin Producer Association) standard values. According to IPPA, the equivalent weight value should range from 600-800 mg. The equivalent weight closest to the IPPA standard was pectin produced by ultrasound-assisted extraction in 0.2 N HCl for 90 minutes.

Table 2. Equivalent weight, methoxyl content, galacturonic content and esterification degree of dragon fruit (Hylocereus polyrhizus) peel pectin.

Samples	Equivalent weight (mg)		Methoxyl content		Galacturonic content		Esterification Degree	
	HCl	CH_3COOH	HCl	CH_3COOH	HCl	CH_3COOH	HCl	CH_3COOH
Control	978.7±12.3	2180.1±112.7	4.6±0.3	5.8±0.3	43.8±0.2	40.8±0.3	59.0±0.4	80.2±0.5
A	1000.2±9.9	1679.3±135.1	5.5±0.8	4.5±0.4	48.9±0.3	36.1±0.6	64.1±0.3	70.9±0.3
B	992.6±15.8	1728.5±188.9	5.8±0.5	4.9±0.2	50.4±0.5	38.1±0.5	64.8±0.5	73.2±0.4
C	990.9±21.8	3428.7±88.9	5.5±0.3	5.1±0.4	49.0±0.3	36.2±0.6	63.7±0.3	85.8±0.2
D	943.9±32.8	3814.2±122.1	5.8±0.4	5.1±0.2	51.7±0.2	39.1±0.4	63.9±0.6	88.1±0.5
E	921.8±25.9	2977.9±77.8	5.9±0.4	5.4±0.4	52.5±0.5	36.5±0.6	63.6±0.5	83.7±0.6
F	902.2±77.5	3210.6±215.8	6.1±0.3	5.6±0.4	54.4±0.3	37.2±0.8	63.8±0.5	85.1±0.3

Note: A=ultrasound-assisted extraction in 0.1 N acid solution for 30 minutes; B=ultrasound-assisted extraction in 0.2 N acid solution for 30 minutes; C=ultrasound-assisted extraction in 0.1 N acid solution for 60 minutes; D= ultrasound-assisted extraction in 0.2 N acid solution for 60 minutes; E=ultrasound-assisted extraction in 0.1 N acid solution for 90 minutes; D= ultrasound-assisted extraction in 0.1 N acid solution for 90 minutes.

Almost all previous research produced pectin from dragon fruit peel had a lower equivalent weight value than our result (Ismail *et al*., 2012; Nguyen & Pirak, 2019). That is because the acid concentration that we used was low. Table 2 shows that pectin produced by ultrasound-assisted extraction in 0.2 N CH_3COOH has a higher equivalent weight than pectin extracted in 0.2 N HCl since CH_3COOH is a weak acid. The extraction in weak acid produced pectin that did not partially degrade.

The measurement of methoxyl content aims to determine the number of esterified methyl groups in pectin from dragon fruit peel. According to Constenla and Lozano (2003), the ability of pectin to form gels, sensitivity to metal ions, and functional properties of pectin solutions depend on methoxyl content. Table 2 showed that the levels of methoxyl produced ranged from 4.55% to 6.14%. The result of Ismail *et al*. (2012) and the study of Nguyen and Pirak (2019) were similar, wherein the value of methoxyl content was less than 7 %, indicating low methoxyl pectin. Low methoxyl pectin was suitable in the jams and jellies industry and the heavy metal industry as an absorbent. According to Ismail *et al.*(2012), low methoxyl pectin can perform gel in low sugar concentration even without sugar.

The galacturonic concentration can affect the structure and texture of the pectin gel. The higher the concentration of galacturonic results in higher pectin quality (Constenla & Lozano, 2003). Table 2 showed the concentration of galacturonic pectin ranged from 35.98 to 54.4%. According to IPPA, the standard for galacturonic content should be at least 35%. All the pectin produced by ultrasound assist extraction has a value of galacturonic content that meets the standard requirement. Pectin from the ultrasound-assisted extraction in CH_3COOH had a lower galacturonic concentration than in HCl. That was due to the increase of hydrolysis reaction when protopectin changed into pectin and evidence that strong acid hydrolyzed better than weak acid.

The pectin esterification degree was from the methoxyl and galacturonic acid content. Table 2 shows that the value of esterification degree ranges from 59.0% to 88.14%. According to the esterification degree (above 50%), the pectin from our research was pectin of high ester. It was the same with the study of Woo *et al.* (2010) that produce high ester pectin (58.9% - 71.0%). The research of Nguyen and Pirak (2019) showed that ultrasound-assisted extraction had a lower esterification degree than ours, where the value was $34.78 \pm 2.15 – 44.31 \pm 0.47$. Ismail *et al.* (2012) and Chua *et al.* (2020) consisted to Nguyen and Pirak (2019), where both studies produced low ester pectin.

The pectin produced by ultrasound-assisted extraction in CH_3COOH has a higher esterification degree than pectin extracted in HCl. The study by Woo *et al.* (2010) confirms that the increase of pH results in a higher degree of esterification. Since CH_3COOH is a weak acid, the same concentration of HCl results in higher pH. According to the data of esterification degree, ultrasound-assisted extraction in diluted acid solution (CH_3COOH) is suitable for pectin production from red dragon fruit peel.

4. Conclusions

The ultrasound-assisted extraction yielded higher pectin than the conventional method (control). The ultrasound-assisted extraction in 0.2 N HCl solvent for 90 minutes produced the highest yield value. The pectin has the highest yield, which was 2.71%, the yellowish-white color, 2.22 g/cm.s viscosity value, 902.2 mg equivalent weight, and 6.14% methoxyl content. The pectin was a low methoxyl pectin type, suitable as an absorbent in the heavy metal industry. The galacturonic content of pectin was 54.4%, and the esterification degree was 63.8%.

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