Extraction of Pectin from Breadnut (*Artocarpus altilis* Fosberg.) using Ultrasound-Assisted Extraction (UAE)

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Abstract. Breadnut (*Artocarpus altilis*Fosberg) is an Indonesian plant that serves as a source of pectin. The purpose of this study is to evaluate and characterize isolated pectin from breadnuts. Extraction was carried out with the aid of ultrasound using citric acid as a solvent (pH 2, 60ºC, 30 and 60 mins). The organoleptic pectin consisted of a coarse and fine powder, blackish-brown in color, and odorless. Water content, ash content, and equivalent weight ranged between 11.36 – 11.00%, 5.28 – 5.65%, and 2295.09 – 2121.20g/Eq, respectively. Low-methoxyl content, galacturonic acid content, and esterification range was 0.44 – 1.14%, 10.19 – 14.78%, and 24.71 – 43.79%, respectively. The results showed that variation of pectin extraction time from breadnut using UAE citric acid solvent method affects the yield, equivalent weight, methoxyl level, galacturonic acid level, and degree of esterification, but it does not affect water and ash content.

Keywords: breadnut; citric acid; extraction; pectin; ultrasonic-assisted extraction

1 Introduction

Gelatin is a special hydrocolloid that can take a gel form and is widely used in various industries such as food, cosmetics, and pharmaceuticals. Currently, the primary source for commercial gelatin is limited to pigskins or cow skins and bones [1]. The use of gelatin made from pork is a problem for Muslims, especially in Indonesia. Muslim is prohibited from eating pork and its derivatives. Halalness is a crucial point that must be considered in the manufacturing of a product.

The raw materials are selected from plants to eliminate doubts among the Muslim population regarding the halal nature of gelatin made from animals. Several types of plant substances can form a gel, such as pectin [1]. The use of pectin is widespread due to its ability to form gels and emulsifying stabilizers. The thickness and stability of pectin, which is similar to gelatin, enables it to be packaged into capsules. Lower prices and no religious restrictions are among the advantages of using pectin [2].

The search for pectin sources is still being explored, mostly from plants found in Indonesia. Indonesia has an abundance of *Artocarpus* plants [3]. The extraction of pectin from jackfruit (*Artocarpus heterophyllus*) and chempedak (*Artocarpus integer*) rinds via conventional method assisted using variations of solvents from citric acid, nitric acid, and sulfuric acid (1:25 g/ml) with a pH level of 2 at 90°C for 60 minutes produces 14.8-18.6% and

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17.6-20.5% yield of pectin [4]. When compared with commercial sources of pectin such as orange peels, which have a pectin content of 10-30% [5], the *Artocarpus* plant has the potential to be a source of pectin.

Breadnut (*Artocarpus altilis*) is one of the robust tropical plants found in Indonesia and commonly used as a vegetable. Research on pectin extraction from the breadnut is still limited. Extracted pectin from breadnut skin via the conventional method produced a 48.30% yield of pectin at 90°C over 120 minutes [6]. The disadvantage of conventional methods is that it is time-consuming and operates at a high temperature. Therefore, it is necessary to develop more efficient extraction methods.

Ultrasound-Assisted Extraction (UAE) method is an extraction technique that makes use of ultrasonic waves. So far there has been no research related to pectin extraction from breadnuts using ultrasonic waves. The optimum conditions for extracting pectin from jackfruit skin using UAE method with citric acid solvent (1:15 g/ml) pH 1.6 produced a yield of pectin of 14.5% at 60°C over 24 minutes [7]. Based on the equations of the *Artocarpus* genus, research on the extraction of pectin from breadnut would be carried out using the UAE method with citric acid (1:15 g/ml) pH 2 at 60°C for variations in sonication time of 30 and 60 minutes.

2 Method

Materials and Chemical Reagents

The breadnut was obtained in July 2019 from the Spice and Medicinal Crops Research Institute (Balittro, Indonesia). The chemicals used in this study are citric acid pro analysis (SMARTLAB), NaOH pro analysis (MERCK), HCl pro analysis (MERCK), oxalic acid pro analysis (MERCK), NaCl pro analysis (MERCK), ethanol 96%, ethanol 80%, ethanol 70%, ethanol 60%, distilled water, KBr, and phenolphthalein indicators (ROFA).

Sample Preparation

Fresh breadnut was washed with water and chopped up. Next, 96% ethanol was added at a ratio of 1: 2 (g/ml) and heated at 80°C for 15 minutes to inactivate the enzymes found in fruit [4]. The sample dried in direct sunlight for ± 8 hours [8]. Dried samples were then ground into a powder and sieved with a size 20 mesh. The powder was added with 80% ethanol at a ratio of 1: 4 (g/ml) and heated at 80°C for 45 minutes. It was filtered and washed with 60% ethanol three times and ethanol 96% once to remove pigments and free sugars [9], and then air-dried at room temperature (± 25°C). The resulting powder is called AIS (Alcohol Insoluble Solid) powder.

Pectin Extraction

Pectin was extracted according to the method employed by Leong et al. [4], with slight modifications. Extraction was carried out using the UAE method with 37 kHz sonicator bath. AIS powder was added with citric acid pH 2 at a ratio of 1:15 (g/ml). The extraction process was carried out with a temperature setting of 60°C for a time variation of 30 minutes and 60 minutes. After that, filtering was carried out in a warm state by using a cotton filter cloth. The pectin filtrate was added 96% ethanol at a ratio of 1: 1 (ml/ml) to precipitate pectin and then allowed to stand overnight (± 15 hours) at room temperature (± 25°C). Next, the precipitate was filtered. Pectin was washed using 70% ethanol twice and ethanol 96% once. Wet pectin was dried in an oven at a low temperature of 40°C for ± 12 hours to obtain dry pectin, which was subsequently reduced into powder using a blender. The pectin powder obtained was stored in a dry and tightly closed container. The yield of pectin obtain was calculated by using the equation:

$$\text{Yield (\%)} = \frac{The\ weight\ of\ pectin\ obtained}{The\ weight\ of\ the\ AIS\ powder} \times 100 \quad \textbf{(1)}$$

Characteristic Evaluation of Pectin

The pectin characteristic evaluation carried out in this study included: Organoleptic, Water Content [10] [11], Ash Content [12] [13], Equivalent Weight [14], Methoxyl Level [14], Galacturonic Acid Level [14], Degree of Esterification [15], and Fourier Transform Infrared Spectroscopy (FTIR) [16].

Statistical Analysis

All analysis was done in triplicates and the data obtained were analyzed statistically using SPSS version 22 so that it can be seen whether there are significant differences between quantitative data.

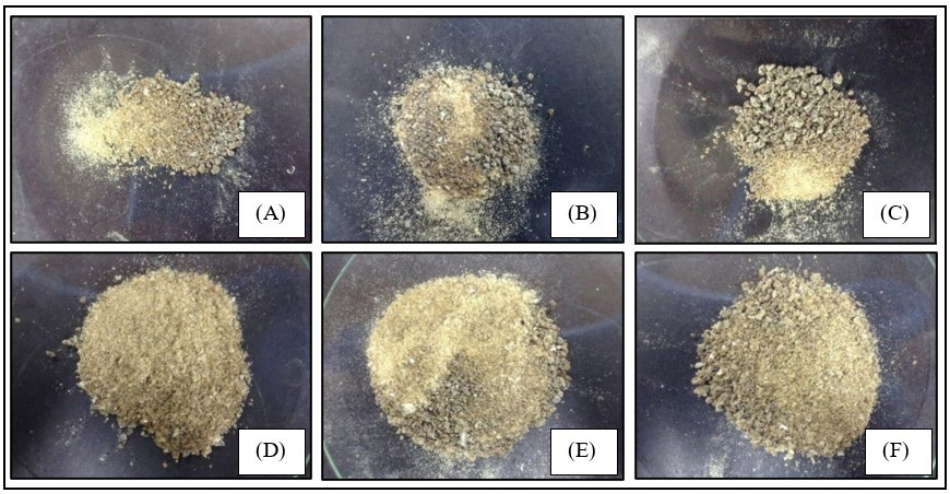
3 Results

Table 1. Average Breadnut Pectin Yield

Sample	n	Mean ± SD (%)	P-value
Variation 1	3	1.19 ± 0.18	0.021
Variation 2	3	1.66 ± 0.14	

Variation 1: pH 2, 60°C, 30 minutes; Variation 2: pH 2, 60°C, 60 minutes

Organoleptic



Note : (A) = pH 2, 60°C, 30 minutes, 1st repetition; (B) = pH 2, 60°C, 30 minutes, 2nd repetition; (C) = pH 2, 60°C, 30 minutes, 3rd repetition; (D) = pH 2, 60°C, 60 minutes, 1st repetition; (E) = pH 2, 60°C, 60 minutes, 2nd repetition; (F) = pH 2, 60°C, 60 minutes, 3rd repetition

Figure 1. Organoleptic Breadnut Pectin

Physicochemical Characteristics

Table 2. The Physicochemical Characteristics of Breadnut Pectin

Characteristics	n	Mean (± SD)		P-value	Requirements	
		Variation 1	Variation 2		IPPA [17]	Indonesian Pharmacopoeia V [11]
Water Content (%)	3	11.36 ± 0.57	11.00 ± 0.57	0.490	< 12	< 10
Ash Content (%)	3	5.28 ± 0.65	5.65 ± 0.82	0.580	-	-
Equivalent Weight (g/Eq)	3	2295.09 ± 63.85	2121.20 ± 41.62	0.017	-	-
Methoxyl Level (%)	3	0.44 ± 0.03	1.14 ± 0.10	0.000	< 7: low-methoxyl > 7: high-methoxyl	≥ 6.7
Galacturonic Acid Level (%)	3	10.19 ± 0.18	14.78 ± 0.66	0.000	≥ 65	≥ 74
Degree of Esterification (%)	3	24.71 ± 1.74	43.79 ± 2.08	0,000	< 50: low-ester > 50: high-ester	-

Variation 1: pH 2, 60°C, 30 minutes; Variation 2: pH 2, 60°C, 60 minutes

4 Discussion

The extraction yield calculation was intended to identify the amount of pectin content that has been successfully extracted from breadnut using the UAE method using citric acid (1:15 g/ml) pH 2 at 60°C for 30 and 60 minutes. The average yield of pectin obtained over 30 minutes was 1.19 ± 0.18%, lower than for 60 minutes, which was 1.66 ± 0.14%. These results show significant differences ($p < 0.05$). The longer the extraction time, the more pectin produced. Extraction time can prolong the contact between the sample and the solvent [18]. However, it should be noted that the addition of extraction time is not proportional to the yield obtained, and therefore must be done at an optimum time [19].

Extracted pectin obtained in this study showed the same description of the two-time variations in each repetition, namely in the form of a coarse and fine powder, blackish-brown, and odorless (Figure 1). When compared with IPPA [17] and Indonesian Pharmacopoeia V standards [11], there is a similarity between the description of pectin produced with the standard, although there is little difference in the color of the final pectin result. As shown in Table 1, the average water content of breadnut pectin at 30 minutes variation was 11.36 ± 0.57%, while at 60 minutes time variation was 11.00 ± 0.57%. The longer the extraction time, the less water content obtained. The data showed no significant difference in water content ($p > 0.05$). The average ash content of breadnut pectin at 30 minutes variation was 5.28 ± 0.65%, while at 60 minutes it was 5.65 ± 0.82%. There were no significant differences in the ash content resulting from the extraction time of 30 and 60 minutes ($p > 0.05$).

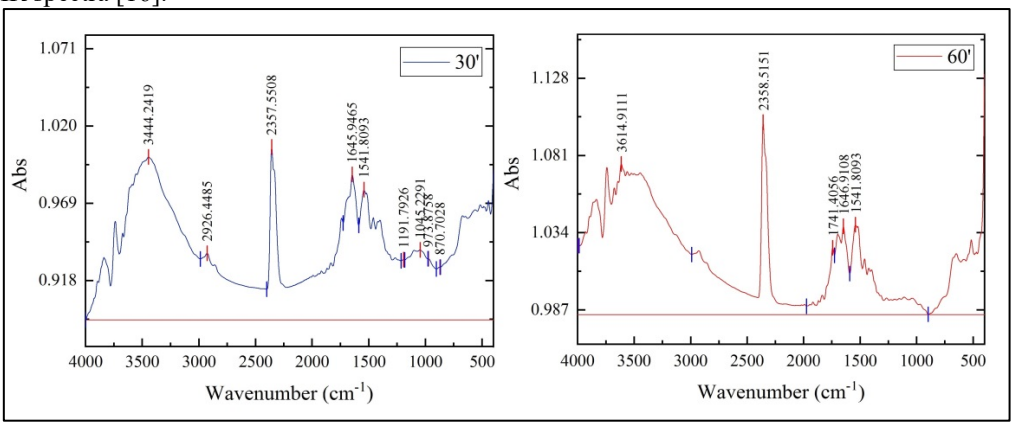
The average equivalent weight variation of breadnut pectin over 30 minutes was 2295.09 ± 63.85g/Eq, greater than over 60 minutes, which was 2121.20 ± 41.62g/Eq. These results show a significant difference ($p < 0.05$). It shows that the longer the extraction time, the lower the

equivalent weight. The process of de-esterification from pectin to pectic acid will occur over a longer extraction time, resulting in a higher number of free acid groups that will reduce the equivalent weight [18].

Examination of methoxyl level is necessary because it affects the type of pectin produced for its application. The average level of methoxyl pectin of breadnut variation over 30 minutes was 0.44 ± 0.03%, smaller than the 60 minutes time variation of 1.14 ± 0.10%, showing a significant difference ($p < 0.05$). From these data, the longer the extraction time, the higher the methoxyl level. The length of time of extraction can increase esterified free carboxyl groups, resulting in higher methoxyl [18] Based on IPPA standards [17], pectin from breadnut can be classified as low-methoxyl pectin. According to Indonesian Pharmacopoeia V standards [11], the resulting methoxyl level is too small for pharmaceutical grade pectin so that it is more suitable for application in the food sector.

The average levels of galactonic acid pectin in breadnut at a variation of 30 minutes was 10.19 ± 0.18% lower than the 60 minutes time variation of 14.78 ± 0.66%. There is a significant difference between the results obtained ($p < 0.05$). Based on these data, the longer the extraction time, the higher the levels of galactonic acid. The higher levels of galacturonic acid, the stronger the gel formed and the smaller the organic content such as arabinose, galactose, rhamnose, and other types of sugar, resulting in a higher level of purity of pectin produced [20].

The degree of esterification is the percentage of D-galacturonic acid residue in which the carboxyl group is esterified with ethanol. The average degree of esterification of breadnut pectin at 30 minutes variation was 24.71 ± 1.74%, lower compared to over the 60 minutes variation of 43.79 ± 2.08%. There is a significant difference between the results obtained ($p < 0.05$). These results indicate that the longer the extraction time, the higher the degree of esterification. IR spectrum of breadnut (Figure 2) showed similarities in shape and bands with the standard pectin IR spectra [16].



Note: 30 ' = pH 2, 60°C, 30 minutes; 60 ' = pH 2, 60°C, 60 minutes

Figure 2. Breadnut Pectin IR Spectrum

5 Conclusion

Based on the research conducted, it can be concluded that variation of pectin extraction time from breadnut using UAE citric acid solvent method affects the yield, equivalent weight, methoxyl level, galacturonic acid level, and degree of esterification ($p < 0.05$), but it does not

affect water content and ash content ($p > 0.05$). Characteristics of breadnut pectin obtained in this study that meets the requirements according to IPPA are organoleptic, water content, methoxyl level, and degree of esterification, where the pectin produced based on these standards are classified as low-methoxyl pectin, while the only characteristic that meets the Indonesian Pharmacopoeia V requirements is organoleptic. The UAE method is proven to be able to extract breadnut pectin at a lower temperature and faster time. Therefore, further research is needed related to the search for the optimum conditions for the extraction of breadnut pectin using the ultrasonic method by varying the amount of citric acid solvent, temperature, and pH. In addition, it is necessary to find a drying method other than oven drying to produce a better colour of breadnut pectin.

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