Extraction and characterization of pectin from the fruit peel of Benincasa hispida (Thunb.) cogn

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ABSTRACT

Pectin is a polysaccharide and a major component of most plant cell walls and serves as an adsorbent, emulsifying agent, gelling agent, stabilizer, and gelling agent. Pectic can be obtained from the fruit peel of Bligo (Benincasa hispida (Thunb.) Cogn). This study aims to determine the presence of pectin in the peel of Bligo fruit, which can be used as a gelling agent, and to obtain the best solvent concentration and time for the extraction and characterization of pectin from the peel of Bligo fruit by using a completely randomized design (CRD) using 4 variations. Concentration of citric acid solvent (5%,7%,9% and 11%) with extraction time consisting of 4 levels (30, 60, 90 and 120 minutes). The characteristics of the pectin produced were determined based on its water and ash content, equivalent weight, methoxyl content, galacturonic acid content, and degree of esterification. The results showed that the highest yield was obtained from 7% citric acid concentration with an extraction time of 60 minutes, with the value of 9.8%. The resulted pectin had a water content of 10.4%; ash content of 8.75%; an equivalent weight of 779.243 mg; methoxyl content of 2.515%; galacturonic acid content of 114.685%; and esterification degree of 12.445%. The characterization of functional groups using FTIR showed groups -OH, -CH3, -C=O, -C-H, and -R-OR with wavelength respectively of 2958.93 cm⁻¹; 1396.52 cm⁻¹; 1724.36 cm⁻¹; 1416.78 cm⁻¹; and 1220.99 cm⁻¹.

Keywords: pectin, Bligo fruit peel extract, characterization, FTIR

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INTRODUCTION

Pectin is a functional ingredient because of its gelling, thickening, stabilizer, texture, and rheological properties in the food industry. In the health sector, pectin has been used for various therapeutic uses, such as prebiotics, antioxidants, anticancer, antimicrobial agents, and treatment of hypercholesterolemia, and contains a high level of fiber. In the last decade, the thickening and stabilizing properties of acidic proteins have made pectin an important additive in food and beverage production. The confectionery business uses high-density pectin in particular, whereas yogurt and fruit juice are made with medium- and low-density pectin. Pectin is also used in cosmetics, personal care products, pharmaceuticals, and nutraceutical products (Freitas et al., 2021).

The main component of pectin comes from the wall of non-wood plant cells. Vegetables and fruits are the most abundant sources. In general, pectin polymers contain between 300 and about 1000 saccharide units (molecular weight 150 kDa). Pectin chains, which are rich in rhamnose, can enhance molecular interactions between cells and polysaccharides, while galactose from hairy and branched parts promotes the formation of entangled structures. The degree of methylation (DM) is different for each pectin. Under acidic conditions, high methoxyl (HM) pectin forms a gel. Low methoxyl (LM) forms a gel by interacting with divalent cations such as Ca2+ (pH ranges from 3 to 7) (Ciriminna, 2012).

The conventional method by combining acid hydrolysis at temperatures up to 363,15 K and a long extraction period of up to 6 hours yields approximately 70% esterified polysaccharides. Pectin is produced from dried apple pomace, orange, and lime peel, sugar beet (Ciriminna et al., 2016), Garcinia binucao (Robrigado et al., 2019), Opuntia robusta (Mota et al., 2020), passion fruit (Liew et al., 2014), and cocoa husks (Chan & Choo, 2013). Other extraction techniques include lowering the temperature, using enzymes, and employing ultrasound (US) and microwave (MAE), both of which have been extensively studied for their ability to speed up the extraction process, lower the temperature, increase yield, decrease acid waste, and use less solvent. With the help of this study, low and high methoxyl pectins (LM, HM) will be produced while the extraction source's structural and functional characteristics are preserved. A new procedure for obtaining high-purity pectin from the final product uses microwave-assisted hydrodistillation and solvent-free microwave-assisted hydro diffusion (MHG) (Ciriminna, 2012).

In addition, alternative sources, such as food waste streams and agricultural by-products, have also been studied for pectin production. Benincasa hispida (Thunb.) Cogn. has been known as Bligo in Indonesia. Bligo can be found in the yard of the house, planted on trellises or grown on roofs, and cultivated as a commercial crop in the fields (Churiyah & Darusman, 2009; Du et al., 2005; Suryanti et al., 2018). Benincasa hispida (Thunb.) Cogn contains many proteins, enzymes, vitamins B1 and C, flavonoids C-glycosides, terpenes, phenolic acids, and free sugars such as glucose, β -sitosterol, rhamnose, mannitol, uronic acid, metals, peptic polysaccharides (sequential extraction), while phenolic compounds, such as astilbin, catechins, and naringenin (Bimakr et al., 2012; Busuioc et al., 2020; Churiyah & Darusman, 2009; Doshi et al., 2015; Du et al., 2005; Mazumder et al., 2004; Sameeksha & Nirmala, 2021).

Benincasa hispida has a rich source of functional and therapeutically important bioactive such as triterpenes, phenolics, sterols, and glycosides. Pharmacological studies show that Benincasa hispida has a wide range of pharmacological effects, including antimicrobial, antioxidant, anti-inflammatory, analgesic, diuretic, nephroprotective, and central nervous effects (anxiolytic, muscle relaxant, antidepressant, in the treatment of Alzheimer's disease to minimize signs of opiate withdrawal) (Abass & Sakran, 2020; Bimakr et al., 2012). Nevertheless, due to the content of Benincasa hispida (Thunb.) Cogn can be a potential source of pectin with attractive features and a viable product with different applications.

MATERIALS AND METHOD Materials

The main material used in this is the peel of the Bligo fruit. Bligo fruit was collected from Rasuna Said Cipete Plantation, Tangerang City, Banten, Indonesia in November 2020. The species were

Extraction and characterization ... (Rasydy et al.,)

identified at the Herbarium of the Indonesian Institute of Science, Research Center for Plant Conservation Botanical Gardens, Cibinong, Indonesia, and a voucher specimen was deposited (No. 1171/IPH/1.01.If.07/XI/2020). Chemicals used for pectin extraction included 5% citric acid, 7% citric acid, 9% citric acid, 11% citric acid, aqua dest, sodium hydroxide (NaOH), sodium chloride (NaCl), hydrochloric acid (HCl), powder KBr, 96% ethanol, phenolphthalein indicator, pure pectin, filter paper, universal indicator, and aluminum foil.

Methods

Sampel preparation

Bligo fruit simplicia powder is generally extracted chemically, while pectin can be extracted from plant tissue using an acid solution which functions to hydrolyze protopectin into water-soluble pectin and can free pectin from bonding with other compounds such as cellulose (Nurhaeni et al., 2019). The clean bligo fruit peel was dried in an oven at 50°C for 5 hours. The dried Bligo peel was grinded and sieved with a 60 mesh sieve to obtain a fine powder. Bligo peel was sievedto obtain small particles to facilitate the pectin extraction process (Nurhaeni et al., 2019). 10 grams of powders were blended with 500 mL of citric acid with concentrations of 5%, 7%, 9%, and 11%. Pectinwas extractedby way of heating at a temperature of 85-90°C for 30, 60, 90 and 120 minutes with a stirring speed of 600 rpm, then, liquid mixture was filtered using a Buchner funnel. The obtained filtrate was evaporated at a temperature of 95°C until the volume reached a half, then cooled and added with 96% ethanol with a volume ratio of 1:1 (v/v) in the filtrate. It stood for 24 hours at room temperature and the filtrate was separatedfrom the residue. The sediment obtained then dried in an oven at 50°C for 24 hours (Nurhaeni et al., 2019).

Pectin content test

The resulted 0.5 grams of pectin extract was put it into a 10 mL beaker and added with 5 ml of distilled water. 1 mL of pectin solution in a beaker was taken and put into a test tube and added with 1 mL of 96% ethanol. Then, the gel formation was observed as a feature of the presence of pectin (Demsi et al., 2019).

Pectin characterization

Determination of water content

The 0.25 g of pectin in a porcelain dish was dried in an oven for 3 hours at 105°C, then cooled in a desiccator and weighed. This method was repeated until the weight remained constant. The water content was determined by the formula (Latupeirissa et al., 2019).

water content =
$$\frac{initial \ weight - final \ weight}{initial \ weight} \ge 100\%$$
 (1)

Determination of ash content

Total ash content of simplisia powder (2 g) in a crucible that had been tared was put at incandescent at 800oC for 6 hours, then cooled and weighed. Total ash content was calculated to the initial powder weight in % w/w (Kemenkes, 2020).

Ash content =
$$\frac{ash weight}{extract weight} \ge 100\%$$
 (2)

Determination of equivalent weight

The 0.5 g of pectin was added to 2 mL of 96% ethanol and dissolved in 2.5% NaCl. The mixture was dripped with 5 drops of phenolphthalein indicator and titrated with 0.1 N NaOH until a pink color was formed. The volume of titrant used was recorded to calculate the equivalent weight of pectin using the formula (Latupeirissa et al., 2019).

295

Equivalent Weight = $\frac{sample \ weight \ (mg)}{V \ NaOH \ (ml)x \ N \ (NaOH)}$ (3)

Determination of methoxyl content

The solution resulting from the equivalent weight analysis was added with 25.0 mL of 0.2 N NaOH solution, stirred, and allowed to stand for 30 minutes in a closed state at room temperature. Then 25.0 mL of 0.2 N HCl solution was added, 5 drops of Phenolphthalein pH indicator was added, then titrated with 0.1 N NaOH solution until a pink color was formed. The used titrant volume was then used to determine the methoxyl content (Latupeirissa et al., 2019).

Methoxyl Content =
$$\frac{V \text{ NaOH } x \text{ N NaOH } x \text{ 31}}{\text{sample weight } (mg)} x 100\%$$
 (4)

Determination of galacturonic acid content

The galacturonic acid content was calculated from the weight equivalent of NaOH obtained from the determination of the equivalent number with the following formula (Latupeirissa et al., 2019).

$$Galacturonic Acid Content = \frac{(Meq NaOH dari BE + Meq NaOH metoksil) x 176}{Bobot sampel (mg)} x 100\%$$
(5)

Determination of the degree of esterification (DE)

The measurement of the degree of esterification was calculated from the methoxyl content and the resulting galacturonic acid content (Latupeirissa et al., 2019).

$$DE = \frac{176 x \% metoksil}{31 x \% galakturonat} x100\%$$
(6)

Identification of the FTIR spectrum

The 0.3 grams of KBr and 0.015 grams of pectin were mixed and compressed in a pellet press with a hydraulic compression force of 10 torr connected to a vacuum pump. The pellet mixture of KBr and the sample was placed between 2 slits through which the infrared light beam passes. The spectrum was set in the wave number range of $4000-500 \text{ cm}^{-1}$ before it was analyzed by FT-IR spectrophotometer.

RESULT AND DISCUSSION

Pectin extraction was carried out by acid hydrolysis using citric acid with various concentrations. The citric acid was the best solvent used in the extraction of pectin in the peel and dami of cempedak fruit with a yield of 38.85% (Nurhaeni et al., 2019). This is because citric acid is a weak organic acid. Strong acids are not very good at extracting pectin compared to weak organic acids because they will only hydrolyze pectin. Pectin (Figure 1) is a polysaccharide type carbohydrate obtained from plant tissue generally using an extraction method with an acidic solvent (Febriyanti et al., 2018).

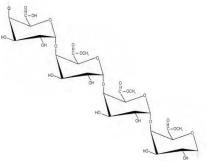
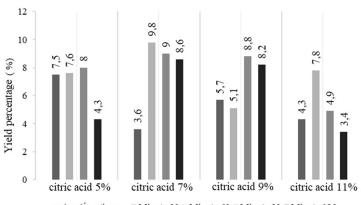


Figure 1. A repeating segment of pectin molecule and functional groups (Sriamornsak, 2003; Yujaroen et al., 2008)

Pectin extraction with citric acid was carried out with various concentration and time variations. This was done to see the effect of concentration and time on the amount of produced pectin yield. Extraction was carried out with four variations of citric acid concentration of 5%, 7%, 9% and 11% using various extraction times of 30, 60, 90 and 120 minutes at a temperature of 85-90°C. In general, the extraction temperature of pectin is between 60°C to 100°C. This is because the higher the temperature, the more calcium and magnesium protopectin that the produced hydrogen ions can substitute, which will be hydrolyzed and produce more pectin (Demsi et al., 2019). The appearance of peptin isolated from Bligo were presented in Figure 2.



Figure 2. Pectin peel of Bligo (Benincasa hispida (Thunb.) Cogn) fruit



extraction time : ■ Minute 30 ■ Minute 60 ■ Minute 90 ■ Minute 120

Figure 3. Pectin yield percentage at various temperatures and times

The results of Bligo fruit peel extraction revealed that the highest yield in Figure 3 was obtained at 7% citric acid concentration for 60 minutes of extraction time, with the yield value of 9.8%, while the lowest pectin yield was obtained at 11% citric acid with an extraction time of 120 minutes with a yield value of 3.4%. The pectin obtained was brownish, and odorless (Nurhaeni et al., 2019). Pectin extraction is not only affected by solvent concentration, but also by extraction time. Extraction time can optimize the yield of pectin, but a long time also causes a decrease in the amount of yield. This is because the longer the extraction time, the more degraded the pectic acid and the lower the pectin content (Zahrotun et al., 2013).

Pectin yield is the pectin content found in the peel of Bligo fruit (Nurviani et al., 2014). The dried pectin obtained brownish color, which was due to the influence of the raw material used, namely the yellow-brown color of Bligo fruit peel powder. The dried pectin precipitate was in the form of powder

after grinding using a mortar. Because only a small amount of dry pectin was produced, the grinding was done using a mortar.

(Indib.) Cogn) Huit		
Pectin Quality Standard	Pectin	Sample
	(IPPA, 2002)	
Water Content	<12%	10.4%
Ash Content	<10%	8.75%
Equivalent Weight	600-800 mg	779.243 mg
Methoxy content:		
a. High Methoxy Pectin	>7,12%	
b. Low Methoxy Pectin	2.5-7.12%	2.515%
Galacturonic acid levels	Min 35%	114.685%
Degree of esterification		
a. High Ester Pectin	>50%	
b. Low Ester Pectin	<50%	12.445%

 Table 1. Pectin Characterization isolated from the peel of Bligo (Benincasa hispida (Thunb.) Cogn) fruit

The characteristic test in Table 1 revealed that the pectin of the bligo fruit peel met all the requirements, including water content and ash content. Determination of equivalent weight was carried out to determine the amount of free galacturonic acid groups contained in the pectin molecular chain and obtained 779.243 mg. The fewer free acid groups, the higher the equivalent weight, and the longer the extraction time, the lower the yield value (Tuhuloula et al., 2013). Bligo fruit pectin has a low methoxy content of 2.515%. The methoxyl content of pectin is divided into 2 types, high Methoxyl Pectin and Low Methoxyl Pectin. The amount of methoxyl pectin in the pectin solution plays a significant effect in determining the functional characteristics of the solution and can influence the structure and texture of the pectin gel (Antika & Kurniawati, 2017).

The galacturonic pectin levels were 114.685%. The predetermined galacturonic level is at least 35% (IPPA, 2002). Galacturonic levels can affect the texture of the pectin gel. The greater the galacturonate in pectin, the more galacturonic polymer chains and the higher the molecular weight of pectin. The galacturonic content tends to increase with time due to the increase in the hydrolysis reaction of protopectin into pectin, and its base component is D-galacturonic acid. The value of the degree of esterification obtained in this study was 12.445%. The quality standards of pectin have a low esterification degree of less than 50%, and pectin has a high esterification degree of more than 50%. The pectin produced low methoxyl content and had a low esterification degree according to its methoxyl content. The degree of esterification decreased with the increasing temperature and extraction time. Glycosidic bonds of pectin compound chains in acidic solvents will tend to hydrolyze to produce galacturonic acid. The use of high concentrations of acid will turn pectin into pectic acid, where the free galacturonic acid is obtained from the methyl ester groups (Budiyanto & Yulianingsih, 2008).

The results of the FTIR spectrum measurement showed functional groups and provided structural information on pectin extracted from the raw material of Bligo fruit peel using an extraction solution in the form of 7% citric acid for 60 minutes. The extracted pectin FTIR spectrum was compared to the standard pectin spectrum. The identification of the measurement of the pectin functional groups ware carried out using an infrared spectrophotometer (FTIR) with a range of wave numbers of 4000-500 cm⁻¹. Identification of the main functional groups in pectin is usually located in the wave number area of 1000-2000 cm⁻¹. The free carboxyl bonds were in the area of 1630-1650 cm⁻¹ and 1740-1760 cm⁻¹ indicating an esterified carboxyl group (Ismail et al., 2012).

The FTIR spectrum of pectin show in Figure 4, at a wave number of 2925.17 cm⁻¹ for standard pectin and 2958.93 cm⁻¹ for extracted pectin indicated the absorption of the carboxyl group (-OH)

(Latupeirissa et al., 2019). The wave number area of 1591.34 cm⁻¹ on standard pectin and the extracted pectin found in the wave number region of 1732.15 cm⁻¹ indicated the presence of a carboxyl group (C=O), in which both standard pectin and extracted pectin had the same absorption. The carboxyl group (C=O) was located at 1724.36 cm⁻¹. The absorption band at wave number 1416.78 cm⁻¹ on standard pectin and extracted pectin was found at wave number 1396.52 cm⁻¹, which indicated the presence of - C-H bonds (Budiyanto & Yulianingsih, 2008). The wave number of the -C-H bond lied in the range of wave number 1450-1375 cm⁻¹ (Donald, 2008). There was an absorption from the ether (R-O-R) on standard pectin at a wave number of 1058.00 cm⁻¹ and the extracted pectin found at a wave number of 1220.99 cm⁻¹. Absorption (-O-) was at a wave number of 1151.29 cm⁻¹. (Tuhuloula et al., 2013) stated that the wave number of the ether bond (R-O-R) liedin the spectrum range of 1050-1260 cm⁻¹ (Tuhuloula et al., 2013). The results of the functional groups produced in the FTIR spectrum with each absorption in a certain wave number region show the suitability of the pectin structure. There are OH vibrations, - C-H bonds, carboxyl groups (C=O), and ether groups (-O-) (Antika & Kurniawati, 2017).

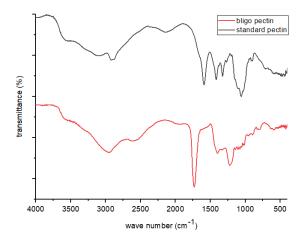


Figure 4. FTIR spectrum standard pectin and bligo fruit peel pectin

CONCLUSION

Bligo fruit pectin can be extracted under optimum conditions using 7% citric acid for 60 minutes, with the following characteristics of pectin: containing water of 10.4%; ash of 8.75% equivalent weight of 779.243 mg; methoxyl of 2.515%; galacturonic acid of 114.685%, and esterification degree of 12.445%. The functional groups in pectin were characterized by FTIR to show group vibrations with - OH, -CH3, -C=O, -C-H, and -R-OR in wavelength respectively at 2958.93 cm⁻¹; 1396.52 cm⁻¹; 1724.36 cm⁻¹; 1416.78 cm⁻¹; and 1220.99 cm⁻¹.

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