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Analysis of Metoxyl and Galacturonate Contents from Pectin of Banana Peel Waste Combined with Sappan Wood

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Abstract

The production of banana plants (*Musaceaea* sp.) ranks first in agricultural output in Indonesia, but this is not balanced by the processing of waste from banana peels, which is a large amount. Therefore, an attempt is made to use banana peel waste as raw material for making pectin. Pectin is used as a functional component in the food industry because of its ability to form thin gels and stabilize proteins. Pectin is also used as a filler in the paper and textile industries, and as a thickener in the rubber industry. This research aims to determine the effect of extraction time and temperature on the quality of pectin from Kepok banana peels. The method used is extraction through Ultrasonic Assisted Solvent Extraction (UASE) in 40 kHz. Banana peels that had been dried and ground into powder were extracted with 0.1 N HCl at temperatures of 70 °C, 80 °C and 90 °C. The variables used in this extraction are variations in extraction time, namely 60 minutes and 80 minutes. The extracted solution is filtered and thickened to half the volume of the original filtrate by heating, then the pectin is thickened using acid ethanol. The pectin precipitate was washed using 96% alcohol until it was free of chloride and the precipitate was separated using a vacuum and then dried in an oven then to analysis of pectin using methoxyl and galacturonate as method to determine pectin quality form banana peel waste. The research results showed that the pectin quality was optimum at a temperature of 90 °C for 80 minutes with a methoxyl content of 4.12% (low methoxyl) and a galacturonic acid content of 79.96%.

INTRODUCTION

The potential for horticultural crops to become one of the important commodities in Indonesia, especially banana plants. Banana production in South Sulawesi Province has increased significantly every year, where in 2021 it reached 161,550 tonnes of banana production with one of the largest banana producing districts being Bone Regency which reached 29,434 tonnes of banana production with a production increase of 2,244 tonnes from 2020 to 2021 (BPS, 2022). One of the most widely cultivated banana varieties is kepok banana (*Musa paradisiaca* L.) because in terms

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of price it is relatively expensive compared to other types of banana and consumer demand is very high to be used as processed food such as chips, sale and various other traditional preparations (Wikantika et al., 2022; Halim & Darmawan, 2022). However, the general characteristics of fruits including bananas are that they are prone to permanent damage (defects) due to storage and the climacteric nature (Pham et al., 2022) of bananas with a short shelf life with a very high respiration rate after being picked (Pham et al., 2023) also results in fruit shrinkage and deterioration (Zainuddin et al., 2023). Post-harvest handling by increasing the shelf life of bananas is important research so that losses for banana farmers are minimized. The shelf life of fruit or food can be said to be a period where safety and minimal loss of sensory attributes can be guaranteed, usually determined by microbial (biological) phenomena where Fresh Cut Product (FCP) can spoil quickly, microbes can grow quickly (Kumar et al., 2022) and cause sensory defects (off-odor, off-tastes, acidification and also visual defects) which lead to opportunities for rejection of produce by consumers (Fadiji et al., 2023; Kawurian & Silvia, 2022; Ruggeri et al., 2020), so preservation treatment is needed to avoid major losses.

Several methods are used to preserve fruit, namely storing it in a refrigerator, in a pressurized room and modifying the room atmosphere (Kim et al., 2022; Garnida & Taufik, 2022). However, this method has weaknesses such as cooling and storage which require high investment costs and have the potential to damage the environment due to the impact of the freon used, so other alternatives are needed to overcome these problems. One effort that can be used to extend shelf life is by using natural preservatives that come from nature by using pectin as an edible coating (Asriah et al., 2022; Emragi et al., 2022). Kepok bananas (*Musa paradisiaca* L.) have around 40% of the fruit skin of the total banana fruit which contains sulfur, glucose and fructose and can be used as a good carbon source, also contains many nutrients such as carbohydrates, vitamins, minerals, fiber and including pectin (Han et al., 2019; Okorie et al., 2015). Pectin from the extraction of fruit skin waste including seeds can be used as an alternative source of polysaccharides in making environmentally friendly edible films or organic coatings (Sigiro et al., 2022; Ratna et al., 2019; Megawati & Machsunah, 2016; Gupta et el., 2022). Edible coating is considered very good (semipermeable barrier) because it is able to prevent respiration which can cause fruit shrinkage due to the breakdown of compounds such as carbohydrates by producing CO_2, energy and water which evaporate through the surface of the fruit skin (Pandey et al., 2022; Aiemcharon et al., 2022), so that the presence of edible coating can prevent damage to the fruit (Andriani et al., 2018), as well as extend shelf life because there is a reduction in the population of spoilage and pathogenic microorganisms (Khamidah et al., 2022).

The potential of Sappan wood (*caesalpinia sappan* L.) is quite large to be combined with banana peel waste as an edible coating because Sappan wood extract contains flavonoids and polyphenols which have the potential to reduce free radicals (Freitas et al., 2021; Hemthanon & Ungcharoenwiwat, 2022) and prevent the formation of reactive oxygen species which inhibit the activity of the xanthine oxidase enzyme and catalyze the formation of superoxide radicals with activity of 89.9% (Settharaksa et al., 2019; Asfar & Asfar, 2021). Brazilin from Sappan extract is effectively used as an anti-inflammatory and antibacterial, especially *Staphylococcus aureus* and *Escherichia coli* bacteria (Asfar & Yasser, 2018; Pattananandecha et al., 2022). Apart from that, it also contains flavonoids and tanninswhich functions as an antioxidant to ward off free radicals and is able to improve edible coating formulas (Rosniawaty et al., 2023; Romruen et al., 2022; Eckardt et al., 2022), while tannins can react with proteins to form soluble complex compounds which have antibacterial properties. and astringent (Bravo et al., 2019; Asfar & Asfar, 2020).

Over the past few years, research on the use of agricultural and industrial waste as a source of pectin has increased. This is due to concern for sustainable issues and efforts to reduce waste produced by industry. Banana peel waste and Sappan wood are two examples of materials that have not been fully explored as sources of pectin. Analysis of methoxyl and galacturonate levels in pectin is a key step as method in determining the quality and application potential of the pectin. Methoxyl content refers to the number of methoxyl groups (CH_3) in the pectin structure, while galacturonate refers to an important basic unit in the pectin structure. The quality of pectin is greatly influenced by the ratio between these two components. Therefore, this research aims to explore the potential of banana peel waste combined with sappan wood as a valuable source of pectin. Analysis of the methoxyl and galacturonate levels as method to determine of the pectin extracted from these two materials will provide important insight into the quality of the pectin produced. The results of this research can be a basis for developing wider applications of pectin in various industries, as well as making a positive contribution to environmental conservation efforts by utilizing agricultural and industrial waste more efficiently.

MATERIALS AND METHOD

Materials

The materials used in this research were kepok banana peels obtained from banana farmers in Maggenrang Village and sappan wood collected from sappan wood collectors in Matajang Village, where both villages are part of Kahu District, Bone Regency, South Sulawesi Province, Indonesia. HCL 0.1 N (Emsure 37%), Aquades (Onemed Water One), Ethanol (Emsure 99.5%), AgNO3 (Merck 99.8%), NaOH 0.1 N (Merck 99% Pellet), and Methyl Orange Solution 0.1% (Merck).

Method

The production process pectin from Kepok banana peels in this research using Extraction Method using Ultrasonic Assisted Solvent Extraction (UASE) consists of several stages, namely preparation, extraction, pection deposistion, washing, and drying.

In the Preparation stage, the kepok banana peel was prepared to be studied. The kepok banana peel was cut finely, then it was dried in an oven at 40°C. Once it was dried, it was then grinded into powder. The extraction stage was carried out to determine the effect of variable temperature and extraction time on the pectin produced using UASE method. Kepok banana peels that have been dried and turned into powder were weighed at ±15 grams. pH adjustment was carried out by adding 250 ml of 0.1 N HCl (pH=1.5). Extraction was carried out above the *heater* by using optimum temperature and varying times as treatment. The extraction time used in this research was 60 minutes using temperature variations of 60^0C, 80^0C and 90^0C. The extracted mixture was then filtered using a thin filter cloth and squeezed to separate the filtrate from the dregs. It was then thicken until the volume becomes half the original volume by heating using ultrasonic bath in 40 kHz. The thickened filtrate was cooled to room temperature and then the pectin was then precipitated by adding ethanol which had been acidified by adding 2 ml of HCl per one liter of ethanol. The ratio of filtrate to added acid ethanol is 1:1.5. The deposition process was carried out for 12 hours. The pectin precipitate formed was filtered using a vacuum to separate the pectin precipitate from the acid ethanol and water solution. The pectin precipitate obtained was washed using 96% alcohol until it was free of chloride. Separation of pectin precipitates from ethanol used from washing is carried out using vacuum. To determine the presence of chloride, a few drops of silver nitrate ($AgNO_3$) solution was added to the used washing liquid. If chloride is still present, a white precipitate (AgCl) will form. The final stage, the washed wet pectin was dried in an oven at 40^0C.

Results Analysis

The dry pectin obtained was tested qualitatively by looking at the description and carrying out an identification test. After that, a quantitative testing was carried out by determining the levels of methoxyl and galacturonic acid with the characteristics were fine powder, yellowish white and almost odorless.

Identification process was conducted by adding 1 gram of pectin and 9 ml H_2O, a stiff gel will then formed when cooled. By a ratio of 1:100 ethanol was then added with a ratio of 1:1 vol., a clear precipitate will then form. 5 ml solution was taken (1 in 100) and added by using 1 ml of NaOH 2N, it was then left at room temperature, then a gel will form. The gel was then acidified with 3N HCl, it was then shaked, and after precipitation process a gelatin (colorless) will form with a white color and clump shape.

Determination of Methoxyl Group content obtained by adding 500 mg of pectin powder, then it was dissolved in 2 ml of 96% ethanol and 100 ml of distilled water. It was then titrated by using 0.1 N NaOH and 5 drops of MO indicator. Once it reached the final point with a pink color, it indicated the volume used was the V1. 20.0 ml of 0.1 N NaOH was then added, and then it was shake and rest for 15 minutes, 20.0 ml of 0.1 N HCl was added again, and it was shake until the pink color disappears. An MO indicator was then added and continued by titration process using 0.1 N NaOH. The final point obtained is Seulas Pink, it was assumed to be the obtained V2. The Methoxyl content was then calculated by using Eq. (1) where the BM of Methoxyl ~ 31 gr/mol –OCH_3.

$$\text{Methoxyl Content} = \frac{V_2 \times N_{NaOH} \times BM_{Metoksil}}{berat\ pektin} \times 100\% \quad (1)$$

Determination of Galacturonic Acid Levels can be determined by determining methoxyl levels, namely by adding up the volume in the first titration (V1) and the volume in the second titration (V2) whereas (BM Galacturonate ~ 194 gr/mol $C_6H_{10}O_7$). Calculation of Galacturonate Acid content was done by using Eq. (2).

$$\text{Galacturonate Content} = \frac{[(V_1 \times V_2)] \times N \times BM_{galakturonat}}{berat\ pektin} \times 100\% \quad (2)$$

RESULTS AND DISCUSSION

The results of this study were the observations of pectin yield, methoxyl content and galacturonic acid content by extracting kepok banana peels weighing ± 15 grams with variations in time as shown in Table 1.

Table 1. Results of ash content analysis.

Extraction Time (Minute)	Extraction Temperature (°C)			Food Chemical Codex Standards
	70	80	90	
60	8.37	8.22	7.89	12%
80	8.02	7.88	7.28	12%

The results of the water content analysis are also shown in Table 2.

Table 2. Water content analysis.

Extraction Time (Minute)	Extraction Temperature (°C)			*Food Chemical Codex Standards*
	70	80	90	
60	0.24	0.28	0.31	1%
80	0.30	0.34	0.36	1%

The results of the ash content analysis showed that the highest water content was obtained at an extraction time of 80 minutes with a temperature of 90°C, while the lowest ash content was at an extraction time of 60 minutes with an extraction temperature of 70 °C. The overall results obtained were that the ash content in the samples was still below the 10% required by IPPA.

The highest pectin water content was at an extraction temperature of 70°C with an extraction time of 60 minutes compared to the pectin content at a temperature of 90 °C with an extraction time of 80 minutes. The water content results show that the water content is at the standard required by the International Pectin Producer Association (IPPA), which is no more than 12%. The high water content of the pectin produced is influenced by the degree of drying of the pectin which is not optimal, so that the water contained in the material is not completely evaporated.

Methoxyl levels

Methoxyl content is defined as the amount of methanol contained in pectin. Based on its methoxyl content, pectin is divided into 2 types, namely (Tambunan et al., 2022) (1) High methoxyl pectin, i.e. methoxyl content >7% and (2) Low methoxyl content, that is, the methoxyl content is <7%

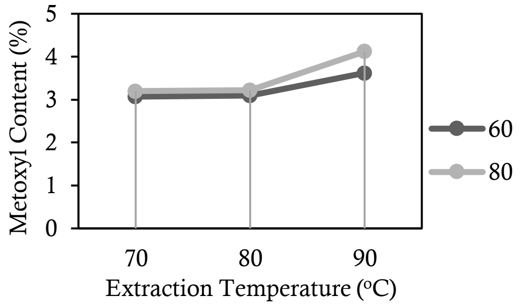

Figure 1. Methoxyl content of banana pectin.

The results from Figure 1 were determining the methoxyl content obtained the highest methoxyl content at a temperature of 90°C with an extraction time of 80 minutes. Methoxyl levels increased with increasing temperature and extraction time. This is because the free carboxyl groups being esterified are increasing (Sigiro et al., 2022). Figure 1 shows that the methoxyl content is below 7. The higher the methoxyl content in pectin will indicate that it is more easily soluble in water. Pectin with a high methoxyl content can dissolve in cold water if sufficient stirring is done, while pectin with a low methoxyl content requires heat to dissolve in water.

Equivalent Weight

Equivalent weight in Figure 2 is the content of free, unterified galacturonic acid groups in the pectin molecular chain. The equivalent weight price is determined based on the saponification reaction of the carboxyl group by NaOH where the equivalent weight will be inversely proportional to the volume of NaOH used to react with the carboxyl group. Pure pectic acid is a pectic substance which is composed entirely of polygalacturonic acid which is free from methyl ester groups or does not undergo esterification. The lower the pectin content, the lower the equivalent weight (Hari et al., 2022).

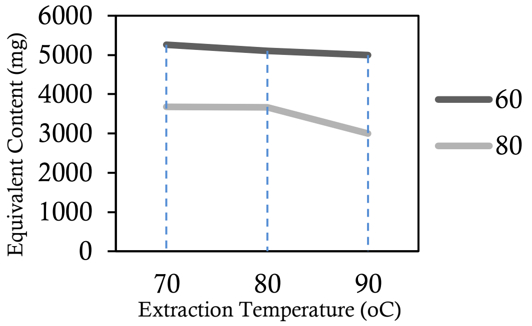

Figure 2. Equivalent content of banana pectin.

The results of the pectin in Figure 2, characterization analysis showed that the equivalent weight did not meet existing standards because the IPPA standard showed a range of 600-800 mg. This is because it is likely that the banana peel waste used is not fully ripe and the titration treatment carried out.

Galacturonic Acid Levels

The results of calculations based on equivalent weight and methoxyl content obtained the highest galacturonate content or galacturonic acid levels in Figure 3, at an extraction time of 80 minutes with a temperature of 90°C, the percentage of galacturonate was 79.96% and the highest degree of esterification in the same variation, namely 80 minutes with a temperature of 90°C, was 29.25%.

The results obtained in Figure 3, indicate that the galacturonate content and degree of esterification still meet the IPPA requirements (Sigiro et al., 2022). Galacturonate content describes the purity of pectin because galacturonic acid is the basic framework of pectin compounds. Galacturonic acid levels increase with increasing temperature and length of extraction time due to the hydrolysis of protopectin into pectin whose basic component is *D*-Galacturonic acid. The glycosidic bonds of the methyl ester groups of pectin tend to be hydrolyzed and produce galacturonic acid. This causes galacturonic acid levels to increase (Azizet al., 2018).

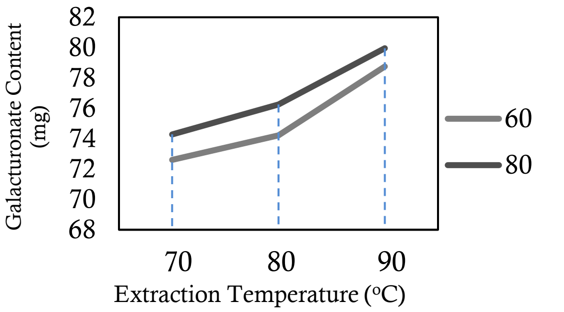

Figure 3. Galacturonate content of banana pectin.

CONCLUSION

The results of the overall research can be concluded that Kepok banana peels, which often become waste, can actually be used to make pectin flour. The optimum conditions obtained from the extraction of Kepok banana peel are at a temperature of 90⁰C for 80 minutes, with a methoxyl content of 4.12% (low methoxyl) and a galacturonic acid content of 79.96%. This can prove that banana peel waste can be used as pectin as a basic ingredient in making edible coatings. To obtain maximum results, the manufacture of edible coatings can be combined with sappan wood extract to improve the performance of banana peels which can become semipermeable coatings by reducing cellular respiration (O_2 and CO_2), as well as the rate of oxidative reactions and suppressing the population of pathogenic microorganisms.

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