Extraction of pectin from banana (*Musa acuminata x balbisiana*) peel waste flour using crude enzymes secreted by *Aspergillus niger*

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**Abstract.** Intensive cultivation has maintained annual banana production in Indonesia at about 7 million tons since 2015. Banana fruits bear about 35 - 40% weight of peel waste, which contains 20.28 g pectin/100 g the dry matter. The peel waste may trigger serious environmental problems. This study aimed to investigate the effect of substrate concentrations and time on the pectin yield extraction from banana peel waste flour using crude pectinase secreted by *Aspergillus niger*. Crude pectinases were prepared through batch fermentation of banana peel flour using *A. niger*. The enzymes were used to hydrolyze banana peel waste flour at various substrate concentrations and time at 323 K. The results indicate that the increase of both extraction parameters leads to increase the pectin yield to some extent and then level off. However, further increase of both extraction parameters cause inhibition by galacturonic acid. The highest pectin yield (10.8% weight) was achieved at 323 K and 0.067 g/mL substrate concentration for 120 minutes. Applications of this result are expected to increase the economic value of banana peel waste. However, further study in the effect of other extraction parameters such as temperature, enzyme loading, pH, flour size and pectin purification are required.

1. **Introduction**

Indonesia has successfully encouraged its farmers to cultivate Kepok Kuning banana (*Musa acuminata x balbisiana*) as one of most preferred horticultural products. Therefore, the annual production of this banana species in Indonesia has been very stable at more than 7 million tons since 2015 [1]. However, upon consumptions either as fresh fruit or in the form of processed food products, banana fruits leave about 35 – 40% weight of peel waste [2-3]. Indeed, these peels are usually just left as solid waste, used as fertilizer or disposed in most countries, which may trigger serious environmental and aesthetic problems [4]. Surprisingly, the peel of culled bananas and plantains could be a rich, low-cost source of dietary fibre, mainly hemicelluloses and pectin polysaccharides [4], which are higher than those of other fruit peels in all stages of maturity [4]. Banana peels also harbour a large amount of high methoxyl pectins (9-24.08%) [5-7], which offer possibility to form jelly without the addition of artificial gel additives. Jelly is a preferable dessert almost by all age groups, primarily due to its excellent digestibility.
and attractive texture. Unfortunately, banana peel is still an underused source of food materials because it brings poor flavour and texture.

As a naturally complex hetero polysaccharide, pectin can be easily found in the primary cell wall of dicotyledonous plants and has been extensively used as a gelling agent, thickener, stabilizer, emulsifier and edible coating in food industry as well as wide application in cosmetic and pharmaceutical industries. It is composed of D-galacturonic acid, L-rhamnose, L-arabinose and D-galactose. They are linked by α (1 to 4) linkages [8]. Recovery of pectin is an important unit operation in food industry in order to cope sufficient supply for the increasing demand. Although pectin is commonly extracted at high temperature by hydrolysis of proto-pectin into pectin at industrial scale, but there have been some novel views in pectin production [9]. This conventional extraction method is carried out by two main steps, i.e. acid hydrolysis of proto-pectin in to pectin and followed by subsequent precipitation with ethanol [10]. However, acid hydrolysis has several limitations, such as large water usage, environmental problems and high wastewater treatment cost and corrosion of the equipment due to the use of large volume of hot strong acid [11]. This issue has caused novel methods such as microwave assisted extraction, enzymatic extraction, supercritical water extraction and ultra sound extraction have become more popular [12]. Microwave assisted extraction exhibits large handling capacity, short processing time and good purity. The enzymatic extraction offers more attractive advantages over other extraction methods because it provides higher yields by breaking down the complex structure of raw material, ability to selectively remove undesirable components of raw material, high catalytic efficiency and preserves the original efficacy of natural products and reduces the time of extraction and volume of solvent used. This method also shows its superiority over other extraction methods as it is environmentally friendly and saves more energy because it can be effectively performed at low to moderate temperatures [13-14].

Enzymatic extraction of pectin is usually conducted by employing pectinases, which are a group of related enzymes responsible to the breakdown of pectin from various plant resources. These enzymes are grouped based on their preferred substrate (pectin, pectic acid or oligo D-galacturionate), the degradation mechanism (transelimination or hydrolysis) and the type of cleavage (random [endo-] or terminal [exo-]) [15-16]. A number of publications related to the production of pectinolytic enzymes using various botanical sources and the effect of extraction parameters, such as temperature, aeration rate and type of fermentation are available in the literature [17]. In addition, different types of microorganisms have been applied for the production of pectinolytic enzymes. Those enzymes have been reported to be produced by a large number of bacteria and fungi such as Bacillus spp., Penicillium spp., Clostridium spp., Aspergillus spp., Monilla laxa, Fusarium spp., Pseudomonas spp., Verticillium spp., Sclerotinia libertiana, Polyporus squamosus, Coniothyrium diploidiella, Thermomyces lanuginosus, etc. [18-19]. It has also been reported that the commercial enzymes originated from a selected strain of Aspergillus niger exhibit mixed activities of pectinlyase, pectinesterase and polygalacturonase [20]. Unfortunately, enzymes derived from A. niger are vulnerable to retardation by their monomeric product, especially the D-galacturonic acid, which is a strong comparative inhibitor of polygalacturonase enzyme [21]. The D-galacturonic acid is usually produced in a lengthy hydrolysis time. In addition, the pectinases may also experience inhibition by the substrate, which usually takes place at high substrate concentration [22].

Considering the promising advantages of enzymatic extraction method compared to other extraction methods and the environmental issues related with the disposal waste by food processing industries, this current research aims to investigate the effect of extraction time and substrate concentration on the yield during extraction of pectin from banana peel wastes at 323 K using crude enzymes secreted by the fungus of Aspergillus niger. It is highly expected that the successful extraction of pectin from banana peel waste flour could be applied by small and medium food enterprises as well larger food industries for pectin productions from any pectin rich agricultural residues.
2. Materials and method

2.1. Materials

The peels of mature banana were collected from student cafes nearby the Department of Chemical Engineering, Faculty of Engineering - Universitas Diponegoro, Indonesia that serve fried banana as the main dish. They were washed three times with fruit detergent to remove dirt by employing flowing water. The clean banana peels were then dried in a natural convection oven (DVS402; Yamamoto Co., Tokyo, Japan) for 48 hours to dryness before being ground with a commercial electric spice grinder (Nima NM-8300, Shenzhen Zhiyuan Technology Co. Ltd, China) into 60 mesh size and stored at -15°C in airtight polypropylene plastic containers for no longer than one week. The chemicals used in this research were analytical grade (purity ≥ 98% w/w) being the product of Sigma-Aldrich, Singapore. They were procured from local authorized chemicals distributor in Semarang, Indonesia and directly used without prior treatment. The isolated strain of Aspergillus niger NRRL A-II,264 mold was purchased from Research Center for Biotechnology, Universitas Gadjah Mada, Indonesia. Pure cultures were obtained by subculturing and further subculturing on potato dextrose agar (PDA). These pure cultures strains were maintained on potato dextrose agar (PDA) slants and were further sub cultured periodically during the course of this research.

2.2. Solid-liquid extraction

The extraction of pectin from banana peel wastes flour was performed using a solid-liquid extraction system employing crude enzyme secreted by Aspergillus niger as the degrading enzyme. The details of the procedure of the experiments are as follows:

2.2.1. Preparation of culture media. The culture media used for pectinase production was a mixture of 50 mL mineral solution, 20 g of banana peel wastes flour as the sole carbon source and 150 mL distilled water as suggested by Khairnar et al. with slight modification [20]. The mineral solution was composed of (g/L): (NH₄)₂SO₄, 4.50; K₂HPO₄, 1.50; MgSO₄·5H₂O, 0.05; CaCl₂, 0.025; and FeSO₄·5H₂O, 0.10. The pH of the solution was adjusted to 5.5 before sterilization.

2.2.2. Preparation of crude enzymes. Prior to submerged fermentation, the culture media was autoclaved at 121 °C for 20 minutes for sterilization purpose. Shake flask cultivations were carried out according to the method developed by Khairnar et al. [20]. The cultivations were performed in seven 250-mL Erlenmeyer flasks of 200 mL working volume, which were inoculated with one disc of actively growing Aspergillus niger from a 96 hours-old culture medium containing approximately 4 × 10⁴ spores/mL. The inoculated flasks were plugged properly and incubated in a temperature-controlled rotary shaker (Innova 4080, New Brunswick Scientific, Edison, NJ, USA) at 150 rpm and 30 °C for 72 hours. The aliquot of the crude enzyme extract was withdrawn and filtered using Whatman No.1 filter paper. The filtrate was further centrifuged at 10,000 rpm for 10 minutes at 27 °C in test tubes to obtain supernatant for use as the source of crude enzyme in the extraction of pectin.

2.2.3. Extraction of pectin. A carefully measured crude enzyme (10 mL) was introduced to 250 mL Erlenmeyer flasks and 140 mL of 0.05 M sodium acetate buffer (pH 5.5) was added to obtain 150 mL extraction media. This solution was incubated in a shaking incubator (HB-201SL, Hanbaek, Bucheon, South Korea) at 323 K at 150 rpm as suggested by Dinu et al. [23] and Locatelli et al. [24]. Upon the achievement of desired temperature, a carefully weighed banana peel wastes flour (5 g) was introduced to the extraction media and let to hydrolyze for 360 minutes. The hydrolysates were taken at 30 minutes interval for pectin analysis after being filtered using Whatman No. 2 filter paper. Similar procedure was applied for the study of the effect of substrate concentrations (5 to 18.5 g banana peel flour), which coincide with 0.03 to 0.13 g/mL substrate concentrations.
2.2.4. Analysis. The total extractable pectin was quantified from the extraction media via ethanol precipitation method as suggested by Jeong et al. [12]. The mixture of enzymatic hydrolysate and 95% ethanol (1:3 (v/v)) was stirred for 5 h and centrifuged at 13,000g for 10 min. After the supernatant was discarded, the sediment was further washed with 65% ethanol and centrifuged at 10,000g for 15 min. The washing and centrifuging processes were repeated 3 or 4 times and then the obtained pectin was lyophilized and ground into flour. A functional groups identification of the extract sample using Fourier-transform infrared spectroscopy (FTIR) was performed using the protocol previously described by Khamsucharit et al. to ensure that pectin was obtained from the proposed enzyme assisted extraction [25]. The pectin yield was determined by the ratio of the weight of the extracted pectin to the initial banana peel waste flour.

3. Results and discussion

As the fermentation process goes by, it was obvious that the banana peel wastes flour underwent hydrolysis by the enzyme. Visual observation revealed the dissolution of soluble portion of banana peel waste flour in the extraction media.

3.1. Effect of extraction time

The profile of pectin yields during enzymatic hydrolysis of banana peel wastes flour using crude enzymes as proto-pectin degrading agent at various extraction time (0 to 360 minutes) is depicted Figure 1. Figure 1 shows that the yield of pectin obtained from extraction process through enzymatic hydrolysis of banana peel wastes flour increases almost linearly from the beginning of the extraction and reaches a maximum value (10.80 % w/w) after 120 minutes. Haslaniza et al. reported that a longer hydrolysis time would promote enzyme to degrade pectic substance more extensively, which finally results in higher yield of pectin [26]. However, prolonged hydrolysis time to 270 minutes causes significant decrease of the yield of pectin in the reverse way. Furthermore, extension the hydrolysis time also caused gradually reduction in the yield of pectin. The decrease in the yield of pectin can be due to the depletion of nutrients in the medium [27]. Similar observations were reported by several authors [28-31] where the degree of hydrolysis increased with the increase in hydrolysis time. The increasing degree of hydrolysis was also caused by the increased cleavage of 1,4-glycosidic bonds which then increases the pectin solubility in water [31, 36].

![Figure 1. Effect of extraction time on the yield of pectin.](image-url)
3.2. Effect of substrate concentration.
The ability of crude enzyme secreted by *Aspergillus niger* to extract pectin from its precursors in the banana peel flour can also be affected by the amount of banana peel flour in the hydrolysis system. The profile of pectin yield obtained at various substrate concentration (0.0333 to 0.1233 g/mL) is depicted in Figure 2. As expected, the increase of banana peel flour concentration from 0.03 g/mL to 0.10 g/mL leads to logarithmic increase of the yield of pectin and reaches a maximum value of 10.8 % w/w. Enzymes will function maximally if there is adequate amount of substrate. As the concentration of the substrate increases, so does the rate of enzyme activity. Unfortunately, the rate of enzyme activity will not increase forever. This is because a condition will be achieved when the enzymes become saturated and no more substrates can fit at any one time although there is plenty of substrates available. An optimum rate is reached at the enzyme’s optimum substrate concentration. Bhaskar et al. also found that the degree of hydrolysis enters a stationary phase at enzyme levels beyond 0.015 g/mL in their optimizing work of protein hydrolysate from viscera waste protein of catla by using alcalase [28].

![Figure 2](image_url)

**Figure 2.** Effect of substrate concentration on the yield of pectin.

As seen in Figure 2, further increase of banana peel flour concentration from 0.10 to 0.13 % w/v in the extraction media gradually decreases the yield of pectin. This is because the activity of the enzyme decreases when substrate concentration increases beyond 1%; this may be due to the fermentation of other metabolites during fermentation and also because of increase in viscosity of the hydrolysis media [27]. Enzyme activity may decrease as a result of substrate and product inhibitions on the active sites of the enzyme through the binding of enzyme and the substrate so that they cannot react with the remaining unbound substrate [33]. The decrement of enzyme activity leads to slower the rate of cleavage of 1,4-α-D-glycosidic of the pectic acid and pectin, which in turn reduces the yield of pectin [34]. Gummadi and Panda also proved that pectinase might undergo a combined mechanism of protective diffusion mechanism due to high substrate concentration and substrate inhibition [22].

3.3. Pectin identification
To confirm the chemical structure of the pectin obtained from this work, the pectin extract was subjected to an FTIR analysis. FTIR spectra in the wavenumber range of 800 and 1300 cm\(^{-1}\) are used as the ‘fingerprint’ zone for carbohydrates, which enable an ascertainment of major chemical groups specific for distinctive polysaccharides [35]. Purcell and Fishman suggested that pectin should produce an ester carbonyl band (C=O) at 1730 –1760 cm\(^{-1}\) and a carboxylate (COO\(^{-}\)) antisymmetric stretching band at
1600 –1630 cm\(^{-1}\) [36]. Hence, the transmittance bands perceived at 1732.13 cm\(^{-1}\) and 1627.18 cm\(^{-1}\) in the FTIR spectrum obtained in this work are regarded to be the functional identity of pectin, which respectively correspond to the stretching vibration of esterified carbonyl groups and free carboxyl groups [37].

4. Conclusion
An enzymatic extraction study of pectin from banana peel waste flour using *Aspergillus niger* in a batch fermenter at various substrate concentrations and extraction time has been studied. Both substrate concentrations and extraction time lead to increase the yield of pectin to some extent and then level off. It was supposed that both high substrate concentration and lengthy extraction time triggered product inhibition by galacturonic acid. The best operating condition for this extraction process is at 328K and 0.13 g/mL substrate concentration for 120 minutes to obtain 10.8% w/w yield of pectin. Prior to commercial applications of these results, further studies in the effect of other extraction parameters such as temperature, enzyme loading, pH and flour particle size as well as the purification methods of the pectin are still needed.

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