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Pectin Derived from Hydrolysis of Ripe *Kepok Kuning* Banana Peel Powder Employing Crude Pectinases Produced by *Aspergillus niger*

(Pektin Terhasil daripada Hidrolisis Serbuk Kulit Pisang Kepok Kuning Matang menggunakan Pektina Mentah yang Dihasilkan oleh *Aspergillus niger*)

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ABSTRACT

Banana fruits consumption generates about 35% weight of peel waste containing approximately 10.61 to 24 w/w% of pectin. Hence, improper banana peel waste management may induce various environmental and health issues. The objectives of this work were to study the effect of substrate concentration, pH, temperature, and duration on the yield of pectin extracted from enzymatic hydrolysis of banana peel powder. In this work, the crude enzymes were obtained via submerged fermentation of *Kepok Kuning* banana peel powder utilising *Aspergillus niger* and directly used without prior purification. Pectin extraction from banana peel powder was performed through hydrolysis using crude pectinases at various substrate concentrations (0.033 to 0.123 g/mL), pH (4.0 to 6.0), and temperature (40 to 70°C) for 180 min. The increase of extraction parameters enhanced the pectin yield to a maximum value and then declined. High substrate concentration, temperature, pH, and monomeric pectin compounds formation at long hydrolysis duration were found to reduce enzyme activity. A recommended extraction condition is using 0.103 g/mL substrate concentration, pH 5.0, and 55 ℃ for 120 min to achieve 10.80% weight yield. Commercial implementations of the results can be worthwhile in solving the environmental problem and enhance the economic value of pectin-rich fruit peels and other agricultural wastes.

Keywords: Banana peel; crude pectinase; extraction condition; hydrolysis

ABSTRAK

Penggunaan buah pisang menjana kira-kira 35% berat sisa kulit yang mengandungi kira-kira 10.61 hingga 24 w/w% pektin. Oleh itu, pengurusan sisa kulit pisang yang tak wajar boleh menyebabkan pelbagai isu alam sekitar dan kesihatan. Objektif kertas ini adalah untuk mengkaji kesan kepekatan substrat, pH, suhu dan tempoh ke atas hasil pektin yang diekstrak daripada hidrolisis enzim serbuk kulit pisang. Dalam kajian ini, enzim mentah diperoleh melalui penapaian terendam serbuk kulit pisang Kepok Kuning menggunakan *Aspergillus niger* dan digunakan terus tanpa penulenan terlebih dahulu. Pengekstrakan pektin daripada serbuk kulit pisang dilakukan melalui hidrolisis menggunakan pektinase mentah pada pelbagai kepekatan substrat (0.033 hingga 0.123 g/mL), pH (4.0 hingga 6.0), dan suhu (40 hingga 70 ℃) selama 180 min. Peningkatan parameter pengekstrakan meningkatkan hasil pektin kepada nilai maksimum dan kemudian menurun. Kepekatan substrat yang tinggi, suhu, pH dan pembentukan sebatian pektin monomer pada tempoh hidrolisis yang panjang didapati mengurangkan aktiviti enzim. Keadaan pengekstrakan yang disyorkan adalah menggunakan kepekatan substrat 0.103 g/mL, pH 5.0 dan 55 °C selama 120 min untuk mencapai hasil berat 10.80%. Pelaksanaan hasil komersial boleh memanfaat dalam menyelesaikan masalah alam sekitar dan meningkatkan nilai ekonomi kulit buah yang kaya dengan pektin dan lain-lain sisa pertanian.

Kata kunci: Hidrolisis; keadaan pengekstrakan; kulit pisang; pektinase mentah

Introduction

Kepok Kuning banana or widely known as Saba banana (*Musa acuminata x balbisiana*) is a preferential horticultural commodity. Hence, Indonesia has established its annual banana production at more than 7 million tons since 2015 (BPS 2018) and placed Indonesia as one of the world's major banana producers along with India, China, Philippines, Ecuador, and Brazil (Padam et al. 2014). Unfortunately, consumption of banana fruits both as fresh fruit or processed foods generates about 35 - 40% weight of peel waste (Naggarajaiah & Prakash 2011). In some countries, this fruit waste is predominantly just left as solid waste, utilized as organic fertilizer, or disposed of offhandedly, which can provoke numerous environmental and aesthetic issues. Surprisingly, the culled plantains and banana peels could be a plentiful and inexpensive dietary fibre source, primarily hemicelluloses and pectin polysaccharides, which are greater than any other fruit by-products in all degrees of maturity (Zhang et al. 2005). Based on their study on five different species (AA, AAA, and ABB genotypes) of banana, Khamsucharit et al. (2018) showed that unripe banana peels bear a large amount of high methoxyl pectin (15.89 - 24.08 w/w%), whereas Barman et al. (2015) found slightly lower (13.05 w/w%) pectin content of dried ripe banana (AAA genotype) peel powder. Castillo-Israel et al. (2015) observed that pectin yields were 11.87 and 16.54%, respectively, for ripe and unripe Saba banana (ABB genotype) peels. However, there were different equations used by Khamsucharit et al. (2018) and Castillo-Israel et al. (2015) to calculate pectin yield. When the pectin yields are recalculated using the equation of Khamsucharit et al. (2018), the pectin yields of ripe and unripe Saba banana peels reported by Castillo-Israel et al. (2015) were 10.61 and 14.19 w/w%, respectively. Pectin has the ability to form jelly even without the addition of synthetic gelling agents, emulsifiers, and thickeners. Jelly is a highly popular dessert of almost every age range, predominantly caused by its superior digestibility and excellent texture. Nevertheless, banana peels remain underused food materials due to their unpleasant flavour and texture.

Being a natural complex heteropolysaccharide, pectin comprises of D-galacturonic acid, L-rhamnose, L-arabinose, and D-galactose, which are connected by α (1 to 4) linkages (Girma & Worku 2016). Large-scale production of pectin is one the foremost unit operations in food manufacturing to address the worldwide increasing demand of pectin. Although commercially pectin is extracted through proto-pectin hydrolysis using high temperature dilute mineral acid at pH 2 for 2 - 4 h, some newer methods have also been developed to produce pectin (Kanmani et al. 2014). Generally, the traditional pectin production method is performed by two consecutive stages, namely, the acid hydrolysis of proto-pectin in to pectin and followed by precipitation of pectin using antisolvent, either ethyl or isopropyl alcohol (Wang et al. 2014). Unfortunately, acid hydrolysis suffers from serious shortcomings, which cause the newer methods, such as ultrasound-assisted extraction, enzymatic extraction, supercritical water extraction, and microwave-assisted extraction have demonstrated more attractive prospects (Jeong et al. 2014). Both microwave-assisted and ultrasound-assisted extractions have been reported to exhibit vast operating capacity, shorter duration, and high purity. Nonetheless, the enzymatic extraction provides even higher attractiveness than any other method due to its higher yield by decomposing the complex structure of the starting material, excellent selectivity, high efficiency, and remarkable ability to preserve the biological activity of the products and shortens the extraction time and reduces the solvent volume. In addition to its lower energy requirement, the enzymatic extraction is also more environmentally benign (Liew et al. 2015). Commonly, the enzymatic extraction of pectin utilises pectinases, which are specific pectinolytic enzymes for the degradation of pectic substances from the cell wall of various botanical sources. These pectinolytic enzymes are classified according to their target substrates (either pectin, pectic acid, or oligo D-galacturonate), the decomposition route (hydrolysis or trans-elimination) and the mechanism of molecular chain cleavage (random [endo-] or end [exo-]) (Kashyap et al. 2001). Numerous previous researches on the synthesis of pectinolytic enzymes from different plant sources and the influence of extraction parameters, namely, the type of fermentation, aeration rate, and temperature have been reported in previous publications (Antov & Peri˘cin 2001). In addition, various kinds of microorganisms have been employed to produce pectinolytic enzymes, which are mainly bacteria and moulds including *Aspergillus* spp., *Bacillus* spp., *Clostridium* spp., *Coniothyrium diplodiella, Fusarium* spp., *Monilla laxa, Penicillium* spp., *Polyporus squamosus*, *Pseudomonas* spp., *Sclerotinia libertiana, Thermomyces lanuginosus,* and *Verticillium* spp. (Sandarani 2007). The commercial enzymes originating from a chosen strain of *A. niger* demonstrate cocktail activities of pectinlyase, pectinesterase, and polygalacturonase (Khairnar et al. 2009). The highest pectinase production (8.0 U/mL) using *A. niger* ABT-5

was achieved at 72 h of incubation in medium containing 0.2 g/mL wheat bran as substrate, pH 6.0 and temperature of 30 °C (Abdullah et al. 2018), while Barman et al. (2015) obtained 6.7 U/mL polygalaturonase (PG) when 8.07 w/v% banana peel powder was used as substrate for incubation at pH 5.8 and 32.37 °C for 65.82 h. Endo-PG catalyses random cleavage of the substrate, while and exo-PG catalyses hydrolytic cleavage at the non-reducing end of the substrate to produce monogalacturonate or digalacturonate in some cases are more active in acidic or neutral medium (pH 4.0 - 7.0) at temperatures between 40 and 60 °C (Dinu et al. 2007). Although partial purification could increase pectinase activity more than three times than that of these crude pectinolytic enzymes, this process requires a long precipitation time and a higher operating cost (Barman et al. 2015).

Bearing in mind the predominant performance of enzymatic extraction compared to any other extraction methods and the increasingly stringent environmental regulations related to the disposal of agriculture-based industry wastes, this present work aims to study the influence of concentration, pH, and temperature on the yield during the extraction of pectin from banana peels powder using crude pectinases produced by *A. niger*. Applications of the results in commercial pectin extraction process and equipment design are expected to be worthwhile in solving the environmental problem and enhance the economic value of banana and similar fruits and other pectin-rich agricultural wastes.

Materials and Methods

Materials

The ripe *Kepok Kuning* banana (*Musa acuminata* × *Musa balbisiana*, ABB genotype) peels were obtained from some food stalls nearby Universitas Diponegoro Campus (Indonesia) that sell various banana-based dishes. They were washed several times using vegetable detergent to get rid of the dirt with the help of continuous flowing water (Kumoro et al. 2020). The peels were then dehydrated in a laboratory-scale oven (DVS402; Yamamoto Co., Tokyo, Japan) at 45 °C to about 5% moisture content, pulverised using a spice grinder (Nima NM-8300, Shenzen Zhiyuan Technology Co. Ltd, China) to obtain banana peel powder with 80 mesh average particle size and deposited it in zipped polyethylene plastic bags at 5 °C for further use. The analytical grade chemicals (Sigma-Aldrich with purity ≥ 98% w/w) used in this work were bought from an authorized chemical store in Semarang, Indonesia, and immediately utilised without pre-treatment. The pure strain of *A. niger* NRRL A-II 264 fungus was procured from Pusat Antar Universitas-Bioteknologi, Universitas Gadjah Mada, Indonesia. Pure cultures were reproduced by sub-culturing them on potato dextrose agar (PDA). These pure culture strains were kept on the slants of PDA and were subsequently sub-cultured occasionally along the period of the study (Kumoro et al. 2020).

Methods

Preparation of Cultivation Medium

The cultivation media used for pectinases production was prepared by mixing of 7.5 g of *Kepok Kuning* banana peel powder, 50 mL mineral solution, and 50 mL aquadest as previously used by Barman et al. (2015). The mineral solution contained 0.04 (g/mL) $(NH_4)_2SO_4$, 0.02 (g/mL) KH_2PO_4, 0.01 (g/mL) $MgSO_4.5H_2O$, 0.001 (g/mL) $MnSO_4$, and 0.10 (g/mL) $FeSO_4.5H_2O$. The solution acidity was maintained at a pH of 5.8 using sodium acetate buffer solution before autoclaving it at 121 °C for 20 min for sterilisation.

Preparation of Crude Pectinases

Shake flask cultivations were conducted following the method previously employed by Barman et al. (2015). Seven sets of parallel cultivation with 200 mL working volume were carried out by inoculating one disc of actively growing *A. niger* from 96 h old culture media harbouring about 4 × 10^8 spores/mL using 250 mL Erlenmeyer flasks. The inoculation flasks were thoroughly lidded and incubated in an isothermic rotary shaker (Innova 4080, New Brunswick Scientific, Edison, NJ, USA), which was operated at 32.5 °C, pH of 5.8 and 150 rpm for 72 h. The extract of the crude pectinases was collected and strained using Whatman No.1 filter paper. The supernatant was separated from the filtrate in several test tubes using a refrigerated centrifuge (4 °C) at 10,000 rpm for 10 min. The collected clear supernatants, which contained about 6.5 U/mL were directly utilised as the source of crude pectinases employed in the enzymatic hydrolysis (Kumoro et al. 2020).

Banana Peel Powder Enzymatic Hydrolysis for Pectin Extraction

A carefully prepared crude pectinases (6.2 mL) was placed in 250 mL Erlenmeyer flasks and followed by addition of 140 mL of 0.05 M sodium acetate buffer (pH 4.5) to obtain 150 mL extraction medium. This mixture was incubated in a shaking incubator (HB-201SL,

Hanbaek, Bucheon, South Korea) operated at 150 rpm and 40 °C (Dinu et al. 2007). Once the desired temperature has been achieved, a precisely weighed banana peel powder (12.5 g) was gently poured into the extraction medium to give a substrate concentration of 0.083 g/mL and allowed to undergo hydrolysis for 180 min. For the study of hydrolysis duration, the hydrolysate samples were withdrawn every 15 min interval and filtered using Whatman No. 2 filter paper for pectin content determination. The one factor experimental design was utilised throughout the study where only one parameter was varied for each hydrolysis. The enzymatic hydrolysis experiments consisted of the investigation of the influence of substrate concentrations (0.033, 0.043, 0.053, 0.063, 0.073, 0.083, 0.093, 0.103, 0.113, and 0.123 g/mL), pH (4.0, 4.5, 5.0, 5.5, and 6.0), and temperature (40, 45, 50, 55, 60, 65, and 70 °C) using the optimum hydrolysis time obtained from hydrolysis duration study. Those hydrolysis parameters were chosen based on the fact that their effects were more pronounced than the mixing conditions and particle size when enzyme loading was fixed (Kumar et al. 2016).

PECTIN YIELD AND CHARACTERISTICS DETERMINATIONS

The total extractable pectin was determined from the extraction broth using the ethanol precipitation method as previously used by Khamsucharit et al. (2018) with necessary modifications. To ensure that all the extracted pectin can be completely precipitated, a preliminary experiment was performed by varying the ratio of the volume of enzymatic hydrolysate and 95% ethanol containing 0.05 M hydrochloric acid used for pectin precipitation, which resulted in a ratio of 1:3 (v/v) was sufficient to facilitate complete pectin precipitation. The enzymatic hydrolysate and 95% ethanol containing 0.05 M hydrochloric acid (1:3 (v/v)) mixture was agitated for 5 min and left to stand for 24 h to allow the entire pectin to precipitate. After the supernatant was removed, the precipitates were further washed three to four times with 95% ethanol before being dried at 40 °C in an air oven to dryness and pulverized into powder. The yield of pectin was defined as the weight ratio of the extracted pectin to the initial weight of banana peel waste powder. The equivalent molecular weight (EMW) and methoxyl content (MeO) of the pectin were examined by a method previously used by Khamsucharit et al. (2018), the anhydrouronic acid content (AUA) and degree of esterification (DE) were further calculated using the formula of Castillo-Israel et al. (2015), respectively. The functional groups contained in the pectin sample were identified employing the Fourier-transform infrared spectroscopy (FTIR) according to the procedure as previously described by Khamsucharit et al. (2018).

The pectin yield, equivalent molecular weight, degree of esterification, and methoxyl and anhydrouronic acid contents were determined from triplicate measurements. The collected numerical data were then statistically analysed using Statistical Package for the Social Sciences (SPSS) version 28 for analysis of variance (ANOVA test). The differences among the means were tested for significance *($p < 0.05$)* using the Duncan's multiple range tests (DMRT).

RESULTS AND DISCUSSION

As the incubation procedure occurred, it was evident that the *Kepok Kuning* banana peel waste powder experienced enzymatic hydrolysis. Perceivable inspection exhibited a noticeable separation of the soluble portion of banana peel waste powder in the extraction broth.

INFLUENCE OF HYDROLYSIS DURATION

Among the pectin hydrolysing enzymes, (endo) polygalacturonases have been reported to be the predominant biocatalysts produced by *A. niger* cultivated in banana peel powder medium (Barman et al. 2015). Both (endo-PG and exo-PG) polygalacturonases are only capable to degrade pectin with a degree of esterification of less than 50 - 60%. Figure 1 presents the yield of pectin during hydrolysis of banana peel waste powder using crude enzymes produced by *A. niger* at different extraction times.

The pectin yield increased sharply from the beginning of the hydrolysis and the increase continued to be significant ($p<0.05$) to the achievement of a maximum value (5.36 w/w%) at 120 min. The result proved that a longer hydrolysis time provides a higher probability for the enzyme to be in intensive contact and subsequently degrade the pectic substances, resulting in higher pectin yields. Similar observations were also reported by other researchers where the degree of hydrolysis was enhanced with the increase of hydrolysis duration (Haslaniza et al. 2010; Ovissipour et al. 2009; Salwanee 2013). The increasing degree of hydrolysis was the result of increasing cleavage of α-1,4-glycosidic bonds of pectin (a polymer of galacturonic acid with some methyl-esterified carboxyl groups) and/or pectic acid (a polymer of polygalacturonic acid) by polygalacturonase enzymes (PG) producing D-galacturonate (Liu & Kokare 2017),

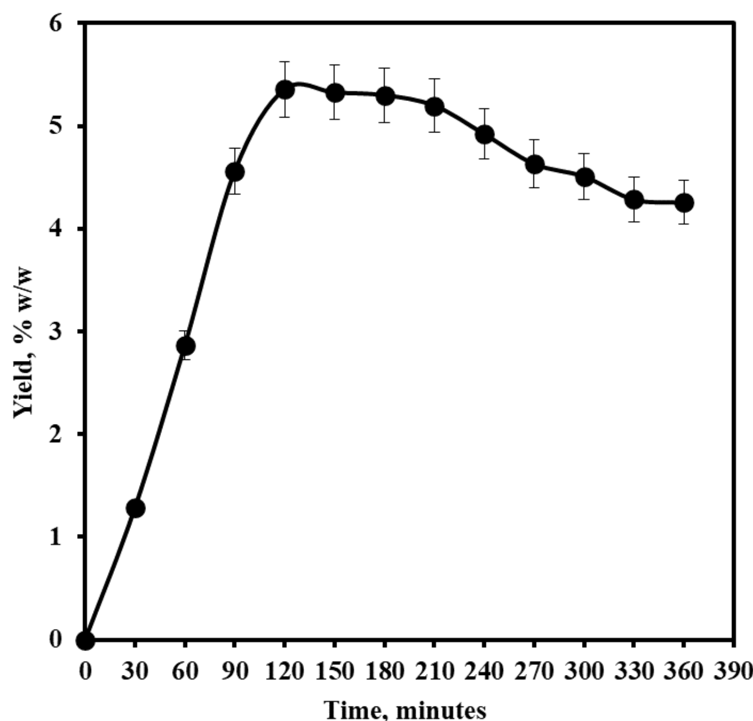

FIGURE 1. Profile of pectin yield obtained from 0.083 g/mL banana peel powder extracted using 4v/v% crude enzyme at 40 °C and pH 4.5 with 150 rpm stirring

which in turn enhances aqueous solubility of pectin (Bélafi-Bakó et al. 2007; Montecalvo et al. 1984). The highest pectin yield achieved in this work was only about a half of that reported by Castillo-Israel et al. (2015) who attained 10.61 w/w% pectin yield when they extracted pectin from ripe Saba banana peel powder at 90 °C using 0.5 N hydrochloric acid solution at pH 1.5 for 360 min. However, further extension of hydrolysis duration from 120 to 180 min leads to a remarkable decrease ($p<0.05$) of the pectin yield. A similar observation was reported by Kumoro et al. (2020). Baciu and Jördening (2004) explained that prolonged hydrolysis duration could induce the production of pectic substance monomer (i.e. D-galacturonic acid), which strongly reduced the activity of PG enzyme from *A. niger* (Bélafi-Bakó et al. 2007). Lengthy hydrolysis may facilitate the degradation of the methyl glycoside of polygalacturonic acid of the pectin by polymethyl-galacturonases (depolymerizing hydrolytic enzymes) into low molecular weight polyuronides (Baciu & Jördening 2004), and finally, reduce the pectin yield. This is because the banana peel waste powder hydrolysis was conducted at pH 5.0, which is very close to the range of optimum pH (5.5 - 6.0) for pectin depolymerase to effectively act as a biocatalyst (Matus 1948). The endo type of pectin depolymerase or pectin lyase (PL) exhibits a high affinity to long, highly methylated chains and depolymerizes methylated α-1,4 homogalacturonan via β-elimination to produce C4-C5 unsaturated oligo-uronides (Ahele 2007). Pectin methylesterase (PME) gets rid of the methoxyl groups from pectin and simultaneously reduces PL affinity for this type of substrate. This condition induces methanol production and the formation of a smaller amount of high methylated pectin. The PME from *Aspergillus* possesses a great attraction toward highly methoxylated pectin and performs its functions following the multichain mechanism (Ahele 2007). Demethylation with PME produces free carboxylic acid groups, which causes the pectin to turn out to be negatively charged. Furthermore, such a decrease in pectin yield could be attributed to the depletion of pectic substances in the raw material available in the hydrolysis medium (Palaniyappan et al. 2009).

INFLUENCE OF SUBSTRATE CONCENTRATION

The ability of crude enzymes produced by *A. niger* to hydrolyze pectic substances may be greatly influenced

by the concentration of banana peel powder as substrate in the hydrolysing medium. Figure 2 illustrates the profile of pectin yield attained at different substrate concentrations.

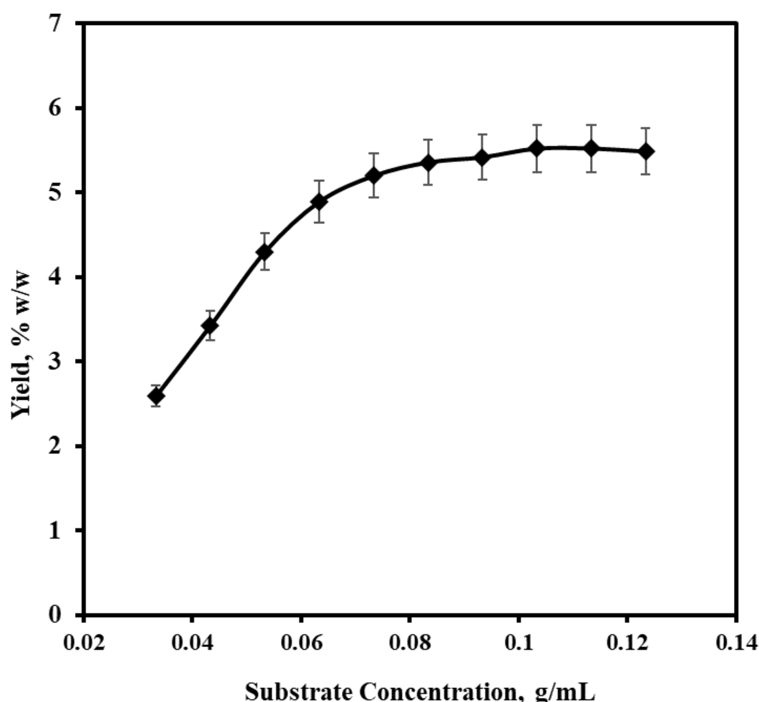

FIGURE 2. Pectin yield at various substrate concentrations at 40 °C and pH 4.5 using 4 v/v% crude enzyme with 150 rpm stirring for 120 min

As targeted, the enhancement of substrate concentration from 0.033 to 0.103 g/mL promotes the significant increase (*p<0.05*) of pectin yields following a logarithmic pattern and reaches a peak value of 5.52 w/w%. The finding of this work is in good agreement with the established theory that any enzymes should perform their maximum function if there is enough substrate available in the reaction medium. Therefore, enzymes activity will increase following the increase of substrate concentration. Unfortunately, the activity of the enzymes will not enhance further due to the attainment of saturation of their active sites by the substrate available in the reaction medium. Bhaskar et al. (2008) also reported that the degree of hydrolysis enters the stationary phase at substrate concentrations beyond 0.015 g/mL in their optimisation study of enzymatic hydrolysis of protein hydrolysates from *Catla viscera* waste protein using alcalase (Palaniyappan et al. 2009).

As clearly illustrated in Figure 2, further increasing the concentration of banana peel powder from 0.103 to 0.123 g/mL in the hydrolysing medium significantly reduced the pectin yield (*p<0.05*). This phenomenon could be the result of the decline of enzyme activity when the concentrations of the substrates were beyond 10 w/v% (Kumoro et al. 2020). Palaniyappan et al. (2009) suggested that the occurrence of this phenomenon was due to the co-hydrolysis of other components in the substrate and the escalation of the viscosity of the hydrolysis medium. Enzyme activity may decline due to inhibition by substrate and products on the active sites of the enzyme through the binding of the enzyme and the substrate so that the enzyme is unable to react with the excess unbound substrate (Shen & Larter 1994). The reduction of enzyme activity can further slowdown the rate of cleavage of 1,4-α-D-glycosidic of pectic acid and pectin, which finally decreases the pectin yield (Lehninger et al. 2017).

INFLUENCE OF pH OF EXTRACTION MEDIUM

Enzyme activity is appreciably affected by pH as the attachment of the substrate and active sites of the enzyme are altered by the charge distribution on the substrate

and enzyme molecules at which an optimum pH for a specific enzyme should exist and depends on where it usually works (Ahmed & Sohail 2020). The profile of pectin yields obtained at various pH of the hydrolysing medium is presented in Figure 3.

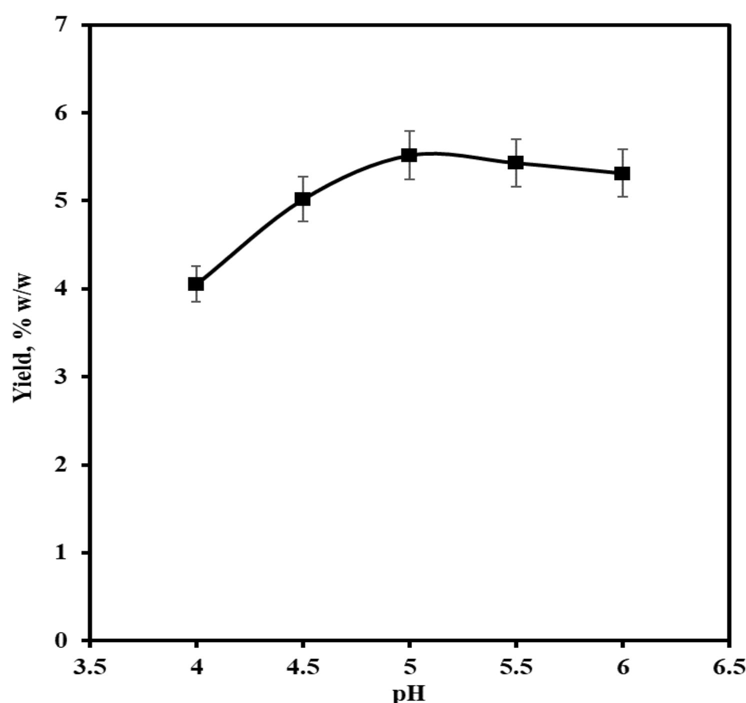

FIGURE 3. Pectin yield at various pH from 0.103 g/mL banana peel powder extracted using 4 v/v% crude enzyme at 40 °C with 150 rpm stirring for 120 min

It is clearly shown in Figure 3 that as the pH increases from 4.0 to 5.0, the hydrolysis of the pectic substance increases substantially leading to a steady and significant increase ($p<0.05$) in pectin yield. However, further increase in pH beyond 5.0 to close towards the neutral condition causes a gradual decline in the enzyme's activity and significantly reduces ($p<0.05$) the pectin yield due to a higher tendency of the denaturation of their protein structure. The result is in good accordance with Blanco et al. (1994) who found that most of the pectinases produced by yeasts possess an optimum pH range between 3.5 and 5.5. In addition, Ahmed and Sohail (2020) also reported that pectinase from *Geotrichum candidum* AA15 exhibited the highest enzyme activity at pH 5.0. During enzymatic hydrolysis of pectic substances, such pH changes are expected to give irreversible impacts on the enzyme activity due to the modification of the molecular structure of the essential groups of amino acids of the enzyme. In industrial applications, enzymes with stable activity at a wider pH range are preferable.

INFLUENCE OF EXTRACTION TEMPERATURE

In addition to the pH of the extraction medium, the activity and stability of an enzyme can also be strongly influenced by the temperature of the extraction medium (Ahmed & Sohail 2020). The profile of pectin yield acquired at elevated temperature obtained in this work is illustrated in Figure 4.

Similar to the common chemical reactions, the rate of enzymatic hydrolysis of banana peel powder studied in this work was found to increase with the increase in temperature, ranging from 40 to 55 °C. As seen in Figure 4, the increase of the rate of enzymatic hydrolysis of banana peel powder resulted in a nearly exponential enhancement ($p<0.05$) of pectin yield. This finding confirmed the well-established theory that such an increase in temperature leads to increase enzymes activity. However, as the temperature was further increased to beyond 55 °C, a remarkable decline ($p<0.05$) in pectin yield was observed. This phenomenon is likely due to the change of molecular structure of the enzymes

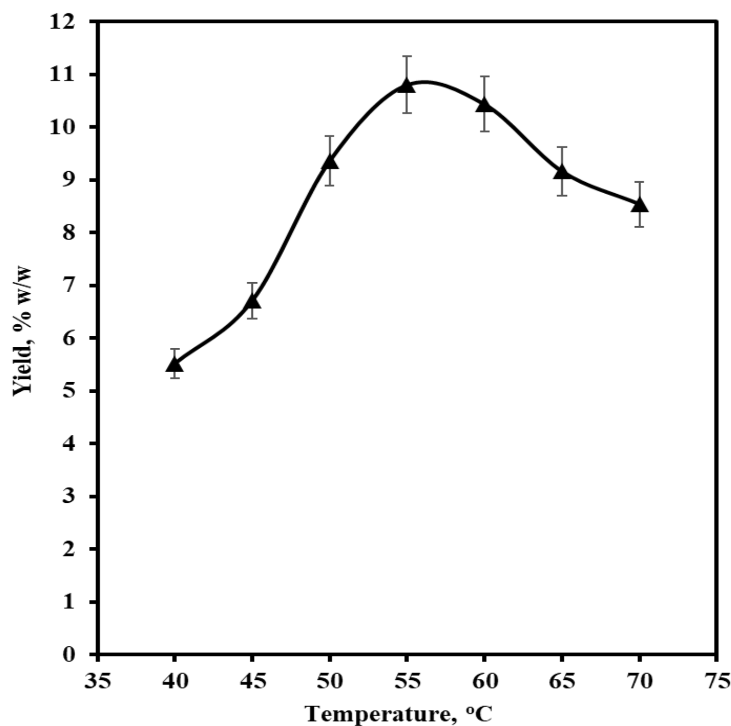

FIGURE 4. Pectin yield at elevated temperatures obtained from 0.103 g/mL banana peel powder extracted using 4 v/v% crude enzyme at pH 5.0 with 150 rpm stirring for 120 min

triggered by thermal stress (thermal denaturation), which subsequently reduces enzyme activity (Ahmed & Sohail 2020; Amin et al. 2013). Depending on their botanical sources and nature, pectinases can be very active at temperatures ranging from 50 to 70 °C. The mold-derived pectinase enzymes, particularly from *Aspergillus* species, are generally active in the temperature range of 30 to 55 °C (Dinu et al. 2007). According to Galiotou-Panayotou et al. (1997), pectinases from *Aspergillus* species suffer from activity decline as a result of the denaturation of their protein molecular structure at temperatures beyond 50 °C.

Khatri et al. (2015) observed that the partially purified alkaline thermostable pectinase enzyme isolated from *A. niger* strain MCAS2 exhibited optimum activity at 50 °C and pH 8.2, which is lower than the optimum temperature found in this work. As seen in Figure 4, the crude pectinase obtained from *A. niger* NRRL A-II 264 used in this work demonstrated maximum activity at 55 °C and pH 5.0. Although pectinase stability at elevated temperatures can be an important attribute for their potential applications in the pectin industries, however, from technical and economic considerations, processing of foodstuffs at low temperature is preferable to preserve their essential nutrients and to reduce the consumption of energy.

IMPORTANT CHARACTERISTICS OF BANANA PEEL PECTIN

To ensure the obtainment of pectin, in this work, an FTIR analysis was performed and the result is presented in Figure 5, where its comparison with the spectra of commercial citrus pectin can be observed. The commercial citrus pectin was selected as a reference because it is the most widely used pectin in food applications.

The FTIR spectra observed in the wave number range of 800 and 1300 cm^{-1} are regarded as the finger print region for carbohydrates, which enable the ascertainment of major functional groups, which belong to typical polysaccharides (Muhammad et al. 2014). Indeed, the vibration bands of pectin in the region of 1000 - 700 cm^{-1} are very complicated in nature. The peak at 776.48 cm^{-1} can be regarded as the characteristic of γ(C–OH) ring. The bands observed between 1100 and 1200 cm^{-1} are attributed to the presence of R–O–R

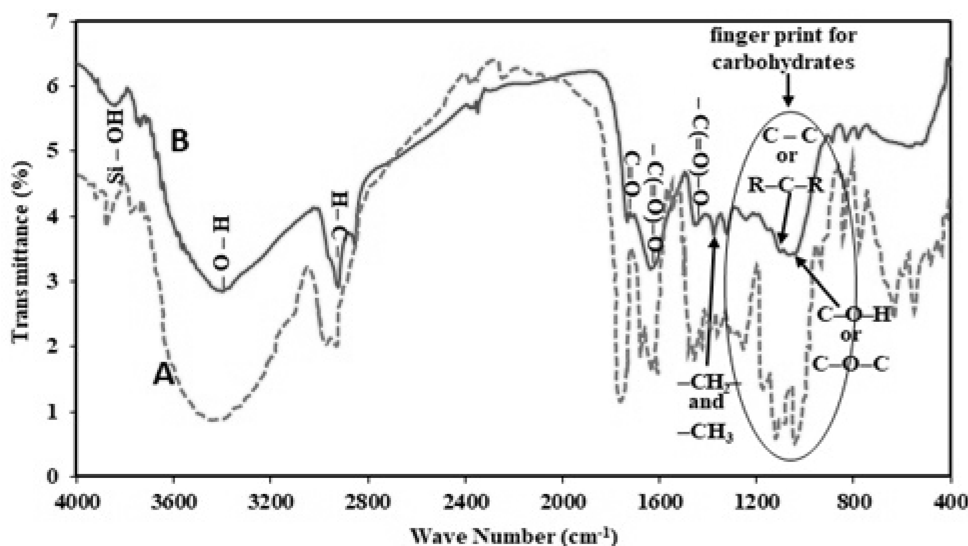

FIGURE 5. The FTIR spectrum of commercial citrus pectin (A) and *Kepok Kuning* banana peel pectin (B)

ether and cyclic C–C bonds in the pectin ring structure (Liu & Kokare 2017). Therefore, the peaks observed at 1101.86 - 1246.46 cm^{-1} can be assigned as the C–O–C stretching type of the bonds in the glycosidic bonds, while the other peak appeared at 1329.52 cm^{-1} should represent the O–C–H bending of pectin molecules. Tipson et al. (1959) explained that a carbon-oxygen stretching band exists at 1637 - 1600 cm^{-1} that distinguishes carboxylate anions from the C=O stretching band of esters, and a band (medium strength) at 1420 - 1300 cm^{-1}. Based on this reference, the transmittance peaks at 1424.38 and 1329.52 cm^{-1} are attributed to the anti-symmetric and symmetric stretching modes of C=O–O– functional group and $–CH_2–$ and $–CH_3$ groups of the pectin. Purcell and Fishman (1987) also proposed that pectin should exhibit an ester carbonyl band (C=O) at 1730 - 1760 cm^{-1} and a carboxylate (COO–) antisymmetric stretching band at 1600 - 1630 cm^{-1}. Therefore, the transmittance bands perceived at 1732.13 and 1627.18 cm^{-1} in the FTIR spectrum obtained in this work are considered the unique identity of pectin, which respectively belongs to the C=O stretching vibration of esterified carbonyl groups and/or free carboxyl groups and the characteristic of O–H bending (Muthukumaran et al. 2017; Singthong et al. 2005). A small broad shoulder near 2600 cm^{-1} can be identified as the O–H stretching vibration in free carboxylate groups (C=O–O–H) bonded by hydrogen bonds of dimers (Synytsya et al. 2003).

The unique bands detected between 2955 and 2895 cm^{-1} confirm the existence of the symmetrical and asymmetrical C–H stretching vibration in unison with bending vibrations around 1424.38 cm^{-1} of aliphatic chain groups ($–CH_2$ and $–CH_3$) and highly probable that the peak centered at 2924.57 cm^{-1} could be attributed to the methyl group stretching vibrations from the methyl esters of galacturonic acid (Mckendry 2002; Scabio et al. 2007). The region between 3200 and 3600 cm^{-1} appertains to the presence of free and intermolecular bonded hydroxyl groups associated with a high amount of O–H group vibrations of carboxylic and alcoholic groups in pectin as well as to the symmetric and asymmetric stretching vibrations related to H_2O molecules and (Zapata et al. 2009). Based on this reference, the broad band identified at 3409.25 cm^{-1} in the FTIR spectrum belongs to the OH groups due to hydrogen intra- and intermolecular hydrogen bonding of pectin (Muthukumaran et al. 2017; Seslija et al. 2016). A narrow but highly intense band at 3788 cm^{-1} could be assigned to surface isolated silanols (Si–OH) as a consequence of the presence of Si in the original sample (Pietrzyk et al. 2007). Accordingly, very weak bands about 3900 - 3700 cm^{-1} can be assigned as the rota-vibrational band of water vapour.

Since the FTIR analysis of *Kepok Kuning* banana peel pectin sample depicted in Figure 5 confirmed the existence of the specific functional groups of the commercial citrus pectin, the extracted polysaccharide from *Kepok Kuning* banana peel obtained in this study is assured to contain pectin with some impurities. A weaker transmittance of esterified carbonyl groups with a stronger transmittance of the carboxyl stretching band in the FTIR

spectra confirms that banana peel pectin falls in the high methoxyl pectin category, with a degree of esterification higher than 50%. The intensity of these two transmittance bands of pectin extract from banana peels was strong, which can be due to the lower content of anhydrouronic acid in the sample. According to Gnanasambandam and Proctor (2000), a little variation in the structure and composition of a molecule can cause considerable changes in the transmittance peaks. Therefore, pectin from the same botanical source can have lower FTIR spectral differences as found in this investigation.

The chemical characteristics of *Kepok Kuning* banana peel pectin obtained from extraction at 55 °C and pH 5.0 using 0.103 g/mL substrate concentration for 120 min and their comparison with those obtained by other researchers and commercial citrus pectin are summarized in Table 1.

TABLE 1. Chemical characteristics of ripe *Kepok Kuning* banana peels pectin

Characteristics	This work	Khamsucharit et al. (2018)	Castillo-Israel et al. (2015)	Commercial citrus pectin
EMW (g/mol)	1168.4 ± 12.58	1380	953.89	577.72
MeO (%)	5.93 ± 0.73	3.86	6.40	9.06
AUA (%)	51.82 ± 0.94	34.56	57.32	82.05
DE (%)	64.97 ± 1.12	63.15	63.67	62.83

Based on the degree of esterification (DE), pectin is classified into two categories, namely, high methoxyl pectin (with a DE higher than 50%) and low methoxyl pectin (with a DE lower than 50%) (Mesbahi et al. 2005). Hence, the banana peel pectin obtained in this work falls in the high methoxyl pectin category. This result is in good agreement with previous works (Castillo-Israel et al. 2015; Khamsucharit et al. 2018). The content of anhydrouronic acid (AUA) represents the purity of pectin, where FCC (1996) suggested AUA values of no lower than 65% for pectin used in food or pharmaceutical applications. Unfortunately, the AUA content of *Kepok Kuning* banana pectin obtained in this study is far below the standard for commercial pectin set by the Food Chemical Codex. Again, the result is in accordance with previously published works (Castillo-Israel et al. 2015; Khamsucharit et al. 2018). The presence of undesirable compounds (usually sugars, ash, and acids) must be completely removed to obtain pure pectin for further applications. Lampitt et al. (1947) suggested precipitation, dialysis, ionic exchange, nitration, as well as their combined methods to purify pectin. Precipitation is the most common pectin purification method for the pectin contained in an aqueous extract by sequential washing with alcohol or acetone. Due to high pectin yield and reasonable operating cost, the use of alcohol is preferred to acetone for pectin purification at both laboratory and commercial scales. In addition, Guo et al. (2016) found that stagewise ethanolic precipitation is a more efficient method for pectin purification than one-stage ethanolic precipitation with the efficiency being strongly dependent on the pectin structure and ethanol concentration. However, the purity of pectin obtained only by alcoholic precipitation was lower than the pectin purified by ultrafiltration and metal ion-binding precipitation (Yapo et al. 2007). Garna et al. (2011) proposed a purification technique for electrically charged polysaccharides using protein (sodium caseinate). Although metal ion-binding precipitation offers a higher selectivity towards undesirable components, its application at an industrial scale suffers a serious limitation due to the generation of abundant harmful effluents that require a complicated treatment prior to their disposal to the environment. By considering technical, environmental, and economic issues, Yapo (2009) also suggested that it would be more beneficial to apply a membrane purification technique (such as ultrafiltration-diafiltration) before alcohol precipitation for a commercial-scale process to effectively remove pectin impurities that guarantee the achievement of high compositional quality and gelling properties of the pectin produced. The results of this work offer potential commercial applications to produce pectin from any carbohydrate and pectin-containing plant materials, such as pear, apple, papaya, orange, lemon, jackfruit, mango, beets, carrot, and guava peels, which becomes very helpful in solving various problems related

to agricultural wastes as well as enhancing the economic value of the by-products of agriculture-based industries. In addition, the successful use of crude enzyme in pectin extraction from any carbohydrate and pectin-containing plant materials will significantly reduce the enzyme preparation cost, leading to an improved profit of small and medium food enterprises.

CONCLUSION

Banana peel pectin has been successfully extracted from *Kepok Kuning* banana peel powder through enzymatic hydrolysis using crude pectinases derived from *A. niger* in a batch fermenter at different substrate concentrations, pH, temperature, and duration. It was confirmed that all extraction parameters govern the increase of pectin yield to a maximum value and then decline. High temperature and pH caused the reduction of enzyme activity due to the denaturation of the enzyme's molecular structure. High substrate concentration and prolong extraction duration induced the formation of galacturonic acid, which in turn reduces enzyme activity to catalyse the hydrolysis pectic substances of the banana peel powder. The recommended operating condition for the banana peel pectin extraction process is by hydrolysis of 0.103 g/mL substrate with pH 5.0 at 55 °C for 120 min to obtain 10.80 w/w% yield of pectin. The results can be used as the basic information for the design and operation of the commercial-scale pectin production process, which is expected to be one of the environmental problem-solving strategies as well as to enhance the economic value of banana peels, similar pectin rich fruits peels, and other agricultural residues.

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