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Anaerobic Fermentation of Mixed Fruits Peel Waste for Functional Enzymes Production Employing Palm Sugar and Molasses as The Carbon Sources

Diah Susetyo Retnowati[1*)], Andri Cahyo Kumoro[1)], Kristinah Haryani[1)]

[1)]Department of Chemical Engineering, Faculty of Engineering, Universitas Diponegoro
Jl. Prof. Soedarto, SH, Tembalang, Semarang

[*)] Corresponding author: diahsr@che.undip.ac.id

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Abstract

The daily abundant generation of fruit peel waste potentially triggers environmental problems if no appropriate management is performed. Besides, fruit peel waste can be a valuable raw material for functional enzyme production. This study aims to investigate the use of molasses and palm sugar as sugar sources during anaerobic fermentation of banana and papaya peel waste to produce functional enzymes. In this study, biomass mixtures comprise banana and papaya peel at various mass ratios were fermented for 3 months. The feed consisted of sugar:biomass:water, and their mass ratio was kept constant at 1:3:10. Both brown functional enzyme cocktails obtained from the fermentation using palm sugar and molasses as carbon source were acidic with pH of 3.7 and 4.4, respectively. The amylase, protease, and lipase activities of the functional enzyme cocktails was maximum when the biomass mixture contained four portions of banana peel and one portion of papaya peel. In addition, molasses was found to be the better sugar source than palm sugar for producing functional enzymes from aerobic fermentation peel fruits comprises banana and papaya peels. This study proved that fruit peel waste can be converted to highly-valuable functional enzymes as one of the solutions of fruit peel waste management

Keywords: anaerobic fermentation; carbon source; enzyme activity; functional enzyme; organic waste

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INTRODUCTION

Banana and papaya are both categorized as world's popular tropical fruits with their worldwide annual production were 81.2 million and 0.3 million tons, respectively (Liu and Zhang, 2013; Evans et.al, 2020). Consequently, they generate large quantities of residues every year in various countries, including Indonesia. According to the data compiled by the Ministry of Environment and Forestry the Republic of Indonesia, Indonesia generated 28.3 million tons of waste per year. Based on its survey in 2021, approximately 55.35% or 115-184 kg/capita of the waste produced was categorized as organic waste. This fact has become a serious challenge for the government and the community to implement any strategic efforts to ensure that the waste will not harm the environment and public health,

Previous research claimed that functional enzymes can be obtained from the anaerobically submerged fermentation of organic wastes, such as fruit and vegetable waste using various types of sugar as the carbon source for three months (Neupane and Khadka, 2019). Hence, one of the potential solutions to reduce organic waste, specifically banana and papaya peels is by processing them employing anaerobic fermentation to produce functional enzymes with the aid of inexpensive carbon sources, such as palm sugar and cane molasses. Depending on the types of the sugar used as carbon source, the functional enzymes produced from the fermentation of fruit and vegetable waste are generally dark brown in color with a strong sweet and sour aroma. These enzymes can be utilized as multifunctional purposes (Vama and Cherekar, 2020). Normally, the functional enzymes obtained from this process are the mixtures of several naturally occurring enzymes, such as proteases, amylase, and lipase (Samriti et. al., 2019). For examples: Panicker et al. (2021) prepared functional enzyme from mixed fruit peel as the base component of a bio cleaning agent, while Low et al. (2021) manufactured functional enzymes from vegetable and fruit waste as a substrate to treat wastewater prior to its final disposal to the river. Lipase enzymes can be used as biocatalysts in the hydrolysis process of oil-based materials, which are in great demand by the oleo chemical industry. In the digestive system in humans and animals, the enzymes such as lipase, amylase, and protease, play a unique role in the breaking down of complex fats, carbohydrates, and proteins into simple compounds that make these nutrient become easier to be digested, absorbed and used by the human and animals. In addition, functional enzymes can also be used as natural cleaning agents as a substitute for synthetic disinfectants and plant fertilizers.

Eco enzymes preparations from fruits peels have been conducted by many researchers, specifically employing Kapok banana (*Saba banana*) and papaya California (*Carica papaya L*) peels, which are both abundantly available in Indonesia. Previous research revealed that banana peel fermentation produces high amount of crude protein, about 58.62% (Panda et al., 2022), while papaya peel fermentation resulted in 52.4% crude protein (Thiviya et al., 2022). The objective of this study is to produce functional enzymes from a mixture of papaya and banana peels by using cane molasses and palm sugar as the carbon source employing naturally occurring microbes to grow. The effect of mass ratio of banana peel and papaya peel in the feed waste and the type of carbon source on the functional enzymes produced were investigated. Furthermore, the activity of the resulting enzymes, namely the protease, amylase, and lipase activities were also quantified.

Materials and Methods

1. Materials

Papaya and banana peels as the representative of tropical fruit waste were collected from a domestic waste collection unit in Semarang-Indonesia. Meanwhile, palm sugar and cane molasses were purchased from a local minimarket operated in Semarang-Indonesia, which have total sugar respectively 63.5% and 76.25%. The water used for the experiment was mineral drinking water (AMIDIS Brand). To ensure the achievement of a high accuracy quantitative analysis, all chemicals used for the analysis were of analytical grade products (purity ≥ 98% w/w) of Sigma-Aldrich, Singapore**.**

2. Fermentation of fruit peel waste

Prior to fermentation, banana and papaya peels were firstly cleaned with water and chopped into small pieces, approximately 1×1 cm^2. Then, the chopped banana and papaya peels were mixed at various mass ratios (1;1, 1:2, 1:3, 1:4 and 1:5) before being introduced into molasses or palm sugar solution in air-tight plastic containers as the fermentation reactors. The mass ratio between sugar source: biomass: water was fixed at 1:3:10. Subsequently, the mixtures were placed in a cool, dry and completely ventilated room to allow anaerobic fermentation of the organic matter for 3 months (Neupane and Khadka, 2019). Every week during the first month, and once in the second and the third month the cover of the containers was opened and the contained mixtures were stirred with a wooden rod to allow the release of the resulting gas. After 3 months, the crude functional enzyme solution was filtered, the resulting filtrate was centrifuged at 3000 rpm for 30 minutes before being subjected to protease, amylase, and lipase activity analysis.

3.Analytical methods

Initial moisture content (*MC*), ash, crude protein (*CP*), total fat (*TF*), and crude fiber (*CF*) were determined in banana and papaya peels at five stages of maturity following the methods described in the Association of Official Analytical Chemists (AOAC) (AOAC, 2010). The carbohydrate content of the samples was estimated according to the difference method:

$$\text{Total Carbohydrate (\%)} = 100 - (\%\, MC + \%\, CF + \%\, CP + \%\, TF + \%\, \text{ash}) \quad (1)$$

The resulting fermentation filtrates were analyzed for their total protein concentration according to the Lowry protein assay method (Lowry et al., 1951). The method allows the tyrosine fraction in the protein to react with Folin's Ciocalteau reagent and copper sulfate solution to generate a blue color solution with maximum absorption at 620 nm. Then, the protein concentration was calculated by referring to a standard curve prepared at the same time using a carefully known concentration of bovine serum albumin. In addition, the protease, amylase, and lipase activities of the filtrate were analyzed following the protocols previously described by Arun and Sivashanmugam (2015). The value of *PA*, *AA* and *LA* correspond to protease, amylase, and lipase activities, which can be calculated using the following equations:

$$PA = (absorbance\ at\ 620\ nm/(PC \times t) \quad (2)$$

where *PC* and *t* are the total protein concentration (mg/L) and time (minute)

$$AA = M_m \times DF/ V_s \quad (3)$$

where M_m is the maltose released (mg), *DF* is the dilution factor, and V_s is the volume of enzyme sample solutions (mL), respectively. Furthermore, the lipase activity can be calculated using Equation (4):

$$LA = ((V_{NaOHs}\text{-}V_{NaOHb})/(V_S)) \times DF \quad (4)$$

where V_{NaOHs} and V_{NaOHb} are the volume of sodium hydroxide solution used to titre the enzyme sample and blank solutions (mL), respectively. All analyses were conducted in triplicate.

RESULTS AND DISCUSSION

Prior to their use as used as the raw materials in this work, banana and papaya peel were subjected to the proximate composition analysis. The results are tabulated in Table 1.

Table 1. Proximate composition of banana and papaya peel (% w/w, wet basis).

Parameters	Banana Peel	Papaya Peel
Moisture (%)	69.83 ± 0.11	68.59 ± 0.15
Ash (%)	4.82 ± 0.18	3.17 ± 0.21
Crude protein (%)	3.62 ± 0.22	6.87 ± 0.06
Total fats (%)	0.11 ± 0.08	0.24 ± 0.05
Crude fiber (%)	1.15 ± 0.15	11.40 ± 0.20
Carbohydrate (%)	18.50 ± 0.20	9.73 ± 0.16

As seen in Table 1, both banana peels and papaya peels contained a comparable high moisture content, which indicate both fruit waste are highly perishable. However, banana peels bear a higher carbohydrate content than papaya peels. In contrast, papaya peels contain a higher value of crude protein and fiber contents than banana peels. The results suggest that banana peel will be more easily degraded by its naturally occurring microflora via fermented than papaya peel (Yang et al., 2016). The availability of water-soluble carbohydrate will promote a faster fermentation and hence requires a shorter fermentation time.

The functional enzyme solutions obtained in this research were brown color with strong sweet sour vinegary aroma. The unique aroma figures their acidic nature as confirmed by their pH values. The pH values of the crude enzyme solutions obtained from anaerobic fermentation of banana peel, papaya peel and their mixtures employing palm sugar and cane molasses as the sugar source were 3.7 and 4.4, respectively. As expected, the decrease in pH of the solution could be associated to the formation organics acids during fermentation process, especially lactic acid and propionic acid (Odumodu and Inyang, 2006).

Enzyme activities, which are usually characterized by protease, amylase, and lipase activity against biomass ratio of the resulting functional enzyme solution are depicted in Figures 1, 2, and 3. As seen in Figures 1, 2, and 3, the three enzyme activities are strongly influenced by the type of the sugar source used as the substrate and banana peel to papaya peel mass ratio. Generally, the highest amylase, protease, and lipase activities could be obtained from anaerobic fermentation using both substrates can be achieved at a fruit waste mass ratio of 4. However, the results of this research revealed that the increase in the mass

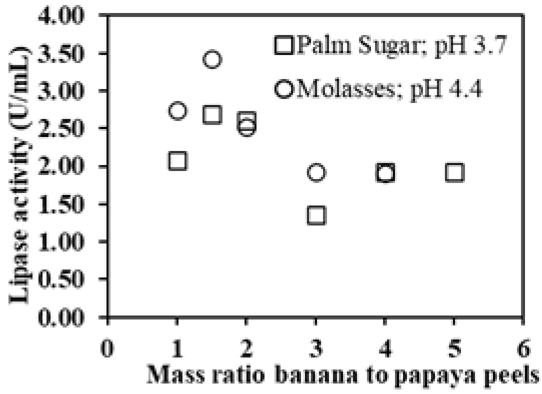

Figure 1. Amylase activity of functional enzyme solution at their original condition

ratio of banana peel to papaya peel led to enhance activity of the enzymes, specifically the amylase and protease activities. In contrast, lipase activity was found to decrease with the increasing mass ratio of banana peel to papaya peel.

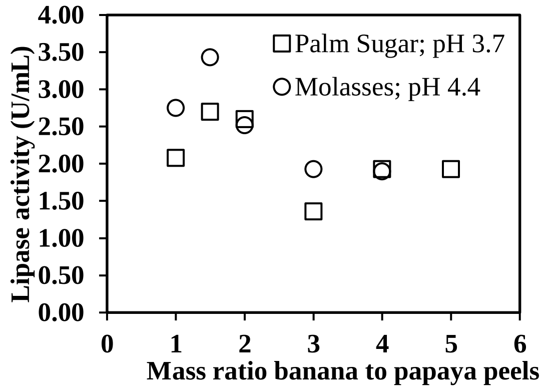

Figure 2. Lipase activity of functional enzyme solution at their original condition

Fermentation reactions that occur during production of functional eco enzyme are very complex. Fruit peels are potential substances to produce enzymes (Thiviya et al, 2022). According to Panda et al., 2017, fruit and vegetable waste could undergo biodegradation following two schemes. In the first scheme, the fruit and vegetable wastes are fermented with prior treatment to produce bioethanol, organic acid, bio hydrogen, and polysaccharide. In the second scheme, fruit and vegetable waste are fermented without any prior treatments, which can be separated into two possible routes. After fermentation process, route one should produce bioethanol, SCP (single-cell proteins)/SCO (single-cell oils), and enzymes as the products. Whereas second route results in biopesticides, bioflavor, SCP, biocolors, and growth regulators. Theoretically, the microflora that naturally present in the fruit peels influences the type and concentration of the enzymes produced when the fruit peels undergo natural fermentation. A previous observation revealed that banana peel contains *bacilli* (*Bacillus substilis, Lactobacillus fermentum, Pseudomonas aeruginosa*) and yeast (*Saccharomyces cerevisiae*) as the naturally occurring bacteria and yeast. In addition, banana peel also contains *cocci* bacteria (*Micrococcus luteus, Staphylococcus aureus*) and fungi (*Aspergillus flavus, Mucor mucedo, Rhizopus stonifer, Aspergillus niger, and Penicillium frequentans*) (Ozabor et al., 2020). Similarly, papaya peel also bears *bacilli* bacteria (*Lactobacillus fermentum, Lactobacillus casei, Lactobacillus perolens, Lactobacillus nagelii, and Lactobacillus plantarum*), *cocci* bacteria, yeast and fungi (Yang et al., 2016), which both bacilli and yeast exhibit excellent ability to produce protease, lipase, and amylase enzymes (Panicker et al., 2021).

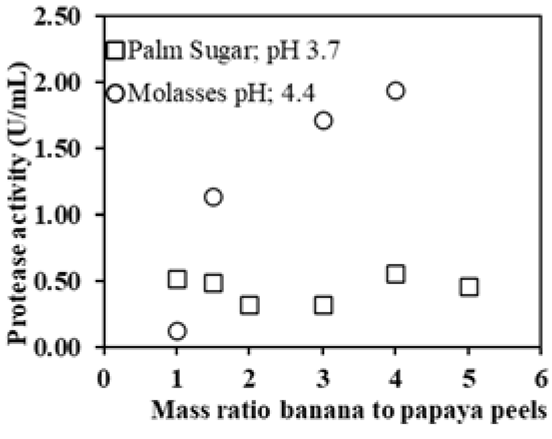

Figure 3. Protease activity of functional enzyme solution at their original condition

In general, *B. substilis* performs better than *S. cerevisiae* in generating the three enzymes (Panicker et al., 2021). However, Bacillus bacteria produce greater amylase and protease activity than *S. cerevisiae*, and to reach maximum lipase activity better using *S. cerevisiae*. The number of *bacilli* (rod shape bacteria) and yeast in papaya peel were reported to be 1,800 cfu/g fresh sample and 7,943 cfu/g fresh sample, respectively (Ozabor et al., 2020). According to Yang et al. (2016), banana peel contained *bacilli* and yeast about 1,585 cfu/g and 2,511 cfu/g fresh sample, respectively. Both banana and papaya peel samples had approximately 650,000 cfu/g lactic acid bacteria (Yang et al., 2016). They also found that the concentration of fungi was less than 100 cfu/g in both fruit residue samples. Therefore, papaya peel contains a larger number of bacteria and yeast than that of banana peel. In addition, the biological reaction rate performed by bacteria to produce enzymes is faster than fungi (Costa et al., 2019). As a result, a higher banana peels mass ratio in the biomass mixture will increase amylase and protease enzyme activity of the resulting crude enzymes. On the other hand, the value of lipase activity will be decreased. In this study, cane molasses resulted in crude functional enzymes with better amylase, protease and lipase activities than those obtained from fermentation using palm sugar as sugar source. The results confirm that enzyme activity strongly depend on the type of the sugar used in the substrate, especially the reducing sugar, such as glucose or fructose, and non-reducing sugar.

In most cases, bacteria, fungi and yeast exhibited their preference to monosaccharide as the carbon source, especially glucose (Crancer et al., 2018) This is because glucose promotes their growth and requires fewer steps and less energy to break down than other types of sugars. Therefore, bacteria consume glucose first in sugar mixtures (Bren et al., 2016). As glucose has been depleted in the fermentation broth, the fungi started producing β-glucosidase and endoglucanases (Jorgensen, 2004). Further, bacteria and fungi begin to use fructose, mannose, arabinose, xylose, and galactose as their carbon source (Song et al., 2013).

Reducing sugars are monosaccharides with a ketone group in its molecular structure, such as glucose, fructose, and galactose. In addition, their aldehyde functional group also triggers the ability to reduce another compound. While, non-reducing sugars are disaccharides that cannot be oxidized by a weak oxidizing agent. Non-reducing sugars do not have free aldehyde or ketone group like sucrose, which consist of glucose and fructose. The two monosaccharide units are held together by a glycosidic linkage between C_1 of α-glucose and C_2 of β-fructose. Therefore, glucose has a simpler molecule structure than sucrose. Basically, the reducing sugar and non-reducing sugar content of cane molasses and palm sugar varies with their location and method of cultivation. In general, palm sugar possesses reducing sugar and non-reducing sugar content than cane molasses. For example, according to Srikaeo et al. (2019), total sugar of palm sugar is 77.81% (w/w) with 5.91% glucose, 6.64% fructose, and 65.26% sucrose. In other hand, cane molasses bears total sugar around 62.4% on dry basis, which comprises 48.8% sucrose, 5.29% glucose, 8.07% fructose, 0.03% raffinose, 0.04% galactose, and 0.01% arabinose (Palmonari et al., 2020). A study of palm sugar in Indonesia by Based on their survey, Radam et al. (2014) revealed that growing conditions (wetland, intermittently wetted land, and dry land) significantly affected the amount of reducing sugar and non-reducing sugar of palm sugar. Sucrose and reducing sugar content in palm sugar, each in the wet area, partially wet, and dry were 75.14 %, 2.39 %, 68.15%, and 0.51%, 88.46%, 1.52%, respectively. However, in this research, total sugar content for palm sugar and molasses were 76.25% and 63.5%, respectively. If sucrose is used as the carbon source in the fermentation to produce functional enzyme, it has to be firstly decomposed into glucose and fructose. Some bacteria and fungi produce extracellular enzymes to degrade polysaccharides and transport partial degradation products into the cell for subsequent processing into fermentable sugars (Shulami et al., 2011). Hence, the time required for anaerobic fermentation by bacteria and fungi to break down fructose as the carbon source will be longer than glucose. For instance, beer industry prefers using glucose as a substrate or carbon source because of the shorter time needed for the fermentation (Crancer et al., 2018)].

In addition, both palm sugar and molasses usually also contain a small amount of Ca^{2+} ions. According to Clarke (2003), the Ca^{2+} ion (as calcium oxide) content in molasses was 0.025 %. Meanwhile, palm sugar contained about 1.35% Ca^{2+} ion (Jamhari et al., 2017). The presence of a higher Ca^{2+} ion concentration retards the growth of live cells, reduces yeast cellular viability and leads to slow down the fermentation rate (Costa et al, 2019). Furthermore, Ca^{2+} ion can also hinder invertase enzyme to breakdown sucrose into glucose and fructose, which optimally occurs at pH 4.5 (Chotineeranat et al., 2010; Jayanti et al., 2019). Previous research proved that the rate of the use of sugar as carbon source for fermentation employing bacteria faster is than that of fermentation using fungi and yeast. The sucrose, glucose and fructose contents in the molasses used in this research was slightly higher than palm sugar as previously reported by Palmonari et al. (2020). In conclusion, cane molasses is the better carbon source (substrate) than palm sugar for fermentation of banana and papaya peels into functional enzymes as proven by the higher value of amylase, protease and lipase activity.

Conclusion

The total amount of the functional enzymes obtained from anaerobic fermentation banana and papaya peels process depend on the native bacteria, yeast and fungi within fruit peels. Amylase and protease activity increase proportional to ratio banana peels to papaya peels, however protease enzyme activity decrease. In addition, mollases is better than palm sugar used as substrate for anaerobic fermentation of peel fruits. In addition, mollases is better than palm sugar used as substrate for anaerobic fermentation of peel fruits.

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