IOP Conference Series: Materials Science and Engineering

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To cite this article: N A M Ridzuan *et al* 2020 *IOP Conf. Ser.: Mater. Sci. Eng.* **991** 012064

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This content was downloaded from IP address 103.53.34.15 on 25/02/2021 at 08:04

IOP Conf. Series: Materials Science and Engineering **991** (2020) 012064 doi:10.1088/1757-899X/991/1/012064

Study on Enzyme Activities in Pineapple Fruit and Pineapple Waste to Be Applied as Poultry Supplement

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Abstract. Pineapple fruit contains essential minerals and vitamins with some medicinal values. Hence, it can be used as a raw material to make poultry supplements. Abundance of pineapple waste also has led to the environmental problems such as producing bad odor and has increased waste capacity in the landfill. In this present work, the potential of pineapple fruit and pineapple waste were investigated to be used as the raw materials for poultry supplement based on their enzyme activities. In this study, the effect of fermentation time and type of pineapple substrates on enzyme activities which were xylanase, total cellulase and protease were studied. Two types of pineapple samples have been used in this study which were pineapple fruit and pineapple waste. Four type of pineapple substrates have been prepared which were pineapple juice, pineapple waste, pineapple juice + probiotic and pineapple waste + probiotic. The purpose of adding probiotic in this study was to enhance the fermentation reaction. The results showed that the fermentation time that have the highest enzyme activities for xylanase, total cellulase and protease were observed at day 2, 4 and 6 respectively, where substrate contains pineapple juice + probiotic recorded the highest enzyme activities value for all the three enzymes. This study had investigated that pineapple fruit and pineapple waste contain digestion enzymes which were xylanase, protease and cellulase that can be applied as poultry supplements.

Keywords: pineapple fruit; pineapple waste; poultry supplements; xylanase; total cellulase; protease; probiotic

1. Introduction

Pineapple is a very well-known tropical plant in Malaysia and also known as the most economically significant plant in the family of *Bromeliaceae*. Pineapple contains considerable amount of calcium, potassium, fibre and vitamin C [1]. Pineapple waste known as the waste that rich in lignocellulosic material, especially the peel and the leaves of the fruit. Pineapple waste such as pineapple peels and core, making about 40-50% of the fresh fruit [2]. Pineapple waste also noted to be an enriched raw material with insoluble fibres, pectins, simple sugars and proteins as the main compounds, followed by a good level of micronutrients such as vitamins, minerals and phenolic compounds [3]. Many agricultural crops were used in poultry feed to prevent disease, improve growth performance, and increase the output of poultry [4]. In pineapple fruit and pineapple waste, there are enzymes that can be used to help the growth of the poultry without giving bad effect to the poultry such as protease, cellulase

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and xylanase. These enzymes are known as digestion enzymes that break down polymeric macromolecules into their smaller building blocks to facilitate their absorption by the body. The fruit waste such as bagasse, husks, and pulp were produced in high amount through industrial food production. The process of waste decomposition generally requires higher cost management to the industry. In addition, the abundance of fruit waste will also affect the environment such as producing bad odor. Therefore, the process of using the pineapple fruit and pineapple waste as the poultry supplements will help to reduce the environmental pollution and increase the sustainability along with obtaining new products with higher added value [5]. The objective of this research is to study the enzyme activities in pineapple fruit and pineapple waste to be applied as poultry supplements.

2. Materials and Methods

2.1. Materials

The materials that were used in this study are DNS solution, 0.05 M phosphate buffer pH 6, 0.05 M acetate buffer pH 4.8, 1% weight/volume xylan solution, 1% (w/v) carboxymethyl cellulose solution, glucose standard stock solution, xylose standard stock solution, 50 mM potassium phosphate buffer pH 7.5, 0.65% (w/v) casein solution, 110 mM tricloroacetic acid solution, 500 mM sodium carbonate solution, 1.1 mM L-tyrosine standard stock solution and Folin-ciocalteau reagent. All of the chemicals used were purchased from Sigma-Aldrich while distilled water was utilized in this whole study as dilution solvent and for the preparation of stock solution. Pineapple fruit (MD2) was purchased at Pekan, Pahang. The microbes used in this study was obtained from probiotic that were purchased at local market in Gambang, Pahang. In this study, three main enzymes activities which were protease, xylanase and cellulase were studied. Pineapple juice is defined as substrate prepared from pineapple fruit while substrate prepared from peels and crown leaves is defined as pineapple waste. It involved several steps which were preparation of samples, the calibration curve (glucose, xylose, and tyrosine standard) and the enzyme activities (xylanase assay, protease assay and total cellulase assay).

2.2. Methodology

2.2.1 Preparation of pineapple substrate

The pineapple substrate samples were prepared using juice extracted from pineapple fruit and pineapple waste. Four pineapple substrate samples were prepared which were pineapple juice, pineapple juice + probiotic, pineapple waste and pineapple waste + probiotic. Firstly, the pineapple fruits and waste were cut into small pieces. A 100g of pineapple fruit and pineapple waste were weighed separately. Then, the pineapple fruit and pineapple waste were blended with water in ratio of 1:10 (pineapple fruit or pineapple waste: water) until it became smoothies. The mixture was filtered and was placed into 4 different water bottles with a volume of 1 L in each bottle. Then, 250 mL of the probiotic was added in the pineapple juice and pineapple waste. Another two bottles contain pineapple and pineapple waste were treated as a control. These mixtures were fermented for 10 days. Three enzymes activities were analyzed which were protease, xylanase and total cellulase.

2.2.2 Analysis of xylanase activities

Xylose working stock was diluted into different xylose concentration of 1 g/L, 0.8 g/L, 0.6 g/L, 0.4 g/L, 0.2 g/L and 0.0 g/L. 0.5 mL of each xylose dilution was pipetted to 1 mL phosphate buffer in test tube with cap and then incubated at 50 °C for 60 minutes in water bath. Then, the test tubes were removed from water bath and cooled downed for 10 minutes. Three mL of DNS reagent was added into the test tubes and mixed well. The test tubes were immediately boiled for 5 minutes in boiling water. After boiling, the test tubes were cooled down under running tap water to quench the reaction and then the absorbance for each xylose diluted solution was then read using UV-Vis spectrophotometer at 520 nm

wavelength. The standard curve of absorbance versus concentration of xylose was then plotted to be used to determine the concentration of xylose in the samples.

Xylanase activity was determined through following method suggested by Kim et al., 2012 [6]. Xylanase activity was measured by using corn core xylan as a substrate. The reaction mixture containing 0.1 mL of crude enzyme, 0.25 mL of 1% xylan solution in 0.05 M phosphate buffer and 0.15 mL of phosphate buffer were added to the capped glass tube and then mixed well. The test tube was then incubated at 50 °C for 10 min. After incubation period, the reaction was stopped by adding 1.5 mL of DNS solution, boiled for 5 min and cooled to ambient temperature under running tap water. Xylose release was estimated by measuring the absorbance using UV-Vis spectrophotometer at 520 nm. The blank contains 0.4 mL of 0.05 M phosphate buffer and 0.1 mL diluted crude enzyme and treated similar as experimental tubes. D-xylose was used as a standard for the preparation of a calibration curve. The estimation of xylose released by the crude enzyme solutions with deduction of the enzyme blank absorbance was calculated based on the xylose standard curve. Xylanase activity was calculated by using equation (2.1). One unit of xylanase activity was defined as the amount of xylanase to released 1 µmol of reducing sugar (xylose) per minute under standard assay conditions.

$$\text{Xylanase activity } \left(\frac{\text{U}}{\text{mL}}\right) = \frac{\text{final absorbance}-\text{c}}{\text{m}} \times \frac{\text{dilution factor}}{\text{sample volume}} \times \frac{1}{\text{reaction time}} \times \frac{1000\ \mu\text{g}}{1\ \text{mg}} \times \frac{1\ \mu\text{mole}}{150.13\ \mu\text{g}} \quad (2.1)$$

2.2.3 Analysis of total cellulase activities

The glucose standard was prepared by diluting glucose stock into different glucose concentration of 1 g/L, 0.8 g/L, 0.6 g/L, 0.4 g/L, 0.2 g/L and 0.0 g/L. 0.5 mL of each glucose dilution was pipetted to 1 mL citrate buffer in test tube with cap and then incubated at 50 °C for 60 minutes in water bath. The test tubes were removed from water bath and cooled downed for 10 minutes and then 3 mL of DNS reagent was added into the test tubes and mixed well. The test tubes were immediately boiled for 5 min in boiling water. After boiling, the test tubes were cooled down under running tap water to quench the reaction. The absorbance for each glucose diluted solution was then read using UV-Vis spectrophotometer at 540 nm wavelength. The standard curve of absorbance versus concentration of glucose was then plotted. The total cellulase assay was conducted by using Whatman filter papers (No. 1) with length of 6 cm and 1 cm width as a substrate and were placed into test tubes. After that, 0.25 mL of diluted enzymes and 0.5 mL 0.05 M acetate buffer (pH 4.8) were added in test tubes containing filter paper and incubated in water bath at 50°C for 60 minutes. 1.5 mL of DNS reagent was then added and boiled for 5 minutes. The test tubes were placed under running tap water to cool down the solution at room temperature and their absorbance was read at 540 nm. Enzyme blanks (0.5 mL 0.05 M acetate buffer + 0.25 mL diluted enzyme) were prepared and treated it as experimental tubes. Glucose was used as a standard for the preparation of a calibration curve. The glucose released by the enzyme solutions with deduction of the enzyme blank absorbance was calculated based on the glucose standard curve. The total cellulase activity was calculated by using equation (2.2) [7].

$$\text{Total cellulase activity } \left(\frac{\text{U}}{\text{mL}}\right) = \frac{\text{final absorbance}}{\text{m}} \times \frac{\text{dilution factor}}{\text{sample volume}} \times \frac{1}{\text{reaction time}} \times \frac{1000\ \mu\text{g}}{1\ \text{mg}} \times \frac{1\ \mu\text{mole}}{180.16\ \mu\text{g}} \quad (2.2)$$

2.3.3 Analysis of protease activity

1.1 mM tyrosine standard stock solution was added with the following volumes in mL: 0.05, 0.10, 0.20, 0.40, 0.50. Once the tyrosine standard solution was added, an appropriate volume of purified water was added to each of the standards to bring the volume to 2 mL. Then, each tyrosine standard solution was mixed with 5 mL of sodium carbonate solution and 1 mL of 0.5 M of Folin-Ciocalteau reagent. The solutions were incubated at 37 °C for another 30 min. After the completion of incubation process, the absorbance of the enzyme samples was measured at 660 nm by (UV-Vis Spectrophotometer). The standard curve of absorbance vs. concentration of tyrosine was then plotted.

The protease assay was conducted by using casein as the substrate following the protocol by Sigma Aldrich. 1.0 mL of 0.65% (w/v) casein in 0.05 M of potassium phosphate buffer (pH 7.5) was added into a 15 mL centrifuge tube that containing 0.2 mL of the crude enzyme extract and partially purified enzyme (for TCA-acetone precipitation method). The tube was capped and swirled slowly, and the mixture was then incubated at 37 °C for 10 min. The enzymatic reaction was terminated by adding 1.0 mL of 110 mM trichloroacetic acid (TCA) into the tube. The mixture was furthered incubated at 37 °C for 30 min. Next, the mixture was centrifuged at 10,000 rpm for 15 min. 0.5 mL of filtrate was mixed with 1.3 mL of sodium carbonate solution and 0.3 mL of 0.5 M of Folin-Ciocalteau reagent. The mixture was incubated again at 37 °C for another 30 minutes. All the enzyme samples were assayed in duplicates. After the completion of incubation process, the absorbance of the enzyme samples was measured at 660 nm by UV-Vis Spectrophotometer. One unit of protease activity represents the amount of enzyme catalysing the production of 1 µmol of tyrosine per minute at 37 °C in the reaction. The L-tyrosine standard curve was constructed for a concentration range from 0.110 – 0.550 µmol to determine the amount of tyrosine liberated from the enzyme reaction in this assay. The protease activity was calculated by using equation (2.3) [8].

$$Protease\ activity\ \left(\frac{U}{mL}\right) = \frac{\text{Tyrosine concentration (µmol)} \times \text{volume of reaction solution (mL)}}{\text{volume of crude enzyme (mL)} \times \text{volume of sample in cuvette (mL)} \times \text{reaction time (min)}} \qquad (2.3)$$

3. Results and discussion

3.1. Effect of fermentation time and type of pineapple substrate on xylanase activity

Xylanase assay were analyzed for four type of samples which were pineapple juice, pineapple juice + probiotic, pineapple waste and pineapple waste + probiotic. Figure 1 shows the result of xylanase activity for all the four samples. It can be observed that at day 2 all of the four samples showed the highest value of xylanase activity. This is because the fermentation time depends on the culture, growth rate and enzyme production [9]. Thus, the time required for enzyme formation in bacteria is generally far less because of their faster doubling rate as compared to fungi [10]. In this study, the highest xylanase activity was shown at day two for all samples. The highest enzyme activity for xylanase was taken from pineapple juice + probiotic sample followed by pineapple waste + probiotic, pineapple juice and finally pineapple waste. The samples contain probiotic showed higher xylanase activity. This might be due to probiotic contains beneficial bacteria such as *Lactobacillus* produces digestive enzyme to facilitate digestion of the poultry [11].

From Figure 1, xylanase activity for all samples showed similar trend where the enzyme achieved highest activity at day 2. After day 2, the enzyme activities have decreased up to day 10. This might be due to the enzyme that has fully reacted on the available substrate. Similar findings were reported by R. Gaur et al., 2015 and Nagar et al., 2011 where the maximum xylanase activities also were determined at 48 hours [9,10]. The optimum period required for incubation depends on growth rate of the microorganisms and its enzyme production pattern where some studies reported that the highest xylanase production was more than 72 hours.

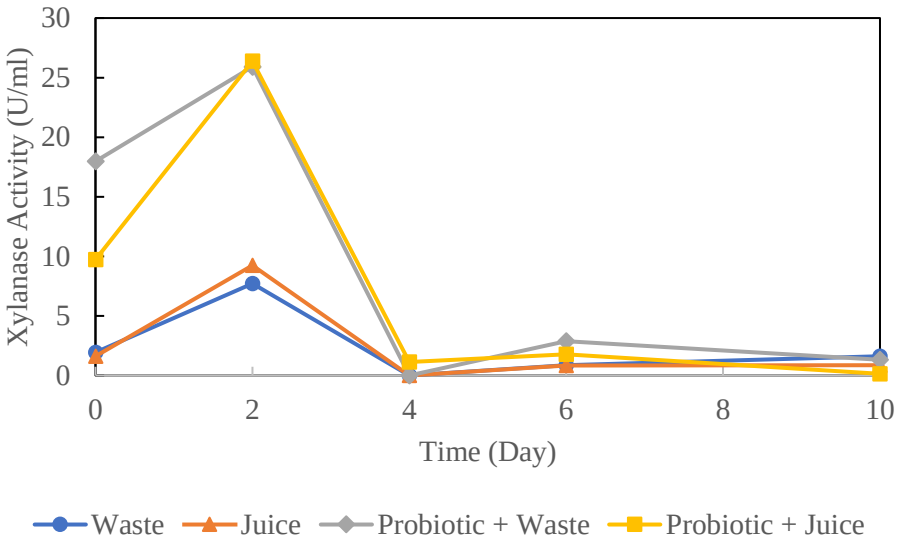

Figure 1: Xylanase activity in pineapple juice and pineapple waste.

3.2. Effect of fermentation time and type of pineapple substrate on cellulase activity

Other than xylanase, total cellulase also have been analyzed in this study as shown in Figure 2. The sample were analyzed until day 10. In comparison to xylanase, the highest cellulase activity was detected at fermentation day 4 (96 hours). Based on the study done by Acharya et al., 2012, the maximum value of cellulase activity was reported at 60 hours [12]. The different might be due to the type of substrate used in the study where they used wheat and rice straw whereas in this study pineapple fruit and pineapple waste were used as substrate. Pineapple juice + probiotic also recorded the highest value of enzyme activity followed by pineapple waste + probiotic, pineapple juice and pineapple waste. This is because, the *Lactobacillus* bacteria have higher content of digestive enzyme. Total cellulase activity show the same trend as xylanase activity where it started to decrease after day 4. This might be due to the depletion of nutrients and production of other by-products in the fermentation medium [12].

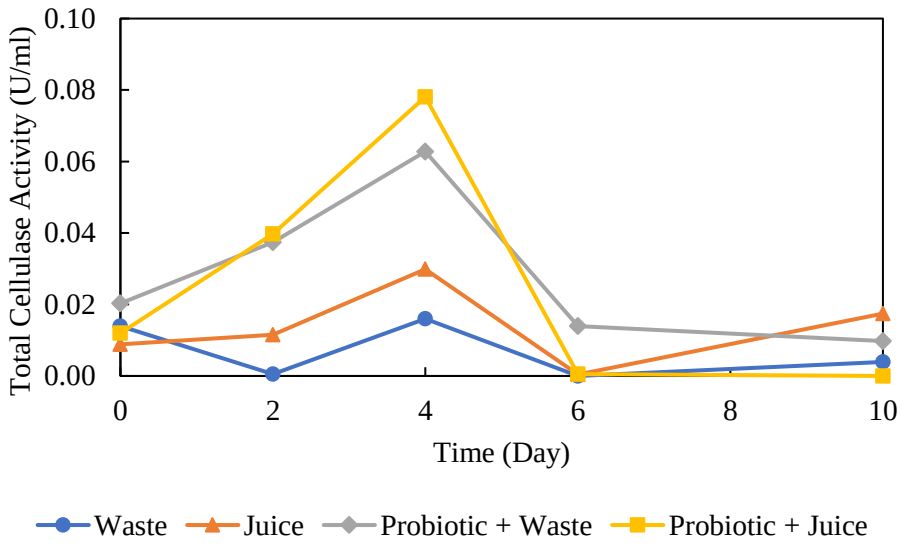

Figure 2: Cellulase activity in pineapple juice and waste.

3.3. Effect of fermentation time and type of pineapple substrate on protease activity

Figure 3 illustrated the protease activity in pineapple juice and pineapple waste. In terms of fermentation time with respect to the highest enzyme activities, protease showed longer fermentation time compared to xylanase and total cellulase which was at day 6. After day 6, the enzyme activities started to decrease up to day 10. Protease showed higher enzyme activities compared to cellulase since pineapple is very well known to contain high bromelain where bromelain is also known as protease enzyme. Due to this reason, protease is higher in samples that contain pineapple juice compares to the samples that contain pineapple waste. In addition, the probiotic used in this study also contains bromelain enzyme that act as digestive enzyme which contributed to the higher value of enzyme activity for pineapple juice + probiotic sample.

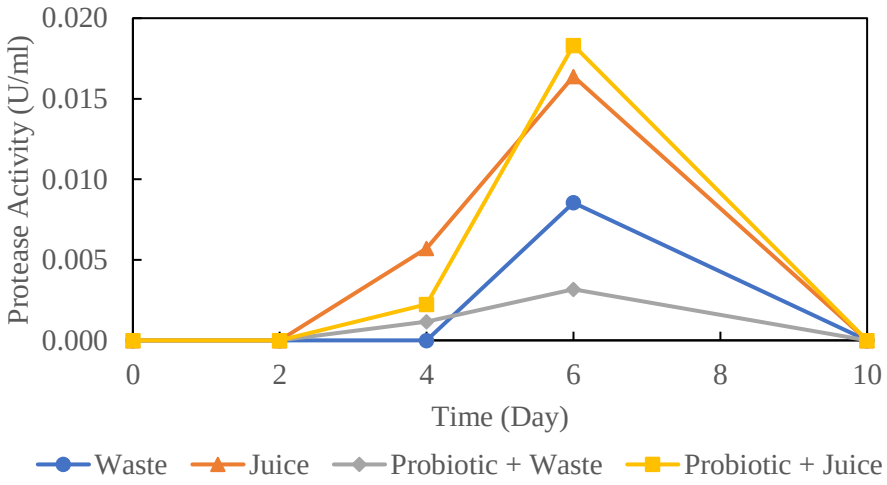

Figure 3: Protease activity in pineapple juice and waste

4. Conclusion

In conclusion, this study showed that the highest enzyme activities in pineapple juice and pineapple waste was shown at day 2 for xylanase, day 4 for cellulase and day 6 for protease. Pineapple juice + probiotic samples showed highest enzyme activities compared to other type of pineapple substrates (pineapple waste, pineapple juice + probiotic, pineapple waste). From the three types of enzymes, xylanase recorded the highest content of enzymes in pineapple fruit. This study revealed that pineapple fruit and pineapple waste can be used a cheap source of digestion enzymes that can be used in poultry.

Acknowledgements

This work was supported by industrial grant under collaboration of Universiti Malaysia Pahang and Aqina Agricultural & Biotech Industries Sdn. Bhd. (grant number UIC200802).

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