POTENTIAL INHIBITORS OF α-GLUCOSIDASE AND α-AMYLASE ENZYMES FROM LOCALLY AVAILABLE FRUIT WASTES BY SOLID STATE FERMENTATION

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ABSTRACT: A therapeutic approach for treating diabetes is to decrease thepost-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract. Inhibition of both enzymes helps to reduce the glucose level in the blood of a diabetic patient. This study was aimed to investigate the production of α-glucosidase and α-amylase inhibitors from local fruit wastes (honeydew skin, banana peel, and pineapple skin) using solid state fermentation. Each of the fruit wastes was fermented with three different types of white rot fungus *Phenarochaete chrysosporium* (PC), *Panus tigrinus* M609RQY (M6) and RO209RQY (RO2) for 7 days. Sampling was carried out starting from day 4 to day 7 to determine the enzyme inhibition activity. The samples were extracted using water prior to enzyme analysis. Most of the fruit samples showed varying degree of percentage inhibition activity depending on the sampling time. Extract of fermented banana peels with RO2 on day 4 showed the higher α-glucosidase inhibition (56.57 ± 0.32 %), followed by honeydew extract fermented with the same fungus on the same day (39.68 ± 0.05 %). Extracts of each fruit waste sample fermented with PC showed the least α-glucosidase inhibition (below 15 %). Meanwhile for α-amylase inhibition activity, the extract from fermented honeydew skins with PC on day 7 showed the highest inhibition activity *i.e.* 98.29 ± 0.63%. The least inhibition activity (43.37 ± 0.54 %) was observed in the extract from honeydew skins fermented with M6 on day 5. All positive results showed that fruit wastes could be the alternative sources for antidiabetic agent especially for α-amylase and α-glucosidase inhibitors.

KEY WORDS: *α-Amylase; Diabetes; α-Glucosidase; Hyperglycaemia, Phenarochaete chrysosporium*

1. INTRODUCTION

Diabetes mellitus is one of the major diseases which had affected human population in the world. Throughout the world, many traditional plants and natural sources are used for treating diabetes and therein lay a hidden wealth of potentially useful natural products for diabetes control [1]. Despite this, few traditional anti-diabetic plants have received

scientific or medical scrutiny, and [2] recommended accordingly that this area warrants further evaluation. One therapeutic approach for treating diabetes is to decrease post-prandial hyperglycaemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract [3]. There are enormous interests in natural anti-diabetics due to their ability to inhibit any of the carbohydrate-hydrolyzing enzymes. The growing interest to eliminate the side effect of the drugs leads to the development of green medicines due to their higher stability, higher potential and low cost of production. Many researchers have been encouraged to find as many as possible fruits, vegetables, plants, agricultural and agro-industrial waste as sources of bioactive compounds.

Recent study shows that α-amylase inhibitor can be produced by using agro waste or fruit waste [4–5]. Sousa and Correia reported in their study that α-amylase inhibition activities were observed in extracts obtained from bioprocessed pineapple and guava wastes. Usually bioactive compounds are recovered from natural sources by solid-liquid extraction employing organic solvents in the system such as soxhlet apparatus [6] and maceration [7–8]. However production and extraction of bioactive compounds by fermentation is also an interesting alternative to be explored. Fermentation technique have the ability to provide high quality and high activity extracts while precluding any toxicity associated to the organic solvents. In this process, bioactive compounds are obtained as secondary metabolites produced by microorganisms after the microbial growth is completed [9].

In this study solid-state fermentation (SSF) was used to improve the quality of fruit waste so that it will enhance α-amylase and α-glucosidase inhibitors activities. The aim of the study was to investigate the potential of α-glucosidase and α-amylase inhibitors from local fruit wastes (honeydew skin, banana peel, and pineapple skin) using SSF.

2. MATERIAL AND METHODS

2.1 Sample Collection and Preparation

The substrates used in this study were collected from the local beverages outlet (pineapple and honeydew skins) and fruits stall (banana peels) and kept for further use in a chiller at 4 °C. The dry samples were prepared by washing the fruit wastes with tap water and dried afterwards at 60 °C for 48 h [10]. Then the dried samples were ground with a dry mill and sieved through 0.2 market sieve mesh size.

2.2 Microorganisms

Three different types of microorganism were used, laboratory stock of *Phenarochaete chrysosporium* (ATCC 20696), locally isolated *Panus tigrinus* M609RQY (M6) and RO209RQY (RO2) (IMI 398363, CABI Europe-UK).

2.3 Inoculum Preparation and Fermentation Process

Spores of *Phenarochaete chrysosporium* were harvested from 7-days old plates with 25 mL of sterile distilled water. Same method was used for *Panus tigrinus* M609RQY (M6) and RO209RQY (RO2), respectively. Each flask was mixed with 6 g of substrate, 12.8 mL of media and 1.2 mL of inoculum. Then it was incubated at the room temperature for 4, 5, 6, and 7 days. All fermentations were done in triplicates.

2.4 Extraction of Fermentation Culture

Extraction of fermentation culture was performed using modification method from Correia [5]. About 60 mL of distilled water was added to fermented sample and the culture was homogenized in a Waring blender for 1 minute, followed by centrifugation at 10000 g at 4 °C for 15 minutes. The samples were filtered through Whatman No.1 filter paper.

2.5 Enzyme Inhibition Assay

2.5.1 α-Amylase Inhibition Assay

The α-amylase inhibition assay was performed using the method adopted from Apostolidis [11]. About 25 µL of samples and 25 µL of 20 mM phosphate buffer (pH 6.9), containing α-amylase at the concentration of 0.5 mg/mL was incubated at 25 °C for 10 minutes. After pre-incubation, 25 µL of 0.5 % starch solution in 20 mM phosphate buffer (pH 6.9) was added. The reaction mixture was incubated at 25 °C for 10 minutes. Then 50 µL of 96 mM 3, 5-dinitrosalicyclic acid (DNS) color reagent was used to stop any reaction. The microplate was incubated in a boiling water bath for 5 minutes and let to cool to the room temperature before the absorbance was measured at 540 nm. The percentage of inhibition was calculated as follows:

$$Inhibition\ (\%) = \left(\frac{Control_{540} - Extract_{540}}{Control_{540}}\right) \times 100 \quad (1)$$

2.5.2 α-Glucosidase Inhibition Assay

The α-glucosidase inhibition assay was performed using the method adopted from Marcia Da [12]. About 50 µL of sample solution, 450 µL of 0.1 M phosphate buffer (pH 6.9) and 250 µL of 5 mM *p*-nitrophenyl-*α*-D-glucopyranoside solution was incubated in a glass tube at 37 °C for 5 minutes. After pre-incubation, 250 µL of α-glucosidase solution was added. Then the reaction mixtures were incubated at 37 °C for 15 minutes. After incubation, the absorbance was read at 400 nm by microplate reader and compared with the control. Percentage inhibition was calculated as follows:

$$Inhibition\ (\%) = \left(\frac{Control_{400} - Extract_{400}}{Control_{400}}\right) \times 100 \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. *α*-Glucosidase Inhibition

α-Glucosidase inhibitors function by interfering with the breakdown of carbohydrates into glucose in the gut. *α*-Glucosidase is an enzyme that acts in the brush border of the proximal intestine to metabolize disaccharides and complex carbohydrates [13]. Inhibition of this enzyme delays the absorption of glucose following starch and sucrose conversion, moderates the postprandial blood glucose elevation, and thus mimics the effects of dieting on hyperglycaemia [14]. Recent studies have shown that plant phytochemicals exert anti-diabetic activity through inhibition of carbohydrate-hydrolyzing enzymes such as α-glucosidase and α-amylase. Thus, fruit wastes could be a potential natural sources of enzyme inhibitors. It is also reported that the phytochemicals of the fruit wastes could be enriched after fermentation with microorganisms [15–16].

In this study, three types of fruit wastes (honeydew skin, banana peel, and pineapple skin) were fermented with three different types of white rot fungus (PC, M6 and RO2) for 7 days by SSF. The percentage of enzyme inhibition over the fermentation period is shown in Fig. 1 (A - C). As the highlight of the study, extract of fermented banana peels with RO2 on day 4 showed the higher potential of α-glucosidase inhibition with 56.57 ± 0.32 %. This was followed by fermented honeydew extract with 39.68 ± 0.05 % of inhibition. Extracts of each fruit waste sample fermented with PC showed the least potential of α-glucosidase inhibition (below 15 %).

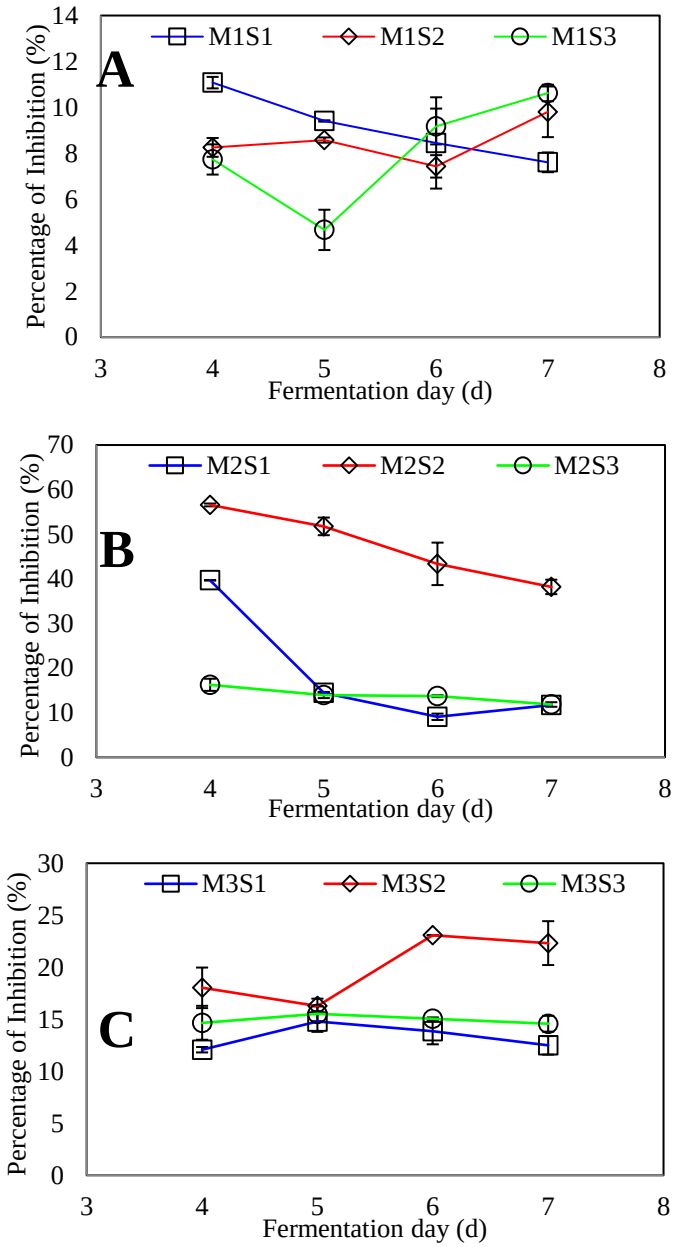

Fig. 1. Percentage of α-glucosidase inhibition of different combination of fungus and substrate. (A) PC (M1) and honeydew (S1), banana (S2) and pineapple (S3), (B) RO2 (M2) and honeydew (S1), banana (S2) and pineapple (S3), (C) M6 (M3) and honeydew (S1), banana (S2) and pineapple (S3).

Previous studies have shown that, the α-glucosidase inhibitory activity could be related to the presence of bioactive compounds, like flavonoids and phenolic in the plant source [17]. The total phenolic content of the honeydew skin, bananas peel and pineapple skin sample were 456.5 GAE mg/L, 305.5 GAE mg/L and 501.5 mg/L, respectively. Apostolidis and Lee [18] concluded on a highly positive correlation between α-glucosidase inhibitory activity and phenolic contents of the extracts. However, it was found that banana peel fermented with the fungus demonstrated a higher α-glucosidase inhibition eventhough it has the lowest total phenolic contents as compared to pineapple and honeydew skins. This suggested that these differences could be due to synergistic of phenolics and also interaction with non-phenolics which may contribute to the total α-glucosidase activity [19]. At the end of the fermentation period, lower α-glucosidase inhibitory activity was observed. This may indicate that modification of phenolics or denaturation of phenolics-associated proteins may contribute to a lowering of α-glucosidase inhibition [5].

3.2. α-Amylase Inhibition

α-amylase is responsible for cleaving the starch during the digestive process, which is important for managing postprandial blood glucose levels. Inhibition of this enzyme will block the absorption of glucose through the inhibition of carbohydrate-hydrolyzing enzymes in digestive tract [3]. Thus, it can be a therapeutic agent in the treatment of diabetes and obesity since it can be a modulator for postprandial blood glucose levels. Recent studies have shown that plant phytochemicals exert anti-diabetic activity through inhibition of carbohydrate-hydrolyzing enzymes such as α-glucosidase and α-amylase. Thus, fruit wastes could be potential natural sources of enzyme inhibitors. It is also reported that the phytochemicals of the fruit wastes could be enriched after fermentation with microorganisms [15–16].

For α-amylase inhibition activity, the extract from fermented honeydew skins with PC on day 7 showed the highest potential inhibition activity of 98.29 ± 0.63 %. The least potential inhibition activity occurred in an extract from honeydew skins fermented with M6 on day 5 showed 43.37 ± 0.54 %. The inhibitory activity of α-amylase was significantly higher (> 80 %) in the early stages of fermentation (days 4) for all combination of fermented fruit waste and fungus as shown in Fig. 2 (A-C). The extracts from the fermented pineapple skin for all samples showed higher percentage of inhibition (> 90 %) until day 7. Sousa and Correia [5] also reported that the fermented pineapple residue with *Rhizopous oligosporus* demonstrated higher percentage of α-amylase inhibition (71.43 % to 100 %) after 10 days of fermentation. However, a significant decrease on enzyme inhibition activity was observed for the fruit waste samples fermented with M6 and RO2. This may indicate that modification of phenolics or denaturation of phenolics-associated proteins may contribute to a lowering of α-glucosidase inhibition [20]. Few extracts from fermented honeydew skin and banana peels with M6 and RO2 also showed the higher negative values (~ 30 %) and this could indicate that the α-amylase is activated rather than inhibited [3].

The higher α-amylase activities shown by the fermented fruit waste extracts could be positively correlated with the higher total phenolics present in the fruit waste samples. The total phenolic content of the pineapple skin was the highest (501.5 GAE mg/L), followed by honeydew skin (456.5 GAE mg/L) and banana peel (305.5 GAE mg/L). Phenolic compounds including flavonoids (such as catechin, quercetin, myricetin and luteolin) have

been reported as potent α-amylase inhibitors. Li [21] has demostrated that the pineapple peel is rich with phenolic compouds such as epicatechin, catechin, gallic acid and ferulic acid, thus in agreement with our results.

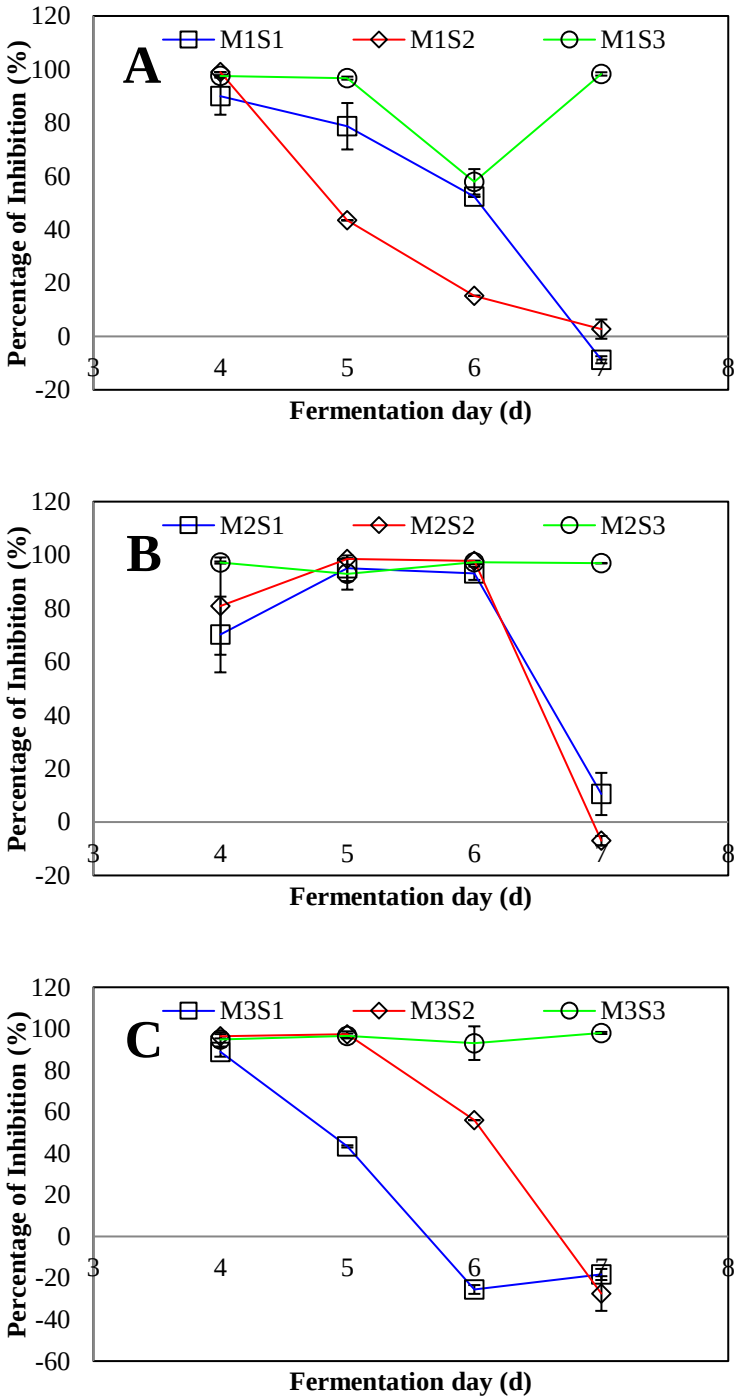

Fig. 2. Percentage of α-amylase inhibition of different combination of fungus and substrate. (A) PC (M1) and honeydew (S1), banana (S2) and pineapple (S3), (B) RO2 (M2) and honeydew (S1), banana (S2) and pineapple (S3), (C) M6 (M3) and honeydew (S1), banana (S2) and pineapple (S3).

4. CONCLUSION

The aim of the study was to investigate the potential of α-glucosidase and α-amylase inhibitors from local fruit wastes (honeydew skin, banana peel, and pineapple skin) using solid-state fermentation. The extract of fermented banana peels with RO2 on day 4 showed the higher potential of α-glucosidase inhibition (56.57 ± 0.32 %). However, the percentage of inhibition of α-glucosidase can be improved by series of media and process optimization of the fermentation conditions. For α-amylase inhibition activity, the extract from fermented honeydew skins with PC on day 7 showed the highest potential inhibition activity 98.29 ± 0.63 %. Overall, the result showed that the selected fruit wastes have the potential to be used as antidiabetic agents in the future. It can also be a novel strategy for waste management for local fruit wastes in Malaysia.

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