Purification and characterization of α-glucosidase enzyme from rice groats

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Abstract. Groat rice is the side product of rice milling process and potentially producing α-glucosidase. The enzyme has an important role in starch degradation process by breaking α-glycosidic bonds to form glucose. This research aimed to understand the optimum activity of the purified α-glucosidase. The research stages are isolation, purification, characterization of substrate concentration, pH level, incubation time, temperature, and effect of metal ion, and dinitrosalicylic acid (DNS) method to determine the activity of α-glucosidase. The result showed the specific activity of α-glucosidase crude extract at 0.003 IU/mg. Fractionation of the highest specific activity on fraction 4 revealed at 0.042 IU/mg and constantly increasing on the dialysis stage at 1.121 IU/mg. α-glucosidase reached optimum activity at substrate concentration 2%, pH of 7, incubation time of 40 minutes, at 37°C, with metal ion Mn²⁺, Co²⁺, Na⁺, Mg²⁺, Ca²⁺ and K⁺ as activators and Zn²⁺ as inhibitor. α-glucosidase could be used to find analog compounds as the cure of type 2 diabetes mellitus.

1. Introduction
α-glucosidase (EC.3.2.1.20) is an enzyme found in the epithelium membrane of the small intestine that breaks down carbohydrate by breaking the bond α-glycosidic into glucose which increases blood sugar level, especially in individuals with type 2 diabetes mellitus [1]. α-glucosidase inhibitors can be used to reduce the increased blood of the sugar level.

Researches focus to find analog compound as α-glucosidase inhibitors are widely available and the availability of α-glucosidase is highly demanded [2]. α-glucosidase capable to be isolated from bacteria, yeast, insects, and plants. Groat rice could be benefitted as an alternative source to fulfill the demands of α-glucosidase.

Groat rice is the side product of rice milling process with average size lesser than 0.2 mm of the whole rice or fractured rice with size 1.7 mm [3]. Groat rice has a similar chemical component with milled rice [4]. α-glucosidase on rice is identified to have an important role in starch hydrolysis that produced glucose which is used by plants as an energy source [5].

The result showed that α-glucosidase can be isolated from groat rice with a large α-glucosidase activity and characterization that is almost similar to α-glucosidase isolated from other sources.
2. Materials and methods
2.1 Materials
The materials of this research were groat rice flour, dinitrosalicyclic, maltose, phosphate buffer 67 mM, and aquades.

2.2 Instruments
The instruments of this study included the analytical balance, centrifuge, stirrer magnetic fisher, blender, micropipette (100-600 μL), and spectronic 20 D+.

2.3 Methods
2.3.1 Isolation of the α-glucosidase enzyme. Groat rice flour was soaked for one night using phosphate buffer 67 mM and centrifugated at 8,000 rpm.

2.3.2 Purification of the α-glucosidase enzyme
2.3.2.1 Fractionation of Ammonium Sulphate. A crude extract of the α-glucosidase enzyme is added ammonium sulphate based on the saturation level table and centrifuged at 10,000 rpm.

2.3.2.2 Dialysis. The highest activity of the α-glucosidase enzyme was inserted into the cellophane membrane and immersed in a 67 mM phosphate buffer solution.

2.3.3 Characterization of α-glucosidase enzymes
The enzyme α-glucosidase activity was tested using the DNS method with variations in substrate concentration 0.5-2.5%. pH 5-9, incubation time 10-50 minutes, temperature 30-55°C and some metal ions.

2.3.4 Test DNS activity method of α-glucosidase enzyme
The activity of α-glucosidase was tested by the dinitrosalicylic acid (DNS) method [6]. 1 mL of phosphate buffer and 1 mL maltose substrate were added into 1 mL of enzyme solution. The mixed solution was incubated for 40 minutes and added with DNS reagent. The mixed solution was heated for 10 minutes before it was cooled. The solution was measured by Spectronic 20 D+. One unit of α-glucosidase activity was declared as μmol of glucose produced from glucosidase hydrolysis for each test condition. The value of α-glucosidase activity was determined based on the following equation:

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\text{Enzyme activity (IU/mL)} = \frac{\text{Sample glucose concentration} \times 1000}{\text{Volume} \times \text{time} \times \text{molecular weight}} 
\]

3. Results and discussion
3.1 isolation of α-glucosidase enzyme
α-glucosidase was isolated using low-concentration phosphate buffer to help the lysis process of cell and organelle that eventually increase the extracted α-glucosidase from its source to phosphate buffer solution [7]. The purification of α-glucosidase was performed in two stages, the first stage was fractionation with ammonium sulphate and the second stage was dialysis.

Table 1 concluded the result of α-glucosidase purification with a specific activity which was increased in each stage. The high activity of α-glucosidase was in contrast with the protein level, this was mainly caused by proteins, other than α-glucosidase, that were deposited on certain ammonium sulphate saturation. Thus, the α-glucosidase protein was best deposited at 60-80% saturation level [8]. The result was in accordance with α-glucosidase that was purified from Moss Hyophilla nymaniana [9].
Table 1. α-glucosidase enzyme activity refining results.

| Sample                | Activity Total (IU/mL) | Total Protein (mg/mL) | Specific activity (IU/mg) | Purification (Fold) |
|-----------------------|------------------------|-----------------------|--------------------------|---------------------|
| Crude extract         | 0.017                  | 4.80                  | 0.003                    | 1                   |
| 0-20% fraction        | 0.044                  | 3.86                  | 0.011                    | 3.666               |
| 20-40% fraction       | 0.037                  | 4.05                  | 0.009                    | 3                   |
| 40-60% fraction       | 0.033                  | 4.41                  | 0.007                    | 2.33                |
| 60-80% fraction       | 0.057                  | 1.35                  | 0.042                    | 14                  |
| Dialysis results      | 0.572                  | 0.51                  | 1.121                    | 373.666             |

3.2 Characterization of α-glucosidase enzyme

3.2.1 Substrate concentration and pH of enzyme activity
α-glucosidase from groat rice was optimum at 2% concentration with activity at 0.433 IU/mL and decreased at 2.5% concentration with activity at 0.422 IU/mL (Figure 1a). The decreased activity was caused by the inability of a substrate to inhibit the active site of an enzyme. The pH level of 8 with activity at 0.459 IU/mL was the physiological pH of α-glucosidase that could increase enzyme activity (Figure 1b).

3.2.2 Incubation time and temperature of enzyme activity
The optimum incubation time for α-glucosidase was 40 minutes with activity at 0.433 IU/mL and decreased at 50 minutes of incubation time with activity at 0.418 IU/mL (Figure 1c). The longer the incubation time resulted in the longer contact between substrate and enzyme. However, the enzyme and saturated substrate resulted in the decreased activity at a certain time [10].

Setting up the temperature at 37°C could increase α-glucosidase activity at 0.448 IU/mL and at 55°C temperature the α-glucosidase activity decreased which was caused by enzyme denaturation (Figure 1d). The obtained α-glucosidase has activity with a slight similar optimum temperature with α-glucosidase obtained from Trichoderma longibrachiatum [10].

Figure 1. Effect of (a) concentration substrate (b) pH (c) incubation time and (d) temperature on the α-glucosidase enzyme of rice groats.
3.2.3 Effect of Metal Ion
Characterization of the effect of metal ions on α-glucosidase was illustrated in (Figure 2). Metal ion Zn$^{2+}$ hindered α-glucosidase at 67.06% percentage whereas Na$^+$, Mg$^{2+}$, Ca$^{2+}$, K$^+$, Co$^{2+}$, and Mn$^{2+}$ increased α-glucosidase activity at the same percentage. Metal Ion Zn$^{2+}$ is reported to hinder α-glucosidase activity from Rhizobium sp [11]. Metal ion K$^{2+}$, Na$^+$, and Mg$^{2+}$ from Moss Hyphillia nymaniana (Fleish) Menzel are reported to increase α-glucosidase activity [8].

![Figure 2](image)

**Figure 2.** Effect of metal ions enzyme activity and relative activity of α-glucosidase from rice groats.

3.2.4 Kinetics parameters
Kinetics study on α-glucosidase is illustrated in (Figure 3), where Km value at 0.4330 g/mL and Vmax value at 8.2×10$^{-3}$ IU/mg protein using maltose as substrate. Silva, et al. reported that α-glucosidase from Aspargillus niveus has a low Km value at 0.07 mg/mL using maltose as substrate, 0.07 mg/mL using starch as substrate, and 0.11 mg/mL using amylopectin as substrate [12].

![Figure 3](image)

**Figure 3.** Lineweaver-Burk curve to determinant of kinetics parameters (Km and Vmax) for α-glucosidase enzymes by using maltose as the substrate.

4. Conclusion
The conclusion of this observation, the optimum activity of the purified α-glucosidase enzyme from the rice groats has optimum concentration of a maltose substrate about 2%, pH of 7, incubation time of 40 minutes, the optimum temperature of 37°C, the metal ions Zn$^{2+}$ as an inhibitor and Na$^+$, Mg$^{2+}$, Ca$^{2+}$, K$^+$, Co$^{2+}$ and Mn$^{2+}$ as activator. The value of Km of 0.4330 mL/g and Vmax values of 8.2×10$^{-3}$ IU/mg protein by using maltose as a substrate.
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