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REVIEW ARTICLE

ALPHA-AMYLASE INHIBITORY ACTIVITY OF INHIBITOR PROTEINS IN DIFFERENT TYPES OF COMMERCIAL RICE

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ABSTRACT

The inhibition of α-amylase enzyme by the inhibitor proteins extracts of all the three types of commercial rice, white rice (WR), brown rice (BR) and glutinous rice (GR) were investigated. Among the three types of rice, the BR has significantly the highest concentration of the inhibitor protein (0.030 ± 0.002 mg/ml) compared to glutinous rice (0.006 ± 0.001 mg/ml) and white rice (0.005 ± 0.001 mg/ml). In term of the percentage of inhibition of the α-amylase enzyme of the *Aspergillus oryzae*, the brown rice shows the highest inhibition (61.22 %) among the three types of rice with the lowest maltose liberated. The acarbose (0.1 mg/ml) which was used as a positive control represent the highest inhibition of the α-amylase enzyme inhibitor among all of the tested samples. Even though inhibitor protein extract of BR is possessed lower ability to inhibit the α-amylase, yet it still can be one of the best option and alternative for the Diabetes Mellitus patients for their daily consumption compared to the WR and GR.

KEYWORDS

α-amylase, rice inhibitor proteins, Diabetes Mellitus

1. INTRODUCTION

Diabetes Mellitus, which is one of the world's oldest known disease has become one of the globally serious occurring case [1]. This disease is characterized by the occurrence of chronic hyperglycemia that disturbed carbohydrate, fat and protein metabolism. The disturbance of these metabolisms is the result of defects in insulin secretion, insulin action or both [2]. This metabolic disorder of multiple aetiology is related to an increased risk of various complications; cardiovascular disease, retinopathy, and nephropathy [3].

Many therapeutic approach has been developed to treat diabetes. One of the approach is by decreasing the post-prandial hyperglycaemia. Through this approach, the carbohydrate –hydrolysing enzymes, α-amylase and α-glycosidase, in the digestive track were inhibited [4]. This approach will delay the absorption rate of glucose and increase the digestion time and eventually reduce the rise in plasma glucose level. Therefore, suppressed the post prandial hyperglycemia [5,6].

As a staple food for most of the world population, rice or Oryza sativa contributes a lot in human dietary intake, including providing energy, nutrition and vitamins where most of this nutritional content is determined by the seed storage proteins (SSPs) content of the rice [7,8]. Apart from being the nutrition contributor, proteins with inhibitory activity against α-amylase or protease have been discovered in most cereals, including rice [9]. A research by Feng et al, 1991 also mentioned that rice was found to have amylase activity and numbers of proteinaceous alpha-amylase inhibitors in which these proteinaceous alpha-amylase inhibitors have not been well characterized [10]. The salt-soluble proteins of ~14 kDa were reported to inhibit the α-amylase enzyme of insect and mammalian.

Due to the high consumption of different types of rice around the globe and they claimed that rice is related to diabetes mellitus, it is important to understand in depth about this plant whether it is good or bad for our health. A study has found out that the germinated brown rice extract was shown to have antidiabetic properties [11]. However, most study focus on the bioactive components of rice that can help in reducing the Diabetes Mellitus effect. There is still lacking the study that focus on the protein extract of different types of commercial rice consumed daily by most people, especially in Malaysia. Therefore, in this study, the relation between the ability of rice inhibitor protein to inhibit the α-amylase of different types of rice is investigated.

2. MATERIAL AND METHODS

2.1 Preparation of Commercial Rice Sample

Available brands of Malaysian commercial rice types; white rice, brown rice and glutinous rice, were randomly selected according to consumers' preference. These samples were made into triplicate and then ground using blender.

2.2 Protein Inhibitor Extraction

The extraction of the inhibitor protein in rice was carried out using the method adopted by Pagnussatt et.al, 2012 with slight modification [12]. About 1g of powdered samples (ground using the mortar and pestle in liquid nitrogen) was homogenized in 3 ml of 95% ethanol according to 1:3 (v/v) ratio. After few minutes vortexing, the incubation of the

homogenates was performed at room temperature on a shaker for 12hr. The samples were sonicated for 30 sec and centrifuged at 14 000 rpm for 15 min. The supernatants were collected as the extracted inhibitor protein.

2.3 α -amylase Inhibition Assay

The α-amylase inhibition assay was carried –out by the modification of the adopted method [4, 13, 14]. The quantification of the reducing sugar (maltose equivalent) amount which were liberated under the assay condition is done to determine the α-amylase inhibition. The enzymatic inhibitory activity was expressed by the decrease of maltose liberated.

Extracted samples (100 µl) containing protein inhibitors was pre-incubated at 37°C for 30 min with 100 µl of 0.05 mg/ml of Aspergillus oryzae α-amylase in 0.02 M potassium phosphate buffer pH 6.9. Before the following incubation, 100µl of 0.5% soluble starch solution. The following incubation was performed at 37°C for 30 min. An amount of 200 µl of dinitrosalicylic acid (DNSA) colour reagent was added to the reaction before the reaction was terminated. The reaction was terminated by transferring into a thermos cycler block of 100°C for 5 min. The reaction was let to cool down to room temperature and 100 µl of the reacted samples were transferred to cuvettes. The mixture was diluted with 1 ml of distilled water and the absorbance of the reaction mixture was measured at 540 nm using a spectrophotometer.

The acarbose (0.1 mg/ml) was used as a positive control. The control incubation, which represents the 100% enzyme activity was carried out by replacing the sample with 95% ethanol. The enzyme solution was replaced with distilled water act as a blank incubation to allow the absorbance produced by the plant extracts.

The absorbance (A) of the generated maltose was calculated as follows:

$$\mathbf{A_{540nm}\ control\ or\ plant\ extract} = A_{540nm}\ \text{Test} - A_{540nm}\ \text{Blank}$$

Using this net absorbance (A) acquired, the concentration of maltose (mg/ml) generated was calculated using the equation from the maltose standard. The percentage (%) of reaction were then calculated as:

$$\textbf{Percentage (\%) of reaction} = \frac{\text{Mean maltose in sample} \times 100}{\text{Mean maltose in control}}$$

From the percentage (%) of reaction obtained, the percentage of inhibition was calculated as:

$$\textbf{Percentage (\%) of inhibition} = 100\ (\%) - \text{Percentage (\%) of reaction}$$

2.4 Statistical Analysis

The statistical analysis was carried out using SPSS version 20 software. The collected data from this research were subjected to the one–way analysis of variance (ANOVA) at level of 5% significance followed by Turkey test to compare the means. Values lower than 0.05 is considered as significant. The statistical test was performed in triplicates.

3. RESULTS

3.1 The Amount of Protein Inhibitors in Different Type of Rice

The quantification of total inhibitor proteins contents of all the three different types of rice presented in Table 1. The amount of inhibitor protein in brown rice (BR) (0.030 ± 0.002 mg/ml) was found to have significantly the highest concentration as compared to white rice (WR) (0.005 ± 0.001 mg/ml) and glutinous rice (GR) (0.006 ± 0.001 mg/ml).

Table 1: The concentration of inhibitor proteins in different types of commercial rice

Rice Types	Concentration of Inhibitor Proteins (mg/ml)
White rice	0.005 ± 0.001[b]
Brown rice	0.030 ± 0.002[a]
Glutinous rice	0.006 ± 0.001[b]

The value was express as means ± SEM of triplicates tests. Different letters (a, b) indicates significant different at $p < 0.05$, (n=3).

3.2 The α-amylase Inhibition Activity of Protein Inhibitors Extract of Different Rice Types

The reaction of the substrate breakdown by the enzyme amylase cause the formation of maltose. In this study, the formation of maltose during the reaction is used to calculate the percentage of inhibition of the enzyme by the sample.

Table 2: The percentage of reaction and inhibition by different types of rice and the positive control (acarbose)

Sample	Reaction (%)	Inhibition (%)
Control	100.00	0.00
Acarbose	38.78	61.22
WR	161.22	-61.22
BR	43.88	56.12
GR	217.35	-117.35

*WR, White ric; BR, Brown rice and GR, Glutinous rice
Percentage of inhibition was calculated as 100 - % reaction; the % reaction= (mean maltose in sample/ mean maltose in control) x 100

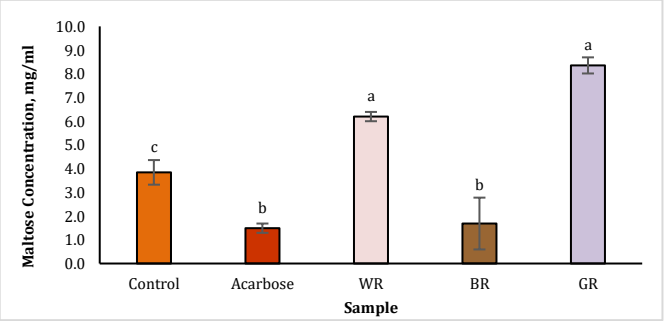

Figure 1: Maltose production in the presence of different rice types inhibitor proteins extract. The result was presented as mean ± SEM. (a, b, c) indicates significant different at $p < 0.05$, (n=3). WR, White rice; B, Brown rice and G, Glutinous rice.

From Table 2, the brown rice shows positive inhibition eventhough it did not give higher inhibition compared to the positive control (acarbose) with 56.12 %. By contrast, in both samples; white rice (- 61.22 %) and glutinous rice (-117.35 %) shows negative inhibition. This negative inhibition was resulted from the significantly high maltose formation by the white rice (6.19 %) and glutinous rice (8.35 %) compared to the control with only 3.84%.

4. DISCUSSION

Cereal grains such as rice has been reported to demonstrate many functional and bioactive properties by their high fiber content of their antioxidant activity. However, the ability of their proteins is usually overlooked [15]. A study done by Pagnussatt et.al, 2012 has established a procedure to extract the inhibitor protein from cereal as such in rice [12]. Due to the fact that protein compounds with the inhibitory activity are

found in the prolamin fraction of the cereals, there were recovered by using an alcohol solution from sources. The 13-kda prolamin has been proved to have the highest level of biological activity due to the presence of the ACE-inhibitor peptides, and also contain of some DPP-IV-inhibitor activity [16]. Another type of prolamin in rice 16-kDa prolamin has been reported to has mostly ACE-inhibitor peptide sequence, as some activity of the DPP-IV-inhibitor, hypotensive and antioxidant [16].

Since brown rice has found to have the highest inhibitor protein content compared to the other type of rice, it provides the best reason on its ability to inhibit the α-amylase enzyme by the Aspergillus oryzae as tested in this study. Even though the inhibition activity of brown rice is lower compared to acarbose (61.22 %), yet it shows no significant different in with acarbose in term of maltose generated. Plus, it shows that the extract contained the bioactive peptide which able to inhibit α-amylase enzyme. This class of proteins possess inhibitory activity of amylolytic enzymes where it becomes part of the defence mechanisms in grains against the attack of pests and germination control [17,18]. Therefore, as for the management of blood glucose which occur in type 2 DM, it may serve as an alternative way for the DM patients.

On the other hand, the negative inhibition bofwhite rice and glutinous rice indicates that the α-amylase enzyme was being activated rather than inhibited and may cause the aggravation of the DM condition if consumed [19] .Not only that, the presence of the intrinsic carbohydrates and /or reducing sugars in the extract could influence the result and/or the inhibition activity [4, 20].

5. CONCLUSION

The study shows that the inhibitor proteins which have the content of prolamin in brown rice have the ability to inhibit the carbohydrate hydrolysing enzyme like α-amylase. Among the commercial rice that usually being consumed by most people; white rice, brown rice and glutinous rice, the brown rice is the best choice for daily consumption especially by the people with various levels of diabetes mellitus condition. Besides that, through the research, we also found out that glutinous rice and white rice have high amount of reducing sugar and their inhibitor protein extracts has the ability to activate the α-amylase rather than inhibited it which might worsen the diabetes mellitus condition if ingested. This study also provides a base for the selection and the amount of rice intake for the consumer in order to maintain the healthy life style.

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