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PROTEOMIC ANALYSIS OF RICE SEED STORAGE PROTEINS IN RELATION TO NUTRIENT QUALITY OF THREE DIFFERENT COMMERCIAL RICE TYPES

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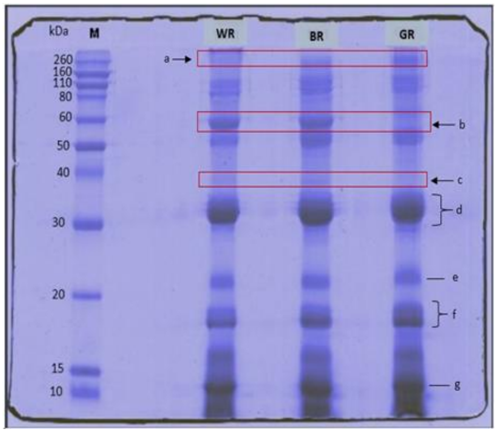
Graphical abstract



Abstract

Seed storage proteins (SSPs) are the most important component in rice, which provides nutrient to consumers. The SSPs content and composition are among the important determinant for rice quality determination specifically for nutritional value. Multiple factors have been identified to give effect to the nutritional value of rice grain including different types of rice. The purpose of this study is to investigate the expression level of SSPs of rice and also relates the nutrient quality of rice with the variability in SSPs expression from three different types of rice commercially available in markets. The SSPs were extracted from three different types of rice; white rice, brown rice and glutinous rice of the local rice brands in markets. Bradford Assay was carried out to determine the total SSPs content and brown rice was found to have significantly higher total SSPs content (9.157± 0.4 mg/100mg seeds) compared to white rice (6.933± 0.8 mg/ 100mg seeds) and glutinous rice (5.388± 0.2 mg/ 100mg seeds). Based on the banding patterns of the SDS-PAGE, there were different level of expressions were observed between the three rice types. The different expression can be observed obviously at the glutelin precursor region and glutelin acidic subunit region and prolamin polypeptides region for each type of rice. Therefore, contribute to the different nutritional value for dietary intake.

Keywords: Rice, nutritional value, seed storage protein (SSPs), protein profile

1.0 INTRODUCTION

Rice or scientifically known as *Oryza sativa* is a staple food for nearly half of the world's population. About more than 90 % of rice production is being consumed by people in Asia including Malaysia. Plus, there is an upward trend of rice consumption for countries outside Asia [1]. This statistic shows that rice is one of the most consumable food in the world nowadays. Thus, more people are concern about the nutritional value of the rice in their dietary intake.

Rice is one of the sources for carbohydrate, vitamins and proteins. However, the determination of the nutritional value of rice depends on its seed storage proteins (SSPs) contents [2]. There are about 50% of storage proteins that made up the total protein in mature cereal [3]. These storage proteins consist of albumin, globulin, prolamin and glutelin. Among those four SSPs, prolamin and glutelin are considered as the major SSPs in rice as these two proteins are the most abundant SSPs in rice seed [4] with about 60-80% of total seed proteins comprises of glutelins whereas 20-30% are prolamin [5]. SSPs of rice are made up of 57 kD glutelin precursors, 40 kD acidic and 20 kD basic glutelin subunits, 26 kD a-globulin polypeptides, and 16, 13 and 10 kD prolamin polypeptides [6].

Despites of its role in providing nutrients to its consumers, proteins in rice are relatively low. These proteins generally are deficient in some essential amino acid and therefore poor in nutritional quality [7]. Rice prolamin is indigestible, causing the reduction of rice protein nutritional quality [8]. Thus, the nutritional value can be raised by increasing the protein content [9], which can enrich the essential amino acids in the rice.

In term of the selection of rice consumed, people around the world have their own preferences in choosing the rice types in their dietary intake. Korean and Japanese people prefer sticky and soft rice. Whereas, India and Malaysian prefer to eat non- sticky rice such white rice. Yet, the selection of type of rice differs in preparation of variety Malaysian cuisines. Plus, the lifestyle of people also influence the intake of rice either the type of rice or the amount taken.

This study aims at investigating the expression level of SSPs from three types of rice and the relatedness of nutrient quality with the variability in SSPs expression. Result from this study will provide people with the information on the rice that they consume daily and thus helping them in choosing the best type of rice and planning for best dietary intake for daily consumption.

2.0 EXPERIMENTAL

Three types local brand of commercial rice (white rice, brown rice and glutinous rice) with three replicates for each rice types available in markets were ground using blender. About 60 mg of the grounded rice were further powdered to finer powder using mortar and pestle in liquid nitrogen. The extraction of total seed storage proteins was done according to [9] with some modifications. The powdered samples were homogenized with 1 ml of lysis buffer (7 M urea, 2 M Thiourea, 2 % CHAPS , 2 % (v/v) pH 3-10 IPG buffer, 20 mM DTT, 10 mM PMSF). The samples were then vortexed vigorously until all of the powder was fully homogenized with the buffer. The homogenates were incubated at room temperature for 2 hours with constant shaking and followed by centrifugation at room temperature at 16 160 x g for 20 mins. The extracted seed storage proteins were isolated from the supernatant collected.

Bradford assay using bovine serum albumin (BSA) was carried out to determine the protein concentration of the extracted samples. The SPSS was used to statistically analyze the concentration of the proteins.

The extracted proteins were separated using SDS-PAGE on the basis of molecular weight. About 8 µg of protein extracts of each samples were loaded in to the wells of polyacrylamide gel (12 % resolving and 4 % stacking gel). The gel electrophoresis was ran with the voltage of 200 V for 45 min. The banding pattern was visualized by staining with Coomassie Brilliant Blue R250.

3.0 RESULTS AND DISCUSSION

3.1 Total Seed Storage Proteins (SSPs)

Based on result of protein quantification, it can be seen that all three types of the rice had the amount of protein contents that were significantly different from each other with the total seed storage proteins of brown rice was significantly the highest, 9.157 ± 0.4 mg/ 100 mg (Figure 1). Meanwhile, the total SSPs content for the other two type of rice, white rice and glutinous rice were 6.933± 0.8 mg/ and 5.388 mg ± 0.2 / 100 mg respectively. Statistically, all type of rice displayed significantly different amount of proteins (Figure 1).

The differences in protein content in rice grains could affect the nutritional quality of the rice. There are multiple factors that could affect the nutritional value of rice or specifically protein in rice which include environmental condition, management system, types of rice, and milling process. Also it was discussed that higher significant phenotypic value of all four traits in rice grown under upland compared to lowland [10]. Growing rice in a non-flooded environment does give effect to the grain quality where it also involved the protein content [2].

The storage proteins are coded by multiple genes, which are specifically and highly expressed in seed [11, 12]. Different type of rice such as brown rice, basmati rice, glutinous rice and long grain rice contain different multiple genes encoding for storage protein that consequently contributed to the variability in rice quality specifically to the nutrient quality [13, 14]. The milling process which alter the appearance of rice to increase in whiteness causing the loose of protein content of the outer part of brown rice [2].

The protein content of brown rice was expected to be higher than the other type of rice especially white rice as it has higher nutrient content compared to white rice [15]. Thus, indicated the higher amount of protein content compared to the other type of rice.

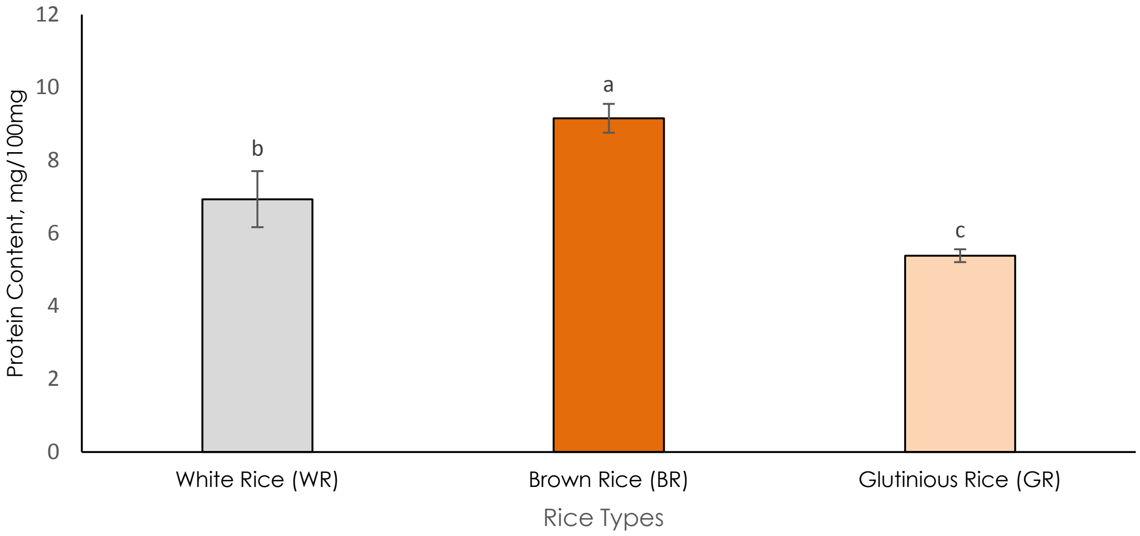


Figure 1 Graph showing the significant different of protein content in all of the three rice types. Different letters indicates significant different

3.2 Banding Pattern of Seed Storage Protein

The protein profile of different type of rice can be visualised from the banding pattern produced. Based on the banding patterns on the SDS-PAGE (Figure 2), there were different levels of protein expression observed between the three types of rice. The protein profile of white rice and brown rice were closely similar compared to the glutinous rice. In white rice, at the protein size between 160 and 260 kDa, no protein band was observed (a). However, a high expression of protein band can be seen at the size of 50 to 60 kDa (b). This condition probably allowed the glutelin acidic subunit region to be expressed lower in white rice (c). There was absence of protein bands (c and b) in glutinous rice compared to the others at the protein size range between 30 to 40 kDa and 50 to 60 kDa. This pattern explained the highest expression of glutelin acidic subunit region in glutinous rice compared to the other type of rice (Figure 2).

The glutelin acidic subunits were expressed between protein size of 30 to 40 kDa appeared to have high band intensity for all of the rice type (d) with slightly different expression level. The formation of glutelin involved the processed of glutelin precursor into acidic and basic subunit by post-translational cleavage [16, 17]. The same glutelin precursor maybe process into different protein subunits through different cleavage patterns and it can be inferred that the different cleavage patterns had caused the different expression level of the glutelin subunits [9].

The low separation of protein bands at the glutelin acidic subunit region (b) caused the low ability to clearly visualize the actual number of subunits presence. There were several subunits of proteins that presence in each of the samples as glutelin being classified into four groups (GluA, GluB, GluC, and GluD) [4]. Analysis by LC/ESI-MS/MS has identified two types of proteins namely hypothetical protein and Glutelin type-A precursor at the size of 34 kDa [9].

The storage proteins are coded by multiple genes, which are specifically and highly expressed in seed [11, 12]. Different types of rice such as brown rice, basmati rice, glutinous rice and long grain rice contain different multiple genes encoding for storage protein that consequently contributed to the variability in rice quality specifically to the nutrient quality [13, 14].The milling process which alter the appearance of rice to increase in whiteness causing the loose of protein content of the outer part of brown rice [2].

As for the prolamin, the banding pattern (g) for all the three type of rice appeared to be similar to each other. However, the glutinous rice seemed to have the highest intensity. The high amount of 13 kDa prolamin in fact did not affect the nutritional quality of the rice as it located at PB-I which is less digestible [16]. Besides, a reduction in prolamins resulted in the enrichment of lysin (essential amino acid), without an equivalent change in grain phenotype [4].

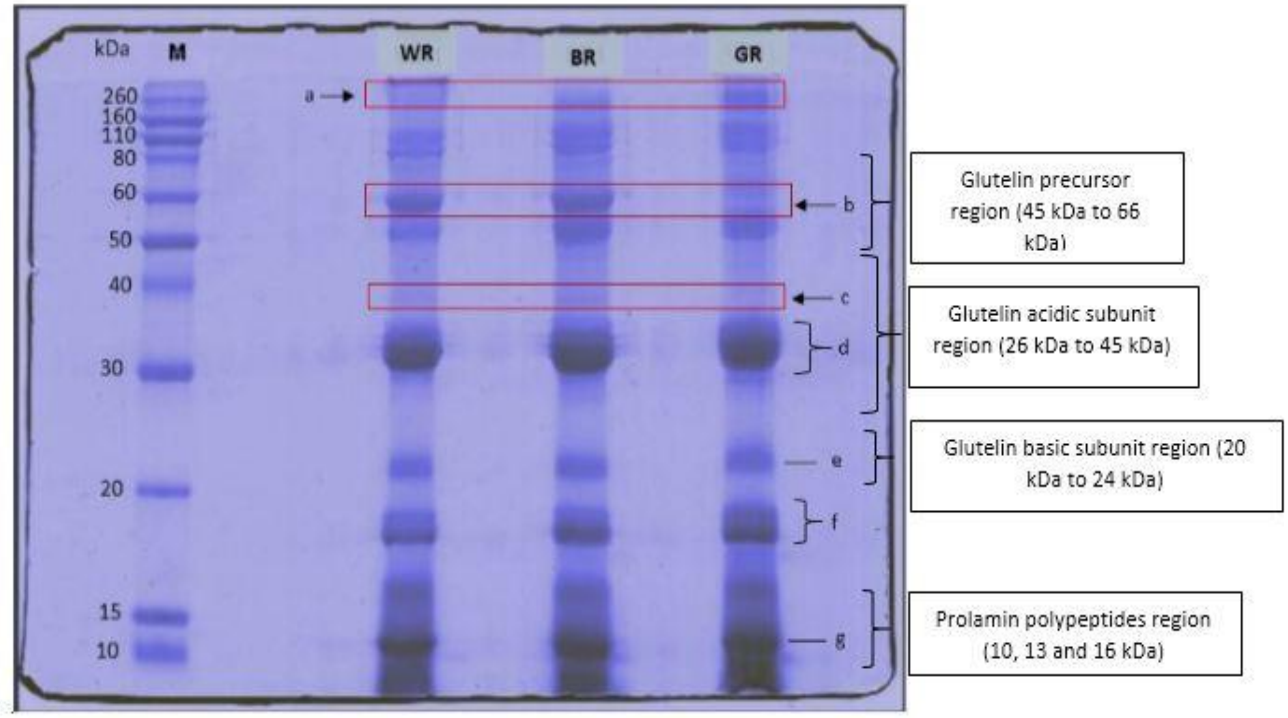


Figure 2 Protein banding pattern for all the three type of rice; M = marker, WR = white rice, BR = brown rice, GR = glutinous rice

4.0 CONCLUSION

Brown rice had significantly the highest SSPs contents among the three types of rice, which contribute to the high nutritional value of rice intake. Different level of protein expressions for glutelin (glutelin precursor, glutelin acidic and glutelin basic subunit) and prolamin of each type of rice contribute to difference in nutritional value for dietary intake.

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