Protein profiling of coloring rice (*Oryza sativa* L.) using SDS-PAGE and experion™260 analysis

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Abstract. Black rice is one of coloring rice and contains high nutrients, minerals, and bioactive compounds. This study provides to investigate the protein profile of rice by using SDS PAGE and Experion™260. Samples were collected from East Java, Central Java and West Java, there were white rice (WREJ), red rice (RREJ), black rice from East Java (BREJ), Central Java (BRCJ) and West Java (BRWJ). Proteins were extracted from rice seeds and run with SDS-PAGE and ExperionTM260. The molecular weight of the protein was determined by the Rf value. Fifteen protein bands were detected on each sample whereas based on ExperionTM260. The range of proteins detected on the SDS page was 8-137kDa, whereas ExperionTM260 detected protein bands from 1.2kDa to 260kDa. There was one protein in WREJ (127kDa), two proteins in RREJ (132kDa & 18kDa) and two proteins in BRWJ (25kDa &21kDa). Whereas the other proteins were not the same in each sample in both analyzes. Several proteins with MW more than 137kDa and less than 8kDa were identified using ExperionTM260, not on SDS-PAGE with CBB stain analyses. Our study suggested that several proteins bands were detected only on specific rice and it could be as biochemical markers for further research.

1. Introduction
Rice is one of the most important crop and food in the world [1,2,3,4,5,6]. Rice belong to the genus *Oryza*, family Poaceae, subfamily Ehrhartoideae, tribe oryzeae [6]. Based on the pigments, rice is classified into red, white, brown and black/purple rice. The four types of rice are included in *Oryza sativa*. *Oryza sativa* is divided into two subspecies, *japonica* and *indica*. Both of them different morphology, *indica* has a long rice and is not sticky while *japonica* has a short and sticky rice character [7]. The distribution of japonica subspecies in East Asia such as Japan and China. *Indica* rice is grown in India, Thailand, and some in Indonesia [6]. The black color of rice distribute in pericarp layer and the other showed the distribution until aleuron layer [8].

Coloring rice become more popular and a lot of attention as raw materials for functional food due to high nutritional value, high antioxidant compound such as anthocyanin. Previous study from Thomas et al. [9] reported that black rice have higher minerals than white and red rice. Those minerals
are Potassium, Sodium, Magnesium, Zinc, and Calcium. Rice generally contains 80% of carbohydrate, 7-8% of proteins, 3% of fats and 3% of fiber [5]. Thomas et al [9] showed that brown rice have higher water-insoluble fiber than black rice, 16.51% in brown rice and 14.49% in black rice. Moreover, coloring rice contains several phytochemical compound such as phenolic, flavonoid, phytosterol, \( \gamma \)-Oryzanol[10]. Those phytochemical compound promote health benefit as antioxidant activity, anti-atherosclerotic, anti-obesity, anti-diabetic and anti-inflammatory [10-14]. Anthocyanin in black rice effectively as antioxidant like blackberry. Anthocyanin inhibit the formation of cell damaging reactive free radicals and scavenge superoxide anion more effectively than hydroxyl radical [14, 15]. The beneficial of coloring rice due to functional protein complex in rice.

Proteomic study of rice have been reported focused on differential expression which are induced by environment [16–18]. Beside that the earlier study reported the functional protein in white rice and japonica rice genotype. Indonesian black rice have been found and classified into eleven varieties [19]. But there was no information about the proteomic study. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) is a useful tool to analyze the proteomic study. SDS PAGE reproducible to analyze the protein profile and the banding pattern can be used to identify and characterize some crop such as rice, maize, wheat and other [16, 20]. Experion™260 is a technology to separate proteins. It need lower volumes of samples and reagent, faster analysis as little as 30 minutes and high resolution for protein separation (Biorad) [21]. Several studies have been characterizing the protein profile of several grains induced by environment and determine the functional protein in wheat. Therefore, in this study investigate the protein profile of coloring rice from Java, Indonesia.

2. Materials and Methods
2.1 Sample collection
Five types of rice grains were collected from Java. There were white rice (WREJ), red rice (RREJ), and black rice (BREJ) from East Java, one black rice from Central Java (BRCJ) and one black rice from West Java (BRWJ). All procedures were approved by ethics committee with registration number 896-KEP-UB.

2.2 Protein extraction
The rice proteins were extracted from seeds using protocol described by Tada et al. [22]. Twenty-five milligrams of rice seeds were homogenized with pre-cooled extraction buffer (62.5 mM Tris-HCl, pH 6.8; 2.5% of SDS; 10% of glycerol; 5% of 2-mercaptoethanol) on ice. The homogenate was incubated on the shaker for 1 hour at room temperature. The homogenate was centrifuged 15,000 rpm for 15 minutes at 4°C. The supernatant was collected and kept in to -20°C.

2.2 Electrophoresis
The electrophoretic gel was done on polyacrylamide gels [23]. The mixture of polyacrylamide contained 3125 \( \mu \)l 30% acrylamide 30%, 2750 \( \mu \)l of 1 M Tris buffer (pH 8.8), 1505 \( \mu \)l of water, 75 \( \mu \)l of 10% SDS, 75 \( \mu \)l of 10% APS and 6.25 \( \mu \)l of TEMED. The protein was solubilized on 1x volume of reducing sample buffer (1M Tris-HCl (pH 6.8), 50% of glycerol, 10% of SDS, 5% of \( \beta \)-mercaptoethanol, water and 1% of bromophenol blue). The mixture was incubated on 100°C for 5 minutes and placed on ice until used. The sample were loaded into well of stacking gel contained 0.45 ml of 30% acrylamide, 0.38 ml of 1M Tris buffer (pH 6.8), 2.110 ml of water, 30 \( \mu \)l of 10% SDS, 30 \( \mu \)l of 10% APS, and 5 \( \mu \)l of TEMED. SDS PAGE were run on 150 V for 30 minutes and stained by Coomassie brilliant blue staining. Moreover, the profile of rice protein also analyzed using Experion Pro260 kit (Catalog Bio-Rad®, Hercules, CA).

3. Result and Discussion
The analysis of SDS-PAGE with CBB staining detected 15 protein bands in every sample (Figure 1). The protein bands were identified in WREJ ranging from 8.6-127.1kDa, five ten proteins were detected in RREJ with molecular weight from 11.7kDa to 132.0kDa. BREJ and BRCJ were identified
protein bands with molecular weight 12.2-137.1kDa, while the protein bands in BRWJ ranged from 12.6 to 137.1kDa. Based on SDS-PAGE analysis, several protein bands identified only on specific sample. The seven protein bands only identified in WREJ (8.6kDa, 10.0kDa, 29.0kDa, 31.3kDa, 57.4kDa, 87.1kDa, and 97.5kDa), five protein bands in RREJ (11.7kDa, 22.3kDa, 26.9kDa, 72.0kDa, and 132.0kDa). BREJ identified five protein bands with molecular weight 15.8kDa, 23.1kDa, 35.1kDa, 101.3kDa and 109.3kDa. Six specific protein bands identified on BRCJ were 13.6kDa, 17.1kDa, 20.7kDa, 24.0kDa, 39.3kDa and 44.0kDa, and five protein bands in BRWJ (12.6kDa, 17.7kDa, 21.4kDa, 25.9kDa and 36.4kDa. Moreover, all black rice detected two protein bands (137.1kDa and 69.4kDa) and WREJ and RREJ have one protein bands with molecular weight 66.8kDa. We though the specific protein bands in black rice was protein related rice pericarp color. Phonsakhan and Kong-Ngern [1] detected several protein spots were analyzed by 2D-PAGE and LCMS/MS. There were six protein spots downregulated and seven protein spots were upregulated in black glutinous rice leaves. Another study from Zhang et al [24] identified the protein bands with molecular weight 54kDa as chloroplast protein in japonica rice. Freire et al [25] predicted three protein in wild rice Oryza glumaepatula as glutelin (34-36kDa), albumin (15-25kDa) and prolamin (15-18kDa).

Figure 1. The protein profile of coloring rice based on SDS-PAGE analysis. a. The rice protein bands were detected on SDS-PAGE with CBB staining, b. molecular weight of rice protein according to gel SDS-PAGE analysis. WREJ: white rice East Java, RREJ: red rice East Java, BREJ: black rice East Java BRCJ: black rice Central Java and BRWJ: black rice West Java.

The protein profiles of rice showed different results using Experion™260 (Figure 2, Table 1). In WREJ was found 47 protein bands RREJ found 52 protein bands, BREJ identified 18 protein bands, BRCJ was 25 protein bands and BRWJ was detected 21 protein bands. Based on Experion™260, two protein bands detected in all sample (1.2kDa and 260.0kDa). Protein bands approximately 88kDa identified in all sample, except in RREJ. Moreover, several protein bands approximately 6kDa, 7kDa, 21kDa, 24kDa, 64kDa, 71kDa, 81kDa, 90kDa, 116kDa, 229kDa and 254kDa identified in WREJ and RREJ. Three black rice didn’t show protein bands with similar molecular weight, but about 36kDa
expressed in BREJ and BRCJ. According to Experion™260 analysis BRWJ expressed eleven protein bands which didn’t detected in another rice, those protein were 25.8kDa, 26.9kDa, 66.1kDa, 76.2kDa, 80.2kDa, 101.8kDa, 179.0kDa, 185.4kDa, 202.5kDa, 243.9kDa and 255kDa. Based on SDS-PAGE and Experion™260 analysis, some protein bands were detected in rice. Those protein bands, approximately 127kDa in WREJ, 18kDa and 132kDa in RREJ, 21kDa and 25kDa in BRWJ.

Figure 2. The protein profile of coloring rice based on Experion™260 analysis. The figure showed protein bands and their peaks, the peak proved the protein expression levels. WREJ: white rice East Java, RREJ: red rice East Java, BREJ: black rice East Java BRCJ: black rice Central Java and BRWJ: black rice West Java.

The differential expression of protein in rice indicated every rice have specific protein bands as character and related with the metabolism in rice, such as protein related the coloring of rice pericarp, protein storage in rice, protein related metabolite synthesized. Our study revealed that the differential protein in every rice could be as biochemical markers for specific rice. Earlier study showed that seed storage protein profiling and the band pattern in SDS-PAGE could be determined the genetic diversity. Those research proved that seed storage protein markers highly polymorphic and their protein profiling can be used to identify the varieties, characterize the germplasm and determine the phylogenetic between different species [20, 26]. Moreover, the protein band in different rice were hypothetically have different function in metabolism. Several studies reported the function of some protein bands. Yang et al [27] detected 480 protein spots which analyzed using 2D with molecular weight between 15-95kDa and the pI were 5-7. The 302 protein spots of them were identified and classified into 12 function. There was as metabolism, protein structure, plant defense/disease, signal transduction, protein synthesis, transporter, cell growth and division. Zhilic et al [28] identified storage protein as albumin approximately 50kDa. Parchin and Shaban [29] found 21-32 protein bands (14kDa-78kDa) in wheat irrigation and non-irrigation treatment. Those study suggested that the irrigation treatment decreased the level of protein in wheat, while the non-stress treatment had highest seed protein levels.
Table 1. The protein profile of coloring rice based on Experion™260 analyses

| Molecular weight (kDa) of rice protein | WREJ | RREJ | BREJ | BRCJ | BRWJ |
|---------------------------------------|------|------|------|------|------|
| WREJ                                  | 260.0| 260.0| 260.0| 260.0| 260.0|
| RREJ                                  | 254.5| 254.7| 249.4| 256.1| 255.4|
| BREJ                                  | 245.2| 252.4| 241.8| 230.7| 243.9|
| BRCJ                                  | 240.1| 248.4| 206.4| 210.6| 213.5|
| BRWJ                                  | 236.3| 242.6| 201.1| 203.8| 202.5|
| WREJ                                  | 231.6| 229.7| 194.9| 183.3| 185.4|
| RREJ                                  | 229.5| 219.0| 188.7| 165.7| 179.0|
| BREJ                                  | 224.8| 213.7| 151.0| 154.5| 138.3|
| BRCJ                                  | 217.6| 208.8| 145.7| 138.1| 135.7|
| BRWJ                                  | 210.8| 206.1| 142.0| 119.0| 101.8|
| WREJ                                  | 201.5| 203.0| 124.7| 111.4| 98.2 |
| RREJ                                  | 187.1| 197.7| 90.6 | 94.6 | 81.6 |
| BREJ                                  | 180.7| 194.5| 88.1 | 93.4 | 80.2 |
| BRCJ                                  | 177.3| 181.2| 36.5 | 92.0 | 76.2 |
| BRWJ                                  | 173.9| 170.9| 24.6 | 90.4 | 66.1 |
| WREJ                                  | 163.7| 164.3| 5.1  | 88.7 | 27.9 |
| RREJ                                  | 151.4| 157.1| 4.6  | 84.1 | 26.9 |
| BREJ                                  | 137.0| 146.8| 1.2  | 82.5 | 25.8 |
| BRCJ                                  | 131.3| 141.1| 56.1 | 21.6 |      |
| BRWJ                                  | 127.8| 138.3| 36.6 | 5.3  |      |
| WREJ                                  | 124.7| 135.4| 22.4 | 1.2  |      |
| RREJ                                  | 119.3| 132.2| 8.1  |      |      |
| BREJ                                  | 116.2| 123.7| 7.5  |      |      |
| BRCJ                                  | 99.2 | 118.9| 3.7  |      |      |
| BRWJ                                  | 90.1 | 116.0| 1.2  |      |      |
| WREJ                                  | 88.7 | 109.1|      |      |      |

4. Conclusion
Several rice protein bands have been detected only on specific rice both of SDS PAGE and Experion™260 analysis. This study suggested the specific protein bands can be used as biochemical markers for rice and further more study to predict their functional protein.

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