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PROTEIN CONTENT AND COMPOSITION OF WAXY RICE GRAINS

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Abstract

Rice is not only an important cereal as a major food worldwide but also valuable sources of nutritional and nutraceutical substances for human health. Protein is one of the major factors determining eating, processing and nutritional qualities of rice. Therefore, we examined effect of cultivar on the content and composition of proteins in waxy rice grains. Grains of eight waxy and one non-waxy Korean rice cultivars used in this study were produced at the N fertilizer level of 7 kg/10a. Crude protein content was higher in waxy cultivars than in the non-waxy cultivar. There was a considerable variation in crude protein contents among waxy cultivars, ranging from 8.6 to 9.7% in brown rice and from 8.1 to 8.5% in milled rice. There was also a significant varietal variation in content of the four protein fractions. The mean contents of albumin, globulin, prolamin, and glutelin fractions of waxy brown rice were 1.86, 0.50, 0.05, and 7.31%, respectively. Those of milled rice were 0.50, 0.30, 0.1, and 8.40%, respectively. Albumin, globulin, prolamin, and glutelin fractions for brown and milled rice were composed of seven, five, one, and five major bands, respectively, and no cultivar- or tissue-specific variations were apparent in the fractional profiles detected on sodium dodecy sulfate-polyacrylamide gels. Thus, the varietal differences of total and fractional proteins were mostly contributed by quantitative rather than qualitative variations.

Introduction

Waxy or glutinous rice is distinguished from non-waxy rice by its opaque appearance and very low starch composition. Countries of Indochina are the world's top producers of waxy rice. Indochina countries produce indica-type, while Asian countries japonica-type waxy rice. Waxy rice is used to make products like tamale, pudding, cake, fermented foods, and oil glutinous rice (Palmiano & Juliano, 1972).

In Korea, japonica-type waxy rice is cultivated and cultivation area is increased up to 49,300 ha which amounts to 5.8% of the total rice cultivation area in Korea in the year 2011. Higher profit of waxy rice compared to that of non-waxy rice is the main driving force for the increase in waxy rice cultivation (Kwon, 2012).

In waxy rice, amylose makes up less than 2% of the starch whereas amylopectin comprises up to 100% of the starch. Waxy rice possesses distinct functional and processing properties mainly due to its unique starch composition. In Korea, waxy rice is mostly consumed for rice cake and fermented food processing (Kum *et al.*, 2010). Recent studies suggest that pasting and cooking properties of waxy rice is affected also by proteins (Xie *et al.*, 2008).

Rice proteins are known as useful because they are colorless, rich in essential amino acids, hypo-allergenic, and hypo-cholesterolemic (Chrastil, 1992). Although rice is generally recognized as having the lowest protein content of about 7.3% among the cereal grains, the net protein utilization of rice protein is highest as about 73.8% among the cereal grains (Bean & Nishita, 1985). Furthermore, rice prolamin proteins possess bioactive properties to activate human anti-leukaemia immunity without inducing unwanted inflammatory diseases (Chen *et al*., 2010).

Rice storage proteins are composed of albumin, globulin, prolamin, and glutelin, and make up most of the total proteins in rice (Chung & Pomeranz, 1985). Glutelin makes up about 80%, (Tecson *et al*., 1971; Padhye & Salunkhe, 1979; Juliano, 1985), albumin 1～5%, globulin 4～15%, and prolamin 2～8% of total protein (Cagampang *et al*., 1966; Houston *et al*., 1968). However, the relative quantities of each protein fraction are affected by cultivation conditions, genotypes, and the analytical methods employed (Huebner *et al*., 1990; Krishnan & White, 1995).

Increased consumer interest on health benefits of rice draws special attention on unique nutritional and nutraceutical properties of waxy rice proteins. However, little information is available on the fractional composition of storage proteins of waxy rice. Therefore, the objective of this study was to examine varietal difference in content and composition of proteins in Korean waxy rice cultivars.

Materials and Methods

Chemicals: All chemicals used in this experiment were purchased from Sigma (St. Louis, USA), unless otherwise indicated. Alcohol and sodium hydroxide were purchased from Merck (Germany), Tris from Amresco (USA), glycine from Bio-Rad (USA), and protein molecular weight markers from Sigma-Aldrich (USA), respectively.

Plant materials: One non-waxy and eight waxy rice cultivars were used in this experiment. The non-waxy cultivar cv. Hopumbyeo (HP) was produced at the paddy field of the Agricultural Research & Extension Service, Iksan, Korea. And waxy cultivars Baekok-chalbyeo, Baeksul-chalbyeo, Bosuk-chalbyeo, Dongjin-chalbyeo, Haepyung-chalbyeo, Hwasun-chalbyeo, Jinbu-chalbyeo, and Sangjoo-chalbyeo were produced at the paddy field of the Experimental Farm, Chonbuk National University, Jeonju, Korea. Milled rice was prepared by removing 8% (w/w) of the outer layer of brown rice kernel. Both brown and milled rice were ground using a ball mill (Retsch, Germany) and the ground samples (10 g) were used for protein extraction. Nitrogen fertilizer was applied at 7 kg/10a.

Extraction and quantification of storage proteins: Extraction of storage proteins was performed according to the procedure described by Ju *et al*., (2001) with minor modifications. Rice flour (10 g) was defatted with 100 mL hexane. The defatted flour was then extracted by stirring in 40 mL of distilled water at room temperature (RT) for 2 hrs to get albumin fraction and centrifuged at 3,000×g for 30 min. After the water extraction the flour residue was extracted with 40 mL of 5% NaCl at RT for 2 hrs to get globulin fraction and centrifuged at 3,000×g for 30 min. The residue was then extracted for prolamin with 30 mL of 70% ethanol at RT for 2 hrs, and followed by glutelin extraction with 75 mL of 0.2 M sodium borate buffer (pH 10) containing 0.5% sodium dodecyl sulfate (SDS) and 0.6% β-mercaptoethanol at RT for 2 hrs (Juliano, 1980; Sugimoto, 1986). Each extraction was repeated two times in order to remove all the protein of each fraction. The extracted proteins were freeze-dried and stored at -70℃.

Total nitrogen of each protein fraction was determined by micro-Kjeldahl method and protein content was calculated using a conversion factor of 5.95 (Anon., 1980).

Protein electrophoresis: Freeze-dried protein samples were resolved in each extraction buffer and separated by SDS polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was carried out by the method of Laemmli (Laemmli, 1970) using a Hoeffer Mini VE system (Bio-Rad, USA). Proteins were separated using a separating gel (12%) with a 4% stacking gel. Proteins were diluted with sample buffer (0.0625 M Tris-HCl buffer, pH 6.8, containing 10% glycerol, 2% SDS, 0.05% bromophenol blue, and 5% dithiothreitol) and denatured by heating at 100℃ for 4 min prior to separation. Protein samples were then loaded onto 1 mm thick gels and run in electrophoresis buffer (1.5% Tris, 7.2% glycine, and 0.5% SDS at pH 8.3) at 200 V. Following electrophoresis, gels were fixed and stained according to the silver-staining procedure (Blum *et al*., 1987). Molecular weights of the protein bands were determined by the method of Weber and Osborn (Weber, 1969) using a molecular weight standard kit (Sigma-Aldrich, USA).

Statistical analysis: Experiments were conducted in the completely randomized design with three replications and mean values were used in comparisons and variance analysis. Analysis of variance was performed using the Student Statistix 9 program (Statistix 9.0, USA).

Results and Discussion

Protein content: There was a considerable variation in total protein content in brown and milled rice of the eight selected waxy cultivars. Total protein content in brown rice of the cultivars ranged from 8.6 to 9.7% with an average of 8.8%, which is about 37% higher than that of the non-waxy cultivar cv. Hopum. Among the waxy cultivars, total protein content was highest in cv. Baekok-chalbyeo (Fig. 1 and Table 1). There was also a significant varietal variation in the content of the four protein fractions in waxy brown rice (Fig. 2A). The mean contents of albumin, globulin, prolamin, and glutelin fractions of waxy brown rice were 1.86, 0.50, 0.05, and 7.31%, respectively (Table 1). Contents of albumin, globulin, prolamin, and glutelin fractions among the cultivars ranged from 1.53 to 2.42%, 0.40 to 0.64%, 0.02 to 0.1%, and 6.3 to 8.89%, respectively. The ratios of prolamin to glutelin ranged from 0.38 to 1.25 (Table 1). Mean contents of Chinese japonica cultivars for albumin, globulin, prolamin, and glutelin are 0.5, 0.34, 0.61, and 6.63%, respectively (Ning *et al*., 2010). Thus, the results indicate that albumin content of Korean cultivars used in this experiment is about three- to four-fold higher but that of prolamin about 6- to 12-fold lower than those of Chinese japonica rice. On the other hand, the contents of globulin and glutelin are similar between Korean and Chinese cultivars. The ratios of prolamin to glutelin range from 8.89 to 9.80 in Chinese cultivars (Ning *et al*., 2010), which is about 9- to 23-fold higher than Korean cultivars.

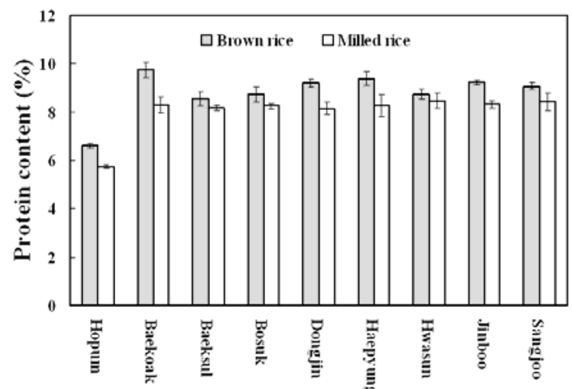

Fig. 1. Content of crude protein in brown and milled rice of one non-waxy (cv. Hopum) and eight waxy cultivars.

Total protein content in milled rice of the waxy cultivars varied from 8.1 to 8.5% with an average of 8.3%, which is about 46% higher than that of the non-waxy cultivar Hopum. Among the waxy cultivars, total protein content was highest in cv. Haepyung-chalbyeo (Fig. 1 and Table 2). There was also a significant varietal variation in content of the four protein fractions in waxy milled rice (Fig. 2B). The mean content of albumin, globulin, prolamin, and glutelin fractions of milled rice was 0.50, 0.30, 0.1, and 8.40%, respectively (Table 2). Contents of albumin, globulin, prolamin, and glutelin fractions among the waxy cultivars ranged from 0.31 to 0.8%, 0.23 to 0.44%, 0.04 to 0.15%, and 6.93 to 9.22%, respectively. The ratios of prolamin to glutelin ranged from 0.43 to 1.97 (Table 2). Mean contents of milled rice of Chinese japonica cultivars for albumin, globulin, prolamin, and glutelin are 0.15, 0.27, 0.56, and 6.31%, respectively (Ning *et al*., 2010). Thus, the results indicate that albumin and glutelin contents of Korean cultivars used in this experiment are about three- and 1.3-fold higher than those of Chinese japonica rice, respectively. The content of globulin is similar between Korean and Chinese cultivars, but that of prolamin is about five-fold higher in Chinese cultivars. The ratios of prolamin to glutelin in Chinese cultivars vary from 8.64 to 9.61 (Ning *et al*., 2010), which is about 5- to 20-fold higher than Korean cultivars.

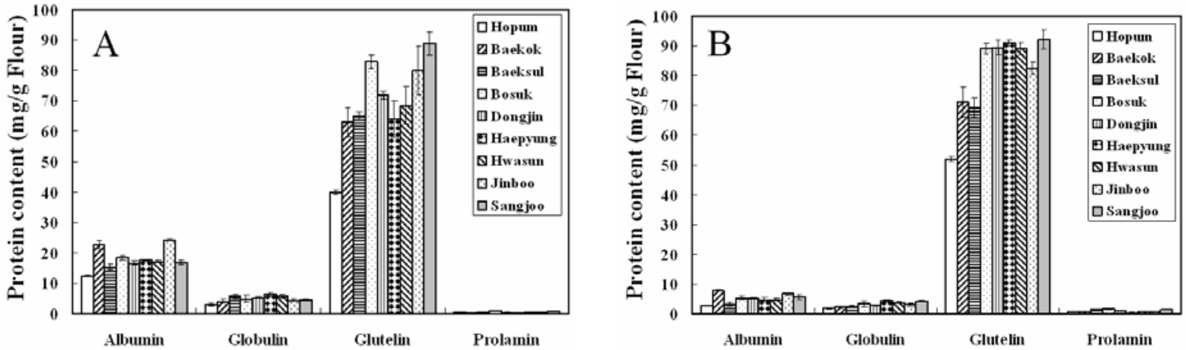

Fig. 2. Content of each protein fraction of the one non-waxy (cv. Hopum) and eight waxy rice cultivars. A, brown rice; B, milled rice.

Table 1. Contents of crude and fractional proteins of brown rice of waxy cultivars.

Cultivar	Brown rice[a]									
	NW[*]	Waxy								
	HP[b]	BO	BS	BK	DJ	HY	HS	JB	SJ	Mean
Crude protein (%)	6.6	9.7	8.6	8.7	9.2	9.4	8.7	9.2	9.1	9.1
Albumin	1.25f	2.25b	1.53e	1.84c	1.67d	1.76cd	1.72cd	2.42a	1.68d	1.86
	(22.3)[c]	(25.1)	(17.7)	(17.2)	(17.7)	(19.9)	(18.7)	(22.2)	(15.1)	(19.2)
Globulin	0.30d	0.40c	0.55b	0.48bc	0.54b	0.64a	0.56ab	0.42c	0.44c	0.50
	(5.3)	(4.5)	(6.4)	(4.5)	(5.7)	(7.3)	(6.0)	(3.9)	(4.0)	(5.3)
Prolamin	0.04b	0.02f	0.06d	0.1a	0.03f	0.03fg	0.04e	0.06d	0.07c	0.05
	(0.8)	(0.3)	(0.7)	(1.0)	(0.4)	(0.3)	(0.5)	(0.6)	(0.6)	(0.6)
Glutelin	4.00f	6.3e	6.51de	8.29ab	7.19cd	6.41de	6.86de	8.00bc	8.89a	7.31
	(71.6)	(70.2)	(75.3)	(77.3)	(76.3)	(72.5)	(74.8)	(73.4)	(80.2)	(75.0)
Prolamin/Glutelin	1.08a	0.38e	0.90c	1.25b	0.48e	0.45e	0.64d	0.76cd	0.80c	0.71
Recovered efficiency (%)	84.6	92.1	101.0	122.8	102.5	94.1	105.1	118.0	122.1	107.2

[*]Non-waxy
[a]Data within a row followed by the same letter are not significantly different ($p<0.05$)
[b]HP, Hopumbyeo (non-waxy cultivar); BO, Baekok-chalbyeo; BS, Baeksul-chalbyeo; BK, Bosuk-chalbyeo; DJ, Dongjin-chalbyeo; HY, Haepyung-chalbyeo; HS, Hwasun-chalbyeo; JB, Jinbu-chalbyeo; SJ, Sangjoo-chalbyeo
[c]Percentage (w/w) to the sum of the four protein fractions

Table 2. Contents of crude and fractional proteins of milled rice of waxy cultivars.

Cultivar	Milled rice[a]									
	NW[*]	Waxy								
	HP[b]	BO	BS	BK	DJ	HY	HS	JB	SJ	Mean
Crude protein (%)	5.7	8.3	8.3	8.3	8.1	8.5	8.4	8.2	8.2	8.3
Albumin	0.27e	0.8a	0.31e	0.54cd	0.53cd	0.46d	0.47cd	0.67b	0.56c	0.50
	(4.8)[c]	(9.7)	(4.0)	(5.4)	(5.4)	(4.6)	(4.8)	(7.2)	(5.4)	(5.8)
Globulin	0.18f	0.23ef	0.25def	0.34bc	0.28cde	0.44a	0.38ab	0.31bcd	0.43a	0.30
	(3.1)	(2.8)	(3.3)	(3.4)	(2.8)	(4.4)	(3.8)	(3.3)	(4.1)	(3.5)
Prolamin	0.09e	0.07d	0.13b	0.15a	0.1c	0.04e	0.08d	0.08d	0.14ab	0.10
	(1.5)	(0.9)	(1.7)	(1.5)	(1.0)	(0.4)	(0.8)	(0.8)	(1.4)	(1.1)
Glutelin	5.19d	7.12c	6.93c	8.93a	8.96a	9.09a	8.92a	8.25b	9.22a	8.40
	(90.6)	(86.7)	(91.0)	(89.8)	(90.9)	(90.7)	(90.6)	(88.6)	(89.1)	(89.7)
Prolamin/Glutelin	1.67d	0.98cd	1.92a	1.63b	1.97c	0.43e	0.88cd	0.93cd	1.55b	1.20
Recovered efficiency (%)	86.8	84.4	89.0	114.00	107.2	106.9	112.7	100.8	114.1	103.6

[*]Non-waxy
[a]Data within a row followed by the same letter are not significantly different ($p<0.05$)
[b]HP, Hopumbyeo (non-waxy cultivar); BO, Baekok-chalbyeo; BS, Baeksul-chalbyeo; BK, Bosuk-chalbyeo; DJ, Dongjin-chalbyeo; HY, Haepyung-chalbyeo; HS, Hwasun-chalbyeo; JB, Jinbu-chalbyeo; SJ, Sangjoo-chalbyeo
[c]Percentage (w/w) to the sum of the four protein fractions

Significant varietal variations were detected in milled rice to brown rice ratios (M/B) of each protein fractions. Mean values of M/B for albumin, globulin, prolamin, and glutelin were 29, 66, 117, and 200%, respectively (Table 3). This result indicates that higher proportions of albumin and globulin are distributed in the outer part of rice grains which may be removed by polishing. On the contrary, prolamin and glutelin are distributed in higher proportions in the inner part of grains. Uneven distribution of albumin and prolamin was more prominent with higher distribution of albumin and prolamin in the outer and inner parts of grains, respectively. Similar uneven distribution of the protein fractions is common in japonica and indica rice cultivars (Chavan & Duggal, 1978; Furukawa *et al*., 2003; Ning *et al*., 2010).

Table 3. Milled to brown rice ratios of the four protein fractions of waxy cultivars.

		(Unit : %)			
		Albumin	**Globulin**	**Prolamin**	**Glutelin**
Non-waxy	HP[a]	21.93ef[b]	59.63c	206.33bc	129.80b
Waxy	BO	35.55a	57.31cd	301.00a	113.13c
	BS	19.99f	44.45e	228.23b	106.57c
	BK	29.01bcd	70.00b	141.03d	107.85c
	DJ	31.73abc	50.94d	281.33a	124.56b
	HY	26.18de	67.94b	140.97d	142.70a
	HS	27.68cd	67.11b	179.27c	130.53b
	JB	27.83cd	71.88b	125.65d	103.73c
	SJ	33.44ab	94.77a	201.26bc	103.73c
	Mean	28.93	65.55	199.84	116.6
	CV	9.70	5.18	10.03	5.01

[a]HP, Hopumbyeo; BO, Baekok-chalbyeo; BS, Baeksul-chalbyeo; BK, Bosuk-chalbyeo; DJ, Dongjin-chalbyeo; HY, Haepyung-chalbyeo; HS, Hwasun-chalbyeo; JB, Jinbu-chalbyeo; SJ, Sangjoo-chalbyeo; CV, coefficient of variation
[b]Data within a column followed by the same letter are not significantly different ($p<0.05$).

Higher milled to brown rice ratios of the protein fractions are nutritionally more advantageous as milled rice is predominantly used for human consumption. Albumin M/B values of the waxy cultivars ranged from 20 to 36% with an average of 29% (Table 3). Globulin M/B values of the waxy cultivars ranged from 45 to 95% with an average of 66%. Prolamin M/B values of the cultivars ranged from 117 to 281% with an average of 200%. Glutelin M/B values of the waxy cultivars ranged from 104 to 143% with an average of 117%. And the ratio was lowest in cvs. Jinbu-chalbyeo and Sangjoo-chalbyeo and highest in cvs. Dongjin-chalbyeo, Hwasun-chalbyeo, and Haepyung-chalbyeo (Table 3). These results indicate that protein compositions of cvs. Dongjin-chalbyeo, Hwasun-chalbyeo, and Haepyung-chalbyeo are nutritionally more advantageous for human consumption.

Nutritional value of prolamin is inferior to glutelin for its low digestibility by human and lower lysine content (Ogawa *et al*., 1987; Tanaka, 1980). Furthermore, prolamin could affect negatively on the quality of cooked rice as prolamin increases the hardness of cooked rice (Furukawa *et al*., 2003). Interestingly, rice prolamin possesses pharmacological activities such as effective activation of human anti-leukaemia immunity (Chen *et al*., 2010). Among the cultivars tested, prolamin content and prolamin to glutelin ratio were higher in waxy rice cultivars. Prolamin contents of cvs. Baeksul-chalbyeo, Bosuk-chalbyeo, and Sangjoo-chalbyeo were 1.5- to 2.5-fold higher than non-waxy cultivars (Tables 2 and 3). These results suggest that cvs. Baeksul-chalbyeo, Bosuk-chalbyeo, and Sangjoo-chalbyeo have higher merits as materials for processed foods and pharmacological use. However, prolamin content is about five-fold higher in Chinese cultivars than in Korean cultivars tested in this study (Ning *et al*., 2010).

Composition of the protein fractions: Compositions of the four protein fractions in brown and milled grains showed little differences between the waxy and non-waxy rice cultivars. There were seven major albumin proteins of 20, 33, 38, 45, 50, 55, and 63 kDa in brown and milled rice (Fig. 3). Rice albumins have a wide range of molecular weights (Juliano, 1972), and the major components have apparent MWs of 18～20 kDa (Houston & Mohammed, 1970). There were five major globulin proteins of 19, 31, 45, 60, and 65 kDa in brown and milled rice (data not shown). Rice globulins consist of α-, β-, γ- and δ-globulins with apparent MWs of 25, 15, 200 kDa, and higher, respectively (Morita & Yoshida, 1968). There was one major prolamin of 16 kDa in brown and milled rice (data not shown). Prolamins consist of three polypeptide subunits with apparent MWs of 10, 13, and 16 kDa (Ogawa *et al*., 1987) and the 13 kDa polypeptide is dominant. There were four major glutelin proteins of 26.5, 36, 45, and 54 kDa in brown and milled rice (data not shown). Glutelin is composed of the two major polypeptide subunits classified as acidic and basic subunits with apparent MWs of 19～25 and 30～39 kDa, respectively (Juliano, 1985; Kagawa *et al*., 1988; Kishimoto *et al*., 1999; Shih, 2004). The differences in SDS-PAGE patterns of rice proteins between the research groups might be due to the differences in the extraction methods and varieties used. The SDS-PAGE results of each protein fraction from this study did not show apparent qualitative differences, indicating that the varietal differences in protein contents are mostly contributed by quantitative variations of each fractional protein.

One of the major objectives of recent specialty rice breeding programs has been developing varieties low in proteins to meet current nutritional and industrial interests. Low-glutelin rice varieties, which are low in crude protein by about 15%, were developed by the conventional breeding approach. Milled grains of low-glutelin rice have decreased total protein and glutelin contents, but increased prolamin content than those of the parental varieties (Furukawa *et al*., 2003). Likewise, variations in protein content and composition in rice germplasms are valuable resources for the development of rice varieties with preferable protein contents and compositions for specific purposes. Therefore, results on content and fractional composition of waxy rice proteins from this study will be valuable information in developing programs and strategies for specialty rice variety with desired content and composition of proteins.

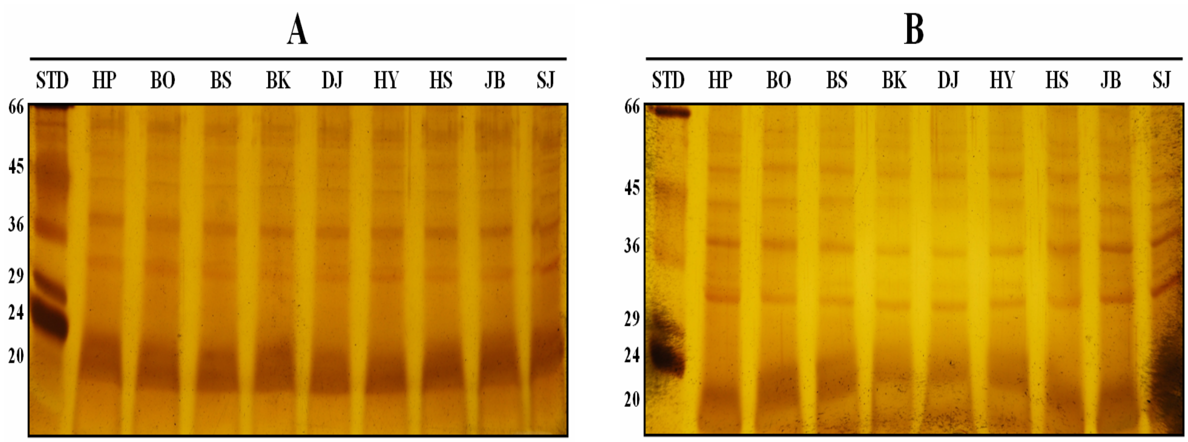

Fig. 3. SDS-PAGE patterns of albumin fractions of rice grains of the nice selected cultivars. STD, standard marker; HP, Hopumbyeo; BO, Baekok-chalbyeo; BS, Baeksul-chalbyeo; BK, Bosuk-chalbyeo; DJ, Dongjin-chalbyeo; HY, Haepyung-chalbyeo; HS, Hwasun-chalbyeo; JB, Jinbu-chalbyeo; SJ, Sangjoo-chalbyeo. A, brown rice; B, milled rice.

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