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Protein Profiles of Major Korean Rice Cultivars

Chuanha Wei[2], Oh Yun Kwon[1], Xi-Wen Liu[2], Hyoung Chin Kim[3], Won Kee Yoon[3], Hwan Mook Kim[3] and Mee Ree Kim[1†]

[1]Dept. of Food and Nutrition, and [2]College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
[3]Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333, Korea

Abstract

The protein profiles among Korean rice cultivars were assessed by total protein determination, solubility fractionation, SDS-PAGE analysis and scanning densitometry. In the extraction of protein, the SDS/urea system at a neutral pH was more efficient than that at alkaline pH. The determination of total protein showed that the protein content was similar among cultivars, ranging from 87.9 to 92.7 mg/g dry weight. Additionally, the water/NaCl-soluble protein fraction, containing 14~16 kDa albumin and 22 kDa globulin α-globulin, was also similar among cultivars, with a range of 9.94 to 11.98 mg/g dry weight. The SDS-PAGE/densitometry of total protein showed that there was no discernable difference in proteins of higher molecular weights among various cultivars, whereas the amount of lower molecular weight proteins (14~16 kDa) is somewhat variable among cultivars. Furthermore, SDS-PAGE analysis of water/NaCl-soluble and propanol-soluble fractions indicates that there is a discernible change in the content of albumin, globulin or prolamin among cultivars. Thus, the PAGE/densitometry method, preceded by solubility fractionation, is useful for examining differences in protein profiles of rice cultivars.

Key words: rice cultivar, protein profile, solubility fractionation, SDS-PAGE

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for more than 60% of the world's population, especially in Asia (1). In particular, with a protein content of ca. 6~8% on a dry weight basis, rice is a primary protein source for numerous people for whom a vegetable-based diet is the major food source. Rice can offer several health-enhancing properties, and is appealing to food processors and consumers (2). Fueled by the rising interest in Asian and Latin cuisines, rice has become the main benefactor of America's new love of grain products (3).

The major rice storage protein, glutelin accounts for more than 80% of total rice protein (4-7). The remaining 20% is composed of albumins (1~5%), globulins (4~15%) and prolamins (2~8%). These proteins have been isolated mainly according to their solubility using the Osborne extraction method (8). In the Osborne extraction procedure, ground rice is defatted and extracted with water to obtain the albumin fraction, followed by sequential extraction with salt solution, alkali and ethanol to obtain globulin, glutelin and prolamin fractions, respectively (9). Nonetheless, this method has a drawback in that the separation of each protein is not complete.

Rice grains, like other cereal grains, have been known to be allergenic (10). Allergenicity was found in the globulin fraction of rice seed proteins, 26 kDa globulin (11) and 33 kDa globulin (12). Additionally, albumin of 14 kDa was also observed to be allergenic (13), and the proteins of 14~16 kDa were found to be alpha-amylase inhibitors as well as antigens recognized by IgE antibodies (14). Furthermore, rice prolamin (14 kDa) also belongs to proteins of low molecular weight, although it, unlike prolamin from other cereals, does not cause celiac disease (15,16). Since prolamin shows a molecular weight similar to that of some parts of albumin, the amount of albumin may not be determined properly by SDS-PAGE, which is based on the molecular behavior. Therefore, the separation of prolamin from the albumin fraction may be important for the accurate determination of allergenic proteins in rice proteins. For this purpose, the solubility fractionation of proteins, followed by SDS-PAGE analysis, may be beneficial.

In this study, the composition and profile of proteins from several rice cultivars, bred in Korea, were examined by SDS-PAGE densitometry. Then the differences in the amount of proteins with lower molecular weights (14~16 kDa) were investigated by SDS-PAGE analysis,

†Corresponding author. E-mail: mrkim@cnu.ac.kr
Phone: +82-42-821-6837, Fax: +82-42-821-8887

accompanied by solubility fractionation.

MATERIALS AND METHODS

Materials

The four different rice cultivars (Keumhobyu, Whaseongbyu, Ohdaebyu and Nakdongbyu) were harvested and supplied by the National Crop Experimental Station, RDA, Suwon in 2003. And seven different rice cultivars (Ilmibyu, Ilpumbyu, Saechuchungbyu, Chuchungbyu, Junambyu, Dongjinbyu and Nampyungbyu) were harvested and supplied from Chungnam Agricultural and Research Extention Services in 2004. Paddy rice was dehulled by a laboratory scale huller (THU 35A, Satake, Japan or SYTH-88, Ssangyoung, Korea), and then the brown rice was milled by laboratory scale whitener (MC-250, Satake, Japan or MC-90A, Tester, Korea) and then polished rice was finally obtained. After milling, the polished rice was stored in a cryogenic freezer (-70°C). In preparation for the experiments, the rice samples were ground in a mill (Perten 3600, Perten Instrument Co., Sweden) and screened through sieve (60 mesh, 250 μm). All other chemicals were of analytical grade.

Preparation of defatted rice flour

Rice grains (10 g) were ground for 3 min in a micro mill (Technilab Instruments, Pequannock, NJ) to make a fine powder as previously described (17), and the ground flour was extracted with 4 vol. of cold acetone (-20°C) for 1 hr according to the procedure of Toshio et al. (18). Then, acetone was removed carefully with a polyethylene pipette. The extraction was repeated three times in the same way as described above. The defatted rice flour was dried for >24 hr in a hood, and used for extraction of protein.

Extraction of total protein from rice sample

Extraction I: Defatted rice flour (40 mg) was suspended in 1.8 mL of 125 mM Tris-HCl, pH 6.8 containing 4% sodium dodecyl sulfate and 6 M urea, and kept under constant sonication in an ultrasonic water bath for 1 hr at room temperature. *Extraction II*: Defatted rice flour (40 mg) was suspended in 1.8 mL of extraction buffer (125 mM Tris-HCl, pH 6.8+4 M urea+4% SDS +5% 2-mercaptoethanol) in a beaker, vortexed briefly and kept under constant shaking for 6 hr at room temperature. *Extraction III*: Defatted rice flour (40 mg) was suspended in 1.8 mL of 0.01 M NaOH solution, and kept under constant sonication in an ultrasonic water bath for 1 hr at room temperature. The supernatant, after centrifugation (15,000 rpm, 20 min), was taken for protein determination and SDS-PAGE analysis.

Fractionation of rice albumin, globulin and prolamin

Defatted rice flour (5 g) was soaked in 4 vol. of distilled water for 1 hr, and the proteins were extracted under sonication in a water bath (Branson sonicator) for 5 min (20~25°C) before centrifugation (15,000 rpm× 10 min). The extraction was repeated three times, and the final extraction was done under shaking (200 rpm, 30 min). Then, the wet flour was extracted three times with 4 vol. of 1 M NaCl in the same way as described above. The combined supernatant, containing albumin and globulin, was kept at 4°C for further study. The remaining residue in a 50 mL centrifuge tube, after removal of albumin and globulin, was washed twice with 5 vol. of distilled water to remove NaCl, and subsequently with 5 vol. of acetone to remove the remaining water. Finally, the sample was dried in a hood overnight. Then, to obtain prolamin, the resulting residue was extracted with 5 vol. of 1-propanol (55%) under shaking (200 rpm×1 hr) at room temperature before centrifugation (15,000 rpm×10 min). This extraction was repeated 4 times. The supernatants were combined (total ca. 100 mL), and an aliquot (0.2 mL) was subjected to vacuum-drying in a cone-type plastic tube in a centrifugal vaporator (speed 5, 24 hr) at 40°C

Determination of total rice protein

Total rice protein was determined by the Kjeldahl method. The percent nitrogen was determined and converted to protein using the factor 6.25 (19). Separately, the amount of rice protein was determined using Bradford method (20). Bovine serum albumin was used as the standard protein.

Determination of albumin, globulin and prolamin

The amount of albumin, globulin or prolamin was determined according to the method of Lowry et al. (21). Bovine serum albumin was used as a standard protein.

SDS-PAGE analysis

SDS-PAGE analysis was conducted using 15% (4%) polyacrylamide gels as described by Laemmli (22). The protein samples were dissolved in sample buffer: 60 mM Tris-HCl (pH 6.8), 25% (v/v) glycerol, 2% (w/v) sodium dodecylsulfate, 0.1% (w/v) bromophenol blue, 5% (v/v) 2-mercaptoethanol at a 1:5 ratio, and the mixture, after 5 min boiling, was centrifuged (1,000 g×5 min). The gels were stained with Coomassie Brilliant Blue (R 250). A molecular weight marker kit (3.5~205 kDa, Koma Biotech) was used as standard molecular weight proteins (myosin, 205 kDa; β-galactosidase, 116 kDa; phosphorylase b, 97.4 kDa; bovine serum albumin, 69 kDa; glutamic dehydrogenase, 55 kDa; lactic dehydrogenase, 36.5 kDa; carbonic anhydrase, 29 kDa; trypsin inhibitor, 20.1

kDa; lysozyme, 6.5 kDa; insulin B chain, 3.5 kDa).

SDS-PAGE densitometry

The electrophoretic behavior of protein subunits was analyzed by a UMAX Power Look 1100 scanner as described before (23). In the computerized densitometric analysis using Total Lab program, relatively small spots (less than 0.5%) were automatically ignored, and the background was subtracted by rolling baseline. The relative density of major peptide subunits was expressed as % of total amount. The R_f of the peaks was denoted as a ratio of migration, and molecular weights were calculated from the standard curve of marker proteins.

Amino acid analysis

Proteins were hydrolyzed with 6 N HCl at 110°C for 24 hr, and analyzed by automated amino acid analyzer (Waters Pico Tag HPLC system, Milford, MA, USA) as described (24). In brief, protein powder (11.9 mg) was subjected to PITC labeling in 400 μL according to PICO-Tag method, and 5 μL was injected to HPLC PICO-tag column (3.9×300 mm, 4 μm), which was eluted with a gradient solvent (140 mM sodium acetate in 6% acetonitrile to 60% acetonitrile). Each peak was detected at 254 nm using a Waters 996 photodiode (PDA).

Statistical analyses

Statistical assessments were performed using a statistical package SPSS 10.0 program (SPSS Inc., Chicago, USA), and significance of each group was verified using one-way ANOVA followed by post-hoc Duncan's multiple-range tests among rice cultivars (25). All data are presented as mean±standard deviation (SD) of three independent experiments. Statistical significance refers to results where $p<0.05$ was obtained.

RESULTS AND DISCUSSION

In an attempt to establish the conditions for maximal extraction of protein from rice flour, the extraction conditions such as extraction buffer or extraction time were changed, and the extraction yields of proteins from four rice cultivars, grown in the same agricultural area, were compared. As shown in Table 1, the extraction yield differed according to the extraction methods. However, under the same extraction conditions, the protein amount was quite similar among the four types of rice. Although the alkaline extraction (method Ⅲ) was efficient as the extraction in the presence of SDS/urea (method Ⅰ or Ⅱ), the latter was favored over the former in consideration of subsequent SDS-PAGE analysis. In the extraction with SDS/urea, method Ⅱ (6 hr extraction) gave a higher yield than method Ⅰ (1 hr extraction); 66~69 mg protein/g *vs.* 31~36 mg protein/g. Furthermore, the macro assay employing 40-fold dilution of protein extract gave higher value than the micro assay employing a 1,000-fold dilution; 90.4 *vs.* 68.01 mg protein/g weight, and the macro assay was more accurate than micro assay on the basis of standard deviation values. Overall, the values for protein content of rice flour were similar to previously reported values (5,6). Based on these results, the macro-assay (50-fold dilution) employing the extraction buffer containing SDS and urea was selected for the extraction of protein from rice flour in further studies.

In a separate experiment to see the quality of rice samples, they were subjected to amino acid analysis. As displayed in Table 2, four samples were quite similar in amino acid composition; overall, the highest concentrations were observed for acidic amino acids such as glutamic acid and aspartic acid, followed by leucine, whereas the concentrations of basic amino acids such as histidine, arginine or lysine was relatively less. Overall, these data are consistent with the previous report concerning amino acid composition of rice protein (26).

Total proteins of various rice cultivars

Based on the previous data, we attempted to find the difference in the protein profiles of seven rice cultivars, cultivated in the Chungnam area. First, rice flour of seven cultivars was subjected to optimal extraction conditions, employing SDS/urea, as described above, and

Table 1. Protein amount of rice cultivars by extraction methods (mg/g dry weight)

Extraction method[1)]	Cultivars			
	Keumhobyu	Whaseongbyu	Ohdaebyu	Nakdongbyu
Ⅰ	36±2[a]	32±5[a]	37±4[a]	35±3[a]
Ⅱ	68±4[b] (90±3)	66±5[b]	68±5[b]	70±6[bc]
Ⅲ	61±8[b]	65±11[b]	60±8[b]	56±7[b]

Rice flour was extracted by each method, and protein amount was determined by the Bradford method as described in Material and Method. Data are expressed as mean±standard deviation (n=3). Microassay (1,000-fold dilution) was used except the value in blanket (macroassay, 40-fold dilution). Different letters in the same column indicate significant differences ($p<0.05$).
[1)]See the Materials & Methods.

Table 2. Amino acid composition of rice protein from different cultivars

Amino acid	Cultivars							
	Keumhobyu		Whaseongbyu		Ohdaebyu		Nakdongbyu	
	ng/mg	%	ng/mg	%	ng/mg	%	ng/mg	%
Cys	819.3^{a}	3.6	1091.4^{b}	3.2	795.7^{a}	2.7	693.6^{a}	2.8
Asp	2241.1^{a}	10.0	3512.4^{b}	10.4	2900.8^{ab}	9.8	2412.6^{ab}	9.6
Glu	5976.8^{a}	26.6	9066.5^{b}	27.0	7818.9^{ab}	26.4	6695.0^{ab}	26.7
Ser	1223.0^{a}	5.4	1893.5^{b}	5.6	1647.4^{ab}	5.6	1451.8^{ab}	5.8
Gly	1308.3^{a}	5.8	1467.0^{a}	4.4	1375.2^{a}	4.6	1231.6^{a}	4.9
His	793.9^{a}	3.5	1213.6^{c}	3.6	1123.1^{bc}	3.8	852.8^{ab}	3.4
Arg	878.3^{a}	3.9	1286.2^{b}	3.8	1194.2^{ab}	4.0	960.6^{ab}	3.8
Thr	917.4^{a}	4.1	1324.9^{b}	3.9	1217.8^{ab}	4.1	1063.4^{ab}	4.2
Ala	1120.8^{a}	5.0	1937.3^{c}	5.8	1731.0^{bc}	5.8	1383.0^{abc}	5.5
Pro	1975.3^{a}	8.8	2222.3^{a}	6.6	2123.1^{a}	7.2	1984.4^{a}	7.9
Tyr	59.6^{a}	0.3	77.4^{a}	0.2	70.7^{a}	0.2	75.0^{a}	0.3
Val	1366.4^{a}	6.1	2176.4^{b}	6.5	1971.8^{ab}	6.7	1640.2^{ab}	6.5
Met	173.8^{a}	0.8	390.9^{b}	1.2	397.9^{b}	1.3	364.3^{b}	1.5
Cys2	1.2^{ab}	0.0	3.2^{b}	0.0	1.6^{ab}	0.0	1.1^{ab}	0.0
Ile	804.6^{a}	3.6	1314.4^{a}	3.9	1169.2^{a}	3.9	938.0^{a}	3.7
Leu	1553.2^{ab}	6.9	2660.8^{b}	7.9	2295.2^{ab}	7.7	1878.6^{ab}	7.5
Phe	972.8^{a}	4.3	1369.6^{a}	4.1	1218.4^{a}	4.1	975.4^{a}	3.9
Trp	8.4^{a}	0.0	58.0^{b}	0.2	54.6^{b}	0.2	76.2^{c}	0.3
Lys	301.5^{ab}	1.3	568.2^{b}	1.7	538.8^{b}	1.8	392.5^{ab}	1.6
Total	22495.9^{b}	100.0	33633.7	100.00	29646.2	100.00	25070.1	100.00

Data are expressed as mean±standard deviation (n=3). Different letters in the same row indicate significant differences ($p<0.05$).

total protein was determined by the Bradford method. As demonstrated in Table 3, the protein concentration, ranging from 87.9~92.7 mg/g, was similar among rice cultivars. However, these values are significantly higher than those (58.2~75.6 mg/g) obtained using Kjeldahl method, although there was a parallel trend of relative values between the two methods. Overall, the values observed here were not much different from values which had been previously reported (5,6). The possible explanation for the different values between two methods may be due to the Coomassie Brilliant Blue staining in Bradford method; SDS used in the extraction buffer may interfere with protein staining in the Bradford method, as reported by Daniel and Stuart (27). Alternatively, it may be due to the difference of amino acid composition, which may determine the interaction between protein and dyestuff. Nonetheless, the Bradford method has more advantages in reproducibility and convenience.

Next, the protein extracts were subjected to SDS-PAGE analysis. In the preliminary study to set up optimal conditions of PAGE analysis, it was found that normal gel PAGE (12.5% or 15% acrylamide gel) achieved better resolution than gradient (4~25%) gel PAGE. Moreover, 15% acrylamide gel was preferable to 12.5% gel for the efficient separation of proteins with lower molecular weights. Therefore, the extract of rice proteins was subjected to 15% acrylamide SDS-PAGE as displayed in Fig. 1. Overall, the respective protein was resolved nicely; glutelin, which appeared as major proteins, was present as 54~57 kDa preglutelin, 32~35 kDa acidic and 19 kDa basic glutelin subunits, and another rice storage protein, prolamin, was presented as 13 kDa polypeptide. The albumin and globulin subunits, known to be rice allergenic proteins, were presented as

Table 3. Amount of total protein and low molecular weight proteins (mg/g dry weight)

Cultivars	Total protein		Albumin +Globulin	Prolamin
	Bradford	Kjeldahl		
Ilmibyu	90.8±1.3^{ab}	58.22±0.01^{a}	10.70±0.27^{ab}	1.31±0.01^{a}
Ilpumbyu	95.7±1.3^{c}	63.20±0.04^{c}	11.98±0.21^{b}	1.80±0.11^{b}
Saechuchungbyu	92.6±1.6^{bc}	72.35±0.07^{f}	11.08±0.49^{bc}	2.20±0.08^{c}
Chuchungbyu	87.9±1.1^{a}	61.12±0.01^{b}	9.94±0.39^{a}	1.64±0.04^{b}
Junambyu	89.9±1.2^{ab}	66.91±0.06^{e}	11.65±0.76^{bc}	1.85±0.10^{b}
Dongjinbyu	91.7±1.6^{b}	64.71±0.01^{d}	10.54±0.62^{ab}	1.60±0.17^{b}
Nampyungbyu	92.7±3.4^{bc}	73.74±0.10^{f}	11.10±0.40^{bc}	1.84±0.21^{b}

Data are expressed as mean±standard deviation (n=3). Different letters in the same column indicate significant differences ($p<0.05$). Low molecular proteins such as albumin, globulin and prolamin were analyzed by fractionation.

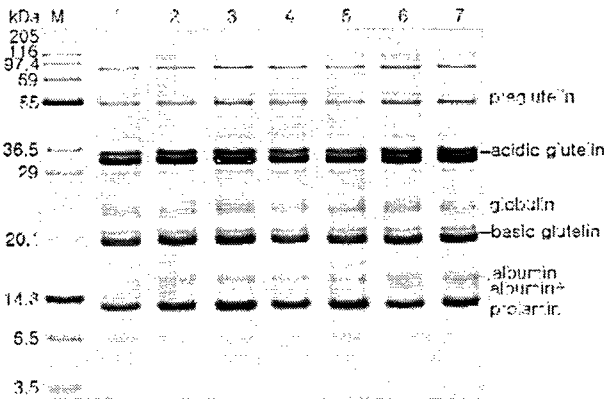

Fig. 1. Profile patterns of total rice protein. Total rice protein was extracted by extraction buffer, and aliquot (ca. 2 μL) was applied to 15%/4% SDS-PAGE. After electrophoresis, the gel was stained with Coomassie brilliant blue R250. Lanes 1 to 7 represent the rice cultivar: 1, Ilmibyu; 2, Ilpumbyu; 3, Saechuchungbyu; 4, Chuchungbyu; 5, Junambyu; 6, Dongjinbyu; 7, Nampyungbyu. Lane M is molecular weight standard. The labeled protein subunits represent major proteins of rice.

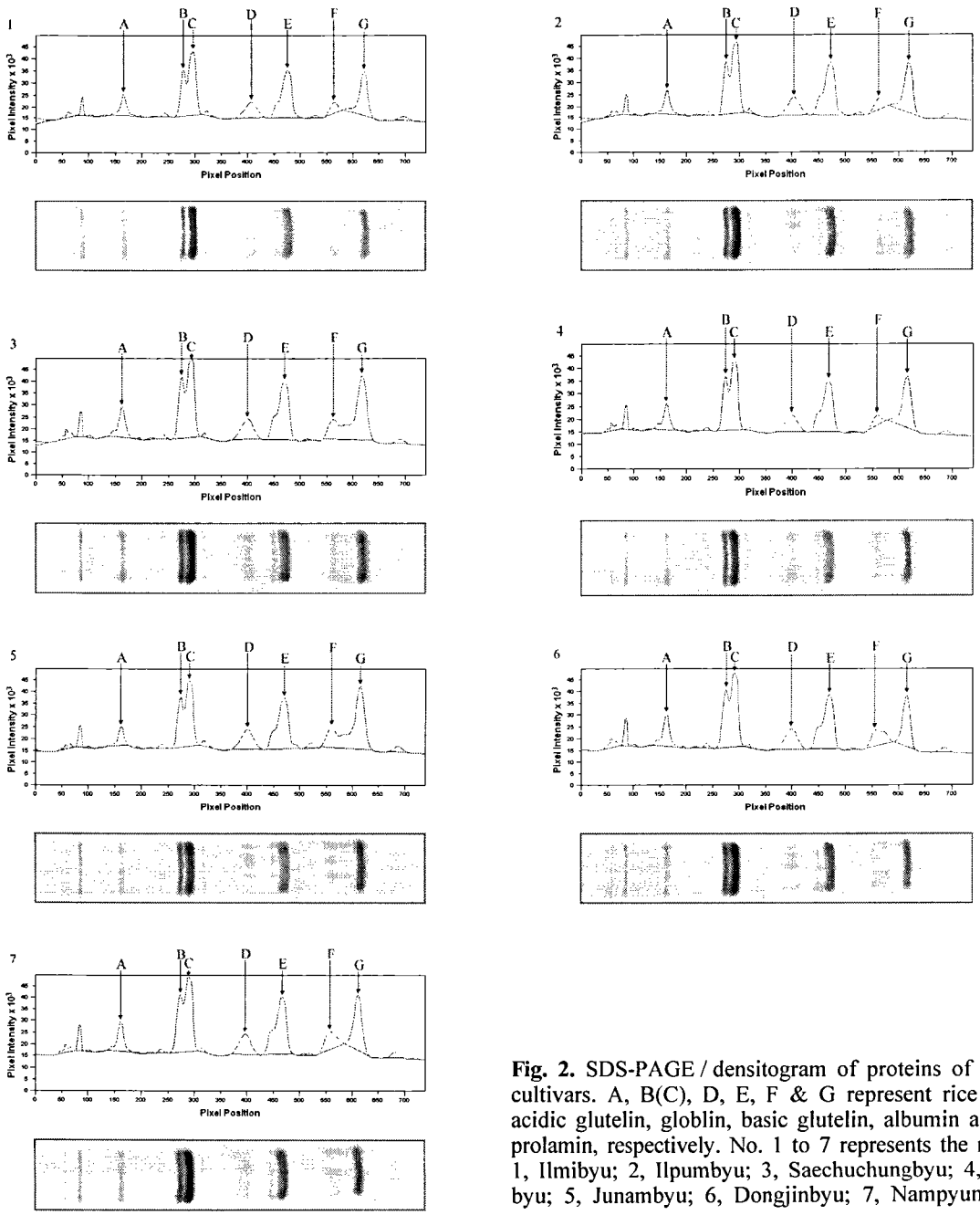

Fig. 2. SDS-PAGE / densitogram of proteins of various rice cultivars. A, B(C), D, E, F & G represent rice preglutelin, acidic glutelin, globlin, basic glutelin, albumin and albumin/ prolamin, respectively. No. 1 to 7 represents the rice cultivar: 1, Ilmibyu; 2, Ilpumbyu; 3, Saechuchungbyu; 4, Chuchungbyu; 5, Junambyu; 6, Dongjinbyu; 7, Nampyungbyu.

a 16 kDa and 22 kDa polypeptides, respectively; corresponding to 14~16 kDa albumin and 26 kDa globulin reported by earlier investigators (14,17). Overall, the protein profile was similar among seven rice cultivars, although the protein profile of protein subunits in the MW range of 13~16 kDa differed depending on rice cultivars. Further study employing densitometry (Fig. 2) indicates that the ratio of the albumin and prolamin subunits is somewhat different according to cultivars (Table 4), and some cultivars (Fig. 2, lanes 1, 2 & 5) contain another trace protein, corresponding to approximately 18 kDa. Additionally, there seems to be a difference in proteins of lower molecular weights (14~16 kDa). Such differences may be caused by the experimental limitations; the albumin and prolamin bands, which constitute a small percentage of total rice protein, are so close that they are not clearly distinguished from one another. Alternatively, albumin and globulin may be partially cross linked with each other as suggested previously (17,28). Moreover, a 14 kDa albumin, which is known to be another albumin allergen, may overlap with the prolamin band, and interfere with the accurate estimation of prolamin content. Therefore, in the following experiment, the further separation of albumins and globulin was attempted by employing sample preparation accompanied by differential solvent solubilization.

Albumin and globulin of various rice cultivars

In an attempt to isolate albumins (14~16 kDa) and globulin (22 kDa) from other rice proteins, Osborne fractionation was performed as previously described (8). In the present experiment, Osborne fractionation was modified to obtain higher extraction yield of albumin and globulin. When rice flour (5 g) of each cultivar was sequentially extracted with 4 volumes of water, followed by 1 M NaCl solution in triplicate, and the content of albumin and globulin in rice was determined by the Lowry method. As shown in Table 4, the combined amount of albumin and globulin is estimated to be around 11 mg/g dry weight, corresponding to about 15% of total rice protein, which was consistent with a previous report (16) that rice albumin is about 1~5%, and globulin is about 5~10% of total protein. Overall, the amount of water/salt-soluble fraction was variable according to cultivar (Table 3).

Next, the protein profile of rice albumin and globulin, resolved by 15% SDS-PAGE, of seven cultivars was evaluated (Fig. 3). Generally, it seems that there is a correlation between Table 3 and Fig. 3; the profile of proteins with MW range of 13~19 kDa is similar among rice cultivars except cultivar N4, which has a relatively lower amount of proteins.

Prolamin of various rice cultivars

The separation of prolamin was carried out using 1-propanol (55%) extraction, which was also by Toshio et al. (18). The prolamin content of different cultivars,

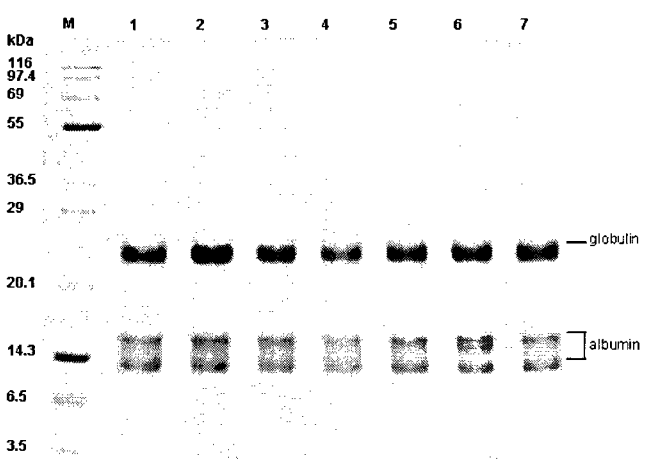

Fig. 3. SDS-PAGE analysis of albumin and globulin fractions. Rice albumin and globulin solution was extracted from rice sample, and then an aliquot (ca. 12 μL) was applied to 15%/4% SDS-PAGE. Lanes 1 to 7 represent the rice cultivar: 1, Ilmibyu; 2, Ilpumbyu; 3, Saechuchungbyu; 4, Chuchungbyu; 5, Junambyu; 6, Dongjinbyu; 7, Nampyungbyu. Lane M is molecular weight standard. The labeled proteins indicate rice major globulin and albumin.

Table 4. Ratio of rice proteins by SDS-PAGE analysis

Cultivars	Preglutelin (54~57 kDa)	Acidic glutelin (32~35 kDa)	Globulin (22 kDa)	Basic glutelin (19 kDa)	Albumin (16 kDa)	Prolamin (13 kDa)
Ilmibyu	15.32±0.62^{a}	27.44±2.21^{a}	11.55±1.54^{a}	27.19±5.33^{a}	6.59±0.75^{a}	11.90±0.76^{ab}
Ilpumbyu	14.61±0.67^{a}	26.75±0.70^{a}	11.32±0.69^{a}	27.24±5.37^{a}	7.41±0.36^{abc}	12.67±0.76^{ab}
Saechuchungbyu	14.73±0.77^{a}	26.37±1.56^{a}	10.75±0.77^{a}	26.93±5.57^{a}	7.41±0.51^{abc}	13.82±0.78^{b}
Chuchungbyu	15.03±0.52^{a}	27.37±0.72^{a}	10.05±0.41^{a}	26.93±4.95^{a}	7.24±0.68^{ab}	13.37±1.22^{ab}
Junambyu	14.81±0.55^{a}	27.55±0.82^{a}	10.40±0.46^{a}	26.76±4.95^{a}	6.88±0.15^{ab}	13.60±1.15^{ab}
Dongjinbyu	15.34±0.31^{a}	28.49±1.01^{a}	11.31±0.40^{a}	23.95±0.47^{a}	9.91±0.49^{d}	11.00±0.38^{a}
Nampyungbyu	14.91±0.75^{a}	27.38±1.01^{a}	10.02±0.90^{a}	28.00±4.75^{a}	8.46±0.84^{bc}	11.23±1.59^{b}

Protein extract of each rice cultivar was subjected to 15% polyacrylamide SDS-PAGE (10 μL/lane). Density of each protein band was determined by scanning densitometry. Data are expressed as mean±standard deviation (n=3). Different letters in the same column indicate significant differences ($p<0.05$).

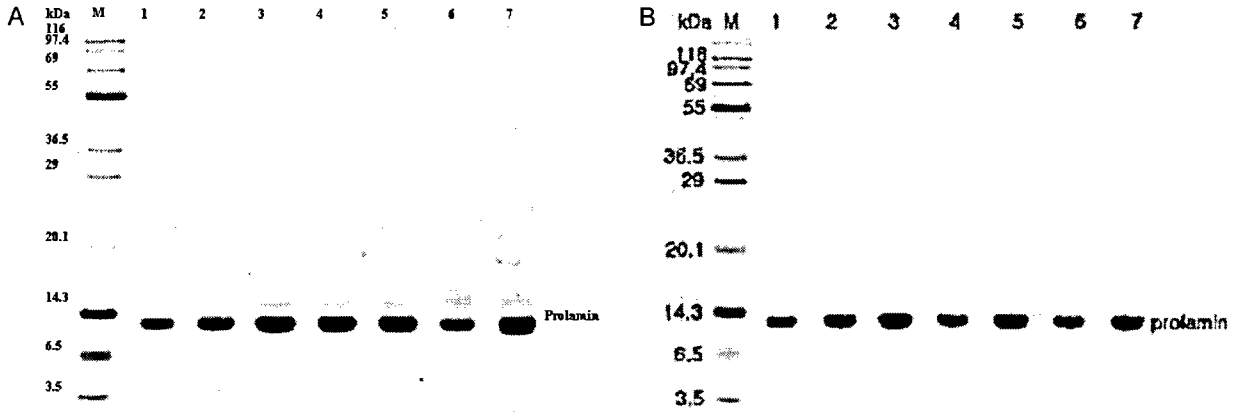

Fig. 4. SDS-PAGE analysis of prolamin fraction for sample A and B. Sample B was the same sample of A, but it was subjected to the wash-out step with distilled water prior to SDS-PAGE analysis. Rice prolamin was extracted from rice sample with 55% 1-propanol, and then an aliquot (ca. 10 μL) loaded onto 15% SDS-PAGE. Lanes 1 to 7 represent the rice cultivar: 1, Ilmibyu; 2, Ilpumbyu; 3, Saechuchungbyu; 4, Chuchungbyu; 5, Junambyu; 6, Dongjinbyu; 7, Nampyungbyu.

determined by Lowry method, is around 1.31~2.20 mg/g rice (dry weight), which is in the range of 1~5% prolamin of total protein (16). This might be suggested from the protein profile of total rice protein in Fig. 1. To selectively isolate prolamin using 1-propanol extraction, the 1-propanol extract was concentrated by vacuum drying, and then the dried sample was applied to SDS-PAGE analysis. As shown in Fig. 4A, the protein profile demonstrates the existence of both albumin and prolamin bands in the protein sample. The amount of prolamin seems to differ according to cultivars. Next, when the same sample was subjected to the wash-out step with distilled water prior to SDS-PAGE analysis, it was found that water-soluble proteins were removed from the 13~16 kDa proteins, leading to the existence of 13 kDa protein only (Fig. 4B). Thus, the wash-up procedure contributes to the resolution of 13 kDa prolamin.

CONCLUSION

The present results indicate that the PAGE/densitometry method, accompanied by solubility fractionation, would be useful to analyze the difference in protein profiles of rice proteins with lower molecular weights, and furthermore to evaluate the protein profile of proteins between genetically modified rice and conventional rice.

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