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Extraction and Electrophoretic Characterization of Rice Proteins

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Abstract

Rice proteins were extracted from brown and milled rice of five varieties: Kwanganbyeo, Daeanbyeo, Daejinbyeo, Surabyeo, Hwaseongbyeo; and their electrophoretic patterns were analyzed by SDS-PAGE. Albumin was extracted with water, globulin with 5% NaCl, prolamin with 70% ethanol, and glutelin with 0.2 M sodium borate buffer (pH 10.0) containing 0.5% SDS, 0.6% β-mercaptoethanol. The ratios of albumin : globulin : prolamin : glutelin in the brown rice were 10.8~14.1 : 12.4~16.4 : 3.6~5.3 : 68.6~72.8, and in milled rice were 4.4~5.6 : 10.6~12.0 : 3.9~5.4 : 75.7~79.8. In albumin seven major bands were observed with molecular weights ranging from 14.9~96.8 kDa, in globulin four bands with molecular weights in the range of 14.4~56.9 kDa, prolamin had only one band with a molecular weight of 14.4 kDa, and glutelin had four bands with molecular weights of 14.4~57.4 kDa. There were no differences in electrophoretic patterns between rice varieties or between brown and milled rice.

Key words: brown rice, milled rice, rice proteins, extraction, SDS-PAGE

INTRODUCTION

Rice is a staple food in many countries and one of the most important cereal grains. Proteins are the second most abundant constituent of cereal grains and can be divided into two broad groups based on their biological function: biologically active enzymes and biologically inactive storage proteins. Rice storage proteins consist of albumin, globulin, prolamin and glutelin, and make up most (up to 80 %) of the total proteins in rice (1). Storage proteins are genotypic (dependent on the genetic background of the plant) and used most often for varietal identification. Moreover, rice protein is valuable because it has unique hypoallergenic properties and ranks high in nutritive quality, especially rich in the essential amino acid, lysine, compared to other cereal proteins (2). The high protein quality and hypoallergenic nature of rice protein isolates or concentrates make them highly desirable as food and beverage ingredients. An effective extraction is essential for the commercial production of rice proteins. The classical fractionation procedure of Osborne (3) has been used for years to divide cereal proteins into four major groups based on their solubilities. In Osborne's scheme, albumins are soluble in water, globulins are soluble in dilute salt solutions but not water, prolamins are soluble in aqueous alcohols but not water or salt solutions, and glutelins are soluble in acid or alkalai but not alcohol, water, or salt solutions. However large differences in estimating the relative proportion of proteins, or the four major solubility fractions, are found in different cereal species; and even within the same species when analyzed by different researchers. The differences between the data reported by different investigators may be due to the use of different rice varieties, but the uncertainties of the extraction procedure may also play a role. Generally, albumin is extracted with water, globulin with 0.2~1.0 M NaCl, prolamin with 70% ethanol or 50% propanol (4-6). For the extraction of glutelin, one of several buffers is typically used (0.01~0.05 N NaOH, borate buffer, 0.1N acetic acid, or SDS-β-mercaptoethanol) by different researchers (7-9). Electrophoretic patterns of rice proteins are one of the methods used to identify rice varieties, as well as for potential end-use quality and molecular weight determinations (10). The objectives of this study were to compare the contents and investigate sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns of four proteins extracted from five rice varieties grown in Korea.

MATERIALS AND METHODS

Rice samples

Five rice varieties: Kwanganbyeo (KA), Daeanbyeo (DA), Daejinbyeo (DJ), Surabyeo (SR), and Hwaseongbyeo (HS) were provided by the National Crop Experiment Station of the Rural Development Administration, Suwon, Korea. Milled rice was prepared by removing 8% (w/w)

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of outer layer of brown rice kernel. Both brown and milled rice were ground to pass through a 60 mesh screen. The ground samples (10 g) were defatted with hexane by Soxhlet extraction for 18 hrs, and then air-dried in a hood for 24 hrs at room temperature (RT).

Extraction of rice proteins

Rice flour (10 g) was deffated with 100 mL hexane (Fig. 1). The defatted flour was then extracted by stirring with 40 mL of distilled water at RT for 4 hrs (albumin extract) and centrifuged at 3,800×g for 30 min. After water extraction the flour was extracted with 40 mL of 5% NaCl at RT for 4 hrs (globulin extract) and centrifuged at 3,800 ×g for 30 min. The flour was then extracted for prolamin with 40 mL of 70% ethanol at RT for 30 min, and followed by glutelin extraction with 75 mL of 0.2 M sodium borate buffer (pH 10) containing 0.5% SDS and 0.6% β- mercaptoethanol at RT for 3 hrs (7,11). 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°C.

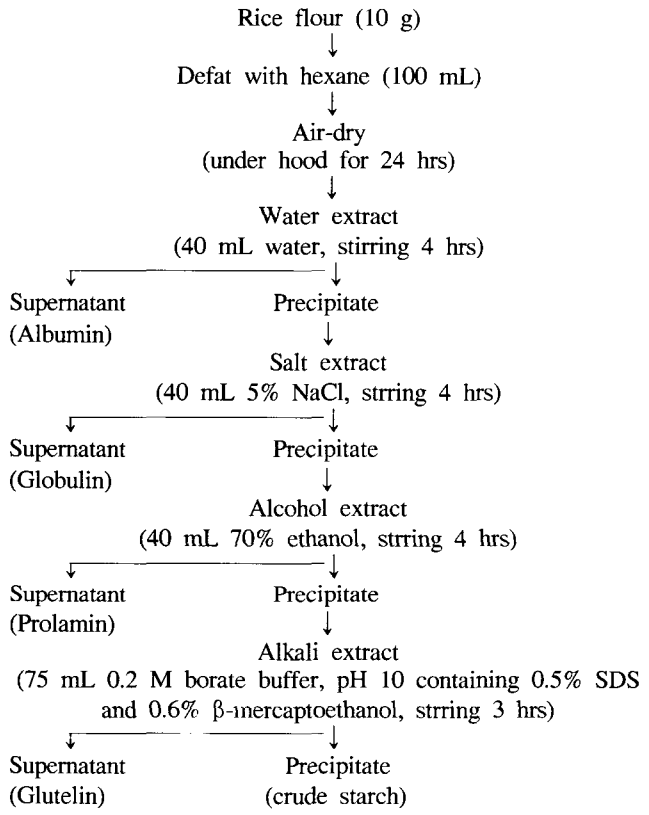

Fig. 1. Flow diagram for extraction of rice proteins.

Crude proteins

Total nitrogen of each rice protein was determined by micro Kjeldahl method (12) and a conversion factor of 5.95 was used for conversion to crude proteins.

Electrophoresis

Freeze-dried protein samples were resolved in each extracted buffer and loaded onto SDS-PAGE gel. SDS-PAGE was carried out by the method of Laemmli (13) using the Hoeffer Mini VE system. The separations of proteins were performed using 12% running gel with a 4% stacking gel. Proteins were diluted with sample buffer (0.065 M Tris-HCl buffer, pH 6.8, containing 10% glycerol, 1% SDS, 0.005% bromophenol blue and 1% 2-mercaptoehtanol) and denatured by heating at 100°C for 4 min. Proteins were then loaded onto 1 mm thick gels and run in a buffer of 0.6% Tris, 2.9% glycine and 0.1% SDS, at pH 8.3 with a current of 120V. Following electrophoresis, gels were stained with 0.1% Coomassie brilliant blue R-250 (Amersham parmacia biotech, Sweden) in 40% methanol and 10% acetic acid, and then destained in 40% methanol and 10% acetic acid. Molecular weights of the protein bands were determined by the method of Weber and Osborn (14) using a molecular weight standard kit (Amersham pharmacia biotech, Little Chalfont Buckinghamshaire, UK) containing: phophorylase b (97 kDa), albumin (66 kDa), oval-

Table 1. Protein contents of rice flour and protein fraction

	Brown rice						Milled rice					
	KA	DA	DJ	SR	HS	Mean	KA	DA	DJ	SR	HS	Mean
Flour	6.7	7.5	8.0	7.0	7.5	7.3	6.1	6.9	7.5	6.6	6.8	6.8
Albumin	0.9 (14.1)[1)]	0.9 (11.7)	0.8 (10.9)	0.9 (12.9)	0.8 (10.8)	0.9 (11.8)	0.3 (5.6)	0.3 (4.4)	0.4 (5.5)	0.3 (4.5)	0.3 (4.9)	0.3 (4.7)
Globulin	0.9 (13.7)	1.0 (12.9)	1.0 (12.4)	0.9 (12.8)	1.2 (16.4)	1.0 (13.5)	0.7 (11.3)	0.8 (12.0)	0.8 (11.5)	0.7 (10.6)	0.7 (10.9)	0.7 (10.8)
Prolamin	0.2 (3.6)	0.3 (4.6)	0.3 (3.8)	0.4 (5.3)	0.3 (4.1)	0.3 (4.2)	0.2 (3.9)	0.4 (5.4)	0.3 (4.4)	0.3 (5.1)	0.3 (4.4)	0.3 (4.4)
Glutelin	4.5 (68.6)	5.4 (70.8)	5.7 (72.8)	4.8 (69.0)	4.9 (68.6)	5.1 (69.2)	4.7 (79.2)	5.2 (78.2)	5.6 (78.6)	4.9 (79.8)	5.3 (79.8)	5.1 (75.7)
Recovered efficiency (%)	98.8	102.4	97.8	100.0	95.2	98.8	98.3	96.1	94.3	94.0	97.4	95.6

[1)]Percentage (w/w) to the sum of four protein fractions.

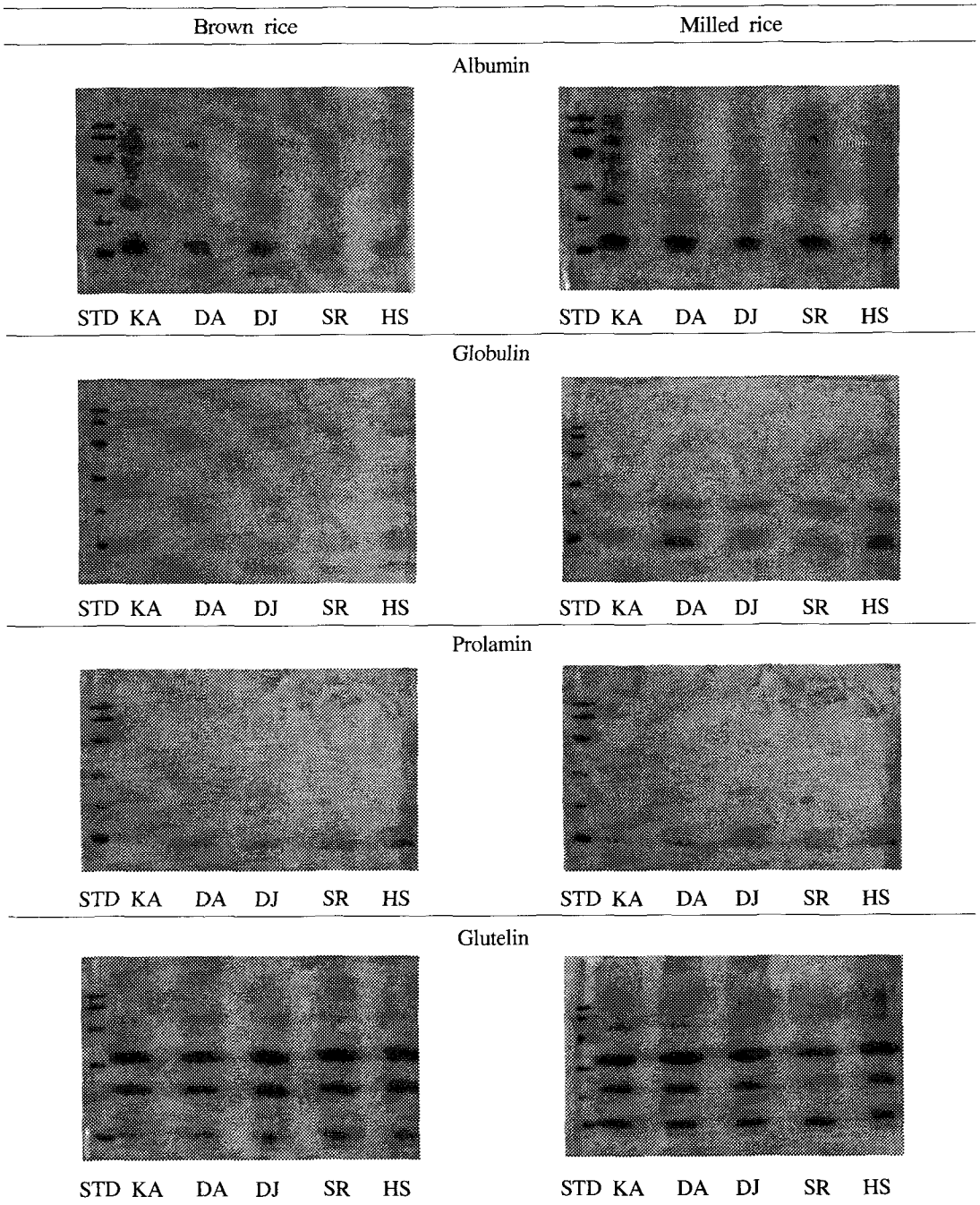

Fig. 2. SDS-PAGE patterns of rice protein fractions. STD: Standard marker, KA: Kwanganbyeo, DA: Daeanbyeo, DJ: Daejinbyeo, SR: Surabyeo, HS: Hwaseongbyeo.

bumin (45 kDa), carbonic anhydrase (30 kDa), trypsin nhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa).

RESULTS AND DISCUSSION

Protein content and their fractionation

The protein content of brown rice ranged from 6.7 to 8.0% while that of milled rice was from 6.1 to 7.5% (Table 1). Among brown rice varieties, Daejinbyeo showed the highest content of protein (8.0%) followed by Daeanbyeo and Hwaseongbyeo (7.5%), Surabyeo (7.0%), and Kwanganbyeo (6.7%). Among milled rice varieties, Daejinbyeo (7.5%) was the richest followed by Daeanbyeo (6.9%), Hwaseongbyeo (6.8%), Surabyeo (6.6%), and Kwanganbyeo (6.1%). The difference in protein content between brown and milled rice ranged from 6.3 to 12.0%. The protein of SR milled rice was decreased about 12.0% among the five varieties after the milling process while that of DJ was about 6%. This suggests that some rice proteins are lost during milling process and the quantity of protein

lost is dependent on the variety. For protein fractionation, the samples were sequentially extracted with water, 5% NaCl, 70% ethanol, and 0.2 M sodium borate buffer (pH 10) containing 0.5% SDS and 0.6% β-mercaptoethanol. Extraction conditions were optimized for high protein recovery efficiency over 94.0%. The mean ratios of albumin, globulin, prolamin, and glutelin for brown rice were 11.8 : 13.5 : 4.2 : 69.2, and those for milled rice were 4.7 : 10.8 : 4.4 : 75.7, among the five rice varieties. These results suggest that the outer tissues of the rice grain are rich in albumin and globulin comparing prolamin and glutelin (15). The rice varieties exhibited ratios of albumin : globulin : prolamin : glutelin that ranged from 10.8~14.1 : 12.4~16.4 : 3.6~5.3 : 68.6~72.8 in the brown rice, and 4.4~5.6 : 10.6~12.0 : 3.9~5.4 : 75.7~79.8 in the milled rice. For protein fractions of brown rice, the ratios of 5~17 : 4~15 : 2~5 : 71~86 were reported by other investigators (16,17). Padhye and Salunkhe (18) reported the ratio of 8 : 10 : 12 : 70 for those protein fractions of milled rice. The wide range of protein content of rice and its fractions is primarily due to such factors as variety, environment, crop season or planting date, differences in extraction procedures, and nitrogen fertilization (16,19,20).

SDS-PAGE patterns of rice proteins

The albumin fraction exhibited seven major bands with molecular weights of 14.9, 17.3, 23.4, 26.6, 43.5, 55.5 and 96.8 kDa (Fig. 2). Globulin had four major bands with molecular weights of 14.4, 16.2, 25.5 and 56.9 kDa. Prolamin had only one band with a molecular weight of 14.4 kDa. The glutelin fraction had four major bands with molecular weights of 14.4, 24.7, 37.9 and 57.4 kDa. There were no differences in the SDS-PAGE patterns of protein fractionation between the five rice varieties or between brown and milled rice. Kim and Jo (21) reported that there were protein bands with molecular weights of 12 and 29 kDa in albumin, 12, 17, 21 and 27 kDa in globulin, 16 kDa in prolamin, and 15, 17, 21 and 35 kDa in glutelin. In other research, SDS- PAGE showed protein bands with molecular weights of 15, 18, 21, 26.5, 30 and 37 kDa in albumin (22), 13, 14.8, 21.3, 24 and 36 kDa in globulin (23), 17 kDa in prolamin (24) and 15, 22 and 38 kDa in glutelin (7,25). The differences in SDS-PAGE patterns of rice protein between the research groups might be due to the different extraction methods, and different varieties of rice, even though there was no difference between the five rice varieties used in this study.

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