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Effect of Acetic Acid Added to Cooking Water on the Dissolution of Proteins and Activation of Protease in Rice

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The effect of acetic acid on the dissolution of proteins in rice was studied to elucidate the mechanism for the textural change induced by the acid by chemical and SDS-PAGE analyses of the rice proteins in the soaking solution. More proteins were extracted with 0.2 M acetic acid (pH 2.7) than with water (pH 6.8). The effect of acetic acid on the protein dissolution increased with increasing temperature. Immunoblotting confirmed that, when rice was soaked in acetic acid, glutelin was dissolved into the soaking solution and degraded by aspartic proteinase. Aspartic proteinase degraded glutelin much more than it did albumin and globulin. It was found that the combined amount of albumin and globulin dissolved into the acetic acid solution was much larger than that of glutelin, despite the smaller amounts present of albumin and globulin than of glutelin. Metal ions were extracted more with acetic acid than with water. In addition, carboxypeptidase was activated under the acidic condition and resulted in an increase in the amount of free amino acids. The main effect of acetic acid on the dissolution of rice proteins was enhancement of the solubility of albumin, globulin, and glutelin, the effect of proteases being minor.

KEYWORDS: Rice; protein; cooking water; acetic acid; aspartic proteinase; glutelin; SDS-PAGE analysis

INTRODUCTION

Rice is cultivated in eastern and southeastern Asian countries as one of the most important foods; rice is the staple food in Japan, as in almost all of these countries. It is well-known that the taste of cooked rice is related to such textural factors as softness and stickiness. The texture of cooked rice is dependent on the variety, storage time, and cooking conditions.

When rice is stored for a long time, the texture of the cooked rice becomes less satisfactory due to increased hardness and decreased stickiness. To avoid such undesirable textural factors, the following procedures have been attempted: (1) the addition of rice vinegar and fruit juice to the cooking water (*1*); (2) high-pressure treatment of the rice (*2*); and (3) enzymatic degradation of the cell wall or proteins (*2*−*4*). Each of these procedures makes the cooked rice softer and stickier. Among these procedures, rice vinegar and fruit juice can be used daily at home, and they are safe and convenient. The addition of acetic and citric acids, which are the main components of rice vinegar and fruit juice, was found to be preferable for improving the texture of cooked rice (*5*, *6*). Although the effects of these added acids have been studied with respect to physical properties, sensory evaluation, and tissue structure, the mechanism for the textural change is not yet well understood.

We have previously reported the effect of acetic acid added to the cooking water on the palatability and physicochemical properties of cooked rice at the phenomenological level (*7*). It was reported that the amount of oligopeptides eluted was larger in the presence of acetic acid than that in its absence. Resting rice seeds contain an aspartic proteinase (*8*, *9*) and a carboxypeptidase (*10*) that optimally hydrolyzed in the acidic pH range. Glutelin, the major storage protein in rice, is dissolved in a dilute acidic or alkaline solution. The increase in the amount of eluted oligopeptides has been suggested to be caused by activation of the enzyme and the dissolution of rice proteins. It remains to be clarified to what extent the proteins are dissolved and the proteases degrade proteins during cooking.

We focus this study on the role of acetic acid in the activation of aspartic proteinase and carboxypeptidase and in the dissolution of glutelin to elucidate the mechanism for the textural change to rice cooked with acetic acid. This is achieved by carrying out an analysis of the soaking solution of the rice containing acetic acid by using protease inhibitors. Generally in Japan, rice is soaked in the cooking water for ∼1 h and then heated until the cooking water has been absorbed by the rice grains. The amounts of rice components such as sugars and amino acids eluted to the cooking water increased with increasing soaking and cooking times (*11*). The stickiness and transparency of the cooked rice might be attributed to the condensed cooking water that covers the surface of the cooked rice grains. Soaking the rice is therefore important for rice

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cooking, and this process has a key role in controlling the dissolution of rice components and the activation of rice enzymes.

MATERIALS AND METHODS

Rice Sample. Rice (*Oryza sativa* L. Japonica cv. Nipponbare) grown in Shiga Prefecture (1999) was used. Brown rice was stored at 4 °C in kraft paper bags and milled by 90% in a polishing machine (MC-90A, Toyoseiki Co.) on the day of the experiment.

Soaking Treatment. Milled rice grains of 3 g were washed three times with 20 mL each of distilled water. The washed rice was then soaked in deionized water or 0.2 M acetic acid for 1 h at 20 or 50 °C. The pH values of the soaking solution with and without acetic acid were 6.8 and 2.7, respectively. The weight ratio of the rice grains to the soaking solution was 1:1.5. The following enzyme inhibitors were added to the soaking solutions: 100 μM pepstatin A, 50 μM leupeptin, and 100 μM E-64 (Peptide Institute); 10 mM EDTA (Wako Pure Chemical Industries); and 5 mM PMSF (Sigma, St. Louis, MO). In the case of PMSF, the sample was soaked in distilled water containing 5 mM PMSF for 1 h at 50 °C and subsequently soaked for 1 h after the addition of acetic acid and pepstatin. After soaking, the rice grains and soaking solutions were separated. Each soaking solution was centrifuged at 3000*g* for 15 min, and the resulting supernatant was used for the chemical analysis.

Measurement of the Protein Content. Nitrogen content was measured according to the Kjeldahl method and multiplied by 5.95 to obtain the amount of crude protein. The protein content in each soaking solution was determined according to the method of Lowry et al. (*12*). A calibration curve was prepared with bovine serum albumin.

SDS-PAGE Analysis. Electrophoresis was carried out on the extracted rice proteins according to the method of Laemmli (*13*), a 10−20% gradient or 15% polyacrylamide gel containing 0.1% SDS being used. Two kinds of SDS-PAGE analysis were carried out: the amount of protein contained in the sample applied was kept constant in one analysis, and the amount of the sample applied was kept constant in the other analysis. The latter result was used to quantitatively determine the relative amount of polypeptides by Scion Image software (Scion Image).

Preparation of Glutelin and Anti-glutelin. Milled rice grains were ground in an electric mill, the resulting flour being sieved through a 355-μm mesh. Acetone was added to the flour to remove the fat, the mixture being kept for 30 min at room temperature with stirring. The ratio of the flour to the solvent was 1:2 (w/v), and after being filtered, the flour was dried at room temperature to remove the solvent. The defatted flour was fractionated into the four portions that respectively contained albumin, globulin, prolamin, and glutelin. These fractions were respectively extracted at 4 °C for 1 h with deionized water, 1.0 M sodium chloride, 55% *n*-propanol, and 2% SDS in a 50 mM Tris-HCl buffer (pH 8.5). The ratio of the flour to each extracting solvent was 1:5 (w/v), and each extraction was conducted three times before centrifugation at 3000*g* for 15 min. Crude glutelin was obtained by carrying out the first and second centrifugations at 3000*g* for 15 min, and the third at 21000*g* for 20 min, before acetone was added to the resulting supernatant; the mixture was left overnight at 4 °C. The residue thus obtained was centrifuged at 12000*g* for 20 min and dried at room temperature. The crude glutelin sample was purified according to the method of Krishman and Okita (*14*). An SDS-PAGE analysis was carried out on the crude glutelin, and gels containing the 29−34 kDa (α-subunit glutelin) and 21 kDa (β-subunit glutelin) polypeptides were sliced off and rinsed with 100 mM Tris-HCl (pH 8.0). These gel slices were mashed and then soaked in 1% SDS and 20 mM Tris-HCl (pH 8.0) overnight with stirring. The proteins eluted from the gels were concentrated by ultrafiltration, the protein concentrations of the α- and β-subunit glutelins being 2.5 and 0.5 mg/mL, respectively. Antibodies of these proteins were commercially produced (Immunoresearch Laboratories Co.).

Immunoblotting. Immunoblotting was performed according to the method of Towbin et al. (*15*). The proteins contained in the SDS-PAGE gels were electrophoretically transferred to a Millipore poly(vinylidene difluoride) membrane. The membrane was blocked with 5% skim milk in 20 mM Tris-HCl (pH 7.5) containing 0.1 M NaCl and then incubated with the anti-α- or anti-β-subunit glutelin antiserum. An anti-rabbit IgG horseradish peroxidase conjugate was used as the second antibody (Sigma). The binding sites of the antibodies were visualized by using solutions of 0.5 mg/mL of 3,3′-diaminobenzidine tetrahydrochloride and 0.05% hydroperoxide.

Measurement of the Metal Ion Content. The potassium, sodium, calcium, and magnesium ions in each soaking solution were determined by atomic absorption spectrophotometry (AA-660, Shimadzu Co.), lanthanum chloride being added in a quantity sufficient to suppress the anionic interference due to phosphoric acid.

Analysis of Amino Acids. The composition of the amino acids was determined with an 835S autoanalyzer (Hitachi Co.). To determine the total amount of the amino acids, the rice extract containing oligopeptides was hydrolyzed by 6 N HCl for 24 h at 110 °C to degrade into free amino acids. The difference in amount between the total amino acids and free amino acids corresponds to the amino acids of oligopeptides.

Table 1. Content of Rice Proteins Extracted with Water and 0.2 M Acetic Acid[a]

	content in soaking solution (mg/100 g of milled rice)	
temp (°C)	water	acetic acid
20	27 ± 2a	130 ± 11b
50	43 ± 3a	573 ± 24b

[a] Mean value ± SD ($n = 3$). Means marked by different letters in the same row are significantly different ($p < 0.001$).

RESULTS AND DISCUSSION

Effect of Acetic Acid on the Amount of Proteins Extracted from the Rice. We examined the amounts of extracted rice proteins in a soaking solution with or without acetic acid. The soaking temperatures used were 20 and 50 °C, the former being the usual temperature for soaking before heating and the latter the optimum temperature for aspartic proteinase (*9*). The molar concentration of acetic acid was 0.2, and its pH value was 2.7. In our previous paper (*7*), when acetic acids of molar concentrations from 0.05 to 2.0 were added to the cooking water, oligopeptides eluted more with increasing molar concentration of acetic acid. To investigate the effect of acetic acid on the rice protein clearly, we used 0.2 M acetic acid as a soaking solution in this experiment. The amounts of proteins in the acetic acid solution were greater than those without acetic acid at both temperatures; see **Table 1**. We have previously reported (*7*) that when rice grains were soaked in the cooking water at temperatures from 40 to 98 °C, the difference in the amount of eluted oligopeptides between with and without acetic acid became larger with increasing temperature. Therefore, the effect of acetic acid on protein dissolution was much stronger at higher temperatures in the temperature region of 20−98 °C.

To examine whether an aspartic proteinase (the optimum pH value was 3.0) in rice grains would be activated or not under the acidic condition, the amount of proteins in the soaking solution with or without acetic acid was measured at 50 °C by using proteinase inhibitors. Pepstatin is an inhibitor of aspartic proteinase, and leupeptin, E-64, and EDTA are inhibitors of cysteine, serine, and metal proteinase, respectively. As can be seen from **Table 2**, when the soaking solution contained no acetic acid, the inhibitors had no effect on the amount of proteins. In the presence of acetic acid, on the other hand, pepstatin reduced the amount of proteins. This result indicates that, among the endopeptidases studied in this work, only aspartic proteinase was activated under the acidic condition and that this enzyme played a key role in the degradation of proteins in the rice cooked with acetic acid.

Table 2. Effect of Inhibitors on the Rice Protein Content of the Extracts[a]

	content in soaking solution (mg/100 g of milled rice)	
inhibitor	water	acetic acid
none	39 ± 3a	582 ± 22a
pepstatin	43 ± 2a	456 ± 27b
leupeptin	43 ± 1a	592 ± 19a
E-64	42 ± 1a	598 ± 17a
EDTA	43 ± 2a	601 ± 20a

[a] Mean value ± SD ($n = 3$). Means marked by different letters in the same column are significantly different ($p < 0.001$). Means in the same row are significantly different ($p < 0.001$).

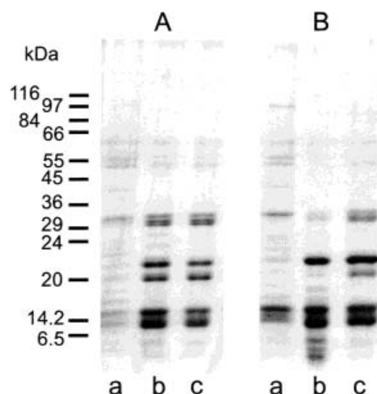

Figure 1. SDS-PAGE analysis of the extracted rice proteins. Rice (3 g) was soaked in distilled water (lane a), 0.2 M acetic acid (lane b), or 0.2 M acetic acid with 100 μM pepstatin (lane c) for 1 h at 20 °C (A) or 50 °C (B). Each lane contains 10 μg of protein. Numbers on the left refer to the apparent molecular weights of the standard marker proteins.

SDS-PAGE Analysis of the Extracted Rice Proteins. It is important to analyze which kinds of rice proteins were dissolved in the soaking solution and how the proteins were degraded by aspartic proteinase activated by the acetic acid. Hence, an SDS-PAGE analysis was carried out on the proteins extracted at different soaking temperatures.

Figure 1 depicts the SDS-PAGE patterns of the rice proteins extracted in the three kinds of soaking solution. At 20 °C, in the case of the sample soaked in water, many bands were detected, although they were unclear as a whole. On the other hand, in the case of the sample soaked in acetic acid, the bands appeared clearly, their molecular weights being 14, 16, 21, 22, 31, and 34K. There was no difference between the results with or without pepstatin, which might be attributable to the low activity of aspartic proteinase at this temperature. In contrast, in the case of the sample soaked at 50 °C in acetic acid, there was a marked difference between the results in the absence of pepstatin and in its presence. When pepstatin was not present, the bands of 21 and 31−34 kDa had substantially disappeared, and the polypeptides of molecular weight smaller than 6K appeared. This indicates that the polypeptides of 21 and 31−34 kDa had been degraded by aspartic proteinase.

The polypeptides of 14 and 16 kDa are a mixture of albumin and globulin, and the polypeptide of 22 kDa corresponds to globulin according to the literature (*16*, *17*), whereas the polypeptides of 21 and 31−34 kDa correspond to glutelin, and the polypeptide of 55 kDa is an unprocessed glutelin precursor polypeptide (*18*, *19*). Rice contains prolamin in addition to these proteins. Although the molecular weight of prolamin, which is an alcohol-soluble protein, has been reported to be 10, 13, and 16 kDa (*20*), it is assumed that the polypeptides of 14 and 16 kDa were due to a mixture of albumin and globulin by considering that these bands could also be seen with water soaking as shown in **Figure 1**.

Figure 2. SDS-PAGE analysis of rice glutelin. The crude glutelin fraction obtained by successive extraction was resolved by SDS-PAGE (right lane). Asterisks indicate the acidic and basic subunits of glutelin. The standard marker proteins are indicated on the left.

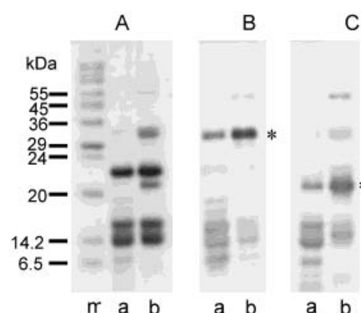

Figure 3. SDS-PAGE and western blot analyses of the extracted rice proteins by using anti-glutelin. Rice was soaked in 0.2 M acetic acid (lane a) or 0.2 M acetic acid with 100 μM pepstatin (lane b) at 50 °C. The extracted rice proteins were subjected to SDS-PAGE at 10 μg/lane in a 15% gel (A), and membranes carrying the blotted proteins were reacted with anti-α-subunit glutelin (B) or anti-β-subunit glutelin (C). Lane m and the numbers on the left refer to the apparent molecular weights of the standard marker proteins. Each asterisk shows the polypeptide reacting strongly with anti-α and anti-β-subunit glutelin.

It is conceivable that the proteins of 21 and 31−34 kDa that had been degraded by aspartic proteinase in our study were glutelin due to its molecular weight. Immunoblotting was therefore carried out to confirm this. **Figure 2** shows the SDS-PAGE pattern of the acidic (α) and basic (β) subunits prepared from the rice glutelin, their molecular weights being 31−34 and 21K, respectively. These fractions were used to obtain each antibody for immunoblotting.

Figure 3 shows the SDS-PAGE and western blots of the rice proteins extracted with an acetic acid solution containing pepstatin or not at 50 °C. In the case of the acetic acid solution with pepstatin, in which aspartic proteinase was not activated, the specificity of the glutelin subunit antibodies can be clearly observed. It can be seen that anti-α- and anti-β-subunit antisera strongly reacted with the polypeptides of 31−34 and 21 kDa, respectively. On the other hand, without pepstatin, when aspartic proteinase was activated, the cross-reactivity against these polypeptides became weaker, instead of stronger, against the polypeptides of <16 kDa. These results confirm that the polypeptides of 21 and 31−34 kDa are identifiable with glutelin and that glutelin was degraded by aspartic proteinase into products with molecular weights of <16K.

To compare the amounts of extracted rice proteins in the different soaking solutions, we kept a constant volume for the samples applied and compared the density of each polypeptide

Table 3. Determination of the Amount of Polypeptides by an SDS-PAGE Analysis[a]

A

temp (°C)	mol wt (K)	soaking solution: W	A	A + P
20	14–16	1.0	16.8	15.3
	21	1.0	25.1	34.5
	22	1.0	21.4	20.1
	31–34	1.0	6.2	8.7
50	14–16	1.0	15.4	10.8
	21	1.0	11.2	48.8
	22	1.0	37.1	41.8
	31–34	1.0	2.9	10.6

B

temp (°C)	soaking solution	glutelin (21 kDa + 31–34 kDa)	albumin + globulin (14–16 kDa + 22 kDa)
20	W	1.0	2.9
	A	1.0	3.1
	A + P	1.0	2.4
50	W	1.0	4.5
	A	1.0	23.8[b]
	A + P	1.0	3.6

[a] The extracted proteins were subjected to SDS-PAGE at 10 μL/lane in a 15% gel. The density of each polypeptide was determined by using computer software. **Table 3A** shows the ratio of the density of the polypeptide band of a sample soaked in the solution containing acetic acid with pepstatin (denoted A + P) or without pepstatin (denoted A) to that of the sample soaked in water (denoted W). **Table 3B** shows the ratio of the density of the sum of albumin and globulin (14–16 kDa + 22 kDa) to that of the sum of the glutelin fractions (21 kDa + 31–34 kDa). [b] This fraction included the decomposition products of glutelin by aspartic proteinase together with albumin and globulin polypeptides.

in the SDS-PAGE pattern. **Table 3A** shows the ratio of the density of the polypeptide bands from the sample soaked in acetic acid containing pepstatin (denoted A + P) or without pepstatin (denoted A) to that of the sample soaked in water (denoted W). This ratio corresponds to the relative amount of polypeptide extracted into the soaking solution.

It can be seen in **Table 3A** that, at 20 °C, each polypeptide was present in greater quantity in sample A + P than in sample W. In particular, the proportions of 21 kDa glutelin and 22 kDa globulin were much larger than those of the other polypeptides. The solubility of each polypeptide in sample A + P increased with increasing temperature. The difference in proportion between sample A and sample A + P reflects the degree of degradation of the protein by aspartic proteinase. The total proportion of 21 and 31−34 kDa polypeptides in sample A was ~24% of that in sample A + P at 50 °C, although the figure was 72% at 20 °C. The reason for this is that 50 °C was the optimum temperature for aspartic proteinase to decompose glutelin, the decomposition being higher at 50 °C than at 20 °C. In contrast, the proportion of 22 kDa globulin was almost the same, despite the presence of pepstatin. These results support the supposition that aspartic proteinase mainly degraded glutelin. The amount of 14−16 kDa polypeptides in sample A was larger than that in sample A + P, suggesting that these polypeptides included the enzyme-catalyzed degradation products of glutelin.

We next compare the amount of glutelin (21 and 31−34 kDa) with the sum of albumin and globulin (14−16 and 22 kDa) in the soaking solutions at 20 and 50 °C. As can be seen in **Table 3B**, all of the ratios are >1.0. This result suggests that more albumin and globulin were eluted than glutelin with or without acetic acid in the soaking solution, although the content of the sum of albumin and globulin in the rice grains was less than that of glutelin (*21*). Glutelin was found to be soluble in dilute acid, indicating that the amount of rice proteins dissolved in the soaking solution was related not only to the solubility of the proteins but to other factors such as the location of the proteins in rice. Cagampang et al. (*22*) have reported that, although albumin and globulin were minor proteins in whole grains, they were concentrated in the bran and at the surface of milled rice. Therefore, when rice grains are merely soaked, albumin and globulin present in the outer layer of milled rice may be easily dissolved. By extracting protein with acetic acid, the starch might easily swell more.

In the case of the sample A at 50 °C, the decomposition of glutelin by aspartic proteinase was included in the 14−16 kDa band, so the proportion was markedly increased.

Table 4. Content of Metal Ions[a]

temp (°C)	soaking solution[b]	metal ion (mM): K	Mg	Na	Ca
20	W	2.3 ± 0.1a	0.13 ± 0.07a	0.39 ± 0.04a	0.01 ± 0.01a
	A	4.0 ± 0.7b	1.08 ± 0.35b	0.82 ± 0.01b	0.32 ± 0.02b
	A + P	4.1 ± 0.7b	1.17 ± 0.38b	0.65 ± 0.05b	0.32 ± 0.04b
50	W	3.4 ± 0.6b	0.48 ± 0.15c	0.48 ± 0.04a	0.17 ± 0.02c
	A	5.4 ± 0.6c	1.36 ± 0.40d	0.91 ± 0.13b	0.49 ± 0.02d
	A + P	5.5 ± 0.7c	1.28 ± 0.39d	1.13 ± 0.05b	0.51 ± 0.05d

[a] Mean value ± SD ($n = 3$). Different letters within the same column indicate significant difference ($p < 0.05$). [b] Notation is the same as in **Table 3**.

Contents of Metal Ions in the Soaking Solutions. To examine why albumin and globulin were extracted more with acetic acid than with water, we measured the contents of metal ions contained in the soaking solutions. As shown in **Table 4**, the amount of all kinds of metal ions determined in this study was greater in the acetic acid solution with or without pepstatin than in water. There was a small increase in the amount of these metal ions with increasing temperature. It has been reported in our previous paper (*7*) that when milled rice was incubated in soaking solutions in the temperature range of 40−98 °C, the amount of soluble components dissolved in the solutions was greater with acetic acid than with water alone. Considering that the content of metal ions in rice grains is highest in the outer layer (*23*), we can conclude that the metal ions were dissolved in the soaking solutions together with the soluble components of the rice grains. The greater amount of metal ions in the soaking solution containing acetic acid brought about an increase in the ionic strength, which can bring about an increase in the amounts of extracted albumin and globulin in the soaking solution (*3*). There was no influence by aspartic proteinase on the amount of metal ions dissolved.

Composition of the Amino Acid Residues of Oligopeptides and Free Amino Acids of the Rice Extract. Finally, we examined the composition of the amino acid residues of oligopeptides and of free amino acids in the extracted rice proteins. As illustrated in **Figure 4A**, the amino acids of the oligopeptides extracted by acetic acid solution without pepstatin were largest in amount at both 20 and 50 °C. In the case of the acetic acid solution with pepstatin, the amino acid amounts were decreased to ~70−80% of the amounts in the absence of pepstatin but were much greater than those in the water extract. It is suggested that the effect of aspartic proteinase on the total amount of dissolved oligopeptides was smaller than that on the dissolution of albumin, globulin, and glutelin under acidic conditions.

On the other hand, the composition of free amino acids differed from that of the oligopeptides. **Figure 4B** shows that the amounts of free amino acids were increased only in the case

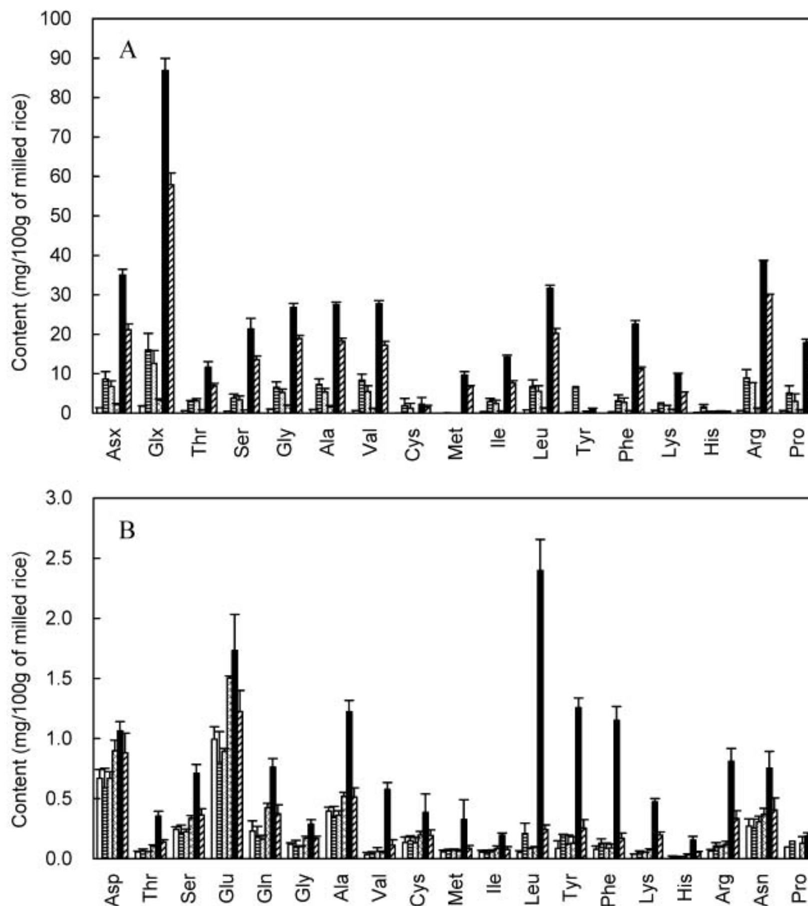

Figure 4. Composition of oligopeptides (A) and free amino acids (B) in the rice extracts: (white bars) water extract at 20 °C; (striped bars) acetic acid extract at 20 °C; (dotted bars) acetic acid with pepstatin extract at 20 °C; (cross-hatched bars) water extract at 50 °C; (black bars) acetic acid extract at 50 °C; (slashed bars) acetic acid with pepstatin extract at 50 °C.

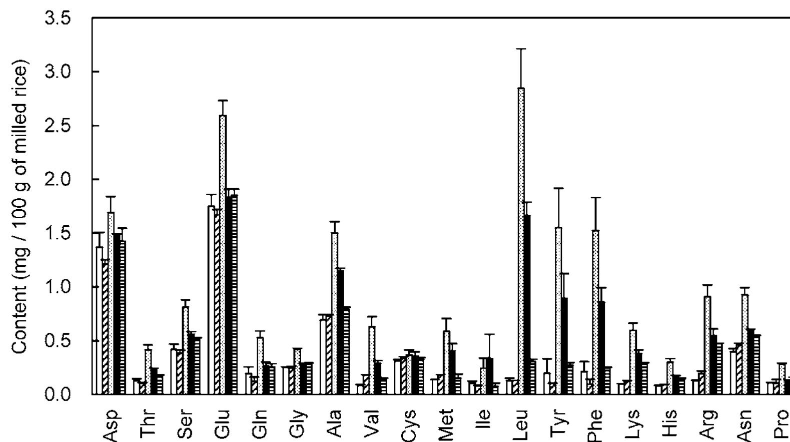

Figure 5. Effect of PMSF on the composition of free amino acids in the rice extracts at 50 °C: (white bars) water extract; (slashed bars) water with PMSF extract; (dotted bars) acetic acid extract; (black bars) acetic acid with PMSF extract; (striped bars) acetic acid with pepstatin extract.

of the acetic acid extract at 50 °C, the most significant increase being in the amounts of such amino acids as leucine, tyrosine, and phenylalanine. This increase was almost completely suppressed by pepstatin, implying that aspartic proteinase was concerned in the increase of free amino acids. We interpret this by considering that an exopeptidase contributed to this increase. It has been reported that several carboxypeptidases exist as an endogenous exopeptidase in rice grains (*10*, *24*) and that the optimum pH for this kind of enzyme is 4.0.

To clarify whether carboxypeptidase was activated or not under the acidic conditions studied here, we examined the distribution of free amino acids in the rice proteins extracted with acetic acid at 50 °C by using PMSF and pepstatin. PMSF is an inhibitor of carboxypeptidase and, as illustrated in **Figure 5**, decreased the amount of free amino acids in the acetic acid extract. There was no difference in the amounts of such free amino acids as glutamic acid and aspartic acid between the cases of extraction with PMSF and with pepstatin. This means that carboxypeptidase was activated under the acidic conditions and that this enzyme reacted with the degradation products by the action of aspartic proteinase. A similar reaction has been reported in the case of wheat gluten (*25*). However, in the case

of such amino acids as leucine, tyrosine, and phenylalanine, the increase was not completely inhibited by using PMSF. This indicates that some exopeptidases other than carboxypeptidase were involved in the increase in amounts of free amino acids in the rice extracted with acetic acid. It thus appears that the role played by aspartic proteinase in increasing the free amino acids of rice was not direct, but indirect.

The results of this study confirm that glutelin was dissolved in the acetic acid solution and degraded by aspartic proteinase, the degradation products then being decomposed by carboxypeptidase. Albumin and globulin were also dissolved in the acetic acid solution, the amount of the sum of albumin and globulin being larger than that of glutelin alone. The main effect of acetic acid on the dissolution of rice proteins was the enhanced solubility of albumin, globulin, and glutelin, the effect of proteases being minor.

ABBREVIATIONS USED

Pepstatin A, isovaleryl-L-Val-AHMHA-L-Ala-AHMHA; leupeptin, acetyl-L-leucyl-L-argininal; E-64, (L-3-*trans*-carboxyoxirane-2-carbonyl)-L-leucyl-agmatine; EDTA, ethylenediamine tetraacetate; PMSF, phenylmethanesulfonyl fluoride; Tris, tris-(hydroxymethyl)aminomethane.

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