Characterization and Digestibility of Basmati Rice (*Oryza sativa* L. var. Dehraduni) Storage Proteins[1]

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Cereal Chem. 72(3):275-280

ABSTRACT

Polished Basmati Dehraduni (BD) rice contained 9.5 ± 0.1% protein (N × 6.25). Albumin, globulin, prolamin, and glutelin fractions accounted for 5.9, 13.8, 5.8, and 74.5% of the total protein, respectively. All four protein fractions showed high amounts of glutamic acid, aspartic acid, and alanine. Threonine was the first limiting amino acid in all the protein fractions and the total rice proteins. The major polypeptides in albumin, globulin, prolamin, and glutelin had estimated molecular masses of 14.1 kDa; 13.9, and 20.3 kDa; 13.6 kDa; and 20.4 and 30.9 kDa, respectively. For each protein fraction there was a positive correlation between in vitro enzymatic digestion time and the extent of polypeptide hydrolysis. Electrophoresis gels of time-course digestions for each fraction revealed more extensive hydrolysis of polypeptides by pepsin than by trypsin, with accelerated hydrolysis rates in the heated protein. Albumin and globulin were most easily hydrolyzed by pepsin and trypsin, while prolamin was the most resistant to in vitro enzymatic digestion.

Rice is the staple food for over 60% of the world's population. It is second only to wheat in terms of annual production for food use. World rice production was 525 million metric tons in 1992 (FAO 1992). Because rice is providing an ever-increasing percentage of the total protein for human consumption worldwide, it is important to understand the physical and nutritional makeup of the protein present in the rice grain.

Milled rice contains an average protein content of 5.9–11.9% (Villareal and Juliano 1978), 8.2–12.1% (Perdon and Juliano 1978), 6.6% (Bandemer and Evans 1963), and 8.0% (McCall et al 1953). The amino acid composition of rice protein is similar to that of other cereal grains, with high levels of the nonessential amino acids glutamic acid and aspartic acid, and corresponding low levels of the essential amino acids lysine and methionine (Bandemer and Evans 1963, Cagampang et al 1966, Houston et al 1969). Determining the protein content and amino acid composition is an important step in determining the nutritional quality of a particular protein. However, because the biological utilization of a protein is primarily dependent on its digestibility by gastric, pancreatic, and intestinal peptidases, the true nutritional quality of a protein can only be determined by measuring its in vivo digestibility. Rapid and relatively inexpensive in vitro assays such as the trinitrobenzenesulfonic acid assay method (TNBS) have been developed that rely on the use of proteolytic enzymes to correlate with the digestion of protein in vivo (Hsu et al 1977, Romero and Ryan 1978, Adler-Nissen 1979).

Basmati Dehraduni (BD) rice is an aromatic, long-grain variety produced on a large scale and is a very popular variety in India. There is a demand for this rice variety because of its "popcorn-like" aroma and flavorful taste. However, the physical characteristics and in vitro digestibility of BD rice protein have not yet been investigated. The objective of this research was to biochemically characterize BD rice protein. Apparent BD rice protein digestibility was determined in vitro by both the TNBS method (Adler-Nissen 1979) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using the proteases pepsin and trypsin.

MATERIALS AND METHODS

Materials

Polished, aged BD rice was obtained from Neil Foods, Pensacola, FL, and milled on a United States Filter Corporation Mikro-Sample Mill (Florida Dept. of Agriculture, Tallahassee, FL) using 50-mesh screen. The resulting rice flour (1 kg) was defatted by using 5 L of cold (4°C) 95% acetone (Fisher Scientific Co., Orlando, FL) for 3 hr. The defatted flour was dried under a fume hood, blended, and stored at 4°C in an airtight container for further use.

Fractionation of Rice Protein

Fractionation of the rice flour protein was conducted by following a modified version of Osborne's (1907) classical fractionation scheme as described by Padhye and Salunkhe (1979). Two 50-g samples of rice flour were sequentially extracted with stirring in 500 ml (flour-to-solvent ratio 1:10, w/v) of 2% NaCl (albumin-globulin), 70% ethanol (prolamin), and then 10% acetic acid (glutelin). Three extractions (using fresh solvent each time) were performed for each protein fraction for both 50-g rice flour samples. Each extraction, except albumin-globulin extracts, was then subjected to processing: 1) centrifugation (using an IEC B-20A centrifuge) for 10 min at 12,000 × *g*; 2) concentration of the supernatant from ~500 ml to ~150 ml to reduce dialysis and freeze-drying bulk (using an Amicon ultrafiltration cell under N_2 pressure, model 8400, MW cut-off 10,000); 3) dialysis of the supernatant against 5 L of distilled water for 24 hr (using Spectra/Por 6,000-8,000 MW cut-off molecular porous membrane) with four water changes; 4) freeze-drying (using a Virtis 10-324 freeze-drying apparatus); 5) collection and weighing of freeze-dried fraction; and 6) pooling each fraction's three respective freeze-dried extracts for storage at 4°C. The albumin-globulin extracts followed the same procedure, except that, after dialysis, the extracts were centrifuged again to separate the albumin (supernatant) from the globulin (precipitate), and then freeze-dried.

Amino Acid Analysis

Five samples (one of rice flour, four of different fractions) of BD rice protein (~0.25 mg each) were analyzed in duplicate for amino acid composition. The analysis was performed using a Waters HPLC physiological fluid column (30 cm). The samples first underwent vapor-phase hydrolysis with 6*N* HCl for 24 hr. The samples were then treated with a 2:2:1 solution of ethanol, triethylamine, and water, and dried. The samples were next treated with a 7:1:1:1 solution of ethanol, triethylamine, water, and phenylisothiocyanate. After being held for 20 min at 25°C in a nitrogen atmosphere, the samples were again dried. Finally,

[1]Presented in part at the Institute of Food Technologists Annual Meeting & Food Expo, June 25-29, 1994, Atlanta, GA.

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the samples were added to 50 μl of 5*M* sodium phosphate buffer (pH 6.4, 40°C) for analysis by HPLC. Tryptophan content was determined by the colorimetric method of Spies and Chambers (1948).

Electrophoresis

SDS-PAGE was performed in a Hoefer Scientific Instruments SE 600 series vertical slab gel unit using the method of Fling and Gregerson (1986). Final gels were 1.5 mm thick, 14 cm long, 18 cm wide, and were comprised of 4% acrylamide stacking and 8–25% continuous acrylamide linear gradient separating gels. Molecular weight standards (Pharmacia electrophoresis calibration kit for low molecular weight proteins) consisted of α-lactalbumin (14.4 kDa), soybean trypsin inhibitor (20.1 kDa), carbonic anhydrase (30 kDa), ovalbumin (43 kDa), bovine serum albumin (67 kDa), and phosphorylase b (94 kDa). Silver staining of the gels was performed by using the method of Wray et al (1981).

In Vitro Protein Digestibility

In vitro digestibility was determined by submitting enzymatically hydrolyzed protein samples to both the TNBS method of Adler-Nissen (1979) and SDS-PAGE. TNBS assay results for the extent of peptide hydrolysis were measured in leucine mM equivalents. For SDS-PAGE, an hydrolyzed sample (15 μg) from each digestion time period (15 sec to 60 min) was placed in each well of a prepared gel. Gels were then run for each enzyme's time course digestion on each protein fraction, heated and unheated. Hydrolysis of both the heated and unheated proteins (casein and individual rice fractions) was initiated by addition of pepsin or trypsin (Sigma Chemical Co., St. Louis, MO) at an enzyme-to-substrate ratio of 1:100 (w/w). Hydrolysis of casein (Sigma) was for comparison purposes in the TNBS method (Adler-Nissen 1979). For the pepsin assays, 500-μg samples of casein, globulin, and albumin were suspended in 225 μl of 0.2*M* HCl buffer (pH 1.5), and 25 μl of pepsin (0.2 μg/μl) in 1 m*M* HCl was added. Samples (1 mg) of prolamin and glutelin were first solubilized in 200 μl of 70% ethanol and 2.5% acetic acid, respectively, and mixed with 250 μl of 0.2*M* HCl buffer (pH 1.5); 50 μl of pepsin (0.2 μg/μl) was then added. Final digestion conditions for pepsin were: 0.02 μg/μl of pepsin concentration, 2 μg/μl of protein sample concentration (1:100), and 0.05–0.1*M* HCl buffer (pH 1.5). Trypsin assays followed a similar procedure, except the buffer used was 0.4*M* Tris-HCl (pH 8.1) containing 40 m*M* $CaCl_2$. Final digestion conditions for trypsin consisted of a 1:100 enzyme-to-substrate ratio in 0.2*M* Tris-HCl (pH 8.1).

Heated and unheated protein controls containing the appropriate sample and buffer with no enzyme, as well as enzyme controls containing the appropriate enzyme and buffer with no protein sample, were included in each assay. Heated protein samples were prepared for digestion by first dissolving them in the appropriate buffer and heating in a boiling water bath at 99°C for 30 min. Time-course digestion studies were done by inactivating the enzymes present in each enzyme digestion mixture at the appropriate time (15 and 30 sec; 1, 2, 5, 10, 20, 30, and 60 min) by adding either 250 μl (for casein, albumin, and globulin) or 500 μl (for glutelin and prolamin) of hot (95°C) 1% SDS sample buffer (0.05*M* Tris-HCl, pH 6.8, 1% SDS, 30% glycerol). After slowly cooling to room temperature, aliquots of the protein sample hydrolyzates were analyzed by SDS-PAGE or TNBS assays. The remainder of the samples were frozen at −20°C until needed.

Analytical Procedures

The micro-Kjeldahl method was utilized to determine the nitrogen content of the rice fractions (AOAC 1960). The protein content of the fractions was obtained by using the conversion factor of 6.25 (Perdon and Juliano 1978).

Statistical Analysis

All experiments were performed at least in duplicate. Data (unless otherwise noted) are reported as mean ± standard deviation. When appropriate, data were analyzed by one-way analysis of variance (ANOVA) using Fisher's Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Protein Content

Polished BD rice contained 9.5 ± 0.1% protein on a moisture-

TABLE I
Amino Acid Composition of Basmati Dehraduni Rice Protein and Individual Fractions[a]

	Protein Fraction				
Amino Acid	Albumin	Globulin	Prolamin	Glutelin	Total Protein
Aspartic acid	9.13 ± 0.22	6.91 ± 0.09	7.34 ± 0.05	9.14 ± 0.42	7.30 ± 1.40
Glutamic acid	12.22 ± 0.03	15.84 ± 0.44	21.84 ± 0.37	17.40 ± 1.66	16.31 ± 1.70
Serine	4.15 ± 0.06	4.86 ± 0.03	4.76 ± 0.30	4.50 ± 0.04	4.99 ± 0.59
Glycine	5.96 ± 0.21	6.23 ± 0.08	2.96 ± 0.18	3.86 ± 0.36	4.42 ± 0.76
Histidine*[b]	1.90 ± 0.20	1.64 ± 0.08	1.17 ± 0.03	1.90 ± 0.27	2.11 ± 0.05
Arginine	7.75 ± 0.30	9.87 ± 0.09	6.14 ± 0.07	9.27 ± 1.08	8.68 ± 0.41
Threonine*	3.12 ± 0.30	2.24 ± 0.18	2.05 ± 0.19	2.69 ± 0.25	3.16 ± 0.45
Alanine	7.61 ± 0.01	6.41 ± 0.11	6.37 ± 0.07	4.99 ± 0.23	6.42 ± 0.26
Proline	5.64 ± 0.28	6.06 ± 0.16	4.44 ± 0.08	4.45 ± 0.14	6.04 ± 0.76
Tyrosine*	4.26 ± 0.10	6.57 ± 0.02	9.60 ± 0.55	5.94 ± 0.33	3.81 ± 0.49
Valine*	6.77 ± 0.11	5.93 ± 0.07	6.24 ± 0.07	6.22 ± 0.08	7.03 ± 0.51
Isoleucine*	3.79 ± 0.02	2.85 ± 0.04	4.90 ± 0.22	4.63 ± 0.24	4.78 ± 0.12
Leucine*	8.07 ± 0.08	7.96 ± 0.17	13.41 ± 0.81	8.93 ± 1.37	9.03 ± 0.74
Phenylalanine*	3.77 ± 0.08	3.82 ± 0.03	6.35 ± 0.36	6.39 ± 0.01	5.64 ± 0.32
Lysine*	10.01 ± 0.61	4.33 ± 0.11	0.50 ± 0.17	6.37 ± 0.17	5.32 ± 0.50
Tryptophan*	2.68 ± 0.06	2.30 ± 0.00	0.76 ± 0.06	1.55 ± 0.28	1.63 ± 0.01
Distribution of amino acids					
Hydrophobic	47.46	47.76	46.61	42.79	48.32
Hydrophilic	11.53	13.67	16.41	13.13	11.96
Basic	19.66	15.84	7.81	17.54	16.11
Acidic	21.35	22.75	29.18	26.54	23.61
Limiting essential amino acid[c]	Threonine	Threonine	Threonine	Threonine	Threonine
E/T %[d]	44.37	37.64	44.98	44.62	42.51

[a] Measured as g/16 g N ± standard error of the mean (SEM).
[b] * = essential amino acids.
[c] Based on the WHO/FAO (1985) amino acid requirement.
[d] Proportion of total essential amino acids and total amino acids as calculated by WHO/FAO (1985).

free basis. This result compares with the rice protein content range of 5.9–11.9% reported by Villareal and Juliano (1978). The total protein yield for two separate extractions was 50.2 ± 4.3%, comparable to the yields of 64.9–86.9% reported by Cagampang et al (1966). The albumin, globulin, prolamin, and glutelin proportions extracted from 50-mesh defatted BD rice flour were 5.9:13.8:5.8:74.5. Mawal et al (1987) reported albumin content to be 5% of total proteins in Basmati rice (var. Basmati 370). The data of Cagampang et al (1966) reveal a similar albumin content (5%) but lower globulin (9%) and prolamin (3%) contents and a correspondingly higher glutelin (83%) content. Lookhart et al (1987) reported that milled rice contains ~5% albumins, 10% globulins, <5% prolamin, and 80% glutelin. It is recognized that, since we had not used 55% isopropanol containing β-mercaptoethanol, prolamin extraction may not have been complete and, consequently, result in higher glutelin estimate (the glutelin content would thus be lower than 74.5%). Lookhart et al (1991) found 60% propanol to be very efficient in solubilizing the rice prolamin. However, the relative proportions of the different protein solubility fractions would not change by a large magnitude since the prolamin content is low in rice. The lower efficiency of protein solubilization (50.2% total yield) is partly due to the particle size (50 mesh) used in this study. Flour particle size of 100 or 200 mesh may improve protein solubilization. The relative proportion of different solubility fractions is unlikely to change however.

Amino Acid Composition

The amino acid composition of BD rice protein and individual fractions are given in Table I. The total protein content was, as expected, high in glutamic acid and aspartic acid. Alanine and leucine levels were high when compared with those of other published reports (Bandemer and Evans 1963, Houston et al 1969, Padhye and Salunkhe 1979). Albumin and globulin had the highest percentage of hydrophobic amino acids in their composition. The majority of the hydrophobic amino acid residues could be "buried" in the inner matrix of the protein, thereby allowing the protein to maintain its solubility in water. Glutamic acid dominated the amino acid profile of prolamin, with a content of 21.84 ± 0.37 g/16 g of N, or almost 22% of the entire profile. The amino acid composition of the albumin and prolamin fractions is comparable to those reported by Mawal et al (1987, 1989) for Basmati 370 rice. The prolamin amino acid results are also similar to those reported by Mandac and Juliano (1978). The proportion of essential amino acids and total amino acids *(E/T%)* for the total rice protein was 42.51, which is comparable to 41.0, the value given for Texas long-grain rice protein (Padhye and Salunkhe 1979). Globulin had the lowest *E/T%* (37.64%) of the four fractions. This is similar to the previous report for Texas long-grain rice globulin (Padhye and Salunkhe 1979). Comparison of the amino acid composition of the protein fractions (Table I) with those of the WHO/FAO (1985) essential amino acid pattern for a weaned child, shows threonine is the first limiting amino acid in all the protein fractions, as well as the total rice protein.

Electrophoresis

SDS-PAGE patterns of total rice protein polypeptides and individual fraction polypeptides are shown in Figure 1. The major polypeptides present (judged subjectively based upon band intensity and width) in the BD total rice protein (lane 2) had estimated molecular masses of 14.5, 20.4, and 33.1 kDa. Slightly higher corresponding molecular masses of 16, 25, and 38 kDa were found by Villareal and Juliano (1978) for the three major subunits present in three indica rice varieties. The albumin fraction of BD rice (lane 3) contained the most heterogeneous polypeptide composition of the four fractions (19 different MW polypeptide subunits), which is consistent with the SDS-PAGE results for other rice varieties (Iwasaki et al 1982). The major subunits were found at ~14.4, 26.9, 29.5, 33.1, and 52 kDa. The globulin fraction (lane 4) consisted of 12 different MW subunits, with three major polypeptides present at ~13.6, 19, and 21.5 kDa. These results compare favorably with those reported by Krishnan et al (1992), in which Lamont rice globulin consisted of two major polypeptides of 16 and 25 kDa. Komatsu and Hirano (1992) reported the 26 kDa polypeptide to be the major polypeptide of globulin in rice variety Norin 29. Prolamin (lane 5) was the most homogeneous of the four fractions (three polypeptide subunits), with a major subunit with an estimated molecular mass of 13.2 kDa and minor subunits at ~16.5 and 18.2 kDa. Similar findings have been reported for the single major subunit of rice prolamin, from 8.3 kDa (Mawal et al 1989, Mawal et al 1990) to 17 kDa (Mandac

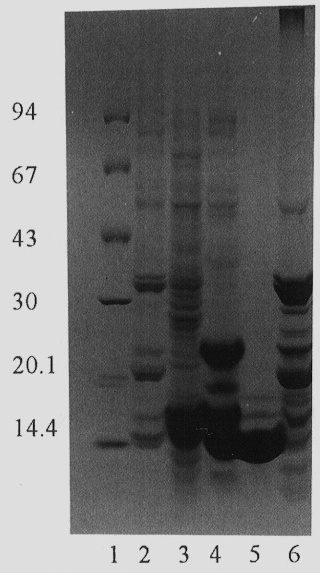

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns (8–25% linear acrylamide gradient) from analysis of native Bamati Dehraduni rice protein and individual fractions in the presence of β-mercaptoethanol. Lane 1, low molecular weight standards containing α-lactalbumin (14.4 kDa), soybean trypsin inhibitor (20.1 kDa), carbonic anhydrase (30 kDa), ovalbumin (43 kDa), bovine serum albumin (67 kDa), and phosphorylase b (94 kDa). Lane 2, total rice proteins. Lane 3, albumin. Lane 4, globulin. Lane 5, prolamin. Lane 6, glutelin. Protein load in each lane was 50 μg.

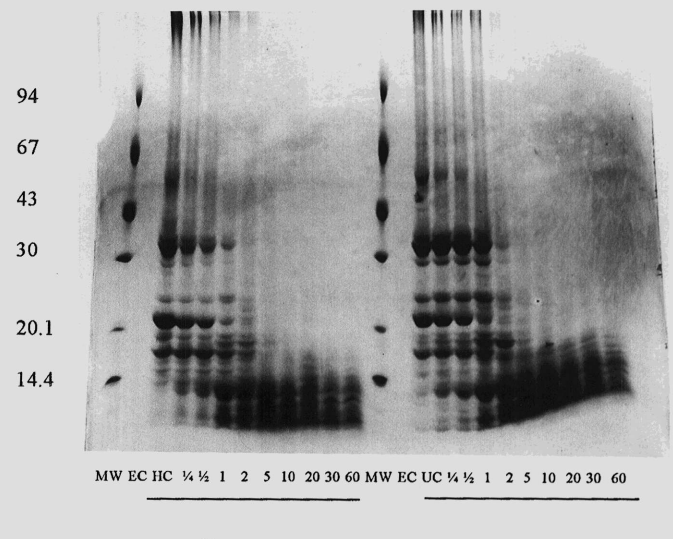

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of heated (H) and unheated (UH) Basmati Dehraduni rice glutelin subjected to pepsin in a time-course digestion. Time of digestion (min) is indicated at the bottom of each lane. Enzyme-to-substrate ratio: 1:100 (w/w). MW = molecular weight markers; EC = enzyme control; HC = heated glutelin control; UC = unheated glutelin control. Protein loads were 25 μg for MW marker lanes and 15 μg for glutelin lanes.

and Juliano 1978). The glutelin fraction (lane 6) was comprised of 13 different MW polypeptides, with major subunits estimated at 15.8, 19.5, 23.4, and 29.5 kDa. Additional gel electrophoretic analyses using heavier protein loads have revealed the presence of several other polypeptides including three additional major polypeptides with estimated molecular masses of 20.4, 30.9, and 51.8 kDa (data not shown). Tanaka et al (1980) observed major polypeptides in the Japonica rice glutelin fraction at slightly higher values of 22 and 37–39 kDa, respectively. The 23.4- and 29.5-kDa subunits visually correspond with the subunits of similar molecular mass in the BD total rice protein lane, due to the predominance of glutelin in the total protein composition of BD rice. Robert et al (1985) reported the MW of major polypeptides of rice glutelins to be 19–22 kDa and 30–36 kDa, and that these polypeptides are linked by disulfide bonds resulting in 50- and 62-kDa species. Several other researchers (Juliano and Boulter 1976, Wen and Luthe 1985, Krishnan and Okita 1986, Snow and Brooks 1989, Zarins and Chrastil 1992) have reported the MW of glutenin polypeptides in the range of 14–51 kDa. Komatsu et al (1993) reported 100 polypeptides in rice (*Oriza sativa* L.) endosperm (cultivars Nipponbare and Norin 29, and a semidwarf line SC-TN 1). A recent report (Tsugita et al 1994) suggests a much more complex pattern (1210 polypeptides by two-dimensional gel electrophoresis analyzed by automatic image analyzer) for rice seed proteins (cultivar Nihonbare of Japonica strain).

In Vitro Protein Digestibility

Time course in vitro pepsin digestions from 0.25 to 60 min of both heated and unheated BD rice glutelin using SDS-PAGE (Fig. 2) indicated that the heated glutelin samples were more rapidly hydrolyzed by pepsin than the unheated glutelin samples. The heated and unheated glutelin controls were both very similar in profile, indicating the heat treatment alone did not affect the polypeptide profile. For the heated samples, the two major subunits of 19.5 and 29.5 kDa were completely digested after 2 min of pepsin incubation, while the same major subunits for unheated samples were not completely hydrolyzed until after 5 min of incubation with pepsin. The proteolytic products of the digestion after 60 min consisted of several polypeptides in the 6- to 12-kDa range. Time-course in vitro trypsin digestions were also performed on BD rice glutelin using SDS-PAGE (Fig. 3). No visible qualita-

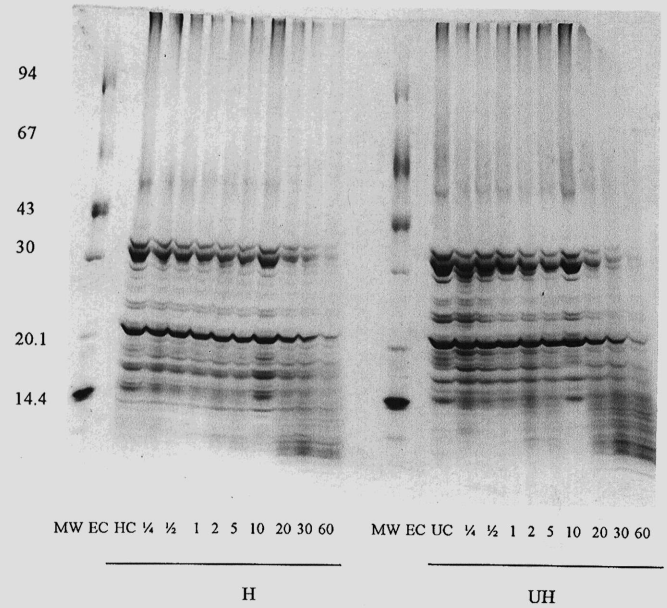

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of heated (H) and unheated (UH) Basmati Dehraduni rice glutelin subjected to trypsin in a time-course digestion. Time of digestion (min) is indicated at the bottom of each lane. Enzyme-to-substrate ratio: 1:100 (w/w). MW = molecular weight markers; EC = enzyme control; HC = heated glutelin control; UC = unheated glutelin control. Protein loads were 25 μg for MW marker lanes and 15 μg for glutelin lanes.

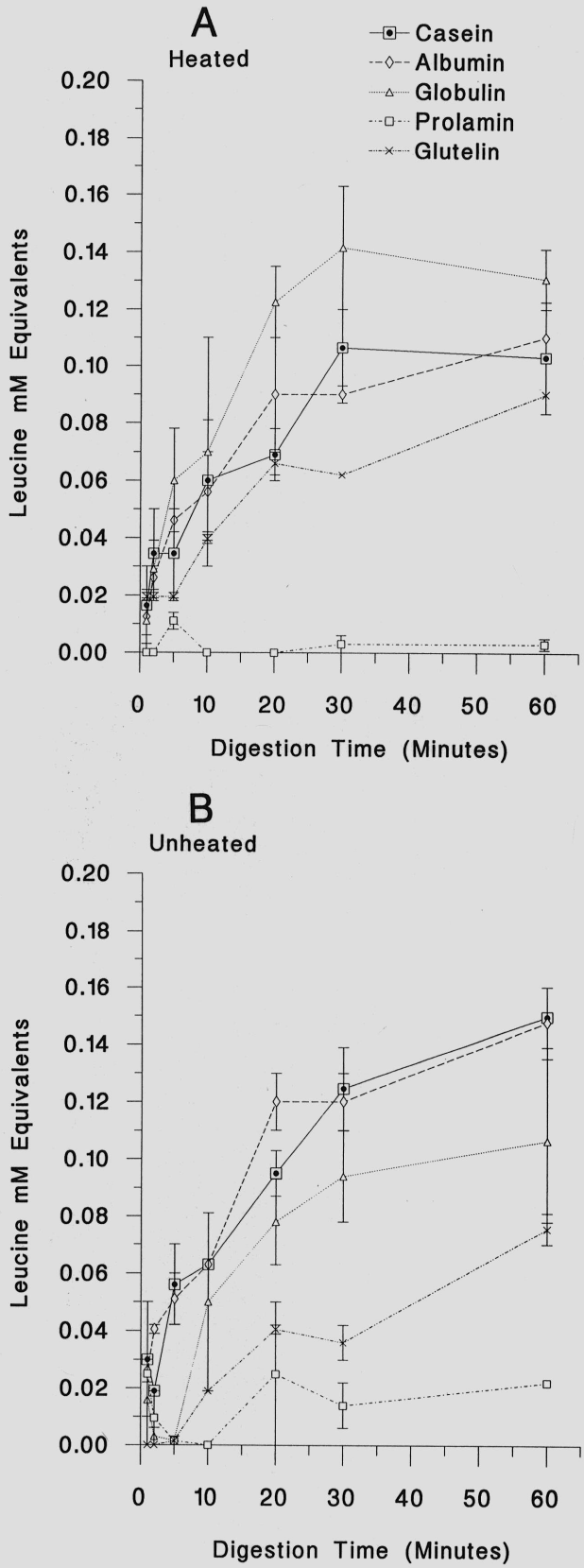

Fig. 4. Comparison of in vitro pepsin digestion of Basmati Dehraduni rice fractions and casein as measured by trinitrobenzenesulfonic acid assay. Leucine mM equivalents = number of free amino groups released as a result of peptide bond hydrolysis. Incubation with pepsin at 1, 2, 5, 10, 20, 30, and 60 min; 1:100 enzyme-to-substrate ratio. **A,** Protein fractions heated 30 min at 99°C before pepsin digestion. **B,** Unheated.

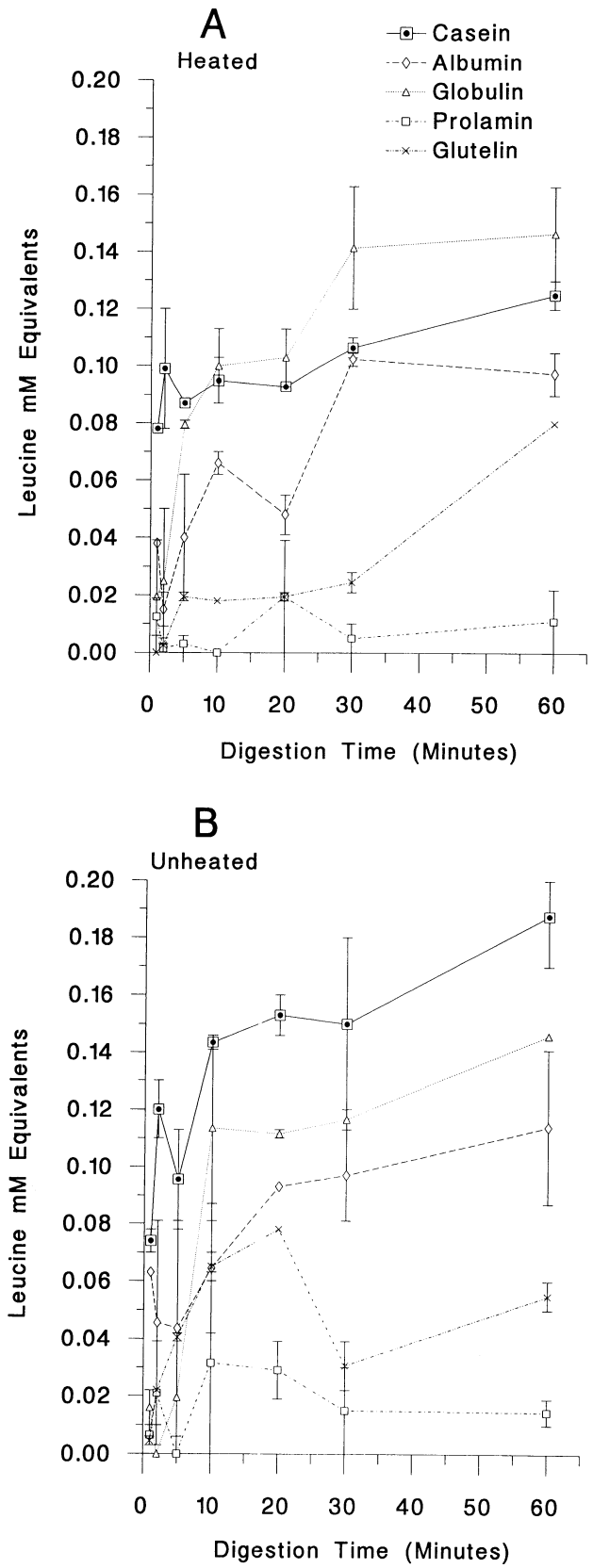

Fig. 5. Comparison of in vitro trypsin digestion of Basmati Dehraduni rice fractions and casein as measured by trinitrobenzenesulfonic acid assay. Leucine mM equivalents = number of free amino groups released as a result of peptide bond hydrolysis. Incubation with trypsin at 1, 2, 5, 10, 20, 30, and 60 min; 1:100 enzyme-to-substrate ratio. **A,** Protein fractions heated 30 min at 99°C before trypsin digestion. **B,** Unheated.

tive differences existed between the heated and unheated time course digestions with regards to polypeptide hydrolysis. The 19.5- and 29.5-kDa main subunits were very slowly hydrolyzed over the entire time-course from 15 sec to 60 min. After 60 min of trypsin digestion, both subunits were still faintly visible. Proteolytic products after 60-min digestions ranged in mass from 8- to 14-kDa.

The 19.5- and 29.5-kDa main subunits of glutelin were hydrolyzed in less than 5 min with pepsin, but were still faintly present after 60 min with trypsin. These results agree with those reported by Monteiro et al (1988), where pepsin hydrolyzed (95.5%) the polypeptides of Italian millet much more thoroughly than did trypsin (26.8% hydrolysis). The limited specificity of trypsin when compared to pepsin is the probable cause for the less rapid hydrolysis of glutelin polypeptides. Similar results were seen for the albumin and globulin fractions. The prolamin fraction had a higher resistance to enzymatic digestion by both pepsin and trypsin than did any other protein fraction (figures not shown). The poor digestibility of the prolamin fraction is consistent with the findings of Resurreccion et al (1993).

A comparison of in vitro pepsin digestion of heated BD rice fractions with casein as measured by the TNBS assay is shown in Figure 4A. There was no significant difference ($P = 0$ 0.05) between heated albumin, globulin, and casein (60-min digestion). Heated glutelin had significantly lower leucine mM equivalent values than heated globulin (60-min digestion), no significant difference from casein and albumin (at all time intervals), and significantly higher values than heated prolamin (at all time intervals). For unheated time course digestions (Fig. 4B), there was no significant difference between albumin and casein, but globulin values were significantly lower (at all time intervals). Glutelin leucine mM equivalent levels were significantly lower than albumin, globulin, and casein at all time intervals.

A comparison of in vitro trypsin digestion of heated BD rice fractions with casein as measured by the TNBS assay is shown in Figure 5A. Albumin was significantly lower than globulin and casein in leucine mM equivalents, but significantly higher than glutelin or prolamin. However, for the unheated trypsin digestions (Fig. 5B), there was no significant difference between albumin and globulin or glutelin and prolamin values.

The TNBS assays for pepsin and trypsin time course digestions suggest that upon heating, albumin, casein, and prolamin in vitro digestibilities are reduced, while glutelin and globulin digestibilities are increased. These results are supported by similar findings by Gauthier et al (1982), who found a lower apparent digestibility in heated casein when compared to unheated casein. Marquez and Lajolo (1981) reported reduced apparent digestibility in the albumin fraction of *Phaseolus vulgaris* after heating, but increased digestibility in the glutelin fraction. Cooked rice should therefore have improved nutritional value, because heating helps improve the in vitro digestibility of glutelin and globulin, the fractions that make up the majority (>88%) of the total protein in BD rice. These data are in agreement with those of Eggum et al (1977), who reported a decrease of 11.1% (decreased from 99.7 to 88.6%) in true digestibility (rats in vivo) of rice proteins on cooking. They noted that this decrease in protein digestibility did not adversely affect the net protein utilization because there was a corresponding increase in biological value (increased from 67.7 to 78.2%, that is, a 10.5% increase). That cooking reduces the rice protein digestibility has been recently reconfirmed by Eggum et al (1994).

ACKNOWLEDGMENTS

We thank K. W. Clara Sze and Bruce Smith for their technical assistance.

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[Received June 6, 1994. Accepted February 16, 1995.]