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NUTRITION

Amino Acid Content and Protein Biological Evaluation of 12 Mexican Varieties of Rice

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ABSTRACT

Protein efficiency ratio (PER) and amino acid content were determined in brown and polished samples of 12 varieties of Mexican rice. All samples of the polished rice presented lysine as the limiting amino acid with a chemical score ranging from 46 to 57. The amino acid content of the brown rice samples was similar to that of the polished rice samples for each variety. Again, the limiting amino acid was lysine, except in Sinaloa A-80 and CICA-6, which presented isoleucine as the limiting amino acid. The protein quality of the brown and the polished rice samples was similar for each variety. Only Sinaloa A-80 and CICA-6 brown rice samples presented a PER lower than that of their corresponding polished rice

Rice, corn, and wheat are the most consumed cereals in the world. Rice has the lowest protein content, but its protein quality is the best. Lysine is the limiting amino acid in the three cereals (Rosenberg and Culik 1957, MacLean et al 1979, Saunders 1979). The high protein quality of rice is due to its high gluteline-toprolamine ratio (Huebner et al 1990). Gluteline has better quality than prolamine because of its higher lysine content (Padhye and Salunkhe 1979).

On the other hand, cereals differ greatly in their protein contents

samples. The adjusted PER for the polished rice samples ranged from 2.23 (CICA-6) to 1.30 (Cárdenas A-80). The adjusted PER for the brown rice samples ranged from 2.20 (Morelos A-83) to 1.47 (Cárdenas A-80). According to these results, the varieties that showed the best protein quality were CICA-6, Morelos A-83, and Navolato A-71 (polished samples). In addition, Navolato A-71 (polished samples) presented the highest protein content (11.6%, db). Thus, Navolato A-71 was the best

due to the environmental conditions, soil composition, genetic improvements, and the use of nitrogen fertilizers (Nishizawa et al 1977, Pérez et al 1990). It has been possible to increase the protein content in corn and wheat using genetic manipulation, but this is always associated with a decrease of protein quality. However, in genetic manipulation of rice, quality is maintained or only slightly decreased (Bressani et al 1971a, Nishizawa et al 1977, Murata et al 1978, Roxas et al 1979, Pereira et al 1981). Different rice varieties cultivated in Mexico have protein contents of 6.9–11.6% (Sotelo et al 1990). Thus, the main goal of the present work was to determine the amino acid composition and the protein quality of 12 Mexican varieties of rice.

variety of the Mexican rices studied and could have an important

nutritional impact if its consumption becomes generalized.

MATERIALS AND METHODS

Twelve varieties of rice were studied: 11 were provided by the Instituto Nacional de Investigaciones Agrícolas, Programa de Arroz, Zona Sur Zacatepec Morelos, México; one was obtained

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in a supermarket in México City. The commercial rice studied was only available as polished rice, the variety was unknown. The other 11 varieties were: CICA-4, CICA-6, Morelos A-70, Morelos A-83, Navolato A-71, Juchitán A-74, Bamoa A-75, Campeche A-80, Sinaloa A-80, Cárdenas A-80, and Culiacán A-82. These samples were evaluated both as brown and polished rice. Samples of each of the 11 varieties of rice were dehulled with a McGill dehusker to obtain the brown rice. A portion of the brown rice was further polished in a McGill-Type miller No. 2 friction-type mill to obtain the polished rice (Sotelo et al 1990). Chemical and biological assays were performed to determine the protein quality of both types of samples.

Chemical Assays

For amino acid determination, 1 g of sample was weighed and 20 ml of 6N HCl were added. The hydrolysis was maintained for 22 hr at 110°C in test tubes with screw caps under a nitrogen atmosphere. Afterwards, the acid was evaporated in all samples using a rotory evaporator. Samples were washed three times with distilled water, and the residue was redissolved in acetate buffer (pH 3.1). After dilution to a known volume, 30 μ l of each hydrolysate was injected into an LKB amino acid autoanalizer (model 4151 Alpha plus). Other authors have found deficiencies of sulfur amino acids and threonine in rice (Bressani et al 1971b, Hegsted and Juliano 1974), which are partially destroyed by direct acid hydrolysis. Therefore, it was decided to oxidize nine samples of polished rice with performic acid before hydrolysis (Spindler et al 1984). The nitrogen content was determined by the Kjeldahl method (AOAC 1980), and tryptophan was assayed using the fluorometric method (Udenfriend 1962). With this information, the chemical score was calculated using the FAO pattern (FAO 1973) as reference.

Biological Assays

Isocaloric (406 Kcal or 1,699 Kjoule) and isoproteic (6% protein) basal diets were prepared using the 11 brown rice and the 12

 TABLE I

 Protein Efficiency Ratio (PER) for Casein

 and Milk-Casein Diets at 6 and 10% Protein

Diet	PER ^a
Casein (6%)	$2.09\pm0.26~\mathrm{c}$
Casein (10%)	$2.50 \pm 0.17~{ m ab}$
Casein-milk (6%)	2.42 ± 0.12 ab
Casein-milk (10%)	2.59 ± 0.17 a

^aDifferent letters indicate significant difference (P < 0.05). Minimal significant difference: 0.32.

polished rice samples, as well as a milk-case mixture as a control diet (50:50 protein). Validation of the control diet demonstrated that the PER value (2.42 ± 0.12) was identical to the PER value of a 10% case in diet (2.50 ± 0.12) (Table I).

Basal diets also contained 64.1% carbohydrates, 14% lipids, 4% mineral mixture, 2% vitamin mixture, and cellulose approaching 100% (Teklad test diet, Madison, WI). The mixture of powdered milk (Nestlé, México City) and casein (Sigma Co., St Louis, MO) was used as the control diet. The protein efficiency ratio (PER) and digestibility were determined (Pellet and Young 1980) using weanling Sprague Dowley male rats (21-23 days old, 50 g average weight). The study was conducted over three weeks under controlled conditions in an animal house at 21-22°C with a 12-hr light-dark cycle. Food and water were provided ad libitum, and food intake and weight gain were recorded twice a week. Protein content of the diets was determined by nitrogen assay (N \times 5.95). Handling simultaneously the large number of animals required for the PER determination of each experimental diet (six rats per diet) was very difficult, so the study was divided in four stages, using the control diets in each one. The PER value of the control diet was adjusted to 2.50 and the PER values of the experimental diets were consequently adjusted for comparison.

Statistical Analysis

The differences between the polished and brown samples for each single variety of rice were evaluated by the paired Student's t test. One-way analysis of variance was used to analyze the differences among the brown or polished samples of all rice varieties (Steel and Torrie 1960).

RESULTS AND DISCUSSION

The amino acid content of the brown and polished rices are shown in Tables II and III; the data are in agreement with those of other reports, with lysine as the limiting amino acid. In both brown and polished samples, the lowest lysine content was observed in the Culiacán A-82 and Sinaloa A-80 varieties. The highest lysine contents in brown samples were detected in CICA-4, Campeche A-80, and Morelos A-83; in polished samples, the highest lysine contents were in Navolato A-71, CICA-6, and Morelos A-83. The lysine content in these rices was similar to those reported for other varieties (FAO 1970, Hegsted and Juliano 1974, Murata et al 1978, Eggum 1981b, Tobekia et al 1981).

The lysine content was 157-199 mg/gN in polished rice and 136-203 mg/gN in brown rice (Table IV). Furthermore, the elimination of the bran did not modify the content of any other amino acid; they were very similar in polished and brown rice samples, although, in certain varieties, minor and nonsignificant differences

Amino Acid Content in Mexican Rice Varieties (Brown Samples)											
Amino Acid (mg/gN)	Bamoa A-75	CICA 4	Juchitán A-74	Culiacán A-82	Navolato A-71	Campeche A-80	Cárdenas A-80	Sinaloa A-80	Morelos A-70	CICA 6	Morelos A-83
Aspartic acid	501	533	444	363	320	358	387	424	449	454	387
Glutamic acid	885	1,030	876	703	796	991	930	726	898	791	917
Threonine	169	197	169	136	205	180	171	142	168	157	172
Serine	223	273	222	185	155	248	247	215	242	234	245
Alanine	234	371	314	240	286	336	235	291	332	316	358
Glycine	242	263	234	175	190	261	237	217	232	239	250
Valine	217	343	284	217	259	295	263	185	273	197	289
Cysteine	66	76	49	45	46	75	49	44	57	47	70
Methionine	133	109	93	86	69	99	101	121	94	130	101
Isoleucine	135	212	178	137	235	183	166	106	173	110	163
Leucine	388	456	390	316	378	424	411	330	404	352	399
Tyrosine	198	145	109	92	163	126	136	162	108	160	132
Phenylalanine	233	264	240	193	248	251	272	218	230	229	269
	174	204	186	136	170	200	185	176	188	167	194
Lysine	108	130	124	86	100	127	109	93	112	98	110
Histidine	349	408	307	235	373	378	357		324		373
Arginine	349 77	408	83	233 74	67	73	73	72	65	63	77
Trytophan Chemical Score ^a	51	60	55	40	50	59	54	42	55	44	57

 TABLE II

 Amino Acid Content in Mexican Rice Varieties (Brown Samples)

"In all varieties, the limiting amino acid was lysine, except in Sinaloa A-80 and CICA-6, which presented isoleucine as the limiting amino acid.

 TABLE III

 Amino Acid Content in Mexican Rice Varieties (Polished Samples)

Amino Acid (mg/gN)	Bamoa A-75	CICA 4	Juchitán A-74	Culiacán A-82	Navolato A-71	Campeche A-80	Cárdenas A-80	Sinaloa A-80	Morelos A-70	CICA 6	Morelos A-83	Commercial
Aspartic acid	489	420	456	380	484	498	445	361	435	506	486	447
Glutamic acid	1,004	888	912	773	951	897	971	788	911	1,023	989	926
Threonine	184	153	173	166	203	171	159	152	161	197	172	166
Serine	266	193	252	206	253	238	213	188	255	270	265	236
Alanine	327	289	295	263	324	306	304	280	312	335	324	304
Glycine	228	211	210	199	242	222	217	195	216	248	227	214
Valine	303	292	281	262	323	254	300	265	248	309	310	290
Cysteine	66	79	64	62	83	70	42	66	59	80	102	49
Methionine	126	95	118	119	126	96	99	97	101	131	128	98
Isoleucine	188	184	194	154	192	165	204	190	164	205	179	187
Leucine	430	382	401	365	423	392	422	370	396	468	426	414
Tyrosine	142	136	216	104	161	131	155	126	100	155	155	148
Phenylalanine	252	242	255	225	287	194	261	231	214	231	248	257
Lysine	179	170	159	157	199	173	180	165	169	193	193	181
Histidine	115	93	104	96	120	98	107	102	92	117	115	122
Arginine	361	387	369			420	405	365	281	484		380
Tryptophan	103	93	100	83	85	87	94	78	96	89	99	90
Chemical Score ^a	53	50	47	46	58	51	53	48	50	57	57	53

^aIn all varieties the limiting amino acid was lysine.

were detected. Previously, Hayakawa et al (1987) indicated that the lysine concentration was greater in the external coats, decreased in the inside coats, and increased slightly in the central part of the grain. This observation is at variance with the results of Tobekia et al (1981) and with the results of the present study. We observed similar lysine contents in most of the polished and

 TABLE IV

 Average of Amino Acid Contents in 12 Mexican Rice Varieties

Amino Acid	Polisl	hed ^a	Brown			
(mg/gN)	$Mean \pm SD$	Range	$\mathbf{Mean} \pm \mathbf{SD}$	Range		
Aspartic acid	451 ± 46	361-506	420 ± 64	320-533		
Glutamic acid	919 ± 78	773-1,023	867 ± 104	703-1,030		
Serine	236 ± 29	188-270	226 ± 33	155-273		
Alanine	305 ± 21	263-335	301 ± 49	234-371		
Glycine	219 ± 16	195-248	231 ± 27	190-263		
Histidine	107 ± 11	92-122	109 ± 14	86-130		
Arginine	383 ± 54	281-484	345 ± 51	235-408		
Tyrosine	144 ± 30	100-216	139 ± 31	92-198		
Cysteine	68 ± 16	42-102	56 ± 13	44–76		
Methionine	111 ± 15	95-131	103 ± 19	69-133		
Threonine	171 ± 16	152-203	170 ± 20	136-205		
Valine	286 ± 24	248-323	256 ± 48	185-263		
Isoleucine	184 ± 16	154-205	164 ± 39	106-212		
Leucine	407 ± 29	365-468	386 ± 41	316-456		
Phenylalanine	241 ± 24	194-287	241 ± 24	193-272		
Lysine	176 ± 13	157-199	179 ± 19	136-203		
Tryptophan	92 ± 8	78–103	72 ± 6	63-83		

^aMean of 12 varieties of rice: 12 polished samples and 11 brown samples. SD = standard deviation. the brown samples. The similarity in the lysine contents is due to the minimal quantity (9% for brown rice) of bran eliminated in the milling process (Sotelo et al 1990). Other nutrients showed different behavior in the brown and polished samples of the same varieties: minerals and vitamins were significantly decreased in polished samples.

The results of hydrolysis and oxidation are presented in Table V. In almost all the varieties analyzed, a decrease of the isoleucine content was observed with direct hydrolysis; the average value was 27% of destruction. The variety most affected was Morelos A-70 (48% of destruction). Sulfur amino acids are most affected by acid hydrolysis. Cárdenas A-80 and Sinaloa A-80 varieties showed a mean decrease of 60% for methionine after direct acid hydrolysis. Almost all the varieties showed destruction of sulfur amino acids (average 53%). However, these amino acids were not limiting in the samples studied, as demonstrated by the chemical scores obtained in the samples treated by direct acid hydrolysis. Lysine and threonine (data not shown), which are the limiting amino acids in rice, were not affected significantly by direct acid hydrolysis. The destruction values were 7 and 9%, respectively.

The PER and digestibility of the samples at each stage of the study are shown in Table VI. The highest PER values were observed in Navolato A-7 (polished), commercial sample (polished), and Campeche A-80 (brown). Although good digestibility for most of the rices was observed (>80%), it was lower than the digestibility reported in the literature (Saunders 1979, Eggum et al 1981a, Pederson and Eggum 1983). Only the Sinaloa A-80 and the Morelos A-83 polished samples showed higher digestibility than the corresponding brown samples. However, in general, the digestibility of the brown and polished samples was similar. Again,

TABLE V
Sulfur Amino Acids and Isoleucine Content in Some Mexican Rice Varieties (Polished Samples) After Direct Acid Hydrolysis (DH)
and Oxidation with Performic Acid Before Hydrolysis (OH)

	Isoleucine			Cysteine	Cysteic Acid		Methionine	Sulphone- Methionine	
Variety	DH	ОН	%Dª	DH	ОН	%D	DH	он	%D
Bamoa A-75	188	240	22	66	141	53	126	180	30
CICA 4	184	240	13	79	151	48	95	206	54
Culiacan A-82	154	273	44	62	155	60	119	234	49
Navolato A-75	192	247	22	83	161	49	126	324	61
Campeche A-80	165	242	32	70	133	47	96	142	32
Cardenas A-80	204	271	42	42	212	80	99	251	61
Sinaloa A-80	190	276	31	66	175	62	97	235	59
Morelos A-70	190	315	48	59	139	58	101	240	58
CICA 6	205	224		80	151	47	131	285	54

^aPercent of destruction (%D) was calculated for the acid hydrolysis values, assuming as 100% the value obtained in the hydrolysis with previous oxidation using performic acid.

 TABLE VI

 Protein Efficiency Ratio (PER) and Digestibility

 of Mexican Rice Varieties^a

	PER		
	Experimental	PER	Digestibility
Sample	Values	(Mean \pm SD)	(Mean ± SD)
Stage 1	2.52 ± 0.44		
Morelos A-70 Brown		1.64 ± 0.38	85.3 ± 2.8
Sinaloa A-80 Brown		1.41 ± 0.35	80.5 ± 2.5
Bamoa A-75 Brown		1.82 ± 0.33	84.6 ± 4.9
CICA 4 Brown		1.71 ± 0.31	78.6 ± 4.8
Cardenas A-80 Brown		1.48 ± 0.32	85.9 ± 3.6
Cardenas A-80 Polished		1.31 ± 0.34	87.6 ± 3.0
Culiacan A-82 Polished		1.79 ± 0.22	82.0 ± 5.2
Stage 2	2.55 ± 0.38		
Morelos A-70 Polished		1.56 ± 0.38 b	84.2 ± 3.1
Bamoa A-75 Polished		1.92 ± 0.32 ab	84.2 ± 5.4
CICA 4 Polished		1.72 ± 0.21 ab	81.6 ± 5.9
Navolato A-71 Polished		2.11 ± 0.20 a	79.7 ± 4.8
Commercial Polished		$2.02\pm0.15~ab$	77.8 ± 1.6
Navolato A-71 Brown		$1.87\pm0.34~ab$	86.8 ± 1.3
Stage 3	2.17 ± 0.33		
Culiacan A-82 Brown		$1.81 \pm 0.25 \text{ ab}$	85.4 ± 3.9
Sinaloa A-80 Polished		1.62 ± 0.30 ab	86.2 ± 2.1
CICA 6 Brown		1.40 ± 0.36 b	81.1 ± 4.0
CICA 6 Polished		1.94 ± 0.36 a	83.7 ± 2.4
Morelos A-83 Brown		1.91 ± 0.23 ab	79.3 ± 1.0
Morelos A-83 Polished		$1.87\pm0.27~ab$	86.3 ± 1.4
Stage 4	2.48 ± 0.34		
Campeche A-80 Brown		1.97 ± 0.14 a	80.7 ± 0.7
Campeche A-80 Polished		1.87 ± 0.14 ab	77.0 ± 3.1
Juchitán A-74 Brown		1.54 ± 0.24 bc	83.8 ± 5.1
Juchitán A-74 Polished		$1.42\pm0.23~c$	84.4 ± 0.9

^aDifferent letter or subscript in each one of the four stages means significant differences (P < 0.05). Stage 1: No significant differences. Stages 2, 3 and 4: minimal significant differences were 0.50, 0.52 and 0.33, respectively.

this can be explained by the low content of bran in the brown rice, which did not greatly influence the digestibility of the total grain.

For comparison of the protein quality of all the varieties of brown and polished rices, the PER values were adjusted with respect to the corresponding value of the milk-casein control diet (PER 2.50). The adjusted values are presented in Table VII. Most of the polished samples exhibited very similar qualities, although the highest PER values were detected in CICA-6, followed by Morelos A-83 and Navolato A-71; the lowest PER values were detected in Morelos A-70, Juchitán A-74, and Cárdenas A-80 $(P \le 0.05)$. Among the brown rice samples, the protein quality of Morelos A-83 was significantly different (P ≤ 0.05) from that observed for Sinaloa A-80, Cárdenas A-80, and Juchitán A-74. The protein quality of the brown and the polished samples was not modified significantly, as previously described by Pedersen and Eggum (1983) and Eggum et al (1981a). Only two varieties of the brown rice, CICA-6 and Sinaloa A-80, had a PER value significantly lower than those of the polished samples. A partial explanation is that the Sinaloa A-80 brown sample also presented less digestibility than the polished sample. In general, the PER values for most of the Mexican rice varieties corresponded with the values reported for other varieties (Elias et al 1968, Saunders et al 1979). Three polished samples presented PER values very similar to that of the control diet (CICA-6, Morelos A-83, and Navolato A-71), confirming the high quality of this cereal (Java and Venkataraman 1979, Chang et al 1986).

Reportedly, rice and other food with less than 8% protein content are difficult to evaluate using the PER method. Nevertheless, many authors reported using 6-8% protein diets (Elias et al 1968, Mitra and Das 1971, Chandrasekarappa 1979, Java and Vankataraman 1979, Devi and Vankataraman 1983). Different studies have also evaluated the protein quality of rice using other indexes, in which increasing quantities of protein are administrated in various diets (Bressani et al 1971a, Hegsted and Juliano 1974, Cabrera-Santiago et al 1986). In both cases, in spite of

TABLE VII
Protein Efficiency Ratio (PER) of Mexican Rice Varieties (Adjusted
According to the 2.5 Value of Milk-Casein Control Diet)

	Adjusted PER ^{a,b}				
Varieties	Polished	Brown			
CICA 6	2.23 a	1.61 a-c			
Morelos A-83	2.15 a	2.20 a			
Navolato A-71	2.08 ab	1.83 a-c			
Commercial	1.98 a–c				
Campeche A-80	1.89 a–c	1.99 a-c			
Bamoa A-75	1.88 a-c	1.81 a-c			
Sinaloa A-80	1.87 a–c	1.40 c			
Culiacan A-82	1.77 a-d	2.08 ab			
CICA 4	1.69 a-d	1.70 a-c			
Morelos A-70	1.54 b-d	1.62 a-c			
Juchitán A-74	1.43 cd	1.55 bc			
Cardenas A-80	1.30 d	1.47 bc			

^aDifferent letter in each column indicates significant differences (P < 0.05).

^bStudent's t test indicated significant differences (P < 0.05) between polished and brown samples for each variety of rice.

deficiency of lysine and threonine, rice presented a good protein quality. Its principal limitation was its low protein content. Hence, the great importance of Navolato A-71 rice, which has both a high protein content and a good protein quality.

A milk-casein mix was used as a control diet instead of casein because a 6% protein casein diet is not as efficient as 10% protein casein diet (Bressani et al 1971a), so the values obtained for the rice diets may be overestimated. Accordingly, in the final part of the study, the protein quality of casein and milk-casein diets at 6 and 10% protein levels were evaluated (Table I). The 6% protein casein diet was determined to have a PER value significantly lower than that of the 10% protein casein diet (2.09 vs. 2.50, respectively). Also, the 6% casein-milk diet had a PER value of 2.42, which was similar to that of the 10% protein casein diet and different from that of the 6% casein. Therefore, it is possible to evaluate the protein quality of rice at a 6% protein level, if an adequate control diet is provided for comparison (10% casein or 6% casein-milk).

It is important to emphasize the good PER value of Navolato A-71 in addition to its high protein content (almost 12% protein, db). This could have an important nutritional impact if its consumption becomes generalized.

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