Table III. Total Amino Acid Content of HVP Filtrates, Pastes, and Powders Derived from Soy Grits (A) and Dehydrated Alfalfa Flour (B) in Comparison with That from Beef Extract (C)

		paste, %			powder, %	
amino acid	B[a]	A	B	C	A	B
asparagine	0.56	4.22	2.00	0.55	4.18	4.26
threonine	0.21	0.92	0.58	0.21	0.98	1.01
serine	0.21	1.90	0.72	0.27	1.92	1.93
glutamic acid	0.67	8.00	2.39	3.51	8.85	2.42
proline	0.20	1.80	0.74	0.90	2.00	0.75
glycine	0.19	2.40	0.57	2.26	2.64	0.60
alanine	0.21	1.52	0.73	1.84	1.70	0.73
cystine				2.30		
valine	0.20	0.90	0.68	0.30	1.06	0.70
methionine	0.04	0.30	0.14	0.10	0.36	0.16
isoleucine	0.19	0.77	0.66	0.23	0.96	0.70
leucine	0.49	2.38	1.71	0.51	2.54	1.75
tyrosine	0.05	0.50	0.16	0.09	0.59	0.18
phenylalanine	0.18	1.44	0.50	0.23	1.67	0.55
lysine	0.24	1.15	0.68	1.33	1.24	0.70
histidine	0.09	0.55	0.19	6.38	0.74	0.21
arginine	0.19	2.80	0.55	1.05	3.57	0.60
tryptophan				0.19		
total amino acids	3.92	31.55	13.00	22.25	35.00	17.25
total essential amino acids	1.56	8.36	5.11	5.49	9.40	5.84

[a] This is the filtrate percent of B.

The chemical and amino acid composition of the HVP from defatted soy grits meets the requirements of Codex Committee on Food Additives (1980) regarding the valuable components in manufacture of seasonings mixtures, and partial substitution for sodium glutamate in the meat industry.

HVP produced from dehydrated alfalfa flour is two times poorer in protein and essential amino acids, its color is far darker in comparison with HVP from soy grits, and it has a specific grass like flavor. However, regarding amino acid composition, this product is similar to beef extract and it would be possible to find uses for it in the manufacture of dark colored food products.

Registry No. NaCl, 7647-14-5; glutamic acid, 56-86-0.

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Lectins in Rice and Corn Endosperm

David S. Newburg* and José M. Concon[1]

Lectins were demonstrated in the albumin and globulin, zein, acid-soluble glutelin, and alkali-soluble glutelin fractions of corn endosperm by means of a modified hemagglutination assay. In rice endosperm only the acid-soluble and alkali-soluble glutelin fraction contained lectins. These activities were not due to cross-contamination from germ lectins. Because the oryzein fraction of rice caused hemolysis in the assay system, definitive testing for lectins was not possible for this fraction. In rice, the acid-soluble glutelin fraction was heat labile, while the alkali-soluble glutelin fraction was stable to heat (110 °C for 30 min). In corn, the acid-soluble glutelin fraction was heat stable, while the other lectin fractions from corn endosperm were partially destroyed by heating under these conditions.

Until recently, lectins in various cereals have been assumed to occur only in the protein fraction of the germ that is soluble in near neutral aqueous solutions (Neucere, 1982; Peumans et al., 1982; Jaffé, 1980; Goldstein and Hayes, 1978). The usual extractants for these substances are water or buffers at near neutral pH; any cereal lectins insoluble in this medium had been overlooked. We devised a modification of the standard hemagglutination assay in which the solvent effectively disperses the buffer-insoluble proteins, but does not interfere with the assay. Using this assay, we had found lectins in the water insoluble gluten fractions of wheat endosperm (Concon et al., 1983).

Köttgen et al. (1982), using laser nephelometry, found that gluten has lectin-like properties. This finding, along with that seen in our modification of the standard hem-

Department of Nutrition and Food Science, University of Kentucky, Lexington, Kentucky 40506–0054.
[1] Deceased.

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Table I. Lectin Activity of Rice and Corn Endosperm Protein Fractions

cereal	fraction	% total protein recovered[a]	unheated units/g	% total activity	autoclaved units/g
rice	buffered saline (albumins + globulins)	5.5	negative		negative
	70% ethanol (oryzeins)	0.9	*b*		*c*
	0.01 N acetic acid (acid-soluble glutelins)	0.7	123000	2.4	negative
	0.075 N NaOH (alkali<<soluble glutelins)	92.9	38400	97.6	38400
corn	buffered saline (albumins + globulins)	7.5	112700	2.4	38400
	70% ethanol (zeins)	52.4	37600	5.7	9400
	0.01 N acetic acid (acid-soluble glutelins)	0.3	18100	0.02	18100
	0.075 N NaOH (alkali-soluble glutelins)	39.7	800000	92.0	200000

[a] Rice: $N \times 5.95$; corn: $N \times 6.75$ (FAO, 1970). [b] No reading can be made because of hemolysis at values greater than 10.2 μg/well. No activity was observed at levels of 10.2 μg/well. [c] Hemolysis occurred at protein level of 16 μg/well or above. No lectin activity was observed at this protein level.

agglutination assay, supports the hypothesis of Weiser and Douglas (1976) that gluten sensitive enteropathy (celiac disease) may be caused by lectins in wheat gluten.

Three closely related grains, i.e., wheat, rye and barley, are omitted from the diets of celiac patients, whereas rice and corn, more distantly related to wheat, are allowed in their diets, as these grains do not seem to exacerbate the symptoms of this disease. We hypothesized that this might be due to the absence of celiotoxic lectins in the gluten fractions of these cereals (Concon et al., 1983). Although Takahashi et al. (1973) found lectins in rice, it is unclear whether the source of the lectins was the endosperm or the germ; also, the possibility of insoluble lectins was not investigated. To our knowledge, the presence of lectins in corn has never been reported, although buffer soluble lectins have been found in sorghum, a closely related grain (Neucere, 1982).

Therefore, we tested for the presence of lectins in rice and corn protein fractions, hypothesizing that their absence might provide a possible explanation of their noninvolvement in celiac disease. Alternatively, if lectins were to be found in the endosperm of such disparate cereals as rice, corn, and wheat, this might indicate that lectins in endosperm proteins are a general property of cereals and that hemagglutination may not be unique or specific to the celiotoxic agents in grains.

MATERIALS AND METHODS

Isolation of Proteins. Whole kernels of normal hybrid corn were washed with distilled water and air-dried. The pericarp and germ were removed from each kernel, and the remaining endosperm was ground to 80–100 mesh in a water-cooled micromill and defatted with 1-butanol. Residual butanol was removed with acetone, and the residual acetone evaporated by air drying.

Commercial long-grained polished rice, washed with distilled water followed by acetone, and air-dried, was ground to 80–100 mesh in a water-cooled micromill and defatted with 1-butanol. Residual butanol was removed with acetone, and the residual acetone evaporated by air drying.

A 20-g portion of each of the above powdered endosperm samples was extracted successively 5 times with 14-mL aliquots of 0.9% NaCl in 0.02 M phosphate buffer (pH 6.81), 70% ethanol, 0.01 N acetic acid, and 0.075 N NaOH. These extracts contained respectively the albumins and globulins, the oryzeins (rice) or zeins (corn), the acid-soluble glutelins, and the alkali-soluble glutelins. After each extraction the slurry was centrifuged at 8000*g* at 15 °C for 10 min, and the combined supernatants for each extractant were dialyzed against distilled water at 5 °C and lyophilized. The protein content of each fraction was determined by a micro-Kjeldahl analysis (Concon and Soltess, 1973); protein recovery from rice was 87% and from corn was 98%. Ten milligrams of each dialyzed and lyophilized protein fraction was dissolved in 0.5 mL of Me_2SO (oryzeins, glutelins) or 0.5 mL of 0.02 N (pH 6.81) phosphate buffer solution (albumins and globulins); 0.5 mL of the alternate reagent was added to each sample such that each 10-mg sample was dispersed in 1 mL of 50% Me_2SO–buffer mixture. Serial dilutions were prepared with the 50% Me_2SO–buffer solvent and each dilution of each protein fraction was tested for hemagglutinating activity. Zeins were dispersed in 60% Me_2SO–buffer solution, and serial dilutions were prepared with the 50% Me_2SO–buffer mixture.

Hemagglutination Tests. Hemagglutination tests were performed on all protein fractions at all dilutions in triplicate, along with blanks containing the Me_2SO–buffer mixtures alone by using a method described previously (Concon et al., 1983). After 6 h, the hemagglutinating activity of each sample was recorded.

RESULTS AND DISCUSSION

Rice endosperm showed lectin activity only in the glutelin fractions (Table I). Lectin activity in the ethanol fraction could not be ascertained because this fraction caused hemolysis, and although some lectins are known to cause hemolysis (Suni Sunita and Singh, 1982), not all hemolytic agents are lectins.

The activity seen in the acid-soluble rice glutelin fraction was quite high. However, this fraction in rice comprised only a small percentage of the total protein, and furthermore, its activity is destroyed by autoclaving at 110 °C for 30 min (total heating time 45 min; 15 min cooling and depressurization).

The alkali soluble fraction, which comprises 93% of the total rice proteins, also shows high activity; the lectins in this fraction are heat stable. It is unlikely that this activity is a residue of the acid-soluble rice lectin fraction, because of the differences in heat stability.

The buffered saline extract of rice endosperm contains no lectin activity. The removal of the germ prevented any contamination from this source.

All of the protein fractions of corn endosperm contain high specific activities of lectins. But, as in rice, most of the total activity is confined to the alkali-soluble glutelin, which is about 40% of the total protein of the endosperm. The lectin activity in the acid-soluble glutelins is stable to autoclaving at 110 °C for 30 min. The lectins in the other fractions are partly destroyed by autoclaving under these conditions; about 66% of the lectins in the albumin and globulin fraction and 75% in the zein and alkali-soluble glutelin fraction are destroyed. Nevertheless, a substantial portion of the lectins in these fractions remains active after autoclaving, which may be sufficient to trigger an immunological response.

Therefore, the high lectin activity which remains even after autoclaving is consistent with reports that corn is considered as one of the most allergenic of foods (Spier,

1976). Corn has more than 10 times the total lectin activity of rice, which is considered one of the foods with the lowest allergenic potential.

The distribution of lectin activity in rice and corn, two grains considered safe in the celiac diet, can be compared to that found in wheat (Concon et al., 1983), the grain most toxic in the celiac diet. The buffered saline extract of rice endosperm (the albumin and globulin fraction) like that of wheat endosperm was devoid of lectin activity, while corn endosperm had a lectin with high activity that was resistant to heat. Wheat had no lectin activity in the alkali-soluable (glutelin) fraction, while both rice and corn had activity in this fraction which persisted after heat treatment. All three grains have lectins of high specific activity in the acid-soluble glutelin fraction, a fraction observed to contain "gly-gli" (Concon et al., 1983), a compound which Douglas (1976) reports to bind strongly to celiac intestinal mucosa. This fraction is heat stable in both wheat and corn, but in rice the lectin activity of this fraction is heat labile. However, in contrast to wheat, both rice and corn contain low levels of this material, perhaps providing an explanation for the ability of most celiac patients to tolerate these grains in their diets, if "gly-gli" should prove to be the offending agent.

Because rice and corn are not excluded from the gluten-free diets of celiac patients, the findings reported herein may provide some basis for speculation regarding the observation that some patients are refractory to gluten-free diets (Congdon et al., 1981; Gryboski, 1981; Hamilton and McNeill, 1972). Thus, if it were established that lectins associated with the gluten of wheat and related cereals are responsible for celiac disease, then it would be possible that some of these refractory patients could comprise a subpopulation who are also sensitive to the lectins in rice and/or corn endosperm, and such individuals might respond favorably to the elimination of all cereals from the diet.

The presence of lectins in the endosperm of rice, corn, and wheat, three disparate cereal grains, raises the interesting hypothesis that endosperm lectins may be common characteristics of all cereal grains. Therefore, hemagglutination does not seem to be an appropriate test for those lectins that might associate with the intestinal lining to cause celiac disease. However, the endosperm of various cereals may prove to be good sources of novel water-insoluble lectins.

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