Molecular Comparison of Alcohol-Soluble Wheat and Buckwheat Proteins

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ABSTRACT

The classification of buckwheat (*Fagopyrum esculentum* Moench) with the cereals in commerce and descriptions of glutenlike proteins in buckwheat endosperm have confused public understanding of the suitability or otherwise of buckwheat for gluten-free diets. Comparisons of buckwheat and wheat proteins according to amino acid composition, electrophoresis, and immunological reaction revealed little or no similarities. Whereas most of the material extracted from wheat flour by 70% ethanol (following salt extraction) was protein in nature, similar extracts from buckwheat contained only 2.4% nitrogen. Unlike wheat gliadin, the corresponding buckwheat fraction was rich in lysine, arginine, and glycine; it was not electrophoretically resolvable into gliadinlike bands on lactate-buffered polyacrylamide gels, and on sodium dodecyl sulfate polyacrylamide gels, it had only minor components in the gliadin molecular size range. Immunological studies with rabbit polyclonal and mouse monoclonal antibodies showed little cross-reactivity between cereal prolamins and buckwheat proteins. Celiac sera with high IgG anti-gliadin titers also reacted very weakly with buckwheat proteins. These results suggest that alcohol-soluble buckwheat proteins (which are only minor components of the endosperm) bear little molecular similarity to wheat prolamins; descriptions of the former as glutenlike are misleading.

Buckwheat (*Fagopyrum esculentum* Moench) is rich in essential amino acids (Pomeranz and Robbins 1972, Pomeranz 1975) and may be milled for consumption in noodles, pancakes, or porridge (Taira 1974, Pomeranz 1983). Interest in buckwheat has been renewed as the increasing popularity of vegetarian diets, the distinctive taste of buckwheat (Marshall and Pomeranz 1982), and its high content of lysine make it a useful adjunct to cereal protein, especially in the vegetarian diet (Sure 1955, Pomeranz et al 1975). Individuals with gluten-sensitive enteropathy (including celiac disease and other gluten intolerances) are unable to tolerate storage protein from wheat, barley, rye, and possibly oats, and they desire high-fiber cereal substitutes such as buckwheat to maintain a normal diet.

Buckwheat grain is the fruit of a dicotyledonous plant, and is thus taxonomically distant from wheat and the true cereals (Watson and Wrigley 1984). In commerce, however, buckwheat has traditionally been classified with the cereals (Pomeranz 1983). This classification has led to some confusion about the toxicity of buckwheat in gluten sensitivity. Although the major endosperm proteins of buckwheat groats are salt-soluble globulins (Taira 1974, Hiller et al 1975), reports have been published of "gluten" proteins being present in grain from this species. Solvent extraction of buckwheat flour by investigators in the U.S.S.R. (Sokolov and Semikov 1969, Belozerskii and Emtzeva 1970), Korea (Byun and Chang 1980), and Yugoslavia (Javornik et al 1981) yielded fractions termed "gliadin" and "glutelin" by these workers. These fractions may total over 20% of the total endosperm protein. In addition, Soda et al (1981) found a protein fraction from buckwheat flour to have rheological properties resembling those of wheat gluten. A survey of Canadian gastroenterologists and written materials available to celiac and other gluten-sensitive patients found that a majority of clinicians and authors recommend caution, or advise against the use of buckwheat in gluten-free diets (Bell et al 1981). Other groups found evidence for immunological amino acid sequence homology between dicotyledonous seed proteins and those in the endosperm of cereals (Shewry et al 1984a, Fabjianski et al 1985).

In these studies, buckwheat and cereal endosperm proteins were compared by amino acid composition, electrophoresis, and immunological means in order to explore the molecular similarity of buckwheat proteins to gluten proteins in wheat.

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MATERIALS AND METHODS

Materials

Mature common buckwheat seeds were ground in a coffee grinder. Bread wheat (cultivar Timgalen) was milled to produce white flour (75.5% extraction rate). Salt-soluble proteins were prepared by extracting twice for 1 hr with 20 volumes of 10% (w/v) NaCl, and crude gliadin was prepared by extracting the residue twice for 1 hr with 20 volumes of 70% (v/v) ethanol at 20° C, dialyzing the extract, and freeze-drying. Glutelin fractions were prepared by extraction (16 hr, 20° C) of the residue from the last step with 10 volumes of aqueous 50% *n*-propanol and 2-mercaptoethanol/1% acetic acid. Monoclonal antibodies to gliadin and glutenin fractions were prepared using methods described previously (Skerritt et al 1984).

Amino Acid Analyses

Amino acid analyses were performed in a modified Beckman Spinco 120C amino acid analyzer. Protein fractions (0.2–0.4 mg) were hydrolyzed in 1 ml of 6*M* HCl for 24 hr in evacuated, sealed tubes at 110° C. Tryptophan and cysteine contents were not determined. Results are not corrected for losses.

Electrophoretic Procedures

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) was performed on proteins extracted with 2% (w/v) SDS, 0.1*M* dithiothreitol, and 62 m*M* Tris-HCl, pH 7.5, using a buffer described earlier (Laemmli and Favre 1973). After preliminary stacking in a 5.5% (w/v) T gel, pH 7.5, proteins were fractionated in a 15% T, 2% C separating gel, pH 8.5 (175 × 155 × 0.7 mm). The running buffer (150 m*M* Tris, 380 m*M* glycine, pH 8.5) contained 0.1% (w/v) SDS; typically the gels were run for 1,000 Vhr.

Immunological Studies

A comparison of the antigenic determinants of wheat and buckwheat proteins was made using a panel of antibodies, including five mouse monoclonal antibodies that had been prepared to wheat storage proteins (Skerritt and Martinuzzi 1986), two rabbit antisera (to whole gliadin and to α-gliadin), and sera from three adult celiac patients previously found to have high IgG antibody titers (2,500–5,500) to gliadin.

The specificities of the monoclonal antibodies were as follows: clone 21/23, α-gliadin; clone 43/6, primarily β- and γ-gliadin (some α- and ω-binding); clone 22/24, ω-gliadin; clone 43/11, broad specificity for gliadin and glutenin proteins; and clone 37/24, γ- and ω-gliadin and some glutenins.

Samples of wheat flour and buckwheat meal were extracted (1 hr) with six volumes of 2*M* urea, and 70% ethanol-soluble fractions from these sources were dissolved in 100 volumes of 1% potassium

hydroxide. Extracts were diluted in the appropriate solution to final concentrations of 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0.003% of the original extracts. Aliquots (1 μl) of the extracts and dilutions were dried onto nitrocellulose strips for 1 hr at 20° C. Nonspecific antibody binding to nitrocellulose was blocked by overnight incubation at 37° C in 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Subsequent antibody incubations were performed using a protocol described elsewhere (Skerritt et al 1984, 1985). Monoclonal antibody culture supernatants for clones 21/23, 37/24, and 43/11 were diluted 1:64 and clone 43/6 diluted 1:16. Ascites fluid for clone 22/24 and rabbit and human sera were used at a 1:100 dilution.

After a 90-min incubation (37° C) with human or mouse antibody, strips were washed (3 × 8 min with PBS containing 0.05% Tween 20 and 0.05% BSA) and incubated 60 min with rabbit-anti-mouse immunoglobulin or rabbit-anti-human IgG immunoglobulins (DAKO, Denmark; diluted 1:80 in 3% BSA in PBS). Rabbit antisera to gliadin were incubated 180 min with antigen. After washing, all strips were incubated 30 min with swine-anti-rabbit immunoglobulins (1:40, DAKO), washed, and incubated 30 min with rabbit peroxidase-anti-peroxidase (1:80, DAKO) before a final wash and addition of enzyme substrate (4-chloro-1-naphthol, [0.05% w/v] hydrogen peroxide [0.024% w/v] in a 17:83 [v/v] mixture of methanol and NaCl [200 m*M*, buffered to pH 7.4 with Tris-HCl, 50 m*M*]). Primary antibody binding to wheat or buckwheat proteins was observed by the development of blue-purple spots within 15 min of substrate incubation.

RESULTS AND DISCUSSION

Yields and Nitrogen Content of Fractions

Bread wheat flours and buckwheat meals were successively extracted with 10% NaCl, 70% ethanol, and 50% *n*-propanol/1% acetic acid/2% mercaptoethanol (selective solvents for wheat albumins and globulins or prolamins and glutelins, respectively; Shewry et al 1984b). The mean total nitrogen contents of the buckwheat and wheat samples used were similar. Recoveries of various fractions were determined by weighing the freeze-dried material obtained after extraction and thorough dialysis. Whereas most solvent-soluble high-molecular-weight buckwheat material (greater than about 8,000 M_r) was extracted by 10% NaCl, sodium chloride solution only extracted a small amount of material from wheat flour (Table I). The recoveries of material in the ethanol and propanol-acetic acid-mercaptoethanol fractions for buckwheat were very low (0.96 and 0.24%, respectively). These results confirm previous findings that the major buckwheat proteins are globulin in nature (Hiller et al 1975, Pomeranz 1983), but salt-soluble proteins are only minor components of wheat flour. Whereas the 70% ethanol-soluble fraction from wheat is mainly protein (N × 5.7 = 83.2%), the analogous fraction from buckwheat contained only 2.4% nitrogen, thus being unlikely to contain over 15% protein. This fraction was invariably yellow-brown in color; possibly polyphenols constitute much of the balance of the material. In contrast, the buckwheat salt-soluble fraction was higher in nitrogen than that from wheat (Table I).

Amino Acid Analyses

The 70% ethanol-soluble fractions from wheat and buckwheat, and the 10% salt-soluble fraction from buckwheat were subjected to amino acid analysis (Table II). The gliadin composition determined for two samples prepared from the wheat cultivar Timgalen in this experiment was in general similar to others reported in the literature (e.g., Bushuk and Wrigley 1974). As noted earlier, the amino acid composition of gliadin differed significantly from that of albumin and globulin. Specifically, gliadin was considerably lower in lysine, arginine, aspartate/asparagine, cysteine, and methionine but higher in proline and glutamate/glutamine. In contrast, the amino acid compositions of the salt- and alcohol-soluble fractions from buckwheat were quite similar to one another, and to the composition of protein from whole, pearled buckwheat endosperm, as reported by Pomeranz and Robbins (1972). Similar amino acid compositions were found by Belozerskii and Emtseva (1970) for buckwheat fractions they termed glutelin and globulin. The amino acid compositions of the 70% ethanol extracts of buckwheat and wheat were quite different. The lysine, arginine, and glycine contents of the buckwheat fraction were much higher than those of prolamins from wheat or any related cereal species (Kasarda et al 1971, Bright and Shewry 1983, Shewry and Miflin 1985).

Glutamine and proline contents are typically high in cereal prolamins, being of the order of 30–40 and 15–20 mol %, respectively, in species within the tribe Triticeae (wheat, rye, and barley) (Shewry and Miflin 1985). The glutamine and proline contents are lower in festucoid (oats and rice) and panicoid prolamins (maize, sorghum, and millet), namely, 19–36 mol % for glutamine and 6–13 mol % for proline. The ethanol-soluble protein fraction from buckwheat contained 14.8 mol % glutamate/glutamine and 9.6% proline, values more reminiscent of proteins from the latter botanical tribes. However, it should be noted that the degree of amidation was not determined for the buckwheat protein fraction. In addition, none of the prolamins from the Gramineae had the combination of high lysine, arginine, and glycine levels found in ethanol-soluble buckwheat protein.

Electrophoretic Studies

An SDS-electrophoretic separation of wheat and buckwheat protein fractions is shown in Figure 1. Total wheat flour had protein components in an apparent molecular weight range of about 15,000–150,000 (lane 1), whereas buckwheat proteins fell into a lower molecular weight range (10,000–80,000). The salt-soluble (albumin plus globulin) wheat protein fraction (lane 2) covered a molecular weight range similar to the overall wheat protein extract, although banding patterns and relative intensities were different. The salt-soluble buckwheat protein profile was similar to that of whole buckwheat meal (data not shown).

Whereas gliadin proteins (lane 3) covered an apparent M_r range of approximately 30,000–70,000 (noted earlier), the buckwheat alcohol-soluble fraction (lane 4) had a lower M_r range (10,000–28,000). Protein extracted from buckwheat meal with 70% ethanol, without prior salt solution extraction, had similar electrophoretic characteristics. While several discrete bands were seen among the alcohol-soluble buckwheat proteins, it did not prove possible to obtain sharper resolution by the use of other buckwheat samples, or by alkylation of cysteine residues with 4-vinylpyridine (Skerritt and Martinuzzi 1986) before electrophoretic separations. Analysis of extracts concentrated 40-fold by ultrafiltration (Amicon YM 10 membranes, nominal cut-off M_r 10,000) revealed minor components of M_r 80,000, 75,000, 68,000, 35,000, and 32,000 in the buckwheat ethanol-soluble fraction.

TABLE I
Yields and Nitrogen Content of Fractions Extracted from Buckwheat Meal and and Wheat Flour[a]

	Extractant			
	NaCl (10%)	Ethanol (70%)	Propanol (50%)/HoAc (1%)/EtSH (2%)[b]	Flour/Meal
Buckwheat meal				
Yields	7.26 ± 0.51	0.96 ± 0.11	0.24 ± 0.02	(100)
n	4	4	4	
% N	13.19 ± 0.88	2.37 ± 0.18	n.t.[c]	1.86 ± 0.19
n	2	2		3
Wheat flour				
Yields	1.65 ± 0.20	4.67 ± 0.16	4.50 ± 0.13	(100)
n	2	2	2	
% N	9.60 ± 0.37	14.59 ± 0.17	n.t.	2.35 ± 0.03
n		2		2

[a] Data shown are percentages by weight and are determined as an "as is" basis uncorrected for moisture content. Nitrogen determined by Kjeldahl analysis.
[b] HoAc = acetic acid, EtSH = mercaptoethanol.
[c] n.t. = Not tested.

TABLE II
Amino Acid Compositions of Wheat and Buckwheat Fractions (mol %)

Amino Acid	Wheat			Buckwheat		
	Albumin[a]	Gliadin[b]	Gliadin (70% ethanol) Extract	Pearled Endosperm	10% NaCl Extract	70% Ethanol Extract
Alanine	4.3	1.5	3.1	6.5	5.0	5.4
Arginine	5.1	1.9	1.0	5.7	9.8	6.9
Aspartic acid or asparagine	5.8	7.9	2.4	10.8	8.6	6.8
Cysteine	6.2	2.7	n.d.[c]	1.9	n.d.	n.d.
Glutamic acid or glutamine	22.6	41.1	43.7	13.6	14.8	14.8
Glycine	3.6	1.5	2.6	7.0	9.8	10.8
Histidine	2.0	1.6	1.2	2.1	2.3	2.3
Isoleucine	3.0	3.2	3.5	5.1	4.9	4.7
Leucine	6.8	6.1	6.5	9.2	7.9	7.5
Lysine	3.2	0.5	0.4	6.1	5.7	5.4
Methionine	1.8	1.0	0.6	3.8	1.5	1.2
Phenylalanine	4.0	6.0	4.6	4.7	5.1	3.6
Proline	8.9	14.3	17.4	4.4	5.7	9.6
Serine	4.5	3.8	4.9	4.3	4.8	4.6
Threonine	3.1	1.5	1.9	4.6	2.9	3.5
Tryptophan	1.1	0.7	n.d[c]	n.d	n.d	n.d
Tyrosine	3.4	2.2	1.8	3.6	2.5	4.6
Valine	4.7	2.7	4.3	6.6	8.5	8.2

[a] From Bushuk and Wrigley, 1974.
[b] From Pomeranz and Robbins, 1972.
[c] From this study; tryptophan and cysteine are known to be present but were not deterined in these experiments (n.d.) Mol percentages in these cases are calculated without considering Trp and Cys.

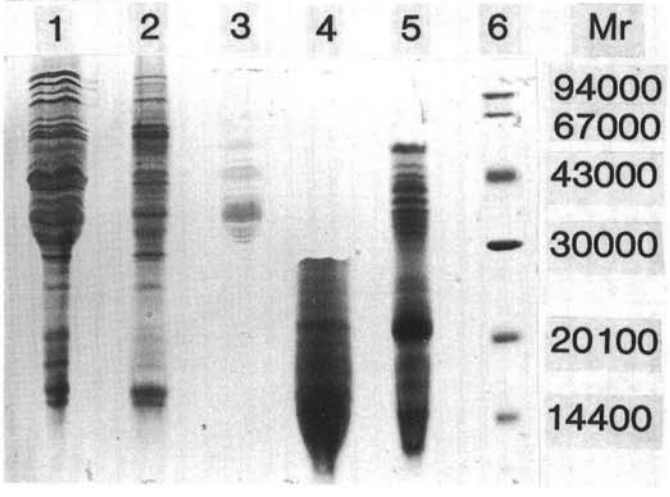

Fig. 1. Analysis of wheat and buckwheat protein fractions by sodium dodecyl sulfate polyacrylamide gel electrophoresis (left to right): Lanes **1,** whole wheat flour extract; **2,** salt-soluble wheat proteins; **3,** wheat 70% ethanol-soluble fraction; **4,** buckwheat 70% ethanol-soluble fraction; **5,** whole buckwheat meal extract; and **6,** molecular-weight-marker proteins: α-lactalbumin (M_r 14,400), soybean trypsin inhibitor (M_r 20,100), carbonic anhydrase (M_r 30,000), ovalbumin (M_r 43,000), bovine serum albumin (M_r 67,000), and phosphorylase (M_r 94,000).

High resolution of gliadins is possible with the use of "non-denaturing" buffer systems, such as sodium lactate, pH 3.1 (Wrigley et al 1982). However, under these conditions, buckwheat alcohol-soluble protein ran as a diffuse streak, and a substantial proportion failed to enter even a 3% gel. Electrophoresis of buffered alcohol (40% *n*-butanol) extracts of buckwheat under alkaline conditions (Tris/borate/ethylenediaminetetraacetic acid, pH 8.3) was also unsuccessful, suggesting that buckwheat alcohol-soluble proteins have low solubilities in these aqueous environments.

Immunological Studies

Studies of the cross-reactivity of proteins with polyclonal and monoclonal antibodies of determined specificities are valuable in assessing whether the proteins under study bear similar or identical amino acid sequences or surface structures (Reichlin 1977, Daussant and Skakoun 1983, Pollock et al 1984).

TABLE III
Specificities of Monoclonal Antibodies for Ethanol-Soluble Proteins in Various Cereals and Buckwheat[a]

Grain	Antibody		
	21/23	43/6	43/11
Bread wheat	1	1	1
Durum wheat	3	1	1
Rye	0.0033	1	1
Barley	0.0033	1	3
Oats	0.0033	0.33	0.0033
Corn	0.033	1	0.01
Buckwheat	0.0003	0.0001	0.0003

[a] Data shown are detection sensitivities determined as the reciprocal of the limit of detection relative to bread wheat. Cereals and buckwheat were first extracted with 20 vol 10% NaCl (2 × 1 hr) before extraction with 20 vol 70% ethanol (2 × 1 hr): these ethanol extracts were dialysed and lyophilized before use in immunological assays.

Accordingly, proteins in extracts of buckwheat and wheat flour were investigated for reactivity with polyclonal antibodies to wheat gliadin raised in two rabbits, human sera from three celiac condition patients, and with five monoclonal antibodies with varying specificities for cereal prolamins. Details of the dot-immunobinding assay used have been published elsewhere (Skerritt et al 1984, 1985); limits of antigen detection were determined as the lowest protein loading that yielded a positive signal after antibody probing. The relative detection sensitivities of three monoclonal antibodies for ethanol-soluble proteins from various cereals and buckwheat are shown in Table III. Clone 21/23 bound strongly to gliadin from bread or durum wheat, from 30- to 300-fold less well to rye, barley, oat and corn prolamins, and 3,000-fold less well to buckwheat protein. Clones 43/6 and 43/11 bound equally to wheat, rye, and barley prolamins, and to varying degrees to corn and oat prolamins; again, binding to buckwheat proteins was barely detectable. Similar results were seen with two other monoclonal antibodies. At the antibody dilutions used, clones 37/24 and 22/24 detected as little as 3 and 100 ng of gliadin, respectively, but failed to detect 10 μg of buckwheat extract assayed at the same time.

Polyclonal rabbit and human antisera also showed little reactivity with buckwheat proteins (Fig. 2). At a 1/100 dilution, anti-gliadin antisera from each rabbit bound 1–10 ng of gliadin;

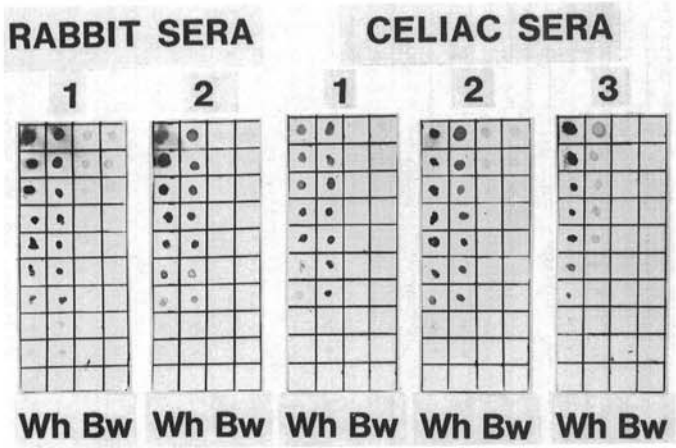

Fig. 2. Reaction of polyclonal antibodies, from two rabbits (left) and from three humans with celiac condition, with wheat and buckwheat extracts. Antigens loaded onto each strip alternate (from left): wheat flour (**Wh**) with 1 *M* urea extract and 70% ethanol-soluble fraction; and buckwheat meal (**Bw**) with 1 *M* urea extract and 70% ethanol-soluble fraction. Samples were diluted from original extracts (166-μg/ml meal or flour in 1 *M* urea, or 10 mg/ml ethanol extract dissolved in 1% KOH) to yield, from top to bottom: 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01, and 0.003% of the original protein concentration before 1-μl aliquots were applied to the strips.

serum from one rabbit (1) showed a weak but significant reaction with urea and ethanol-extracted buckwheat material. It is likely that this antibody response is not a cross-reaction of the gliadin antibody population but reaction of another antibody population formed as a result of dietary immunization with a buckwheat-related species. Sera from three celiacs, which were found earlier to contain high levels of anti-gliadin antibody, also reacted strongly with urea and ethanol extracts of wheat flour but poorly and not at all with the respective extracts from buckwheat. Taken together, these results indicate that the alcohol-soluble proteins from buckwheat bear little immunological resemblance to prolamins in wheat or related cereals.

Severe clinical sensitivity to buckwheat has been noted in several patients (Horesh 1972) but it is of an allergic nature rather than an enteropathy such as celiac condition. In one group of patients with celiac disease, Bahna et al (1980) found elevated IgD antibodies to an aqueous wheat extract but not to a similar buckwheat preparation.

CONCLUSION

Amino acid composition, electrophoresis, and immunological analysis revealed little similarity between proteins in wheat and buckwheat endosperm. In addition, the relative proportions of salt-soluble and alcohol-soluble proteins in wheat and buckwheat differ vastly; the latter form only a minor portion of buckwheat-endosperm protein. While it is possible that components in buckwheat meal (such as polyphenols) may decrease extraction of glutenlike proteins from buckwheat, this is unlikely to account for the failure to observe such proteins, because a wide variety of extractants (acidic, alkaline, alcoholic, and SDS-based) were used in these studies. These findings suggest that the description of buckwheat or other seed protein as gluten, gliadin, or glutelin purely on the basis of solubility properties (Sokolov and Semikov 1969, Belozerskii and Emtseva 1970, Byun and Chang 1980, Javornik et al 1981) is unfortunate and can lead to unnecessary exclusion of valuable sources of dietary protein in gluten-sensitive individuals.

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LITERATURE CITED

BAHNA, S. L., TATENO, K., and HEINER, D. C. 1980. Elevated IgD antibodies to wheat in celiac disease. Ann. Allergy 44:146.

BELL, L., HOFFER, M., and HAMILTON, J. R. 1981. Recommendations for foods of questionable acceptance for patients with celiac disease. J. Can. Diet. Assoc. 42:143.

BELOZERSKII, M. A., and EMTSEVA, I. B. 1970. Comparative study of proteins of diploid and tetraploid buckwheat. Biokhimiya 35:152.

BRIGHT, S. W. J., and SHEWRY, P. R. 1983. Improvement of protein quality in cereals. Crit. Rev. Plant Sci. 1:49.

BUSHUK, W., and WRIGLEY, C. W. 1974. Proteins: composition, structure and function. Page 119 in: Wheat: Production and Utilization. G. E. Inglett, ed. Avi Publ. Co.: Westport, CT.

BYUN, S. S., and CHANG, S. K. 1980. A study on the chemical composition of Korean buckwheat flour. Kolyo. Uikitae. Chapchi 11:17.

DAUSSANT, J., and SKAKOUN, A. 1983. Immunochemistry of seed proteins. Page 101 in: Seed Proteins. J. Daussant, J. Mosse, and J. Vaughan, eds. Academic Press: London.

FABJIANSKI, S., ALTOSAAR, I., LAURIERE, M., PERNOLLET, J. C., and MOSSE, J. 1985. Antigenic homologies between oat and wheat globulins. FEBS Lett. 182:465.

HILLER, A., MLODECKI, H., and TOMCZYK, M. 1975. Preliminary characterization of proteins of buckwheat grain by extraction with selected solvents and molecular filtration on Sephadex G-75 gel. Bromatol. Chem. Toksykol. 8:205.

HORESH, A. J., 1972. Buckwheat sensitivity in children. Ann. Allergy. 30:685.

JAVORNIK, B., EGGUM, B. O., and KREFT, I. 1981. Studies on protein fractions and protein quality of buckwheat. Genetika 13:115.

KASARDA, D. D., NIMMO, C. C., and KOHLER, G. O. 1971. Proteins and the amino acid composition of wheat fractions. Page 227 in: Wheat Chemistry and Technology. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

LAEMMLI, U. K., and FAVRE, M. 1973. Maturation of the head of bacteriophage T4. I. DNA packaging events. J. Mol. Biol. 80:575.

MARSHALL, H. G., and POMERANZ, Y. 1982. Buckwheat: description, production, and utilization. Page 157 in: Advances in Cereal Science and Technology. Vol. 5. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

POLLOCK, R. R., TEILLAUD, J. L., and SCHARFF, M. D. 1984. Monoclonal antibodies: A powerful tool in selecting and analyzing mutations in antigens and antibodies. Ann. Rev. Microbiol. 38:389.

POMERANZ, Y. 1975. Proteins and amino acids of barley, oats and buckwheat, Page 13 in: Protein Nutritional Quality of Foods and Feeds, Part 2. M. Freidman, ed. Marcel Dekker: New York.

POMERANZ, Y. 1983. Buckwheat: structure, composition and utilization. Crit. Rev. Food. Sci. Nutr. 19:213.

POMERANZ, Y., and ROBBINS, G. S. 1972. Amino acid composition of buckwheat. J. Agric. Food Chem. 20:270.

POMERANZ, Y., MARSHALL, H. G., ROBBINS, G. S., and GILBERTSON, J. T. 1975. Protein content and amino acid composition of maturing buckwheat (*Fagopyrum esculentum* Moench) Cereal Chem. 52:479.

REICHLIN, M. 1977. Use of antibody in the study of protein structure. Page 55 in: Molecular Biology, Biochemistry and Biophysics. Advanced Methods in Protein Sequence Determination. Vol. 25. S. B. Needleman, ed. Springer: Berlin.

SHEWRY, P. R., and MIFLIN, B. J. 1985. Seed storage proteins of economically important cereals. Page 1 in: Advances in Cereal Science and Technology. Vol. 7. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

SHEWRY, P. R., LAFIANDRA, D., SALCEDO, G., ARAGONCILLO, C., GARCIA-OLMEDO, F., LEW, E. J. L., DIETLER, M. D., and KASARDA, D. D. 1984a. N-terminal amino acid sequences of chloroform/methanol-soluble proteins and albumins from endosperms of wheat, barley and related species. FEBS Lett. 175:359.

SHEWRY, P. R., FIELD, J. M., FAULKS, A. J., PARMAR, S., MIFLIN, B. J., DIETLER, M. D., LEW, E. J. L., and KASARDA, D. D. 1984b. The purification and N-terminal amino acid sequence analysis of the high molecular weight glutenin polypeptides of wheat. Biochim. Biophys. Acta. 788:23.

SKERRITT, J. H., and MARTINUZZI, O. 1986. Effects of solid phase and antigen solvent on the binding and immunoassay of water-insoluble flour proteins. J. Immunol. Methods. 88:217.

SKERRITT, J. H., SMITH, R. A., UNDERWOOD, P. A., and WRIGLEY, C. W. 1984. Monoclonal antibodies to gliadin proteins used to examine cereal grain protein homologies. J. Cereal Sci. 2:215.

SKERRITT, J. H., DIMENT, J., and WRIGLEY, C. W. 1985. A sensitive

monoclonal antibody-based test for gluten detection. 1. Choice of primary and secondary antibodies. J. Sci. Food Agric. 36:995.
SODA, T., KATO, J., KIRIBUCHI, S., and AOKI, H. 1981. Properties of buckwheat proteins from the standpoint of food processing. Nippon Shokuhin Kogyo Gakkaishi 28:297.
SOKOLOV, O. A., and SEMIKOV, V. F. 1969. Protein complex of polygonum seeds dependent on ploidy and nutrition conditions. Page 80 in: Fiziol. Biokhim. Sorta, Tr. Konf. Fiziol. Biokhim. Rast. Sib. 3rd ed. F. E. Reimers, ed. Vostaka, USSR.
SURE, B. 1955. Nutritive value of proteins in buckwheat and their role as supplements to proteins in cereal grains. Agric. Food Chem. 3:793.
TAIRA, H. 1974. Buckwheat. Page 139 in: Encyclopedia of Food Technology. A. M. Johnson and M. J. Peterson, eds. Avi Publ. Co.: Wesport, CT.
WATSON, L., and WRIGLEY, C. W. 1984. Relationships between plants relevant to allergy. Med. J. Aust. 141(Suppl):S18.
WRIGLEY, C. W., AUTRAN, J. C., and BUSHUK, W. 1982. Identification of cereal varieties by gel electrophoresis of the grain proteins. Page 211 in: Advances in Cereal Science and Technology. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

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