PHYSICOCHEMICAL PROPERTIES OF WHEAT GEL PROTEINS: EFFECTS OF ISOLATION CONDITIONS[1]

M. F. JEANJEAN and P. FEILLET, Institut National de la Recherche Agronomique, Laboratoire de Technologie des Bles Durs et du Riz, 34060 Montpellier, France

Cereal Chem. 55(6): 864-876

ABSTRACT

Gel proteins (GP) were isolated from wheat flour after sequential extraction under different conditions; a number of extractions with combinations of five solvents (H_2O, $0.5M$ NaCl, 60% ethanol, $0.01M$ acetic acid, 70% 2-chloroethanol) were compared. From 11 to 29% of the flour proteins were present in the gel fractions. Polyacrylamide gel electrophoresis in aluminum lactate buffer (PAGE) showed large differences between GP; these differences were reduced or absent when GP are dissolved in $6M$ urea or 0.25% sodium stearate. Molecular weights of GP subunits, determined by sodium dodecylsulfate PAGE, ranged from 12,000 to 136,000; only minor differences occur between samples. In most of the cases, amino acid composition of GP was found to be close to that of glutenin. From the results, GP, a complex of albumin, globulin, gliadin, and specific glutenin fractions, is postulated to differentiate from glutenin only by the strength of hydrogen and hydrophobic bonds between protein units.

A large number of studies have been done to correlate the protein composition and breadmaking quality of wheat (1). In the last ten years, many authors (2–6) have underlined the importance of the acetic acid-insoluble gluten and glutenin fractions. Mecham et al (7) and Cole et al (8) have shown the importance of the so-called gel protein fractions, which are those proteins that occur in the highly hydrated insoluble viscous residue remaining when wheat flour is extracted with dilute acetic acid.

In an earlier work we (9) showed that the glutenin content of flour (mg/ 100 g of flour) is correlated with the Zeleny test and the W alveograph Chopin values. More recently, highly significant correlations were shown between baking score, Zeleny test, alveograph test, and gel protein content of wheat flour, while correlations were not shown between wheat protein and gel protein contents (10,11).

The nature of gel proteins is not yet established. In a previous paper, Mecham et al (7) stated that the gel protein is possibly a fraction of glutenin, but according to Bietz and Wall (12), gel proteins consist both of glutenin and low molecular weight gliadin-like components.

The present study was done to compare the effects of isolation conditions of gel proteins on their physicochemical properties.

MATERIALS AND METHODS

Extraction of Gel Proteins

Gel proteins were extracted from a commercial untreated wheat flour (*Triticum vulgare*) (protein content, 10.5% db) by seven different procedures as summarized in Table I.

[1]Presented at the AACC 62nd Annual Meeting, San Francisco, October 1977.

0009-0352/78/000097$03.00/0
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Gel A was isolated according to the method of Jeanjean and Feillet (11). Water solubles were extracted twice with 30 ml of deionized water from 5 g of flour by gently stirring for 15 min and then centrifuging at 38,000 × g for 15 min. The residue was extracted with 30 ml of 70% 2-chloroethanol (CE), pH 1.8 (70:30 CE/water, v/v) by stirring for 15 min. The gel layer was then recovered from the upper layer of the centrifuged sediment.

Isolation conditions of gels B and C were identical to those of gel A except for the extraction numbers (Table I).

Gel D was extracted using the method of Feillet et al (13) after solubilization of soluble proteins by 0.5*M* NaCl, gliadins by 60% ethanol, and glutenins by 70% CE.

Gel E was prepared according to the method of Inamine et al (14), but without flour delipidation. Wheat flour, 10 g, was suspended in 500 ml of 0.01*M* acetic acid. After stirring, the suspension was allowed to settle for 1 hr; then the supernatant was decanted. The flour was extracted six times under the same conditions. The residue was centrifuged for 15 min at 38,000 × g, and the gel layer was recovered.

Gel F was isolated after three extractions of the flour by 70% CE (5 g flour, 30 ml of 70% CE, 15 min of stirring, and 15 min of centrifugation at 38,000 × g).

Gel G was extracted in the same manner as gel D except for deletion of the 0.5*M* NaCl extraction step.

After extraction, gels were dialyzed against deionized water. Except when specified, soluble and insoluble gels were gathered after dialysis and freeze-dried for subsequent analysis.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was done in aluminum lactate buffer, pH 3.2, with 6*M* urea (PAGUE) (15) or without (PAGE) (16), or in tris-borate buffer, pH 8.9, with 0.1% sodium dodecylsulfate (SDS-PAGE) (17).

Polyacrylamide gel slab (7%) (22.5 × 15.0 × 0.6 cm) was prepared by dissolving 20.37 g of acrylamide (Fluka), 0.63 g of bisacrylamide (Fluka), 1 ml of dimethylaminopropionitrile, and 300 mg of ammonium persulfate in 300 ml of water (PAGE), 6*M* urea (PAGUE), or tris-borate buffer (SDS-PAGE). After polymerization, gel was dipped into buffer for 24 hr.

TABLE I
Gel Protein Extraction Procedures

	Extraction Procedures[a]						
	A[b]	B	C	D[c]	E[d]	F	G
Deionized water	X(2)	(X20)	X(20)				
0.5*M* NaCl				X			
60% Ethanol				X			X
70% 2-Chloroethanol	X(1)	X(1)	X(2)	X		X(3)	X
0.01*M* Acetic acid					X(6)		

[a]Numbers in parentheses are extraction numbers.
[b]According to Jeanjean and Feillet (11).
[c]According to Feillet et al (13).
[d]According to Inamine et al (14).

For PAGE and PAGUE, a dispersion of 50 mg of freeze-dried gel protein in 1 ml of buffer was pipetted (50 μl) into the preformed slots. In some cases, gel proteins (25 mg) were solubilized in 1 ml of 0.25% sodium stearate water suspension by stirring overnight (18). Electrophoresis was done for 2–3 hr with a potential of 7 V/cm across the polyacrylamide gel slab.

For SDS-PAGE, 35 mg of freeze-dried gel proteins were incubated overnight at 40°C in 1 ml of tris-SDS borate containing 1% 2-mercaptoethanol (17). Electrophoresis was done for 3–4 hr with a potential of 6 V/cm across the polyacrylamide gel slab. Cytochrome C (11,700 mol wt), chymotrypsinogen A (25,000 mol wt), ovalbumin (45,000 mol wt), and bovine serum albumin (65,000 and 130,000 mol wt) were used as standard proteins for molecular weight calibration.

After electrophoresis was completed, the gel was stained with Coomassie Brilliant Blue G 250 in 12.5% trichloroacetic acid (19).

Analytic Procedures

Nitrogen content was determined by a semiautomatic Kjeldahl method (20). Amino acid composition of protein fractions was performed by the method of Moore and Stein, with slight modification. All determinations were duplicated.

TABLE II
Percentage (% Total Protein) of proteins Extracted Under Different Procedures

	Extraction Procedures[a]						
Protein Fractions	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Water soluble	20.0	45.3	45.3				
0.5M NaCl soluble				20.8			
60% Ethanol soluble				37.6			55.1
0.01M Acetic acid soluble					79.5		
70% 2-Chloroethanol soluble	30.2	17.3	25.0	18.2		79.4	19.4
Gel protein	20.9	29.0	22.2	18.6	11.3	13.2	18.9
Insoluble	28.9	8.4	7.5	4.8	9.2	7.4	6.6

[a]See Table I.

TABLE III
Water Solubility, Protein, and Moisture Content of Gel Proteins

	Extraction Procedures[a]						
	A	**B**	**C**	**D**	**E**	**F**	**G**
Moisture content (% on wet basis)	94	95	96	96	93	94	93
Protein content (% db)	19.1	18.8	20.1	19.5	9.5	15.1	17.6
Water-soluble proteins (% total gel proteins)	39	25	15	10	14	2	1

[a]See Table I.

RESULTS

Wheat Gel Protein Content

Depending on the extraction conditions, the amounts of gel proteins that have been isolated range from 11.3% (gel E) to 29.0% (gel B) of the flour proteins (Table II). The low content in gel E was probably due to the successive losses that might occur during the decantation of the flour extracts, since the substitution of CE by 0.01 *M* acetic acid in procedure B or C did not modify the gel protein yield. The difference in the number of CE extraction may explain that the yield in gel C was lower than that in gel B. In their work, Mecham et al (7) have found that the gel protein content was 12–28%, depending on wheat varieties.

The amount of residual proteins (insoluble) was low (4.8–9.2%) except in procedure A, where it reached 28.9%. When residue A was extracted by 70% CE, 65% was soluble, 21% formed a gel-like layer, and 14% remained insoluble.

When the flour was extracted 20 times by deionized water (extractions B and C), 45.3% of the flour proteins were soluble as already observed by Baudet and Mosse (21). Under these conditions, gliadin would be extracted after removing phosphate ions by successive water leaching (22). Accordingly, 62.6% and 70.3% of flour proteins were extracted before gel B and C isolation, respectively.

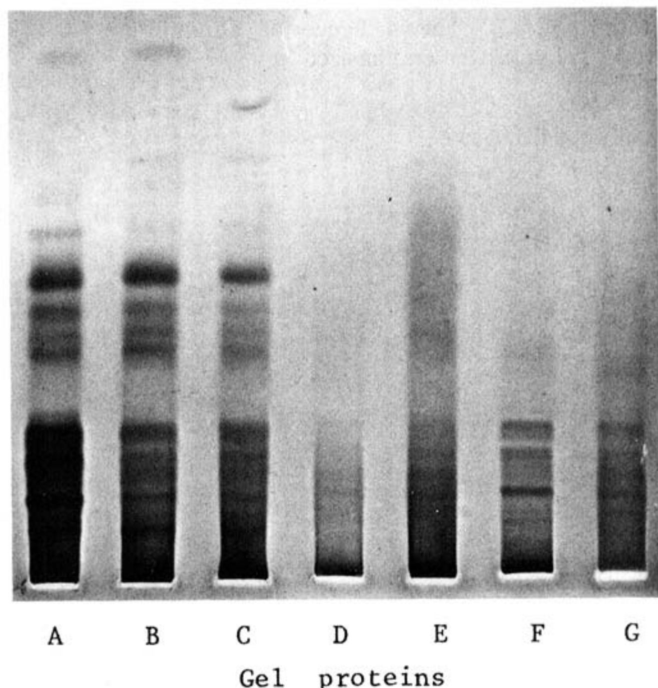

Fig. 1. Polyacrylamide gel electrophoresis (aluminum lactate buffer, pH 3.2) of gel proteins (PAGE). Gel proteins (50 mg) were dissolved in aluminum lactate buffer (1 ml); 50-μl sample deposit.

The solubilization of gliadins by 60% ethanol, with (sequence D) or without (sequence G) a previous salt-soluble protein extraction, had little effect on the gel protein yield. When the flour was extracted three times by 70% CE (sequence F) without any previous treatment, the amount of protein solubilized was higher than with any other condition, but the gel protein yielded only 13.2% of the flour protein.

The protein content (percent db) of the gel protein ranged from 9.5 to 20.1%; its moisture content (percent wet basis) ranged from 93 to 96% (Table III). Protein content of Inamine et al (14) gel was 26%.

PAGE of Gel Proteins

PAGE of gel proteins is shown in Fig. 1. Fast- (globulin-type), medium- (albumin-type), and low- (gliadin-type) moving components were present and well separated in gels A, B, and C; only a small amount of proteins seemed to stay in the starting slot. Gliadin-type fractions were visible in the patterns of gels F and G, while the separations of gels D and E were poor, most of the proteins staying in the slot or producing streaking without any band apparent.

The addition of 6*M* urea in the buffer (PAGUE) improved the separations of gels F, G, and H, which were more like those of gels A and B; separation of gel D was still poor (Fig. 2).

By dissolving the gel proteins in 0.25% sodium stearate to solubilize glutenin as Kobrehel and Bushuk (18) described, all gels did migrate in PAGE and had a similar pattern (Fig. 3). The presence of albumin-like and gliadin-like components in gel D must be emphasized.

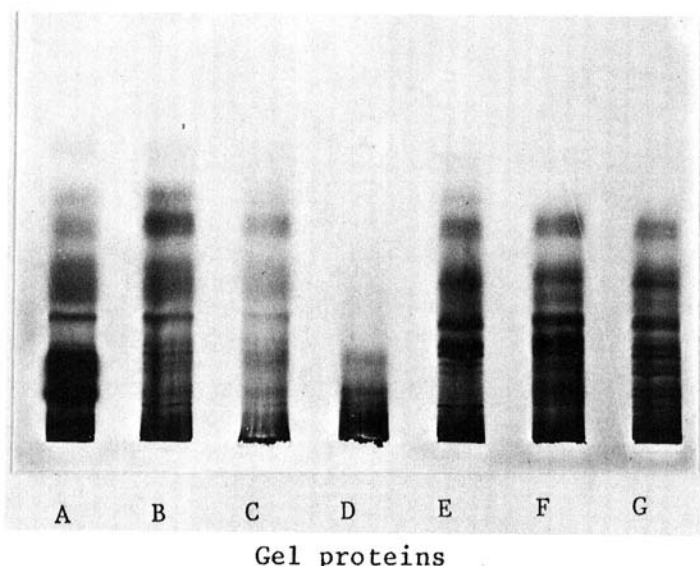

Fig. 2. Polyacrylamide gel electrophoresis (6*M* urea-aluminum lactate buffer, pH 3.2) of gel proteins (PAGUE). Gel proteins (50 mg) were dissolved in 6*M* urea-aluminum lactate buffer (1 ml); 50-μl sample deposit.

Molecular Weight Distribution of Gel Protein Subunits

Reduced gel proteins behaved on SDS-PAGE essentially the same; nevertheless, differences were visible in the electrophoretic pattern (Fig. 4). At least 14 subunits occurred in gel A; 13 in gels B, C, and F; and only 11 in gel D and 12 in gel E. Their calculated subunit molecular weights (Table IV) ranged from 12,000 to 138,000. Subunit 134,000–138,000 was absent from gel E.

Comparison of the SDS-PAGE patterns of albumin-globulin, gliadin, glutenin, and gel proteins (Fig. 5 and Table IV) confirms that each Osborne's protein group has a characteristic pattern and that high molecular subunits (higher than 100,000) were specific for glutenin. The Osborne glutenin as prepared by Feillet et al (13) and the glutenin of Bietz and Wall (17) were much the same. Depending on the electrophoresis conditions and wheat varieties (23), other investigators found the molecular weight of the largest subunit was equal to 152,000 (24), 134,000 (25), 133,000 (17), or even 104,000 (26).

The subunit composition of the gel protein is doubtlessly similar to that of glutenin, but one cannot exclude from these data that albumin-globulin and gliadin might be present in the gel protein fractions.

Amino Acid Composition

The amino acid compositions of gel proteins and those of albumin, globulin, gliadin, and glutenins are shown in Table V. The high glutamic acid and proline content and the low basic amino acid and aspartic content, which are

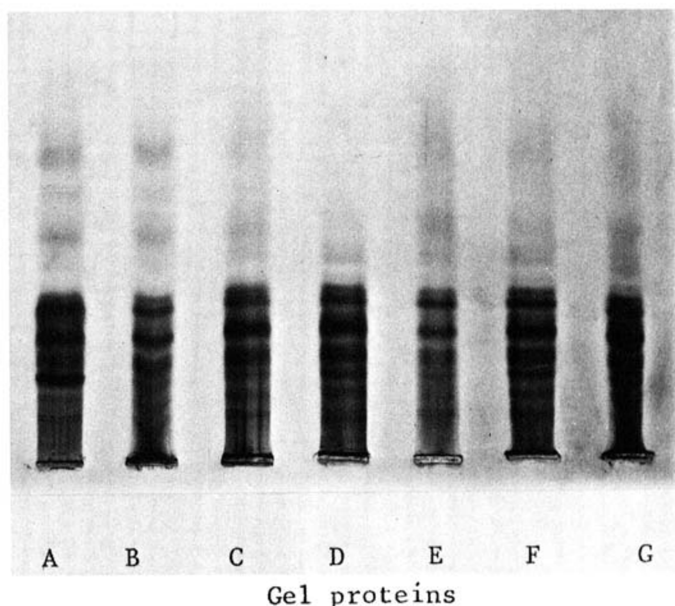

Fig. 3. Polyacrylamide gel electrophoresis (aluminum lactate buffer, pH 3.2) of gel proteins (PAGE). Gel proteins (25 mg) were dissolved in 1 ml of 0.25% sodium stearate in water; 50-μl sample deposit.

characteristics of the gliadin and glutenin composition, are also distinctive features of gel proteins. As glutenins, gel proteins tend to have larger amounts of lysine, arginine, glycine, and alanine than does gliadin, whereas gliadin has more glutamic acid, proline, and phenylalanine. Accordingly, the amino acid composition of gel proteins and glutenins was similar except for gel A, which was higher in proline, and gel D, which was higher in glutamic acid. Qualitatively and, to a considerable extent, quantitatively, Cole et al (28) found the amino acid composition of gel protein to be like that of glutenin. Compared with glutenin, that gel was higher in tyrosine and lower in glutamic acid, phenylalanine, and proline. Though small differences can be found in the various gel protein preparations, these differences were not larger than those that occur between various glutenin samples.

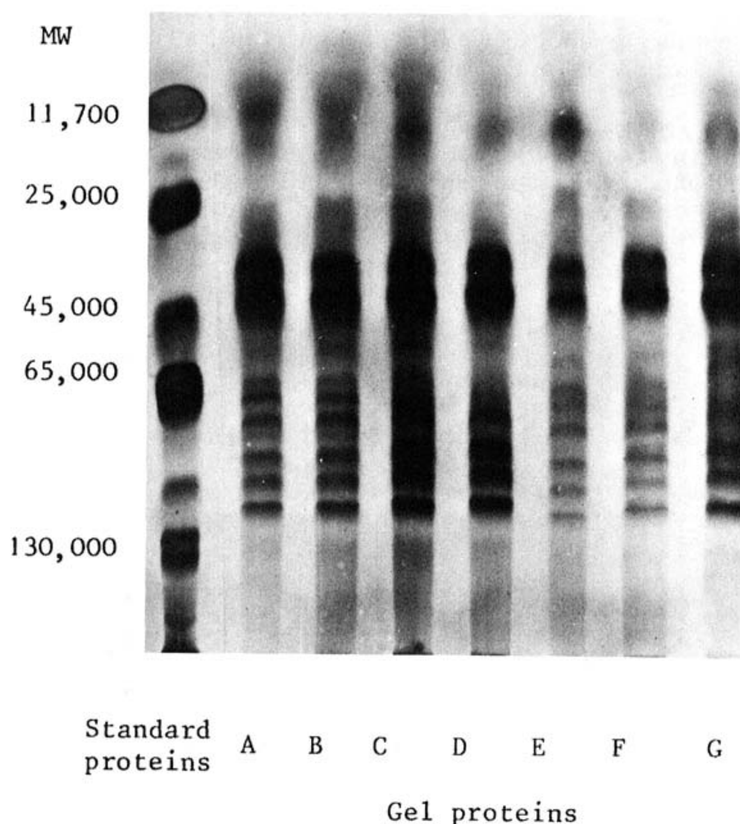

Fig. 4. SDS-polyacrylamide gel electrophoresis (tris-SDS-borate buffer, pH 8.9) of reduced gel proteins (SDS-PAGE). Gel proteins (35 mg) were dissolved in tris-SDS-borate buffer containing 1% 2-mercaptoethanol (1 ml); 50-μl sample deposit. Standard proteins are cytochrome C (CYT), chymotrypsinogen A (CHYM), ovalbumin (OVA), and bovine serum albumin (monomer and dimer) (BSA).

TABLE IV
Molecular Weights (10^3 g) of Gel Protein Subunits Evaluated by SDS-PAGE (pH 8.9)

Osborne's Protein Groups				Gel Proteins									
				A									
Albumin-Globulin[a]	Gliadin[a]	Glutenin[a]	Glutenin[b]	Total	Water Soluble	Water Insoluble	B	C	D	E	F	G	Cole et al (8)
		138	133	136	136	136	134	134	134		138	136	
	(126)[c]	126	124										
	(112)[c]			114	114	114	114	114	114	118	116	114	
102	(100)[c]	104	102	104	104	104	103	102	101	108	104	99	105
		93		93	95	93	92	92	91	94	94		95
89	(85)[c]		87	85	89		85	85		89	88	88	86
77		79	79	81	81	79	79	78	77	83	81	77	80–77
73	(71)[c]	71	71	72	72	72	72	72	72	74	74	73	70
68				67	68	68	67	67		68	67		
64		65	64			64							65
	(61)[c]	59		58	57	58	58	58	58	60	60	59	60–56
52	48		49									57	50–47
42		45	45	44	42	42	44	44	43	43	40	43	43
			42										
	37	37	36	36	35	35	36	35	35	36	35	35	39
30			33										
25			28	26	21		25	25	25	21	22	27	
		18	18	16			15	14	15	14	14	14	16–14
12	12	12	12	12	12	11							

[a]Extracted as described by Feillet et al (13).
[b]According to Bietz and Wall (17).
[c]Traces.

Water-Soluble and Water-Insoluble Gel Proteins

After dialysis against deionized water, the gel proteins have been separated by centrifugation in a water-soluble gel (WSG) and a water-insoluble gel (WIG). Depending on the gel protein extraction conditions, the amount (percent of total gel protein) of WSG ranged from 39 (gel A) to 1 (gel G) (Table III). Gels F and G, which were isolated without previous flour extraction by water or salt solution, had the lowest WSG content.

In PAGE, WIG A (or B and C) gave the same electrophoretic pattern as gel A, while the globulin-like fast component was absent from WSG A (or B and C). As gel D, WSG D and WIG D did not migrate; only streaks were visible on the pattern (Fig. 6). Detecting such large amounts of fast-, medium-, and low-moving protein components in WIG C was rather unexpected, since that fraction, which accounts for only 19% of flour protein, was isolated after 73.4% flour protein already had been extracted by water (48.4%) and 70% CE (25.0%).

The subunit composition of WIG and WSG was similar, with some minor differences, to that of the whole gel protein fraction, whatever the gel extraction conditions (Fig. 5); all of them were like the subunit composition of glutenin. High molecular weight subunits specific for glutenin were still present in the WSG protein.

The amino acid compositions of WSG A and WIG A are given in Table V. The composition of WSG A and gliadin resembled each other more than WSG A and glutenin. Composition of WIG A was almost if not identical to that of the pH-precipitation glutenin (27), which is believed to be a highly purified glutenin.

DISCUSSION

The amino acid content and the subunit composition of gel proteins and glutelins are similar, although minor differences occur between samples. In

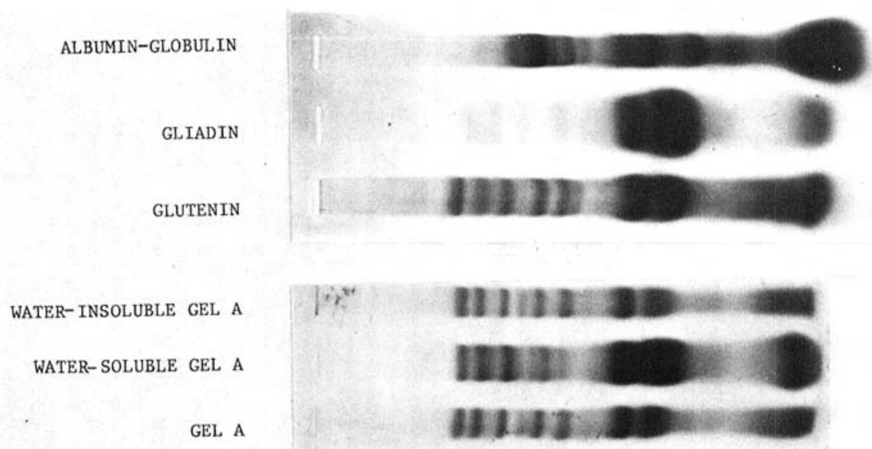

Fig. 5. SDS-polyacrylamide gel electrophoresis of protein subunits in tris-SDS-borate buffer (pH 8.9) (SDS-PAGE). Albumin-globulin, gliadin, and glutenin were extracted using Feillet's (9) method. Gel proteins (35 mg) were dissolved in 1% 2-mercaptoethanol tris-SDS-borate (1 ml); 50-μl sample deposit.

TABLE V
Amino Acid Composition of Gel Protein Fractions
(Number of Residues per 100,000 g of Protein)

Amino Acid	Osborne's Protein Group[a]				Glutenin[b]		Gel Protein Fractions						
	Albumin	Globulin	Gliadin	Glutenin	Gel Filtration	pH Precipitation	A			B	C	D	E
							Total	Water Soluble	Water Insoluble				
Lysine	35	64	7	26	13	19	12	7	19	18	18	13	22
Histidine	20	17	17	16	15	16	12	13	14	14	16	13	15
Arginine	42	54	19	28	21	26	23	18	28	26	31	23	32
Threonine	41	45	23	35	25	26	29	24	33	31	29	30	32
Serine	59	61	56	66	57	52	62	61	66	65	62	66	68
Glutamic acid	162	98	368	249	305	259	282	320	259	271	275	303	258
Aspartic acid	66	82	23	44	23	38	34	22	39	35	31	30	43
Proline	81	48	157	101	110	107	126	140	100	108	98	88	74
Glycine	82	98	42	88	63	79	70	49	80	77	80	80	87
Alanine	80	91	32	57	29	38	42	28	46	42	40	40	56
Valine	65	62	43	53	36	40	38	33	39	39	40	39	43
Methionine	17	16	12	13	12	12	12	8	13	13	12	11	13
Isoleucine	35	36	38	36	28	27	30	30	31	31	31	30	31
Leucine	70	74	65	69	60	60	60	62	66	64	62	62	63
Tyrosine	26	24	21	26	20	28	22	19	24	23	25	24	24
Phenylalanine	28	28	42	34	38	29	31	36	34	33	32	31	31

[a] From Feillet (9).
[b] From Orth and Bushuk (27).

PAGE, the behavior of gel proteins are totally different. Albumin-like, globulin-like, and gliadin-like components are clearly visible in the electrophoretic pattern of gels A, B, and C, while gels D, F, and G behave more like a glutenin fraction. These differences decrease in PAGUE, and disappear when proteins are solubilized with 0.25% sodium stearate.

These results were unexpected and are not easy to explain. The physicochemical properties of the WIGs are even more paradoxical. From the subunit composition and the amino acid distribution, there is strong evidence that WIG is a highly purified glutenin fraction. From PAGE, there is evidence that WIG seems highly contaminated by albumins, globulins, and gliadins. The presence of high molecular weight subunits in the reduced WSG presents another problem.

The presence of free soluble proteins and free gliadins in gel A is possible. With regard to the isolation conditions, it is unlikely in gels B and C and still more in WIG.

Others (12,27,29) have noted the occurrence of soluble proteins and gliadins in glutenin preparations, but whether these proteins were trapped in or bound to the protein matrix or a part of the glutenin structure was not clear. Gueguen et al (30) hypothesized the occurrence of three classes of glutenin (salt soluble-like, gliadin-like, and specific).

Comparing the physicochemical properties of gel proteins to those of glutenins is not easy, because the term "glutenin" has been applied to proteins isolated in many different ways (31). Orth and Bushuk (27) considered the pH precipitation glutenin (pH - G) as a highly purified glutenin preparation. We think that pH - G and WIG likely consist of a complex of the same protein units, which would be

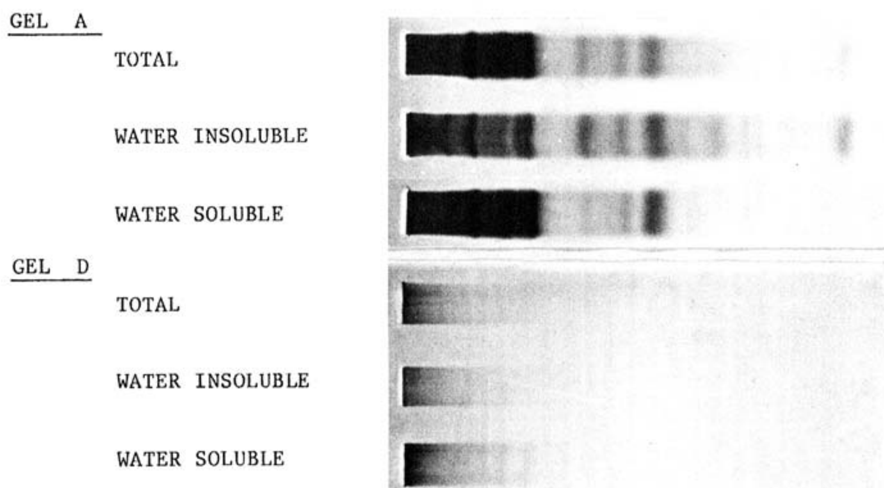

Fig. 6. Polyacrylamide gel electrophoresis (aluminum lactate buffer, pH 3.2) of water-soluble, water-insoluble, and total gel proteins A and D. Gel proteins (50 mg water-insoluble and total gel proteins, 10 mg water-soluble gel) were dissolved in aluminum lactate buffer (1 ml); 50-μl sample deposit.

more or less strongly associated together through hydrophobic and hydrogen bonds. That WIG complex would be dissociated during PAGE into albumins, globulins, gliadins, and high molecular weight, specific glutenin proteins. After reduction by β-mercaptoethanol, the subunits with molecular weights over 100,000 would be released from that specific glutenin fraction. Because of the extraction conditions, and by a mechanism that has yet to be explained, pH - G (and other glutenin preparations) would not be dissociated in PAGE and would not enter into the gel except when sodium stearate is added to solubilize that glutenin.

In water, an equilibrium between the water-soluble and the water-insoluble forms of that complex would occur with possibly higher content of soluble proteins in the water-soluble complex than in the insoluble. Therefore, a part of the specific glutenin protein was found in the supernatant after dialysis of gel proteins against water.

The main differences between glutenin and gel protein preparations are the strength and the nature of the associations between essentially the same protein components. More work is necessary to understand the structure and the properties of that complex and to isolate the specific glutenin protein.

Acknowledgments

We are grateful to Y. Lozano, Groupement d'Etudes et de Recherches pour le Developpement de l'Agronomie Tropicale, Montpellier, France, who performed the amino acid analysis.

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[Received January 18, 1978. Accepted March 2, 1978]