Protein Isolate from an Experimental High-Protein Wheat and Flour

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An experimental high-protein (25% protein, N × 5.7 dry basis) spring wheat (ND 643) and flour from that wheat were used to make protein isolate by alkaline extraction. Optimal extraction of protein was at pH 10.7 in 0.03 N sodium hydroxide with a solvent:solid ratio of 6:1. The alkaline extract after centrifugation was adjusted to optimal pH of 6.2 to yield a precipitate and a supernatant. Bran was separated from starch by screening the second alkaline dispersion. The protein contents of the isolates from ground wheat and flour were 90 and 95%, respectively, and accounted for 65 and 68% of the total nitrogen of the starting wheat and flour. Yields of protein isolate were 18 and 16%, respectively, of starting high-protein wheat and flour compared with 10% of starting wheat and flour from a typical hard red winter wheat. Protein isolate had a nitrogen solubility of 96% at pH 2.6 and a minimum solubility of 5.7% at pH 5.8, a hydration capacity of 2.5, an emulsifying activity of 51%, and an emulsion stability of 50%.

INTRODUCTION

Industrial manufacture of gluten and starch from wheat flour includes the Martin process (Knight, 1965) and the continuous batter process (Anderson et al., 1958, 1960); both processes depend primarily on physical separation of starch and gluten particles formed in neutral systems. Slotter and Langford (1944) used a 24-h sulfurous acid steep of whole wheat kernel to obtain denatured gluten of 26–37% purity and represents 49–55% of the total wheat protein. Alkaline extraction of protein in wheat flour by sodium hydroxide solution (Dimler et al., 1944; Crozier, 1959) and by an ammonia process (Fellers et al., 1969; Johnson and Fellers, 1971; Phillips and Sallans, 1966) was reported. Fellers et al. (1966) and Saunders et al. (1972) described an alkaline method for extracting protein from wheat bran and shorts. Wu and Sexson (1974a,b) prepared protein concentrate from high-protein wheat (17%, N × 5.7 dry basis) by an alkaline extraction process.

Khan et al. (1989) reported that ND 643, an experimental hard red spring (HRS) wheat derived from crosses of *Triticum turgidum* var. Dicoccoides (a high-protein wild tetraploid) with Len and RL 4352-1 (HRS), had substantially higher protein content than its HRS wheat parents. A high-protein wheat or flour will yield more protein isolate per unit weight of wheat or flour than normal protein wheat or flour, if similar percentages of protein are extracted from high-protein and normal protein varieties. This paper investigates the effect of pH and solvent-to-solid ratio on extracting ND 643 wheat protein and the effect of pH on precipitation of wheat protein from the alkaline extract as well as yield, composition, and functional properties of protein isolate and byproducts.

MATERIALS AND METHODS

Wheat and Flour. ND 643 is an experimental high-protein spring wheat from Fargo, ND. Our sample, grown in 1990, had a protein content of 24.6% (nitrogen × 5.7, dry basis). A typical hard red winter wheat from Kansas is Scout 66. Our sample, grown in 1986, had a protein content of 13.8% on a dry basis.

AACC (1983) Approved Method 26-10A suggested 16% moisture content for tempering hard wheats (spring and winter). Each wheat was tempered to 15.5% moisture overnight and then to 16.0% for 0.5 h before it was milled in a Buhler pneumatic laboratory flour mill (Buhler, Uzwil, Switzerland) in a 25 °C and 48% relative humidity milling laboratory. Flour was the combined break and reduction flour fractions from the Buhler mill.

For alkaline extraction, each wheat was ground twice in a hammer mill equipped with a screen containing 1.6 mm diameter holes.

Protein Extraction. Ground ND 643 wheat (5 g each) was stirred magnetically with 30 mL of sodium hydroxide (0.025, 0.03, 0.04, and 0.05 N) for 25 min, and each slurry was centrifuged for 10 min at 3300*g* in a Sorvall RC-5B centrifuge (Du Pont Instruments, Wilmington, DE). A portion of the total supernatant volume was analyzed for nitrogen by micro-Kjeldahl.

Ground ND 643 wheat was extracted at solvent:solid ratios of 3:1 (0.06 N sodium hydroxide), 4:1 (0.045 N sodium hydroxide), 6:1 (0.03 N sodium hydroxide), and 10:1 (0.02 N sodium hydroxide). The pH of each slurry was adjusted between 11.1 and 11.5 to minimize the effect of pH on extraction of wheat protein. After stirring for 25 min, each slurry was centrifuged for 10 min at 3300*g*. An aliquot of the supernatant was analyzed for nitrogen by micro-Kjeldahl.

Alkaline wheat extract (4 mL each) from 0.03 N sodium hydroxide (solvent:solid ratio = 6:1) was pipetted into 50-mL centrifuge tubes, and 0.03 N hydrochloric acid was added to final pH values of 5.6, 6.0, 6.2, 6.4, and 7.0, respectively. The tubes were centrifuged at 3300*g* for 10 min to obtain an acceptable pellet, and a portion of the supernatant was analyzed for nitrogen.

Protein Isolate from Whole Ground Wheat. Ground wheat (25 g) and 150 mL of 0.03 N sodium hydroxide were stirred magnetically for 25 min (Figure 1). The slurry at pH 10.7 was centrifuged at 3300*g* in a Sorvall centrifuge for 20 min, and the supernatant was adjusted to pH 6.2 to precipitate almost all of the protein. The mixture at pH 6.2 was centrifuged at 3300*g* for 20 min to yield a first extract precipitate (protein isolate) and a first extract supernatant.

The alkaline residue from the first extraction was redispersed in 150 mL of 0.03 N sodium hydroxide. This mixture was stirred for 25 min and passed through a bolting cloth with 88-μm openings. The slurry that passed through the cloth was centrifuged at 3300*g* for 20 min to obtain a supernatant, a starch layer, and a layer above starch. The supernatant was adjusted to pH 6.2 to precipitate most of the protein. The mixture was centrifuged at 3300*g* for 20 min to give a second extract precipitate and a second extract supernatant. The bran that remained on the bolting cloth, the starch, and the layer above the starch were each neutralized. Each fraction was freeze-dried without dialysis.

Protein Isolate from Flour. Wheat flour (25 g) and 150 mL of 0.03 N sodium hydroxide were stirred for 25 min magnetically. The slurry was centrifuged at 3300*g* for 20 min, and the supernatant was adjusted to pH 6.2 to precipitate most of the

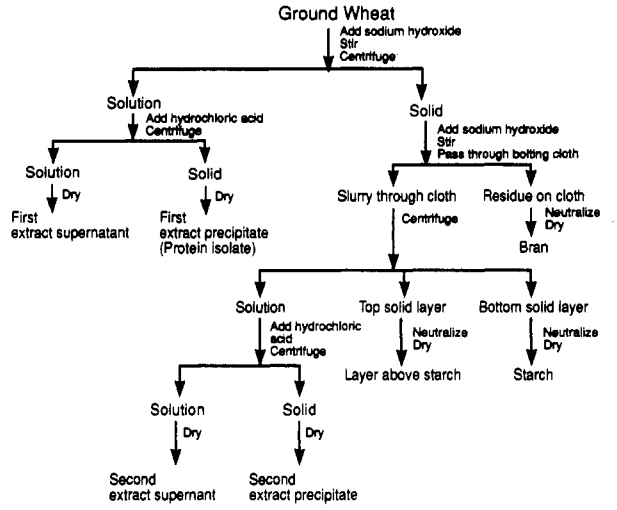

Figure 1. Schematic diagram for preparing protein isolate and byproducts from ground wheat.

protein. The mixture at pH 6.2 was centrifuged to give a first extract precipitate (protein isolate) and a first extract supernatant. The residue after first extraction was dispersed in 150 mL of 0.03 N sodium hydroxide. The mixture was stirred for 25 min and centrifuged to yield a second extract and an alkaline residue. The second extract was adjusted to pH 6.2. All fractions were freeze-dried.

Nitrogen solubility was measured by mixing 0.1 g of protein isolate with 10 mL of water at various pH values by addition of hydrochloric acid or sodium hydroxide solutions. The mixture was stirred magnetically for 25 min and centrifuged at 1300*g* for 20 min, and the supernatant was analyzed for nitrogen by micro-Kjeldahl. Emulsifying activity and emulsion stability were determined according to the method of Yasumatsu et al. (1972) for a simple system; only soybean oil was added to the protein isolate. Hydration capacity was determined according to AACC (1983) Approved Method 56-20.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out on 12% (w/v) acrylamide gel according to a modified Laemmli (1970) procedure. The samples were electrophoresed at 35 mA unitl the tracking dye migrated near the end of the gel. The gel was stained with 0.1% (w/v) Coomassie Brilliant Blue R250 in 12.5% (w/v) trichloroacetic acid and then destained until the gel background was clear for photography.

Analyses. Nitrogen by micro-Kjeldahl, fat by petroleum ether extraction, ash from 2-h treatment at 600 °C, moisture from 2 h at 135 °C, and starch by enzymatic method were determined by AACC (1983) Approved Methods 46-13, 30-26, 08-03, 44-19, and 76-11, respectively. Protein was calculated from triplicate micro-Kjeldahl analysis as N × 5.7. Moisture of wheat grain for milling in a Buhler mill was determined in triplicate by a Brabender moisture/volatiles tester, type SAS (C. W. Brabender Instruments Inc., Hackensack, NJ) after wheat was cracked in an Enterprise Model 00 grain mill (Philadelphia, PA).

Samples for amino acid analyses were hydrolyzed with 6 N hydrochloric acid for 4 h at 145 °C (Gehrke et al., 1987). Cystine and methionine were oxidized by performic acid (Moore, 1963) before hydrolysis. Amino acids were quantified by cation-exchange chromatography using a Beckman 6300 amino acid analyzer (Beckman Instruments Inc., San Raman, CA). After enzymatic hydrolysis by pronase, tryptophan was determined using a colorimetric method (Spies and Chambers, 1949; Holz, 1972).

RESULTS AND DISCUSSION

Effect of pH. Table I shows the effect of pH on extraction of ND 643 wheat protein with a solvent:ground wheat ratio of 6:1. Optimum pH was 10.7 where 74% of total wheat protein was extracted. No increase in percentage of wheat protein was observed at pH 11.2, and a small decrease in percentage of wheat protein extracted was observed at pH 11.6 compared with pH 10.7.

Table I. Effect of pH on Extraction of ND 643 Wheat Protein[a]

NaOH normality	slurry pH	% N extracted[b]
0.025	10.3	57 (3)
0.03	10.7	74 (6)
0.04	11.2	74 (3)
0.05	11.6	72 (4)

[a] 5 g of ground wheat + 30 mL solvent. [b] Standard deviation from micro-Kjeldahl in triplicate is in parentheses. Standard deviation from duplicate extraction with averaged micro-Kjeldahl values was considerably lower than that from triplicate micro-Kjeldahl (0.4 vs 1.5 for solvent:solid ratio of 10:1 in Table II).

Table II. Effect of Solvent:Solid Ratio on Extraction of ND 643 Wheat Protein

solvent:solid ratio	slurry pH	% N extracted
3:1[a]	11.3	44.9 (2.8)[b]
4:1	11.1	64.1 (3.3)
6:1	11.4	67.3 (1.6)
10:1	11.5	72.0 (1.5)

[a] 0.06, 0.045, 0.03, and 0.02 N NaOH were used, respectively, and pH was adjusted between 11.1 and 11.5. [b] Values in parentheses were standard deviations of triplicate micro-Kjeldahl. Standard deviation from duplicate extraction with averaged micro-Kjeldahl values was 0.4 for solvent:solid ratio of 10:1.

Table III. Effect of Precipitation pH on Alkaline Extract[a] of ND 643 Wheat

pH	% N precipitated
5.6	75 (17)[b]
6.0	77 (7)
6.2	80 (14)
6.4	76 (2)
7.0	72 (2)

[a] Solvent:solid ratio = 6:1, 0.03 N NaOH. [b] Values in parentheses were standard deviations of triplicate micro-Kjeldahl.

Effect of Solvent:Solid Ratio. Table II shows the effect of the solvent:solid ratio on extraction of ND 643 wheat protein at pH 11.1–11.5. A large increase of percent protein extracted was observed when the solvent:solid ratio increased from 3:1 to 4:1. Further increase in the solvent:solid ratio resulted in smaller increases of percent protein extracted, because maximum percentage of protein extracted was approached. An optimal solvent:solid ratio of 6:1 was chosen to obtain relatively high percentage of protein extracted without a large volume of solvent.

Table III gives the percentage of nitrogen precipitated from pH 10.7 extract (0.03 N NaOH, solvent:solid ratio = 6:1) of ND 643 wheat. The best pH to recover wheat protein from alkaline extract was 6.2, where 80% of nitrogen was recovered by precipitation.

Products from Wheat. Yield and composition of protein isolate (first extract precipitate) and byproducts from ND 643 and Scout 66 wheats are shown in Table IV. Yield of protein isolate from ND 643 at 18% was much higher than yield from Scout 66 at 10%. The protein content of isolate was also higher for ND 643 compared with Scout 66. The yield and protein content of second extract precipitate were also higher for ND 643 compared with Scout 66. The yields of bran and the layer above the starch were similar for ND 643 and Scout 66, but the yield of starch was considerably lower for ND 643 compared with Scout 66. The protein content of starch and the layer above the starch fractions can be lowered by washing with water before neutralization. Fat and ash contents of corresponding fractions from ND 643 and Scout 66 were in general not greatly different. The relatively high content

Table IV. Yield and Composition of Protein Isolate and Byproducts from ND 643 (ND) and Scout 66 (S) Wheats (Percent Dry Basis)

fraction	yield, %		protein (N × 5.7)		fat		ash		starch		% total N	
	ND	S	ND	S	ND	S	ND	S	ND	S	ND	S
whole wheat			24.6 (0.4)	13.8 (0.1)	2.1 (0.0)	1.6 (0.1)	2.3 (0.0)	1.9 (0.0)	54.9	63.2		
first extract precipitate	17.8 (0.6)[a]	9.8 (0.1)	89.9 (2.6)	85.1 (2.5)	1.7 (0.0)	3.7 (0.1)	0.7 (0.1)	0.9 (0.1)	nd[b]	nd	64.8 (0.5)	60.3 (1.3)
first extract supernatant	7.6 (0.0)	6.6 (0.0)	32.8 (0.6)	27.2 (0.1)	0.3	0.6 (0.0)	13.6 (0.2)	14.3 (0.1)	nd	nd	10.1 (0.1)	13.1 (0.1)
second extract precipitate	3.4	1.3	83.9 (2.0)	70.6 (0.4)	nd	nd	0.6 (0.1)	0.5 (0.1)	nd	nd	11.6	6.7
second extract supernatant	1.6	1.2	41.9 (3.3)	28.6 (0.5)	nd	nd	20.1 (0.4)	23.1 (0.3)	nd	nd	2.7	2.5
bran	21.6	22.5	6.8 (0.4)	5.2 (0.1)	1.5 (0.3)	1.1 (0.4)	3.4 (0.0)	3.7 (0.1)	32.1	39.2	6.0	8.5
layer above starch	13.5	14.5	2.9 (0.4)	1.2 (0.1)	1.4	0.5 (0.0)	3.6 (0.0)	2.3 (0.1)	78.9	77.8	1.6	1.3
starch	35.1	44.2	0.5 (0.0)	0.3 (0.0)	0.3 (0.0)	0.2 (0.1)	1.8 (0.1)	1.0 (0.0)	91.9	87.4	0.7	0.8
total	100.6	100.1									97.5	93.2

[a] Values in parentheses are standard deviations of duplicate extractions (first extract precipitate and first extract supernatant), of duplicate analyses (fat and ash), and of triplicate analyses (nitrogen). [b] nd, not determined.

Table V. Yield and Composition of Protein Isolate and Byproducts from ND 643 (ND) and Scout 66 (S) Flours (Percent Dry Basis)

fraction	yield, %		protein (N × 5.7)		fat		ash		starch		% total N	
	ND	S	ND	S	ND	S	ND	S	ND	S	ND	S
wheat flour			22.1 (1.4)	12.8 (0.1)	0.8 (0.0)	0.8 (0.1)	0.7 (0.0)	0.5 (0.0)	69.2	77.6		
first extract precipitate	15.7 (0.3)[a]	9.9 (0.0)	95.0 (0.3)	97.0 (0.9)	0.5 (0.1)	0.6 (0.0)	0.5 (0.1)	0.5 (0.0)	nd[b]	nd	67.7 (0.9)	74.0 (1.0)
first extract supernatant	5.4 (0.1)	4.5 (0.0)	37.5 (1.2)	32.9 (0.1)	0.5 (0.1)	0.5	17.7 (0.1)	18.2 (0.1)	nd	nd	9.2 (0.5)	11.5 (0.1)
second extract	5.0	2.0	71.7 (0.6)	51.6 (1.6)	nd	nd	6.5 (0.1)	21.7 (0.1)	nd	nd	16.3	7.9
alkaline residue	74.0	83.6	1.3 (0.1)	0.6 (0.0)	0.2 (0.0)	0.2 (0.0)	0.8 (0.0)	0.5 (0.0)	89.2	89.5	4.2	3.6
total	100.1	100.0									97.4	97.0

[a] Values in parentheses are standard deviations of duplicate extractions (first extract precipitate and first extract supernatant), of duplicate analyses (fat and ash), and of triplicate analyses (nitrogen). [b] nd, not determined.

of ash for first and second extract supernatants were derived in part from sodium chloride formed by neutralization. The high protein content of ND 643 wheat was balanced by lower starch content compared with Scout 66. Protein isolate and second extract precipitate from ND 643 accounted for higher percentages of total nitrogen than those from Scout 66.

Products from Flour. Table V lists the yield and composition of protein isolate and byproducts from ND 643 and Scout 66 flours. The yield of protein isolate (first extract precipitate) was much higher for ND 643 at 16% compared with Scout 66 at 10%. ND 643 flour gave higher yield of second extract but lower yield of alkaline residue compared with Scout 66. The higher protein content of ND 643 flour compared with Scout 66 flour was also reflected in higher protein contents for first extract supernatant, second extract, and alkaline residue. The protein content of alkaline residue can be reduced by washing with water. Fat contents of wheat flour and fractions were low and similar for corresponding fractions from both flours. Ash contents of corresponding fractions from both flours were in general similar. Relatively high contents of ash for first extract supernatant and second extract were due in part from sodium chloride formed by neutralization. The high protein content for ND 643 flour was balanced by lower starch content. Protein isolate and first extract supernatant accounted for lower percentages of total nitrogen for ND 643 compared with Scout 66. Second extract accounted for higher percentage of total nitrogen for ND 643 than Scout 66, however.

SDS–PAGE of Wheat, Flour, and Protein Isolates. Figure 2 shows SDS–PAGE of ND 643 wheat, ND 643 wheat protein isolate, ND 643 wheat first extract supernatant, ND 643 flour, ND 643 flour protein isolate, ND 643 flour first extract supernatant, Scout 66 flour, and Scout 66 flour protein isolate. Both wheats, flours, wheat protein isolates, and flour protein isolates had similar patterns including high molecular weight glutenin subunits (MW above 66 000), except ND 643 wheat protein isolate also had some streaking. ND 643 wheat and flour first extract supernatants had similar patterns, and they had

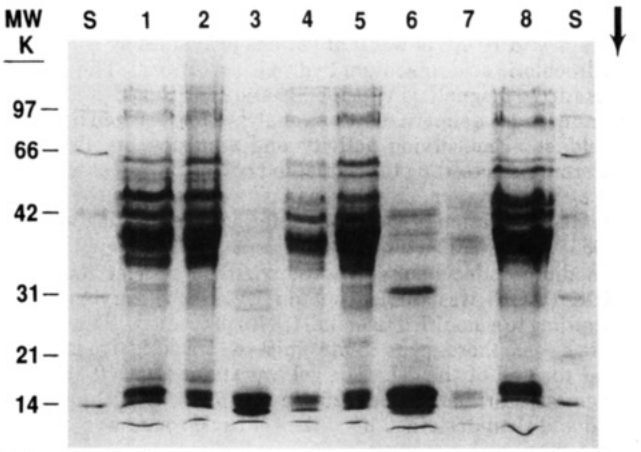

Figure 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis. (Lane S) Standard proteins; (lane 1) ND 643 wheat; (lane 2) ND 643 wheat protein isolate; (lane 3) ND 643 wheat first extract supernatant; (lane 4) ND 643 flour; (lane 5) ND 643 flour protein isolate; (lane 6) ND 643 flour first extract supernatant; (lane 7) Scout 66 flour; (lane 8) Scout 66 flour protein isolate.

relatively more faster moving bands (lower in molecular weights) than the protein isolates. Khan et al. (1992) showed the SDS–PAGE patterns of ND 643 gliadin and glutenin consisted predominantly of bands above 31 000 in molecular weight and the ND 643 albumin and globulins had more bands below 31 000 in molecular weight. Our SDS–PAGE pattern for ND 643 wheat also agreed with that from Khan et al. (1992). Our protein isolates are thus mostly gluten (gliadin plus glutenin), and the first extract supernatants are mostly albumin plus globulin on the basis of our SDS–PAGE patterns and those of Khan et al. (1992). Table III showed that the maximum amount of wheat protein was precipitated at pH 6.2, and Wrigley and Bietz (1988) stated that gluten proteins were usually insoluble near their isoelectric point. Therefore, wheat protein isolates and wheat flour protein isolates are mostly gluten on the basis of solubility properties.

Table VI. Amino Acid Composition[a] of ND 643 Wheat, Flour, and Fractions

	wheat					flour	
			first extract				
amino acid	HRS[b]	whole	precipitate	supernatant	bran	white	first extract precipitate
methionine	1.4	1.5	1.3	1.1	1.5	1.5	1.3
cystine	2.4	2.2	1.8	3.2	2.1	2.4	1.8
lysine	2.4	2.2	2.0	3.1	4.4	1.9	1.7
arginine	4.3	4.3	4.1	5.1	7.5	3.7	3.5
tryptophan	1.2	1.0	1.0	1.1	1.0	1.2	1.0
tyrosine	2.7	2.8	3.5	2.3	3.2	3.2	3.8
threonine	2.6	2.5	2.4	2.8	3.3	2.5	2.4
serine	4.0	3.6	4.1	3.4	3.7	4.0	4.0
phenylalanine	4.4	4.6	4.6	2.9	4.5	5.3	4.8
aspartic	4.9	4.2	3.6	7.5	6.7	3.7	3.3
glutamic	30.0	28.7	30.9	18.1	17.1	34.5	32.8
proline	10.2	11.0	11.3	6.9	6.6	13.4	12.3
glycine	3.8	3.6	3.6	3.7	4.9	3.5	3.4
alanine	3.4	3.1	2.8	4.2	4.7	2.9	2.8
valine	4.1	4.2	4.1	4.1	5.6	4.3	3.8
isoleucine	3.3	3.5	3.5	2.7	3.7	3.8	3.4
leucine	6.3	6.4	6.5	5.2	7.0	7.2	6.6
histidine	2.0	2.2	2.2	2.2	2.7	2.3	2.1

[a] Grams of amino acids per 16 g of nitrogen. [b] Average of 4–10 samples (Drake et al., 1989) of hard red spring wheats.

Amino Acid Composition. Table VI gives the amino acid composition of ND 643 wheat, flour, and fractions compared with the average value for hard red spring wheat expressed in grams of amino acids per 16 g of nitrogen. ND 643 wheat had an amino acid composition close to the average of hard red spring wheat; no significant decrease in lysine content was observed. The first extract precipitate from wheat had an amino acid composition similar to that of ND 643 wheat. The first extract supernatant had higher cystine, lysine, arginine, threonine, aspartic acid, and alanine but lower tyrosine, serine, phenylalanine, glutamic acid, proline, isoleucine, and leucine levels than first extract precipitate. Bran had higher lysine, arginine, threonine, aspartic acid, glycine, alanine, valine, and histidine but lower glutamic acid and proline contents than first extract precipitate.

ND 643 flour had an amino acid composition similar to that of ND 643 wheat except the former had lower lysine but higher glutamic acid and proline contents. First extract precipitate from flour had an amino acid composition similar to that of flour except the former had higher cystine and tyrosine.

Wrigley and Bietz (1988) showed that wheat albumin and globulin had higher lysine but lower glutamic acid plus glutamine levels compared with gluten. Amino acid compositions from Table VI also suggest that first extract precipitates (protein isolate) are mostly gluten and that first extract supernatant is mostly albumin and globulin.

Nitrogen Solubility. The solubility of ND 643 wheat flour protein isolate at various pH values (Figure 3) indicates good solubility of the isolate below pH 4 and above pH 8.5. Minimum solubility was close to pH 6. The excellent solubility of the protein isolate around pH 2.5 suggests that the isolate may have potential for protein fortification of carbonated beverages.

Hydration Capacity, Emulsifying Activity, and Emulsion Stability. The hydration capacity of wheat protein isolate was close to that of wheat flour protein isolate and somewhat lower than that of soy protein isolate (Table VII). Emulsifying activities of wheat protein isolate and of wheat flour protein isolate were better than that of soy protein isolate. Also, emulsion stabilities of wheat protein isolate and of wheat flour protein isolate were better than that for soy protein isolate. The good emulsifying properties of wheat flour protein isolate and wheat protein isolate suggest possible applications in frankfurter, sausage, and emulsified meat. Hydration capacity values of wheat protein isolate and wheat flour protein isolate suggest potential as a water binder.

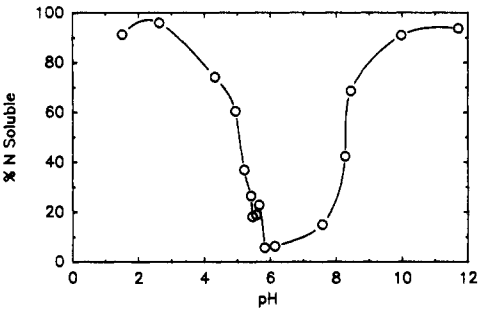

Figure 3. Nitrogen solubility of ND 643 wheat flour protein isolates at various pH values. Protein isolate (0.1 g) was stirred with 10 mL of either hydrochloric acid or sodium hydroxide solution at different pH values.

Table VII. Functional Properties of ND 643 Wheat Protein Isolate, ND 643 Wheat Flour Protein Isolate, and Soy Protein Isolate

	hydration capacity	emulsifying activity, %	emulsion stability, %
wheat protein isolate	2.5 (0.1)[a]	51 (2)	50 (3)
wheat flour protein isolate	2.4 (0.0)	51 (1)	59 (14)
soy protein isolate	3.1 (0.0)	47 (0)	47 (0)

[a] Values in parentheses were standard deviations from duplicate.

Conclusions. ND 643 wheat and flour are attractive as starting materials for protein isolate production, because both wheat and flour have very high protein content compared with normal hard wheats and because a high percentage of protein can be extracted and recovered. The protein isolate has good functional properties including nitrogen solubility and may have potential for various food uses.

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