Preparation of Protein Concentrate from Normal and High-Protein Wheats

Y. Victor Wu* and Kenneth R. Sexson

An alkaline extraction process was developed to produce protein concentrates from high- and normal-protein wheats. Different solvents, various pH values, wheat-to-solvent ratios, and particle size of wheat were studied. Optimum extraction was at pH 10.8 in 0.03 *N* sodium hydroxide with 100 g of wheat per 600 ml of solvent. After centrifugation each of two alkaline extractions was adjusted to pH 6 to yield a precipitate and a supernatant. Bran was removed from starch and protein by screening the second alkaline dispersion. The protein content (nitrogen × 5.7) of the concentrate varied between 83 and 92%, depending on particle size and protein content of the wheat used, and accounted for 52 to 64% of the total wheat protein. Prime starch (0.3% protein) was also produced in good yield. Higher yields of protein were obtained from wheat containing higher protein.

Hammonds and Call (1972) estimated a market potential for functional protein of approximately 3.1 billion lb annually. The relative high cost of animal proteins compared with plant proteins suggests an increasing market for the latter. Wheat gluten is a plant protein used in baked goods and other foods because it has unique functional properties and bland flavor (Anderson and Vojnovich, 1962). However, production of wheat gluten in this country at 30–40 million lb per year (Inglett, 1974) is not enough to meet demand, so about half the domestic consumption is imported. Producing a protein concentrate from high-protein wheat could help meet the domestic requirement.

Currently, industrial manufacture of gluten and starch from wheat flour depends primarily on physical separation of starch and gluten particles formed in neutral systems (Knight, 1965). In the Martin process (Knight, 1965), dough is formed by mixing wheat flour and water, starch is washed away, and the gluten is retained as a single coherent mass. The continuous batter process (Anderson et al., 1958, 1960) disperses the dough in water and recovers the divided gluten particles on a sieve.

Slotter and Langford (1944) developed a method for extracting starch and gluten from the whole wheat kernel, which is analogous to that used in the wet milling of corn. During the 24-hr steep, sulfurous acid denatures the gluten, which has a purity of 26 to 37% and represents 49 to 55% of the total wheat protein.

In one alkaline extraction method explored, the protein in wheat flour is dissolved in a sodium hydroxide solution and then precipitated by neutralization with acid (Dimler et al., 1944; Crozier, 1959). Laboratory-scale protein–starch separation from wheat flour by an ammonia process resulted in a protein fraction with 20 to 40% protein (Fellers et al., 1969; Johnston and Fellers, 1971). Protein concentrate containing 70–80% protein from first clear flour by another ammonia process was reported by Phillips and Sallans (1966). Fellers et al. (1966) described an alkaline method for extracting protein from wheat bran and shorts, and Saunders et al. (1972) also prepared a protein concentrate from wheat shorts by alkaline extraction. These two methods gave products with 40–86% protein and recovered 23–60% of the total protein.

Since whole wheat has a higher protein content and a better amino acid composition than wheat flour and since no practical process has been described to make gluten or protein concentrate from whole wheat, we investigated a number of factors affecting extraction of protein concentrates from whole wheat with normal- and high-protein contents.

Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604.

MATERIALS AND METHODS

Wheat. A high-protein hard wheat, NE 701136, was a gift from V. A. Johnson, Department of Agronomy, University of Nebraska, Lincoln. It had a protein content of 17.4% (nitrogen × 5.7) on a dry weight basis. The wheat is derived from Atlas 66 (soft red winter wheat of high protein) crossed with hard red winter wheats (Comanche and Lancer) from Nebraska, and the seed was produced in 1972 at Yuma, Ariz. The other wheat was Parker, a hard red winter wheat grown also in 1972 but in Illinois near Peoria. It had a protein content of 11.4%, dry basis.

Each wheat was ground in a hammer mill equipped with a screen containing $\frac{1}{16}$-in. holes. Grain ground once in the hammer mill is designated as 1× and that ground twice, 2×. Forty-two percent of the 1× and 64% of the 2× NE 701136 wheat passed through a 100-mesh screen.

Protein Extraction. To test the effectiveness of various extraction solvents, ground wheat was mixed with a solvent at a specified weight to volume ratio, stirred for 25 min, and then centrifuged for 10 min at 3300*g* in a Sorvall laboratory centrifuge. A portion of the supernatant was analyzed for nitrogen by a micro-Kjeldahl method, and the remaining supernatant was freeze dried.

Protein Concentrate. In making the final protein concentrate (first extract precipitate) and by-products, ground wheat (150 g) and 900 ml of 0.03 *N* sodium hydroxide were stirred for 25 min magnetically (Figure 1). The slurry at pH 10.8 was centrifuged at 3300*g* in a Lourdes centrifuge for 30 min, and the supernatant was adjusted to pH 6 with 6 *N* hydrochloric acid to precipitate almost all the protein. The mixture was centrifuged at 3300*g* for 20 min to yield a precipitate and a supernatant. After freeze drying the precipitate and supernatant, the first extract precipitate and supernatant solids were obtained.

The alkaline residue from the first centrifugation was redispersed to original volume and pH by addition of water and sodium hydroxide solution (Figure 1). This mixture was stirred and passed through 100-mesh bolting cloth. The slurry that passed through the cloth was centrifuged to obtain a solution, a starch layer, and a layer above starch. The centrifuged solution was adjusted to pH 6 to precipitate most of the protein. The mixture was centrifuged to yield a precipitate and a supernatant, and they were freeze dried to obtain a second extract precipitate and supernatant solids. The alkaline starch, the layer above the starch, and the bran that remained on bolting cloth were each neutralized and freeze dried.

RESULTS AND DISCUSSION

Solvent. Various solvents were used to extract NE

Table I. Effect of Various Solvents on Extraction of Wheat Protein[a]

Solvent	pH of slurry		% of wheat protein extracted		Protein (nitrogen × 5.7) in extracted solids, 1×, %
	1×[b]	2×	1×	2×	
0.08 *N* hydrochloric acid	2.4		11		25
4 *N* acetic acid		2.7		28	
2 *N* acetic acid	3.1	3.1	20	20	32
Hydrochloric acid[c]	3.1		16		34
1 *N* acetic acid		3.4		20	
0.1 *N* acetic acid	4.1		17		35
Water	6.4		12		29
0.015 *N* sodium hydroxide	9.2		23		52
0.02 *N* sodium hydroxide	9.8	9.9	38	40	65
0.025 *N* sodium hydroxide	10.3	10.5	53	60	62
0.03 *N* sodium hydroxide	10.8		57		68
0.04 *N* sodium hydroxide	11.0		58		66
0.05 *N* sodium hydroxide	11.2	11.2	60	70	61

[a] NE 701136 wheat-to-solvent ratio, 1:6, dry basis. [b] 1×, once-ground wheat; 2×, twice-ground wheat. [c] Hydrochloric acid was added dropwise to the slurry until pH 3.1; exact normality was not known.

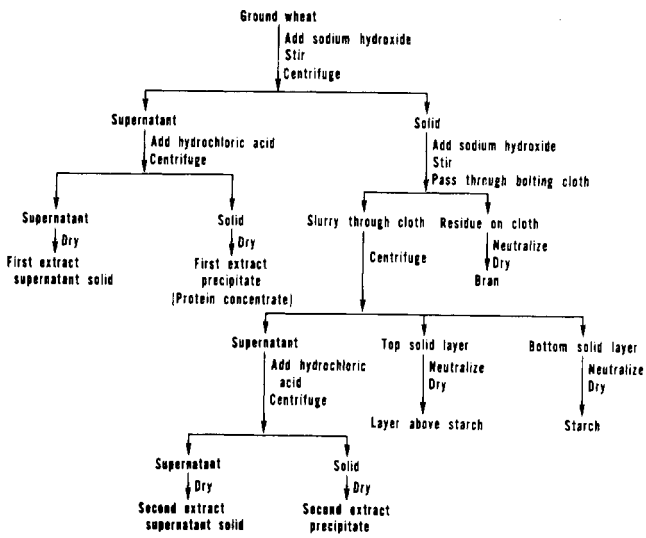

Figure 1. Schematic diagram for preparing protein concentrate and by-products from ground wheat.

701136 wheat protein at a solid-to-solvent ratio of 1:6 (Table I). Water removed 12% of the total protein from once-ground (1×) wheat, and the extracted solids contained 29% protein. Dilute hydrochloric acid and acetic acid of various concentrations at pH 2.4 to 4.1 extracted 11 to 28% of total wheat protein, and the extract solids had 25 to 35% protein. Acetic acid solubilized more wheat protein than did hydrochloric acid at the same pH. Sodium hydroxide (0.015 *N*, pH 9.2) extracted 23% of the wheat protein and the extracted solids had 52% protein. The percentage of wheat protein solubilized increased with increasing pH to 70% at pH 11.2, but the protein in extracted solids reached a maximum of 68% at pH 10.8. The optimum pH for dissolving wheat protein was 10.8. At higher pH values, the percentage of wheat protein extracted increased slightly, and there was more risk of modifying the wheat protein at higher pH values and more chance of starch gelatinization.

There was no difference in percentage of protein extracted between 1× and 2× ground wheats with 2 *N* acetic acid at pH 3.1 (Table I). The effect of particle size was increasingly important at higher pH values so that, at pH 11.2, the percentage of protein extracted increased from 60 for 1× wheat to 70 for 2× wheat which had a smaller particle size.

Table II. Effect of Solid-to-Solvent Ratio on Extraction of Wheat Protein[a]

Solid:solvent ratio	Solvent (NaOH) normality	pH	Protein extracted, %
1:3	0.09	10.9	49
1:4	0.045	10.2	36
1:6	0.03	10.2	60
1:10	0.018	10.2	67

[a] NE 701136, 2×.

Wheat-to-Solvent Ratio. Ground wheat was extracted with sodium hydroxide at various solid-to-solvent ratios from 1:3 to 1:10 (Table II). The normality of sodium hydroxide used was adjusted in order to arrive at the same pH value. At a constant pH of 10.2, the protein extracted increased markedly from 36 to 60% when the solid-to-solvent ratio was increased from 1:4 to 1:6. The percentage of protein dissolved was not increased very much when the solid-to-solvent ratio was further increased to 1:10. Therefore, a solid-to-solvent ratio of 1:6 seemed to be a good compromise between the highest percentage of protein extracted and a minimum amount of extractant needed.

At pH 10.9 with a solid-to-solvent ratio of 1:3, 49% of the wheat protein was solubilized (Table II). The percentage of protein extracted with a solid-to-solvent ratio of 1:6 at pH 10.9 was around 66% by interpolation of data on the 2× wheat from Table I.

Products from Wheat. Seven fractions were obtained from alkaline extraction of whole wheat (Table III). The effect of particle size of wheat on products is shown by comparing 1× and 2× wheat (NE 701136). The 2× wheat (smaller particle size) gave a slightly better yield of first extract precipitate (10.4 vs. 9.9%) and accounted for a little higher percentage of the total wheat protein (54 vs. 52%) compared with the 1× wheat. In addition the 2× wheat had considerably less bran (13 vs. 24%) and considerably more in the layer above the starch (16.4 vs. 10.9%) than the 1× wheat. The protein content of the fractions from 1× and 2× wheat was comparable, except the first extract precipitate, second extract supernatant solids, and the layer above the starch from 1× wheat contained somewhat more protein. The total recovered weight, as well as the total wheat protein accounted for, was greater for the 1× wheat.

Table III. Products from NE 701136 and Parker Wheats[a]

Product	Weight			Protein (nitrogen × 5.7) of solid			Total wheat protein		
	NE 701136		Parker	NE 701136		Parker	NE 701136		Parker
	1×	2×	2×	1×	2×	2×	1×	2×	2×
First extract precipitate	9.9	10.4	8.8	92.0	89.9	82.6	52	54	64
First extract supernatant	7.5	7.2	5.7	27.7	27.8	27.4	12	12	14
Second extract precipitate	1.7	1.6	0.6	74.7	74.6	60.4	8	7	3
Second extract supernatant	1.2	1.7	1.4	35.4	30.8	32.2	3	3	4
Bran	24.0	13.0	16.1	9.2	9.0	6.6	13	7	9
Layer above starch	10.9	16.4	14.2	7.3	6.0	1.1	5	6	1
Starch	42.3	44.0	51.8	0.3	0.3	0.3	1	1	1
Total	97.5	94.3	98.6				94	90	96

[a] Solid-to-solvent ratio was 1:6, pH 10.8, percent dry basis.

The effect of protein content of wheat on extraction results is also shown by the 2× columns in Table III. The high-protein (NE 701136) wheat gave first extract precipitate with both more yield (10.4 vs. 8.8%) and better protein content (89.9 vs. 82.6) compared with that from low-protein Parker wheat; the same trend was observed for second extract precipitates. Yields of supernatant solids from first and second extracts for NE 701136 wheat were also higher than those for Parker. Bran and the layer above starch from NE 701136 wheat had a considerably higher protein content compared with those from Parker. The yield of starch was higher from Parker than from NE 701136 wheat.

Consecutive Extraction. It is generally known that acetic acid is a good solvent for wheat gluten, which accounts for about 85% of the total protein in wheat. The data in Table I show that only 17–28% of the total protein was extracted from wheat by 0.1–4 *N* acetic acid. This small amount may be due to the salt originally present in wheat since the solubility of wheat gluten is known to be sensitive to ionic strength (Beckwith et al., 1963). If the salt present in wheat causes low solubility of wheat protein, then a preliminary water extraction to remove the salt in wheat should enable acetic acid to extract much more wheat protein later.

Consecutive extractions of wheat (NE 701136, 2×) at solid-to-solvent ratio of 1:6 with water and 1 *N* acetic acid and water and 0.03 *N* sodium hydroxide were made. After almost all the salt and 12% of the protein in wheat was removed by water extraction, 1 *N* acetic acid extracted 29% of the total wheat protein. Consecutive extraction of wheat with water and 1 *N* acetic acid removed 41% of the total protein. By comparison, consecutive extraction with water and 0.03 *N* sodium hydroxide removed 12 and 64% of the total protein, respectively. The corresponding figure for a single sodium hydroxide extraction under the same conditions was 66% (Tables I and III). Consecutive extractions with water and sodium hydroxide appeared attractive, and further studies are planned.

Future Potentials. Research on high-protein wheat has already produced commercial wheat having a protein content higher than normal wheat. Further increases in protein content of commercially available wheat can probably be expected. Wheat with a protein content higher than used here will likely yield an even better protein concentrate at a lower cost.

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