Composition and Properties of Protein Concentrate from Normal and High-Protein Wheats

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Protein concentrates and by-products produced from ground wheat were analyzed for amino acid composition, protein, starch, fat, fiber, ash, and various neutral carbohydrates. The concentrates contained from 83 to 92% protein (nitrogen × 5.7), 2.1 to 2.6 g of lysine, 2.5 to 3.5 g of total sulfur amino acids per 16 g of nitrogen, 1.5 to 7.4% fat, 0.9% ash, and from 1.8 to 3.9% total carbohydrate. Protein concentrate had a nitrogen solubility of 97% at pH 2.5 and a minimum solubility of 5.5% at pH 6.2, a hydration capacity of 2.6, had an emulsion stability of 63%, and formed a reasonably strong and elastic gluten ball.

An alkaline process was developed to prepare a protein concentrate and by-products from ground wheat of normal and high protein contents (Wu and Sexson, 1975). We studied the commercial potential of these protein concentrates and byproducts by determining their composition and functional properties. Solubility, hydration capacity, emulsion stability, and gluten ball-forming ability of the concentrates were determined.

MATERIALS AND METHODS

NE 701136 wheat contains 17.4% protein (nitrogen × 5.7), dry basis, and Parker wheat, 11.4%. Ground wheat and 0.03 *N* sodium hydroxide in a 1:6 ratio were stirred and the slurry centrifuged. The alkaline supernatant at pH 10.8 was adjusted to pH 6 and centrifuged to yield a precipitate and a supernatant. After freeze drying the precipitate and supernatant, the first extract precipitate (protein concentrate) and supernatant solids were obtained. The residue from the first centrifugation was restored to original volume and pH by addition of water and sodium hydroxide solution, and the slurry was stirred and passed through 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. After neutralization of starch layer and a layer above starch from centrifuging the alkaline dispersion that passed through the cloth and of residue on bolting cloth, starch, a layer above the starch, and a bran were obtained by freeze drying. The preparation of protein concentrate and by-products has been reported in more detail in a companion paper (Wu and Sexson, 1975).

Protein content was calculated from duplicate micro-Kjeldahl analyses by multiplying percent nitrogen by 5.7 and correcting to dry weight basis. A gas–liquid chromatographic (GLC) procedure was used to determine total neutral carbohydrates of acid-hydrolyzed samples of protein concentrate and by-products (Sloneker, 1971). After other components were solubilized and removed, the cellulose fraction was analyzed by the GLC procedure (Sloneker, 1971). Fat, fiber, ash, and hydration capacity were determined by AACC Approved Methods (1971). Starch was measured by a polarimetric method (Garcia and Wolf, 1972).

Nitrogen solubility was determined by mixing 0.1 g of protein concentrate with 10 ml of a solution of either hydrochloric acid or sodium hydroxide at various pH values. The mixture was stirred magnetically for 25 min, it was then centrifuged at 1300*g* for 20 min, and the supernatant was analyzed for nitrogen by micro-Kjeldahl. Emulsion stability was determined by the method of Yasumatsu et al. (1972) for a simple system (only soybean oil was added to the protein concentrate).

Samples for amino acid analysis were hydrolyzed for 24 hr by refluxing in constant-boiling hydrochloric acid, evaporated to dryness, and dissolved in citrate buffer at pH 2.2. A portion of the hydrolysate was analyzed in a Beckman Spinco Model 121 amino acid analyzer, and data were computed automatically (Cavins and Friedman, 1968).

Vital gluten, Pro 80, and devitalized gluten were supplied by General Mills, Inc., Minneapolis, Minn. A gluten ball was formed by mixing 2 g of either gluten or protein concentrate with 3 ml of 0.1% NaCl at room temperature. The inability to form a gluten ball is a good indication of denaturation.

RESULTS AND DISCUSSION

Composition. The protein, fat, fiber, ash, and starch contents of protein concentrates and by-products from NE 701136 and Parker wheats are shown in Table I. In addition to protein, fat, fiber, ash, and starch, wheat also contains 2.4% reducing sugar and 7.8% pentosans (Aykroyd and Doughty, 1970). For wheat with a protein content of 17.4% (NE 701136), the first extract precipitate (protein concentrate) had a high protein content of 92%. This concentrate had no fiber, lower ash, and about the same fat content as the whole wheat. The second extract precipitate from NE 701136 wheat had lower protein (75%), much higher fat (13%), and somewhat higher fiber and ash compared with the protein concentrate. The protein in the two extract precipitates was probably gluten because little gluten is soluble at pH 6 in the presence of salts originally present in wheat and from neutralization of sodium hydroxide. Commerical gluten contains 75–80% protein, 5%–15% carbohydrates (mostly starch), and 5–15% lipids (Knight, 1965).

The first and second extract supernatant solids from NE 701136 wheat had around 30% protein, low fat, and fiber (Table I). These two fractions contained the albumins and globulins, as well as salt and other water-soluble materials. The ash content of the two extract supernatant solids was high, partly as a result of sodium chloride formed by neutralizing sodium hydroxide solution. The bran had lower protein and starch, similar fat, and much higher fiber contents compared with whole NE 701136 wheat. This fraction contained also starch. The layer above the starch had lower protein, fat, and fiber, but higher starch compared to the bran. Although this layer was mostly starch, it contained some protein and bran also. The NE 701136 starch was 99.3% starch and 0.3% protein. The high starch and low

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Table I. Composition of Protein Concentrates and By-Products from NE 701136 (NE) and Parker (P) Wheats (% Dry Basis)

Fraction	Protein (N × 5.7)		Fat		Fiber,	Ash		Starch	
	NE	P	NE	P	NE	NE	P	NE	P
Whole wheat	17.4	11.4	1.6	1.9	2.7	2.0	1.7	59.5	70.3
First extract precipitate	92.0	82.6	1.5	7.4	0.0	0.9	0.9	[a]	[a]
First extract supernatant	27.7	27.4	0.2	0.2	0.2	18.8	15.1	[a]	[a]
Second extract precipitate	74.7	60.4	12.6	24.5	0.2	1.3	1.0	[a]	[a]
Second extract supernatant	35.4	32.2	[a]	1.8	[a]	20.5	20.2	[a]	[a]
Bran	9.2	6.6	1.5	2.0	11.2	2.9	4.9	43.6	28.8
Layer above starch	7.3	1.1	1.2	0.3	1.0	3.9	1.0	78.6	88.0
Starch	0.3	0.3	0.1	0.1	0.2	0.6	2.8	99.3	94.4

[a] Not determined.

Table II. Neutral Carbohydrates from Hydrolysates of Protein Concentrates and By-Products from NE 701136 (NE) and Parker (P) Wheats (% Dry Basis)

Fraction	L-Arabinose		D-Xylose		D-Mannose		D-Galactose		D-Glucose		Total	
	NE	P	NE	P	NE	P	NE	P	NE	P	NE	P
Whole wheat	0	1.6	3.0	3.0	0	0	0	0	78.0	92.7	81.0	97.3
First extract precipitate	0	0	0	1.8	0	0	0	0	3.9	0	3.9	1.8
First extract supernatant	3.6	0	4.5	3.5	0.6	0	2.9	0	22.3	2.5	33.9	6.0
Second extract precipitate	0	0	0	0	0	0	0	0	0	0	0	0
Second extract supernatant	0	3.9	3.6	5.7	0	0	1.1	0	11.1	1.5	15.8	11.1
Bran	0	1.0	14.6	20.2	0	0.1	0	0.2	59.9	48.6	74.5	70.1[a]
Layer above starch	0	1.3	0	1.7	0	0	0	0	90.2	98.3	90.2	101.3
Starch	0	0	0	0	0	0	0	0	123.0	121.0	123.0	121.0

[a] Includes a 4.6% cellulose fraction of which 3.9% is D-glucose and 0.7% is D-xylose.

protein values for this fraction qualify it as a prime starch.

Parker wheat with lower protein content than NE 701136 wheat gave considerably less protein in the two extract precipitates, bran, and the layer above the starch but comparable protein in the two extract supernatant solids and starch (Table I). Fat in the two extract precipitates of Parker wheat was much higher than that from NE 701136. The higher fat content of the second extract precipitate from Parker compared with that from NE 701136 wheat is a result of the much lower yield of that fraction from Parker. However, the layer above starch from Parker had lower fat than that of NE 701136 wheat. Ash contents of the bran and the starch from Parker were higher than those from NE 701136, but the layer above starch and the first extract supernatant solids from Parker had lower ash. Also, Parker starch had a low protein content of 0.3%. The layer above starch from Parker had a higher starch content than that of NE 701136, but the trend was reversed for bran and starch.

Neutral Carbohydrates. The amount and kind of neutral carbohydrates from hydrolysates of protein concentrates and by-products from NE 701136 and Parker wheats are listed in Table II. The concentrate from NE 701136 yielded 3.9% glucose, whereas Parker concentrate yielded 1.8% xylose. The first and second extract precipitates yielded no neutral sugar. The first extract supernatant solids from NE 701136 yielded 33.9% total sugar in contrast to 6% from that of Parker. This difference between 33.9 and 6% is much higher than the experimental uncertainty. The neutral carbohydrates in extract supernatant solids include water-soluble sugars and pentosans. The first extract supernatant solids from NE 701136 produced small amounts of arabinose, mannose, and galactose that were not detected in the starting wheat, because of the difference in concentration, the yield of the first extract supernatant solids being only 7.5% (Wu and Sexson, 1975).

The bran yielded 49–60% glucose, probably derived mostly from starch, but in Parker a small part came from cellulose. No cellulose was detected in NE 701136 bran. In addition, the bran produced 15–20% xylose, apparently derived from hemicellulose. The layer above starch from NE 701136 wheat yielded only glucose, but that from Parker also produced 3% other sugars in addition to glucose. Parker and NE 701136 starch yield only glucose.

Amino Acid Composition. The amino acid composition of protein concentrates and by-products from the two wheats is listed in Table III. Only significant difference in amino acid composition was mentioned. The amino acid pattern of NE 701136 wheat protein resembles closely that of hard red winter wheat No. 3 (Horn et al., 1958; Waggle et al., 1967). Proteins from the two extract precipitates from NE 701136 wheat had similar amino acid patterns and, in general, were close to that of gluten (Wu and Dimler, 1963) except the latter was considerably lower in lysine. The proteins in the bran and first extract supernatant solids of NE 701136 had considerably higher lysine, aspartic acid, and alanine, and lower glutamic acid and proline contents com-

Table III. Amino Acid Composition of Protein Concentrates and By-Products from NE 701136 (NE) and Parker (P) Wheats (g of Amino Acid/16 g of Nitrogen)

Amino acid	Whole wheat		1st extract precipitate		2nd extract precipitate		Bran		1st extract supernatant		2nd extract supernatant,
	NE	P	NE	P	NE	P	NE	P	NE	P	P
Lys	2.7	3.3	2.1	2.6	2.1	2.7	4.7	5.2	3.3	3.5	3.7
His	2.4	2.6	2.3	2.5	2.2	2.4	2.8	3.0	2.5	2.1	3.1
NH_3	3.4	4.2	3.5	3.9	4.0	3.8	2.1	2.1	3.4	3.2	4.6
Arg	5.1	5.9	4.5	5.1	3.9	5.3	8.1	8.7	5.9	5.5	7.4
Asp	5.0	5.3	4.3	4.3	3.9	4.1	7.0	7.8	9.2	6.6	6.9
Thr	2.7	3.1	2.7	2.8	2.6	2.8	3.5	3.9	3.1	3.1	3.8
Ser	4.5	4.7	4.8	4.6	4.7	4.6	4.5	4.4	4.4	4.0	5.7
Glu	31.4	29.0	36.7	32.1	37.8	30.2	18.9	14.0	25.6	19.2	34.5
Pro	9.6	8.9	11.2	9.8	12.1	9.3	5.9	4.7	6.8	6.3	10.6
Gly	3.9	4.5	4.1	4.0	2.9	3.8	5.4	6.4	4.1	3.9	5.3
Ala	3.3	3.9	3.1	3.2	2.7	3.2	5.0	5.8	4.5	4.4	4.7
Half-Cys	1.4	1.3	1.5	1.8	2.0	1.7	0.8	0.6	3.1	1.7	2.1
Val	4.1	4.8	4.2	4.5	4.2	4.6	5.1	5.6	4.4	4.4	5.7
Met	1.5	1.4	1.0	1.7	1.8	1.9	1.6	1.6	1.7	1.0	1.2
Ile	3.4	3.6	3.5	3.7	4.0	3.8	3.4	3.5	3.2	2.9	4.5
Leu	6.5	6.8	6.8	6.8	7.2	6.9	6.7	6.6	5.8	5.3	7.9
Tyr	3.3	3.3	3.9	3.5	2.6	3.7	3.0	3.2	2.7	2.4	4.6
Phe	4.5	4.5	4.8	4.9	5.8	5.8	4.6	4.3	3.2	3.1	6.8

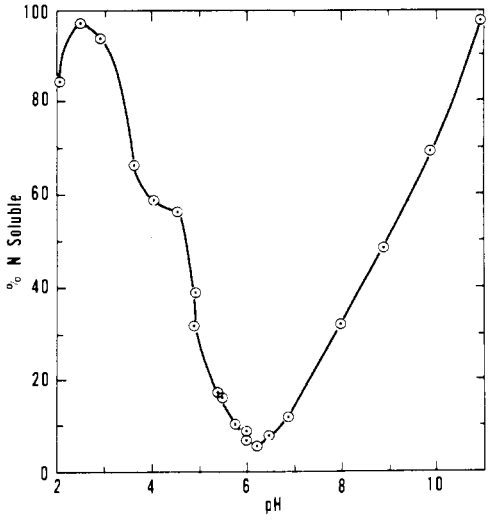

Figure 1. Nitrogen solubility of NE 701136 wheat protein concentrate at various pH values. About 0.1 g of the protein concentrate was stirred with 10 ml of either hydrochloric acid (below pH 6) or sodium hydroxide (above pH 6) solution at different pH values.

pared with the extract precipitates when expressed in g/16 g of nitrogen basis. The protein of bran had almost identical amino acid composition as that of hard red winter wheat No. 3 shorts and bran (Kohler and Palter, 1967) except the former had a lower half-cystine content. The lower lysine content from the protein of the extract precipitates was compensated by the higher lysine values of the remaining fractions compared with NE 701136 wheat protein. A material balance showed that no lysine was destroyed during alkaline extraction.

Parker wheat had a lower protein content (Table I) and its protein had higher lysine compared with NE 701136 wheat. The negative correlation of protein content and lysine for wheat in general was also observed here. Otherwise, the two wheat proteins had similar amino acid compositions. The higher lysine of Parker wheat protein compared with NE 701136 resulted in more lysine in all the Parker fractions. In general, the proteins of the corresponding fractions of Parker and NE 701136 wheat had similar amino acid patterns.

Nitrogen Solubility. One of the important functional properties of a protein concentrate is its solubility at various pH values. Figure 1 shows the percentage of nitrogen soluble at a number of pH values from 2 to 11 for NE 701136 protein concentrate. Minimum solubility was at pH 6.2 where 5.5% of the nitrogen was soluble. When the NE 701136 protein concentrate was prepared by precipitation at pH 6, the ionic strength of the solution due to salt present originally in wheat and due to neutralization of alkali was higher than that used in solubility study here. Since gluten is more soluble at low ionic strength (Beckwith et al., 1963), the protein is slightly soluble at pH 6 instead of insoluble. Solubility increased rapidly as pH increased beyond 6.5; almost all the nitrogen was soluble at pH 11. Nitrogen solubility below pH 6 also was enhanced, and a shoulder in the solubility curve was observed around pH 4. Another rapid increase in solubility was seen below pH 4, and the curve passed through a maximum around pH 2.5 where 97% of the nitrogen was soluble. Solubility dropped to 85% at pH 2. The excellent solubility of the protein concentrate around pH 2.5 suggests that the concentrate may have commercial potential for protein fortification of carbonated beverages.

Hydration Capacity and Emulsion Stability. The hydration capacity of NE 701136 protein concentrate was 2.6 (weight of sediment per weight of dry sample). The emulsion stability of NE 701136 protein concentrate was 63%, and it was better than that of a commercial soybean protein isolate which had a value of 51% under the same experimental condition.

Gluten Ball Forming Ability. Since wheat gluten is frequently used in baked goods, we evaluated the potential of wheat protein concentrates for that purpose. A commercial vital gluten, a laboratory gluten prepared from NE 701136 flour by the dough-ball method, NE 701136 protein concentrate, and a commercial devitalized gluten were compared in their abilities to form gluten balls. The laboratory NE 701136 gluten formed a strong and elastic gluten

ball. The wheat protein concentrate and the commercial vital gluten formed a reasonably strong and elastic gluten ball but not quite so good as that from laboratory NE 701136 gluten. The devitalized gluten could not form a gluten ball. Vital gluten (ability to form a gluten ball) is preferred for baked goods. Since the protein concentrate is about equal to the commercial vital gluten in gluten ball forming ability, likely no appreciable denaturation of the protein resulted from alkaline extraction, and the concentrate can be used in baked goods.

Potential Uses of Protein Concentrate and By-Products. Wheat protein concentrate may find application in conventional wheat foods to improve protein levels in the final product. Other possible uses of this protein concentrate include meat extenders and protein fortification of beverages. Currently, wheat starch has acceptable outlets. Further research will be necessary to evaluate the products from wheat for different food and industrial applications.

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Condensed Tannins in Grain Sorghum: Isolation, Fractionation, and Characterization

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A new procedure was developed for the isolation of condensed tannins. Separation from non-tannins was based upon the finding that tannins were adsorbed to Sephadex LH-20 in 95% ethanol. Following exhaustive washing with ethanol, the tannins were eluted with 50% aqueous acetone. By applying this procedure, highly purified condensed tannins were isolated from grain sorghum. Tannins from Leoti red sorghum and Georgia 615 were further fractionated by gel filtration on Sephadex LH-20 using 50% aqueous acetone. The tannins from both strains were shown to consist of a series of polymeric polyphenols which upon acid hydrolysis generated cyanidin, exclusively. Hydrolyzable tannins were apparently absent since neither glucose nor other sugars were found after hydrolysis.

The hydrolyzable and condensed tannins are two groups of polyphenols widely distributed in the plant kingdom which may be differentiated by their structure and reactivity toward hydrolytic agents. The hydrolyzable tannins are readily cleaved by enzymes as well as by dilute acids, into a sugar such as glucose, and a phenolcarboxylic acid such as gallic acid. In contrast, the condensed tannins are resistant to enzymatic degradation. Upon acid treatment, this group of polyphenolics not only decomposes with the liberation of a small amount of anthocyanidins, but also progressively polymerizes to yield amorphous phlobaphens or tannin reds (Haslam, 1966; Ribereau-Gayon, 1972).

The tannins are characterized by their ability to interact with and precipitate proteins such as gelatin. They appear to be responsible for the astringency of many plant materials and decrease nutritive value when added to the diet or when found naturally in high levels in certain foodstuffs. The presence of tannins in grain cereals is relatively rare, occurring only in selected strains of sorghum and barley. Although the toxic properties of tannins in sorghum have been well documented (Chang and Fuller, 1964; Fuller et al., 1966; McGinty, 1969; Harris et al., 1970), there have been relatively few characterizations of these tannins. Several types of anthocyanidin-generating compounds have been identified in different varieties of sorghum. Blessin et al. (1963) used a procedure based on the adsorption on a strongly basic ion-exchange resin and extracted profisetinidin as the main pigment in Martin sorghum. (The prefix pro is used rather than leuco to designate those colored, polymeric flavonoids which generate anthocyanidins when heated with hot acid (Freudenberg and Weinges, 1960).) In a further report of the chemical nature of the pigment of sorghum, Yasumatsu et al. (1965) obtained propelargonidin by using methanol extracts of seed coats of commercial sorghum. However, neither the profisetinidin nor the propelargonidin was tested for tanning properties.

Bate-Smith and Rasper (1969) reported that the principal tannin of sorghum, based upon their work with the Kafir variety, was a proanthocyanin which, when heated with mineral acid, yielded the very uncommon luteolindin. They also found procyanidin in a rose-brown variety of the grain but not in the red grains examined.

Many of the usual methods for the isolation of tannins depend on the differential solubility of tannins in various solvents (Roux, 1953; Roux and Maihs, 1960; Haslam,

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