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SOLUBILIZATION AND RECOVERY OF PROTEIN FROM WHEAT MILLFEEDS[1]

DAVID A. FELLERS, VERNON SINKEY, ALLAN D. SHEPHERD, AND JAMES W. PENCE

ABSTRACT

Various extractants and conditions were evaluated for effectiveness in solubilizing the protein of common millfeed fractions of wheat (coarse bran, fine bran, and shorts). Soaking with alkali for 1 hr. at pH 10.5, 23°C., and with a solvent:millfeed ratio of 10:1 was very effective. Grinding all of the fractions increased significantly the solubilization of protein by alkali. Much of the solubilized protein could be recovered from alkaline extracts by precipitation at pH 5.5. A little more could be recovered by heating the supernatant liquor. Of the total nitrogen in fine bran, 23.5% (11.8% dialyzable, 11.7% nondialyzable) could not be precipitated isoelectrically (pH 5.5) from alkaline extracts (pH 6.5–12.5). Freeze-drying the precipitate from fine bran yielded a brown material containing 69% protein, 21% fat, 3% ash, and 7% carbohydrate. Drying of this precipitate with ethanol gave a light-grayish product, 87% protein (dry basis). A single assay showed that the protein extracted from fine bran with alkali (pH 10.5) had a protein efficiency ratio of 2.0 compared with 2.2 for untreated fine bran.

Protein concentrates for human food have been and are being developed from a number of sources, such as the soybean, peanut, cottonseed, sesame seed, and other plant materials. Wheat millfeeds (coarse bran, fine bran, shorts, and feed middlings) have been relatively neglected, although the nutritional quality of the protein is good (1,2) and large supplies of low-cost wheat millfeeds are readily available (3). Wheat millfeeds appear to lack toxic substances such as hemagglutinin, trypsin inhibitor, and gossypol, which pose problems in other plant protein sources.

Several earlier publications (4,5,6) reported types and quantities of protein (according to solubilities in various solvents) in branny ma-

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terials. Albumins, globulins, prolamines, glutelins, and alkaline-insoluble protein have been reported. Rather large amounts (9–25%) of nonprotein nitrogen have also been reported.

Pecini (7), through a water-soaking and heating process, produced a water extract from wheat bran which he spray-dried and used as a food additive. Others (8,9) have also obtained extracts from bran, but not necessarily with the objective of a high-protein product in substantial yield. Johnson and Anderson (10) recently patented a process, in which they claim that addition of hydrogen peroxide with heating during alkaline extraction of certain natural materials, including wheat bran, increased protein extraction and improved isoelectric precipitability. The practice of using alkaline hydrolysis for dispersing plant proteins is well known, but specific data for wheat bran or millfeeds seem not to be available. Studies reported here were undertaken to determine the feasibility of producing for human food a protein concentrate from wheat millfeeds by wet-extraction methods.

Materials and Methods

Millfeeds. A Montana hard red spring (HRS) wheat was commercially milled to yield five streams of millfeeds: screenings, coarse bran, fine bran, shorts, and feed middlings. Screenings were excluded from the present work and only limited work (not reported) was carried out on feed middlings. Table I gives data on the composition of the millfeeds used and the whole wheat from which they were milled (11,12,13).

On the basis of the starch content of the various millfeeds (Table I) and 77% starch in the starchy endosperm (dry basis) for a HRS wheat (14), the percent of starchy endosperm in each millfeed was calcu-

TABLE I
PROXIMATE ANALYSIS OF A HARD RED SPRING WHEAT AND MILLFEED FRACTIONS THEREFROM
(Dry basis)

	HRS Wheat	Coarse Bran	Fine Bran	Shorts	Feed Middlings	Reference of Method
	%	%	%	%	%	
Protein	16.4	20.6	22.0	21.1	20.0	11
Pentosan	...	28.4	24.9	19.2	10.9	12
Starch	...	5.3	10.5	19.9	41.5	13
Crude fiber	2.9	12.8	10.5	7.4	3.3	11
Total sugar[a]	3.1	5.5	7.7	7.5	5.5	11
Fat[b]	2.4	5.7	6.9	7.0	4.3	11
Ash	1.8	7.4	6.3	5.1	2.9	11
Undetermined	...	14.3	11.1	12.8	11.6	..

[a] Invertase converted.
[b] Ether-extractable.

lated, along with the amount of protein contributed to the total millfeed protein by the starchy endosperm (see table below).

Millfeed	*Starchy Endosperm*	*Millfeed Protein Contributed by Proteins from the Starchy Endosperm*
	%	%
Coarse bran	6.9	5.0
Fine bran	13.6	9.3
Shorts	25.8	18.3
Feed middlings	53.8	40.4

To obtain finely ground materials, millfeeds were passed through a Bantam Mikro Pulverizer (hammer mill) with a 0.039-in. screen (one pass with air relief) (Table II).

TABLE II
PARTICLE SIZE OF MILLFEEDS

U.S. SIEVE SIZE	COARSE BRAN		FINE BRAN		SHORTS	
	Original	Ground[a]	Original	Ground[a]	Original	Ground[a]
mesh	%	%	%	%	%	%
On 14	45	..	..	..	..	..
On 20	39	..	8	..	1	..
On 30	12	..	54	..	16	..
On 60	4	10	36	10	71	3
On 80	trace	19	2	14	9	12
On 100	..	10	..	8	1	7
On 140	..	15	..	15	2	13
Through 140	..	46	..	53	..	65

[a] Ground materials were washed in ether to allow easier sieving.

Solubilization. Solubilization of any component Z is defined as the ratio of Z in the entire aqueous phase to total Z in the sample. The following formula was used to calculate percent solubilization:

$$\% \text{ Solubilization Z} = \frac{(\text{mg. Z per ml. supernatant})\ (\text{ml. water in system})}{\text{mg. Z in system}} \times 100.$$

Protein and Solids Solubilization. The effect of pH (6.45 to 12.5) on the solubilization of protein (Kjeldahl N $\times$ 5.7) and solids from the various materials employed was determined under the following fixed conditions: solvent-to-millfeed ratio, 10:1; 23°C.; 1 hr. with stirring. The pH was adjusted with sodium hydroxide. Additional sodium hydroxide was added as required to maintain constant pH. Separations were achieved by centrifugation at 850 $\times$ *g* for 15 min.

To ascertain the effect of temperature, solvent-to-millfeed ratio, and time of extraction on protein solubilization, fine bran was treated

at pH 8.5 under variations of the factors in the table below.

Variable	*Range of Variable*	*Time*	*Temperature*	*Solvent-to-Millfeed Ratio*
		hr.	*°C.*	
Temperature	5–90°C.	1	..	10:1
Solvent-to-millfeed ratio	7.5:1–20:1	1	23	...
Time	¼–4 hr.	..	23	10:1

Protein Recovery. Precipitation of protein from alkaline extracts of millfeeds by isoelectric adjustment occurred over a wide pH range from slightly greater than pH 7 down to pH 3 or 4. The composition as well as yield of the precipitate depended on the pH of precipitation. Most protein was recoved at pH 5.5, and this pH at 23°C. was subsequently used throughout this work to obtain precipitates.

Heating the residue liquor *after* isoelectric precipitation yielded additional coagulated protein. Coagulation commenced at about 60°C., and yield of heat-coagulated protein was maximum after the liquor was heated for 10 min. at 100°C. These conditions were accepted as general practice.

In work with the original or ground coarse bran, fine bran, or shorts, extracts for protein recovery experiments were obtained under the fixed conditions previously described. In some cases the residual millfeed was washed with a quantity of water equal to one-half the volume of the original solvent, and the pH of the resuspended material was approximately the same as that of the original suspension. The resuspended material was separated as before, and the supernatant collected. The supernatant from the washing was then combined with the first supernatant for further treatment.

Isoelectric and heat-coagulated precipitates recovered by centrifugation at 850 × *g* for 15 min. from the alkaline extracts contained about 90% water. The precipitates were not washed, but were freeze-dried directly.

Pre-Extraction Treatments of Millfeeds. Several pre-extraction treatments of fine bran were tested to determine effects on protein solubilization and, particularly, on the color and quantity of the recovered precipitate. Treatments tested were: autoclaving for 10 min. at 240°F., dieythl ether wash, 70% ethanol wash, and hydrochloric acid-water washes (pH 2.0–5.5).

Acid Extractants. Protein solubilization from fine bran with various acids (hydrochloric, sulfuric, phosphoric, and acetic) and strengths of acids was determined under the following conditions: 23°C.; solvent-to-millfeed ratio, 10:1; 1 hr. extraction with stirring.

Results and Discussion

pH. Figure 1 shows how solubilization of protein (Kjeldahl N ×

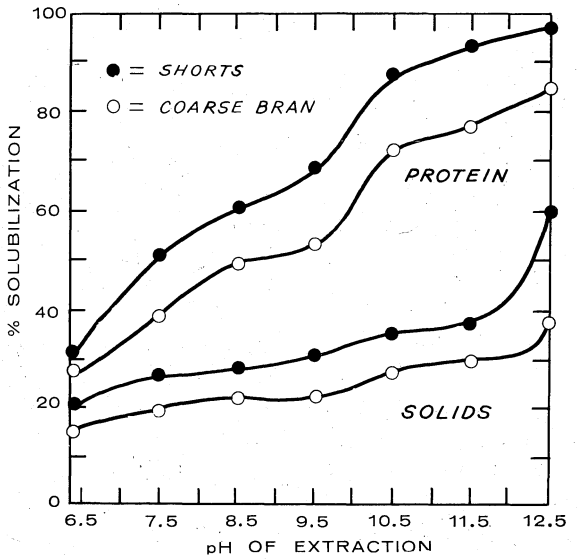

Fig. 1. Solubilization of protein and solids from coarse bran and shorts as affected by pH. Solvent-to-millfeed ratio, 10:1; 23°C.; 1-hr. extraction with stirring.

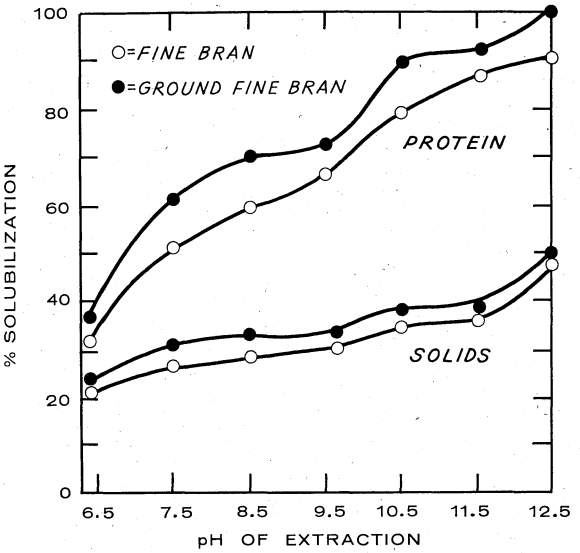

Fig. 2. Effect of grinding on protein and solids solubilization from fine bran over the pH range 6.45 to 12.5. Conditions: solvent-to-millfeed ratio, 10:1; 23°C.; 1-hr. extraction with stirring.

5.7) and solids from shorts and coarse bran varied with pH at 23°C. The curves for fine bran are shown in Fig. 2.

Coarse bran, fine bran, and shorts displayed only slight differences with respect to the proportion of sodium hydroxide to millfeed necessary to maintain a specified pH during extraction. Grinding the various millfeeds, however, resulted in a significant increase in the quantity of sodium hydroxide required at pH values of 7.5–10.5, as seen in the table below.

pH	*Sodium Hydroxide*	
	Original	*Ground*
	mg./g. millfeed (dry basis)	
6.45 (water)	..	..
7.5	3.0	4.3
8.5	5.6	7.1
9.5	9.4	10.7
10.5	16.6	17.2
11.5	28.0	28.0
12.5	104.0	104.0

Grinding. Grinding millfeeds resulted in increased solubilization of both protein and solids at all pH values. The effect on fine bran is depicted in Fig. 2. The increases due to grinding in protein and solids solubilization at the various pH values are summarized in Table III. The greatest effect was on coarse bran and the least effect on shorts.

TABLE III

INCREASE IN PERCENT SOLUBILIZATION OF PROTEIN AND SOLIDS DUE TO GRINDING MILLFEEDS

pH	Coarse Bran		Fine Bran		Shorts	
	Protein	Solids	Protein	Solids	Protein	Solids
	%	%	%	%	%	%
6.45 (water)	6.1	4.0	3.6	2.4	2.6	2.5
7.5	14.4	5.2	10.8	5.0	11.3	4.2
8.5	12.6	3.1	11.0	5.0	8.6	3.2
9.5	16.5	5.1	6.2	3.0	2.0	1.2
10.5	14.9	4.4	10.0	4.2	4.6	2.2
11.5	12.4	3.4	5.0	2.4	5.3	3.1
12.5	12.7	3.1	9.4	2.0	..	..
Av.	12.8	4.0	8.0	3.4	5.7	2.7

Temperature. Solubilization of protein in fine bran at pH 8.5 was greatest at 23°C. Below or above 23°C., protein solubilization was less (Fig. 3). Actually, the decrease in solubilization above 23°C. would have been even more pronounced except that considerably more sodium hydroxide was necessary to maintain pH 8.5 at the higher temperatures (Fig. 3).

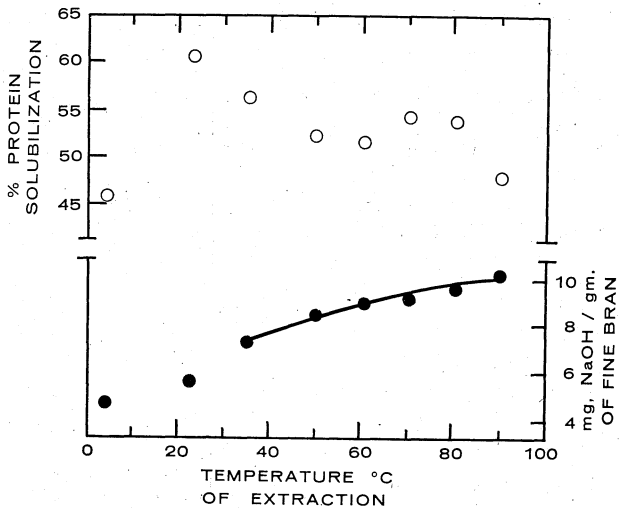

Fig. 3. Effect of temperature at pH 8.5 on protein solubilization from fine bran and effect on the quantity of sodium hydroxide necessary to maintain pH 8.5 for the 1-hr. extraction period. Solvent-to-millfeed ratio, 10:1.

Solvent-to-Millfeed Ratio. Increasing the solvent-to-fine-bran ratio from 7.5:1 to 20:1 did not materially affect the quantity of sodium hydroxide necessary to maintain the pH at 8.5 for 1 hr. at 23°C. Protein solubilization at the 7.5:1 and 10:1 ratios were similar (60%) but at the greater dilutions of 15:1 and 20:1 were about 5% less. Such a drop in protein solubilization at the higher dilutions might occur because the lower specific gravity of the solution made the protein less buoyant. Other factors such as viscosity or ion effect might also play a role.

The 7.5:1 ratio was about the lowest ratio at which the slurry could still be effectively handled as a liquid. Grinding allows the use of lower solvent-to-millfeed ratios than are possible with the original materials.

Time. Extraction for 4 hr. at pH 8.5, 23°C., and solvent-to-fine-bran ratio of 10:1 resulted in a protein solubilization of 64%; 1-hr. extraction yielded 60%; 15 min., 58%. The sodium hydroxide, mg. per g. fine bran (dry basis) necessary to maintain pH 8.5, was 5.9, 5.6, and 4.5 respectively. The majority of protein (better than 90%) that is going to be solubilized at pH 8.5 will dissolve in 15 min. or less. Rapid dispersion is an important characteristic of the millfeed protein; it affects the capacity of any given process designed to recover the protein. Furthermore, because millfeeds are highly contaminated, rapid handling may be important to minimize microbiological problems. Rapid

dispersion raises the possibility of a countercurrent process for recovering millfeed proteins. Grinding millfeeds probably accelerates protein dispersion.

Protein Characterization. In Fig. 4, the protein of ground fine bran

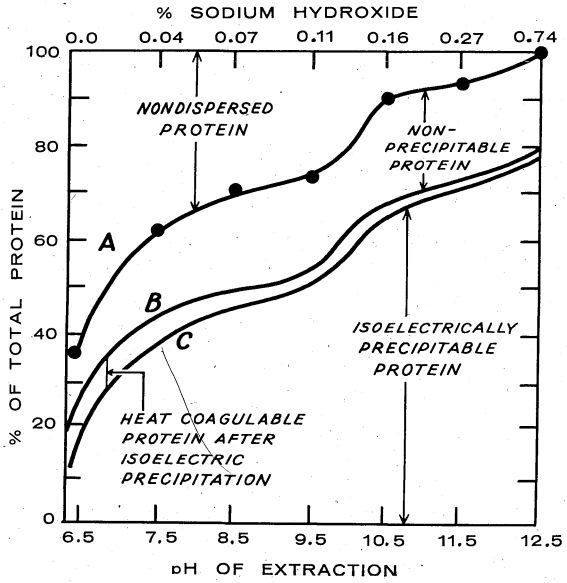

Fig. 4. Classification of the total protein (N × 5.7) of ground fine bran with respect to solubility, isoelectric precipitability (pH 5.5), and heat-coagulability (100°C.) at various pH values.

is classified with respect to solubility, precipitability (pH 5.5), and heat-coagulability (100°C.) of the protein remaining *after* precipitation. Protein solubilization (curve A) was determined experimentally under the fixed conditions previously described. Precipitability (curve C) and heat-coagulability (curve B minus curve C) were determined from experimental data corrected to 100% recovery of the solubilized protein. Thus, all values in Fig. 4 represent maximum possible yields of these various fractions.

The maximum possible yield (%) of isoelectric protein (curve C) equaled the dispersed protein (curve A) minus 23.5%. This was true with the original fine bran as well as with the ground fine bran. This relationship held throughout the pH range of 6.45 to 12.5, indicating that alkaline treatment did not have any effect on the proteins that changed their precipitability by isoelectric adjustment. Fractionation of the 23.5% of protein (N × 5.7) not precipitable at pH 5.5 (obtained

from a water extract, pH 6.45, of fine bran) showed that 11.8% was dialyzable (Visking cellophane tubing). Of the 11.7% nondialyzable protein, 6.4% was precipitated by heating at 100°C. for 10 min., leaving 5.3% of soluble, nondialyzable, nonprecipitable protein.

The maximum possible amount of heat-coagulable protein (curve B minus curve C, Fig. 4) obtainable from the pH 5.5 solution *after* precipitation ranged from 6.4% for the pH 6.45 (water extract) down to about 2% for the higher pH extracts. This indicates that most all the heat-coagulable protein is extracted at neutral pH (6.45) and that alkaline treatment results in a modification of these proteins that renders them less heat-coagulable.

On shorts and coarse bran, experiments were carried out at pH 8.5 and 10.5 only. The data indicate that the protein not precipitable by isoelectric adjustment is 33% for coarse bran compared with 23.5% for fine bran. The results with shorts were similar to those with fine bran.

Protein Recovery. The recovery of protein is effected in three steps: 1) collection of supernatant liquor, including any washings of the insoluble residue; 2) precipitation (pH 5.5); and 3) heat-coagulation at 100°C. of the solution remaining after precipitation (not reported).

Actual yields of protein in the supernatants, precipitates, and heat-coagula were somewhat less than the maximum possible yields described in Fig. 4. Table IV depicts yields of supernatant protein (step 1).

TABLE IV

YIELDS[a] OF SUPERNATANT PROTEIN FROM CENTRIFUGATION[b] AT 850 × *g* FOR 15 MINUTES

pH	Coarse Bran		Fine Bran		Shorts	
	Original	Ground	Original	Ground	Original	Ground
	%	%	%	%	%	%
6.45 (water)	17.3	25.9	22.6	26.7	23.1	27.5
7.5	22.8	41.5	35.1	49.9	41.8	52.4
8.5	29.5	49.7	41.5	56.4	46.1	57.5
9.5	31.5	52.7	44.6	58.4	52.7	58.5
10.5	43.6	68.5	52.7	71.3	68.6	75.6
11.5	45.6	68.8	57.7	69.4	75.2	79.7
12.5	38.0	64.2	58.5	66.1	68.4	...

[a] Based on total protein in the millfeed.
[b] Of 10:1 solvent:millfeed slurries previously agitated 1 hr. at 23°C.

Whereas protein solubilization increased steadily with pH from 6.45 up to 12.5, as indicated in Figs. 1 and 2, the yield of supernatant protein (Table IV) tended to peak at pH 10.5–11.5. This phenomenon occurred because increased hydration of the insoluble residue at the higher pH values decreased the volume of supernatant.

Grinding greatly increased the yield of supernatant protein (Table IV), partly because the more compacted insoluble residue held less solution. The remainder of the increase was due to the greater protein solubilization of the ground millfeeds. None of the residues from centrifugation was very tightly packed, and more supernatant could easily have been recovered by squeezing or pressing the residue.

At pH 12.5, the 10:1 slurries were quite viscous, and separation of the insoluble millfeed residue was not easy, particularly the shorts. The dispersion of starch at this high pH was probably the cause of the increased viscosity.

One wash of the insoluble residue with a volume of water equal to half the original solvent increased the protein yield at step 1 substantially. This is demonstrated in Fig. 5 where protein solubilization

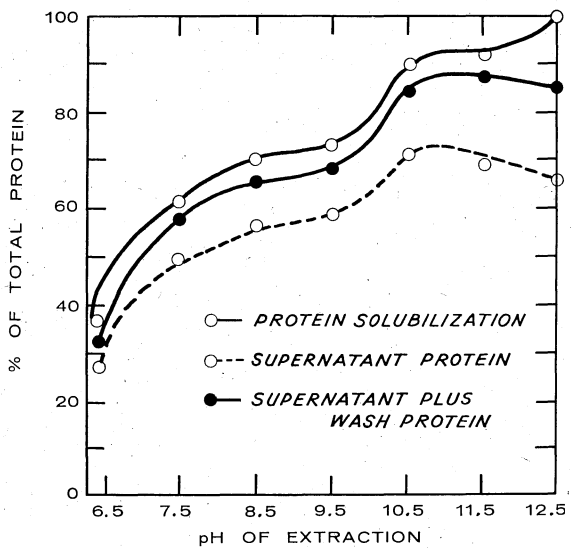

Fig. 5. Yield of supernatant and wash-water protein at various pH values compared with theoretical yield (protein solubilization). Conditions: solvent-to-ground-fine-bran ratio, 10:1 (wash-water, 5:1); 23°C.; 1-hr. extraction with stirring; separations at 850 × *g* for 15 min.

of ground fine bran, protein yield in the initial supernatant, and protein yield as a result of the combined initial supernatant and one wash of the residue are compared.

In the precipitation process (step 2) it was necessary, with the higher pH supernatants, to have very good agitation and to use a fairly dilute acid (0.5*N*); otherwise, gelling would occur around the drops of acid, causing clots which were difficult to disperse.

The actual yields of freeze-dried, isoelectric protein (not washed) from fine bran, coarse bran, and shorts (original and ground) are reported in Table V. These yields were from the supernatants described in Table IV.

TABLE V

YIELDS[a] OF ISOELECTRIC PROTEIN FROM 10:1 ALKALINE EXTRACTS OF MILLFEEDS

pH	Coarse Bran		Fine Bran		Shorts	
	Original	Ground	Original	Ground	Original	Ground
	%	%	%	%	%	%
6.45 (water)	...	...	5.0	5.9	...	...
7.5	...	...	16.5	26.9	...	...
8.5	10.1	19.4	27.5	37.4	26.4	35.5
9.5	...	...	31.2	40.8	...	...
10.5	23.4	43.6	39.2	53.0	50.0	60.4
11.5	...	...	42.0	50.5	...	...
12.5	...	...	43.8	49.5	...	...

[a] Based on total protein in the millfeed.

One washing of the insoluble residue millfeed increased the resultant yield of isoelectric protein substantially. For example, for ground fine bran extracted at pH 10.5, one washing increased the yield of protein from 53 to 62.4%. Referring to Fig. 4, curve C, the 62.4% yield represents about 93% of the maximum possible yield at pH 10.5.

Nature of the Isoelectric Precipitates. The composition (dry basis) of freeze-dried precipitates arising from pH 6.45–11.5 extracts of fine bran were similar: protein, 69%; fat, 21%; ash, 3%; and, by difference, 7% carbohydrate. The precipitate from the pH 12.5 extract contained only 60% protein. A single analysis of a precipitate obtained from a pH 10.5 extract of fine bran indicated 4% pentosan.

The composition (dry basis) of the isoelectric precipitates from coarse bran supernatants made at pH 8.5 and 10.5 only was: protein, 86%; fat, 1.2%; ash, 2.5%; and, by difference, 10.3% carbohydrate. For shorts, the values were: protein, 66%; fat, 18%; ash, 2.4%; and, by difference, 13.6% carbohydrate.

These precipitates were tan to light gray when precipitated from the alkaline supernatants with hydrochloric acid. The use of sulfur dioxide to reduce the pH to 5.5 had no effect on the color of the precipitate. When frozen, the precipitates turned brown and stayed brown after freeze-drying. The freeze-dried precipitates developed rancid odors at room temperature in the presence of air, usually within a week.

Washing the freeze-dried fine bran products with ether, acetone,

95% ethanol, or water did not greatly change the brown color. Organic solvents reduced the fat content, which resulted in more-powdery products higher in protein. Rancid odors could be delayed by solvent washes, but complete removal of fat is difficult and, eventually, rancid odor developed.

Solvent drying (ethanol) of the fresh precipitate from a pH 10.5 extract of fine bran resulted in a product of a light-gray color and 87% protein (dry basis). Acetone drying yielded a similar product of 97% protein. When suspended with water, these solvent-dried materials produced slurries of lighter color (grayish) than the freeze-dried products (brownish yellow).

With the use of hydrogen peroxide with heat on alkaline slurries, alkaline extracts, or precipitates of fine bran under conditions similiar to those reported by Johnson and Anderson (10), precipitates were obtained that were straw-yellow in color when freeze-dried, but of reduced yield.

Nutritional Quality. A 1-hr., 23°C., pH 10.5 fine bran extract was adjusted to pH 5.5 and freeze-dried (without separation of the precipitate). The product contained 44% protein (dry basis) and, when fed to rats, showed a protein efficiency ratio (15) of 2.0. The ratio of the untreated fine bran was 2.2. Thus, alkaline treatment damaged the nutritional quality only slightly.

Pre-Extraction Treatments. Autoclaving ground fine bran for 10 min. at 240°F. reduced to half both the protein and solids solubilization at pH 10.5. The freeze-dried precipitate was brown and of reduced yield.

Prior washing of ground bran with ether reduced protein solubilization at pH 10.5 from 90 to 80%. The product recovered by precipitation and freeze-drying was a brown, powdery material containing 90% protein.

Exhaustive washing of ground fine bran with 70% ethanol removed 15% of the protein (N × 5.7) and 16% of the solids. Extraction of the washed, dried (40°C.) residue at pH 10.5 for 1 hr. dissolved an additional 43% of the protein and 16% of the solids. The freeze-dried precipitate obtained from the alkaline extract was brown; it had 92% protein; and its yield was comparatively small.

Washing fine bran with water at various pH values (5.5, 4.0, 3.0, and 2.0; adjusted with hydrochloric acid) prior to extraction with alkali at pH 10.5 only slightly affected color and composition of the freeze-dried precipitates. Yield was lowered if the pH of the wash-water was greater than 3.0.

Acid Extractants. At 23°C., acid solutions solubilized less protein

from fine bran than did alkaline solutions. At pH 2.0 (0.1% hydrochloric acid), protein solubilization was 23%; at pH 1.0 (0.25% hydrochloric acid), it was 31%; with 2% sulfuric acid, it was 30%. Protein solubilization observed with phosphoric and acetic acids was similar.

Acknowledgment

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