Protein Concentrates by Dry Milling of Wheat Millfeeds. II. Compositional Aspects[1]

DAVID A. FELLERS, ALLAN D. SHEPHERD, NANCY J. BELLARD, ALBERT P. MOSSMAN, PHILIP H. JOHNSTON, THEODORE WASSERMAN, Western Regional Research Laboratory, ARS, USDA, Albany, California 94710

Protein concentrate (PC) was prepared by dry-milling and sifting hard red spring wheat shorts of 9% moisture. The Protein Efficiency Ratio of the PC was 1.9, the same as the shorts from which the concentrate was prepared. Lysine content of the PC was 4.0 g./16 g. nitrogen (4.1 in the shorts). Thiamine, folic acid, and choline were concentrated in the PC. Riboflavin and vitamin B-6 were reduced slightly in the PC; niacin and pantothenic acid were decreased about 50%, compared to the original shorts. All these vitamins were present in the PC at 3 to 38 times the concentration found in white flour of 75% extraction. The iron content of the PC was 8.5 mg./100 g., about 20% less than that of the starting shorts. Other minerals in the PC also were reduced. Moisture of shorts at the time of milling and sieve size for separation of PC's were investigated as factors affecting yield of PC and its content of protein, crude fiber, thiamine, riboflavin, and certain amino acids. Results indicate that many differing PC's can be produced with respect to both yield and nutritional qualities. Generally the PC's may be described as natural wheat food products, high in B vitamins and good-quality protein, and of moderate fiber content.

Previous work (1) has shown that a high-protein flour can be stripped from wheat millfeeds by use of a roller mill and recovered by sifting. Moisture content of the millfeed at time of milling greatly affected yield and composition of the protein concentrate (PC). Sullivan (2) has used impact milling followed by sifting to achieve similar results.

Wheat millfeeds are known to be rich in B vitamins (3,4) and minerals (4,5). Kent et al. (6) described a Canadian process used during World War II to prepare a vitamin concentrate: low-grade flour was stripped from wheat millfeeds by hammer-milling and recovered by sifting. This vitamin concentrate was then added to 75%-extraction white flour to yield what was known as "Vitamin B White Flour (Canada Approved)." In a later paper, Kent et al. (7) described various experimental methods used to recover scutellum (portion of germ extremely rich in thiamine) (8) from certain millfeed streams; these methods used the principles of differential friability, air-classification, and differential adhesion on a moist surface. It was expected that PC's milled from wheat millfeeds (1) would also contain substantial quantities of B vitamins.

Amino acid composition for various wheat millfeeds has been reported (4,9, 10). Generally, the balance of essential amino acids is better than in flour, and this may be explained by the presence of substantial amounts of germ and aleurone (a layer of cells with high metabolic activity) in the millfeeds. Stevens et al. (11) determined the lysine content of aleurone cell contents to be about 4.2 g./16 g. N, compared with 1.8 g. for flour in a Manitoba wheat. Waggle et al. (4), working with nine wheat blends, found the range of lysine content in "commercial" germ to be 5.4 to 7.4 g./16 g. N. Shorts, which contains much germ tissue, had a range of lysine content of 4.2 to 5.5 g./16 g. N.

---

[1]Presented at the 52nd annual meeting, Los Angeles, Calif., April 1967. Contribution from the Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, Calif. 94710.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Kohler (12) has noted a direct, positive correlation between lysine content and protein efficiency ratio (PER) for wheat products. Thus the PER values of the millfeed fractions of wheat run two to four times that of white flour, which has a very low lysine content. The present work was undertaken to determine the levels of vitamins, minerals, and amino acids in PC and to determine the effect of milling moisture on those levels. We were also interested in the sieve size distribution of PC's and any shifts in composition of the fractions produced. The ability to produce PC's with various characteristics by a simple procedure such as sifting would enhance the flexibility of the process and allow a processor to meet broader consumer requirements.

The present work was carried out in two separate phases. The first entailed extensive analysis of a single, specific protein concentrate. The second phase was a study of the effect of moisture content of shorts at time of milling on compositional aspects of protein concentrates produced.

MATERIALS AND METHODS

Extensive Analysis of a Single Protein Concentrate

Preparation of Protein Concentrate. Montana HRS wheat shorts (obtained from a commercial mill) were dried in a forced-air dryer at 130°F. to 9% moisture, equilibrated 24 hr., and passed once through the reduction rolls of a Brabender Quadrumat Senior flour mill (C. W. Brabender Instruments, Inc., South Hackensack, N.J.). The yield of protein concentrate (PC), through 7XX bolting cloth, was 26.9%. Residue yield was 73.1%.

Analytical. Samples of the shorts, PC, and residue were taken for nitrogen, fiber, fat, ash, and PER determinations.

Samples of the shorts and PC were hydrolyzed with 6N hydrochloric acid at 110° C. under vacuum for 24 hr., and amino acids were determined on a Phoenix automatic analyzer. Methionine and cystine were determined by the performic acid oxidation method of Moore (13).

Samples of the shorts and PC were assayed microbiologically for riboflavin, folic acid, niacin, Vitamin B-6, pantothenic acid, and choline chloride. Riboflavin was also measured chemically to determine the extent of any interfering fluorescing compounds and thus the suitability of using the chemical method in other portions of this work. Thiamine was determined by the thiochrome method. Calcium, copper, iron, magnesium, manganese, potassium, sodium, and zinc content of shorts and PC were determined by atomic absorption spectrophotometry. Phosphorus was determined colorimetrically (14).

Effect of Milling Moisture on Protein Concentrates

Moisture Adjustment and Milling Procedure. Ten batches of shorts of about 1,500 g. each were weighed out and adjusted to 3, 5, 7, 8, 9, 10, 11, 12, 13, and 15% moisture as described previously (1). A 500-g. and a 1,000-g. sample of each particular moisture level were prepared. The 500-g. sample was used to condition the Brabender Quadrumat Senior flour mill. The 1,000-g. sample was then milled (reduction rolls only) three times, separating flour (7XX bolting cloth) after each milling yielding first, second, and third milling PC's and a final residue.

Total Protein Concentrate. Portions of the PC's from the first, second, and third millings at a particular moisture were combined, in proportion to their yield, to give a sample designated as Total PC.

Particle-Size Classification. A quantity of Total PC (100 g.) was sifted 15 min. on a Rotap with clean U.S. 100- and 140-mesh screens. The sifting operation yielded three fractions: − 7XX + 100, − 100 + 140, and − 140.

Analytical Tests. Nitrogen, crude fiber, thiamine, and riboflavin determinations were made on all PC's including fractions obtained by sifting on the Rotap. Riboflavin was determined by AACC Method 86-71 (Simplified Fluorescence) and thiamine by Method 86-80 (Thiochrome) (15). Certain PC's from the 3, 5, 9, 11, and 15% moisture samples were hydrolyzed and amino acids determined as described previously.

RESULTS AND DISCUSSION

Extensive Analysis of a Single Protein Concentrate

Analyses of shorts, PC, and residue (remainder of shorts after removal of PC) on moisture-free basis (m.f.b.) are presented in Table I. PER values for shorts and PC were the same, 1.9, indicating that there must have been very little shift in lysine between the milled products, because lysine is the limiting amino acid for wheat.

TABLE I
Composition and PER Values for Shorts, Protein Concentrate, and Residue, MFB

	SHORTS	PC	RESIDUE	HRS-L SHORTS[a]
Protein (N X 5.7), %	19.6	23.4	18.0	19.7
Crude fiber, %	7.7	2.0	10.2	7.9
Crude fat, %	6.5	6.8	6.4	7.3
Ash, %	4.9	3.5	5.5	4.7
Adjusted PER[b]	1.9	1.9	1.8	

[a]Data presented by Waggle et al. (4) for HRS wheat shorts.

[b]Product and casein standard fed at 10% protein level. Results adjusted to give a PER value of 2.5 for casein diet.

To observe the effect of a heat-process on PER, a 24% slurry of PC was cooked at 212°F. for 10 min. and then half of it was freeze-dried and the other half drum-dried at 260°F. The PER's of the respective products were 1.6 and 1.8. The heat-processing resulted in only slight damage to the nutritional quality of the protein as determined by this test.

The amino acid analyses verified our observation with PER results that there was little in lysine to or from the PC. Amino acid data for shorts, PC, and residue are shown in Table II. Values for HRS-L flour and shorts (from a HRS wheat milled to 76.7% extraction) (4) are shown for comparison. There was a 17 and 12% increase respectively in glutamic acid and proline content in the PC compared with the shorts; in large part this can be attributed to the concentration of starchy endosperm in the PC.

One might ask why lysine content of the PC did not decrease more if indeed endosperm is being concentrated in the PC. Kent (16) reported a layer of endosperm cells bordering the aleurone in a HRW wheat which is very high in protein (as

TABLE II
Amino Acid Composition of Shorts, Protein Concentrate, Residue, and Flour
(g./16 g. N)

AMINO ACID	SHORTS[a]	PROTEIN CONCENTRATE[a]	RESIDUE[a]	HRS-L SHORTS[b]	HRS-L FLOUR[b]
Ala	4.88	4.55	5.14	5.56	2.94
Arg	6.84	6.39	7.23	7.16	3.27
Asp	7.03	6.30	7.57	2.58	3.82
Cys	2.08	1.91	...	2.25	2.67
Glu	20.38	23.86	18.19	16.81	32.41
Gly	5.25	4.70	5.63	5.56	3.62
Hist	2.58	2.47	2.81	2.55	1.84
Ileu	3.31	3.31	3.37	3.02	3.44
Leu	6.15	6.10	6.13	6.16	6.89
Lys	4.06	3.96	4.21	5.03	1.81
Meth	1.68	1.79	...	1.66	1.65
Phen	3.79	3.84	3.96	3.61	4.95
Pro	6.28	7.03	5.82	5.45	12.00
Ser	4.16	4.14	4.11	4.56	4.82
Thre	3.22	3.13	3.36	3.61	2.62
Tyro	2.61	2.63	2.83	2.78	2.73
Val	4.83	4.66	4.89	4.44	3.70

[a]Average of duplicate determinations.
[b]Data presented by Waggle *et al.* (4) for HRS wheat shorts and flour of 76.7% extraction.

much as 50%) and represents about 10% of the wheat kernel. Because subaleurone fraction adheres directly to bran particles, it is likely that a large portion of endosperm found in millfeeds will be of the subaleurone high-protein, low-lysine type. Some compensating factor or factors must be entering in to offset the lysine-diluting effect of endosperm on the PC, such as a high-lysine-content aleurone and/or germ fraction. The magnitude of this may be surmised from estimates on endosperm content in shorts *vs.* PC (calculations based on starch content) (1). In shorts, there was a 1:3 endosperm: "bran" ratio; in the PC the ratio was about 1:1, indicating the much greater contribution of endosperm in the PC.

The content of B vitamins (single values) found for shorts and PC is reported in Table III. The value for HRS-L unenriched flour (4) is given for comparison.

TABLE III
Vitamin Content of Shorts, Protein Concentrate, and Flour, MFB

VITAMIN	SHORTS	PC	HRS-L SHORTS[a]	HRS-L FLOUR[a]
	γ/g.	γ/g.	γ/g.	γ/g.
Thiamine	27.9	46.1	25.4	1.62
Riboflavin (chemical)	4.7	2.6	5.2	0.41
(microbiological)	3.6	3.2	...	...
Niacin	165.2	67.8	139.2	11.3
Pantothenic acid	30.9	19.7	28.4	3.84
Folic acid	1.6	1.6	2.0	0.14
Vitamin B-6	7.5	6.4	...	0.49
	mg./g.	mg./g.	mg./g.	mg./g.
Choline	3.0	4.0	2.3	1.14

[a]Data presented by Waggle *et al.* (4) for HRS wheat shorts and flour of 76.7% extraction.

The concentration of thiamine in the PC indicates a preferential grinding of scutellum, which is reported to contain 52 to 65% of all thiamine in the wheat kernel (8). Heathcote et al. (17) reported the niacin content in various anatomical wheat parts: 82% of the niacin in the aleurone, 12% in the endosperm, 4% in the pericarp and testa, and only 2% in the germ. The large reduction of niacin in this PC indicates that it is much lower in aleurone content than the shorts.

From the vitamin assays it appears that endosperm and germ were concentrated in the PC whereas pericarp and aleurone were reduced.

Mineral contents of shorts and PC are given in Table IV, with values for HRS-L shorts and flour (4) for comparison.

TABLE IV
Mineral Content of Shorts, Protein Concentrate, and Flour
(Moisture-free basis)

MINERAL	SHORTS	PC	HRS-L SHORTS[a]	HRS-L FLOUR[a]
	mg./100 g.	mg./100 g.	mg./100 g.	mg./100 g.
Fe	10.8	8.5	6.4	0.42
Na	6.5	4.4	15.0	< 6.0
K	1021.7	760.8	942.0	85.0
Ca	71.7	47.8	66.0	< 29.0
Mg	478.3	304.3	337.0	22.0
Cu	1.7	1.6	4.3	0.07
Zn	12.0	10.9	9.3	0.45
P	994.6	833.7	942.0	113.0
Mn	17.4	10.9	14.8	0.41
Total	2,614.7	1,982.9	2,333.8	256.4

[a]Data presented by Waggle et al. (4) for hard red spring wheat shorts and flour of 76.7% extraction.

The reduction in ash content from shorts to PC was 28.6% (Table I). Of the individual minerals, Mn, Mg, Ca, and Na decreased the most (37—32%); K, Fe, and P decreased moderately (26—16%; and Zn and Cu decreased the least (9 and 6% respectively). The weighed average reduction for all the minerals assayed was 24.2%.

Hinton (18) reported the ash content of the aleurone of a hard red Canadian wheat as 17.2% (14% moisture basis). He further determined that the aleurone represented only 6.7% of the whole kernel but contained 60.2% of the ash of the wheat kernel. The reduction of ash in the PC from the shorts is a further indication that aleurone tissue content is reduced in the PC.

Effects of Milling Moisture on Protein Concentrates

The yields of PC from shorts milled at various moisture levels and the distribution of Total PC into three particle-size groupings as determined by sifting on a Rotap are shown in Table V. The percentage of Total PC in each particle-size group was relatively constant (29, 26, and 45% respectively for −7XX+100, −100+140, −140) at all milling moistures except 15%.

Figure 1 compares Total PC (—7XX bolting cloth) with that portion of total PC which passed through a 140-mesh screen, with respect to yield and fiber and protein contents. Little difference was noted between protein contents of the two PC's. However, when the same milling and sifting procedure was carried out on

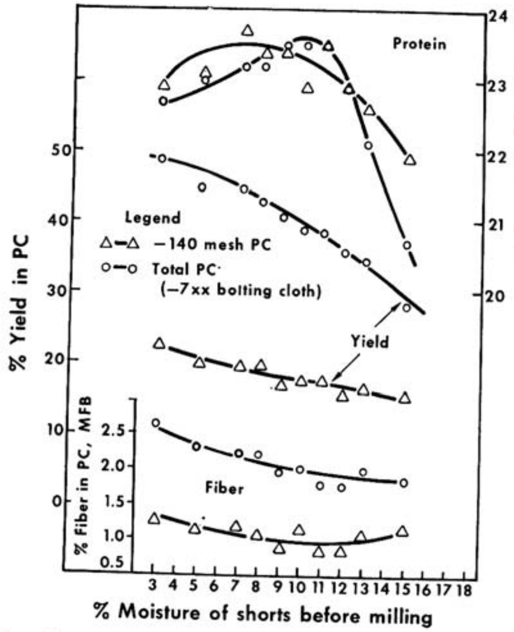


Fig. 1. Effect of moisture content of shorts at time of milling on fiber, protein, and yield of protein concentrates of two different sieve sizes.

TABLE V
Yields of Protein Concentrate from Shorts at Various Moisture Levels and Their Particle-Size Distribution

Moisture at Time of Milling	Yield of PC: 1st Milling PC	2nd Milling PC	3rd Milling PC	Total PC	Distribution of Total PC: −7XX Bolting Cloth+100 Mesh	−100+140 Mesh	−140 Mesh
%	%	%	%	%	%	%	%
3	33.2	10.3	5.3	48.8	28	27	45
5	29.7	9.9	5.1	44.7	28	28	44
7	29.5	9.8	5.2	44.5	30	27	43
8	28.9	9.1	4.9	42.9	30	24	46
9	26.6	9.0	4.9	40.5	35	24	41
10	25.0	8.5	5.1	38.6	26	28	46
11	21.6	10.2	6.5	38.3	29	26	45
12	19.3	9.9	6.5	35.7	29	28	43
13	21.6	7.5	5.6	34.7	28	25	47
15	18.2	6.2	3.6	28.0	18	28	54

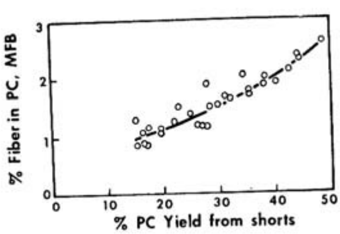
shorts and fine bran at 9% moisture from a different sample of HRS wheat, there was a definite concentration of protein in the finer sieve fractions (Table VI). Differences in wheat such as variety, growing conditions, and maturity may have an effect on how the protein of PC's will be distributed among various particle-size groupings.

Selection of sieve size and milling moisture in the preparation of PC's provides some control over its fiber content. For example, fiber content of the through

TABLE VI
Yield and Protein Content of Various Total PC's and Sieve Fractions Therefrom

SAMPLE	YIELD	PROTEIN
	%	%
SHORTS		20.9
Total PC (−7XX bolting cloth)	37.2	25.1
Sieve fractions of Total PC:		
−7XX+100	12.0	22.6
−100+140	12.8	25.3
−140	12.4	27.4
FINE BRAN		20.6
Total PC (−7XX bolting cloth)	27.1	28.4
Sieve fractions of Total PC:		
−7XX+100	5.8	25.5
−100+140	9.5	32.1
−140	11.8	30.9

140-mesh PC (Fig. 1) was about half that of the Total PC, but yield was slightly less than half. Figure 2 shows the relation between yield and fiber content of PC's (first, second, third milling PC's, Total PC's, and the various sieve fractions of Total PC's). In general, the higher the yield, the more rapidly fiber content increased. The variation from the trend line (Fig. 2) was due to the method of obtaining a particular yield; thus, some ways were better than others for obtaining the same yield, at least with respect to fiber.



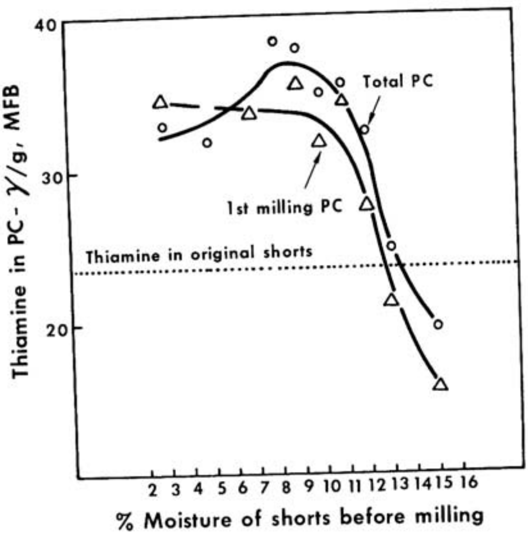
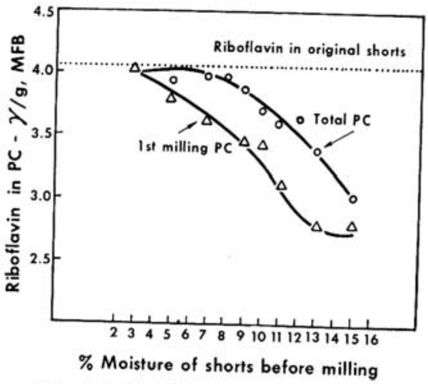


Fig. 2 (left). Relation between fiber content and yield of protein concentrates.
Fig. 3 (right). Effect of moisture content of shorts at time of milling on thiamine content of protein concentrates.

The effect of moisture at time of milling on thiamine content of PC's is shown in Fig. 3. The horizontal line at 23.5 represents γ thiamine/g. in the original shorts. There was a sharp increase in thiamine in the PC as milling moisture was reduced from 15 down to about 11%, indicating that at about this 11% moisture level the scutellum was quite friable and was readily broken down into flour (− 7XX bolting cloth).

Figure 4 shows the effect of moisture at time of milling on riboflavin content of PC's. The horizontal line at 4.05 represents γ riboflavin/g. of starting shorts. As milling moisture decreased, riboflavin content of PC's increased, but none of the PC's contained more riboflavin than the starting shorts. Since a large portion of the riboflavin of the wheat kernel is located in the aleurone, it would appear that the aleurone increases in friability only gradually with decrease in moisture and never becomes as friable as the endosperm or germ.



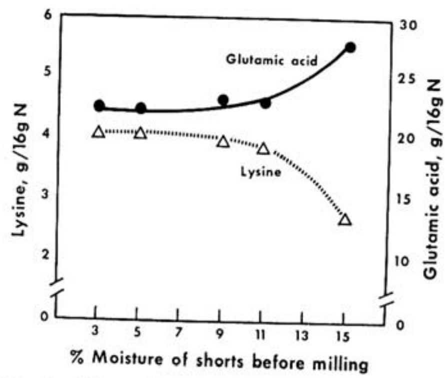


Fig. 4 (left). Effect of moisture content of shorts at time of milling on riboflavin content of protein concentrates.

Fig. 5 (right). Effect of moisture content of shorts at time of milling on lysine and glutamic acid content of protein concentrates.

When Total PC's were further fractionated by sifting, the finest fraction (−140-mesh) had about 20% lower thiamine content than Total PC, whereas the two coarser fractions (−7XX+100 and −100+140) were enriched in thiamine. Riboflavin also averaged lower in the finest fraction by about 24% compared with Total PC.

Lysine content of Total PC's increased, and conversely, glutamic acid decreased with decreasing milling moisture of shorts (Fig. 5). Proline and, to a lesser extent, phenylalanine and leucine followed a pattern similar to that of glutamic acid. Arginine, histidine, alanine, aspartic acid, and glycine showed a less pronounced tendency to follow the lysine pattern. Isoleucine, serine, threonine, tyrosine, and valine remained essentially unchanged, regardless of milling moisture.

The Total PC produced at 15% moisture was whiter and higher in starch, and obviously contained more starchy endosperm than Total PC's produced at lower moistures. Since gluten of the endosperm is low in lysine and high in glutamic acid, the results displayed in Fig. 5 were as expected.

The sharp increase in lysine content from the 15%- to the 11%-moisture Total PC indicates that a fraction with very high lysine content was being reduced to flour at 11% moisture but not at 15% moisture. Separate milling experiments where "commercial" germ was milled (reduction rolls of a Brabender Quadrumat Senior) one time showed a germ flour (−7XX bolting cloth) yield of 12% at 15% moisture, 30% at 12% moisture, and 60% at 9% moisture. Friability of the germ increased tremendously with decrease in moisture. This evidence, along with supporting evidence from the thiamine determinations and the fact that germ has a high lysine content, indicates strongly that germ tissue is substantially broken down at 11% moisture but not at 15%.

TABLE VII
Composition and Yield of Various Total PC's and Sieve Fractions Therefrom

Milling Moisture of Shorts	Yield of Total PC	Yield of −140-Mesh PC	Fiber (MFB)	Lysine	Glutamic Acid
%	%	%	%	g./16 g. N	g./16 g. N
3	48.8		2.6	4.1	22.4
3		22.0	1.3	3.2	28.1
9	40.5		2.0	4.0	23.1
9		16.6	1.0	3.2	27.6
15	28.0		1.9	2.8	27.9
15		15.1	1.2	2.6	31.5

Fractionation of Total PC's by sifting may provide products of significantly different amino acid compositions, as shown for selected samples in Table VII. The lysine content was about 20% lower in the through 140-mesh PC (except at 15% moisture) compared with Total PC. Conversely, glutamic acid (and proline) was higher in the through 140-mesh PC. The results point to a further concentration of endosperm in the finest sieve fractions compared with Total PC at the expense of germ, aleurone, and pericarp.

The Brabender Quadrumat Senior flour mill used in this work is a laboratory mill with some rather atypical product-flow characteristics compared with those of commercial flour mills. Particularly unusual is the passage of stock through three sets of rolls before any sifting is carried out. However, similar results were obtained on a single roll stand where sifting could be carried out after each pass. The stand had rolls 9 in. in diameter by 6 in., 38 corrugations per in., Getchell cut, dull-to-dull grinding action, and ½-in. spiral per ft. It was operated at a 2 to 1 differential with the fast roll speed at 760 r.p.m. and 0.001- to 0.0015-in. gap. Protein concentrate was separated through a 88-mesh stainless-steel screen in a laboratory sifter.

LITERATURE CITED

1. FELLERS, D. A., SHEPHERD, A. D., BELLARD, NANCY J., and MOSSMAN, A. P. Protein concentrates by dry milling of wheat millfeeds. Cereal Chem. 43: 715–725 (1966).
2. SULLIVAN, BETTY. Wheat-based products for world use in United States Aid Programs. Am. Miller & Processor 95 (6): 11, 12, 30 (1967).
3. BRADLEY, W. B. Bread as a food. Northwest. Miller 272 (4): 17—19 (1965).
4. WAGGLE, D. H., LAMBERT, M. A., MILLER, G. D., FARRELL, E. P., and DEYOE, C.W. Extensive analyses of flours and millfeeds made from nine different wheat mixes. II. Amino acids, minerals, vitamins, and gross energy. Cereal Chem. 44: 48—60 (1967).
5. CZERNIEJEWSKI, C. P., SHANK, C. W., BECHTEL, W. G., and BRADLEY, W. B. The minerals of wheat, flour, and bread. Cereal Chem. 41: 65–72 (1964).
6. KENT, N. L., SIMPSON, A. G., JONES, C. R., and MORAN, T. High vitamin flour: Developments and recommendations in milling techniques. Milling 103: 294–298, 300 (1944).
7. KENT, N. L., THOMLINSON, J., and JONES, C. R. Scutellum and its separation in milling. Milling 113: 46—54 (1949).
8. HINTON, J. J. C. The chemistry of wheat germ with particular reference to the scutellum. Biochem. J. 38: 214—217 (1944).
9. HEPBURN, F. N., CALHOUN, W. K., and BRADLEY, W. B. The distribution of the amino acids of wheat in commercial mill products. Cereal Chem. 37: 749—755 (1960).

10. HORN, M. J., FIFIELD, C. C., BLUM, A. E., and WARREN, Helen W. The distribution of amino acids in wheat and certain wheat products. Cereal Chem. 35: 411—421 (1958).
11. STEVENS, D. J., McDERMOTT, E. E., and PACE, J. Isolation of endosperm protein and aleurone cell contents from wheat, and determination of their amino-acid composition. J. Sci. Food Agr. 14: 284—287 (1963).
12. KOHLER, G. O. Effect of processing on the biological value of wheat proteins. Cereal Sci. Today 9: 173—174, 176, 178, 186 (1964).
13. MOORE, S. On the determination of cystine as cysteic acid. J. Biol. Chem. 238: 235—237 (1963).
14. ALLEN, R. J. L. The estimation of phosphorus. Biochem. J. 34B: 858—865 (1940).
15. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (7th ed.). The Association: St. Paul, Minn. (1962).
16. KENT, N. L. Subaleurone endosperm cells of high protein content. Cereal Chem. 43: 585—601 (1966).
17. HEATHCOTE, J. G., HINTON, J. J. C., and SHAW, B. The distribution of nicotinic acid in wheat and maize. Proc. Roy. Soc. (London) B139: 276—287 (1952).
18. HINTON, J. J. C. The distribution of ash in the wheat kernel. Cereal Chem. 36: 19—31 (1959).

[RECEIVED DECEMBER 4, 1967. ACCEPTED MAY 6, 1968]