PREPARATION OF PROTEIN CONCENTRATES FROM WHEAT SHORTS AND WHEAT MILLRUN BY A WET ALKALINE PROCESS[1]

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ABSTRACT

Protein concentrates were prepared from wheat shorts and millrun by a wet process. The millfeed was suspended in water, adjusted to a specific alkaline pH, and extracted for a specified time interval. The mixture was squeezed to separate the liquid phase, which also contained starch granules, from fibrous material. Fractions rich in protein, starch, and fat and low in fiber were prepared from the squeezed juice by heat coagulation or acid precipitation. The yield and composition of concentrates as a function of extraction pH are described. The concentrates generally contained about 30 to 40% protein, 36 to 60% starch, and 7 to 11% fat, and were obtained in yields of 15 to 25%. Removal of starch from the squeezed juice before precipitation of the protein yielded products containing approximately 64% protein and 19% fat. After removal of the precipitated concentrates the residual juice was recycled to be used as the extracting medium for another batch of millfeed; an eight-time recycling experiment is described. Addition of bisulfite during processing resulted in lighter-colored products.

The search for protein sufficiency and the manufacture of protein concentrates has stimulated investigation of many different sources of protein. At the present time cereal grains represent the most plentiful source of protein suitable for man's needs. A conventional procedure for manufacturing products with high protein content from whole cereal grains is a combination of fine-grinding and air classification. Flours with relatively high protein content have been prepared from rice (1), oats (2), wheat (3,4), sorghum (5,6), and triticale (7) in this manner. However, the yields have been low and the protein content usually less than 25%. Through further milling and sifting, wheat millfeeds provided flours with a protein content of about 25% and a fiber content of about 2% (8), in yield of 30 to 40% in the case of shorts.

Protein concentrates have also been obtained from whole cereal by wet processing. Reiners et al. (9) described several products with high protein content that were obtained during wet milling of corn. Further processing of corn gluten meal yielded concentrates with protein contents of 70 to 75% and 90 to 95% (10). Wet milling of sorghum yielded products with high protein content (11). Wet milling of oats and oat millfeeds by an alkaline extraction process has been described (12). Depending on the fraction extracted and the experimental conditions, products containing up to 89% protein were obtained. A similar alkaline extraction process has been used to isolate a concentrate containing 73% protein from corn germ meal (13).

In a publication which appeared in 1966, wet alkaline extraction of wheat bran or shorts followed by isolation of the protein gave products high in protein and fat, and low in fiber (14). No further work has been done using this process to prepare protein concentrates from wheat millfeeds, although the same technique has since been applied to defatted rice bran (15).

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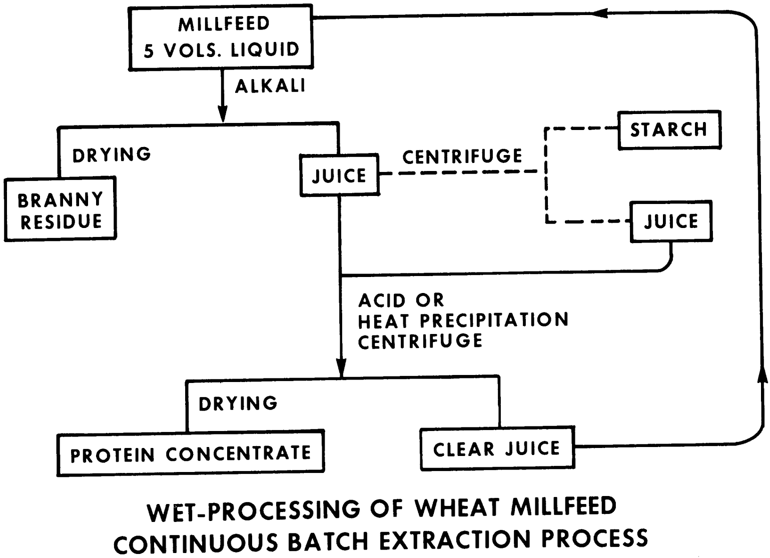

Fig. 1. Flow diagram illustrating preparation of protein concentrate by wet-processing of wheat millfeed.

Nearly five million tons of wheat millfeeds, containing almost one million tons of protein are produced annually in the U.S. alone. The vast majority of this is used in feeds for animals, mainly ruminants, since the high fiber content prevents widespread use in human food. The protein nutritive value of millfeeds is superior to endosperm protein (16), the fats are highly unsaturated, and the mineral and vitamin content are high (17)—all desirable qualities for food or feed. Wet alkaline processing of wheat millfeeds has been investigated further with the objective of establishing experimental conditions and developing a concentrate rich in the desirable nutrients but low in fiber. It is expected that the concentrates would find immediate use in high-energy nonruminant feeds and ultimately in human foods.

Wet alkaline processing of millfeeds is described in this study, along with several different products obtained from wheat shorts and millrun.[2] Subsequent papers will describe nutritional aspects and food uses of the concentrates, and pilot-scale operations.

MATERIALS AND METHODS

Wheat shorts from hard red spring wheat were donated by International Multifoods through the courtesy of William R. Johnston. Wheat millrun from hard red spring wheat was donated by ConAgra-Montana Inc. The proximate analyses, calculated on a moisture-free basis, were: Shorts, 3.64% nitrogen, 7.46% fat, 5.48% ash, and 9.77% fiber; millrun, 3.02% nitrogen, 5.56% fat, 5.50% ash, and 10.34% fiber.

[2]Millfeed is a general term applied to any one of the fractions of the kernel left after flour removal. Shorts is the fraction of millfeed left after removal of coarse bran and most of the red dog and germ. Millrun is the total residue left after flour removal.

TABLE I
Yield and Composition of Heat- or Acid-Precipitated Protein Concentrates from Wheat Millrun and Shorts by Wet-Processing at Different pH Values

Material	Conditions[a]			Yield[b]	Composition				
					Protein[c]	Fat	Fiber	Ash	Starch
				%	%	%	%	%	%
Millrun	NaOH	7	Heat	15.0	26.1	7.5	0.6	4.2	52.1
	NaOH	8	Heat	18.4	27.2	7.7	0.8	4.5	49.3
	NaOH	9	Heat	20.1	26.2	7.0	0.8	4.1	47.7
	NaOH	9	Acid	17.5	28.1	8.9	0.5	2.0	61.3
	NaOH	9	Acid[d]	15.0	31.3	10.5	0.6	1.1	...
Shorts	None	5.4	Heat	5.5	33.6	2.8	1.0	4.6	46.3
	NaOH	8	Heat	20.0	35.2	6.9	0.4	5.1	...
	NaOH	8	Acid	21.8	30.9	...	...	...	...
	NaOH	9	Heat	22.3	35.1	11.1	0.4	3.9	36.5
	NaOH	9	Acid	22.2	38.6	...	...	...	...
	NaOH	9	Heat[d]	18.8	41.1	10.0	0.5	2.1	47.0
	NaOH	10	Heat	25.1	40.7	10.9	0.3	4.1	38.4
	NaOH	10	Acid	24.8	38.0	...	...	...	...
	NaOH	11	Heat	31.8	42.2	10.2	0.4	5.1	35.8
Shorts	NH_4OH	7	Heat	17.6	33.5	6.4	0.7	5.0	...
	NH_4OH	8	Heat	20.3	38.2	9.7	0.3	4.9	40.5
	NH_4OH	9	Heat	19.7	43.0	11.7	0.8	4.4	39.4
	NH_4OH	10	Heat	25.2	38.2	8.5	0.2	2.7	38.6

[a]Alkali used; extraction pH; method of precipitation.
[b]In all tables analyses are on a dry basis.
[c]N × 6.25.
[d]Product washed.

Preparation of Protein Concentrate

Figure 1 is a flow sheet describing the experimental procedure. One hundred grams of millfeed was mixed with 500 ml. of water, and the mixture was treated with 3N NaOH solution as required to maintain a pH of 8.6 to 9.0. In cases where ammonia was used instead of NaOH, 15% NH_4OH solution was used. The mixture was extracted by stirring for 15 min. at room temperature (20° C.) in a Hobart mixer. Where necessary, experiments were run at other pH values. After extraction the mixture was placed in a Norwalk nylon bag and squeezed in a portable Norwalk juicer (hydraulic press) at pressure gradually increasing to about 40 p.s.i.g. After juice was extruded (<5 min., 400 to 420 ml.) it was subjected to either of two different processes depending on the particular demand. In one process the pH was lowered to 6.0 by dropwise addition of 1N HCl, steam was injected to attain a temperature of 80° to 85° C., and the precipitated protein concentrate was centrifuged (3,050 × g, 10 min.) of filtered (referred to as "heat-precipitated"); alternatively, the pH was lowered to 4 to 5 by dropwise addition of 1N HCl, and the protein concentrated was collected by centrifugation (3,050 × g, 10 min.)(referred to as "acid-precipitated"). In the second process, the extracted juice was centrifuged (1,465 × g, 5 min.) to precipitate the starch as a

TABLE II
Yield and Composition of Protein Concentrates from Wheat Millrun and Shorts by Wet-Processing at pH 8.6 to 9.0. Starch Has Been Removed during the Process

Material (Precipitation Method)	Yield %	Protein[a] %	Fat %	Fiber %	Ash %
Millrun (heat)	7.6	64.1	19.2	0.4	4.7
Millrun (acid)	7.1	62.8	19.1	0.5	3.6
Shorts (acid)	11.2	65.3	17.7	0.5	3.6
Shorts (acid)[b]	9.7	79.6	0.7	0.4	4.0

[a]N × 6.25.
[b]Defatted with diethyl ether.

separate product the supernatant was then treated as in the first process to precipitate the protein. From each process the final "clear juice" plus any washes could be recycled to the next batch of millfeed. All products after centrifugation or filtration could be washed if desired. This was done by washing with 400 ml. of water adjusted with dilute HCl to the particular pH of isolation. All products were lyophilized. This is the basic process, and is shown in Fig. 1.

The Effect of Bisulfite

In concentrates containing starch the effect of bisulfite was studied as follows. Concentrates were prepared as described in the basic process except that sodium bisulfite (0, 0.1, 0.2, 0.5, 1.0 g.) was added to the extractant water. The color of the products was read on a Hunter Lab D25 Color Difference Meter using as a comparison standard color D33C-157 from Hunter Associates Lab., Inc., Fairfax, Va.

In concentrates devoid of starch, the effect of bisulfite on color was studied as above except that the squeezed juice was centrifuged at 1,465 × g for 10 min., thereafter the basis process was followed. Colors were read as above.

Recycling of "Clear Juice"

A concentrate containing starch was prepared by heat precipitation as described in the basic process. After removing 10 ml. for solids determination, the final clear juice was diluted to 500 ml. with water and added to another 100-g. batch of shorts, and the basic process repeated. This procedure was repeated so that a total of eight batches of shorts was involved in the recycling process. The concentrate from each stage was lyophilized.

For proximate analyses, protein was calculated by multiplying N by 6.25. Correction was made for added ammonia where appropriate. Starch was determined with pig pancreatic α-amylase as described by Saunders et al. (18). Sugars in the clear juice were determined by chromatography on Dowex 50 (K^+) followed by routine analyses as described for wheat bran sugars (19).

RESULTS AND DISCUSSION

Composition and Yield of Concentrate

A flow-sheet describing the preparation of protein concentrates by wet alkaline extraction of wheat millfeeds is shown in Fig. 1.

Variables such as pH of extraction, extraction time and pH, and pH-temperature interactions were examined to optimize extraction and precipitation of the protein. As a result, conditions of extraction were generally chosen to be pH 8.6 to 9.0 with an extraction time of 15 min. at room temperature. Longer extraction times or increased temperature caused little increase in yield of concentrate, whereas higher pH, though increasing the yield, would necessitate in commercial operation higher costs for acid and alkali, but perhaps more importantly, could cause sulfur-amino acid destruction and racemization. The optimum conditions for precipitation were determined to be either at the isoelectric point (pH 4 to 5), or lowering the pH to 6.0 and heating to 85°. At pH ⩾7 little, if any, protein precipitated with heat.

The same process has been successfully applied to manufacture protein concentrates from milling by-products of rice[3], rye[3], triticale (20), and brewer's spent grains[3].

The yield and composition of protein concentrates extracted from wheat shorts and millrun by wet-processing at different pH values are shown in Table I. The products from shorts at pH 10 and 11 are included to show that yield and protein extraction not only increase from pH 7 to 9 but continue to increase at higher pH. Ammonium hydroxide was as effective as sodium hydroxide as an extractant. Ammonium hydroxide would be advantageous when the product or residual extracted residue was to be fed ruminants since any residual ammonia would be utilizable. The protein concentrates from shorts contained more protein and were obtained in higher yield than concentrates from millrun. The yield and composition of products prepared at pH 8.6 from shorts and millrun where the starch was removed as a separate product during the process are shown in Table II. In addition a product obtained by subsequent defatting with ether is described.

It is apparent that products with varying compositions of protein, fat, and starch can be prepared from wheat millfeeds by this process. Compared to the protein concentrates (WPC) prepared from shorts by the dry-milling process (8), the wet alkaline process products have higher protein and starch content, similar fat and ash, and lower fiber. Products can be washed to lower ash. In the wet alkaline process, unlike the dry-milling process, starch can be manufactured as a separate product.

Composition and Yield of By-Products

The yield and composition of the by-products produced during the isolation of a protein concentrate from shorts are shown in Table III. The composition of the residue is similar to that of wheat bran except that the fat content is lower. This by-product could presumably find use as a ruminant feed. The starch is almost 90% pure and could be purified further, if necessary. The sugars in the clear juice have been identified as glucose, fructose, sucrose, raffinose, neokestose, and maltose. The sugars are similar to those described in wheat bran except that maltose was not found earlier (19). Glucose and fructose are at higher levels in the processed material. It is possible that maltose and higher contents of monosaccharides have appeared as a result of amylase and invertase activity on starch and sucrose during the initial wetting of the bran. Concentration of the

[3]Saunders, R. M., and Connor, M. A., unpublished work.

TABLE III
Yield and Composition of By-Products of Wet Processing of Wheat Shorts at pH 9

Material	Yield %	Protein[a] %	Fat %	Fiber %	Ash %	Starch %	Sugar %
Pressed residue (dried)	65.5	15.9	1.5	14.8	4.5	14.2	...
Starch	7.9	3.9	0.4	0.4	5.4	88.9	...
Clear juice	15.3	17.0	...	0	8.0	0	44.0

[a]N × 6.25.

clear juice with a concomitant increase in sugar content should make it suitable for an animal feed supplement or a growth medium for cellular organisms.

Recycling Experiment

Data obtained from the recycling experiment with shorts are shown in Table IV. The products obtained from an eight-batch operation do not differ appreciably in yield or composition, except for the fat content which decreased after the first cycle. Experimentally no difficulties were encountered. The solids level of the recycling juice increased initially but reached an equilibrium level by the fifth pass. It would be advantageous for large-scale processing systems to use recycling since this would minimize water usage and drying costs.

Effect of Bisulfite

The protein concentrates are light tan in color. This color was lightened when bisulfite was incorporated into the process. The yield, protein content, and color of the products obtained from shorts when sodium bisulfite was added during the process are shown in Table V. ΔE is the color difference compared to the standard color, higher ΔE indicates a darker color. Color was diminished with increasing concentration of bisulfite. Yield was enhanced in products containing starch with increasing bisulfite levels, although no statistical data are available.

Nutritive Value

The theoretical metabolizable energy of the protein concentrates for chicks, calculated by the method of Carpenter and Clegg (21) compares favorably with present commercial poultry feeds. For example, the calculated metabolizable energy of the concentrates containing starch prepared from shorts or millrun at pH 9 was 1,600 to 2,000 kcal. per lb., dry basis, as compared to values of 1,450 and 1,150 kcal. per lb., dry basis, for fishmeal and soybean meal, respectively.

In a preliminary feeding study with rats using materials prepared on a pilot plant scale from millrun, protein digestibility in excess of 90% was found for all materials whether freeze-dried, drum-dried or spray-dried. Compared to a PER value of 2.50 for casein, PER values from 1.70 to 2.00 were obtained. It is unlikely that the mild alkaline conditions employed in this process would have caused formation of lysinoalanine, which has been associated with adverse clinical symptoms in feeding trials. Severe alkali treatment (pH 12, 40° to 60° C., 4 to 8 hr.) of protein is typical of conditions necessary for lysinoalanine formation (22,23).

TABLE IV

Yield and Composition of Protein Concentrates from Wheat Shorts by Wet-Processing at pH 8.6 to 9.0, during an Eight-Batch Recycle Operation; Also Solids Content of the Recycled Juice

Recycle Batch No.	Yield %	Protein[a] %	Fat %	Fiber %	Ash %	Juice Solids %
1	21.7	36.8	9.2	0.2	4.9	3.0
2	19.6	34.6	7.0	0.3	5.4	4.3
3	20.4	33.0	6.3	0.3	5.4	5.7
4	21.1	31.6	6.0	0.4	6.3	6.4
5	20.6	29.7	6.2	0.3	5.4	7.3
6	22.4	32.3	5.1	0.4	5.8	7.3
7	21.7	30.2	5.1	0.3	6.6	7.4
8	21.2	32.7	5.4	0.4	6.2	7.3

[a]N × 6.25.

TABLE V

Yield, Protein Content, and Color Comparison of Protein Concentrates Prepared by Wet-Processing of Wheat Shorts at pH 8.6 to 9.0 with Addition of Sodium Bisulfite[a]

	Concentrates Containing Starch						Concentrates Without Starch					
	Acid			Heat			Acid			Heat		
Bisulfite %	Yield %	Protein[b] %	Color ΔE	Yield %	Protein[b] %	Color ΔE	Yield %	Protein[b] %	Color ΔE	Yield %	Protein[b] %	Color ΔE
0	16.6	43.8	23.92	16.8	40.2	25.50	7.4	70.3	37.98	7.7	67.9	37.22
0.1	18.7	38.6	16.78	18.5	38.0	19.77	8.2	73.7	28.74	7.8	76.4	31.56
0.2	18.8	39.4	14.22	18.9	37.4	15.97	8.3	72.0	23.16	8.0	70.9	25.51
0.5	19.4	39.2	12.42	20.0	38.3	12.84	8.1	74.2	22.95	8.5	72.1	21.99
1.0	20.6	40.4	11.02	20.6	37.4	14.40	7.2	74.6	22.81	7.2	67.9	18.97

[a]All values shown are average values from duplicate experiments.
[b]N × 6.25.

The concentrates were found to contain a high concentration of a natural antioxidant. The millrun concentrate, for example, contained about 0.03% by weight. The prevalent antioxidant has been identified as 5-*n*-heneicosylresorcinol[4].

Other studies underway on the nutritional potential of these materials include protein digestibility and protein quality measurements, metabolizable energy measurements, fatty acid compositions, and mineral and vitamin compositions. Several functional property tests have been completed. Tests on the incorporation of these products into foods are underway. These studies will be reported in their entirety in an ensuing publication.

[4]Burkhardt, H. J., unpublished work.

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