THE CHEMICAL, FUNCTIONAL, AND NUTRITIONAL CHARACTERIZATION OF PROTEIN CONCENTRATES FROM DISTILLER'S GRAINS[1]

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ABSTRACT

Protein concentrates having potential food use were obtained from distiller's fermented wheat and corn. The yield of distiller's protein concentrate (DPC) from fermented wheat was optimal using a pH 12.2 extraction at 23°C. Optimal yield of DPC from distiller's fermented corn was obtained by a pH 12.2 extraction at 80°C. Nutritional and amino acid analyses of both DPCs produced showed them to be almost identical to their starting grains. Analysis of each DPC for ribonucleic acid (RNA) showed each to contain less than 0.1% RNA, well below the safe level of 2%. Metal ion analyses showed that neither DPC contained an accumulation of lead, but both were found to be significantly lower in calcium, magnesium, manganese, and zinc, when compared to soy isolate. The functional characteristics of each DPC were identified for use in bread, extruded puffed snacks, and meat emulsions. When both DPCs were compared to soy protein isolate, wheat DPC was found to surpass corn DPC and soy isolate in baking and extrusion properties. Corn DPC possessed an emulsion stability almost equal to that of soy isolate and far exceeding that of wheat DPC.

The principal raw materials used for the manufacture of grain alcohol in the U.S. are grains, with corn the primary grain used (1). More than 427,000 tons of distiller's feeds, by-products of the fermentation procedure, are produced each year. Of this amount, 85% is dried for livestock feed, whereas 15% is fed to livestock wet (2).

The composition and nutritional quality of distiller's feeds are dependent upon the grain fermented, the cooking procedure used, the type of enzyme conversion employed, and numerous other processing factors (3). Stillage, the fermented grains which have had all ethyl alcohol removed by distillation, yields two feeds: dried solubles and dried grains, both of which possess from 26–30% protein (4). The limiting essential amino acids in both products are lysine for fermented wheat, and lysine and tryptophan for fermented corn and corn-sorghum mixtures (5).

Distiller's grains are known to be good feed materials for broilers and other livestock (4,6).

The primary role of the grain in the production of grain alcohol is to provide a carbohydrate source for yeast fermentation. The grain protein going into the fermentor goes unused, and is complemented with high-lysine yeast protein. Much is known about the protein composition and nutritive quality of the grains used for fermentation, but little is known about the protein that can be recovered from the stillage. Ten per cent of the corn kernel is protein, with a protein efficiency ratio (PER) of 1.36 and a biological value (BV) of 60 (7,8). Corn meal, void of the germ fraction, has a BV of 54. Wheat has been shown by Boas-Fixen

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et al. (7) to have a PER of 1.36 and by Mitchell and Block (8) to have a BV of 70. Wheat white flour, void of the germ and bran fractions, has a BV of only 47. Corn and wheat have protein digestibilities of 87.6 and 89.6%, respectively (9).

The purposes of this study were to investigate the feasibility of obtaining a protein concentrate from wheat and corn stillage and to further characterize each concentrate.

MATERIALS AND METHODS

Grains Fermented

This investigation involved the grains, corn and wheat, as the carbohydrate sources for fermentation. Several sources of corn, ranging in protein content from 7.80 to 10.12%, were used throughout this study. Only one wheat, hard red winter (HRW) which had 13.86% protein, was used in this study.

Laboratory Fermentation Procedure

A laboratory procedure was developed for the fermentation of hammer-milled wheat and/or corn. This procedure was designed to simulate the U.S. industrial fermentation procedure as closely as possible, with the following exceptions: 1)

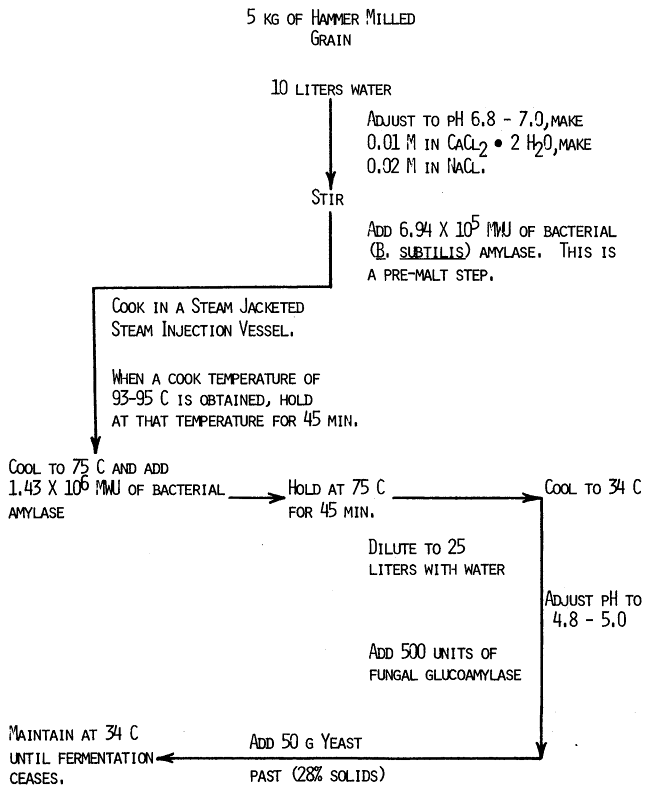

Fig. 1. The laboratory procedure used for the fermentation of corn and wheat.

the industrial procedure involves high-temperature-pressure continuous cooking, but in the laboratory a batch-type cooking was performed; and 2) the industrial procedure uses a direct heat distillation for the beer still, whereas the laboratory used steam distillation for the beer still.

The detailed diagram in Fig. 1 shows the entire fermentation procedure for both corn and wheat. All enzymes used were obtained from the Marschall Division of Miles Laboratories, Inc., Elkhart, IN 46514.

Protein Extraction Procedure

The alkaline extraction of both corn and wheat was studied, using varying concentrations of NaOH. Figure 2 shows the entire extraction procedure for both corn and wheat that had gone through the fermentation described in Fig. 1. Because of the different chemical composition of the two grains, each required differing amounts of NaOH to titrate the fermented wet solids to pH 12.2.

A second alkaline extraction of the wet solids was routinely performed after a

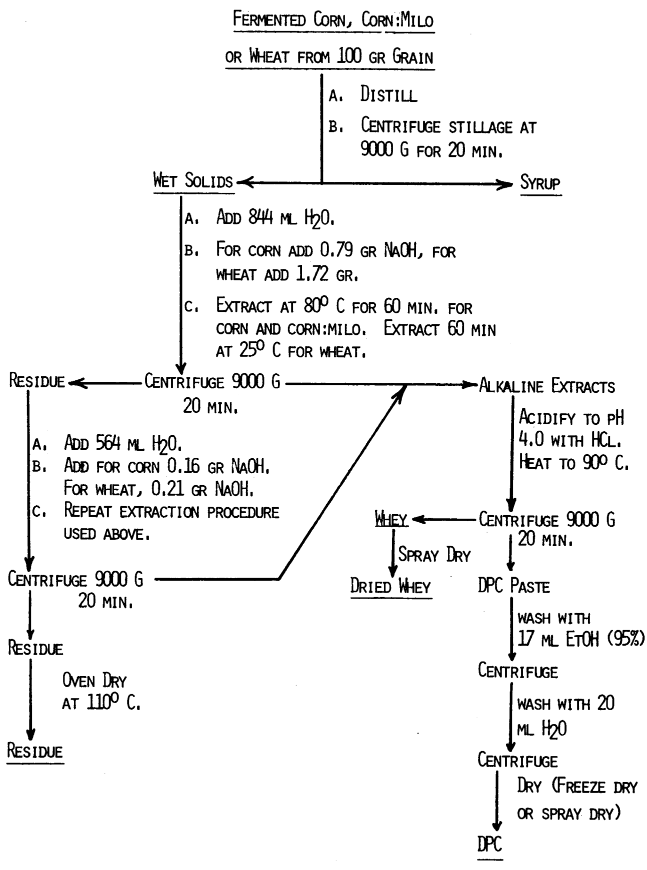

Fig. 2. The laboratory procedure for the alkaline extraction of fermented corn and wheat to obtain a distiller's protein concentrate (DPC).

preliminary investigation indicated it would increase final product yield by approximately 25%.

Proximate Composition

Proximate analyses were performed on all starting grains and all fractionation products using AOAC techniques (10). A Kjeldahl factor of 6.25 was used for all protein concentrations, while a factor of 5.7 was used for all other products.

Amino Acid Composition

All starting grains and final distiller's protein concentrates (DPCs) were analyzed for total amino acid composition using the procedures listed below. All acidic, neutral, and basic amino acids, with the exception of cysteine and tryptophan, were released by hydrolysis in 6*N* HCl at 110°C for 24 hr and then analyzed on a Beckman 120 C amino acid analyzer. Quantitative determination of cysteine was performed using a performic acid preoxidation to cysteic acid and then quantitative determination on the amino acid analyzer (11). Quantitative determination of tryptophan content of the various samples required $Ba(OH)_2$ hydrolysis and subsequent analysis on the amino acid analyzer (12).

All amino acid compositions reported are in units of g amino acid/100 g protein.

Nutritional Analyses

The nutritive quality of the starting grains and DPCs obtained from the fermentation of these grains was measured by determining the PER and apparent protein digestibility using 21-day-old male weanling rats. The rat diets were formulated to contain 12% protein when the protein concentrates were tested and 8% protein when the grains were tested. A control diet of casein at either 12 or 8% protein was included with each experiment.

The PER was determined as follows:

$$\text{PER} = \frac{\text{weight gain (g)}}{\text{protein consumed (g)}}$$

over a 28-day trial period. The apparent protein digestibility of each sample was measured as follows:

$$\text{Apparent Protein Digestibility} = \frac{\text{Nitrogen in diet (g)} - \text{Nitrogen in feces (g)}}{\text{Nitrogen in diet (g)}} (100)$$

over an 8-day period. All PERs reported are in relationship to casein, which was adjusted to a PER of 2.50.

Analysis for Ribonucleic Acids

Because of the presence of yeast from the fermentation step, all protein concentrates obtained were analyzed for the presence of ribonucleic acid (RNA). The procedure used to measure RNA content was that of Fleck and Begg (13). The RNA content of distiller's protein concentrates is reported as g RNA/100 g protein concentrate.

Elemental Analyses

The concentrations of the following elements were determined on the DPCs and on soy isolate: Ca, Mg, Zn, Fe, Cu, Pb, and Mn. The procedure used involved dry-ashing 6.5 g of each sample overnight at 550° C, then dissolving the ash in 30 ml of 6*N* HCl. The solubilized ash was then further diluted with glass distilled water to achieve the desired concentration of each element prior to measuring the exact elemental concentration on a Perkin Elmer Model 303 atomic absorption spectrophotometer. To inhibit interference from other ions affecting the determination of Ca and Mg concentrations, the diluted samples for these two elements were made to contain 1% lanthanum (w/v).

The element Ca is reported as mg Ca/g protein concentrate, whereas all other elements are reported as μg/g.

Determining Functional Properties

Thermoplastic extrusion. The effect of protein concentrate addition to the puffing ability of corn meal upon thermoplastic extrusion was measured. The protein concentrates were added to corn meal (20% moisture) to give final protein concentrations of 12, 14, 16, 18, 20, and 22%. Puffing ability was determined by dividing the cross-sectional area of the die into the cross-sectional area of the corn meal collet after extrusion.

Extrusion was performed using a Brabender Laboratory Model Extruder® with a 2-chambered barrel, a heated 3-mm diameter cylindrical die, and a 3/4-in. screw with a 20:1 ratio. Barrel and die temperatures were maintained at 148° C. Screw speed was 100 rpm during extrusion.

Bread fortification. A bread-baking (straight-dough microloaves) procedure similar to that described by Marnett *et al.* (14) was used. The flour used was HRW wheat. Various amounts of both corn and wheat protein concentrate were

TABLE I
Protein-Fortified Breads (30% Moisture Basis), their Protein Content and Loaf Volume

Sample Numbers	Actual Protein Level Obtained	Loaf Volume
Corn DPC		
1 (Control)	9.59	165
2	12.40	151
3	13.24	130
4	15.82	95
5	18.23	85
Wheat DPC		
1 (Control)	9.64	165
2	12.41	145
3	13.90	126
4	16.34	110
5	18.84	108
Soy isolate		
1 (Control)	9.43	165
2	11.14	122
3	13.48	80
4	15.16	66
5	18.27	61

added to the formulation to yield the desired protein content. All treatments are shown in Table I. Each treatment was performed in duplicate, and the volume of each loaf was measured by displacement of rapeseed.

Emulsification characteristics. The emulsification characteristics, stability and capacity, were determined for both protein concentrates using the procedure of Townsend *et al.* (15) and a modification of the procedure of Satterlee *et al.* (16), respectively. The emulsion capacity procedure was modified by using protein concentrations of both 3 and 10 mg of protein per ml buffer.

RESULTS AND DISCUSSION

Protein Extraction Procedure

In order to determine the optimal NaOH concentration for the extraction of

TABLE II
The Yield of Protein from Corn Stillage Solids by Extraction with Varying Concentrations of NaOH at 23°C

Alkaline Extraction (pH)	Per Cent of Total Corn Protein Extracted
11.8	18.47
12.2	23.36
12.5	22.09

TABLE III
The Effects of Different Alkaline[a] Extraction Conditions on the Yield of Products from Corn and Wheat Stillage Solids

Treatment	Per Cent of the Original Grain Protein Found in the Various Fractions				
	Protein concentrate	Residue	Syrup	Whey	Washes
Corn					
Extracted at 23°C	23.36	53.22	12.65	10.30	0.64
Extracted at 60°C	28.39	54.53	9.48	7.14	0.44
Extracted at 80°C	53.98	16.35	10.20	19.46	0.70
Extracted at 90°C	40.18	24.00	16.42	18.61	0.57
Extracted with sonication at:					
80°C	55.72	17.05	12.96	14.16	0.09
90°C	53.93	11.83	14.45	18.42	1.37
Extracted with:					
papain pretreatment	15.15	30.26	9.97	43.60	0.43
sulfite pretreatment	12.07	66.11	13.27	2.75	1.10
Wheat					
Extracted at 23°C	36.38	10.45	35.83	17.19	0.13
Extracted at 60°C	28.30	5.35	34.01	30.50	0.40
Extracted at 80°C	24.63	1.87	32.62	40.30	0.56
Extracted at 90°C	24.35	0.99	31.62	42.98	1.14
Extracted with sonication at:					
80°C	26.44	1.68	33.38	37.48	0.99
90°C	20.69	1.81	36.16	38.96	2.35

[a]All extractions were performed with pH 12.2.

protein from corn or wheat stillage, the solids from the corn or wheat stillage were equilibrated to pH 11.8, 12.2, and 12.5*N* NaOH. The yield of protein concentrate expressed as percentage of protein extracted from the stillage solids at the varying NaOH concentrations is given in Table II. The optimal NaOH concentration for extraction yield was found to be pH 12.2 NaOH.

Other modifications to the alkaline extraction procedure for the stillage solids were: heated extractions at 60°, 80°, and 90°C; sonication; pretreatment with papain; and pretreatment with 1% sodium sulfite. Table III lists the various additional treatments as applied to corn and wheat stillage wet solids (see Fig. 2) and their effect on protein concentrate yield. As can be seen, the best yield of the original corn grain protein can be obtained using the 80°C-pH 12.2 NaOH extraction. The best yield of protein concentrate can be obtained from wheat stillage using a pH 12.2 NaOH extraction at room temperature.

Other modifications to the extraction procedure either did not sufficiently solubilize the grain protein (pretreatment with papain or sodium sulfite, extraction at 60° and 90°C) or gave results equal to those obtained with the 80°C extraction (extraction at 80° and 90°C with sonication).

This study sought extraction methods that would yield the greatest amount of protein, not necessarily that with the best nutritive quality. Even though the lysine, methionine, and half-cystine contents of the protein concentrates were greater than those of the starting grains (Table IV), neither the amount of loss of these amino acids nor the formation of lysinoalanine during alkaline extraction was determined.

Chemical Composition of the Corn and Wheat Extraction Components

Proximate analysis of the components obtained from the extraction of wheat and corn stillage solids is given in Table V. As noted, the protein concentrate

TABLE IV
The Amino Acid Composition of Corn and Wheat Grains and their Respective Protein Concentrates

	Amino Acid Content (g/100 g Protein)			
Amino Acid	**Corn**	**Corn protein concentrate**	**Wheat**	**Wheat protein concentrate**
Aspartic acid	6.31	6.95	4.90	6.06
Threonine	3.70	2.65	2.84	3.65
Serine	4.52	1.93	4.42	3.94
Glutamic acid	20.28	20.10	31.63	20.01
Proline	7.79	6.71	8.64	9.30
Glycine	3.50	4.22	3.68	4.22
Alanine	6.69	7.16	3.15	6.13
Half-cystine	0.68	0.74	0.41	3.46
Valine	4.62	5.58	4.37	5.14
Methionine	0.42	1.74	0.71	2.15
Isoleucine	4.46	3.94	3.14	3.23
Leucine	10.90	12.03	5.81	9.64
Tyrosine	4.96	4.46	2.76	4.36
Phenylalanine	4.99	5.23	3.53	4.14
Lysine	2.75	3.47	2.81	3.09
Histidine	2.40	2.13	2.40	2.68
Ammonia	3.64	2.07	4.37	1.84
Arginine	2.00	4.61	5.43	4.92
Tryptophan	0.57	0.65	1.44	1.59

from corn stillage solids contains a large amount of lipid prior to the final alcohol wash. The lipid content of the corn and wheat protein concentrates was substantially reduced by the alcohol wash. The final lipid content of both concentrates could create shelf stability problems, primarily because of the unsaturated nature of the fats present. The final protein concentrates from both sources are rich in protein (81–87% protein) and possess an amino acid composition similar to that of the starting grain (Table IV). The lysine content of the protein concentrates is slightly higher than that of the starting grains because of the small amount of yeast protein (9.08 g lysine/100 g yeast protein) contributed to the protein concentrate by the anaerobic fermentation process.

Elemental Composition of the Protein Concentrates

The elemental composition of the protein concentrates from wheat and corn stillage, as well as that of soy protein isolate, is given in Table VI. The corn and wheat proteins are lower in Ca, Mg, Zn, and Mn, and similar in Fe content. Wheat protein is much more abundant in Cu than is the corn or soy protein. All three are low in Pb.

TABLE V
Proximate Composition of Corn and Wheat Alkaline Extraction Components

Component	Moisture %	Ash %	Lipid %	Protein %	Carbohydrate[a] %
Corn					
Protein concentrate:					
Before alcohol wash	2.15	5.14	26.90	61.02	3.07
After alcohol wash	3.84	5.00	6.49	81.06	3.97
Residue	3.14	10.50	4.10	29.32	52.94
Syrup	9.74	49.97	2.98	4.10	33.21
Whey	3.62	70.90	2.02	6.55	20.49
Wheat					
Protein concentrate:					
Before alcohol wash	8.96	0.80	10.31	59.76	20.17
After alcohol wash	6.01	0.98	2.16	87.87	2.98
Residue	5.64	6.27	1.28	29.13	57.68
Syrup	15.06	23.12	0.33	29.90	31.59
Whey	5.66	66.15	1.68	13.20	13.13

[a]Carbohydrate content determined by difference.

TABLE VI
A Partial Elemental Analysis of Protein Concentrates from Corn, Wheat, and Soy Protein Isolate

Protein Concentrate	Element Concentration						
	Calcium mg/g	Magnesium μg/g	Zinc μg/g	Iron μg/g	Copper μg/g	Lead μg/g	Manganese μg/g
Corn DPC	0.11	10	30	70	5	30	1
Wheat DPC	0.12	30	20	70	60	10	2
Soy isolate	2.83	91	70	80	20	10	7

Ribonucleic Acid Content

The RNA content of proteins extracted from the corn and wheat stillage is much less than the safe limit of 2 g/ 100 g protein concentrate (Table VII). This is because anaerobic fermentation produces only a small amount of yeast which, in combination with the alkaline procedure, results in low RNA contents in the two products. Alkaline extraction dissolves RNA which then remains soluble during the acid precipitation of the protein.

Nutritional Evaluation of the Protein Concentrates

Nutritional analyses of the corn and wheat grains using the white rat tests indicated that they have comparable nutritional quality (Table VIII), as measured by their respective PERs (1.40, 1.44) and apparent digestibilities (78.32 and 78.02%).

The protein concentrate obtained from the fermented corn had a PER of 1.45 and an apparent digestibility of 78.00%. The protein concentrate obtained from the fermented wheat had a PER of 1.26 and an apparent digestibility of 82.80%. The corn protein concentrate is identical to its starting grain, whereas the wheat protein concentrate is of slightly lower nutritional value when compared to the starting material, whole wheat.

Functional Characteristics of the Protein Concentrates

Thermoplastic extrusion. The addition of either corn or wheat protein concentrate to corn meal does cause a progressive reduction in puff volume with increasing protein levels. The effect of protein concentrate on puff volume is shown in Fig. 3. Wheat protein concentrate had the least detrimental effect on puff volume. Both protein concentrates could be incorporated into the corn collet giving acceptable puff volumes up to a final protein concentration of 22% for the wheat and 18% for the corn protein concentrate. Both proteins exceed soy protein isolate in their performance upon extrusion.

TABLE VII
The Determination of the Ribonucleic Acid (RNA) of the Protein Concentrates from Fermented Corn and Wheat

Protein Concentrate	g RNA/100 g Protein Concentrate
Wheat DPC	0.09
Corn DPC	0.07

TABLE VIII
The Nutritive Quality of Various Grains and Protein Concentrates

Material Tested	PER	Protein Digestibility
Grains:		
Corn	1.40	78.32
Wheat	1.44	78.02
Protein concentrates:		
Corn DPC	1.45	78.00
Wheat DPC	1.26	82.80
Casein (control)	2.50	91.70

Bread fortification. The addition of either corn or wheat protein concentrate to the basic bread formulation resulted in the successive depression of loaf volume as the amount of added concentrate increased. Table I lists the protein content and loaf volume of loaves fortified with corn and wheat protein concentrates and soy protein isolate (91% protein). Wheat protein concentrate, because of its gluten content, has the least detrimental effect on loaf volume even though the alkaline extraction procedure could have lowered its elasticity. Soy isolate has the greatest detrimental effect on loaf volume.

Emulsification characteristics. The emulsifying characteristics of both the corn and wheat protein concentrates, nonfat dry milk (33% protein), and soy isolate (91% protein) are given in Table IX. The emulsion capacity of corn protein concentrate exceeds that of nonfat dry milk and wheat protein concentrate, but is less than that of soy isolate.

The emulsion stability of the corn protein concentrate was equal to that of nonfat dry milk, greater than that of wheat protein concentrate, but less than that of soy isolate.

CONCLUSIONS

Protein concentrates were produced from fermented wheat and corn grains by alkaline extraction. A heated (80°C) extraction greatly enhanced the yield of corn protein concentrate, but was ineffective in enhancing the yield of wheat

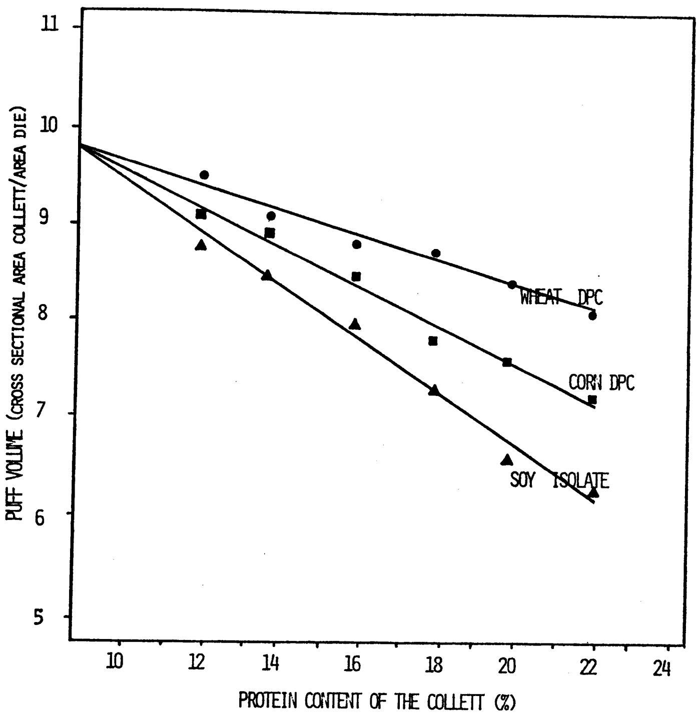

Fig. 3. The effect of DPC and soy protein addition on the puff volume of corn meal collets.

TABLE IX
Emulsification Characteristics of Various Protein Sources

Protein Concentrate	Emulsion Capacity	Emulsion Stability	
	ml Oil emulsified / 100 mg Protein	ml Oil released / 10 g Emulsion	ml H_2O and Solids released / 10 g Emulsion
Nonfat dry milk	12.00	0.07	1.60
Soy isolate	22.20	0.03	1.40
Protein concentrates from:			
Corn	18.90	0.06	1.89
Wheat	8.90	0.10	2.72

protein concentrate. Amino acid composition and nutritional evaluation of the protein concentrates show them to be almost identical in protein quality to their starting grains. Evaluation of the functional properties of both protein concentrates indicates that wheat protein concentrate works well in extruded corn meal snacks and in bread. The corn protein was an excellent emulsifier in meat emulsions.

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