Corn Germ Protein Isolate—Preliminary Studies on Preparation and Properties[1]

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ABSTRACT

Commercial corn germ was extracted with hexane and ground to a meal. Protein was solubilized by two extractions in a high-speed blender at either a 10:1 or a 5:1 solvent:meal ratio. The first solvent was water containing 10 mg. sodium hydroxide per g. germ meal (pH 8.7); the second was water only. Protein in the extract was precipitated by adjusting to isoelectric pH (4.7). The protein precipitate, after being washed with water, was adjusted to pH 7.0 and freeze-dried. The neutralized isolate extracted at a 5:1 solvent:meal ratio was dialyzed against distilled water for 48 hr. to reduce ash content before freeze-drying. Proximate analysis of the dialyzed isolate was 74% protein (N X 5.4); 4.3% ash, most of which was phosphate; and 0.08% fiber. The protein in the isolate contains 6% lysine with a good balance of other essential amino acids. Mild flavor, light tan color, solubility at neutral and low pH, and ability to stabilize an oil-in-water emulsion are some properties of this corn germ protein isolate that indicate its potential for many food uses.

Germ constitutes about 12% of a normal dent corn kernel (1). It is a by-product of both the wet- and dry-milling corn industries. The proteins of wet-milled germ are, however, altered during steeping. Previous work (1) has shown that corn germ contains about 19% protein on a moisture-free basis, or about 29% protein on a moisture-free and an oil-free basis. The proteins in corn germ are mostly albumins and globulins with a good amino acid balance (2). Feeding studies (3,4) have shown the protein in whole corn germ to be almost as nutritious as animal proteins. Wall et al. (5) demonstrated that the nutritional value of corn germ protein is not reduced if oil is removed by solvent extraction rather than expeller processing, which subjects the germ to high temperatures. Methods have been described for making corn germ products suitable for addition to human foods (6,7).

Protein isolates have been made from several plant sources, such as soybean (8), dehulled cottonseed (9), wheat germ (10), coconut (11), rapeseed (12), and alfalfa leaf (13). In general, these isolates were made by aqueous extraction at neutral or mildly alkaline pH. Proteins in the extracts were then precipitated at isoelectric pH (near pH 4.5), washed, adjusted to neutral pH, and gently dried. We adapted these procedures in making a protein isolate from corn germ obtained as a by-product of dry milling.

MATERIALS AND METHODS

Oil-Free Corn Germ Meal

An air-dried sample of dry-milled corn germ stream was supplied by Illinois Cereal Mills, Paris, Ill. It contained 66.5% germ as determined by flotation on 1,2-dichlorethane (ρ = 1.257) (14). After the corn germ stream sample was flaked, oil was removed by eight extractions with commercial hexane at room temperature. Solvent was removed by drying at room temperature, after which the material was

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ground through a 0.02-in. screen on a Fitzpatrick hammer mill. This oil-free corn germ meal, which served as starting material for extraction experiments, contained 19% protein and 0.35% oil on a dry basis. Protein contents for corn germ and corn germ isolate are based on N × 5.4, a factor which was calculated from amino acid composition of the materials by a procedure published by Tkachuk (15). Nitrogen was determined by AACC micro-Kjeldahl Method 46-13 (16). Moisture was determined by drying to constant weight at 105°C., and fat by AACC Method 30-26, based on petroleum ether extraction (16).

Extraction and Isolation

To determine extractability of corn germ protein as a function of pH, five samples were prepared, each consisting of 1.0 g. of oil-free corn germ meal and 10 ml. of 10% sodium chloride. The pH of each slurry was 6.0. Two samples were adjusted to pH 2.1 and 3.8 with N HCl and two to pH 8.7 and 9.9 with N NaOH. The five samples were stirred separately on magnetic stirrers for 1 hr. at 600 r.p.m. and 33°C. Nonsolubilized material was removed by centrifugation at 3,000 r.p.m., after which the nitrogen content of two aliquots of the supernatant liquid from each sample was determined. Nonprotein nitrogen was determined after an equal volume of 20% trichloracetic acid had been added to another portion of each supernatant liquid. Results are given in Fig. 1.

The effect of sodium chloride concentration on extractability of corn germ proteins was investigated, as well as the effect of high-speed mixing. This experiment was performed with 0, 5, and 10% aqueous sodium chloride with and without 0.025M sodium hydroxide. After 200 ml. of solvent was added to 20 g. of de-oiled corn germ meal, the system was blended at high speed for 5 min.; blending caused heating from 30° to 45°C. Nondispersed material was removed by centrifugation at 3,000 r.p.m., and the nitrogen content of the supernatant liquid was determined (Table I).

Preliminary isolation experiments compared 0.025M calcium hydroxide (pH 8.7) and 0.025M sodium hydroxide (pH 9.0) as extractants. Oil-free corn germ meal was extracted first with base and then with water at a 10:1 solvent:meal ratio for 5 min. in a high-speed blender which caused heating to 45°C. Protein in the extract was precipitated by adjusting to pH 4.7 and the precipitate was washed once with water. Aliquots of the supernatant liquid were adjusted to pH 4.2 and 5.2 and were then boiled to see if whey protein would precipitate.

One isolation experiment was also run in which the meal was first washed with 2.5 parts of water at pH 4.7, after which protein was isolated with 0.025M sodium hydroxide as described above. Criteria for evaluating these isolation procedures were the amount and purity of the protein isolated.

The isoelectric pH of corn germ protein was determined by exhaustively dialyzing the isolates against distilled water after which the pH of material inside and outside of the dialysis membrane was measured.

Corn Germ Protein Isolate

A larger amount of corn germ protein was isolated by extracting 200 g. of oil-free germ meal with 2 liters of water plus 2.0 g. sodium hydroxide (pH 8.7) for 5 min. at full speed in a 5-qt. blender. The temperature of the system rose from 29° to 50°C. during extraction. A second 200-g. batch of oil-free meal was extracted as above. The residue from each extraction was removed by centrifugation at 3,000

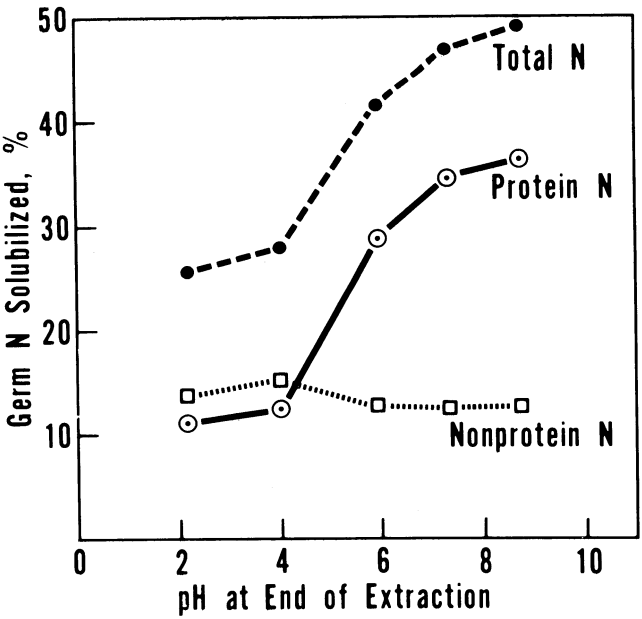

Fig. 1. Solubility of oil-free corn germ meal nitrogen constituents as a function of pH after stirring for 1 hr. at 33°C. Top curve (• - - •) is percentage of total germ N solubilized. Lower curve (□ . . . . □) is nonprotein nitrogen, i.e., N remaining soluble in 10% trichloracetic acid. Middle curve (⊙——⊙) represents protein nitrogen solubilized, i.e., difference between other two curves.

TABLE I. EFFECT OF SODIUM CHLORIDE CONCENTRATION ON AMOUNT OF CORN GERM PROTEIN NITROGEN SOLUBILIZED BY HIGH-SPEED BLENDING WITH AND WITHOUT DILUTE SODIUM HYDROXIDE

Sodium Chloride Concentration %	No Sodium Hydroxide		0.025M NaOH	
	pH	N Solubilized %	pH	N Solubilized %
10	5.9	61	7.3	60
5	6.0	60	7.6	61
0	6.7	54	8.8	75

r.p.m. The two residues were combined and re-extracted with 2 liters of water followed by centrifugation. The supernatant liquids from the extractions were combined and adjusted to pH 4.7 from 8.7 with HCl to precipitate corn germ protein, which was isolated by centrifugation and then washed with 500 ml. of water. One-half of the isolated protein was neutralized to pH 7.0 with N sodium hydroxide; then both parts of the batch were freeze-dried.

A second batch of corn protein isolate was made by the same procedure but with the initial extraction at a 5:1 solvent:meal ratio; i.e., 400 g. of oil-free meal was extracted with 2 liters of water plus 4 g. NaOH (pH 8.5). The isolated protein was adjusted to pH 7.0, dialyzed against several changes of water for 48 hr. at 5°C., and then freeze-dried.

Properties of Isolates

Most of the procedures used to characterize the two larger batches of corn germ isolate were standard AACC Methods (16). Total ash was by Method 08-01; fiber was by Method 32-17, which involved extraction by boiling dilute sulfuric acid and then boiling dilute sodium hydroxide and phosphorus by Method 40-57. The water solubility of the isolate as a function of pH was determined by a scaled-down version of Method 46-23, which involves stirring for 2 hr. under defined conditions. Results are given in Fig. 2. The solubility of dialyzed corn germ isolate was compared at neutral pH to that of a commercial soybean protein isolate using a scaled-down version of Method 46-24, which involves high-speed blending for 10 min.

Emulsion-stabilizing capacity of the corn germ isolates was compared to that of soybean isolate by a modification of the procedure of Inklaar and Fortuin (17). In this modified procedure 1.0 g. protein is dissolved in 20 ml. water in a 125-ml. Erlenmeyer flask by stirring at 1,000 r.p.m. for 15 min. with a 1.5-in. magnetic bar. After adding 0.6 g. NaCl, 10 ml. of corn oil is added over a period of 5 min. with stirring at 1,000 r.p.m., and stirring is continued 1 min. more. The system is

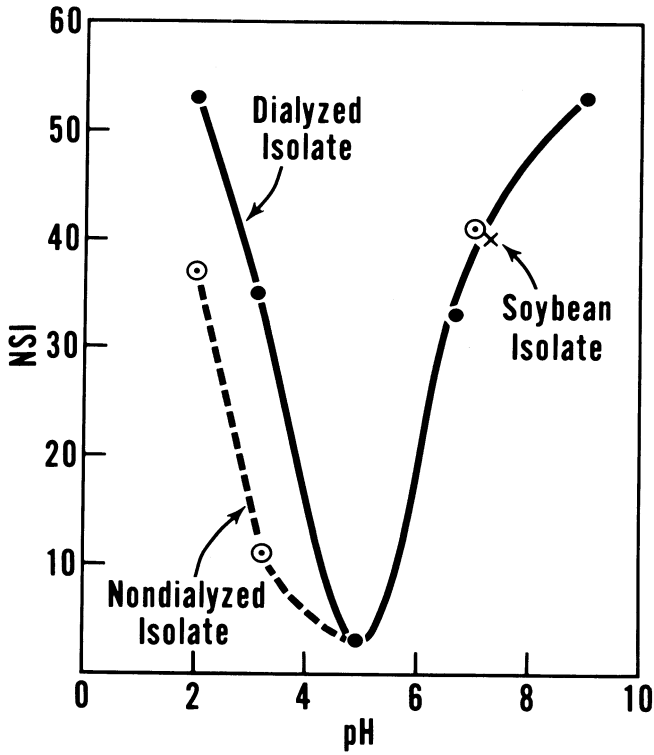

Fig. 2. Percent water-soluble protein in corn germ isolate as a function of pH as determined by stirring for 2 hr. at 30°C. Solid curve (●———●) represents isolate extracted at a 10:1 solvent:meal ratio; broken curve (⊙ - - - ⊙) represents isolate extracted at a 5:1 solvent:meal ratio followed by dialysis.

TABLE II. COMPOSITION OF OIL-FREE CORN GERM, DIALYZED CORN GERM ISOLATE, AND SODIUM CASEINATE COMPARED TO IDEAL PATTERNS OF ESSENTIAL AMINO ACIDS

Component	Oil-Free Corn Germ Meal	Corn Germ Isolate (Dialyzed)	Sodium Caseinate[a]	FAO Reference Pattern[b]
Proximate analysis, %[c]				
Protein[d]	19	73	94	
Starch	32	3	0	
Crude fiber	4.6	0.08	0	
Cellulose	3	0	0	
Sugars	6	0.8	...	
Ash	9.2	4.3	3.8	
Fat	0.35	...	1.2	
PO_4	...	3.6	...	
Amino acids, g./16 g.N				
Lysine	6.2	6.3	8.3	4.2
Histidine	3.3	3.2	3.0	
Ammonia	2.0	1.3	1.9	
Arginine	8.4	9.5	3.8	
Aspartic acid	8.2	7.7	7.3	
Threonine	3.7	3.8	4.8	2.8
Serine	4.7	4.8	6.2	
Glutamic acid	14.6	13.3	22.5	
Proline	5.0	5.0	11.8	
Glycine	5.8	5.5	2.0	
Alanine	6.3	6.2	3.1	
Cystine	1.3	1.5	0.36	2.0
Valine	5.2	5.9	6.9	4.2
Methionine	1.7	1.9	2.8	2.2
Isoleucine	3.1	3.5	5.5	4.2
Leucine	7.3	7.3	9.7	4.8
Tyrosine	3.0	3.3	5.9	2.8
Phenylalanine	3.8	4.5	5.3	2.8
Tryptophan	1.2	1.3	1.6	1.4

[a]Proximate analysis of sodium caseinate (personal communication, Erie Casein Co., Erie, Ill. 61250.)
[b]FAO/WHO (24).
[c]Dry basis.
[d]N X 5.4 for corn germ and corn germ isolate, N X 6.38 for sodium caseinate.

transferred to a 40-ml. centrifuge tube, heated in an 85°C. bath for 15 min. with occasional stirring, and then cooled 15 min. in 25°C. water. The tube is finally centrifuged at 3,000 r.p.m. for 15-min. periods until the volume of oil separated from the emulsion is constant. Results are expressed as percentage of oil that separates from the emulsion layer.

Samples for amino acid analysis were hydrolyzed for 24 hr. under reflux with 2 ml. of 6N hydrochloric acid per mg. sample. Amino acids were determined by the method of Benson and Patterson (18), and results were calculated by an automated procedure developed by Cavins and Friedman (19). This procedure adds methionine and methionine sulfone and reports the sum as methionine. Tryptophan was determined by the procedure of Opieńska-Blauth et al. (20). Results are in Table II.

Carbohydrates in corn germ meal and in the isolates were determined by the method of Sloneker (21) which was modified to quantitate sugars extracted by 80% ethanol. Flavor and color values were judgments of the authors.

RESULTS AND DISCUSSION

Extraction and Isolation

Figure 1 summarizes the effect of pH on the amounts of total nonprotein and protein nitrogen solubilized in 10% sodium chloride by stirring for 1 hr. at a 10:1 solvent:meal ratio at room temperature. From 10 to 15% of the nitrogen in corn germ meal is nonprotein, based on its solubility in 10% trichloracetic acid. The small increase in nonprotein nitrogen at pH 2 and 4 may be caused by a protease in corn germ active at acid pH values (22). Minimum amounts of protein are extracted at pH values of 4 and below, the low protein solubility being consistent with the idea of phytic acid forming an insoluble complex with corn germ proteins at acid pH values (22). As shown in Fig. 1, optimum extraction of protein occurs above pH 7.

The amounts of corn germ nitrogen solubilized by high-speed blending at various sodium chloride concentrations with and without added dilute sodium hydroxide are given in Table I. (Extraction time was 5 min. at a 10:1 solvent:meal ratio.)

More nitrogen is solubilized in a high-speed blender than by stirring. This increase is probably caused by greater intensity of mixing, plus heating from room temperature to 50°C. Table I shows that adding sodium chloride increases extractability of protein when no sodium hydroxide is present (salting in) but decreases the amount of protein extracted when dilute sodium hydroxide is present. The maximum amount of protein is solubilized in dilute sodium hydroxide without added sodium chloride. This probably results from increased charge on the protein at higher pH values. The added sodium chloride decreases solubility at higher pH by screening the charged groups on the protein molecules. In subsequent experiments, alkaline-extraction media without added sodium chloride were used.

Preliminary isolation experiments comparing sodium hydroxide (pH 9.0) and calcium hydroxide (pH 8.7), in which germ meal was extracted twice in a high-speed blender and in which protein in the extract was precipitated by adjusting pH to 4.7 with hydrochloric acid, indicated that dilute sodium hydroxide was a better extracting medium. With sodium hydroxide, 52% of the germ meal nitrogen was present in the protein isolate and the isolate was 69% protein; whereas with calcium hydroxide, only 41% of the germ meal nitrogen was present in the isolate and the isolate was only 59% protein. It was thought that the calcium ion would precipitate phytic acid and give a purer protein, but this idea was incorrect. Preliminary washing of the germ meal at pH 4.7, followed by extraction with dilute sodium hydroxide, produced no improvement over extraction with dilute sodium hydroxide without prior washing. With prior washing at isoelectric pH, 54% of the germ nitrogen was present in the isolate and the isolate was 62% protein.

Results of the dialysis experiment indicated isoelectric pH values for corn germ isolate ranging from 4.2 to 5.2 with an average of 4.7. For this reason, 4.7 was chosen as the pH to precipitate corn germ isolate. Attempts to coagulate proteins present in the supernatant liquid by heating to boiling at pH 4.2, 4.7, or 5.2 produced no more precipitate.

Properties of Isolates

Properties of corn germ isolates were determined on the two larger batches prepared earlier. In the batch in which oil-free corn germ meal was extracted with dilute sodium hydroxide at a 10:1 solvent:meal ratio, 46% of the nitrogen in the

starting material was present in the isolate; 26% of the nitrogen was in the final residue; and 16% was in the supernatant liquid, about one-half of the nitrogen in the supernatant liquid being nonprotein nitrogen. In the other batch, germ meal was extracted with dilute sodium hydroxide at a 5:1 solvent:meal ratio, and the isolated protein was dialyzed against distilled water which reduced its ash content from 8.9 to 4.3%. Forty-one percent of the nitrogen in the starting material was present in the isolate. The properties of the two batches were almost identical, except that the protein extracted at a 10:1 solvent:meal ratio and not dialyzed had an ash content of 7.1%.

Proximate analysis and amino acid composition of dialyzed corn germ isolate and oil-free corn germ meal are given in Table II. The table also gives for comparison the composition of food-grade sodium caseinate (23) and the FAO/WHO essential amino acid reference pattern (24). Corn germ isolate has a good balance of essential amino acids. The content of corn germ isolate exceeds the FAO/WHO reference pattern for all essential amino acids except cystine, methionine, and isoleucine, which are 75, 86, and 83%, respectively, of the FAO/WHO reference pattern. Feeding studies (3,4,5) have shown that the nutritional value of the protein in corn germ approaches that of animal proteins. Since the amino acid composition of corn germ protein isolate is quite similar to that of oil-free corn germ meal (Table II), one would expect corn germ isolate to be equally nutritious.

Corn germ isolate is light brown both as a powder and a paste as compared to the cream color of soybean isolate. When tasted as a paste, dialyzed corn germ isolate was similar to soybean isolate having a bland flavor with a weak cardboard-like note and a slightly bitter aftertaste. The mouth-feel was smooth. The corn germ isolate extracted at a 10:1 solvent:meal ratio (not dialyzed) had, in addition to the other flavors described, a slight salty taste.

Figure 2 presents the water solubility of the two samples of corn germ protein isolate as function of pH as determined by stirring. Both isolates have similar solubility values at neutral and alkaline pH, reaching a maximum of 54% at pH 9 and having a minimum of 4% at pH 5. The isolate prepared by extraction at a 5:1 solvent:meal ratio followed by dialysis is more soluble at acid pH values presumably because of its lower phytic acid content. When the water solubility of dialyzed corn germ protein isolate was determined by high-speed blending, a solubility of 65% was obtained at pH 7.2 which was 25% higher than the value obtained by stirring at the same pH. Soybean protein isolate was 79% soluble under the same conditions. Sodium caseinate is claimed to be almost completely soluble at neutral pH. High solubility seems to be one of the important functional properties of a protein intended as a food additive.

Another important property of a protein intended as a meat additive is ability to bind fat or to stabilize an oil-in-water emulsion. Inklaar and Fortuin (17) developed a laboratory procedure for determining emulsion-stabilizing capacity which they claim correlates well with results of sausage-making practice. A modification of their procedure was used to compare the two corn germ isolates with a commercial soybean isolate. The corn germ isolate extracted at a 5:1 solvent:meal ratio and then dialyzed had the same emulsion-stabilizing capacity as soybean isolate (56 to 59% of the added oil separating). The corn germ isolate extracted at a 10:1 solvent:meal ratio and not dialyzed was not so good, because 71% of the added oil separated.

Preliminary calculations made from the laboratory preparation of corn germ protein isolate indicate that it would cost about 8 cents per lb. more to make than soy isolate. This figure suggests that corn germ isolate could be priced significantly lower than the current price of sodium caseinate.

Corn germ isolate in its present state of development seems to have good nutritional and functional properties. However, it is not as pure nor as light-colored as sodium caseinate or soy isolate, and its flavor is not as bland as that of sodium caseinate. Work is currently under way to improve the purity, flavor, and color of corn germ isolate.

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