# Functional Properties of Protein Extracted from Flaked, Defatted, Whole Corn by Ethanol/Alkali During Sequential Extraction Processing[1]

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**Functional properties (solubility, foaming capacity and stability, emulsifying capacity, emulsion stability, heat coagulability, heat gelation and film formation) of protein extracted by 45% ethanol/55% 0.1 M NaOH from flaked, defatted, undegermed corn during the Sequential Extraction Process (SEP) were evaluated and compared with those of a laboratory-prepared soy protein concentrate. SEP is a new approach to corn fractionation that recycles the ethanol produced from the fermentation of cornstarch to upstream steps of protein extraction and the simultaneous extraction of corn oil and dehydration of the ethanol. Freeze-dried corn protein extracts contained at least 80% crude protein (dry basis), which is indicative of protein concentrates. SEP protein concentrates had solubilities in water of greater than 80% at pH values of 7 or above and were significantly more soluble than the soy protein concentrate at pH above 3. SEP corn proteins also showed better heat stabilities and greater emulsifying capacities and emulsion stabilities. Dilute dispersions (0.1%) of corn protein produced substantial but less stable foams. Corn proteins produced films similar to zein and soy protein films but were unable to form heat-induced gels. These results indicate that SEP produces a protein concentrate with functional properties suitable for food and industrial uses.**

KEY WORDS: Corn, corn protein, functional properties, maize, protein concentrate.

Proteins provide a variety of useful functions in food applications. In addition to providing needed nutrients in the diet, they also contribute to the improvement of sensory, stability and shelf-life requirements that consumers and food processors demand. Some of these functional attributes include emulsification, foaming, water and fat binding, gelation and thickening, and film formation.

The importance of proteins in the food industry is evidenced by the growth and size of the protein ingredient market. In 1989, the protein ingredient market was estimated at 1.3 billion lb with a 4% annual growth rate (Frost & Sullivan Inc., personal communication). Traditional proteins from animal sources, such as casein, whey and egg, are still widely used with an estimated value in 1989 of over $500 MM. Vegetable proteins, however, were used in significantly greater amounts and showed the greatest overall growth rate (4.5% vs. 2.1%). The estimated value, however, was almost equivalent to those from animal sources. The factors that determine whether a protein will be used in a specific food application are the protein's functional properties, availability and cost (1). In light of this statement, the greater use and growth rate of proteins from vegetable sources can be accounted for by the lower price relative to animal sources and the improved functionality of these proteins through increased knowledge in processing and protein modification.

Soy proteins are the undisputed leaders in the amounts used in food products. In 1989, it was estimated that soy protein accounted for over 75% of all proteins used as food ingredients and for 90% of all vegetable proteins used (Frost & Sullivan Inc., personal communication). The unique functional attributes and the availability of soy proteins, in addition to improvements in processing and functionality, account for their position as the industry leader. Other vegetable proteins of note include wheat gluten and pea.

Notably absent from this list of vegetable proteins used in food applications are those derived from another major commodity grown in the United States, corn. There are several key attributes of most proteins derived from corn processing that makes them unsuitable for a majority of food applications. First of all, the process by which most corn proteins are produced, wet-milling (2,3), renders them unsuitable for a majority of food applications (4). Wet milling is the preferred method for obtaining starch from corn. The starch is converted into other products for rapidly growing markets, such as high-fructose corn syrup and fuel ethanol. A key ingredient, $SO_2$, is used during the steeping process to facilitate the separation of the starch from the starch–protein matrix in the endosperm. The $SO_2$, unfortunately, negatively affects the functional and edible properties of the proteins and makes them unsuitable for food use (4). The proteins are sold primarily in the commodity feed markets as corn gluten feed (21% protein minimum) and corn gluten meal (60% protein minimum) (2,3).

Secondly, corn proteins are not very soluble in water. One of the major proteins in corn is zein, which comprises 41% of the total protein in corn and 50% of the endosperm proteins (5). Zein is classified as a prolamine, a protein that is soluble in aqueous ethanol and relatively insoluble in water. The relatively high level of this protein limits corn protein solubility in water, thereby limiting its use in water-based systems such as food products.

Finally, corn protein has a yellow color and a relatively strong "corn" flavor associated with it. For many food uses, the protein should not impart any additional flavor and color that could cause problems in the formulation of the product.

A radically different corn processing method, the Sequential Extraction Process (SEP), extracts a corn–protein fraction with quality and functional attributes that are well suited for some food applications. In SEP, the protein is extracted with a solvent mixture of 45% ethanol/55% 0.1 M NaOH from undegermed, flaked corn previously defatted with 95% ethanol. Preceding research by Hojilla-Evangelista *et al.* (4,6) has shown that, compared with corn gluten meal, this product (i) has a greater protein content, 80% vs. 60%; (ii) contains more of the limiting amino acid lysine and other essential amino acids; (iii) is light in color; (iv) has a bland

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flavor; and (v) is considered food-grade because all chemicals employed in the process were food-grade, and no $SO_2$ was used.

Although the aforementioned attributes clearly show the potential for food uses, no data on functional properties of the SEP corn proteins have been reported. The objectives of the present work are to characterize selected functional properties of the SEP protein concentrate and to compare them with the properties of a soy protein concentrate.

## EXPERIMENTAL PROCEDURES

*SEP.* SEP of corn was performed according to the procedure of Hojilla-Evangelista *et al.* (4,6). After oil extraction, the protein was extracted from the marc by using 45% ethanol/55% 0.1 M NaOH (15 mL per gram of corn). The mixture was ground for 1.5 min at full speed and then allowed to soak for 2 h. After soaking, additional solvent was added for a total ratio of 15 mL solvent per gram of corn. The mixture was blended further for 30 s and then transferred to centrifuge bottles. The capped bottles were placed in a shaking water bath at 55°C for 2 h and then centrifuged at 1050 × *g* for 5 min to separate the protein-containing supernatant and the fiber/starch residue. The protein extract was dialyzed against water, ultrafiltered through a 10-kdal membrane, and then freeze-dried to recover the protein-rich solids.

*Moisture and protein analysis.* The moisture content of the protein was determined by the Karl Fischer titration method (7). The protein contents were determined by both the Kjeldahl (AACC Standard Method 46-08) (8) and Biuret methods (9).

*Functional properties.* The tests to evaluate solubility, foam capacity and stability, and heat coagulability of the protein were modifications of procedures reported by Balmaceda *et al.* (10).

*Solubility.* Aqueous solutions containing 1% protein (dry basis) were stirred for 7 min, adjusted to pH 3.0, 4.5, 6.0, 7.0, 8.5 or 10.0 and centrifuged at 10,000 × *g* for 15 min. The amount of protein in the supernatant was determined by the Biuret method. The percent soluble protein was calculated from the amount of soluble nitrogen (g N × 6.25) in the supernatant.

*Foaming capacity and stability.* The method (10) was modified for small sample volumes. Five mL of a 1% protein solution, adjusted to pH 7.0, was pipetted into a graduated column equipped at the bottom with a coarse fritted disk. Air was introduced into the column bottom at a flow rate of 100 mL/min at 20 psi. Timing started at the first appearance of air bubbles, and foam volume after 1 min was used to measure foaming capacity (mL). Foam stability was obtained from the percentage of the original foam remaining after 15 min.

*Heat coagulability.* Solutions containing 2% protein (dry basis), adjusted to pH 7, were centrifuged at 10,000 × *g* for 15 min. The protein content of the supernatant was determined by the Biuret method. A 10-mL aliquot of the supernatant was heated at 90–100°C for 20 min, cooled to room temperature and again centrifuged at 10,000 × *g* for 15 min. The supernatant was filtered through a Whatman No. 2 filter paper, and the amount of protein in the filtrate was measured by the Biuret method. Heat coagulability was expressed as the percentage loss in solubility after heating.

*Film-forming ability.* The film-forming properties of the protein were evaluated by following precisely the procedures outlined by Balmaceda *et al.* (10).

*Emulsifying capacity.* The analysis of the emulsification capacity of the protein was largely based on the procedure of Hung (11). Fifty mL of 1% protein solution at about 0°C was mixed with oil at 12,000 rpm by a Biomixer™ (Model M 122; Biospec Products, Bartlesville, OK) until inversion of the emulsion occurred. Emulsifying capacity was the amount of oil (g) needed for 1 g of protein to reach its capacity.

*Emulsion stability.* The stability of the emulsions were measured by following the procedures outlined by Pearce and Kinsella (12).

## RESULTS AND DISCUSSION

*Protein analyses.* The data in Table 1 show the results of the protein analyses of the freeze-dried protein concentrate. Data from the Kjeldahl analyses verified previous results by Hojilla-Evangelista *et al.* (4) that the protein content was greater than 80%, which classifies the protein as a concentrate. Due to the small amount of corn and soy protein available for analysis in some functionality experiments (solubility and heat coagulability) the Biuret method was used in place of the Kjeldahl procedure. The results in Table 1 show that the Biuret test had higher standard deviations and lower total protein measured, but these results were not significantly different from the Kjeldahl values. The data also showed that the soy protein concentrate has a significantly higher protein content than those from corn.

*Solubility profile.* Functionality has been defined as "any property of a food or food ingredient, except nutritional ones, that affect its utilization" (13). Solubility has been judged by many to be the single most important factor affecting protein functionality in foods (14). Soluble protein is the amount that dissolves completely and disperses thoroughly (10,15). The SEP corn protein concentrates were markedly more soluble than the freeze-dried soy protein concentrate at pH values above 3. More than 80% of the corn protein remained soluble in water at pH above 7 (Fig. 1). This result is probably due to the presence of albumin and globulin proteins as well as glutelins (4). The high degree of solubility also indicates that little denaturation of the proteins occurred during protein recovery. This behavior was surprising because most proteins are denatured and insolubilized when exposed to hot aqueous ethanol. The data indicate that we have recovered a corn protein concentrate that is highly soluble within the pH range found in most food systems.

*Foaming properties.* Foaming is important for proteins in some food applications, such as whipped toppings, baked products and frozen desserts. The foam volumes produced by dilute (0.1%) solutions of corn protein concentrates were significantly greater than that produced by the same concentration of soy protein concentrate (Table 2). Increasing the concentration to 1% reduced the foam volumes for the corn protein but substantially increased that of the soy protein concentrate (Table 2). The foam volumes produced by 0.1% corn protein solutions were nearly equal to that produced by 1% soy protein concentrate. The corn protein foams, however, were unstable and collapsed readily (Table 2). Little or no corn protein

TABLE 1

**Moisture and Protein Contents of Freeze-Dried Sequential Extraction Process (SEP) Corn Protein Concentrates and Laboratory-Prepared Soy Protein Concentrate[a]**

| Protein concentrate source | Moisture content (%) | Crude protein content (% db) | |
|---|---|---|---|
| | | Kjeldahl[b] | Biuret |
| Soft dent corn (SEP) | 5.3 ± 4.1 | 83.1 ± 0.6 | 81.4 ± 1.7 |
| Medium-hard dent corn (SEP) | 4.4 ± 3.2 | 81.8 ± 0.7 | 80.3 ± 4.0 |
| High-lysine corn (SEP) | 3.6 ± 2.1 | 82.9 ± 1.5 | 77.8 ± 4.2 |
| Soybean (acid-washed) | 7.0 ± 1.2 | no data | 85.5 ± 3.0 |

[a]Grand mean of three analyses.
[b]% N × 6.25.

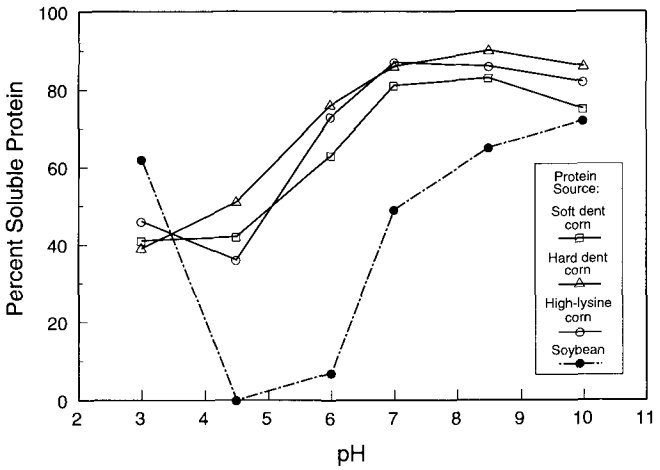


**FIG. 1. Solubility profiles of freeze-dried corn and soy protein concentrates (values are means of duplicate determinations).**

foam remained after 15 min. Increasing the corn protein concentration to 1% only marginally improved foam stability, and there was no corresponding increase in foam volume.

For an adequate foam to form, the interfacial behavior of the protein must be such that it interacts with water (polar) and air (nonpolar) to reduce the high surface tension between the water molecules surrounding the air bubbles (13,16). The interfacial protein layer then confers stability to the foam after formation. This requires some denaturation of the protein during the "whipping" phase and the need for the appropriate amount and correct ordering of hydrophobic and hydrophilic amino acids. The foaming properties of the SEP protein suggest that the amino acid composition and protein conformation can develop a foam with adequate volume; however, the same conformational structure and amino acid residues may not be sufficient to stabilize the foam.

*Emulsifying properties.* Emulsifying capacity is defined as the maximum amount of lipid emulsified by a protein dispersion (17). The protein concentrates from soft (Pioneer 3377) and medium-hard (Pioneer 3732) dent corn had markedly greater emulsifying capacities than the protein concentrate from either high-lysine corn or soybean (Table 2).

Emulsion stability index (ESI) is defined as the unit weight of lipid stabilized per unit weight of protein (11). ESI values indicated that the corn protein emulsions were stable, with soft dent corn protein showing the greatest emulsion stability (Table 2). Soy protein ESI could not be

TABLE 2

**Selected Functional Properties of Soy and Sequential Extraction Process Corn Protein Concentrates[a]**

| Functional property | Protein concentrate source | | | |
|---|---|---|---|---|
| | Soft dent corn | Hard dent corn | High-lysine corn | Soybean |
| Foam stability[b] (%) | | | | |
| 0.1 | $2^{b} \pm 2$ | $2^{b} \pm 1$ | $0^{b} \pm 1$ | $10^{a} \pm 1$ |
| 1.0 | $11^{c} \pm 6$ | $35^{b} \pm 8$ | $6^{c} \pm 1$ | $98^{a} \pm 1$ |
| Foam capacity[c] (%) | | | | |
| 0.1 | $144^{a} \pm 0$ | $146^{a} \pm 5$ | $124^{b} \pm 12$ | $53^{d} \pm 3$ |
| 1.0 | $107^{b,c} \pm 24$ | $98^{c} \pm 4$ | $70^{d} \pm 0$ | $147^{a} \pm 4$ |
| Emulsifying capacity[d] | $878^{a} \pm 12$ | $861^{a} \pm 43$ | $710^{b} \pm 39$ | $638^{b} \pm 8$ |
| Emulsion stability index[e] | $198^{a} \pm 3$ | $119^{b} \pm 11$ | $105^{b} \pm 4$ | no data |
| Heat coagulability at 100°C[f] | $5^{b} \pm 1$ | $5^{b} \pm 2$ | $5^{b} \pm 3$ | $36^{a} \pm 0$ |

[a]Values are means of duplicate determinations. Means across columns followed by the same superscript are not significantly different at $P < 0.05$.
[b]Percent remaining foam after 15 min.
[c]mL of foam.
[d]Grams oil/g protein.
[e]Minutes.
[f]Percent loss in solubility.

determined because its emulsion formed two layers shortly after blending.

For proteins to stabilize emulsions, the protein must be able to adsorb into the oil–water interface and rearrange its conformation such that the hydrophobic residues are in the oil phase and the hydrophilic residues are in the water phase. Furthermore, the protein forms a film around the oil and suspends it in the water phase in the form of droplets. The emulsification data indicate that the SEP protein concentrates have these attributes to an even greater extent than the freeze-dried soy protein concentrate.

*Heat coagulability.* Heat coagulability of the protein was expressed as the percentage loss in solubility after heating at 100°C for 20 min. The SEP corn protein concentrates showed greater heat stabilities than did the soy protein concentrate (Table 2), another indication of the solubility of the protein as well as its stability during heating.

*Heat gelation.* Protein gelation is typically caused by the partial denaturation of the protein followed by reaggregation or reassociation (13,17). Factors such as protein conformation, disulfide linkages, calcium content and hydrophobicity have all been reported to play a role in a protein being able to form a gel.

The soy protein concentrate formed a firm, solid gel, while the SEP corn protein concentrate formed only a viscous liquid. The protein classes needed for gel formation may be present in insufficient amounts or are entirely lacking in the SEP corn protein concentrate.

*Film formation.* Glossy, translucent films similar to those made from zein or soy protein were produced by corn protein concentrates dissolved in water at pH 7. The SEP corn protein films were also more brittle than either the zein or soy protein film.

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