Sequential Extraction Processing of Flaked Whole Corn: Alternative Corn Fractionation Technology for Ethanol Production[1]

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ABSTRACT

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A new approach to fractionating dried, flaked corn (*Zea mays* L.) by using ethanol was studied. The process involved the simultaneous extraction of crude oil and dehydration of ethanol. Protein was extracted by using a mixture of alkali and ethanol. The procedure provided a means for recycling the alcohol from ethanol fermentation to upstream steps of protein and oil extractions. Ethanol extracted more than 90% of the oil from medium-hard dent corn (Pioneer 3732), soft dent corn (Pioneer 3377), and high-lysine corn. These recoveries were significantly greater than the estimated recovery by wet milling corn and prepress hexane-extraction of the germ. The moisture adsorption capacities of the flaked whole corn (initially at less than 2% moisture content) were 22, 20, and 18 g/kg of soft dent corn, medium-hard dent corn, and high-lysine corn, respectively. These capacities were sufficient to dry 35 g of 95.0% ethanol per 100 g of corn (2.5 gal/bu) initially at less than 2% moisture content to 99.0% ethanol. Ethanol drying could be accomplished simultaneously with oil extraction in a percolation mode. The alcohol-alkali mixture removed as much as 65% of the available corn protein. The freeze-dried protein extracts from the three corn types contained about 80% crude protein (db). The type of corn did not significantly affect the oil and protein yields. The sequential extraction of corn with ethanol was technically feasible and may have considerable economic potential when producing ethanol by corn-starch fermentation.

Wet grain milling is used to recover starch from corn (*Zea mays* L.), and this process has not changed significantly over the last 50 years. Corn starch is used in the manufacture of high-fructose corn syrups and for fermentation into industrial solvents and fuel ethanol. Wet-milling techniques are preferred to dry milling because the starch is recovered in greater yield and purity. However, current wet-milling methods use considerable amounts of capital and energy. These factors have impeded the expansion of the wet-milling industry caused by the increased demand for fuel ethanol and high-fructose corn syrups. In addition, the traditional feed markets are becoming saturated with the coproducts from wet corn mills, resulting in lower prices for corn gluten meal, corn gluten feed, and corn germ meal. More cost-effective methods to process corn into starch and starch-derived products are necessary if these and related industries are to remain competitive and expand.

One such method is the sequential extraction process (SEP) (Fig. 1). It is a new approach to corn milling for ethanol production that aims to reduce operating costs for processing, increase the yields of high-value products, and upgrade the values of coproducts. The coproducts of today's wet corn mills are produced in a manner that makes them suitable only for feed, even though corn proteins possess properties that have potential uses in the food industry and for industrial products. SEP has three novel steps: 1) simultaneous extraction of corn oil and drying of the alcohol, 2) use of alcohol-alkali to extract protein and to produce a food-grade protein concentrate, and 3) recycling of ethanol from fermentation of corn starch to upstream extraction steps.

Oil Extraction Using Alcohols

Ethanol and isopropanol occasionally have been used to commercially extract vegetable oils (Johnson and Lusas 1983). Solubility of vegetable oil in these alcohols varies greatly with temperature and water content of the alcohol. Oils are completely miscible in anhydrous ethanol at its boiling point, substantially soluble (7-10%) in boiling aqueous ethanol azeotrope, and only slightly soluble at temperatures lower than the boiling point (Harris et al 1947, 1949; Beckel et al 1948; Rao and Arnold 1956a,b). SEPs used ethanol to extract oil and aflatoxin from cottonseed (Karnofsky 1981, Hassanen et al 1985) and isopropanol to extract oil and toxic simmondsin from jojoba (Hassanen et al 1985).

Alcohol Dehydration

Ladisch and Tsao (1982) developed an energy-efficient recovery process for anhydrous ethanol that involved the partial distillation of 12% alcohol to a 70-90% alcohol concentration followed by adsorption of water by using cellulose, corn residue, or cracked corn. Ladisch et al (1984) designed a pilot-scale adsorber that used corn meal to dry ethanol vapors. Simultaneous dehydration of 95% ethanol and extraction of crude oil from dried ground corn was accomplished by Robertson and Pavlath (1986) and by Chien et al (1988) using a column extraction process.

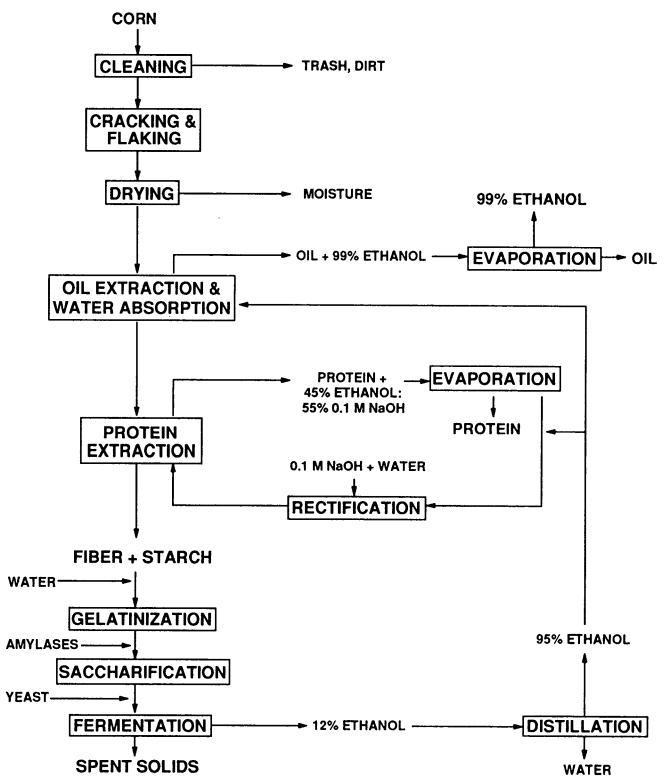

Fig. 1. Sequential extraction processing of corn.

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Protein Extraction Using Ethanol

Substantial amounts of zein are soluble in alcohols and can be extracted with aqueous ethanol (Swallen 1941). Ethanol concentrations ranging from 55 to 70% (Turner et al 1965, Russell and Tsao 1982) and temperatures close to 25°C (Turner et al 1965, Chen and Houston 1970, Concon 1973) have been identified as the optimum conditions for extracting corn endosperm proteins with ethanol. Russell (1980) reported total protein recoveries of 80% from corn endosperm by using a process that combined elements of dry milling to separate fiber and germ followed by extraction with ethanol and then alkali to remove zein and glutelin, respectively. Lusas et al (1985) reported that extraction efficiency from degermed corn can be as much as 85% with proper pH adjustment of the aqueous phase. Lawhon (1986) reported that sonication improved protein yields from degermed corn. Recently, Chen and Hoff (1987) developed a milling process that integrated these elements to produce oil, edible protein, fiber, and starch from cracked corn, which was the adsorbent for drying ethanol.

This study was undertaken to evaluate the feasibility of a sequential extraction approach to milling corn for ethanol production by using ethanol to extract oil while simultaneously drying the alcohol in a countercurrent system and to extract protein from other components of dried, flaked, whole corn.

MATERIALS AND METHODS

Preparation of Corn

Soft dent corn (Pioneer 3377, Pioneer Hi-Bred International, Inc., Johnston, IA), medium-hard dent corn (Pioneer 3732, Pioneer Hi-Bred), and high-lysine corn (Crow's Hybrid Seed Co., Milford, IL) were used in this study. Soft dent corn was selected because it is the type of dent corn that is typically used in the wet-milling industry. Dent corn of intermediate hardness was evaluated because of its theoretically higher crude protein content, which, in turn, could result in increased SEP protein yields. Classification on the basis of hardness was performed by Pioneer Hi-Bred. Laboratory verification of dent corn hardness properties was conducted by using the method of Dorsey-Redding et al (1991). High-lysine corn was tested because it has a nutritionally better amino acid profile than that of dent corn.

Twenty-five batches, each weighing 350 g, were prepared for each type of corn. Each batch was cracked and then flaked to 0.5 mm (0.02 in.) by a Roskamp roller mill (model K, Roskamp Mfg., Inc., Waterloo, IA). The flaked corn samples were placed in aluminum pans and dried at 50°C in a forced-air convection oven to a moisture content of less than 2%. Each dried sample was stored in a polyethylene bag (0.0675 mm [2.7 mils] thickness) and kept in a desiccator at ambient temperature until used.

Proximate Analyses

All batches of corn were analyzed for crude free fat and crude protein by using AACC standard methods 30-20 and 46-08 (AACC 1983), respectively. Moisture contents were determined by Karl Fischer titration by using ASTM standard method E 203-75 (ASTM 1975). Volatile matter was determined using AACC method 44-31 (AACC 1983). These methods were also used to analyze the fractions produced by SEP of corn.

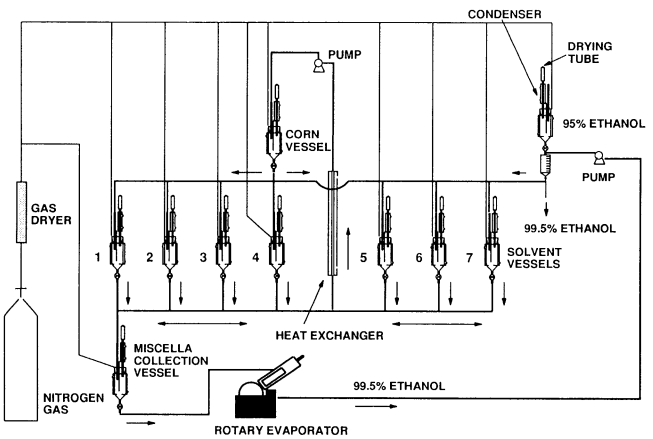

Fig. 2. The countercurrent oil-moisture extraction system.

Solvent Preparation

The seven ethanol concentrations at start-up of the countercurrent extraction were determined on the basis of 1) the exponential relationship between oil extractability and alcohol concentration; 2) the amount of ethanol retained in the marc (solvent-laden defatted flakes), which was experimentally determined to be 65% of the weight of the corn; and 3) the amount of ethanol produced from the fermentation of 1 bu of corn (15% moisture content), which is 2.5 gal, or 35 g of ethanol per 100 g of corn at 2% moisture content. These concentrations (in order of newest to oldest solvents, vessels 7–1) were 97.2, 98.4, 99.0, 99.2, 99.5, 99.5, and 99.5% (v/v). The water content was measured by Karl Fischer titration (ASTM 1975). Fifteen extraction trials were completed to obtain miscellas that were at steady state.

Sequential Extraction Processing of Corn

The extraction system (Fig. 2) was modified from the laboratory extractor-simulator used by Hassanen et al (1985) by using multiple solvent-holding vessels for the seven ethanol concentrations. Dried nitrogen gas was flushed through the system to prevent moisture contamination from the atmosphere. Desiccants were attached to the condenser vents to prevent entry of atmospheric moisture into the vessels. A rotary evaporator was incorporated into the system to separate dry ethanol and oil from the miscella without exposure to air. A diaphragm pump was used to circulate the solvent through the heat exchanger and the flaked-corn bed. A peristaltic pump transferred the ethanol from the rotary evaporator into the graduated separatory funnel. The miscellas were preheated and maintained at 75°C by circulating heated water through the jacketed glass vessels.

Solvent (650 ml) for each stage was placed in the appropriate jacketed, glass solvent-holding vessel. This amount was sufficient for a 2:1 ratio (w/w) of solvent to corn. Dried, flaked corn was placed in the extraction vessel and was subjected to seven extraction stages. In each stage, the solvent percolated through the flakes for 10 min and then drained by gravity for 5 min. Except for the solvent in vessel 1, the contents of each vessel were pumped into the previously emptied vessel after percolation, thus advancing the miscella and simulating countercurrent solvent flow. After the first extraction stage (oldest miscella), the miscella was drained into the recovery vessel and drawn by vacuum into a preweighed sample flask of the rotary evaporator. The alcohol was evaporated, condensed, and then pumped into a graduated separatory funnel, where its volume was measured. Volume equivalent to 35 g of dry ethanol per 100 g of corn extracted was taken out to represent the amount of ethanol produced from the fermentation of 350 g of corn. The equivalent of 100 g of dry ethanol per 100 g of flaked corn was then drained into solvent vessel 7. The rest of the dry ethanol was emptied into a preweighed screw-capped vial and analyzed for moisture content. Volume equivalent to 100 g of 95% ethanol per 100 g of flaked corn was mixed with the recovered dry ethanol in vessel 7. This amount of 95% ethanol represented the solvent holdup (65%) and the 35 g of dry ethanol per 100 g of corn taken out earlier.

Small portions (less than 5 g) of the defatted flakes were removed for determining moisture, volatile matter, residual oil (crude free fat), and crude protein contents. The remaining flakes were weighed into six blender cups in amounts equivalent to 25 g of dry corn. A mixture of 45% ethanol-55% 0.1*M* NaOH (v/v) (concentrations were determined to be optimum in an earlier study [Hojilla-Evangelista 1990]) was added at a total ratio of 15 ml/g of dry corn in two grinding steps. In the first grinding, the smallest volume of ethanol-NaOH needed to cover the defatted corn (1.5 ml/g of dry corn) was used to allow maximum shearing and cutting action by the blades. The contents of each cup were ground in a Waring Blendor at full speed for 1.5 min and then allowed to stand for 2 hr to soften the corn particles and to facilitate

protein extraction. After soaking, more ethanol-alkali was added at a ratio of 13.5 ml/g of dry corn, the mixture was blended for another 30 sec, and then the contents of the blender cups were transferred to centrifuge bottles. The bottles were capped tightly, placed in racks, and then immersed in a water bath maintained at 55°C. The bottles were shaken for 2 hr at 130 rpm. After protein extraction, the bottles were centrifuged at 1,050 $\times$ *g* for 5 min. The supernatant was analyzed for crude protein content, and the extraction efficiency was calculated. The residues (fiber + starch) were analyzed for moisture content by Karl Fischer titration. Ethanol and moisture were evaporated by drying the residues in an oven at 105°C before analysis of residual oil and crude protein contents.

The sample flask from the rotary evaporator was also disconnected and set aside for measuring oil yields. The oil and solids were separated by washing with petroleum ether, filtering the washings into a preweighed flask, and evaporating the ether by using a hot-water bath. The cleaned extraction vessel and a new rotary evaporator sample flask were then replaced in the system for the succeeding extraction. Twenty extraction trials were performed, the first 14 of which were used to establish system equilibrium. Data from the last six trials were used to evaluate the process.

Statistical Analyses

The data were analyzed by using a Statistical Analysis System program (SAS 1987). Significant differences among treatment means were identified by least significant difference. Probability levels of $P \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Stenvert Hardness of Dent Corn Samples

Stenvert hardness values (at 15.5% moisture basis) for Pioneer 3377 and 3732 were 12.4 ± 0.4 cm and 11.6 ± 0.4 cm, respectively (significantly different means). These values were within the range of Stenvert hardness data reported by Dorsey-Redding et al (1991) from tests performed on 1988 maize hybrids. The results of the Stenvert hardness test indicate that Pioneer 3377 conforms to the soft dent corn type, whereas Pioneer 3732 may be classified as dent corn of medium hardness.

Oil Extraction with Ethanol

The countercurrent system provided oil yields that were far superior to the 72% estimated recovery (80% of the oil in corn found in the germ $\times$ 90% extraction efficiency) for conventional prepress hexane extraction (Table I). The oil recoveries of more than 90% from our countercurrent simulation of continuous extraction were similar to those obtained by the batch process of Chen and Hoff (1987) and were also not significantly different from oil recoveries obtained from the earlier percolation extraction trials (Hojilla-Evangelista 1990). The type of corn had no significant effect on the amount of crude oil extracted.

The profile of oil concentrations in the miscellas for each extraction stage is given in Table II. These values were determined after the fifth steady-state extraction trial for each type of corn. The greatest oil concentrations were obtained in the first two stages of extraction. This was because, in countercurrent extraction, the fresh corn containing the maximum amount of oil for extraction comes in contact first with the oldest solvents (miscella 1). Towards the last extraction stages, very little oil is available for recovery by the fresh solvent (miscella 7). In addition, the miscellas from the first two extraction stages had the smallest moisture contents and were closest to anhydrous levels (Table III) where oil solubility is high. Thus, oil is easily extracted from flaked whole corn by using percolation extraction principles.

TABLE I
Oil Recoveries from Dent Corn and High-Lysine Corn Extracted with Ethanol[a]

Corn Type	Initial Crude Fat (% db)	Residual Oil (% db)	Oil Yield (%)
Pioneer 3377 (soft dent)	3.28 ± 0.19 a	0.30 ± 0.08 a	90.8 ± 2.2 a
Pioneer 3732 (medium-hard dent)	3.83 ± 0.33 b	0.37 ± 0.07 a	90.3 ± 2.3 a
High-lysine corn	3.93 ± 0.30 b	0.24 ± 0.13 a	93.7 ± 3.7 a

[a] Grand mean of five extraction trials per corn type. Means within a column followed by a common letter are not significantly different at $P < 0.05$.

Ethanol Dehydration

The moisture contents of the three corn types significantly increased during oil extraction (Table IV), indicating the simultaneous adsorption of water from the solvent by the flaked corn. More water was adsorbed by the dent corn types (Pioneer 3377 and 3732), but their water adsorption capacities were not significantly different from that of high-lysine corn. The marked reduction in the moisture content of the ethanol recovered from the evaporation of the full miscella further verified ethanol dehydration during the oil extraction process. All three corn types dried 95% ethanol to about 99%, but Pioneer 3732 dehydrated the alcohol to a greater degree than did Pioneer 3377 or the high-lysine corn. The difference may have been due to slightly greater starting moisture contents of the latter corn types.

Protein Extraction

The crude protein contents of the dent corn and the high-lysine corn at various stages of SEP are presented in Table V. Some nonoil solids were coextracted with the crude corn oil, with more being taken out of soft dent corn than either high-lysine or medium-hard dent corn. The solids appeared as yellow, flaky residues after rotary evaporation of the ethanol from the miscella and were separated from the oil by washing with a small volume of petroleum ether and filtration. The recovered solids contained 25–30% protein, accounting for about 10% of the protein initially present in corn. Ethanol is capable of solubilizing and extracting small amounts of protein during oil extraction, and a slight reduction in crude protein content was expected.

More than 57% of the total protein was extracted by the ethanol-alkali from the three corn types, a recovery comparable to that observed by Chen and Hoff (1987) when a mixture of 50% ethanol and 0.08*N* NaOH was used to extract protein from ground corn. The type of corn did not significantly affect protein yields. These protein yields were less than the earlier protein recoveries (about

TABLE II
Oil Concentration Profile of Miscellas

Miscella Number	Oil Content, g/100 g of miscella: Pioneer 3377 (Soft Dent Corn)	Pioneer 3732 (Medium-Hard Dent Corn)	High-Lysine Corn
1 (full)	3.00 ± 0.08	3.34 ± 0.04	2.50 ± 0.06
2	1.62 ± 0.02	2.23 ± 0.07	1.64 ± 0.10
3	0.79 ± 0.01	0.76 ± 0.02	1.01 ± 0.03
4	0.44 ± 0.09	0.49 ± 0.05	0.67 ± 0.08
5	0.26 ± 0.02	0.39 ± 0.11	0.41 ± 0.13
6	0.15 ± 0.05	0.44 ± 0.10	0.26 ± 0.08
7	0.06 ± 0.00	0.08 ± 0.01	0.08 ± 0.00

TABLE III
Moisture Content Profile of Miscellas

Miscella Number	Moisture Content, % volume basis: Pioneer 3377 (Soft Dent Corn)	Pioneer 3732 (Medium-Hard Dent Corn)	High-Lysine Corn
1 (full)	1.74 ± 0.02	1.30 ± 0.06	1.65 ± 0.01
2	2.14 ± 0.08	1.38 ± 0.04	1.78 ± 0.01
3	2.08 ± 0.07	1.70 ± 0.05	1.79 ± 0.03
4	2.31 ± 0.08	1.78 ± 0.01	1.79 ± 0.00
5	2.36 ± 0.14	1.88 ± 0.01	1.85 ± 0.01
6	2.54 ± 0.12	1.94 ± 0.01	1.95 ± 0.00
7	3.11 ± 0.02	2.04 ± 0.02	2.11 ± 0.02

TABLE IV
Moisture Contents (MC) of Flaked Corn and Ethanol After Oil Extraction[a]

Corn Type	Initial MC (%)	MC After Oil Extraction (%)	MAC[b] (g H_2O/kg dry corn)	Ethanol MC (% vb[c])
Pioneer 3377 (soft dent)	1.12 ± 0.04 a	3.28 ± 0.14 a	21.6 ± 1.3 a	1.67 ± 0.10 a
Pioneer 3732 (medium-hard dent)	0.97 ± 0.18 a	2.96 ± 0.14 b	20.0 ± 1.5 ab	0.99 ± 0.02 b
High-lysine corn	1.39 ± 0.16 b	3.17 ± 0.09 a	17.8 ± 1.9 b	1.27 ± 0.03 c

[a] All values are means of five extraction trials. Means within a column followed by a common letter are not significantly different at $P < 0.05$.
[b] Moisture adsorption capacity.
[c] Volume basis.

TABLE V
Crude Protein (CP) Yields of Dent Corn and High-Lysine Corn During Sequential Extraction Processing[a]

	Pioneer 3377 (Soft Dent Corn)	Pioneer 3732 (Medium-Hard Dent Corn)	High-Lysine Corn
Initial CP in flaked corn, % db	8.3 ± 0.2 a	8.6 ± 0.8 a	8.7 ± 0.4 a
CP extracted with oil			
Residue with oil, g/100 g of dry flaked corn	3.2 ± 0.1 a	2.8 ± 0.4 b	3.1 ± 0.1 ab
CP with residue, % db	29.4 ± 2.0 a	28.7 ± 4.1 b	25.5 ± 2.2 b
Percentage of total CP with oil, db	11.6 ± 1.2 a	8.7 ± 2.1 b	9.0 ± 1.1 b
CP extracted by ethanol-NaOH			
CP in ethanol-NaOH, % db	5.1 ± 0.2 a	5.6 ± 0.8 a	5.6 ± 0.4 a
Percentage of total CP extracted, db	66.1 ± 1.1 a	60.1 ± 5.9 b	57.6 ± 2.5 b
CP in freeze-dried extract, % db	79.6 ± 3.1 a	75.3 ± 2.7 b	78.5 ± 3.9 a
Residual CP in fiber-starch			
Residual CP, % db	1.7 ± 0.1 a	2.9 ± 0.6 b	3.2 ± 0.1 b
Unrecovered CP, %	21.8 ± 0.9 a	31.2 ± 7.6 b	33.4 ± 1.5 b

[a] Grand mean of five extraction trials. Means across columns followed by a common letter are not significantly different at $P < 0.05$.

72%) obtained by Hojilla-Evangelista (1990), but they were still significantly greater than the 48% expected protein recovery estimated from an earlier protein solubility study (Hojilla-Evangelista 1990). The protein from the dent corn and high-lysine corn solubilized in ethanol-alkali were dialyzed against water and then freeze-dried to recover the protein in solid form. The freeze-dried corn protein extracts contained 75–80% crude protein (db, Table V). This amount of protein was significantly greater than the typical 60–62% protein content of corn gluten meal. SEP protein concentrate was white, had a very mild corn flavor, and could have been considered food grade because all chemicals used in SEP are allowed for processing food. Its light color (compared with the yellow of corn gluten meal) is a potential advantage in food applications because little added color will be imparted to the product.

A small amount of protein still remained in the fiber and starch residue after extraction with ethanol-alkali. The starch from this new process is expected to be of poorer quality than the starch from conventional wet milling because of its greater residual protein content (about 2% compared with less than 0.3% in wet-milled starch). The high residual protein content also makes the SEP starch unsuitable for marketing as food or industrial starch or for conversion into syrups, because the protein causes undesirable browning reactions. However, the starch can still be used as substrate for ethanol fermentation.

CONCLUSIONS

The sequential extraction of dried, flaked whole corn was technically viable and may have considerable economic potential in producing fuel ethanol from the fermentation of corn starch. Ethanol extracted 90% of the oil in the corn, a recovery significantly greater than the 72% estimated for the conventional prepress hexane-extraction process. The moisture adsorption capacities of nearly 20 g/kg of corn at an initial moisture content of less than 2% were sufficient to dry 35 g of 95% ethanol per 100 g of corn at less than 2% moisture content (2.5 gal/bu) to about 99% ethanol. The ethanol-NaOH mixture extracted more than 57% of the available protein in the corn. The protein concentrate contained almost 80% crude protein (db). The type of corn had no significant effect on the oil and protein extraction efficiencies.

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