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Protein Distribution in Commercial Wet- and Dry-Milled Corn Germ

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To identify high-valued coproducts from commercially processed corn germ, it was necessary to determine the effect of processing conditions on corn germ proteins. We found that significantly less protein was extracted from commercial wet-milled as compared to dry-milled corn germ using Tris, sodium dodecyl sulfate (SDS) buffer containing 14 mM 2-mercaptoethanol at 100 °C for 10 min. SDS−polyacrylamide gel electrophoresis (PAGE) revealed a number of proteins with molecular masses ranging from approximately 10 to 66 kDa for the dry-milled corn germ as compared to only a few significant protein bands centered around 23 kDa in the wet-milled corn germ. The protein content of the wet- and dry-milled corn germ was approximately the same; however, nonprotein nitrogen values were significantly greater for the wet-milled than for the dry-milled germ. The distribution of fractionated germ protein freshly excised from the embryo of yellow dent corn kernels was more similar to that of dry-milled than wet-milled corn. SDS−PAGE of laboratory preparations of wet-milled corn germ more closely resembled commercial dry- than wet-milled corn germ, which could be attributed to limited microbial growth during steeping in the laboratory preparations.

KEYWORDS: Corn germ; commercial wet-milled; dry-milled; laboratory preparation

INTRODUCTION

Corn wet milling is a process developed to obtain high yields of starch from corn kernels for the production of sweeteners and ethanol by fermentation. It is designed to separate germ, fiber, protein, and starch constituents from the kernel. Separation of these components is facilitated by initially steeping the corn kernels in solutions containing SO_2, which softens the kernel and aids in the separation of the starch and protein (*1*−*3*). Corn dry milling, on the other hand, was developed to obtain food grade grits, hominy, and other food fractions. It involves removing the germ and bran to produce products with a longer shelf life and lower oil fiber. Corn germ obtained from wet mills usually contains about 40−50% oil, and corn germ from dry mills contains approximately 20−25% oil on a dry basis (*4*). The protein content of the germ from both processes is roughly between 14 and 16%, but there is a significant loss of water- and salt-soluble proteins in the wet-milling process due to steeping (*2*) and an increase in the amount of α-zein, contributed from the germ endosperm, during dry milling. Unfortunately, little information is available regarding the fate of corn germ proteins as a result of processing conditions. As part of a project to develop new environmentally safe aqueous/enzymatic processes to extract edible oil from corn germ, the objective of this research was to develop bioprocesses to enhance the value of proteins from the deoiled germ.

MATERIALS AND METHODS

Materials. Corn degerminator was from Beall Degerminator Co. (Decatur, IL). Dialysis tubing, with a nominal molecular mass cutoff of 3500 Da, was from (Fisher Scientific, Pittsburgh, PA). The protein assay kit used was a Bio-Rad DC protein assay (Hercules, CA), and a Polytron model PT 10/35 homogenizer was from Binkmann (Switzerland). All bench scale laboratory experiments were carried out using a single hybrid of yellow dent corn (Pioneer 33G26). The corn was grown at the University of Illinois experimental station during the 2002 season, field-dried, and combine-harvested. The corn was hand-cleaned to remove broken kernels and foreign materials, weighed into polyethylene bags (1 kg wet weight, with approximately 14% moisture content), and stored at 4°C until use.

Commercial Milling Procedures. For comparative purposes, samples of wet-milled corn germ were obtained from two different commercial corn wet mills A and B. Samples of dry-milled corn germ were obtained from a commercial corn dry mill. All germ samples were stored in sealed containers at 4 °C. Wet-milled samples from commercial mill A were prepared using continuous countercurrent steeping for 30 h in 0.15−0.2% SO_2. Wet-milled samples from commercial mill B were continuously batch steeped for 24 h in 0.15% SO_2. Commercial dry-milled samples were tempered for 5 min. The moisture content of the tempered corn was between 18 and 20%, and the samples were degermed using a Beall degerminator.

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Corn Dry-Milling Germ Recovery. The laboratory dry-milling procedure was carried out as previously described by Johnston et al. (*5*). Triplicate samples (1 kg) were tempered for 18 min after the addition of 8.5% (by weight) water to the corn. Tempered corn was passed through a horizontal drum degerminator, which impacts and abrades the corn, resulting in partial separation of germ and fiber from endosperm. The product was dried for 1 h at 49 °C to approximately 15% moisture. A subsample (10−15 g) was used to determine the moisture content using the two-stage convection oven method (AACC 2000: method 44-18). Materials were sieved using a laboratory sifter (model P1202, Great Western Manufacturing Co., Leavenworth, KS). The fraction that passed over a standard five mesh sieve (+5 mesh) was roller milled to flatten the germ and aspirated (model: 6DTA, Kice Metal Products Co., Wichita, KS) (0.4−0.5 in. of water vacuum) to remove the pericarp fraction. The material not removed by aspiration ("heavy" fraction) and was sifted on a standard 10 mesh screen to remove the flattened germ particles. The remaining endosperm fraction was weighed and identified as "large grit". The portion passing through the five mesh sieve also was roller milled, aspirated, and sifted on a 10 mesh sieve. The lighter material removed by the aspirator was added to the pericarp fraction. The heavier material from the aspirator was sifted on a standard 10 mesh sieve. Material passing over the 10 mesh sieve (primarily germ particles) was added to the germ fraction, and material passing through the 10 mesh sieve (primarily endosperm particles) was sifted on a standard 24 mesh sieve. The yield expressed as % dry matter was 12.71 ± 0.98.

Corn Wet-Milling Germ Recovery. Laboratory conventional corn wet milling and germ recovery were done in triplicate using the 1 kg laboratory corn wet-milling procedure as outlined by Eckhoff et al. (*6*). The yield expressed as % dry matter was 7.55 ± 0.16.

Defatted Commercial Corn Germ. For wet- and dry-milled corn germ, 100 g was ground to a size less than 3 mm and extracted twice with 500 mL of hexane at room temperature for 30 min. The oil content (%) was determined after the combined supernatants were decanted and dried under nitrogen.

Protein Extraction from Commercial Corn Germ. A modification of two methods Landry and Moureaux (*7*) and Wu and Hojilla-Evangelista (*8*) was used to sequentially extract the germ proteins. For defatted wet- or dry-milled germ, 20 g was extracted twice at room temperature with 300 mL of 0.5 M NaCl for 1 h. The mixtures were combined and centrifuged at 13000*g* for 20 min. The supernatant was dialyzed for 4 days at 4 °C with several changes of water and centrifuged. The globulin precipitate was dried to constant weight under nitrogen, and the supernatant, containing the albumin, was lyophilized overnight. The residue, from the salt extraction, was extracted with 300 mL of 70% ethanol (v/v) at room temperature for 1 h and centrifuged. The supernatant, containing the zein proteins, was dialyzed for 4 days at 4 °C and lyophilized overnight. Glutelins were extracted twice from the residue with 300 mL of 0.1 N NaOH at room temperature for 1 h. The combined solutions were neutralized with 1 N HCL, dialyzed for 4 days at 4 °C, and centrifuged, and the supernatant was lyophilized overnight.

Protein Extraction from Kernel Embryo. A modification of the procedure used to excise the corn kernel embryo and extract albumin and globulin proteins has been reported (*9*). The kernels were soaked overnight in water at 4 °C, and embryos were removed with a scalpel. Protein isolation procedures were carried out between 0 and 5 °C. Five grams of embryo was homogenized twice in 30 mL of acetone and centrifuged at 10000*g* for 10 min, and the precipitate was dried under nitrogen. The precipitate was homogenized with 0.5 M NaCl and 14 mM 2-mercaptoethanol (2-ME) and centrifuged as before, and the pellet was reextracted with saline solution as before. The combined supernatants were centrifuged at 30000*g* for 15 min, and the supernatant was dialyzed extensively. Zein and glutelin fractions were isolated as described above but in the presence of 14 mM 2-ME.

Protein Analysis. The total protein content of corn germ was determined in duplicate by the Bio-Rad DC protein assay. Trichloroacetic acid (TCA)-precipitated protein and nonprotein nitrogen (NPN) of clear supernatants was determined after treatment of samples with a TCA solution to a final concentration of 6% (w/v), shaken for 20 min, and then centrifuged at 12000*g* for 10 min at 4 °C (*10*). The lowest value of NPN occurred below 20% TCA. Above this concentration, germ proteins were soluble and interfered with the assay.

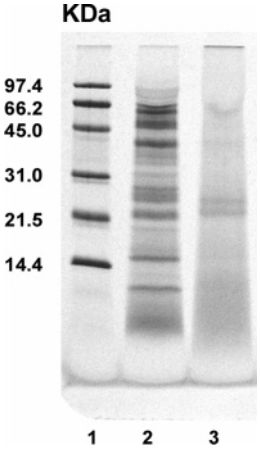

Figure 1. SDS-polyacrylamide gel electrophoresis of commercial corn germ proteins extracted from dry- and wet-milled corn germ. Lane 1, molecular weight standards; lane 2, protein extracted from dry-milled corn germ; and lane 3, protein extracted from wet-milled corn germ (A).

Gel Electrophoresis. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) of corn germ proteins was carried out on a phast System Pharmacia (Piscataway, NJ) with a phast gel of 20% acrylamide. Dried protein samples from wet- and dry-milled corn germ were solubilized in 200 μL of protein solvent system (0.44 M Tris, 1 mM EDTA, and 10% SDS, pH 8.0) plus 40 μL of 2-ME, and the mixtures were heated at 100 °C for 10 min. Gels were stained with 0.2% (w/v) Coomassie R350 dye. Molecular mass standards (Bio-Rad, Richmond, CA) and their corresponding molecular masses were as follows: phosphorylase b, 97400; bovine serum albumin (BSA), 66200; ovalbumin, 45000; carbonic anhydrase, 31000; soybean trypsin inhibitor, 21500; and lysozyme, 14400.

RESULTS AND DISCUSSION

In an attempt to explain differences in the properties of commercial wet- and dry-milled corn germ, we compared their protein content and distribution. After extraction with Tris, SDS buffer containing 2-ME at 100 °C for 10 min, we found significantly less protein extracted from wet-milled as compared to dry-milled corn germ. Gel electrophoretic patterns of dry-milled germ samples indicated a number of proteins with molecular masses ranging from approximately 10 to 66 kDa and only a pair of significant protein bands, centering around 23 kDa, for wet-milled corn germ (A) (**Figure 1**, lanes 2 and 3). This could be attributed primarily to removal of some proteins with the steep liquors and to a lesser extent during cake pressing and/or aggregation during drying of wet corn gluten. Protein aggregates, sufficiently large in the wet-milled sample, did not enter the separation gel as shown in **Figure 1**, lane 3.

A comparison of total protein, TCA-precipitated protein, and the NPN content of commercial wet- and dry-milled corn germ is shown in **Table 1**. Although commercial wet-milled corn germ (A) contained less precipitated protein than commercial dry-milled germ, its NPN content was approximately twice that of the dry-milled germ. Similarly, another commercial wet-milled germ (B) with total protein content equivalent to the commercial dry-milled corn germ contained approximately three times the amount of NPN present in the commercial dry-milled corn germ.

The data from **Table 1** have been analyzed by analysis of variance to determine any treatment differences. There was evidence of a significant treatment effect for each of the four variables: total protein, TCA ppt protein, NPN, and the sum

Table 1. Total Protein, Precipitated Protein, and Nonprotein Nitrogen in Wet- and Dry-Milled Corn Germ

germ	wt/% total protein[a]	wt/% TCA ppt protein[a]	wt/% NPN[a]
	commercial		
wet-milled A	11.02b (9.76)[b] c	4.38 c	5.38 b
wet-milled B	14.97a (15.28) a	6.28 b	9.00 a
	bench scale		
wet-milled	12.22b (11.46) bc	7.27 b	4.19 b
	commercial		
dry-milled	14.74a (13.81) ab	10.64 a	3.17 c
	bench scale		
dry-milled	9.97c (10.93) c	7.59 b	3.33 c

[a] Values followed by different letters are significantly different at $p < 0.05$ using the Bonferroni LSD multiple comparison method. [b] Values in parentheses are the sums of TCA ppt protein + NPN.

of TCA ppt protein + NPN. Examination of the four treatments means showed evidence that for total protein, commercial wet milling B and dry milling had significantly higher total protein values than the other three. There was also a significantly higher total protein value in the wet-milled bench scale samples than in the dry-milled samples. The TCA component resulted in a significantly higher response for the commercial dry-milled sample and a significantly lower response for the commercial wet-milled sample A. Both commercial wet-milled samples showed significantly higher NPN result than most, if not all, of the other samples with wet-milled B exceeding all others. Finally, the sum of TCA ppt protein and NPN exhibited higher values in commercial wet-milled B and dry-milled samples with commercial dry-milled samples being no different from the bench scale wet-milled samples.

The Bradford protein assay was used in this study because the sum of the TCA ppt protein + NPN more closely agreed with the total protein values **Table 1** than did protein values obtained using the Dumas combustion method. Also, sugars, present in dry-milled corn germ, resulted in artificially high NPN values using the Pierce method but not the Bradford assay. In addition to proteolysis and the loss of water-soluble protein during steeping, Craine and Fahrenholtz (*11*) reported that water-soluble proteins from whole kernels react with phytate, which can change the fractionation of albumins and globulins. Phytic acid may make up almost 1% of the dry weight of the total kernel, and about 88% of the phytate is found in the germ. O'Dell and de Boland (*12*) reported that phytate was extracted from corn germ with water but found that the proteins extracted were not complexed with phytate.

We found that the albumin, globulin, and glutelin proteins sequentially extracted from commercial dry-milled corn germ each represented approximately 30% of the protein and zein about 5% (**Table 2**). SDS−PAGE patterns of the albumin and globulin fractions revealed a number of protein bands between 10 and 66 kDa and some low molecular mass peptides primarily in the albumin fraction (**Figure 2**, lanes 2 and 3). Only α-zeins were present in the alcohol extract, and a number of poorly resolved bands were present in the alkaline extract or glutelin fraction (**Figure 2**, lanes 4 and 5). SDS−PAGE revealed a significant amount of proteolysis and a decrease in the amount of albumin, globulin, and zein proteins sequentially extracted from wet-milled as compared to dry-milled corn germ (compare **Figures 2** and **3**). In addition, there was a significant increase in the percent glutelin in commercial wet-milled germ (**Table 2**).

Table 2. Distribution of Protein in Commercial Dry- and Wet-Milled Corn Germ and Kernel Embryo[a,b]

fraction	dry (wt/%)	wet (wt/%)[c]	embryo (wt/%)
albumin	34.1	22.6	36.7
globulin	28.0	2.3	34.6
zeins	4.6	0.9	4.6
glutelin	33.3	74.2	24.2

[a] Proteins were sequentially extracted as described in the Materials and Methods. [b] Weight percent protein fraction isolated from corn germ. [c] Wet-milled corn germ A.

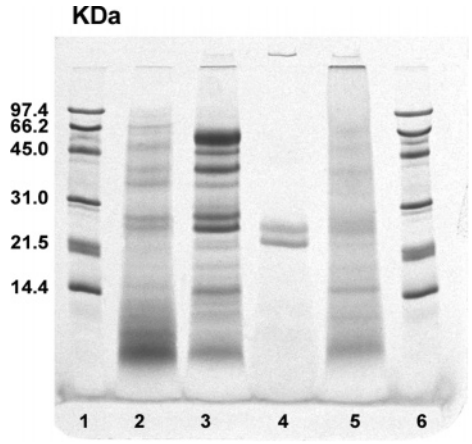

Figure 2. SDS-polyacrylamide gel electrophoresis of fractionated germ proteins from dry-milled corn. Lanes 1 and 6, protein molecular weight standards; and lanes 2–5, albumin, globulin, zein, and glutelin proteins, respectively.

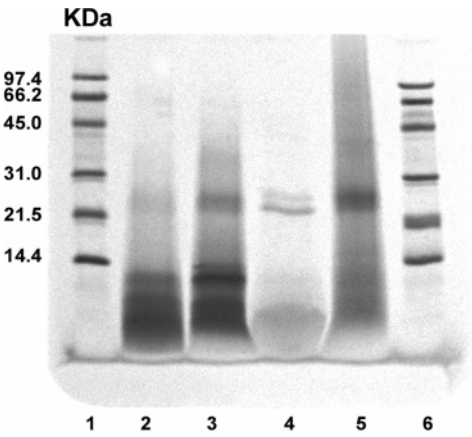

Figure 3. SDS-polyacrylamide gel electrophoresis of fractionated germ proteins from wet-milled corn. Lanes 1 and 6, protein molecular weight standards; and lanes 2–5, albumin, globulin, zein, and glutelin proteins, respectively.

Comparison of commercial and laboratory corn germ preparations indicated that considerably less proteolysis and protein aggregation occurred in wet-milled germ prepared in the laboratory as compared to the commercial product (**Figure 4**, lanes 3 and 4). In addition, many protein bands, with similar migration patterns, were present in both laboratory preparations of the dry- and wet-milled corn germ (**Figure 4**, lanes 2 and 3).

To critically address germ protein proteolysis during commercial processing, germ proteins were carefully excised from the embryo of commercially available yellow dent corn kernels followed by homogenization between 0 and 5 °C to minimize

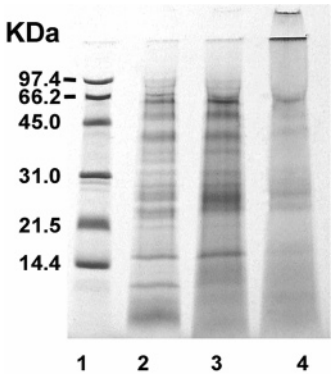

Figure 4. SDS-polyacrylamide gel electrophoresis of laboratory-prepared wet- and dry-milled corn germ. Lane 1, protein molecular weight standards; lanes 2 and 3, dry- and wet-milled corn germ, respectively; and lane 4, commercial wet-milled corn germ (B).

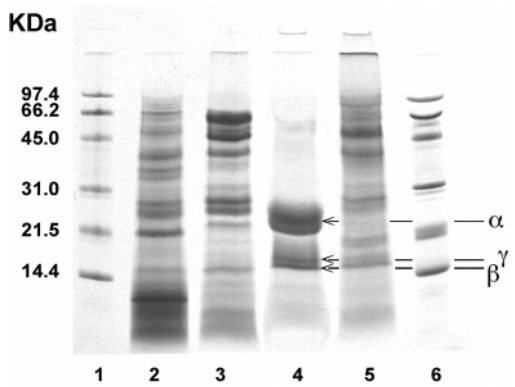

Figure 5. SDS-polyacrylamide gel electrophoresis of fractionated germ proteins from kernel embryo. Lanes 1 and 6, protein molecular weight standards; and lanes 2–5, albumin, globulin, zein, and glutelin proteins, respectively.

proteolysis. Protein fractionation resulted in a distribution more closely resembling that of commercial dry-milled than wet-milled corn germ (**Table 2**). The albumin and globulin fractions represented the major portion of the germ protein (*13*). Because of the distinctive peptide patterns and the ease of isolation of embryo storage globulins, Cross and Adams (*9*) have suggested their use in establishing the identity and ancestry of inbred lines. SDS−PAGE patterns of the germ albumin and globulin proteins consisted of a large number of bands (**Figure 5**, lanes 2 and 3). The albumin and globulin contents of the dry-milled and embryo germ were 62.1 and 71.3%, respectively, and both contained the same amount of zein proteins (**Table 2**). Depending on the technique used, the total water-soluble albumin and salt-soluble globulin proteins and amino acids can account for >75% of the total protein of the germ (*7*). Tsai (*14*) found that 10% of the germ proteins migrated as zein on SDS−PAGE but believed it was due to contamination with zein from the endosperm since no means to produce zein in the embryo or cross-reactions between embryo tissue and antibodies against zein have been found (*15*). Embryo zein extracted from the germ, in the presence of 2-ME, consisted of a mixture of α-, β-, and γ-zeins and a small amount of δ-zein (**Figure 5**, lane 4). Landry et al. (*16*) reported an increase in α-zein and a decrease in α-, β-, and δ-zeins extracted from the endosperm without and with reducing agent due to the presence of SO_2 in the steeping liquor. We found that the zein fraction extracted from commercial dry- and wet-milled corn contained only the α-zein; however, when the extraction was carried out in the presence 14 mM 2-ME, SDS−PAGE revealed additional bands attributed to γ- and δ-zein from the dry-milled corn germ (figure not shown). SDS−PAGE of the glutelin fraction revealed well-resolved proteins and little evidence of protein aggregation (**Figure 5**, lane 5).

In conclusion we have shown that proteins present in commercial wet-milled corn germ are significantly altered as compared to the proteins that are present in fresh embryo. This is a result of proteolysis or protein aggregation and a significantly greater amount of NPN than found in the dry-milled corn germ. In addition, sequential extraction of proteins from wet-milled corn germ resulted in an alkaline soluble glutelin fraction about three times greater than was extracted from the germ embryo. The fraction may be similar to the glutelin-like protein fraction previously observed (*16*) whose disulfide was not cleaved during steeping due to its poor solubility in acid medium. In contrast, proteins sequentially extracted from commercial dry-milled corn germ were similar to those freshly extracted from corn germ embryo and very little proteolysis was indicated by gel electrophoresis.

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