Fractionation of Transgenic Corn for Recovery of Recombinant Enzymes

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ABSTRACT: Two germ-separation methods, dry-milling and density separation by flotation, were evaluated for recovering recombinant β-glucuronidase (rGUS) that accumulated primarily in the germ of transgenic corn. The dry-milling process consisted of (i) seed tempering, (ii) degerming with a horizontal-drum degermer/dehuller, (iii) particle size fractionation with standard sieves, (iv) germ and endosperm separation by roller milling and sifting, and (v) removal of hulls by aspiration. Sieves nos. 5, 6, and 7 retained the majority of germ, and subfractions from these sieves were pooled as a germ-rich fraction. Mass balances showed that the germ-rich fraction, which constituted 17% of the total dry-milled corn weight, contained 49% of rGUS activity and 64% of the total recoverable oil. Germ fractionation by flotation was tested as a proof-of-concept method aimed at separating corn fractions based on their difference in specific gravity (sp gr). The process consisted of impact-grinding of corn kernels followed by density separation using 1.15 or 1.3 specific gravity sodium nitrate solution. The oil-containing germ fraction floated, whereas the heavier endosperm fraction sedimented. The flotation method was simpler and resulted in higher enzyme recovery, that is, the germ-rich fraction was 20% (w/w) of the initial corn weight, and accounted for 80% of rGUS activity and 77% of total oil. The sodium nitrate solution did not have an adverse effect on the enzyme activity.

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KEY WORDS: Dry milling, flotation, germ recovery, recombinant enzyme, transgenic corn.

Developments in plant biotechnology have created opportunities for improving quality and composition of corn seed *(Zea mays* L.) and diversified the number of products that can be obtained by plant transformation. Transgenic corn and other transgenic crops are being generated and evaluated for their abilities to efficiently synthesize a variety of therapeutic and industrial proteins (1–5). The impetus for using plant crops as natural bioreactors includes factors such as established cultivation, storage and processing infrastructure, and reduced health risks from human and animal pathogens. Transgenic corn plants that express a recombinant product in the seed are currently the best system to fulfill economic and safety requirements for producing biopharmaceuticals and industrial proteins.

The economics of producing pharmaceutical molecules (high price and low volume) in transgenic corn is primarily affected by the expression level, the quality of the expressed protein (glycosylation, folding, activity), and downstream purification cost, however, the cost of the transgenic raw material has a relatively small effect (6,7). At the other end of the cost spectrum are high-volume, low-priced industrial products whose economic viability is a strong function of the accumulation level as well as the costs of the transgenic seed and protein extraction; the cost of transgenic corn alone could be as high as 50% of the manufacturing cost. Therefore, the economical production of industrial products from transgenic plants calls for more efficient utilization of transgenic biomass and byproduct revenues. One approach, primarily advocated and applied by ProdiGene (1), is to target the recombinant protein synthesis to a plant tissue or organelle that could be readily separated from the rest of the plant material and utilized as starting raw material.

In collaboration with ProdiGene Inc., our group demonstrated that recombinant β-glucuronidase (rGUS) preferentially accumulated in the corn germ (8). The extraction of hand-fractionated corn kernels showed that 93% of the extracted enzyme activity was localized in the germ and 7% in the endosperm. Because germ constitutes about 10% of the kernel weight, a ninefold enrichment of rGUS concentration in the crude extract was achieved by extracting germ instead of the whole kernel meal (8). Besides the greater concentration of recombinant protein in the extract, protein recovery from the germ meal would (i) require 8–10-fold less buffer and other consumables compared with the whole kernel meal, and (ii) allow selling starch-rich endosperm as animal feed or fermentation feedstock.

The preliminary screening of traditional corn wet-milling indicated that steeping with 0.2% sulfurous acid and/or 0.5% lactic acid was detrimental to rGUS activity. Therefore, we proceeded with investigating alternative methods for germ isolation. Two germ isolation processes were evaluated: dry fractionation and density separation (germ flotation). The dry fractionation process consisted of (i) seed tempering, (ii) degerming, (iii) particle size fractionation by sifting, and (iv) germ and endosperm separation using roller mills, sifting, and aspiration. Germ recovery by density separation involved (i) impact-grinding and (ii) density separation of germ and endosperm particles by using concentrated sodium nitrate solutions. In this investigation we used transgenic corn expressing rGUS enzyme as a model system to evaluate the effect of the proposed dry-milling and density fractionation methods on (i) the rGUS concentration in each of the fractions, (ii) the effect of the proposed fractionation methods on the extractable enzyme activity, and (iii) the overall rGUS yield.

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MATERIALS AND METHODS

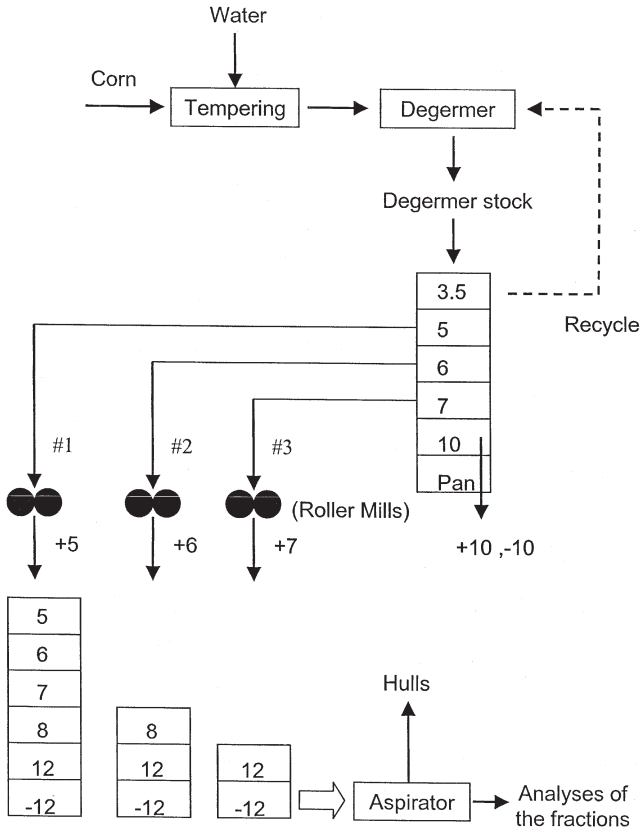
Raw materials. Two different lots of transgenic corn containing recombinant rGUS were used in this study. The first lot (ZS95) was provided by Pioneer Hi-Bred International (Johnston, IA), and the second (NE98KK) was supplied by ProdiGene, Inc. (College Station, TX). The two lots were derived from the same rGUS event, but from the different production years 1995 and 1998, respectively. The average extractable rGUS activity from ZS95 and NE98KK was 1494 ± 192 and 2840 ± 312 units/g of fat-free dry solids, respectively. A twofold improvement of rGUS expression measured in the seed produced in 1998 was a result of the standard transgenic corn selection (Delaney, D., personal communication). Transgenic corn ZS95 was kept in a refrigerated storage room (10ºC) for approximately 1 yr before use in the dry fractionation study. The NE98KK corn, which was used in the flotation experiments, was produced in the fall of 1998, stored at 10°C, and used in the spring of 1999. No change of rGUS activity was detected for either lot during prolonged storage. The average moisture and crude fat contents as measured with a grain analyzer (Grainspec, Eden, MN) were 13% (wet solids) and 3.8% (dry solids), respectively.

Dry-milling of transgenic corn. The process-flow diagram of the dry-milling process developed at the Center for Crops Utilization Research, Iowa State University, Ames, is illustrated in Scheme 1.

Tempering and degerming. Five to seven samples (1 kg) were drawn from different locations in the 150-kg drum containing transgenic corn. The samples were combined, mixed well, divided into 1-kg subsamples, and kept in sealable Ziploc® bags.

To determine the optimal tempering conditions, one-step tempering to a final moisture content of 17, 21, and 24% (w/w) was first investigated (9). Distilled water was sprayed onto the corn samples to bring the moisture content to the desired level. The plastic bags were stored at ambient temperature and were occasionally mixed until the corn completely absorbed the surface moisture. At the end of the 2-h tempering period, the corn was degermed within 20 min by using a horizontal drum impact rotor degermer/dehuller at a constant feed rate of 3.5 kg/h and rotational speed of 1800 rpm. To prevent moisture loss, the degermer stock was kept in a plastic bag between processing steps. The oil recovery in the germ-rich fractions (+5, +6, +7) and the weight of the recycle fraction (+3.5) were utilized as an index of the dry-milling efficiency. The corn tempered to 17% moisture had the worst dry-milling performance; it resulted in the greatest recycle (+3.5 fraction) weight and lowest oil yield from the germ-rich fractions. Because the dry-milling performances of transgenic corn samples tempered to 21 and 24% moisture content were similar and significantly better than those at 17% moisture, one-step tempering to 21% final moisture with 2 h of incubation was used in this study.

First fractionation. Degermer stock was mixed well and three subset samples of equal size were screened for 10 min using a rotary-screen shaker (Ro-Tap; W.S. Tyler, Inc., Mentor, OH) with U.S. standard sieves (3.5, 5, 6, 7, 10, and Pan)



SCHEME 1

(Scheme 1). To determine the germ distribution among the fractions, each fraction was analyzed for moisture content, oil content, and rGUS activity. From the oil content of each fraction it was determined that the majority of the germ was retained on sieves 5, 6, and 7. Fraction +3.5 contained mostly unbroken and partially broken kernels and hulls. Fraction +3.5 and particles that passed through sieve 7 (–7 fraction), containing small grits and flour, were not further processed.

Roller milling and second fractionation. To further break endosperm particles and to remove endosperm fragments that remained attached to the germ, three pairs of corrugated roller mills (Witt Corrugating, Inc., Wichita, KS) were used to grind fractions +5, +6, and +7 (Scheme 1). The gaps of the roller mills were set at 0.24, 0.21, and 0.21 in. for mill 1, 2, and 3, respectively (Scheme 1). The outflow from the roller mills was fractionated as before into subfractions by using the rotary screen shaker (Ro-Tap). Each subfraction was aspirated by a lab aspirator (Kice Industries, Carter-Day Co., Minneapolis, MN) to remove the hulls.

Germ separation by flotation. Germ separation from the degermer stock by flotation is illustrated in Scheme 2. Transgenic corn NE98KK samples (3–4 kg) without tempering were broken using an impact-type degermer (Entoleter Inc., Hamden, CT) at a feed rate of 1640 kg/h and 2600 rpm. Sodium nitrate solution at a specific gravity (sp gr) of 1.15 or 1.3 was prepared in a measuring cylinder by using tap water and a hydrometer. Aliquots of 500 g were taken from the degermer stock, fed into the cylinder with sodium nitrate solution, and thoroughly stirred for about 1 min. Germ particles

that floated to the surface were skimmed and passed through a wire screen. The remaining suspension containing mainly endosperm particles was decanted through a wire screen into another container. Germ and endosperm fractions were rinsed with water for a few seconds, and the screens were set aside for less than 1 min until dripping stopped. The samples were analyzed the same day for rGUS activity. The flotation procedure was repeated three times at each specific gravity.

Preparation of aqueous extracts. The various samples designated for rGUS and soluble protein analysis were milled using a Retsch mill (Brinkmann Instruments, Karlsruhe, Germany) equipped with a 0.5-mm screen. Two grams of milled sample was extracted with 20 mL sodium phosphate buffer, pH 7.5, by stirring with a paddle mixer for 20 min at 600 rpm. To eliminate the possibility of sample overheating during milling and to reduce sample cross-contamination, we changed the protein extraction method to aqueous homogenization. Dry-milled corn samples (8–10 g) were mixed with 50 mM sodium phosphate buffer (pH 7.5) at a 1:10 ratio, and the slurry was continuously homogenized (Ultra-Turrax T25; Janke & Kunkel, Staufen, Germany) for 1 min at 13,500 rpm and ambient temperature. The slurry was centrifuged at 13,500 g for 25 min at 4°C, and the supernatant clarified by filtration through two layers of cheesecloth. The supernatant was then assayed for rGUS activity, total soluble protein, and rGUS content by ELISA. The data reported in Tables 1 and 2 were generated by using the paddle mixer, and those in Tables 3 and 4 by slurry homogenization. No significant difference between the two methods was observed, and the switch to homogenization was made mainly for convenience (i.e., faster rGUS activity and protein content analysis).

Analysis of aqueous extracts. For analysis of enzyme activity, the initial reaction rate was determined by measuring an increase in absorbance at 405 nm resulting from the hydrolysis of *p*-nitrophenyl-β-D-glucuronide (Sigma N-1627) to *p*-nitrophenol. One unit (U) of enzyme activity was defined as the amount of enzyme that liberated 1.0 nmol *p*-nitrophenol/min at 37°C and pH 7.0 in the presence of 1 mM EDTA (Fisher Scientific, Philadelphia, PA) and 10 mM mercaptoethanol (Sigma M-6250) in the reaction buffer (8). A spectrophotometer was programmed to calculate the enzyme activity based on the following formula:

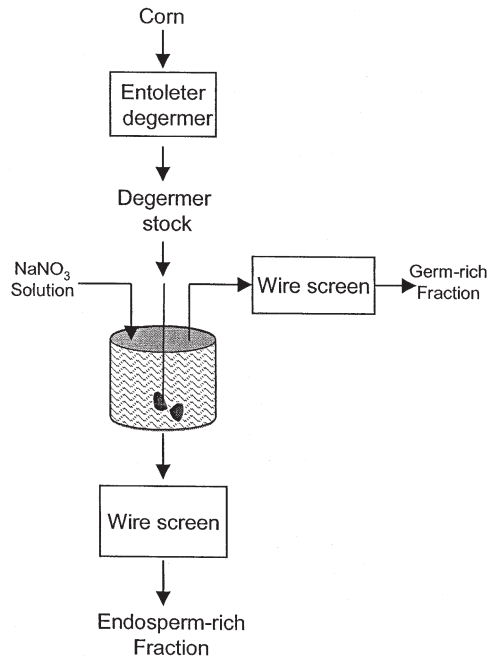
$$\text{U/mL} = \Delta A_{405\ \text{nm}}/\text{min} \times 1000/18 \times \text{total volume (mL)/sample volume (mL)} \times \text{dilution factor} \quad [1]$$

A molar extinction coefficient for *p*-nitrophenol at 405 nm of 18,000 M^{-1} cm^{-1} was used.

Total soluble protein in aqueous extracts was determined using a Coomassie Blue protein analysis kit (Pierce, Rockford, IL).

For quantification of rGUS in the extracts, sandwich-type ELISA was used. Polyclonal antibodies supplied by ProdiGene Inc. as a crude serum, were purified in our laboratory by using affinity chromatography. The primary antibody was from sheep anti-GUS antibodies from a 12/12/95 bleed; the secondary antibody was from rabbit serum from a 11/23/95 bleed. Anti-rabbit alkaline phosphatase (Boehringer-Mannheim, Indianapolis, IN) was the enzyme-attached antibody used as conjugate. Commercial GUS (Sigma G-8271) was utilized as antigen to construct the standard curve. The assay was conducted in 96-well microplates (Nunc Maxisorp; Fisher Scientific) and a microplate reader recorded the absorbance (Model 550, Bio-Rad Laboratories, Hercules, CA).

Oil and moisture analysis. A Goldfisch apparatus was used to determine the oil content of ground samples by using petroleum ether "B" according to AACC Method 30-26 (10). The amount of extracted oil was measured gravimetrically. The moisture content of the various fractions and subfractions was measured according to AACC Method 44-15A (10).



SCHEME 2

RESULTS AND DISCUSSION

Table 1 summarizes the oil content, rGUS activity, and total soluble protein of each fraction obtained after fractionating the ZS95 degermer stock. Almost 70% of rGUS activity was found in fractions +5, +6, and +7, and 20% in unbroken and partially broken kernels (recycle stream +3.5), which would be returned to the degermer. The amounts of oil and water-soluble protein in the same fractions were about 62% each, with 25% oil and 30% soluble protein remaining in fraction +3.5. The rGUS activity, water-soluble protein, and oil contents in the fractions retained by sieves 5, 6, and 7 were positively correlated, because the majority of water-soluble proteins in the kernel, rGUS, and corn oil were located in the transgenic germ (8,11). The data suggest that fractions from sieves 5, 6, and 7 warrant further processing, as illustrated in Scheme 1, to increase the purities of the germ-rich fractions and the average rGUS concentration in fat-free dry solids. Large standard errors for activity and total protein (Table 1)

TABLE 1
Compositions of Degermer Stock Fractions from ZS95 Corn[a]

Degermer stock fraction	Weight (% ds)	Oil content (% ds)	Total oil (%)	rGUS activity (U/g-ffds × 10^{-3})[b]	Total activity (%)	Soluble protein (mg/g-ffds)[c]	Total soluble protein (%)
+3.5	15.6	6.1	24.9	2.2 ± 0.5	20.1	13.3 ± 4.8	30
+5	34.5	4.3	39.2	2.0 ± 0.3	41.6	6.9 ± 1.6	35
+6	23.3	3.1	19.1	1.6 ± 0.01	22.4	6.9 ± 1.0	23
+7	7.7	1.6	3.2	1.0 ± 0.06	4.9	3.7 ± 0.3	4
+10	4.3	1.5	1.7	0.5 ± 0.1	1.4	1.6 ± 0.2	1
−10	8.2	1.7	4.1	0.4 ± 0.02	2.5	3.7 ± 0.1	5
Hulls	6.2	3.0	4.9	0.4 ± 0.08	1.4	4.0 ± 0.7	3
Total	99.0		97.2		94.3		101

[a]Abbreviations: ds, dry solids; ffds, fat-free dry solids.
[b]Units/g fat-free dry solids.
[c]mg/g fat-free dry solids.

reflect the interference of corn extractables as well as the additional error introduced by expressing these values on a fat-free dry basis. Fractions +3.5, +5, and +6 have similar specific activities, whereas fraction +7 contains almost twofold lower GUS concentration than the top three fractions. The hulls and smaller particle fractions that passed through sieve 7 during the first fractionation (i.e., +10 and −10) were excluded from further processing for two reasons: first, they contained less than 6% of total rGUS; second, they had significantly lower GUS concentrations than the other fractions. Although fraction +7 could have also been excluded from further processing at the expense of 5% GUS loss, we decided to further process it to determine if roller milling and second fractionation could yield an enriched rGUS fraction.

Table 2 summarizes weight and component balance of various subfractions obtained after the roller-milling and second sifting of fractions +5, +6, and +7 (Scheme 1). Subfraction weights, oil content, and protein content have been added and expressed as subtotals in Table 2. The total weight of all subfractions was within 2% of the original weight of fractions +5, +6, and +7. The subtotal oil contents of subfractions that originated from fractions +5, +6, and +7 were 92, 114, and 102%, respectively. Likewise, soluble protein subtotals were 97, 121, and 125% of the protein content of fractions +5, +6, and +7, respectively.

Total rGUS activity of all subfractions was 80% of the fractions that entered the roller milling. Specifically, the activity subtotals of subfractions from +5, +6, and +7, accounted for 72, 91, and 90% of the respective activity before the roller milling. We have continuously encountered an underestimation of subfraction activities but not of the +5, +6, and +7 fractions. Our attempts to determine what caused this discrepancy did not yield conclusive results (9). We investi-

TABLE 2
Oil, Protein, and Enzyme Activity Contents of Subfractions[a]

Fraction/ subfraction	Weight (% ds)	Oil content (% ds)	Total oil (%)	rGUS activity (U/g-ffds × 10^{-3})[b]	Total activity (%)	Soluble protein (mg/g-ffds)[c]	Total soluble protein (%)
+5							
+5	3.0	21.4	16.7	8.2	12.0	34.4	12
+6	1.2	14.2	4.5	7.5	4.9	25.9	5
+7	1.6	7.5	3.2	3.4	3.2	14.2	3
+8	4.4	3.0	3.5	1.6	4.2	6.7	4
+12	12.6	1.4	4.6	0.5	3.9	3.5	7
−12	9.4	1.4	3.5	0.3	2.0	1.9	3
Subtotal	32.2		36.1		30.2		34
+6							
+8	2.2	22.8	13.3	10.9	11.7	46.3	16
+12	3.4	3.6	3.2	2.9	6.0	12.6	7
−12	16.8	1.2	5.4	0.3	2.7	2.4	5
Subtotal	22.4		21.9		20.4		28
+7							
+12	1.4	6.5	2.4	4.1	3.4	13.5	3
−12	6.4	1.0	1.7	0.3	1.0	1.9	2
Subtotal	7.8		4.1		4.4		5
Total	65.6		62.3		68.9		62

[a]See Table 1 for abbreviations.
[b]Units/g fat-free dry solids.
[c]mg/g fat-free dry solids.

gated the effect of tempering, temperature, and the inhibition of GUS activity spiked into germ and endosperm extracts. Spiking of purified GUS from *Escherichia coli* (Sigma) to whole corn extracts (9) and the adsorption chromatography of GUS-corn extracts on ion-exchange resin (8) indicated the enzyme inhibition in the corn extracts that accounted for as much as 25% of the original activity. At low GUS concentrations, nontransgenic germ extract seemed to exhibit a greater inhibition of the spiked enzyme than did the endosperm extract (9). None of the other factors investigated significantly affected the GUS activity. By using ELISA, the GUS concentration estimates in the subfractions increased by 5–10% but still underestimated the overall initial activity.

The activity data of the subfractions given in Table 2 indicated that after the second fractionation, subfractions +5/+5, +5/+6, +5/+7, +6/+8, and +7/+12 had greater GUS concentrations (U/g-ffds) suggesting greater purity of the germ-rich fractions. The overall conclusion from the dry-milling process is that by combining the above subfractions, 35% of the initial rGUS activity and 40% of the oil can be captured in 9.4% of the original kernel weight. Adding the contribution of weight and activity from degerming the recycled +3.5 fraction would amount to approximately 49% GUS activity and 64% oil in 17% of the original kernel weight.

The dry-milling experiment was repeated about a year later with the higher-expressing GUS corn (NE98KK) using the same tempering and degerming conditions. In this experiment, fraction +3.5 was recycled to the degermer, processed, and then added to the rest of the degermer stock before the first fractionation (Scheme 1). After the first fractionation, fractions +5, +6, and +7 contained 83 and 81% of the initial rGUS as estimated by activity and ELISA assay, respectively (data not shown). After the roller milling, the recovered activity in the subfractions was 66% of the initial rGUS. The GUS recovery measured by ELISA was about 10% higher (77%) than that based on the activity. In any case, rGUS distribution among the fractions and subfractions and its recovery were similar to those from the previous experiment, which used ZS95 corn.

Based on the oil content and estimated activities in the different fractions and subfractions, we formed three pools simulating the streams that would be collected in a dry-milling plant (Table 3). Our objective was to generate a germ-rich pool with high specific activity (U/g-ffds) to be used for recombinant protein production and an endosperm-rich pool for ethanol production. The hulls, depending on the residual starch content, could be combined with the endosperm-rich pool or used directly as an animal feed ingredient. Fractions +10 and −10 and the various subfractions of +5, +6, and +7, which contained more than 3% oil, were pooled into a germ-rich fraction. Hulls, tip caps, and (−12) subfractions were pooled into a hull-rich fraction, and the rest into an endosperm-rich fraction. Because our major interest in this study was rGUS recovery, pooled fractions were analyzed for rGUS content by both activity and ELISA (Table 3). The germ-rich fraction accounted for 57% of the total extractable rGUS activity. The endosperm-rich fraction and the hulls yielded 19 and 2% of the total extractable activity, respectively. ELISA revealed that 63% of the total rGUS was located in the germ-rich fraction, 21% in the endosperm-rich fraction, and 6% in the hulls fraction. The oil recovery in the germ-rich fraction at 65% again correlated well with that of rGUS (data not shown) and, therefore, could serve as an additional measure of the effectiveness of the dry-milling process and germ purity. Overall, the total extractable activity was 78% based on activity assay and 90% based on ELISA (Table 3). Again, ELISA seems to give more accurate estimates of rGUS in the extracts than the enzymatic assay. Nevertheless, we still prefer using the activity assay because it measures the active fraction of the enzyme.

Germ separation by flotation. The objective of this set of experiments was to establish whether a two-step processing method consisting of breaking nontempered corn by a degermer and separating the degermer stock by density would yield a viable method for recovering transgenic germ (Scheme 2). The idea originated after finding that rGUS activity was positively correlated to oil (germ) recovery; thus, any method that would lead to germ enrichment should theoretically yield an enriched enzyme fraction, assuming that there is no adverse effect on activity from the flotation medium. Density separation of corn germ with hydroclones is currently practiced in the corn wet-milling industry (12), whereas germ flotation in concentrated sodium nitrate solutions has often been used as an analytical tool to evaluate dry-milling performance and product purity (13,14). Our main concern in this study was the stability and possible leaching of rGUS into the aqueous salt solution during flotation. Table 4 summarizes the weight, oil content, and rGUS activity of the germ- and endosperm-rich fractions. When the sodium nitrate solution with 1.15 sp gr

TABLE 3
rGUS Activities and Concentrations in the Pooled Fractions[a]

		Activity assay		ELISA		Soluble protein	
Fraction	Weight (% ds)	rGUS activity (U/g-ffds) (10^{-3})[b]	Total activity (%)	rGUS (μg/g-ffds)	Total rGUS (%)	Soluble protein (mg/g-ffds)	Total soluble protein (%)
Germ-rich	21	8.4 ± 1.0	57	105 ± 17	63	3.10 ± 0.40	72
Endosperm-rich	71	0.7 ± 0.2	19	10 ± 2	21	0.25 ± 0.03	24
Hulls	8	0.8 ± 0.1	2	22 ± 3	6	0.79 ± 0.07	5
Total	100		78		90		101

[a]See Table 1 for abbreviations.
[b]Units/g fat-free dry solids.

TABLE 4
Germ and Endosperm Separation by Flotation (NE98 KK corn)[a,b]

Specific gravity[c]	Fraction	Weight (%)	rGUS activity (U/g-ffds × 10^{-3})[d]	Total activity (%)	Oil content (% ds)	Total oil (%)
1.15	Germ-rich	14	15.5 ± 1.1	73	16.0 ± 0.0	71
	Endosperm-rich	83	0.7 ± 0.1	26	0.9 ± 0.1	23
	Total	97		99		94
1.30	Germ-rich	20	11.7 ± 0.4	80	12.5 ± 0.3	77
	Endosperm-rich	77	0.4 ± 0.2	12	0.7 ± 0.3	18
	Total	97		92		95

[a]See Table 1 for abbreviations.
[b]Mean of two runs at each level specific gravity.
[c]Specific gravity of sodium nitrate solution.
[d]Units/g of fat-free dry solids.

was utilized to float the oil-containing particles, the total weight of the germ-rich fraction was 14% on a dry basis. The total weight of the same fraction increased to 20% when 1.30 sp gr solution was used to float the lighter germ fraction. The total oil and enzyme yield also increased from 71 to 77% and from 73 to 80%, respectively. The oil and rGUS activity showed the opposite trend to specific gravity: The oil content of the germ-rich fraction dropped from 16.0 to 12.5% and specific enzyme activity from 15.5 to 11.5 (U/g-ffds) when the specific gravity of the solution increased from 1.15 to 1.30. The oil content and specific activity of rGUS (U/g-ffds) indicated that the greater total oil and rGUS yields were achieved at the expense of purity. It is important to note that neither activity nor oil balance was significantly affected by flotation. The activity balance was 99 and 92%, and that of the oil was 94 and 95%. Both estimates were slightly lower than theoretical and, considering 97% weight yield, were still within the range of the experimental error (Table 4).

Density separation seemed to be a higher-yielding and simpler method for rGUS recovery in the germ-rich fraction than dry-milling. For example, approximately 20% of dry weight contained 80% of the original activity compared with 57 or 63% (ELISA) activity recovered from the pooled dry-milled fractions (Tables 3 and 4). More important, the germ-rich fraction obtained by flotation had greater purity as demonstrated by the specific activity of the respective germ-rich fractions of 8.4 (U/g-ffds) (Table 3) vs. 11.5 (U/g-ffds) (Table 4). Our data suggest that density separation using hydroclones could be a feasible method for fractioning degermer stock. rGUS is a rather stable and large intracellular protein (270 kDa) that, as we have demonstrated, does not leach out from the germ during flotation. The applicability of this method to other recombinant enzymes should be tested before application. Some of the considerations should include tissue and subcellular localization, protein size, protein stability, contact time, and temperature of solution.

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