J Am Oil Chem Soc (2009) 86:927–932
DOI 10.1007/s11746-009-1429-9

ORIGINAL PAPER

Foam Separation of Oil from Enzymatically Treated Wet-Milled Corn Germ Dispersions

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Received: 9 January 2009 / Revised: 14 May 2009 / Accepted: 13 June 2009 / Published online: 3 July 2009

Abstract More than 9 billion gallons of ethanol were produced in 2008, mostly from dry grind corn fermentation plants. These plants are a potential source of substantial amounts of corn oil, if an economical method of separating it can be developed. In this work, oil was separated from corn germ by aqueous enzymatic extraction (AEE). Batches of wet-milled corn germ in water were preheated in a pressure cooker, ground in a colloid mill, and churned in a vertical column/mixing vessel system, after the addition of enzyme. Nitrogen gas was then bubbled through the column removing an overflowing foam fraction which was subsequently centrifuged to separate free oil. Using a newly commercialized enzyme complex it was found that 80% of the oil could be recovered using a w/w ratio of enzyme solution to germ of 1:80. The low dose and low price of the enzyme complex leads to a cost estimate of AEE of corn oil from germ, similar to the wet-milled germ extracted, cost competitive with expelled oil (with the separation and drying of the foam protein), and feasible for commercialization in a dry grind plant retrofitted to separate germ.

Keywords Aqueous enzymatic extraction · Corn germ · Dry grind modifications · Foam separation · Oil-seed processing

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Introduction

Corn to ethanol plants are financially beset by rising corn prices that often outpace increases in the price of ethanol. Dry grind processes that produce a single co-product, distillers dried grains with solubles (DDGS), are being reconsidered. If corn germ and other DDGS components can be separated and sold at the higher prices driven by the same forces that raise corn prices, it may be worth the separation cost to retrofit dry grind plants to recover them. If 1.0 kg of oil could be recovered from each bushel of corn converted by dry grind plants producing 7.5 billion gallons of ethanol (close to the current output) then 400 million gallons of oil could be recovered. Processes have been proposed to separate corn germ prior to fermentation in a dry grind ethanol plant. Quick germ [1] and enzymatically milled germ [2] have been produced in laboratory quantities with 30 and 39% oil, respectively. Oil is currently removed from dry-milled germ or wet-milled germ by crushing for 35–45 $/ton of germ or by hexane extraction for 20–40 $/ton [3]. These costs, as well as the capital to build an oil recovery facility onsite have been significant hurdles to separating corn germ, and then oil, from dry grind ethanol plants. The unrefined product oil was worth about $0.35 lb^{-1} on October 2008 [4]. Oil yields of 65 wt.% were recovered from wet-milled or dry-milled germ by laboratory scale pressing [5], press cake from commercial-scale full pressing of corn germ has 6% residual oil, which for germ with 40% oil corresponds to an oil yield of 91% [6] and 72% from wet-milled germ by aqueous enzymatic extraction (AEE) using cellulase [7]. More recently wet-milled corn germ AEE was carried out using a bubble column to remove most of the oil, by centrifuging a (foam) fraction of the aqueous dispersion [8] and proteases were used to extract oil from extruded

soybean flakes [9]. A major component of AEE cost has been the cost of the enzyme. A recent study of AEE of rapeseed indicated an optimum (oil recovery) enzyme solution-to-seed v/w ratio of 1:25, in two stages [10], and a 1:10 w/w ratio of cellulase solution to corn germ was found to be optimal based on trials with several candidate enzymes [11]. Recently the enzyme manufacturer released a less expensive cellulase complex intended for biomass hydrolysis prior to fermentation. We expected that use of this new enzyme complex might significantly reduce AEE cost. Our experiments were designed to determine the lowest effective dose of the new enzyme complex and whether its use would impair transfer of oil in the germ dispersion to a foam fraction, because of enzymatic degradation of foam stabilizing compounds in the dispersion.

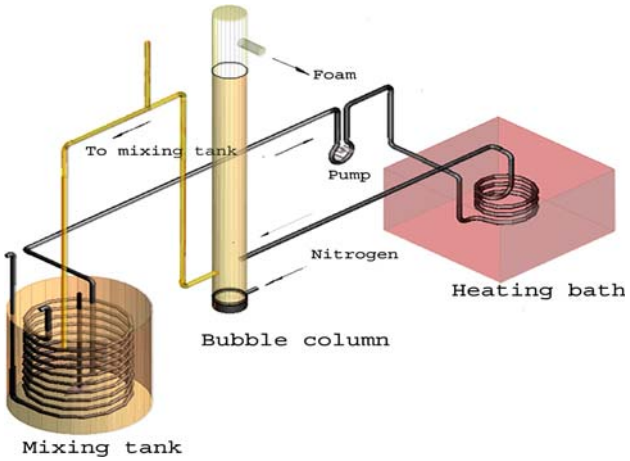

Fig. 1 Diagram of equipment

Materials and Methods

Materials

Samples of wet-milled corn germ (dried at the mill to about 3% moisture), hereinafter called germ, were obtained from a commercial mill. Based on the total oil and protein yield of the products the germ contained 42.4 ± 2.96% oil and 10.72 ± 0.65% protein. The germ used was stored in a sealed drum at 4 °C and, on the day of the extraction, the material to be extracted was removed from storage and allowed to equilibrate to room temperature (22 °C), before extraction. Bottles of Accellerase™ 1,000 cellulase complex (Genencor International) were stored in a refrigerator and the amount for each extraction taken as needed from the appropriate bottle. The complex contains multiple enzyme activities; mainly exoglucanase, endoglucanase, hemi-cellulase and beta-glucosidase derived from a genetically modified strain of *Trichoderma reesei*. The endoglucanase activity per Genecor is 2,500 CMC units/g and beta-glucosidase activity 400 pNPG units/g.

Dispersion Preparation, Separation and Free Oil Separation

A dispersion of 800 g of germ in either 8 kg of (3 wt.%) acetate buffer (pH 4.1) or 8 kg of water was cooked at 2 atm (122 °C) in a pressure cooker for 20 min. It was weighed, ground in a colloid mill (Eppenbach 4535, Long Island City, NY) set at its narrowest blade/stator clearance, for 3 min. It was then cooled for 30 min, and approximately 160 mL of buffer or water added to bring the dispersion mass back to its original 8.8 kg. Accellerase™ 1,000 complex solution was added to the dispersion and it was mixed in a 20-L stirred polypropylene pail (mixing tank, Fig. 1) at 50 °C for 24 h. The bubble column shown in Figure 1 consisted mainly of a vertical cylindrical acrylic tube of 7.0 cm i.d. and 1.16 m height mounted above an 8-cm ceramic disk with a pore size of 10–16 μm (42% pore volume). The pressure differential across the disk was 10 mbar. Nitrogen gas was admitted from a continuous supply through a parallel bank of seven independently adjustable 0.47 L/min maximum rotameter/flow controllers. The flow rate was adjusted from an initial low rate of 0.235 L/min to enough to keep the liquid height 2–3 cm below the foam outlet port.

Buoyant drops, droplets and particles were carried out of the bubble column with foam which flowed from the bubble column through the port shown in Figure 1. The collected foam was centrifuged after about 200 g was collected which was a convenient volume for filling the centrifuge bottles. After centrifugation to settle any solid particles, the collected collapsed foam was frozen and the free oil separated by scraping from the top of the frozen ice. The oil was then lyophilized weighed and analyzed as described in [5]. The centrate was dried by evaporation over a hot plate, then lyophilized and analyzed. Free oil was the oil that could be obtained by centrifugation, freezing and lyophilization. Fines oil was oil in the aqueous fraction of the foam that was identified from solvent extraction of the dried aqueous and solid particle fractions of the foam. The same definitions (free oil/fines oil) applied to the non-foam fraction of the dispersion that remained in the system after the foaming ceased.

A diagram of the equipment used is shown in Figure 1.

Estimation of the Molecular Weight of Foam Protein, Gel Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein was carried out on a Phast System Pharmacia (Piscataway, NJ) with a Phast gel of 20 wt.%

acrylamide. A foam sample from which the free oil was removed by the process described [8] and which contained 1.9 wt.% protein and 3.5 wt.% oil was concentrated by evaporation to a concentration of 6 wt.% protein. It was then diluted with seven parts of the protein-solvent system (0.44 M Tris, 1 mM EDTA, 10 wt.% SDS, pH 8.0), centrifuged at 14,000 × *g*, 5 min and the clear supernatant analyzed. Gels were stained with 0.2% (w/v) Coomassie R350 dye and molecular weights of protein bands were determined by comparison with molecular weight standards (Invitrogen Corp., Carlsbad, CA).

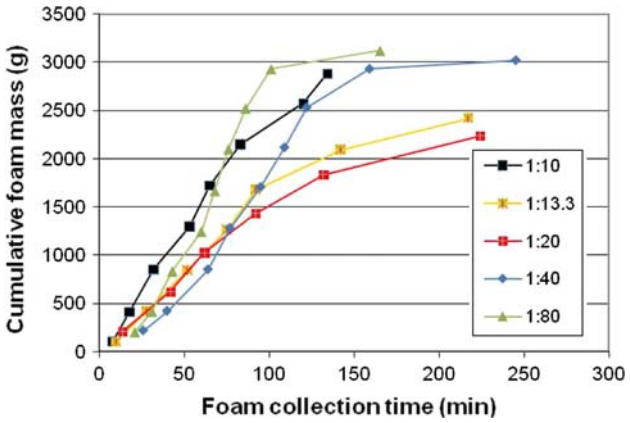

Fig. 2 Effect of enzyme complex: germ mass ratio on foam accumulation

Results and Discussion

Previous experiments in which oil was extracted using AEE from 300 to 400 g batches of wet-milled corn germ recoveries of 80% of the oil were obtained, mostly as free oil, used an enzyme solution to germ mass ratio of 1:10 [7]. Lower ratios could not be ground to a smooth dispersion in the colloid mill. Initial extractions using Accellerase™ 1,000 complex solutions and Multifect GC, a cellulase used in prior AEE, showed that better yields were obtained with the new complex (shown by comparing the first two lines of Table 1). The oil was centrifugally separated from the entire churned dispersion in the prior work, whereas only the foam fractions were centrifuged in the separations reported here. To use the foam method and reduce the mass that must be centrifuged requires that the enzyme not degrade the foam stabilizing components in the germ to the extent that foam collection is impaired significantly.

Foam collection is influenced by the enzyme, as shown in Figure 2, and the foam yields shown in Table 1 indicate that lower enzyme doses led to collecting greater amounts of foam; however no foam was produced when no enzyme was used. It appears that a relatively small amount of enzyme (1:80 for example) releases enough foam stabilizer to support foam. The variation of foam yield with enzyme:germ ratio suggests that effective stabilizer availability may be the sum of opposed enzyme influences (1) release from the germ and (2) attack and reduction of the stabilizing capability on released stabilizer. Thus foam yield drops as the ratio increases from the minimum, until 1:20, where the increase in stabilizer release overpowers the enzymatic degradation of the released stabilizer. An alternative hypothesis has been suggested: buffer masked the beneficial influence of lowering enzyme dose at the higher doses and its removal allowed the improvement in foam yield seen at lower enzyme doses. The runs began with 1:10 and the dose was reduced until a minimum was reached; buffer addition was eliminated after the 1:20 run showed it was unnecessary. The absence of foam formation, without any enzyme, indicates that enzyme is needed to release stabilizer from the germ.

The table shows that addition of buffer to control pH did not improve oil yield. Without buffer, which had been used in earlier extractions, the pH stayed within the range of 4.1–4.2 and the projected process cost of acetate buffer use would exceed the enzyme cost at its new low dose.

The change in the slope of the yields plotted in Figure 3 admit of several explanations: (1) a change in the foam structure, as a result of foam stabilizer depletion in the initial solution, (2) depletion of the free oil droplets, (3) depletion of the free oil droplets in the column above the port connecting the column to the mixing tank. None of the explanations can be ruled out but the gradual nature of the slope change seems to indicate (3) a drop in free oil collection limited by depletion of the foam stabilizer in the

Table 1 Free oil yield produced from 800 g of wet-milled germ using different amounts of enzyme solution and buffer solution (or water)

Enzyme complex (g)	Acetate buffer (%)	Foam fraction (g)	Non-foam fraction (g)	Total free oil (g)
Multifect GC (80)	3	197	3.9	200.9
Accellerase (80)	3	240.2	22.1	262.4
Accellerase (60)	3	248.6	15.8	264.4
Accellerase (40)	0	216.7	44.9	261.6
Accellerase (20)	0	242.5	12.5	255.0
Accellerase (10)	0	270.2	0.2	270.4
Accellerase (0)	0	0	0	0

A mass ratio of 10:1 buffer solution or water to germ was used

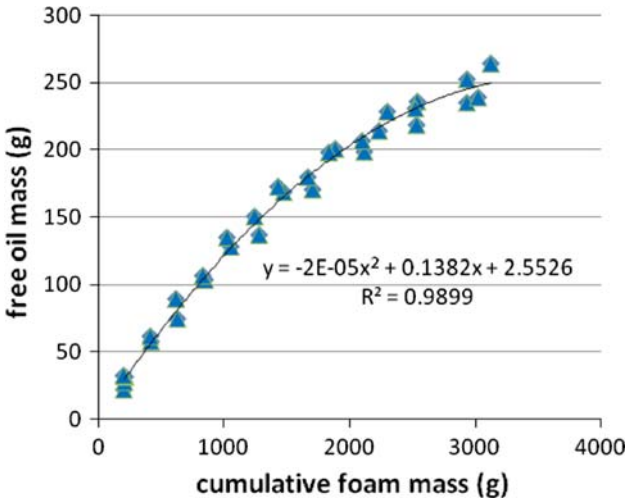

Fig. 3 Cumulative free oil obtained from collected foam, enzyme complex to germ mass ratio from 1:13 to 1:80; R^2 fit for early data = 0.9735, R^2 fit for later data = 0.885

dispersion in the mixing tank. The linearity of the samples taken from the first 1,200 g of foam suggests that the foam was saturated with oil up to that point but thereafter lower foam stabilizer concentrations produced visibly thinner foam with larger bubbles, which did not transport as much oil.

The plots of foam oil in Figure 4 show that, for the three higher enzyme dose runs, the free oil content rose for samples corresponding to lower cumulative foam mass before declining. For the two lowest enzyme dose runs, the oil content declined monotonically. These trends are consistent with enzyme, for the highest enzyme dose run (1:10), producing so much stabilizer that the stabilizer molecules aggregated and limited oil droplet numbers on the bubbles' surfaces. As the stabilizer was depleted by foam collection, less aggregation allowed more oil to be collected on the bubbles' surfaces raising the oil content of the foam. When the oil droplet number in the column dropped to the point that the oil on the bubbles was no longer limited by surface availability the oil content of the foam dropped. For runs with lower enzyme doses, less stabilizing compound was released so there was less aggregation and the highest yield point for the curves in Figure 4 was higher. The initial rising segment of the curve-corresponding to a purge of the aggregated stabilizing compound was shorter, since there was less of it. At the lower enzyme dose runs, there was no stabilizer aggregation so there were no initial rises for these two curves.

The higher free oil contents of the lowest enzyme dose runs were accompanied by high solute and fine particle content as shown in Figure 5, despite the small differences in foam collection rates shown in Figure 2.

In order to estimate the effect of the initial solution concentration on the separation process, we calculated ratios of the foam and solution compositions (averages of before- and after-foam sample collection) from which the foam had originated. Concentration factor (CF) was defined as the ratio of the solute and fine particle concentration (not including free oil or large particles removed by centrifugation) in the foam to the solute and particle concentration in the initial dispersion. Showing the ratio of concentrations rather than amounts of solute in the two phases separately gives a picture of the filtering capability of the foam and its trend as the dispersion changes with foam removal. Figure 5 shows that CF increased with decreased enzyme dose suggesting that the higher enzyme doses reduced the foam's ability to preferentially retain the solute enriched solution that stabilized the foam structure. Initial foam samples, for each run, were composed of fine bubbles and thus had a higher solute mass fraction close to

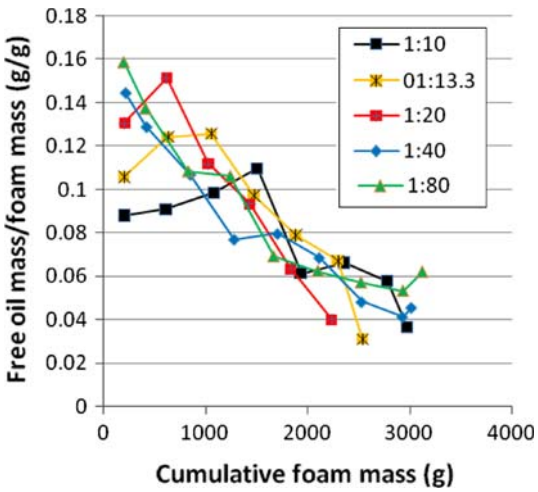

Fig. 4 Free oil fraction of foam samples, enzyme complex to germ mass ratio from 1:13 to 1:80

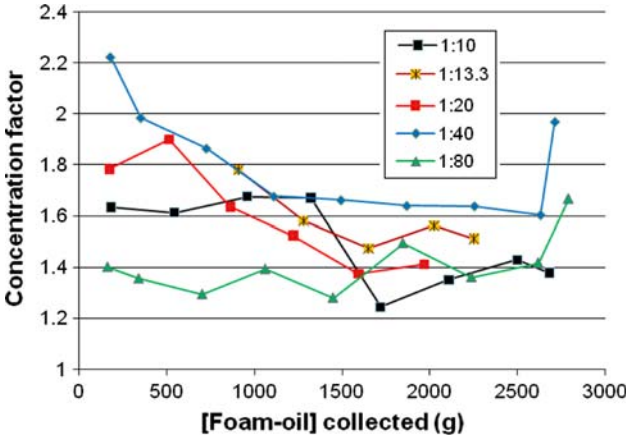

Fig. 5 Concentration factor, enzyme complex to germ mass ratio from 1:10 to 1:80; Concentration factor is the solute and fines concentration in foam (not including free oil)/solutes and fines concentration in the centrate of the dispersion left after foaming

the bubbles whose film would have contained foam stabilizing compounds (a fraction of the solutes). Later foam samples were comprised of larger bubbles and were less stable, possibly due to reduction of foam stabilizer. The CF of all samples exceeded one, and therefore foam stabilizing compounds in the dispersion batch were depleted as foaming continued. However there was not a marked drop in CF with foam collection (for the higher enzyme doses). This suggests that a pool of stabilizer—possibly in the mixing tank or transition from aggregated to single stabilizers in the foam layers, preserved the foam's CF despite depletion of components enriched in the foam. The scatter in CF values for a run (given enzyme dose) is probably controlled by the inaccuracy inherent in collecting samples from a dispersion of oil in water, with a wide range of oil droplet sizes.

A similar protein concentration factor, PCF, is the ratio of protein concentrations in the foam and in the corresponding dispersion. PCF variation with foam collection is shown in Figure 6.

The PCF values are about twice those of the CFs indicating that protein (or peptides) collected in the foam about twice as much as total solutes (everything left when water was evaporated from the de-oiled foam or dispersion samples and used to calculate CF), which would support the argument that some proteins are stabilizers or integral to the bubbles. The initial and final protein fractions of the foam and dispersions are listed in Table 2. The PCF rise for the later samples may be due to reduction in oil drops in the dispersion which had reduced foam stability by countering stabilizers at the bubble surfaces [12]. Earlier studies of foam concentration of soluble proteins, at about 10 × higher concentration than examined here, found PCF values in the range 1.5–2.5 [13]. Prior studies of beer foam showed that proteins coat the surfaces of the bubbles, stabilizing the foam [14]. Candidates to stabilize corn germ dispersion foam are enzymatically generated residues of lipid transfer proteins with molecular masses of 9 kDa (nsLTP1) and 7 kDa (nsLTP2) [15]. These residues may retain the preference for adsorption on hydrophobic interfaces noted for non-specific LTPs, which have a predominance of aliphatic residues lining the hydrophobic cavity in the intact protein [16]. Additional stability may result from other compounds which form bridges between adsorbed LTP residues [17]. Protein in a de-oiled corn germ foam sample was characterized by SDS-PAGE resulting in the identification of a band with a molecular weight range from 5 to 17 kDa. This band is consistent with a mixture comprised of slightly reduced nsLTPs and double aggregates. However, there are many proteins in germ, including zein and olesins, which have hydrophobic sequences that may act as foam stabilizers. It was suggested that oil may have been emulsified by surfactant proteins in the mixture during preparation of the dispersion. We agree that may have occurred. It could, perhaps, be verified by preparation using a device with less shear than the colloid mill we used.

Table 2 Protein fraction of aqueous liquid of foam and dispersions from which the foam was collected

Sample	Foam	Dispersion
1:13.3 initial	0.0185	0.0063
1:13.3 final	0.0169	0.0066
1:20 initial	0.0180	0.0083
1:20 final	0.0137	0.0060
1:40 initial	0.0190	0.0079
1:40 final	0.0149	0.0049
1:80 initial	0.0197	0.0074
1:80 final	0.0172	0.0042

The dispersion fraction was calculated by adding the foam samples' content to the final dispersion content. Free oil and centrifuge precipitated solids were subtracted from all contents

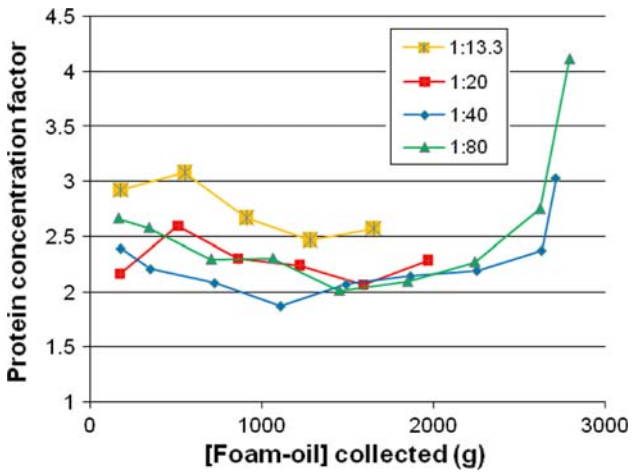

Fig. 6 Protein concentration factor, enzyme complex to germ mass ratio from 1:13 to 1:80; Protein concentration factor is the protein concentration in foam (not including free oil)/protein concentration in the centrate of the dispersion left after foaming

Using the enzyme complex to germ mass ratio, 1/40, and a cost of \$2.50 kg^{-1}, enzyme cost can be estimated for a model 40 million gallons/year dry grind plant retrofitted to separate germ and extract oil from the germ. Annual enzyme cost would be: 26.2 million × (1/80) × \$2.50 = \$0.82 million would extract oil from 3,033 kg of germ/h. If germ separated by new processes can be extracted as easily as the wet-milled germ used here, 80% of the oil can be recovered and 8.38 million kg of oil/360 d will be obtained with an enzyme cost of \$0.098 kg^{-1} of oil. Other process costs including plant labor, utilities and depreciation charges amount to about \$0.13 kg^{-1} of oil based on our preliminary cost estimate for a 3,033 kg/h germ fed process. The cost

advantage of foam separation is evident in this estimate; a bubble column is estimated to cost $25,000 and the remaining centrifuges $450,000 (this centrifuge cost is 0.28× that of the cost of centrifuges required to separate oil from the entire dispersion). The total cost, $0.23 kg^{-1} is higher than the cost estimated for expelling, $0.17–18 kg^{-1} [5]. Thus the AEE oil is about $50 ton^{-1} more expensive than expelled oil but AEE offers the opportunity, not available to expelling, to dry the centrate and create a protein-rich product more valuable than as a component of DDGS. The protein mass in the foam is about 15% of the free oil mass, so the value of the protein product would need to be ~$(50 ton^{-1})/0.15 or $0.33 kg^{-1} plus the cost of separation and drying, to compensate for the cost difference between oil by AEE and expelling. Last year corn gluten meal (60% protein) cost about $0.50 kg^{-1}. To calculate the cost of separating the germ from corn we assumed that the protein value of the germ was preserved and set the value of the products other than oil equal to the cost of the germ. Thus the cost of separating germ from corn in a retrofitted 40 MGY dry grind plant is estimated to be $0.224 kg^{-1} of germ; 45% of this cost is the reduced ethanol production at $2.00 gal^{-1}, assuming the same corn input. The principal uncertainty in the AEE cost estimate was germ grinding cost, which was based on a rate of 8,665 kg/h, 35% germ particles, and 48.5 kW of electric power, using a rotating plate mill, which is commonly used for corn milling.

Conclusions

The extractions described here show an optimum corn germ:enzyme mass ratio, to extract free oil from corn germ using AEE and Accellerase™ 1,000, near 80:1. This dose of enzyme greatly reduces the projected cost to separate oil from corn germ using AEE compared to prior estimates. The free oil separation cost, by AEE and foam, will be cost competitive with expelling when the protein in the foam can be recovered as a dry product, and lower than the current corn oil price. Foam concentration of free oil from an enzymatically treated corn germ dispersion is enabled by an endogenous foam stabilizer likely to be a protein or protein residue. There is no clear evidence that the foam stabilizer is degraded by the enzyme complex but if not, then excess enzyme may release too much stabilizer so that it aggregates on bubbles impairing their ability to entrain oil droplets.

Acknowledgments Michael F. Dallmer carried out the experiments and made significant improvements to the equipment and procedures and Neil Goldberg and the mechanical group refurbished and tested the colloid mill and modified the bubble column. John Minutolo carried out the gel electrophoresis and Paul Pierlott prepared the figures. Genencor® provided ample quantities of Accellerase™ 1,000 cellulase complex and Cargill Inc. supplied dried, wet-mill germ.

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