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ORIGINAL PAPER

Using Microwave Heating and Microscopy to Estimate Optimal Corn Germ Oil Yield with a Bench-Scale Press

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Abstract The increase in ethanol production from corn has prompted development of processes to separate corn germ. The corn germ co-product would be a source of corn oil if a practical oil separation process were also developed. We carried out bench-scale corn-germ-pressing experiments to determine the maximum potential oil recovery which were then used to estimate commercial germ crushing costs. Corn germ was preheated in a microwave oven and oil was then extracted with a bench-scale press. Preheating the germ was necessary to obtain good oil yields. The uniform heating of the microwave oven more closely resembles compressive heating of commercial scale presses than does oven heating. Three different microscopic techniques were used to examine the effects of microwave and conventional-oven heating on corn germ. Microscopy revealed that microwave heating heated oil in the germ more quickly than the other components of the germ. Heating by both methods destroyed lipid body membranes and oil coalesced and pooled. Less oil could be pressed from germ initially containing 3–6% moisture than germ containing 15–20% moisture. Maximum oil recovery of about 65% was obtained for all germs tested when the optimum press temperature and germ feed moisture were used.

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Introduction

Dry milling of corn produces various food-grade products including flaking (corn) grits used to make corn flakes. Germ is usually a co-product and oil is extracted from germs with hexane or by pressing or by pressing followed with hexane extraction. Hexane extraction is capital intensive and carried out using corn in only a few large facilities. Dry grind plants produce fuel ethanol and distillers dried grains (DDG) or distillers dried grains with solubles (DDGS) for animal feed. Removing oil from either product may increase income for dry grind plants, because extracted corn oil is more valuable than oil retained in DDG or DDGS. Extraction of oil from the DDGS with ethanol was examined [1] but the results were not encouraging. VeraSun Energy Co. (Brookings, SD, USA) has announced plans to centrifuge oil from the thin stillage at four dry-grind plants and use the oil to make bio-diesel fuel [2].

Several germ separation methods that potentially could be used in dry-grind plants have been evaluated: ''Quick Germ'' [3], enzymatic milling [4, 5] and mechanical [6]. The oil contents of dry-milled germs vary from 20 to 25% compared to as much as 50% for wet-milled germ. A method to recover oil from dry-milled germ may suggest further development of a feasible process to recover oil directly from corn.

Recently, the effect of heating wet- and dry-milled corn germ prior to pressing [7], the effect of the moisture content of dehulled crambe seed on pressing yields [8] and the energy dissipated in screw pressing of flaxseed [9] have

been studied with bench- and lab-scale screw presses. Heating oilseeds prior to mechanical extraction has been practiced ever since screw presses became widely used; cottonseed, for example, is heated with minimal drying for 20 min at 87 °C [10]. For some oilseeds, pressing is followed by solvent extraction, and prepress optimization differs from that for pressing alone.

It is difficult to make conclusions gathered from studies with different presses and different oil seeds. The previous corn-germ study [7] reported that, with dry-milled corn germ, microwave heating was superior to conventional oven-heating and, with wet-milled corn germ, conventional oven-heating resulted in higher pressed oil yields. The flaxseed study [9] found that decreased moisture led to significant increases in both oil and meal temperature. In the dehulled crambe seed study [8] preheating was unimportant but the yields improved as the moisture content decreased from 9 to 3%.

These studies are consistent with water evaporation dissipating a significant amount of the heat generated during pressing. Under the best yield conditions, evaporation can keep the pressing from burning the seed. Preheating will, in addition to cooking the seed, allow the seed to enter the smaller presses used in these studies near its maximum temperature. A full-scale screw press would usually generate more than enough heat to reach and surpass (without cooling) the optimum pressing temperature. Bench-scale tests may be useful if they identify pressed germ maximum oil yield, which can then be used to estimate large-scale pressing costs.

We hypothesized that careful experiments using a bench scale press could predict the performance of a full-scale screw press. Microwave oven-heated corn germ better simulates heating in an expeller than a convection oven because pressing heats germ as it is compressed without the delay and temperature gradient in particles that is inherent in conduction heating. Furthermore, we expected that rapid microwave heating would sufficiently reduce oil viscosity for the oil to drain during pressing before the outer material in the germ particles was 'cooked' enough to increase its rigidity. We also wanted to determine if germ dried sufficiently for ambient storage could be pressed efficiently or, if not, if it could be rehydrated and efficiently pressed. Storage will be necessary if the germ from several dry-grind plants is pressed in a single facility.

Experimental Procedures

Heating and Pressing Corn Germ

Experiments were carried out with one wet-milled and three dry-milled corn germ batches (A, B and C) provided by commercial mills. Although our goal was to develop a process to remove oil from dry-grind germ, none was available when the initial experiments were carried out. Wet-milled germ contained 3.4% moisture and dry-milled germs A, B and C had moisture contents of 17, 5.6 and 18%, respectively. Most of the testing was done with dry-milled germ A that was separated from the corn kernels using Beall degerminators. Typically, 500 g of germ was weighed, spread evenly in a 2.6-l, rectangular Pyrex dish and heated at full power in a household microwave oven with a rotating stand (Panasonic model S954WF, 1,250 W, 2,450 MHz, Secaucus, NJ, USA). To measure microwave energy absorption, the temperatures and masses of water, oil and corn germ were each measured before and after heating in a Pyrex dish for 4 min. Using published specific heats [11], we calculated that water absorbed 683 W and commercial corn oil 293 W. The temperature of corn germ increased to 93 °C and 23.5 g of water evaporated. Using the germ specific heat [12] the sensible heat gain was 4.5 kJ. The absorbed energy used to evaporate water from the germ was 95.4 kJ and the total energy absorbed was 100 kJ (416 W).

When a series of heated pressings was carried out, a batch of germ was heated so that the heating period would end less than 1 min before the pressing of the preceding batch finished. Except for one run, only a single collection of cake and oil products for a pressing was made. After cooking, the germ was weighed again, rapidly sampled and immediately poured into the feed hopper of a small laboratory screw press (Taby Type 20, Skeppsta Maskin AB, Orebro, Sweden). The germ descended from the hopper to the screw barrel inlet by gravity. The 20 mm shaft press had an electrically heated section just before the 0.78-mm outlet die; the heater was controlled to produce a 100 °C outlet temperature. The press was preheated for 90 min prior to beginning a series of germ pressings. After each series, the press was disassembled and cleaned, and the distance between the die face and the end of the screw was adjusted to 15 mm. The screw rotational speed was always 95 rpm. The rate of germ pressing varied with the heated germ batch from 20 to 50 g/min; the germ that pressed more rapidly produced more oil. Oil draining from the eight 1 mm drain holes and extruded germ pellets were collected in plastic containers and weighed. The oil was subsequently filtered (Whatman #1 paper) in an aspirated Buchner funnel and weighed.

The germ was used as received (after sampling) for three runs. In the other runs, the germ was dried in a 140-l vacuum tumble dryer (Patterson Kelly Co., Inc., East Stroudsburg, PA,USA). Another run was made to determine the time to reach steady pressing conditions. Two 500-g batches of dry germ A (4% moisture) were heated for 4 min prior to pressing, and the second batch was added

to the press hopper after 7.5 min of pressing the first batch. The oil and pressed germ cake was collected in six equal batches. One series of pressings used 4.5 kg of dried germ A (4% moisture) that was mixed with 0.45 kg of water in the tumble dryer (no heat or vacuum) and mixed overnight. This re-hydrated germ contained 12.1% moisture. Heating and pressing of this germ was the same as previously described. In another series, dried germ A was mixed with water in mass ratios of 1:2 and 1:138 and, after a day of soaking, the germ was sieved from the water, tumble dried, microwave heated and pressed using the usual procedure. The germ A batches that were soaked in various proportions of water gave similar pressing results and were lumped together as rehydrated dry-milled germ A in later analyses.

Chemical Analysis

Analyses of pressed crude oil samples that were mostly liquid were performed on the upper liquid layer of the samples. Oil content was determined by extracting with hexane in a separatory funnel. The upper hexane layer was collected into tared beakers and the oil was determined gravimetrically. The oil contents of solid samples were determined by hexane extraction [13]. A known volume of liquid sample was dried under a stream of nitrogen gas, weighed and the solid material collected for protein determination. The pyrolysis protein nitrogen method conformed to standard methods [14, 15]. Moisture contents of solid samples were determined using AACC method 44-19 [15] and weight change after 2 h at 135 °C. Starch was determined using an enzymatic assay conforming to AOAC method 46-30 [14] and AACC method 32-32 [15].

Microscopic Examination of Germ Samples

Heat-treated and control samples of germ fractions were prepared for microscopy by immersing in a solution of 2.5% glutaraldehyde buffered with 0.1 M imidazole (pH 7.2) and stored in sealed vials. The organization of cellular structures in the scutellar tissue of the samples was visualized by autofluorescence induced by glutaraldehyde reaction products using a TCS-SP laser scanning confocal microscope system including an IRBE inverted optical microscope (Leica Microsystems, Exton, PA, USA). Particles of the germ fraction were cross-sectioned using a stainless-steel razor blade and the cut surfaces were mounted in microwell dishes (MatTek Corp., Ashland, MA, USA). Excitation from the 488 nm line of an Argon laser and emission in a green fluorescence channel (500–550 nm) were used to outline the cellular profiles in the scutellar tissue. Samples were stained with Nile Red [16] to visualize the distribution of oil in optical sections of cells and tissue in a red fluorescence channel (620–670 nm). The distributions of oil within tissue cells of the scutellum and other tissues of the germ fraction were localized in overlay digital images of the two fluorescence channels.

Germ was prepared for transmission electron microscopy to examine structural changes on a finer scale. Whole particles of germ fraction were immersed in 2.5% glutaraldehyde–0.1 M imidazole buffer solution (pH 7.2) and stored in sealed vials. For embedding and thin sectioning, particles were sliced longitudinally with a stainless-steel razor blade into sections around 1 mm thick, washed in imidazole buffer, immersed for 2 h in a solution of 2% osmium tetroxide in imidazole buffer, washed in distilled water, dehydrated in a graded series of ethanol solutions and finally embedded in an epoxy resin mixture. Cured blocks were trimmed to expose the regions of scutellum around the embryo and semi-thin sections were cut and stained according to methods described by Richardson et al. [17] for light microscopy. Digital images of the semi-thin sections were made with model DC 200 charge coupled device camera coupled to a model IRBE inverted optical microscope (Leica Microsystems, Inc., Exton, PA, USA). Thin sections of selected areas of embedded tissue were cut with a diamond knife, stained with solutions of uranyl acetate and lead citrate and photographic images were made of the ultrastructure in tissue cells with a model CM12 scanning-transmission electron microscope (FEI Inc., Hillsboro, OR, USA) operated in the bright field mode.

Results and Discussion

Early tests led us to conclude that the time to attain steady-state would be an appreciable fraction of the time needed to press 500 g of germ. Therefore, two consecutive 500 g batches of germ were pressed that had been preheated for 4 min. The hopper was refilled with the second batch of preheated germ just before the first batch of germ completely drained from the hopper so that steady state pressing was maintained. Oil drained at the rate 6.9 filtered g/min after 9 min of pressing. The protein content in the heated germ, 13.4%, increased to 17.7% after pressing. The increased protein content as a result of pressing the germ was consistent with the conservation of the protein, confirming absence of germ protein degradation by cooking.

Moisture reductions for the wet- and dry-milled germ as a result of heating in the microwave oven were linear with heating time (for positive moisture), as shown in Fig. 1. Dry-milled germ A with an original composition of 15.9% starch, 13.2% protein, 17.2% moisture and 14.8% oil was pressed after heating and the trend of oil yield with heating time is shown in Fig. 2. Oil yield was calculated as the

weight of oil recovered divided by sum of the weights of the oil and pressed germ cake recovered times 100. The pressed oil yield increased with heating time. Continued heating of germ with 0% moisture content led to lower oil yields and germ burning. It also reduced the weight of the '0%' moisture germ since the pressing conditions were more dehydrating (probably higher temperature) than those specified in the standard AACC method (135 °C/ambient).

One explanation for the observation that increased germ heating (time) increased oil yield is that cooking weakens the lipid body membranes or the germ matrix around the lipid bodies and makes them susceptible to rupture during compression. This was not the major, or only, cause as was shown by an experiment in which a batch of germ was pressed after heating for 4 min and cooling overnight. Germ that was allowed to cool after heating produced no oil when pressed by the usual procedure, but when re-heated produced usual oil yields. The drier the germ fed to the press, the less moisture lost during pressing.

Water evaporation from germ removes some of the energy from absorbed microwave radiation. Therefore, the correlation of oil recovery with the moisture of germ fed to the press was a consequence of oil yield increase with net energy absorption. The oil yield versus moisture of heated germ samples is shown for several runs in Fig. 3. The runs were made with 500 g of germ heated for 3–9 min. The ordinate in Fig. 3 is fraction of the total oil recovered, pressed oil/(pressed oil and oil in cake). Showing the yield as a fraction brings the yields for the germs in each group into alignment by weighting them for total oil content.

The runs shown in the figure can be assembled into two groups: (1) a low expressed oil fraction group comprised of the germ B and germ A dried, and (2) a higher expressed oil fraction group comprised of germ A and rehydrated germ A and germ C. Moisture $\leq 6.5\%$ appears to correlate with the maximum oil recovery (65%), by pressing. One study of a dry-mill process with and without oil and fiber separation used an efficiency (equivalent to oil recovery) of

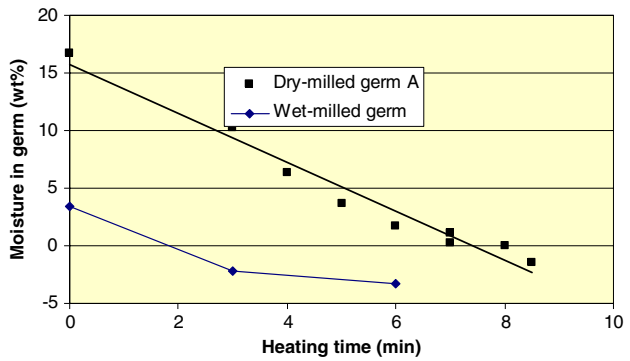

Fig. 1 Microwave drying trends for two corn germs with different initial moisture content

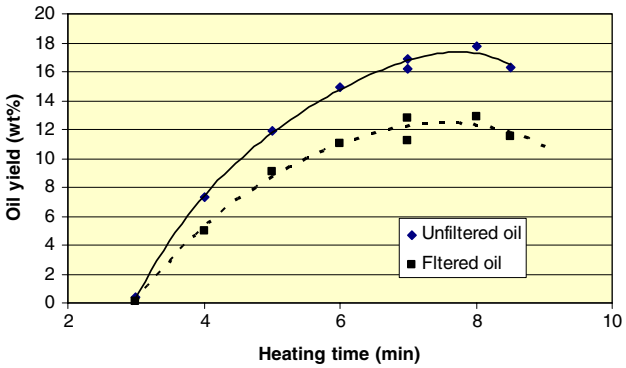

Fig. 2 Effect of microwave heating time on expressed oil yield from dry-milled germ, before and after filtration

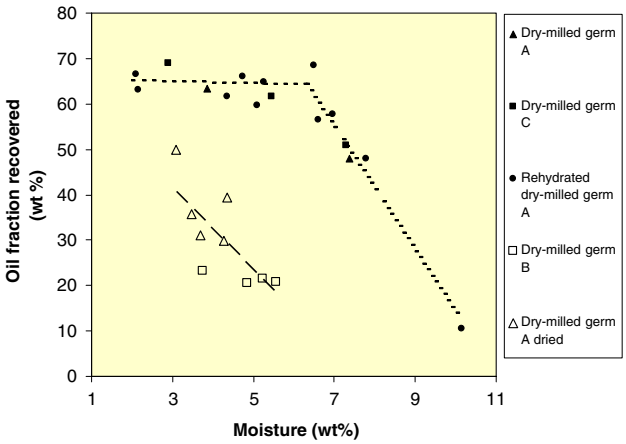

Fig. 3 Variation of oil fraction expressed with germ type and moisture content

the screw press block of 63% in the Aspen Plus model [18]. A simple explanation of the plot is that germ wetter than 6.5% moisture contains enough moisture to keep the steady temperature profile in the press below the level needed to liberate all expressible oil from the germ. The open-symbol extractions in Fig. 3 were roughly parallel to the slope of the filled-symbol extractions (dry-milled germ A, dry-milled germ C and rehydrated dry-milled germ A) with >6% moisture, suggesting a similar mechanism behind the relation between moisture and oil recovery for the two groups. One cause is that oil in the germ extracted in runs represented with open symbols (dry-milled germ B and dry-milled A dried) was held by overheated germ. The initial moisture of this (before heating) germ was 4–5.6%, whereas the germ extractions represented by filled symbols (dry-milled germ A, dry-milled germ C and rehydrated dry-milled germ A) contained 12–18% moisture. The open symbols (dry-milled germ B and dry-milled A dried) coincide with the trend line of the filled points with >6% moisture if the open points were moved +5 moisture % to the right. The maximum fractional oil recovery limit of the

open-symbol group was estimated to be 50%, the value of the (dashed line) extrapolated to 0% moisture, where the germ begins overheating during pressing.

Heat transfer by microwave radiation to the germ interior is not hindered appreciably by dry germ outer layers and, as elaborated below, it is likely that oil bodies are hotter than adjacent germ material during heating and when pressed promptly. As shown by microwave heating of mixtures of corn oil and water, oil in water (o/w) emulsions heated faster than mixtures in which there was less interfacial area [19]. Fine o/w emulsions heated most rapidly with the smallest samples, which would not be limited by the microwave power limit, showing two to three times faster heating than a weighted average rate of separate water and oil samples. The reason for the greater heating rate for the emulsions was microwave reflection at phase interfaces, creating higher fields within the oil droplets. Corn germ with oil bodies is a natural mixture similar to o/w emulsions.

The effect of microwave versus conventional oven heating was then studied using three different microscopic techniques. For these studies, we carefully dissected small pieces of control and heated germ, and used low-resolution light microscopy to examine the various types of cells in the germ samples. We noted that several types of oil-rich cells were present in the germ sample, and we decided that since the cellular structure of the scutellum cells was uniform, we would limit our microscopic examination of heating effects to the scutelum cells. As shown by confocal microscopic examination of germ scutelum cells heated in either a conventional oven or microwave oven (Fig. 4), the heating processes were visibly different although similar oil yields for the two heating methods were reported in a previous paper [7], which we now attribute to inadequate pressing time. The photomicrographs show oil-rich droplets, made visibly distinct by Nile Red staining, inside some of the smoothly distorted cells that appeared to have been burst, possibly by expansion of the contents of the cell membrane. The cells were green (when viewed in color), except for the coalesced droplets inside, compared to the yellow of the intact cells resulting from the air heating. It appeared that the oil collected in some of the cells and not in others, the coalesced droplets were larger than would result from melting a single lipid body or spherosome. The germ heated in the 180 °C convection oven did not show distortion or rupture of the cells and only lipid was visible in spaces between the closely packed, intact cells.

The cellular structure of the germ scutelum cells shown by light microscopy after methylene blue staining (Fig. 4) also showed differences between the two heating methods and the control. In this view, the microwave heating has distorted the original structure more than the 180 °C convection oven and the (white) oil droplets appear more smoothly bounded, possibly as result of melting.

The same three types of sample were examined via transmission electron microscopy at 100,000× magnification. In the control sample, the spherical oil bodies (approximately 1 μm in diameter) were each surrounded by an intact biological membrane. When heated at 180 °C, there was some destruction of oil body membranes and the oil contained in the lipid bodies coalesced. In the microwave-heated samples, there was extensive destruction of the oil body membrane. Large portions of the cells contained a continuous pool of oil and small granular material (probably aggregates of oil body membranes) were observed.

Increasing the corn oil temperature from 24 to 100 °C reduced oil viscosity from 0.052 to 0.0066 Ns/m^2 [20]. According to Darcy's law, the flux through a packed bed, such as crushed corn germ, should be inversely proportional to the viscosity of the oil. Thus, heating with constant permeability of the porous medium and constant pressure gradients should increase oil flow about tenfold. The steady increase in yield with moisture decrease precludes either decomposition of the oil body membranes or surrounding material and was more consistent with a gradual improvement that simply results from greater oil fluidity and germ softening. Germ softening allowed the particles to deform and reduced the volume available to the oil during compression. Compression and drainage of a slurry of non-deformable solid particles will leave a solid/liquid product of no less than 50% liquid, whereas the pressed germ cake contained as little as 8% oil. High oil fluidity is consistent with the fact that the same fraction of oil in corn germ produced in a dry-milling process (which has about one-half as much oil as corn germ produced in a wet-milling process) can be pressed out as the fraction that can be pressed out of the wet mill germ. This showed that the oil did not cling to the germ during pressing. If it had, the pressed dry-milled germ, with much higher non-germ to oil mass ratio, would have had a lower oil fraction expressed than the pressed wet-milled germ. The heated germ usually contained less oil in the heated germ than in the products. Although the product oil must have been present initially, pressing was necessary to enable the extracting hexane used in the analytical determination to reach some oil trapped in bodies. The protein content of the heated germ feed matched that of the pressed products, consistent with little protein destruction during pressing.

On average, 10 g/min of water was evaporated from 500 g of dry-milled germ (initially 17% moisture) during microwave cooking; at a power consumption of 5.39 kcal/min (0.376 kW). It took about 7 min to dry 500 g of germ. At $0.05/kWh, the electric power cost to heat the germ was $0.04/kg of germ. If 17% of the heated germ mass can be recovered as oil, the heating cost for the oil was $0.022/kg of oil. Although the microwave oven was rated for

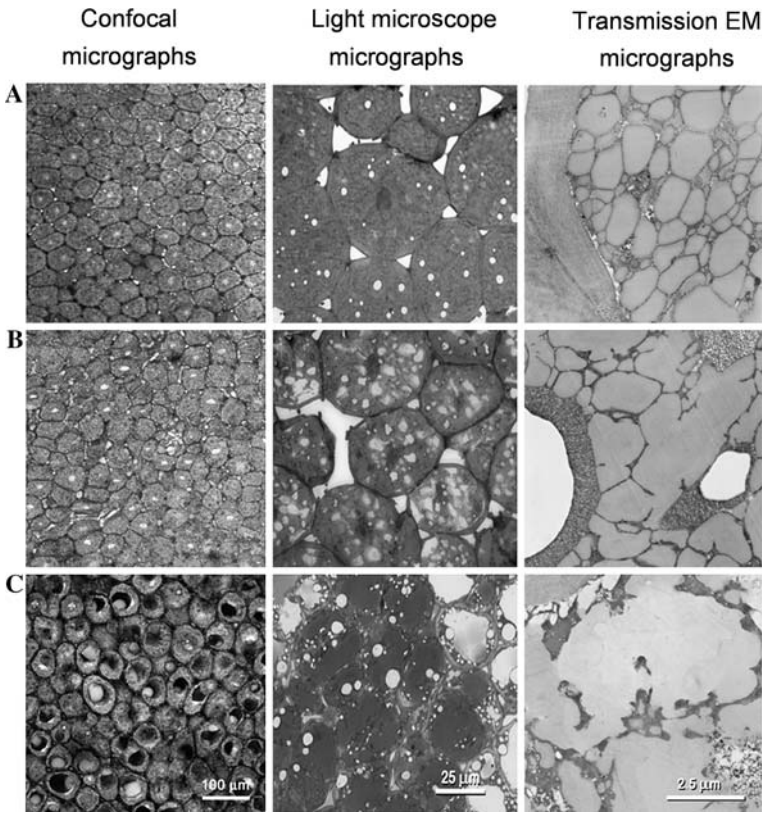

Fig. 4 Effect of heating on the ultrastructure of dry-milled corn germ A as studied with confocal (100 μm), light (25 μm) and transmission electron (2.5 μm) microscopy. **a** control, uncooked corn germ; **b** corn germ cooked in a convection oven at 180 °C for 6.5 min; **c** corn germ cooked in a 1,500-W microwave oven for 4.5 min

1.25 kW, our germ absorbed less than one-third of this power. In a commercial plant, the press itself would provide most of the heat needed and it is unlikely that germ preheating would be a significant cost.

A feasibility design for a 300 Mt/day soybean-crushing mill which extracts 12.3 thousand Mt/year of de-gummed soybean oil (330 day/year) by pressing can be used to estimate the cost of mechanically extracting corn oil from the germ. Such a facility would cost about $14 million and would have annual processing costs, excluding the soybean feedstock, of $3 million or approximately $4.0/Mt of beans processed (Stroup R. L. personal communication).

Facilities for mechanically extracting oil from corn germ will be less expensive than soybean processing facilities. If the oil extraction process was part of the ethanol facility, the germ can be fed directly into the extraction units. The pressed germ can be blended with the DDGS. This will eliminate the need for large feedstock and meal storage facilities that are needed for soybean processing since these will already be part of a dry-grind ethanol plant that separates germ. The soybean tempering, cracking and dehulling systems will also not be required, and the common systems, such as steam generation, process control and electrical distribution, will be integrated with dry-grind ethanol plant systems. This should reduce the capital costs for a 300 Mt/day corn oil extraction unit to about $2.5 million and the oil extraction costs to about $22/Mt of germ or $170/Mt of oil extracted. A model study of integrated dry-grind plants with co-products used a screw pressed cost of oil of $176/Mt [18].

In conclusion, the highest oil recovery was obtained by pressing germ that had been dried and rehydrated to 2–6.5% moisture before final heating and pressing. Germ with less than 2% moisture overheated during pressing, as determined by reduced oil recovery, and visible burning in extreme cases. This suggested that it would be preferable to expel germ commercially with sufficient moisture so that the germ temperature will not exceed that experienced by germ of 6.5% moisture fed to the press. Commercial presses are cooled to remove heat of compression, but increasing the germ moisture, to the extent possible, would be cheaper. A pressing cost estimate was made showing that pressing oil from corn germ generated by a single dry-grind plant was marginally feasible at current corn oil prices. Since we have shown that germ can be dried (for storage) and rehydrated to obtain good pressing yields, a large plant could feasibly press stored germ provided by several nearby dry-grind plants.

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