

Isolation of Zein Using 100% Ethanol

John W. Lawton¹

ABSTRACT

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Traditionally, zein is isolated and recovered from corn gluten meal (GCM) using aqueous alcohol as the solvent. Recovery of zein from this solvent is inconvenient and costly. Zein is insoluble in 100% ethanol at room temperature, but it is soluble at 120°C in ethanol. Absolute ethanol effectively extracted zein from CGM, distillers dried grains (DDG), and ground corn. Zein was extracted from CGM with absolute ethanol in a high-pressure reactor at 130°C. After extracting at 130°C for 45 min, the solution was pumped out of the extractor and allowed to cool. Upon cooling, the zein precipitated from solution. The precipitate was removed from the solution and air-dried, resulting in 14% recovery of the starting

material. The recovered precipitate had an average protein content of >90% on a dry basis, accounting for ≈20% of the CGM protein and recovered ≈35% of its zein. No differences were seen in the amount of zein extracted from CGM samples that were hand-collected off the dewatering screen and gently dried, versus commercial CGM samples. The commercial CGM did produce a greater amount of solubles. The extraction procedure also worked at temperatures as low as 90°C. The lower temperature did produce lower yields of extracted zein. The zein extracted at the lower temperatures was less brown, but zein extracted at either temperature was almost fully soluble in traditional zein solvents.

Zein was first extracted by Gorham (1821) from whole corn using aqueous ethanol. Osborne (1891) was granted the first patent for a commercial method for zein extraction. Commercial production of zein was not achieved until 1938 when the Corn Products Refining Company built a small pilot plant (Anon. 1939). A much larger plant was built by Corn Products Refining Company in 1943 (Anon. 1944). These plants recovered zein by spraying the aqueous alcohol zein solution into cold moving water (Horesi et al 1941). Today, zein is recovered from the aqueous alcohol solvent by chilling the extract to –10 to –20°C (Lawton 2002). Zein precipitates from aqueous alcohols at these low temperatures. Spray drying or vacuum drying have been suggested as methods to recover zein from aqueous alcohol solutions (Shukla and Cheryan 2001; McInnis and Tang 2003).

The problem with using aqueous ethanol is that zein remains in solution at room temperature. To recover the zein, it either has to be precipitated from the solvent, or large amounts of solvent have to be driven from zein in the drying step. Potential problems are encountered using a spray dryer to recover zein from an aqueous alcoholic solution. Alcohols are explosive under heated closed systems such as a spray dryer. Zein is a film former and has a tendency to form films as it exits the sprayer of the dryer. It also has to be totally dry by the time it hits the wall of the spray dryer. If not, it will either form films on the wall or stick to it. Either way, the zein remains in the heated dryer too long. To save time and expense, it would be desirable to find a single component solvent for zein where the solvent could easily be removed or precipitated. Zein is soluble in 100% ethanol at 120°C (Manley and Evans 1942). Once the solution is cooled to <120°C, zein precipitates and can be recovered. The objective of this work was to see if 100% ethanol at elevated temperatures would be an effective extractant for zein.

MATERIALS AND METHODS

Corn gluten meal (CGM) was obtained from Aventine Renewable Energy (Pekin, IL). CGM was obtained either dry (commercial

product) or wet (from their dewatering screen). Wet CGM was air-dried and ground through a pin mill. Corn grits were donated by Bunge Milling (St. Louis, MO) and distillers' dried grains were donated by Big River (Burlington IA). Ethanol and petroleum ether were reagent-grade and purchased from Sigma-Aldrich (St. Louis, MO).

Samples were defatted with petroleum ether using a soxhlet extractor. Extractions were run for ≈55 hr. Nitrogen analysis was performed using a PerkinElmer 2400 Series II Dumas-type elemental analyzer. Approximately 2–3 mg of material was used for each measurement and each sample was run in triplicate. The instrument was calibrated using an acetanilide standard. Protein content was calculated from the nitrogen analysis using a 6.25 factor. Samples that were vacuum-dried before extraction were dried overnight at 35°C and 70 mm of Hg to <1% moisture. Moisture percent of samples was determined using the modified Approved Method 44-15A (AACC International 2000). Because of the amount of material extracted, some samples were dried using 1 g or 0.5 g.

Crude zein recovered from the ethanol extraction was redissolved in either 70% aqueous ethanol (v/v), 88% aqueous isopropanol (v/v), dimethyl formamide, 2-ethoxy ethanol, or ethyl lactate. Crude zein (1 g) was mixed with 10 mL of solvent and stirred in a closed beaker for 16 hr (overnight). After stirring overnight, the zein-solvent mixture was transferred to a tared centrifuge tube and centrifuged for 20 min at 2,000 rpm. The liquid was discarded and the solids in the tube were air-dried in the hood for 48 hr, followed by drying at 100°C for 2 hr in a forced-air oven. The solubles percent was calculated by subtracting the starting weight from the remaining weight and was an average of three solubilization runs.

Extraction Method

Samples (37.5 g) were either vacuum-dried or used as received and were extracted using 300 mL of 200 proof ethanol in a pressure reactor (model 4842, Parr Instrument Co., Moline, IL). The extraction was performed at 130°C unless otherwise stated and stirred at 150 rpm for almost 1 hr. Approximately 10–15 min was needed for the reaction vessel to reach temperature. After reaching the set temperature, the substrate was extracted for an additional 30 min. After 30 min, the stirrer was turned off and the sample was allowed to settle to the bottom of the reactor for 15 min. The reactor was equipped with a valve and a tube that extended about halfway down the reactor's vessel. This tube was fitted with a fine mesh screen to keep the extracted sample separate from the solution. Upon opening the valve, the difference in the pressure inside the vessel (≈70 psi) and the outside pressure allowed the liquid to be easily pumped into a beaker. The zein

¹ United States Department of Agriculture, Agricultural Research Service, Plant Polymer Research Unit, National Center for Agricultural Utilization Research, 1815 N. University Street, Peoria, IL 61604. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable. Phone 309-681-6419. E-mail: lawtonjw@ncaur.usda.gov

solution was allowed to cool to room temperature ($\approx 23^{\circ}\text{C}$). After cooling, the solution was centrifuged at 2,000 rpm for 15 min (Bechman Coulter Allegra 6R centrifuge). The zein was collected from the bottom of the centrifuge tube and air-dried. The supernatant was put in a 600-mL beaker and left to evaporate in a laboratory hood. The wet sample was removed from the reactor vessel and the ethanol was allowed to evaporate in the hood. After drying, all materials were weighed (zein, extracted sample, solubles).

Extraction of CGM at 90°C was performed using the Parr 4842 pressure reactor. The extractions were performed as previously described. The pressure inside the reactor reached only ≈ 17 psi because of the lower extraction temperature, and this pressure was not sufficient for pumping the solvent out of the reactor. The reactor was attached to a compressed air tank to achieve greater pressure inside and was charged with ≈ 50 psi of air before heating the reactor. During the heating stage, the pressure reached ≈ 65 psi. The valve to the compressed air tank was left open to optimize the discharge of solvent from the reactor.

RESULTS AND DISCUSSION

Absolute ethanol can be used to extract zein from corn and its coproducts, provided the ethanol is heated above its boiling point. The initial extractions took place at 130°C and extracted on average ≈ 6 g of crude zein from 37.5 g of dried CGM (Fig. 1). The nitrogen content of the extracted crude zein was $\approx 15.5\%$, yielding a protein content of the extract of $\approx 96\%$ ($\text{N} \times 6.25$). This percent protein is about what commercial zein is, with only one extraction step. The average dry matter yield is $\approx 15\%$ of the starting dry CGM (Fig. 2). The protein recovered as the crude zein accounted for $\approx 20\%$ of total protein in the starting CGM. The yield of extractable zein based on an estimated zein content of CGM was $\approx 35\%$. Zein content of CGM was estimated to be 60% of the total protein. Landry et al (1999) showed that CGM has 62–66% zein. A conservative estimate of 60% was used in the calculation for yield.

Using air-dried, hand-collected CGM (HC-CGM) from dewatering screens over commercially dried CGM did not have an effect on the amount of crude zein recovered. No statistical difference was seen in the amount of crude zein recovered from HC-CGM and commercial CGM. Defatting the HC-CGM with petroleum ether before extraction did not increase the amount of crude zein extracted. A more polar solvent may have been a better choice as the crude zein extracted remains yellow. This color retention, at the very least, indicates the presence of xanthophylls and β -carotene. Defatting commercial CGM did significantly increase the amount

of crude zein recovered over commercial zein that was not defatted. Defatted commercial zein also gave the greatest yield in protein and extractable zein (Fig. 2). It is unclear why the defatted commercial CGM gave better yields in extractable protein and zein. The starting protein content of the commercial defatted CGM was $\approx 5\%$ lower than the HC-CGM. This accounts for the greater protein and zein yields on a mathematical basis but does not explain why the extraction process extracted more zein from the defatted commercial CGM. Defatting the CGM before extraction did slightly increase the protein content of the crude zein collected but not significantly over CGM that was not defatted.

Commercial samples of CGM produced greater amounts of solubles than did the HC-CGM. The solubles are the remaining dry matter in the supernatant after the ethanol has been evaporated. The HC-CGM extraction solvent contained ≈ 1.8 g of dry matter while the commercial CGM contained 3 g. The greater amount of solubles produced by the commercial CGM is probably due to fermented corn extractives or corn germ meal being added to commercial CGM (Weigel et al 1997). The solubles from both types of CGM contained considerable amounts of soluble proteins that did not precipitate upon cooling and centrifuging. The HC-CGM solubles averaged $\approx 62\%$ protein, while the protein content of commercial CGM averaged $\approx 56\%$.

The extracted crude zein compared favorably with commercial zein when both were evaluated by SDS-PAGE containing dithiothreitol (Fig. 3). The two prominent bands seen in the gel at $\approx 22,000$ are α -zein, the major proteins in commercial zein. Some minor bands show up in some of the samples, but these are also seen in the commercial zein sample. The soluble protein left in the ethanol after cooling also appears to be zein. It is unclear why all of the zein does not precipitate upon cooling because zein is not soluble in absolute ethanol at room temperature. The amount of soluble protein left in the solubles is 1.2–1.6 g, depending on the starting CGM. The volume of the solubles after centrifuging and before drying is ≈ 200 mL. It is possible that an ethanol-lipid solution could dissolve a small amount of zein.

There was concern that the high temperatures used to extract zein would affect its solubility when redissolved in some of its traditional solvents. However, as Table I shows, the solubility of the extracted crude zein was unaffected by the high temperature used in the extraction process. The zein readily dissolved in the solvents with only a small amount remaining. Only ethyl lactate solvent did not efficiently redissolve high-temperature-extracted zein. However, our laboratory sometimes has trouble dissolving commercial zein in ethyl lactate. We usually have to stir the mixture for >24 hr to get zein into solution. The initial extraction of CGM using high-temperature ethanol only recovered $\approx 35\%$ of

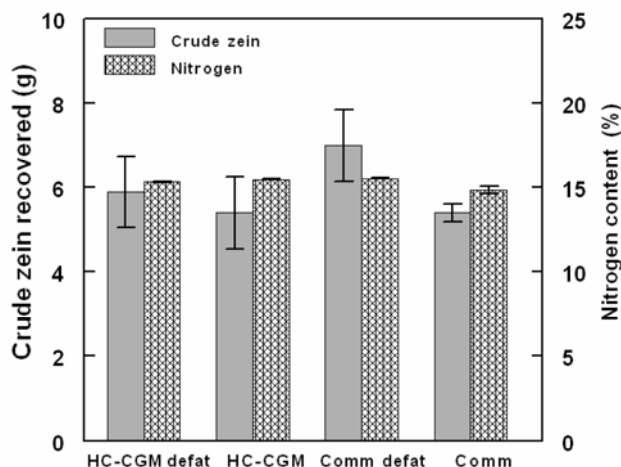


Fig. 1. Amounts recovered and percent nitrogen of crude zein extracted at 130°C with 100% ethanol. Error bars indicate ± 1 SD.

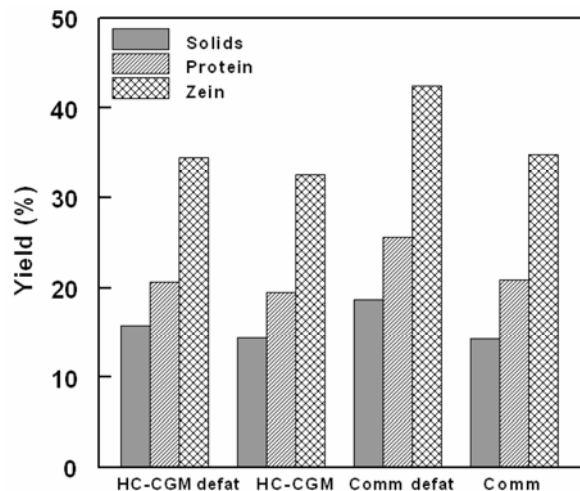


Fig. 2. Yields of crude zein extracted at 130°C with 100% ethanol.

the available zein in CGM. To evaluate whether the zein solution becomes saturated during extraction, the HC-CGM was extracted as described earlier and then reextracted four more times. The amount of recovered crude zein for each extraction is shown in Fig. 4. The initial extraction recovered more crude zein than was extracted for samples shown in Fig. 1. The reason for greater recovery is not known. The CGM used for this set of extractions was hand-collected at a later date and therefore was not the same starting material. The amount of recoverable crude zein decreased with each extraction. The sequential extraction does show that solvent saturation may be the cause of not fully extracting all of the zein on the first extraction. An additional 3.6 g (db) of crude zein was extracted on the second extraction. Additional extractions did not remove any significant amount of zein, with only 0.2 g (db) recovered by the fifth extraction. This is probably because once two extractions have occurred, there is not much zein left to extract. The starting HC-CGM had a protein content of 74.8% (11.96% N), meaning there are ≈ 27 g of total protein in the starting material before extraction. Using a conservative estimate of 60% zein for this HC-CGM, a theoretical yield of ≈ 16 g of zein is possible. The calculated extracted protein for the first two crude zein extractions and their collected solubles was 13.3 g. After five extractions, the calculated protein recovered from the crude zein extract and their solubles was 15.7 g.

The initial samples were dried before extraction to $<1\%$ moisture content. The reason for this was to ensure that there was no water in the substrate to change the absolute ethanol into an aqueous ethanol solution. It was believed that aqueous ethanol, even with low water contents would not allow the zein to fully precipitate upon cooling. However, drying substrate in a commercial operation to $<1\%$ moisture content would be impractical. CGM samples were extracted as received to study the effect of the absorbed water on the extraction of zein. The moisture content of the CGM was 9.5%. No differences were seen in the amount of protein extracted or on the amount of solubles recovered. This was

TABLE I
Solubility of Extracted Zein

Solvent	Percent Dissolved
70% aqueous ethanol	96 \pm 1.2
88% aqueous isopropanol	94 \pm 0.2
Dimethyl foramide	98 \pm 1.6
2-ethoxy ethanol	95 \pm 0.3
Ethyl lactate	50 \pm 1.6

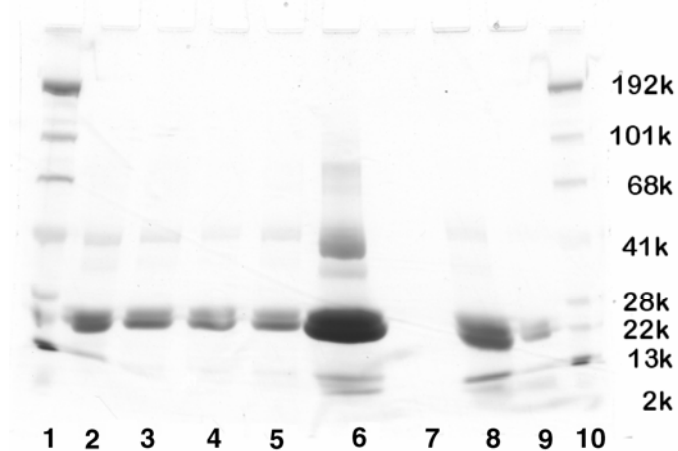


Fig. 3. SDS-PAGE of extracted zein and solubles Lanes 1 and 10: MW Std; Lane 2: HC-CGM; Lane 3: commercial CGM; Lane 4: defatted HC-CGM; Lane 5: defatted commercial CGM; Lane 6: commercial zein; Lane 8: HC-CGM solubles; Lane 9: commercial CGM solubles.

probably because the small amount of absorbed water in the CGM coupled with the much greater amount of absolute ethanol used in the extraction. The water in the CGM accounted for only 3.6 g of water, making the final ethanol concentration 98.8%. This concentration of aqueous ethanol is not an effective solvent for zein at room temperature. For this reason, it is believed that CGM with moisture levels as high as 14% would probably not adversely affect the zein recovery as long as the solvent-to-CGM ratio was maintained. Using CGM at 14% moisture content would add 5.25 g of water to the ethanol, giving a final aqueous ethanol concentration of 98.3%. Zein would not be soluble in 98.3% aqueous ethanol at room temperature (Manley and Evans 1942).

In a commercial zein operation, the extraction would probably not use fresh ethanol for each extraction but instead use ethanol from previous extractions. To observe how well previously used solvent extracted fresh CGM, the CGM was extracted as before but ethanol that had been used was substituted for fresh ethanol. No differences were seen in the amount of zein extracted (Fig. 5). There was a slight increase in the amount of solubles recovered, which would be expected because this solvent would contain the solubles from two extractions.

This extraction procedure works on corn grits and distillers' dried grains. However, the yields are reduced because of the lower protein content of the starting material. The yields for corn grits and DDG were ≈ 1 and 2% respectively. Extraction amounts were 15% of the protein in corn grits but only 7.4% of the protein in DDG. Zein is more difficult to extract from DDG (Wolf and Lawton 1997). The DDG produced more solubles than did CGM or corn grits. DDG extraction solvent contained 5.4 g of dry matter. Because DDG is sold with solubles produced from the fermentation process, it was thought that better yields could be obtained by removal of these solubles before extraction. DDG was washed 3 \times with 10 \times its volume of water before extraction. This washing increased the dry matter yield slightly to 3%, and 10% of the protein was extracted in the washed DDG. Washing DDG also decreased the amount of solubles recovered to 2.61 g. The crude zein extracted from DDG was only 85% protein. Crude zein extracted from DDG was the only isolated zein that had protein content $<90\%$ on a dry matter basis.

While experimenting with a continuous zein extraction using 100% ethanol, it was observed that zein could be extracted at $<130^\circ\text{C}$ (data not shown). A benefit of using lower temperature extractions was that the recovered zein was the more familiar

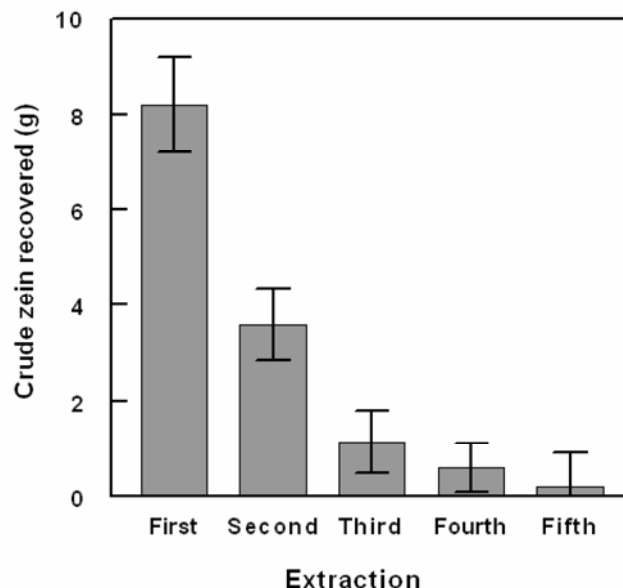


Fig. 4. Recovered crude zein after sequential extraction of HC-CGM at 130°C with 100% ethanol. Error bars indicate ± 1 SD.

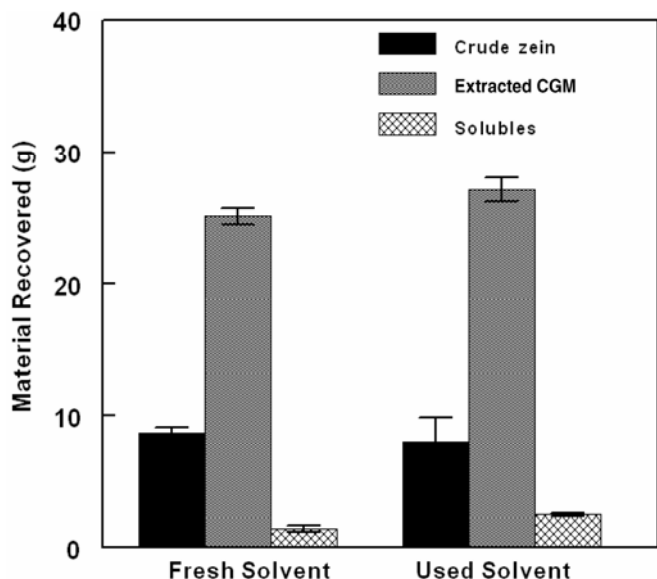


Fig. 5. Effect of used solvent on the material recovery after the extraction of HC-CGM at 130°C. Error bars indicate ± 1 SD.

yellow color instead of a brownish yellow that was seen at higher temperatures. To examine the low-temperature extraction more thoroughly, the Parr 4842 pressure reactor was used but only heated to 90°C. The lower temperature used for extraction did not produce sufficient pressure inside the apparatus to efficiently pump out the zein solution. A compressed air tank was added to the apparatus and charged with enough air to bring the pressure inside the reactor to 50 psi. The pressure rose inside the reactor to ≈ 65 psi during the heating. This is about the same pressure that was seen when the reactor was heated to 130°C. At 90°C, lower amounts of crude zein were extracted than with the high-temperature extraction method, yielding 4.2 g for HC-CGM and 4.8 g for commercial CGM. The lower temperature did not affect the purity of the crude zein extracted from HC-CGM and commercial CGM, they had percent proteins levels of 97.5% (15.6% N \times 6.25) and 96.3% (15.4% N \times 6.25), respectively. The lower temperature used did not extract as much crude zein as the extraction done at 130°C. This may reflect a lower extraction efficiency because of the lower temperature or may reflect the inefficient removal of the zein solution from the reactor. Even with the compressed air to aid in pumping, there was still solvent left in the reactor. Zein that precipitated could be seen on the surface of the CGM when the reactor was opened. The lower temperature extraction produced about the same amount of solubles as those at higher temperature extractions. The HC-CGM produced 1.9 g of solubles and, as with the high-temperature extraction, the commercial CGM produced slightly more at 3.3 g.

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CONCLUSIONS

Zein can be extracted with 100% ethanol at elevated temperatures. Once the ethanol is cooled, the zein precipitates, making its recovery easier than traditional recovery methods. The current method extracts $>20\%$ of the CGM protein and $\geq 35\%$ of its zein. The extraction method is very dependent on the starting material and yields can vary greatly. The procedure also produces a zein product that has average protein contents of 96% without any further purification steps, other than allowing the zein to precipitate, and followed by centrifugation. The initial work done was with dry CGM, but CGM with normal moisture contents can be extracted without leaving more zein in the solubles than was seen with dry CGM. Zein can also be extracted using 100% ethanol at temperatures as low as 90°C. The procedure also worked with ground corn and DDG. The yields were predictably lower with these materials because of their lower protein contents. The DDG also produced a greater amount of solubles, as anticipated, because fermentation solubles are added back to DDG.

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