REVIEW

Zein Extraction from Corn, Corn Products, and Coproducts and Modifications for Various Applications: A Review

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ABSTRACT

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Corn can be fractioned to produce starch, fiber, oil, and protein in relatively pure forms. The corn kernel contains 9–12% protein, but half of this is an industrially useful protein called zein. Dry milled corn (DMC), corn gluten meal (CGM), and distiller's dried grains with solubles (DDGS) are all coproducts from corn that contain zein and are used for zein extraction. Because it is insoluble in water, zein has found uses in many products such as coatings, plastics, textiles, and adhesives. Newer applications are taking advantage of zein's biological properties for supporting growing cells, delivering drugs, producing degradable sutures, and producing biodegradable plastics. This review covers zein characteristics and nomenclature, past and current practices in processing and extraction of zein from corn products and coproducts, and the modifications of zein for various applications.

ZEIN EXTRACTION AND APPLICATIONS: AN OVERVIEW

Maize or corn is a major cereal grain throughout the world; it also is one of the most dominant crops in the Unites States (Anonymous 2010). Yellow dent variety of corn has become the most utilized. It varies greatly from the sweet corn for human consumption. The endosperm and germ contain the bulk of the dry mass of the kernel at 81.9 and 11.9%, respectively (Earle et al 1946). The bran and tip cap constitute the remaining portion of the kernel. Starch is the main component of the endosperm at 86.4% of its mass (db). The starch can be extracted in pure form for various food and industrial uses. Starch has been used mostly in the food sweetener market. Oils extracted primarily from the germ can be utilized in cooking oils or in other food products. Proteins are located mainly in endosperm and germ. Different types of proteins are found in the two main constituents: albumins and globulins centralized primarily in the germ, and prolamin-type proteins found mostly in the endosperm.

These prolamin proteins provide nitrogen for growing corn kernels during germination. Zein, the main prolamin in corn, was first discovered by Gorham in 1821 in the product *zea*, otherwise known as "Indian corn" (Gorham 1821). It was classified by Osborne (1924) as a prolamin and shown to be extractable in aqueous alcohol such as ethanol. As production of zein was commercialized in 1939, many potential uses for zein were identified. Because of zein's insolubility in water, resistance to grease, and glossy appearance, it was ideal for adhesives, plastics, and fiber applications. As the protein structure and properties of zein have become known, there has been a surge in zein-related research. However, commercial production of zein has been low with mainly two companies producing it: Freeman Industries (Tuckahoe, NY) now owned by Flo Chemical Corp. (Ashburnham, MA) and Showa Sangyo (Tokyo, Japan). Recently POET Inc. (Sioux Falls, SD) and Prairie Gold Inc. (Bloomington, IL) have introduced zein prepared using different processes. The POET product called Inviz is extracted from POET's Dakota Gold HP distillers' grains. COPE-zein from Prairie Gold Inc. is extracted from ground corn before the dry-grind process. Zein has normally sold for $10–40/kg with higher purities commanding higher prices. Until new extraction methods or new products, such as the two listed here, can prove themselves as economically viable, zein will not likely be able to compete with synthetic plastics that have a very low market price of ≈$2/kg.

Zein is a protein that is found only in corn; however, there are proteins that share prolamin characteristics similar to that of the zein found in corn. Other cereals such as wheat, barley, rye, and sorghum each contain prolamins with similar characteristics to zein. The extracted prolamin proteins from these cereals each have industrial importance, but zein is favored because of higher yields and the large volume of corn coproducts available for extraction. Corn is processed using four different methods and zein extracted from these products/coproducts could differ in properties and end uses. The four corn processing methods are wet-milling, dry-milling, dry-grind processing, and alkaline treatment. Corn wet-milling produces a protein-rich coproduct called corn gluten meal (CGM) from which zein has been extracted commercially. Dry-milled corn (DMC) separates fibrous material from grits. Dry-grind ethanol process is grinding of corn and the subsequent saccharification and fermentation of glucose to ethanol, leaving behind the coproduct distillers' dried grains with solubles (DDGS). Because of the conversion of starch to sugars and subsequently ethanol, fractions such as cellulosic materials and protein are concentrated in DDGS. Alkaline-treated corn has been mainly utilized for human consumption and has little basis for zein extraction. Zein has been extracted from all these coproducts, but commercial zein is normally produced from CGM. Most zein extractions have been based on aqueous alcohol extractions, but many other solvents can solubilize zein. Zein extraction schemes have been optimized for different corn products and coproducts because of differences in protein concentrations and processing conditions.

Zein extractions are a complex balance of yield, quality, and purity. Yields refer to the amount of zein extracted. Until recently, commercial zein has been composed primarily of α-zein, and purity is the amount of protein contained in extracted zein (Pomes 1971). Commercial zein tends to be of high quality and purity, but yields are low. The production and properties of zein have been reviewed in the past. Two recent excellent reviews are by Shukla and Cheryan (2001) and Lawton (2002). The review by Shukla and Cheryan (2001) has an overview of zein properties, extractions, and applications. The review by Lawton (2002) includes data on extractions and zein-solvent interactions, but it mainly

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focuses on the numerous industrial applications for zein. Lawton reviews the emphasis of zein extraction that has shifted toward the dry-grind ethanol processes, which are emphasized through new commercial zein products. Also, the practical uses of zein demand a product with less pigment and odor. The objective of this review is to critique and update the recent literature on zein extraction from corn, various corn products, and coproducts. It also discusses the corn zein classification and properties, treatments to increase zein extraction yields, and zein modifications for industrial applications.

CORN PROTEINS AND THEIR CLASSIFICATIONS

Corn production in the Unites States was ≈335 billion kg in 2009 (Dougherty and Honig 2010). Oils, starch, fiber, and proteins are the major constituents of corn, with protein at 9–12% w/w of a corn kernel (Earle 1977). Based on the production of the 2010 harvest, ≈30.2–40.2 billion kg of corn protein was available for zein extraction or to supplement animal feed.

Endosperm, germ, bran, and tip cap are four main components of the corn kernel; each contains protein in varying amounts. The largest part of the seed is the endosperm, which constitutes 82% of the total mass and contains 86.4% starch and 9.4% protein (db). The germ constitutes 12% of the mass of the total seed and contains 34.5% oil, 18.8% protein, 10.1% ash, 10.8% simple sugars, and 8.2% starch (db). The bran and tip cap are ≈6% of the total mass of the seed and contain mostly fiber (db). The bran contains 7.3% starch, 3.7% protein, and 1.0% oil (db). The tip cap contains 5.3% starch, 9.1% protein, and 3.8% oil (db) (Earle et al 1946). According to Wilson (1987), the endosperm contains the prolamin zein, which accounts for 60% of the total protein, glutelins account for 26% of total protein, and albumins and globulins account for 6% of total protein. The germ protein contains mainly albumins and globulins, each at 30%, glutelins 23%, and prolamins 5% (Lasztity 1979). Zein exists only in the endosperm; prolamin that is found elsewhere is either due to contamination or another prolamin protein that is not zein.

Classification for cereal grain proteins was first established by Osborne (1924), who reported four different kinds of protein based on solubility. Albumins dissolve in pure water; globulins do not dissolve in pure water, rather in dilute salt solutions; prolamins dissolve in 70% ethanol; and glutelins are soluble in dilute acid or base. The albumins and globulins are considered biologically active proteins. They regulate and control seed metabolism. The prolamins and glutelins are major storage proteins that contain nitrogen for seed germination (Tsai et al 1980b). These two categories of protein include 80% of the nitrogen in the corn kernel (Tsai et al 1980b). Even with newer protein models, these four main categories of proteins are still the basis for corn protein identification.

The solubility model that Osborne (1924) developed was far from perfect. Many of the proteins dissolved in more than one solvent, some could not dissolve at all, or proteins such as β- and γ-zein dissolved in aqueous alcohols with reducing agents were classified as glutelins (Lawton and Wilson 2003) (Table I). An important improvement upon Osborne's method was accomplished by Landry and Moureaux (1970). Their method extracted all but 5% of the total proteins and took Osborne's method further by using the reducing agent 2-mercaptoethanol to help extract glutelins in an aqueous alcohol system. Later, other extraction procedures were developed to separate and understand the prolamin and glutelin fractions (Paulis and Wall 1971, 1977; Sodek and Wilson 1971; Paulis et al 1975). As studies showed that prolamins could be extracted by aqueous alcohol or aqueous alcohol with reducing agent, confusion in nomenclature arose because glutelins could also be extracted under the same conditions as prolamins with reducing agent (Lawton and Wilson 2003).

Zein is a family of many similar proteins that are most commonly classified based on solubility and identified by molecular weights against known standards in gel electrophoresis. Zeins were thought to be prolamin proteins, but with modern classification models, zein embodies both prolamin and proteins that are soluble in aqueous alcohol and a reducing agent. Because these protein fractions also are part of the zein protein body, they are included within the zein nomenclature (Wilson 1991). These classification methods were created by Wilson (1985) and Esen (1987).

The first method devised by Wilson (1985) used two different solubility profiles based upon zein solubility in aqueous alcohol and aqueous alcohol with 2-mercaptoethanol. Zeins were characterized based on molecular weight using SDS-PAGE. The highest molecular weight (MW) protein was A-zein (21–26 kDa) followed by B-zein (18–24 kDa). These two bands were both soluble in aqueous alcohol without reducing agent. The two bands soluble only in aqueous alcohol with reducing agent were C-zein (15–18 kDa) and D-zein (9–10 kDa). Proteins soluble in water after being reduced were identified as reduced soluble protein (RSP). RSP-1 had MW ≈ 27 kDa and RSP-2 ≈ 58 kDa. Later RSP-1 was renamed as E-zein (Wilson 1991).

The second method introduced by Esen (1987) classified zein based on the protein's solubility in reduced aqueous 2-propanol. Similar to Wilson's classification scheme, zeins were identified with gel electrophoresis. Whole zein dissolved in 60% 2-propanol v/v with 1% β-mercaptoethanol from corn endosperm. The α-zein was the fraction that was soluble in 50–95% (v/v) 2-propanol but also insoluble in 30% (v/v) 2-propanol with 30 m*M* sodium acetate at pH 6.0, and with MW bands within 21–25 kDa and one at 10 kDa. β-Zein was soluble in 30–85% 2-propanol (v/v) with reducing agent and was insoluble in solutions of 90% 2-propanol and 30% 2-propanol with 30 m*M* sodium acetate at pH 6.0 with MW bands at 17–18 kDa. The γ-zein was the fraction soluble in 0–80% 2-propanol with reducing agent; it was also soluble in 30% 2-propanol with 30 m*M* sodium acetate unlike both α- and β-zein. The γ-zein had MW bands at 27 kDa. Later, Esen (1990) reclassified the 10 kDa α-zein band as δ-zein and changed the 18 kDa β-zein band to be considered γ-zein (Fig. 1). The band in Fig. 1 at ≈48 kDa, considered γ zein, is a dimer of some of the smaller MW proteins.

There are large disparities when comparing the two nomenclature systems. Esen (1987) assigned α-zein as accounting for both A-zein and B-zein in Wilson's (1985) classification. The β-zein by Esen (1987) corresponded to the C-zein by Wilson (1985). Esen's (1987) γ-zein was the equivalent of Wilson's (1985) RSP-1,

TABLE I
Corn Protein Fractionation Based on Osborne Solubility Principles

	% of Total Protein				
Protein Class	**Osborne and Mendel (1914)**	**Mertz and Bressani (1957)**	**Paulis et al (1969)**	**Hansel et al (1973)**	**Wall and Paulis (1978)**
Albumin	7.8	12.4	7.8	2.3	4.7
Globulin	–	–	–	2.3	3.5
Prolamins	50.0	33.9	37.6	57.5	45.8
Glutelins	38.2	36.8	43.6	31.2	38.0
Residue	4.0	16.9	11.0	5.8	9.0

which was not considered a prolamin. Finally, the δ-zein classified by Esen (1990) corresponded to the D-zein by Wilson (1985). The Esen classification has been further refined to no longer represent only 2-propanol solublilities (Esen 1990). Zeins are now classified primarily on SDS-PAGE migration, amino acid structure, and complimentary DNA (Mohammad and Esen 1990). Of the four zein fractions that were proposed by Esen (1987), α-zein is the most prevalent followed by γ-, β-, and δ-zein. These proteins make up ≈71–85%, 10–20%, 1–5%, and 1–5%, respectively of the total zein (Wilson 1991).

ZEIN STRUCTURE AND PROTEIN BODY

α-Zein, which is the most abundant prolamin in corn, is also the most widely used. It was determined that α-zein was the only zein present in zein produced industrially (Wilson 1988). This fraction has a unique amino acid sequence and structure that allows for many industrial uses. α-Zein contains >50% nonpolar amino acid residues and contains 9–10 tandem repeats of helical segments of these nonpolar residues linked by polar turns high in glutamine (Argos et al 1982).

The proposed tertiary structure of hydrogen-bonded α-helices and the tandem repeats has been evaluated by surface adhesion with zein using hydrophobic and hydrophilic surfaces binding zein (Wang et al 2008). A recent study of the α-zein Z19 species by Momany et al (2006) proposed a three-dimensional model in alcohol and water that showed a protein with nine helical repeats with ≈35–60% helical character and an oblong structure with an aspect ratio of ≈6:1. The helical repeats form a triple superhelix where lutein is within the core to stabilize the protein. Even though most characterizations of α-zeins show that they contain two electrophoresis bands considered Z19 and Z22, it has been known for some time that these α-zein bands hide a much more complex protein array that can be seen using two-dimensional electrophoresis (Consoli and Damerval 2001).

Two-dimensional electrophoresis techniques show that there are many different types of zein, however, it falls short in characterizing true molecular weights. In recent years, mass spectrometry has become important in identifying protein molecular weights, including those of zein. α-Zein analyzed by matrix-assisted laser desorption time-of-flight (MALDI-TOF) mass spectrometry (Adams et al 2004) showed nine different α-zein proteins ranging in true MW of 23,359-27,128 Daltons. Five of these were considered Z19 zeins, and four were Z22 zeins. Data from capillary electrophoresis mass spectrometry discerned even more Z19 and Z22 α-zeins (Erny et al 2007). Eleven Z19 zeins were accounted for, along with eight different Z22 α-zeins.

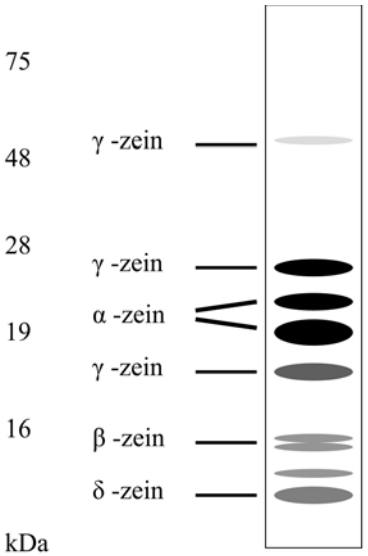

Fig. 1. SDS-PAGE fractionated zein from corn endosperm using extraction method of Wallace et al (1990); adapted from Hamaker et al (1995).

Protein bodies are the means for prolamin storage in corn kernels. The spatial distribution of zeins has been postulated in these bodies based on zein degradation during germination (Mohammad and Esen 1990). The use of zein specific antibodies indicated that certain zeins degraded at different rates during germination. The γ-zein degraded the fastest, placing them on the outside of the protein body. β-Zeins were the next slowest, putting them in a second layer. δ-Zein and α-zein both degraded gradually, placing them most likely together at the core of the body. Earlier work (Lending and Larkins 1989; Thompson and Lending 1989) used immunostaining and microscopy methods to determine the placement of the zein proteins within the protein bodies. The studies found that protein bodies within the subaleurone layer of cells of the corn endosperm contain small protein bodies with mainly β- and γ-zeins with little or no α-zein. The protein bodies found in cells further from the aleurone cell layers were larger and had an outer layer containing β- and γ-zeins and an inner core of α-zein.

PROCESSING CORN PRODUCTS AND COPRODUCTS HIGH IN ZEIN

Dry-Milled Corn

Dry-milled corn (DMC) is water-tempered corn grits where the corn endosperm has been separated from germ and pericarp through the milling process (Rausch et al 2009). It is a good material to extract zein because it has not been exposed to high heat, which may affect the zein protein. A negative aspect is that DMC contains a low amount of protein. Total protein content of DMC was 6.8–8.0% of the milled corn based on hybrid. Endosperm protein concentrations for two inbred corn varieties, W64A+ and W64Aae, which is homozygous for the recessive gene ae, have high amounts of protein (Wolf et al 1975). The protein concentrations were ≈13.1% in W64A+ and as high as 18.7% in W64Aae (Wolf et al 1975; Landry et al 2002). Commercial corn dry-milling includes three different processes: full-fat milling process, bolted milling process, and tempering-degerming milling process (Duensing et al 2003). The tempering-degerming milling process has the potential to produce a wide array of products (Fig. 2). The process involves tempering corn with water to increase moisture to ≈22%; this increase in moisture aids the separation of germ from corn when using a degerminator. The corn is ground, and then a series of sieves separates grits or small pieces of corn endosperm from fiber and germ. The method separates the dry-milled corn into five different fractions: large grits, small grits, fines, germ, and pericarp. Whole dry-milled corn is the sum of all five fractions, and endosperm is the sum of the grits and fines. Many zein extractions have used whole ground corn (Shukla et al 2000; Selling and Woods 2008). The corn is dried in a low-temperature convection oven (49°C), not high enough to alter zein physiology. The physical grinding would also not harm the protein, owing to the fact of the minute size (1.4–1.8 μm of the protein bodies (Wolf et al 1969). These protein bodies house the extractable zein in corn products (Duvick 1961). When whole DMC is extracted with 70% (v/v) ethanol without reducing agent, mainly α-zein proteins along with small amounts of β-zein are extracted because the disulfide bonds in the zein have not been broken with a reducing agent (Tsai 1980a). The extract was called "native" zein and contained α-zeins along with dimers (≈50 kDa) and trimers (≈75 kDa). However, this "native" zein should not be confused with zein in a truly native state, which would be packed within the protein body. There is evidence that extracting DMC without a reducing agent can extract small amounts of β-zein along with the α-zeins (Parris and Dickey 2001). This extract can be reduced to the α-zein constituents when a reducing agent is used to treat "native" zein (Tsai 1980a). Reducing agents used

with 55% (v/v) aqueous 2-propanol extracts zein profiles that are much broader (Wilson 1985). This fraction, called total zein, extracts not only α-zein, but also β-zein, γ-zein, and δ-zeins. If extraction of only α-zein is the goal, "native" zein extractions are ideal, but yields are low. Total zein extractions yield more zein, but the other three fractions of zein are also extracted along with α-zein, reducing its purity.

Corn Wet-Milling and CGM

Corn wet-milling is another process that creates a coproduct that is rich in zein. The gluten meal is the component from wet-milling, which contains the greatest percentage of protein along with zein. While commercially prepared samples can contain 62–74% protein (Wu et al 1997b), pilot-plant-scale operations can produce gluten meal that usually has 50–54% protein (Wu et al 1997a). The commercial wet-milling process produces many high-value products besides the gluten meal, which contains nearly all of the zein (Fig. 3). The process of wet-milling alters the zein protein in a multitude of ways that affect both extractability and properties of the protein. The steeping process helps facilitate the separation of fiber and germ. When steeping the corn, reducing agents such as SO_2 are used to break the disulfide linkages between proteins which help weaken the endosperm and allow better starch separation (Cox et al 1944). Zein proteins along with others become modified with the reduction of these disulfide bonds. Drying of CGM also affects zein properties; the redness in color of CGM is correlated with degree of drying. Excessive drying can reduce the yield of α-zein (Wu et al 1997b). The steeping solution does not fully penetrate the kernel so as to reduce all proteins, but the cleavage of disulfide bonds affects the ability of solvents to extract zein (Landry et al 1999). This incomplete reduction does not effectively allow all zeins to be extracted from CGM without a reducing agent. To do so, a solvent such as 60% (v/v) 2-propanol and a reducing agent such as 2-mercaptoethanol would be necessary (Parris and Dicky 2001). Increasing the solvent concentration of 2-propanol to 90% precipitated β-zein and γ-zein along with a fraction of the α-zein. The data suggested that only high concentrations of alcohols that solvate α-zein should be used so that extraction of β-zein and γ-zein are minimized from zein extractions.

Dry-Grind Ethanol

Dry-grind ethanol is a process utilized to produce ethanol from corn. The coproduct of this process is called DDGS (Kwiatkowski et al 2006). This process is used for fuel ethanol and beverage alcohol. Cereal grains such as corn are as much as 60–75% starch and this makes them ideal candidates for fermenting and producing ethanol (Singh et al 2002). The conventional dry-grind ethanol process is quite severe and could affect zein in a many ways. Milled corn is combined with water, thermally stable α-amylase, ammonia, and lime to form a slurry. The mixture is sent to a liquefaction vessel where the starch is cooked and gelatinized; the time-temperature combinations for liquefaction vary, e.g., up to 165°C for 3–5 min for very high-temperature cooking or 90–105°C for high-temperature cooking (Robertson et al 2006; Whitlock, unpublished) with further holding at 90°C for 1–3 hr. The cooked mash is then cooled to 60°C and glucoamylase is added to produce glucose during saccharification before fermentation by yeast. After the fermentation, the composition of the DDGS is different than the original corn. These DDGS constituents can vary from plant to plant and also on process variations, if any. One study of DDGS from six different dry-grind plants of both fuel and beverage ethanol variety showed that oil was 7.9–15.1%, protein 28–30%, neutral detergent fiber 38–49%, acid detergent fiber 14–19%, and ash 3.7–4.6% (Singh et al 2002). During the dry-grind ethanol process, the corn components change considerably from ground corn to DDGS (Han and Liu 2010) (Table II). Han (2010) found that yeast protein contributed ≈20% to the pro-

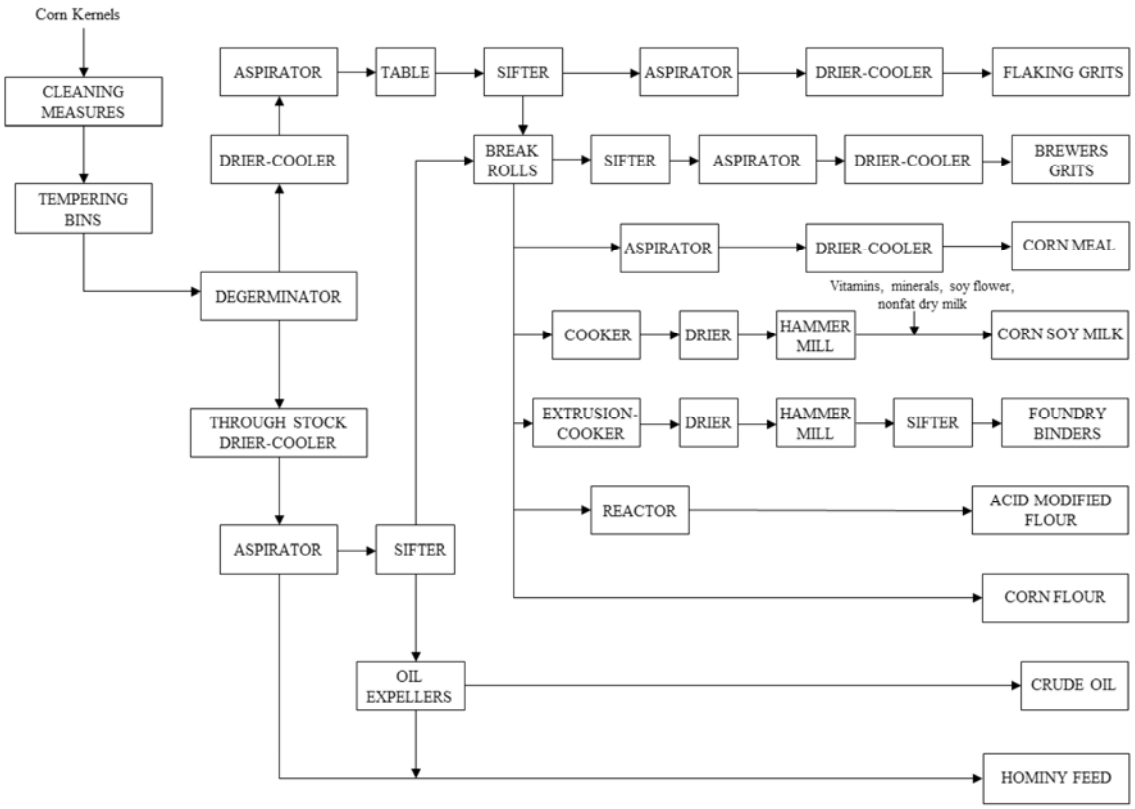

Fig. 2. Flow diagram of corn tempering-degerminating process (adapted from Wells 1979).

tein in DDGS. Modifications have been proposed to the dry-grind ethanol process by Singh et al (2005). The modifications were to remove intact germ and fiber before the dry-grind process to reclaim high quality coproducts. The germ and fiber contribute little to the fermentation process because starch is the corn component utilized to produce ethanol. The modifications by Singh et al (1999; 2005) were termed quick germ and quick fiber processes. After the germ and fiber removal, the dry-grind ethanol process was performed the same as the conventional method. The E-mill process went further than the quick germ and quick fiber processes. Enzymes, such as amylase and protease, were added to the ground corn and water and incubated. Germ and pericarp fiber were removed and then the slurry was sieved to collect endosperm fiber before the rest of the process, which was conventional. The protein concentrations in the recovered DDGS were 28, 36, 49, and 58% for the conventional, quick germ, quick germ and quick fiber, and E-mill process (Singh et al 2005). This DDGS material has a protein content nearly that of CGM and much greater than DDGS from the conventional dry-grind process (Kim et al 2008). There are no data for extraction of zein from DDGS produced through the quick germ, quick fiber, or E-mill processes; however, the quality of the zein extracted from DDGS of E-mill processes could be inferior because of hydrolysis or modification to zein from the proteases used to separate protein bodies from starch. It is not clear whether the zein from this DDGS can be used industrially until further study to characterize the proteins is conducted. If the zein could be used industrially, it may contain unique properties due to the fact that it has not undergone a sulfite steep like zein obtained from CGM. The breaking of disulfide bonds in zein may change the conformation of solvated zeins in solution by opening up their native conformation and thus alter the properties of the extracted zein. If the disulfide bonds are not broken, less concentrated solvents such as 70% (v/v) ethanol can extract α-zein without worry of extracting γ-zeins (Tsai 1980a). Dry-grind ethanol process involves drying of DDGS at higher temperatures, which could affect the zein extractability. Kwiatkowski (2006) detailed a conventional dry-grind ethanol process (Fig. 4). At ≈90°C, the shape of the protein bodies are not altered, it would take mechanical means such as extrusion or pressing to cause leakage of α-zein or merging of protein bodies (Batterman-Azcona and Hamaker 1998).

Cooking up to 70°C did not greatly affect protein extractability, but when cooking at 100°C the extraction yield of protein decreased (Batterman-Azcona and Hamaker 1998). The fermentation with yeast is also another potential setback for degradation or alteration of zein in the process. The fermentation yeast converts starches to ethanol, but yeast need nitrogen also for vigorous growth so urea or ammonia have been added as supplements (Jiranek et al 1995). Recently, proteases may be added to the fermentation to break down corn protein to help aid yeast uptake of nitrogen considering their lack of producing their own proteases

TABLE II
Distribution of Protein in Various Steps of Dry-Grind Ethanol Process in Commercial Plants[a]

Ethanol Processing Streams	Protein Content (%)		
	Ethanol Plant 1	Ethanol Plant 2	Ethanol Plant 3
Ground corn	7.7	7.8	7.5
Cooked slurry	9.3	8.9	8.1
Liquified mass	9.8	9	8.1
Saccharified mass	12.1	9	na
Fermented mass	29.4	26.6	27.2
Whole stillage	29.5	26.8	26.6
Thin stillage	22.9	17.2	21.1
Distiller solubles	21.3	17	21.2
Distiller grains	33.4	32	30.2
WDGS	27.7	na	26.9
DDGS	29.5	29.4	26.7

[a] Protein contents of corn samples, intermediate products, and ethanol coproducts from processing streams produced in three dry-grind ethanol plants. Data adapted from Han and Liu (2010).

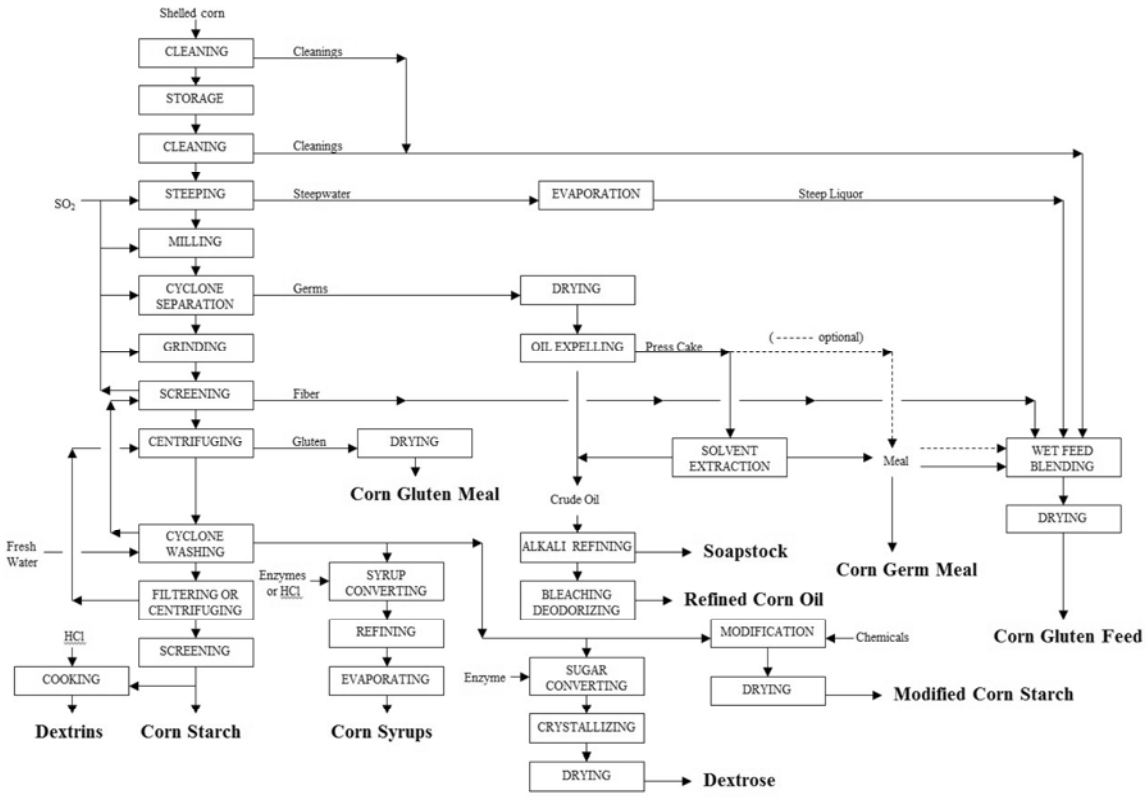

Fig. 3. Wet-milling process flow diagram (Courtesy of L.A. Johnson, the Center for Crops Utilization Research, Iowa State University).

(Bothast and Schlicher 2005). This proteolytic activity may hydrolyze the zein protein also. Papain can hydrolyze zein into low molecular weight peptides with molecular masses <10 kDa (Saito et al 1988). The rest of the brew after fermentation/distillation is separated into thin stillage and distiller's grains. The thin stillage is evaporated and combined with distillers' grains before drying to become DDGS. Drying distiller's grains can further degrade zein because of a harsh drying step that quickly reduces the moisture from 65 to 10–12% (Bothast and Schlicher 2005); the high heat conditions can induce cross-linking of zein protein in their native state in the protein body. With all the potential for changes to zein in DDGS, it could be the least preferred source to extract zein among the three methods described above. Zein proteins have been extracted in small scale from DDGS and characterized by SDS-PAGE (Wolf and Lawton 1997; Xu et al 2007).

EXTRACTIONS OF ZEIN FROM CORN PRODUCTS AND COPRODUCTS

Zein Extraction Solvents

Many different solvents can be used to extract zein. Much of the zein solvent solubility was determined based on the solubility of commercial zein. There are three different types of solvents for extracting zein: primary solvents, secondary solvents, and ternary solvents (Evans and Manley 1941, 1944; Manley and Evans 1943) (Table III). A primary solvent is a compound that could dissolve zein alone in a concentration >10% (Evans and Manley 1941). To stand alone as a solvent for zein, it needs to be able to interact with the amino acids of zein so that it could simultaneously dissolve both the polar and nonpolar amino acids in zein. Secondary solvents are organic compounds in two different classes: one group must be added to water and the other added to a lower aliphatic alcohol to gain solvation power (Manley and Evans 1943). These solvents rely on the organic compound to provide interaction with the nonpolar amino acids and water to interact likewise with the polar amino acids. Ternary solvents are similar to the secondary solvent's two classes of compounds. The ternary solvents must be a combination of solvent, water, and lower aliphatic alcohol.

A comprehensive list of primary, secondary, and ternary solvents can be found in the zein review by Lawton (2002). Most commonly, binary solvents of alcohols such as ethanol and 2-propanol are used for extracting of zeins. These two solvents are easy to separate from zein to aid in an easy recovery (Swallen 1941).

When evaluating a solvent or solvents, it is important to understand recovery and recyclability of solvents. If the final material is a zein concentrate, solvent cost would be very important because of solvent being lost with product. Mckinnery (1958) noted that α-zein is soluble in 95% (v/v) ethanol and 85% (w/w) 2-propanol. α-Zein, β-zein, γ-zein, and potentially δ-zein was soluble in 60% (v/v) ethanol. This combination of zein proteins in solution caused gelation (Pomes 1971).

Zein Extraction from DMC

Most of the zein extractions have been modeled off of dry-milled corn. The first zein extractions were from dry-ground corn as described in a patent by Osborne (1891). Dry-ground corn is all of the milled components of the kernel. In laboratory-scale operations, zein could be dissolved in 80–85% ethanol and concentrated by evaporating the alcohol. The zein then was redissolved in 90% ethanol and reduced and added to absolute ethanol to remove the pigment and lipid. Osborne noted that the extraction was not commercially practical due to only 6–7% zein yield of the total corn meal. His method of extraction was similar to the laboratory-scale method of the time, but used gluten meal which contained higher protein content. He extracted the zein by means of 95% (v/v) ethanol and recovered the dissolved zein by pressing the spent gluten meal, and precipitated the zein from the solvent by adding it to water. This method extracted mainly α-zein and lipid impurities were precipitated out by cooling.

A more recent extraction by Shukla et al (2000), using ethanol as solvent, identified many parameters for optimal zein extraction from milled corn before dry-grind ethanol processing using whole ground corn obtained from a dry-mill facility. Processes optimized were based on zein extraction time, temperature, ethanol concentration (% v/v), solvent-to-solids ratio, and a number of extractions.

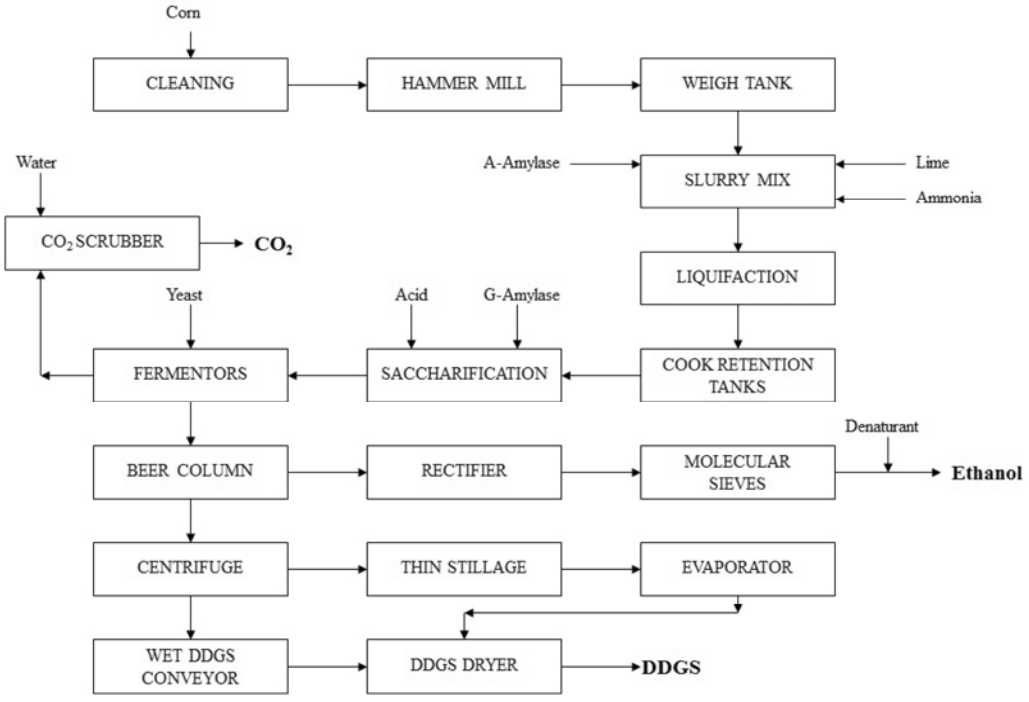

Fig. 4. Dry-grind ethanol flow diagram (adapted from Kwiatkowski 2006).

They utilized a quadratic mathematical model to identify optimal temperature (45.6°C), ethanol concentrations (68.1%), solvent-to-solids ratio (7.8 mL/g), and time for extractions (54.8 min).

The yield was 60% of the total zein with 50% protein purity. However, quality of the zein extracted was questionable because 1) the extraction solvent potentially extracted small amounts of β-zein, and 2) the prepared SDS-PAGE gel was indiscernible to allow for meaningful characterization of proteins extracted. Because the corn was not steeped, the β-zein may not be detrimental to the final zein quality, but there was no mention of zein solubility or functional properties. Protein purity of the extracted zein was low at 50%; lipids were co-extracted with zein. Purity would likely be improved if the corn was defatted before the extraction.

An extraction of DMC by Parris and Dickey (2001) used various pretreatments of substrate to explore differences in zein extraction and analyze film qualities. The pretreatments were 0.5% sulfuric acid, 0.55% lactic acid plus 0.2% sulfur dioxide, 0.5% sodium bisulfite, and 0.5% sodium sulfite at 50°C in water for 6 hr. When extracted, zein from pretreated DMC using 70% ethanol at 60°C for 2 hr yielded 1.8, 2.7, 1.3, and 2.1% for 0.5% sulfuric acid, 0.55% lactic acid plus 0.2% sulfur dioxide, 0.5% sodium bisulfite, and 0.5% sodium sulfite treatments, respectively. The isolate from these zein extractions were 80–85% protein, 15–20% lipid, and <0.25% starch. The authors also studied the solubility of zein extracted from DMC using 70% (v/v) ethanol under extraction temperatures of 23 and 60°C along with NaOH. They found that zein solubilities were 64 and 76% when extracted at 60°C with and without NaOH, respectively. At 23°C, zein solubilities were 96 and 95% with and without NaOH, respectively. A gradient was set up to study the SDS-PAGE protein profile of zein from DMC using extraction solvents that were 95, 90, 80, 70, 60, and 50% (v/v) aqueous ethanol. There was a gradual increase in presence of β-zein in the samples as the proportion of alcohol in the solvent dropped. These solubility characteristics showed that extraction procedures as in Shukla et al (2000) with 70% (v/v) aqueous ethanol from dry-milled corn may leave much as 24% recovered zein rendered insoluble. Absent from characterization were SDS-PAGE profiles of zein extracted from CGM at solvent concentrations of 50, 60, 70, 80, 90, and 95% (v/v) ethanol concentrations similar to that for ground corn. Detail of how β-zein in low concentration ethanol solutions will affect zein solubility.

Extraction of oils from milled corn is important for producing a useful oil stream and increasing a purity of extracted corn protein. Early work exploring the solubility of vegetable oils in anhydrous and azeotropic ethanol and 2-propanol was done by Harris et al (1947, 1949), Beckel et al (1948), Rao et al (1955), and Rao and Arnold (1956ab). A patent by Chen and Hoff (1987) used 90–100% aqueous ethanol to remove oil from cracked corn and subsequently extract protein from the remaining residue. They used 50–70% ethanol with 0.05–0.15*N* NaOH at 50–70°C. If only zein proteins are to be extracted, NaOH should not be used. Zein extraction was done at 50°C and 40% of the total corn protein was extracted with a solution concentration of 2.8% w/v zein. A second extraction using 50% ethanol and 0.08*N* NaOH extracted an additional 20% of corn protein. This procedure was successful in extracting both oil and protein using a single solvent. Higher titers of ethanol were able to extract oil while not extracting zein. A drawback to this procedure is that it extracted protein with an ethanol solution which may not be optimal for zein extraction. Zein in dry-milled corn extracted well with 70% ethanol, but at 50% ethanol, α-zein along with its dimers and trimers are not highly soluble (Parris and Dickey 2001). Treating with NaOH may help facilitate the protein extractions, but with a potential to co-extract nonzein proteins.

Another similar procedure by Hojilla-Evangelista et al (1992a,b) used a sequential oil extraction process that simulated countercurrent oil extraction. The process extracted >90% of the oil, which was superior to the estimated 72% oil extracted from conventional hexane prepress extraction. Nonoil materials were co-extracted with the oil. These materials were 25–30% protein and accounted for ≈10% of the protein initially in the corn. A 50% ethanol and 0.08*M* NaOH solvent extracted 57% of the total protein (Hojilla-Evangelista et al 1992b; Meyers et al 1994).

Zein extraction from defatted DMC was reported by Hojilla-Evangelista and Johnson (2003). Method A used a 4:1 70% aqueous ethanol-to-DMC ratio at 60°C for 1 hr, liquid solvent was collected and cold precipitated at –18°C, redissolved, and subsequently precipitated at –18°C once more and dried. In Method B 4:1 70% aqueous ethanol was added to ground corn at 60°C for 1.5 hr. The supernatant was collected and concentrated through a 10-kDa regenerated cellulose membrane by ultrafiltration, solids were air-dried, then further dried in vacuum oven. When comparing the two extraction methods, the Method A yielded 24% of the extractable zein while B had a 70% yield. They mentioned that when utilizing 80% ethanol in both methods, there was a decrease in yield down to 14% for Method A, and 44% for Method B, indicating a decrease of zein solubility with increasing ethanol concentration. The extracted LMW proteins, based on the SDS-PAGE analysis, were different even though zein was extracted using both Methods A and B using the same extracting solvent. Method A had two bands at 14.2–18.4 kDa and Method B showed a band between the α-zein and 18.4-kDa marker. The two bands at 14.2–18.4 kDa from Method A were considered to be nonzein protein, which showed that the chilled extract contained more impurities. The comparison between these two methods seems flawed in that extraction times were different by 0.5 hr along with a 7,000 × *g* variation in centrifugation. It would seem that a true comparison would synchronize the time of the extraction of the two methods employed. They do show some merit though, with Method B obtaining a higher yield of extractable zein. Method A employed cold precipitation, which caused the zein to leave solution, the protein precipitated on the walls of the vessel or in solution. But the zein also could stay dissolved into the solution or not completely precipitate out of solution. With such a low amount of zein extracted from dry-milled corn, it may be more prudent to follow Method B and use ultrafiltration to recover zein.

Zein Extractions from CGM

Commercial extractions of zein have classically utilized CGM because protein contents are 61.5–74% (db) with 60–71% zein proteins (Wu et al 1997b). One of the first commercial zein extractions from CGM was described in a patent by Swallen and Haute (1938). A well-defined extraction process was later patented by Swallen (1942) detailing extraction from CGM with 85% 2-propanol in a solute-to-solvent ratio of 1:3.5 at 60°C. The extracted zein solution and gluten were separated and the zein solution cooled to 15°C and filtered. The solution had much of the yellow pigment extracted in a mixture with 80 parts of hexane to 100 parts of zein solution. The hexane could be removed and the zein precipitated in water. The precipitated zein was then placed into ring dryers. The yield of zein from this method was 50% of the protein in the CGM, which is considered very high. The higher yield of zein was probably due to the use of a countercurrent extraction method rather than batch extraction. Even after hexane extraction, the zein was not completely decolorized but still was pale yellow due to residual pigment. The one major problem with this method was that it employed two extraction solvents that must be separated and recycled to be profitable. This incurs large costs and the hexane and 2-propanol separation to decolorize zein can carry some zein out of the 2-propanol layer, decreasing yield. Also, based on the current measurements of α-zein extractability from commercial CGM using a similar solvent and method, yields are only 21–32% (Wu et al 1997b) in comparison to the 50% reported by Swallen (1942).

TABLE III
Categories of Solvents That Extract Zein[a]

Solvents for Zein	Class A Primary Solvent[b]	Class B Secondary Solvent[c]	Class C Secondary Solvent[d]	Class D Ternary Solvent[e]	Class E Ternary Solvent[f]
Acetaldehyde			•	•	
Acetamide	•				
Acetic acid	•				
Acetone		•	•	•	
Acetonyl acetone		•		•	
2-amino-2-ethyl-1,3-propandiol	•				
2-amino-2-methyl-1-propanol	•				
Aniline	•				
Benzene			•	•	
Benzyl alcohol	•				
Benzyl Cellosolve	•				
1,3-Butanediol					•
1,4-Butanediol					•
2,3-Butanediol					•
n-Butanol		•			
t-Butanol		•			
s-Butanol		•			
Butylamine	•				
Butyraldehyde				•	
Butyl tartrate	•				
Butyl lactate			•		
1,3-Butylene glycol	•				
Chloroform			•		
o-Cyclohexylphenol	•				
Diacetone alcohol				•	
1,3-Diaminopropanol	•				
Di[-β-hydroxyethyl]aniline	•				
Dichloromethane			•		
Diethanolamine	•				
Diethylene glycol	•				•
Diethylene glycol monoethyl ether	•		•		
Diethylene glycol monomethyl ether	•				
Diethylenetriamine	•				
Diglycolchlorohydrine	•				
Diisopropanolamine	•				
Dioxalane		•			
Dioxane		•		•	
Dipropylene glycol	•				•
Ethanol		•			
Ethyl ether tripropylene glycol	•				
Ethyl lactate	•		•		
Ethylpheylethanolamine	•				
Ethylene chlorohydrine	•				
Ethylene dichloride			•		
Ethylene glycol	•		•		•
Ethylene glycol monoethyl ether	•			•	
Ethylene glycol monomethyl ether	•				
Ethylenediamine	•				
Formaldehyde				•	
Formic acid	•				
Furfuryl			•		
Furfuryl alcohol	•				
Glycerol	•				
Glycerol furfuryl	•				
Glycerol-α-γ-dimethyl ether	•				
Glycerol-α-monochlorohydrine	•				
Glycerol-α-methyl ether	•				
Glycerol-α-phenyl ether	•				
Hexylene glycol					•
β-Hydroxyethylaniline	•				
Hydroxyethylethylenediamine	•				
2-Hydroxymethyl-1,3-dioxolane	•				
Isobutanol		•			
Isopropanol		•			
Lactic acid	•				

(continued)

[a] Table created based on solvent classes as categorized in Lawton (2002) and references therein.
[b] Primary solvent: compound that alone dissolves zein in a concentration >10%.
[c] Secondary solvent: dissolved zein when solvent was combined with water.
[d] Secondary solvent: dissolved zein when solvent was combined with a lower aliphatic alcohol.
[e] Ternary solvent: dissolved zein when solvent was combined with water and a lower aliphatic alcohol.
[f] Ternary solvent: dissolved zein when e solvent was combined with water, and another class-E solvent. Compiled data of CPSC (1949), Evans and Manley (1941, 1943, 1944).

TABLE III (*continued*)
Categories of Solvents That Extract Zein[a]

Solvents for Zein	Class A Primary Solvent[b]	Class B Secondary Solvent[c]	Class C Secondary Solvent[d]	Class D Ternary Solvent[e]	Class E Ternary Solvent[f]
Methanol	•	•			
Methyl acetate				•	
Hydroxyethylethylenediamine	•				
2-Hydroxymethyl-1,3-dioxolane	•				
Isobutanol		•			
Isopropanol		•			
Lactic acid	•				
Methanol	•	•			
Methyl acetate				•	
Methyl ethyl ketone			•		
Methyl Lactate	•				
Methylene Chloride			•		
Monoethanolamine	•				
Monoisopropanolamine	•				
Morpholine	•				
Morpholine ethanol	•				
Nitroethane			•	•	
Nitromethane			•	•	
Phenol	•				
Phenylethanolamine	•				
n-Propanol		•			
Propionic acid	•				
Propylene chlorohydrin	•				
Propylenediamine	•				
Propylene glycol	•		•		•
Pyridine	•				
Resoricinol monoacetate	•				
1,1,2,2-Tetrachloroethane			•		
1,2,3-Tetrachloroethane			•		
Triethanolamine	•				
Triethylenetetramine	•				
Tetrahydrofurfuryl alcohol	•				
Toluene			•		
Triethylene glycol	•				
Triisopropanolamine	•				

[a] Table created based on solvent classes as categorized in Lawton (2002) and references therein.
[b] Primary solvent: compound that alone dissolves zein in a concentration >10%.
[c] Secondary solvent: dissolved zein when solvent was combined with water.
[d] Secondary solvent: dissolved zein when solvent was combined with a lower aliphatic alcohol.
[e] Ternary solvent: dissolved zein when solvent was combined with water and a lower aliphatic alcohol.
[f] Ternary solvent: dissolved zein when e solvent was combined with water, and another class-E solvent. Compiled data of CPSC (1949), Evans and Manley (1941, 1943, 1944).

Carter and Reck (1970) proposed an extraction process that is considered to be the most common commercial method based on Swallen's work (1938, 1942). Carter and Reck's method extracted zein using 88% (w/w) aqueous 2-propanol with 0.25% NaOH for 1 hr at 1:4 solute to solvent ratio and 55–65°C. The resulting zein solution was separated from the spent CGM and subsequently chilled to –15°C. The zein precipitated into a taffy-like solid and the supernatant was discarded. One-half of the solid was used to produce a low quality zein when the solids were dried at 0.06 atm and 50°C. Redissolving the second one-half of the zein solids from the first precipitation in 88% aqueous 2-propanol and performing a second precipitation produced high-quality zein when dried similarly. The yield of the total zein from both parts was ≈22% of the CGM mass used for the extraction. This method has problems mostly with the means of extraction and the cold precipitation. Extractions using higher titer alcohols such as 95% (v/v) ethanol are good at extracting just α-zein, but the yields are low. 2-Propanol used at 85% (v/v) has a solvation potential for zein similar to 92% (v/v) ethanol (Swallen 1942). Without extracting the outer layers of the protein body, which are not soluble in those extraction solvents, the amount of α-zein extracted from the core of the protein body may be low. With commercial CGM containing 36–47% α-zein, the extraction is not very efficient, but still obtains a lot of zein because of the high amount of protein (Wu et al 1997b). A cold precipitation step works better for extraction from CGM than from DMC, but still has potential for not precipitating all zein from solution. This precipitation may further decrease the zein yield coupled with poor solvent. Even with the low extraction efficiency, this zein has good solubility characteristics and is of good quality for commercial use.

The zein produced by Carter and Reck (1970) had a yellow hue; Cook et al (1996) invented a process that removed pigments. Their extraction destarched the CGM first and then washed it several times with absolute ethanol to remove pigment and oil. The CGM was then washed with water and extracted with 80% ethanol. The extract was treated with activated carbon to remove flavors and pigments. Water precipitated the zein, which was dried for pharmaceutical use. Cook et al (1993) mentioned that after extracting the CGM with 70–90% ethanol (v/v), the extraction cake can be purified and a glutelin by-product can be collected, which can be used to make products such as vegetable protein supplements. This method can extract zein without pigment. However, copious amounts of ethanol must be used to wash the pigments from the CGM and the multiple separations. The pellet was resuspended as many as five times in two volumes of 100% ethanol to remove all the pigment. In addition to the large amount of solvent, ≈2% of zein was lost in the pigment extraction. The solvent used may affect the quality of zein extracted. Zein extracted with high titer alcohol concentrations, such as 90–95% ethanol, produced high quality zein (Swallen 1942). Solvents with

less ethanol may extract the other zein fractions, which impairs resolubility of extracted zein with the benefit of increased yield.

Another method for extracting and decolorizing zein was patented by Takahashi and Yanai (1994) of Showa Sangyo, Japan. The method extracted zein from CGM using 70% (v/v) aqueous acetone at 40°C for 4 hr at a solute-to-solvent ratio of 1:5. The solution was separated from the solids and concentrated by evaporation. Absolute acetone was added to the precipitate to render it into honey-like consistency. The syrup was added drop-wise to an absolute acetone solution to precipitate the zein. The method recovered 20.4% yield of white zein to starting CGM. This method performed better than that of Carter and Reck (1970) in that the zein was mostly depigmented. Using size-exclusion chromatography of Showa zein and analyzing the eluted solvent with absorbance spectroscopy, small peaks matched those of xanthophylls (Cheryan et al 2007; Kale et al 2007). Zein may have appeared depigmented in its dry form, but when observed in solution it still contained pigments (Sessa et al 2003). The authors stated that zein obtained was of high purity, but protein purity was not stated, also the solubility of the zein was uncertain. Evans and Foster (1945) had extracted zein using similar aqueous acetone solutions and found a different solubility than alcohol-extracted zein. There was no evidence that the zein was actually low in solubility, but only that it may have a different solubility profile because of the difference in the extracting solvents.

While most extractions of zein are done with aqueous alcohols, a method by Selling and Woods (2008) showed that glacial acetic acid could be used to extract large quantities of zein from CGM, and to a lower degree zein from ground corn and DDG. The method extracted zein from the materials using 25 g of dry solids in 75 g of acetic acid. The extraction was done at 25°C for 1 hr, and the supernatant was separated from the solids by centrifugation. The zein yields were 37.2, 1.2, and 3.2%, for CGM, ground corn, and DDG, respectively. The percent protein content of the zein from CGM, ground corn and DDG was 84, 67, and 20%, respectively. These higher yields were due mainly to lipids and pigments extracted with the zein. They concluded that zein extracted with acetic acid had a SDS-PAGE profile similar to zein extracted using other solvents and commercial zein. This method seems to get large yields of what appears to be zein, based on the SDS-PAGE which showed protein bands from the acetic acid extraction to be nearly homologous to commercial zein and zein extracted with 80% ethanol. They also demonstrated that the films of zein extracted with acetic acid had a tensile strength of 18 MPa, 11% elongation, and 293 MPa Young's Modulus. The film prepared from commercial zein had a tensile strength of 43 Mpa, 13% elongation, and 777 MPa Young's Modulus. This showed that the films prepared from acetic acid zein extract had physical strength properties lacking in comparison to the commercial zein. Also, solubility characteristics were only demonstrated for acetic acid and no other solvent, which does not make it clear whether the zein would be soluble in aqueous alcohols, or show a similar profile for solubility. Other deleterious properties of using this solvent are that zein binds the acetic acid and may hold a pungent acid odor after a potentially expensive solvent removal.

Zein Extractions from DDGS

In recent years, as ethanol dry-grind processes have become widely utilized, DDGS has become much more available. Wu et al (1981) gave insight into the composition of the protein fractions of DDGS and materials at the base of still after corn ethanol distillation. Four consistent extraction methods followed with two different methods of extraction with reducing agent were employed and compared to determine protein solubility based on Landry and Moureaux (1970). The four consistent extraction procedures consisted of a water extraction, a sodium chloride extraction, a 70% ethanol extraction, a 70% ethanol plus dithiothreitol (DTT) extraction. These first four extractions used for both methods extracted 14% of the total protein. The first reducing method utilized borate, SDS, and DTT extracted only 30% of the DDGS protein while 51% of the total protein was left in residue. The second method with NaOH and DTT at pH 11.9 extracted 28% of the protein; the next step using NaOH with SDS and DTT extracted an additional 26% protein and 18% was left in the residue. Extractions using methods one and two could not extract more than ≈45 or ≈70% of the total DDGS protein, respectively. The zein protein was just fractionated and no attempt was made at characterization of zein proteins. The reasoning provided for the low protein yield in comparison to corn was attributed to denaturation of the protein during alcohol distillation.

Further work on DDGS extractions with reducing agents was done by Wolf and Lawton (1997). Nine different materials were extracted and compared in this work; among them were corn flour, CGM collected in a centrifuge and air-dried, CGM commercially dried in a drum dryer, whole stillage from the dry-grind ethanol process, a DDGS sample of each dry and wet material, all wet samples of whole stillage that had been freeze-dried, and three other DDGS materials that were commercially dried. The yields of crude zein from the extractions were 3.2–6.6%, but protein contents of these yields were only 37–57%; lipids and pigments co-extracted were to blame for decreased protein content. SDS-PAGE showed that DDGS contained faint bands of α-zein. The authors concluded that because of the low protein purity of the samples, integration of the zein from DDGS into biodegradable materials was still far off. This extraction showed that extracting zein from DDGS was possible, although the yields were low, even with a reducing agent. Defatting DDGS would most likely be a remedy for the low purity of the extracted zein proteins.

Extraction of zein from ethanol-defatted DDGS under acidic and basic conditions with reducing agent was done by Xu et al (2007). Zein was extracted from DDGS using 70% ethanol and 0.25% sodium sulfite with the pH altered using HCl or NaOH. The optimal yield of zein solid obtained was ≈90% protein and a recovery of 44% of the protein in DDGS was attained at pH 2. The high purity of protein was promising and the method also extracted a higher yield. The quality of zein thus extracted is of concern with potential non-α-zein proteins extracted. The use of a reducing agent may also liberate glutelin proteins. The SDS-PAGE of the protein in DDGS extracted at pH 2 had a similar protein profile to that of commercial zein and contained a large degree of α-zein.

One successful extraction of an utilizable zein from a dry-grind method wasn from DDG not DDGS. POET (Sioux Falls, SD) has been able to produce an edible zein product called Inviz extracted from POET's Dakota Gold HP distillers' grains using the BFRAC dry-mill ethanol process (*personal communication,* Summary of Characteristics of INVIZ, POET representative, Sioux Falls, SD). The resultant DDG from the method contained ≈40% protein. The zein had many different properties from that of conventional commercial zein extracted from CGM; it contained not only α-zein, but also β- and γ-zein. Possibly, the zein contained these fractions because it had not been steeped, giving the zein properties similar to that of zein extracted from dry-milled corn that can be directly used to make films (Boundy et al 1967). The steeping process reduces intermolecular disulfide bonds that can cause β- and γ-zein proteins containing many cysteine residues to unfold and change conformation when the thiol groups are reoxidized. The Inviz zein was described as being slower at dissolving in aqueous alcohols than commercial zeins containing just α-zein (*personal communication,* POET representative). No information is available as to whether a reducing agent was used to facilitate more complete extraction of zein from the DDG.

Another recent release of a commercial zein that has been implemented within the dry-grind ethanol process has been made by Prairie Gold (Bloomington, IL). Their product is called corn oil

and protein extracted (COPE) zein or COPE-zein and is extracted from the ground corn at the front end of the dry-grind ethanol process whereas the POET process is a back end extraction process (Cheryan 2009). The COPE process simultaneously produces both high-quality corn oil with beneficial nutrients and commercial quality zein. This extraction from ground corn uses 90–100% aqueous ethanol that extracts mainly corn oil and small amounts of zein. A second extraction of ground corn with 60–90% aqueous ethanol yields a majority of the zein from the corn. Ultrafiltration and nanofiltration of the extracts allows oil and zein to be collected and solvent to be recycled into the system (Cheryan 2002). Size-exclusion chromatography can be used with this technology to further purify and separate zein from the pigments and oils (Cheryan et al 2007). The benefit of such zein is that it has not been altered by either steeping or fermentation (Cheryan 2009).

ZEIN PURIFICATION

Zein extracted conventionally with aqueous alcohols or aqueous acetone contains carotenoids including β-carotene, zeaxanthin, and lutein that give zein its yellow hue (Quackenbush et al 1961; Blessin 1962; Kurilich and Juvik 1999). Decolorized zein commands higher prices and has more uses than conventional yellow zein. Sessa et al (2003) investigated the ability of conventional procedures to discolor zein, such as partitioning and activated carbon (Mason and Palmer 1934; Swallen and Haute 1938; Pearce 1941; Starling et al 1951). They compared the conventional methods to the newer processes of column chromatography, supercritical fluid extraction with CO_2 (SFE-CO_2), ultrafiltration and diafiltration, and subcritical propane extraction. Combination of sephadex LH-60 in column chromatography and ultrafiltration and diafiltration removed pigments effectively. The best method was activated carbon, but as the zein in solution was dilute, other procedures such as SFE-CO_2, and column chromatography also removed nearly the same amount of pigment. Recently, Sessa (2008) reported that zein could also be deodorized as well as decolorized with activated carbon at various temperatures and that the odor component was diferuloylputrescine. When zein in solution was heated to 55°C, color and odor compounds that bound to the activated carbon were significantly increased. This increase was attributed to a denaturation of the α-helical nature of zein, which houses lutein (Momany et al 2006). The removal of the odor improves zein's marketability for applications such as gum (Sessa and Palmquist 2008). Besides activated carbon, Sessa and Palmquist (2009) used zeolites to bind color and odor components. The activated carbons and zeolites both adsorbed protein as well as the color and odor components, thus reducing the efficiency of the zein purification.

Other zein decolorizing work using column chromatography obtained a high-value pure xanthophyll stream from zein before dry-grind ethanol processing (Cheryan 2001, 2002), whose method to purify xanthophylls used extensive ultra- and nanofiltration and diafiltration to obtain zein at >90% purity. To simplify xanthophyll extraction, Kale et al (2007) and Kale and Cheryan (2009) used LH-20 resin in a column. Zeins eluted first, nonzein impurities second, and xanthophylls last with good resolution. Most membrane separation methods such as ultra- and nanofiltration had lower zein purity and yield; however, with size-exclusion chromatography, both higher yield and purity >90% were possible.

ZEIN MODIFICATIONS

Zein Plasticization

Zein protein without a plasticizer produces brittle solids, thus it must be plasticized to provide flexibility. The α-zein protein contains a majority of nonpolar residues ≤53.2% but many other residues are polar, the most prevalent being glutamine (Geraghty et al 1981). An understanding of the amino acids present and the relative polarity determines what compounds can be used to plasticize zein. Parris and Coffin (1997) showed that a combination of glycerol and poly(propylene glycol) (PPG) increased zein film flexibility. They compared the water vapor permeability of films; low water vapor permeability values are ideal for packaging applications.

Water vapor permeability (WVP) for 15% glycerol, 30% glycerol/PPG, and no plasticizer were 1.01, 1.06, and 0.62 (g·mm/kPa·hr·m^2), respectively. Films without plasticizer had nearly double the WVP. This corresponded with the plasticized zein being less effective at blocking water vapor migration. Many zein plasticizers such as glycerol are not beneficial to zein solids because they are polar and migrate to the surface of the matrix (Parris and Coffin 1997). Initially, plasticized zein is pliable, but as the glycerol bleeds to the surface the zein becomes brittle. One plasticizer often overlooked is water. Zein plasticized with water has increased flexibility, and again becomes brittle when dehydrated, similar to other plasticizers (Wu et al 2003).

Fatty acids like oleic acid can be used to plasticize zein because of interaction with nonpolar amino acids such as proline and leucine (Geraghty et al 1981; Lai and Padua 1998). Flexibility of extracted zein formed into film after the removal of solvent most likely originates from endogenous corn pigments and lipids (Parris and Dickey 2001; Selling and Woods 2008). Parris et al (2002) found that presence of endogenous oils could have drastic effects on tensile strength and elongation to break zein films. Oleic acid was used as a plasticizer for zein to decrease WVP (Lai and Padua 1998). Wang et al (2004) characterized oleic acid and zein resins and showed formation of structured alternating layers of zein and lipid at nanoscale that led to improved barrier properties. Wang et al (2003) studied the binding of oleic acid to zein for plasticization using thermal conditions. They found that by extruding oleic acid and zein, films showed a higher degree of plasticization and decreased phase separation over films without extrusion. Another study by Wang and Padua (2006) found that oleic acid and zein films had different WVP depending on ambient temperature. Films at 4°C had lower WVP because of crystallized oleic acid and films at 25°C had higher WVP because of oleic acid in its liquid phase. Rakotonirainy and Padua (2001) studied fusion lamination and the effect of drying oils on oleic acid and zein films. Lamination produced films that were clearer, tougher, smoother, and more flexible than untreated oleic acid and zein films. Both oleic acid and zein films that had either the drying oil coating or lamination had increased tensile strength, % elongation, and toughness, but had a decreased modulus. The films with either lamination or drying oils both had decreased O_2 and CO_2 permeability, but only drying oils decreased WVP. Kleen et al (2002) found that as the oleic acid oxidized, the films lost color, became brittle, and had off-smells. They used butylated hydroxyanisole (BHA) as the antioxidant and found that at 4,000 ppm it protected the loss of natural zein pigments in the film over the control. Wang and Padua (2004) also showed that extrusion and plasticization of zein with oleic acid lowered water adsorption over zein powder alone. Zein nanocomposites are also of interest because of their inherent ability to decrease WVP and oil permeation (Arora and Padua 2010).

Zein Modification and Cross-Linking

Zein films are inherently water resistant, but gradual absorption of water decreases zein's utility as a packaging material. Biswas et al (2009) proposed a method to modify the surface chemistry of zein by derivatizing the film with octenyl succinic anhydride and alkyl and alkenyl ketene dimers. These compounds react with the surface residues and successfully decrease the ability of the film to absorb water during immersion. Wang and Padua (2005) showed that moisture absorption of the film can be reduced by using drying oils such as flax or tung oil, which can be cured on the films with UV light or γ-radiation. In a wetting test using water for 10 days, films with oil coatings did not allow penetration. Films without the oils allowed water to penetrate within one day. Com-

pounds such as polycaprolactone (PCL) have been successful copolymers with zein to improve water-resistance (Wu et al 2003). Incorporating PCL and plasticization with dibutyl L-tartrate in compression-molded zein sheets improved water resistance, tensile strength, and elongation.

Cross-linking of zein protein matrix can increase the strength and water resistance of zein films. Proteins such as zein have a wide variety of reactive side groups such as amide (53%), amine (1%), carboxyl (4%), hydroxyl (24%), and phenolic (8%) (Spence 1994). Many different compounds such as formaldehyde, glutaraldehyde, and epichlorohydrin are used to cross-link zein proteins (Parris and Coffin 1997). When these compounds were cross-linked with zein in aqueous ethanol, tensile strength and modulus significantly increased over the control. The opposite was true with films produced in aqueous acetone. The increased strength of cross-linked over noncross-linked zein in ethanol solution may be because the zein protein stays folded in solution. The steric effects of side groups do not allow the zein to efficiently align and form strong films (Yang et al 1996). Sessa et al (2007) studied the effects of cross-linking zein with glutaraldehyde in acetic acid. Zein was cross-linked with glutaraldehyde in a closed system and formed a gel that was not soluble in solvents that normally dissolved zein. Before testing, the gel bars were placed in boiling water for 10 min or 24 hr in room temperature water. The bars that were cross-linked retained their shape both in 10 min of boiling and 24 hr of standing in water; they showed increased strength, ductility, and stiffness compared to untreated zein samples. Sessa et al (2008) also cross-linked zein using glutaraldehyde while compression molding at a pressure of 12,500 psi at 99°C. Bars produced with and without compression molding were similar in tensile strength, ductility, and stiffness over unmodified controls. The benefit of compression molding was that it could reduce solvent use and improve recovery of acetic acid (Sessa et al 2008). The effects of time, temperature, and concentration of glutaraldehyde used for curing of electrospun zein fibers was explored by Selling et al (2008). Zein fibers that were derivitized with glutaraldehyde before spinning had increased tensile strength and were not soluble in standard zein solvents, while fibers produced without glutaraldehyde were still soluble. Heat promoted cross-linking, but did not improve tensile strength of the fibers. Woods and Selling (2007, 2008) and Selling et al (2009) cross-linked zein with glyoxal using extrusion in the presence of base. Woods and Selling (2007) cross-linked zein with glyoxal, formaldehyde, and methylglyoxal. Zein bars cross-linked with formaldehyde and glyoxal had significant increases in tensile strength over control and were also resistant to boiling water. Methylglyoxal did not increase the tensile strength. Woods and Selling (2008) evaluated effect of concentration of base, glyoxal, and melt temperature on compression-molded zein bars. Varying temperature and time during the melt processing step before compression molding did little to change the solubility of the zein bars. The tensile strength of the zein bars did increase with increased processing time and temperature. Selling et al (2009) used a twin-screw extruder to cross-link zein with glyoxal during the extrusion; the bars resisted dissolution in acetic acid whether they were injection-molded or compression-molded. The compression-molded samples had higher tensile strength than those that were injection-molded. Incorporating glyoxal improved resistance to acetic acid but not tensile strength of samples. Also, even though these cross-linking agents impart strength, many of them are toxic (formaldehyde) and may have limited practical use with zein unless rinsed away or rendered inert during processing. Milder cross-linking reagents like 1-[3-dimethylaminopropyl]-3-ethyl-carbodiimide hydrochloride and *N*-hydroxysuccinimide were used to cross-link zein (Kim et al 2004).

These reagents are zero-order cross-linking reagents that lose atoms during the reaction and link the carboxyl and amine groups of two different protein molecules. Cross-linking decreased the aggregation of the zein and improved the tensile strength of zein; they could also be washed away with water for a nontoxic zein. It is also possible to sufficiently cross-link the zein using safer compounds such as water. Pelosi (1997) mixed water and zein together and heated the mixture to 150°C for 22 min in a press with a total force of 4,500 kg, and cooled the mixture to 60°C with pressure before removal. This product was cross-linked sufficiently enough to not dissolve in 50% acetic acid, which was a good indicator of complete cross-linking. However, a solubility test in 50% acetic acid may not be a good indicator as zein is readily soluble in a single solvent (primary solvent) such as acetic acid (Selling and Woods 2008) and data are lacking on whether acetic acid and water would be a good zein solvent. Most likely, an acetic acid and water mixture would not appreciably dissolve uncross-linked zein (Lawton 2002) and would most likely not be a good indicator of cross-linking.

ZEIN APPLICATIONS

Zein has had a variety of applications: plastics, coatings, inks, chewing gum, adhesives, and fibers, etc. (Sturken 1938; Coleman 1939, 1941; Croston et al 1945; Lougovoy 1949; Simonds et al 1949). When synthetic materials became cheaper in the 1950's, zein products were not cost-effective and lost use. Rathman (1954) compiled a comprehensive literature search of patents from 1891-1953 that is a good source for those seeking information on zein applications before 1954.

Currently, much of the zein from CGM is used for food and pharmaceutical coatings (Shukla 1992). Being mostly nonpolar in nature, zein films have been explored for coatings in numerous food applications. Rakotonirainy et al (2001) used three-ply pressed oleic acid zein resin sheets laminated with tung oil for broccoli preservation. Both the zein film and polyethylene films allowed retention of broccoli firmness and color after six days in refrigerated storage; broccoli in zein-only films lacked off-smells. Another method used zein to help preserve the integrity of a turkey product (Ilter et al 2008). The turkey was dusted with zein and soy protein isolate before frying. Zein's film-forming properties were credited for helping reduce the uptake of oil. Zein coatings have even been considered as a means to control undesirable seed germination. Broccoli and sugar beet seeds germinated later and more slowly when dressed with a light zein coating (Assis and Leoni 2009. The slow germination was attributed to the coating preventing moisture permeation.

In recent years, hydrophobity of zein in water dispersions has been widely studied. Microspheres and nanospheres of zein have a wide variety of uses in the food and drug sector. Stark and Gross (1991) detailed the controlled production of microparticles of zein and showed that it could be used as a substitute for most dietary fats as protein has lower caloric density than lipids. The size of fat substitute particles was ≈4.0 μm, giving the apparent mouthfeel of fats and lipids. Micro and nanoparticles of zein have been studied as carriers of nonpolar drugs; microspheres of zein have been produced that contain Ciprofloxacin, an antibiotic (Fu et al 2009). They showed that these antibiotic-laden zein spheres inhibited bacterial growth compared to control spheres.

Recently, biomedical field and controlled self-assembly has seen newer applications of zein. Many of these new processes need purified decolorized and deodorized zein. Dong et al (2003) grew human liver cells (HL-7702) and mice fibroblast cells (NIH3T3) on zein films and used polylactic acid (PLA) and Corning microplates as control. Zein films were produced from zein particles that agglomerated upon drying. The zein film with the smallest zein particles produced from the solvent (0.3% w/v) showed the best results for proliferation of both cells after three days. The films produced from zein particles 100–2,500 nm did not show significant differences in cell proliferation. Zein is promising for tissue work because it has high tensile strength to support the cells. The film and scaffold dissolution after about two weeks is

beneficial when replacement cells have taken hold and no longer need the support scaffold. More elaborate three-dimensional porous zein scaffolds for tissue support produced by Wang et al (2007) had ≈80% porosity and 100–380 μm diameter pores. They were implanted into 15 rabbits over a period of 242 days. The state of the scaffold degradation and tissue growth were observed at 7, 28, 91, 183, and 242 days by euthanizing rabbits. The rabbits showed good tissue compatibility; blood vessels could form within the scaffolds and the scaffolds were completely degraded at the end of 242 days. Tu et al (2009) studied the growth of bone tissues on zein scaffolds to repair the radius bone of the rabbit. They studied bone repair with X-ray imaging of control (without assistance), repair with zein scaffold, and repair with zein scaffold and rabbit mesenchymal stem cells (MSC) at 2–12 weeks. It was apparent through gross observation at the end of 12 weeks that the control bone was still highly damaged; the zein scaffold supported bone was partially repaired; and the bone with scaffold and MSC was nearly repaired. Electrospun zein fibers cross-linked with citric acid were prepared by Jiang et al (2010) for growth with NIH 3T3 mouse fibroblast cells. The fibers were prepared as mats and treated with phosphate-buffered saline (1xPBS, pH 7.4) for analysis of cell growth on electrospun zein scaffolds cross-linked and treated with PBS, electrospun uncross-linked zein scaffolds; electrospun cross-linked zein scaffolds with (SHP), and electrospun PLA.

Zein scaffolds that were cross-linked and treated with PBS grew cells that had the best attachment, spreading, and proliferation. The zein fiber scaffolds supported cell proliferation better than film-based zein scaffolds because of higher porosity in mats (Jiang et al 2010). The observation of higher cell growth with higher porosity is consistent with the results of Dong et al (2003). Zein scaffolding have also been produced to coat surfaces by taking advantage of its chemical affinities (Wang et al 2008). They showed that by patterning certain hydrophobic or hydrophilic compounds on gold sheets and allowing zein solution to self-assemble on them produced zein overlays on these patterns that interacted differently based on the water affinity of compounds. This ability to produce controlled zein structures would be very important for the consistent production of high-ordered zein scaffolds.

SUMMARY

Zein is a protein biopolymer that is renewable and can be extracted from corn and corn coproducts. The ability for zein to be renewable is important now that other synthetic polymers are tied to increasing prices in oil. Also enhancing the importance of zein is its inherent water insolubility and ability to be plasticized and cross-linked, which can impart desired flexibility, strength, toughness, permeation resistance, and solvent insolubility. New interest in utilizing renewable polymers has helped spur interest in zein extractions and applications. As more corn is being used in dry-grind ethanol process, there is more interest in zein extraction from both front- and back-end coproducts. New technologies have allowed the production of DDG with higher percentages of protein. There has also been renewed work improving the purity and functionality of zein for new applications. The ability to produce decolorized and deodorized zein has allowed application of extremely pure zein in the medical field for tissue technology and cell growth. Other new uses have been in the packaging field, various food applications, and also producing spheres that can bind drugs and other labile compounds for time-released purposes.

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