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Zein: the industrial protein from corn

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Abstract

Zein is the major storage protein of corn and comprises ≈45–50% of the protein in corn. It was first identified in 1897, based on its solubility in aqueous alcohol solutions. Zein isolate is not used directly for human consumption due to its negative nitrogen balance and poor solubility in water. Current zein manufacture is limited to ≈500 tonnes per year from corn gluten meal. Zein sells for ≈US$10–40 per kilogram, depending on purity. The ability of zein and its resins to form tough, glossy, hydrophobic grease-proof coatings and their resistance to microbial attack have been of commercial interest. Potential applications of zein include use in fiber, adhesive, coating, ceramic, ink, cosmetic, textile, chewing gum and biodegradable plastics. These new applications of zein appear promising, but requires the development of low-cost manufacturing methods. This paper reviews the present status of the chemistry, properties, uses and methods of manufacturing zein. The characteristics of zein are discussed in terms of its composition, structure, solubility in various solvents and gelation properties.

Keywords: Corn; Extraction; Protein; Solubility; Zein

1. Introduction

Corn or maize (*Zea mays* L.) is the only cereal crop indigenous to the Americas and one of the most important food and industrial crops in the US. It is a warm-season crop, requiring warmer growing temperatures than other grains. World annual production is ≈560 million metric tons, of which the US alone produces about one-half. The 'Yellow Dent' is the major variety grown for animal feed, food ingredients and industrial products. The major parts of the corn kernel are the endosperm and the germ, which contain most of the starch and oil, respectively (Fig. 1). The distribution of the major components of corn is presented in Table 1. Also shown in Table 1 is the proximate composition of the pericarp and tip cap, as well as the major protein coproducts of corn processing.

Corn is processed primarily by four methods: dry milling, alkaline processing, wet milling and the dry grind process for ethanol production (Fig. 2). Alkaline processed and dry milled corn go directly for human consumption (Watson and

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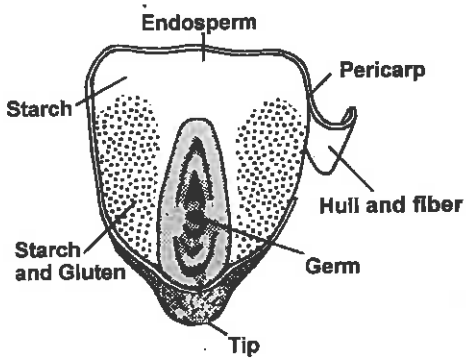

Fig. 1. Cross-section of corn kernel showing location of major components.

Ramstad, 1987). The primary products from wet milling are starch and oil, whereas the main product of dry grind ethanol plants is ethanol. The protein byproducts from corn wet milling are corn gluten meal (CGM) and corn gluten feed (CGF). In a dry-grind ethanol process, the protein ends up in the distillers dried grains (DDG) or DDG with 'solubles' (DDGS). At present, these protein byproducts, with varying protein contents (Table 1), are incorporated into animal feed.

The protein content of different corn varieties is 6–12% on a dry basis. About 75% of the protein is contained in the endosperm tissue. The remainder is distributed between the germ and bran. Zein alone determines the hardness of corn endosperm. Four major classes of protein in corn are defined primarily by their solubility in selected solvents (Table 2). Zein belongs to the characteristic class of proteins known as prolamines, which occur specifically in cereals (the equivalent of hordein in barley and gliadin in wheat). It was named by John Gorham in 1821 who first identified it by infusing water in *zea*, known as 'Indian corn' in the US (Gorham, 1821). Almost all the zein is present in the endosperm, whereas glutelin is distributed between the endosperm and the germ. The albumins and globulins are present mainly in the germ (Table 2). Among the wet milling protein coproducts, the endosperm proteins are found mostly in CGM, whereas the germ proteins occur mostly in CGF.

This paper focuses on the alcohol-soluble protein, zein. Zein has been of scientific interest since its isolation in 1821. It is deficient in essential amino acids, such as lysine and tryptophan and this makes it poor in nutritional quality. Its insolubility in water limits its use in human food products. Thus, the main focus since the mid-20th century has been on its possible utilization as an industrial polymer. Several attempts have been made to develop a commercial, cost effective process for zein. Commercial production of zein from corn gluten meal began in 1939. However, current zein production worldwide does not exceed 500 tons per year and it is produced by only two companies (Freeman Industries, USA and Showa Sangyo Corp., Japan). It is a high value product,

Table 1
Distribution of major components in corn and some corn processing by-products[a]

Component	Whole kernel (%)	Dry weight of components (%)						
		Endosperm	Germ	Pericarp	Tip Cap	CGF	CGM	DDGS
Starch	62.0	87	8.3	7.3	5.3	27	20	–
Protein	7.8	8	18.4	3.7	9.1	23	65	27
Oil	3.8	0.8	33.2	1	3.8	2.4	4	13
Ash	1.2	0.3	10.5	0.8	1.6	1	1	4
Others*	10.2	3.9	29.6	87.2	80.2	46	10	56**
Water	15.0	–	–	–	–	–	–	–

[a] CGF, corn gluten feed; CGM, corn gluten meal; DDGS, distillers dried grains with solubles. Data sources: Watson and Yahl (1967); Reiners et al. (1973); Anonymous (1982, 1997, 1999); Neumann and Wall (1984); Watson and Ramstad (1987); Singh and Cheryan (1998).

* By difference. Includes fiber, nonprotein nitrogen, pentosans, phytic acid, soluble sugars, xanthophylls.

** Also includes glycerol, organic acids and other byproducts of ethanol fermentation.

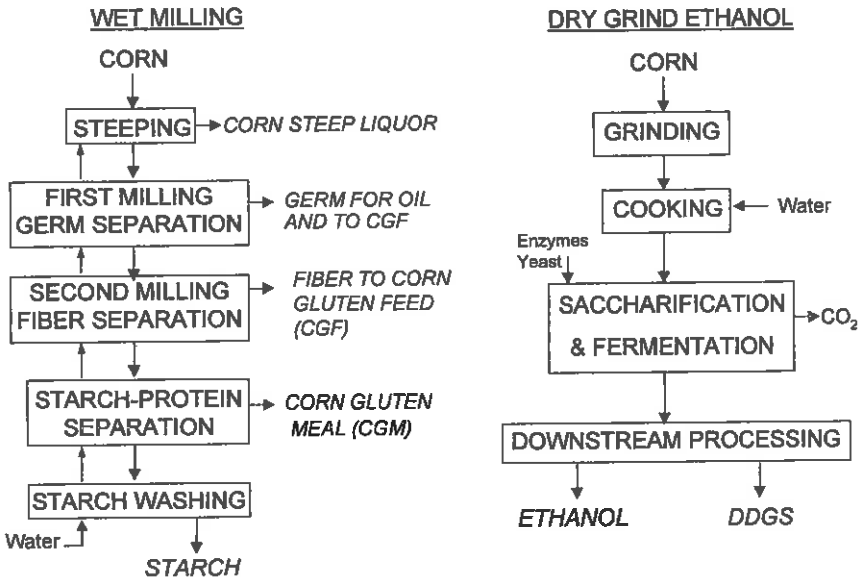

Fig. 2. Process flow sheets for corn wet milling (left) and dry grind ethanol production from corn (right).

with prices varying from $(US)10–40 per kg, depending on purity. However, while this price represents a very high value addition to corn, it also limits more widespread utilization of zein. Methods must be found to significantly lower the cost of manufacturing and to increase the utilization of zein.

2. Characteristics of zein

2.1. Composition

Zein is located in 'zein-bodies' of ~1 μm distributed uniformly throughout the cytoplasm of corn endosperm cells between starch granules of 5–35 μm (Duvick, 1961). Zein's defining characteristic is insolubility in water except in the presence of alcohol, high concentrations of urea, high concentrations of alkali (pH 11 or above) or anionic detergents. This is due to its amino acid composition (Table 3). Zein is particularly rich in glutamic acid (21–26%), leucine (20%), proline (10%) and alanine (10%), but deficient in basic and acidic amino acids. The notable absence of tryptophane and lysine in zein accounts for its negative dietary nitrogen balance. The high proportion of nonpolar amino acid residues and deficiency in basic and acid amino acids is responsible for the solubility behavior of zein. In whole corn, zein occurs as a heterogeneous mixture of disulfide-linked aggregates having a weight average molecular weight of 44000 Da (Mossé, 1961; Pomes, 1971).

Zein is actually a mixture of different peptides of various molecular size, solubility and charge. Two major fractions of zein, α and β, were first described by McKinney (1958). α-Zein was defined as that prolamine of corn soluble in 95% ethanol and represents ≈80% of the total prolamine present in corn. This protein closely resembles the zein available commercially before 1957 (Turner et al., 1965). α-Zein contains less his-

Table 2
Distribution of protein fractions in corn (% dry basis)

Protein	Solubility	Whole kernel	Endosperm	Germ
Albumins	Water	8	4	30
Globulins	Salt	9	4	30
Glutelin	Alkali	40	39	25
Zein	Alcohol	39	47	5

Table 3
Amino acid composition of zein (g amino acid/100 g zein)

Class	Amino acid	Native zein Mossé (1961)	Commercial zein Pomes (1971)
Nonpolar	Glycine	0	0.7
	Alanine	10.52	8.3
	Valine	3.98	3.1
	Leucine	21.1	19.3
	Isoleucine	5	6.2
	Phenylalanine	7.3	6.8
	Tryptophane	0.16	NR*
	Proline	10.53	9.0
–OH	Serine	7.05	5.7
	Threonine	3.45	2.7
	Tyrosine	5.25	5.1
–S	Methionine	2.41	2.0
	Cysteine	0.83	0.8
Basic	Lysine	0	NR
	Arginine	4.71	1.8
	Histidine	1.32	1.1
Acidic	Aspartic acid	4.61	NR
	(as aspargine)	NR	4.5
	Glutamic acid	26.9	1.5
	(as glutamine)	Nr	21.4

* NR, not reported.

tidine, arginine, proline and methionine than β-zein. When zein extracts of corn were analyzed by starch gel electrophoresis, there were four distinct bands that migrated into the gel and another major fraction remained at the origin. The components migrating into the gel were labeled α-zein, whereas those remaining at the origin were called β-zein (Pomes, 1971). By proximate analysis, Paulis et al. concluded that ≈35% of the total zein was α-zein, which had two major bands with molecular weights of 24000 and 22000 and an amino acid and peptide composition similar to whole zein (Paulis et al., 1969; Paulis and Wall, 1977; Wall and Paulis, 1978; Paulis, 1981).

β-Zein is soluble in 60% ethanol and insoluble in 95% ethanol. This zein is relatively unstable, precipitating and coagulating frequently and consequently, was not a constituent of commercial zein preparations. Pomes (1971) suggested that β-zein could be a high molecular weight protein formed from disulfide-linked α-zein molecules. For example, after application of a reducing agent, β-zein migrated in the starch gel and displayed three major bands with molecular weights of 24000, 22000 and 14000 Da.

Moureaux and Landry (1968) and Paulis et al. (1969) identified other fractions of zein using reducing agents. Gianzza et al. (1976) fractionated zein into two broad categories, one soluble in isopropanol (termed 'Z_1') and the other soluble in isopropanol containing 2-mercaptoethanol ('Z_2'). The Z_2 fraction was further resolved into four bands by SDS-electrophoresis with molecular weights of 23000, 21000, 13000 and 9600. However, the Z_1 and Z_2 components were nearly indistinguishable from each other by isoelectric focusing. These components were further resolved into at least 15 fractions between pH 5 and 9 by staining (Soave et al., 1975; Gianzza et al., 1976), and ≈20 fractions by TCA precipitation (Landry et al., 1983).

Zein has also been fractionated by precipitation, which involves adding water to solutions of zein in ethanol or using cellosolve (Watson et al.,

1936; Gortner and MacDonald, 1944), cation exchange chromatography (Craine et al., 1961, Landry and Guyon, 1984a), differential solubility (Osborne, 1924; Gortner and MacDonald, 1944; Mertz and Bressani, 1957; Mertz et al., 1958; Esen, 1987), cryo-precipitation, charcoal filtration and gel filtration (Danzer and Rees, 1971, Mossé and Landry, 1980; Landry and Guyon, 1984b). The various fractions have been referred to as glutelin-1, alcohol-soluble reduced glutelin (ASG), zein-2, zein-like, γ-zein, C-zein, D-zein and reduced-soluble protein (Paulis et al., 1969; Paulis and Wall, 1977; Wilson, 1985; Esen, 1986, 1987). These fractions contain varying amounts of sulfur amino acids, such as cystine and methionine (Sodek and Wilson, 1971). Depending upon the protocol used for protein fractionation, zein content range of corn is as low as 35% (Reiners et al., 1973) to as much as 60% (Hamaker et al., 1995) of the total protein of corn, of which up to 74% is in the endosperm alone.

The large number of protein fractions identified by various techniques by several researchers in this period led to confusion on the nomenclature of zein polypeptides. At least five systems of zein nomenclature are in use, e.g. those proposed by Osborne (1924), Moureaux and Landry (1968), Landry and Moureaux (1970), Wilson (1985), Esen (1987) and Wallace et al. (1990). The nomenclature proposed by Esen (1986, 1987) provides the best understanding of the various fractions. Esen fractionated whole zein into three separate fractions by differential solubility in solutions containing between 0 and 95% isopropyl alcohol (IPA), with or without reducing agents and/or buffers. The three fractions so obtained were labeled α-zein, β-zein and γ-zein. α-Zein was the fraction soluble in 50–95% IPA, but insoluble in 30% IPA/30 mM Na-acetate. It accounted for 75–85% of the total zein in corn depending on the genotype and is primarily made up of 21000–25000 MW polypeptides plus a 10000 MW peptide. Samples of commercial zein available in the market today closely resemble α-zein.

β-Zein was the fraction soluble in solutions of 30–95% IPA that contained a reducing agent, but insoluble in both 90% IPA (without the reducing agent) and 30% IPA/30 mM Na-acetate. It contains two 17000–18000 MW methionine-rich polypeptides and accounts for 10–15% of the total zein. This fraction, however, is not the same β-zein described by McKinney (1958). γ-Zein is soluble in 0–80% IPA in the presence of a reducing agent as well as soluble in 30% IPA/30 mM Na-acetate. It constitutes 5–10% of the total zein.

Savich (1991) showed that the hydrophobic properties of zein are primarily due to the larger peptides. Lower molecular weight peptides have lower mean hydrophobicity and fewer nonpolar amino acids. (This is could be the reason these lower molecular weight subunits were originally identified as 2-glutelins and not a part of zein in corn).

2.2. Structure

A helical wheel model for zein was proposed by Argos et al. (1982) where nine homologous repeating units are arranged in an anti-parallel form stabilized by hydrogen bonds resulting in a protein molecule which was only slightly asymmetric. Circular dichroism and optical rotatory dispersion measurements indicate the helical content of zein varies between 33.6 and 60% in 50–80% ethanol (Gortner and MacDonald, 1944; Danzer et al., 1975; Argos et al., 1982; Matsushima et al., 1993) with α- and β-zein displaying nearly the same content. This helical content suggests that zein has a globular structure in non-aqueous solutions with conformational properties similar to conventional globular proteins such as insulin and ribonuclease (Danzer et al., 1975). Conformational changes take place as the ethanol concentration is reduced from 80 to 50%. However, they were unable to establish the presence of a β-sheet structure (the pleated sheet configuration) as had been hypothesized earlier from infrared studies. Recently, Matsushima et al. (1993) revised the Argos et al. model based on small-angle X-ray scattering measurements and proposed that reduced α-zeins exist as asymmetric particles of 13 nm in length and an elongated molecular structure with an axial ratio of 6:1.

3. Extraction of zein

The first step in zein manufacture is its extraction from corn or corn gluten meal using a suitable solvent. Based on the predominance of nonpolar amino acids in zein (Table 3), it is possible to predict that solvents for zein should possess mixed characteristics, containing both ionic and nonionic polar groups as well as nonpolar groups, either by their structure (in case of pure solvents) or by their composition (for mixed solvents). Dill (1927) used the term 'critical peptization temperature' to describe limiting conditions for which zein would be soluble. Zein is considered 'soluble' if $> 0.5\%$ (w/v) of the protein dissolves in the solvent and gives a visually transparent solution at room temperature (20–25°C). By this definition, there are several methods to solubilize zein from corn as described below.

3.1. Nonaqueous solvents

Nonaqueous solvents for zein are usually of two types: (a) a mixture of an organic compound with water; or (b) a mixture of two anhydrous organic compounds. An exhaustive list of about 70 likely solvents for zein is given by Evans and Manley (1941, 1944) and Manley and Evans (1942, 1943). Aqueous alcohol solutions have been used extensively for commercial production of zein (Table 4). Zein is soluble in 50–90% ethanol, but not in anhydrous alcohol solutions (except methanol). However, zein is dispersible in high concentrations of alcohol and in absolute alcohol when temperatures are above the normal boiling point of the solvents. Similarly, zein can be solubilized in 40% ethanol at high temperatures. At lower concentrations of ethanol, zein tends to denature before reaching the temperatures required for it to disperse (Manley and Evans, 1942). Zein is also soluble in ketones (e.g. methyl ethyl ketone, acetone), amide solvents (e.g. acetamide), in high concentrations of salt (NaCl, KBr), in esters and glycols.

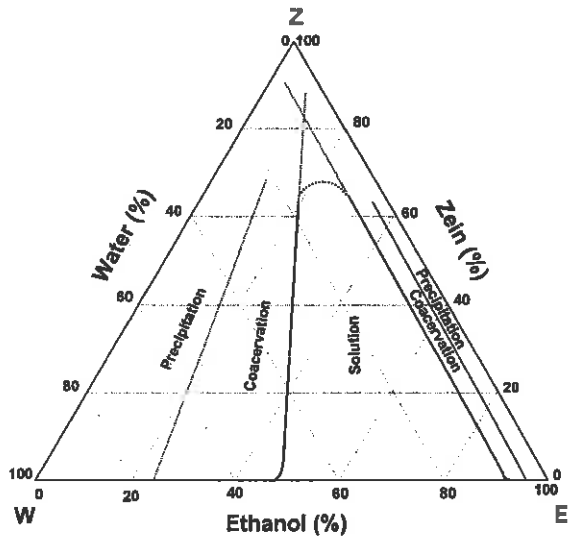

Fig. 3. Ternary phase diagram for the solubility of zein in ethanol and water. Adapted from Mossé (1961) ©INRA, Cedex, France.

The solubility behavior of zein is shown in the form for a ternary phase diagram in Fig. 3. At constant temperature, the solubility of zein varies between 2 and 60% (w/w), depending on the ethanol concentration. At lower ($< 40\%$) and higher ($> 90\%$) concentrations of ethanol, two liquid phases appear, both containing zein, water and ethanol. This phenomenon has been referred to as appearance of a 'taffy' layer and is widely used to recover zein after extraction from CGM, as described in Section 4. It corresponds to a transition state between complete solubilization and precipitation of zein.

From Fig. 3, it appears that it is impossible to prepare solutions containing $> 65\%$ zein without raising the temperature. Higher temperatures increase the solubility curve and the maximum rises and disappears. This phenomenon has been used to form zein films. Heating a zein solution to evaporate the ethanol gives an increasingly concentrated solution without discontinuity. The solution progresses from a thick transparent syrupy state to a glassy state in a thin coat, permitting zein to form glaze-like films with remarkable properties (Mossé, 1961). Formation of precipitates at low ethanol concentrations can be suppressed by low temperatures, removal of pigments and other ether-soluble compounds, and reduction of disulfide bonds (Abe et al., 1986).

Table 4
Processes for manufacture of zein[a]

Source	Extraction process	Method of protein recovery	Results	Reference
Corn	Destarched, extracted with alcohol in presence of heat and temperature. Ratio 1:1	Filtration, cooling and evaporation	First known process for zein recovery	(Osborne, 1891)
Corn	Degermed, extracted with alcohol–alkali mixture	Vacuum distillation. Pigments are washed off with benzene	Color free zein is produced	(Wulkan, 1902)
Corn	Dry milling, 55% EtOH, 30°C, Ratio 15:1–8.8:1		Near complete solubilization of zein after 18 h extraction	(Russell, 1980)
Corn	Grinding, two-step extraction with 55–60% EtOH + 40–45% 0.1 N NaOH with sonication, Ratio 10:1		Yield of 50–80%. Both zein and non-zein proteins are extracted	(Lawhon, 1986)
Corn	Wet attrition milling to loosen starch–protein matrix, EtOH extraction at pH 11.4	Membrane filtration	82% extraction of both zein and non-zein proteins, purity >90%	(Kampen, 1995)
Corn	Milling, 70% EtOH, ambient temperature, Ratio 4:1	Dilution and centrifugation. Precipitation at 3°C	80% purity, low cost ($3/kg). Contains oil and oil solubles	(Dickey et al., 1998, Dickey et al., 1999)
Corn	Dry milling, extraction with 70% EtOH at 50°C in batch or continuous mode. Ratio 1:1–8:1	Membrane filtration	50–60% of zein is extracted. Purity ≈60%	(Cheryan, 1999)
Defatted corn	80% EtOH, 6 h near boiling point. Ratio 3:1	Precipitation in 6 vol of water		(Donard and Labbé, 1903)
Defatted corn	Flaking, extraction with 45% EtOH + 55% 0.1 M NaOH at 55°C. Ratio 15:1		57% of total protein (zein and non-zein) extracted, purity 25–30%	(Hojilla-Evangelista, 1990; Hojilla-Evangelista et al., 1992)
Defatted corn	60% EtOH, 50–70°C. Ratio 1:1		40% of total corn protein recovered	(Chen and Hoff, 1987)
Defatted corn	Enzyme hydrolysis of corn starch, extraction with 65% EtOH, 65°C. Ratio 1:1		95% of zein extracted	(Cao et al., 1996)
CGM	85–95% EtOH for several days	Precipitation in 1% NaCl and decolorization in ethylene dichloride/ether or acetone and air dried	White zein is produced	(Mason and Palmer, 1934)
CGM	Continuous extraction with 80% EtOH, *n*-butanol or IPA at 62°C	Alkali treatment, acidification and precipitation in cold water	>97% pure zein. Higher purity (up to 100%) obtained by extraction of zein with benzol, carbon tetrachloride or ether	(Buron and MacDonough, 1936)
CGM	85% IPA, 60°C. Ratio 4:1	Alkali treatment and hexane clarification	50% yield	(Swallen and Haute, 1938)

Table 4 (Continued)

Source	Extraction process	Method of protein recovery	Results	Reference
CGM	Extraction with 80% EtOH	Precipitation by dilution with 5 vol of water. Oil and impurity removal by treating zein precipitate with 20–25% toulol or benzol	EtOH recovery by distillation. Fine granular zein obtained free from oil-solvent phase	(Pearce, 1941)
CGM	85% IPA or 92% EtOH, 60°C continuous extraction. Ratio 3.5:1	Alkali treatment, filtration, acid treatment, hexane extraction, water displacement and spray drying	Zein powder containing some pigments and impurities. 50% yield based on total protein. High solvent losses	(Swallen, 1942)
CGM	55–65% IPA, alkali treatment, followed by aging for 1 h and cooling	Filtration and water displacement to 35% IPA, acid treatment and water precipitation	Quality and stability of zein is poor	(Swallen, 1943)
CGM	Extracted with a mixture of five parts 37% formaldehyde and 20 parts 55% EtOH or 80% IPA. 120°C for 15–30 min. Ratio 2.9–3:1	Cooled and filtered through diatomaceous earth.	55% of protein from crude gluten was recovered	(Manley and Evans, 1944)
CGM	Extraction with 91% IPA at 82°C for 30 min. Ratio 4:1	Filtration and stabilization with propylene glycol at 250°F	Zein solutions produced can be directly used as coatings and are stable against gelation	(Coleman, 1944)
CGM	80% IPA, 30 min, 50–60°C. Ratio 3:1	Washing and precipitation of fines at 4–10°C and oil/pigment removal by petroleum ether	90% of protein extracted	(Evans et al., 1945)
CGM	Extraction with 40–60% EtOH at 75–85°F followed by filtration with diatomaceous earth, halogen treatment and bleaching for pigment removal. Ratio 3:1	Chilling and water precipitation	Zein that is soluble in ammonia and in low (40%) alcohol concentrations is produced	(Walsh et al., 1944)
CGM	28–33% IPA with 6% lime, 70°C to boiling, 15–30 min. Ratio 7–8.5:1		75% yield, higher purity obtained by 10–20 repeated extractions. No oil, color or non-zein proteins are extracted	(Morris et al., 1956; Morris and Wilson, 1959)
CGM	88% IPA + 0.25% NaOH, 55–65°C. Ratio 4:1	Chilling extract to −10 to −20°C to precipitate proteins	Low yield (20–24%), easy pigment and oil removal. Variable quality of product. Multiple extractions with fresh IPA for added purity	(Carter and Reck, 1970)
CGM	Deoiled gluten extracted with 85% MeOH at 130°C for 2 min, cooled, filtered, NaOH added at 100°C. Ratio 5:1	Solution is cooled and filtered below −25°C. pH is adjusted. Cooled further to −35°C. Zein precipitate is purified by washing several times with methanol	Colorless, pigment-free zein	(Reiners et al., 1974)

Table 4 (Continued)

Source	Extraction process	Method of protein recovery	Results	Reference
CGM	Extraction with 3% NaCl at 4°C for 30 min followed by extraction with 60% EtOH at 60°C for 30 min. Ratio 10:1	Zein precipitated in equal volume of 1% NaCl and centrifuged	Good quality of zein	(Kawata et al., 1989)
CGM	Enzymatic starch hydrolysis, alkaline treatment, alcohol washing and alcohol extraction	Zein precipitated in cold water, dried and ground	Defatted, decolored zein, high purity (>96%)	(Cook et al., 1993, 1996)
CGM	70% acetone, 40°C for 4 h. Ratio 5:1	Evaporation, concentration, precipitation	30% of zein recovered in white porous granular form	(Takahashi and Norimasa, 1994)
CGM	95% EtOH, 70°C. Ratio 8:1	Centrifugation and precipitation at −10°C. Washing zein with hexane	30% recovery of zein. Color pigments obtained useful for food processing	(Takahashi and Yanai, 1996)
CGM	88% IPA, pH 12.5. Ratio 4:1	Precipitating by chilling to −18°C	21–32% recovery with 80–87% purity	(Wu et al., 1997a,b)
DDGS	60% EtOH with 0.1% dithiothreitol, 60°C, Ratio 5–10:1		Low yields 1.5–6.6%. Protein purity was low (37–57%)	(Wu et al., 1981; Wu and Stringfellow, 1982; Wolf and Lawton, 1997)

[a] CGM, corn gluten meal; DDGS, distillers dried grains with solubles; EtOH, ethanol; IPA, isopropyl alcohol; MeOH, methanol. Ratio, volume of solvent (ml)/weight of solids (g).

The presence of –OH, $-NH_2$, $-CONH_2$ or –COOH groups in the dissolving solvents is important. Rees and Singer (1956) reported about 15 additional organic solvents for zein and found a remarkable similarity in solubility behavior of insulin and zein. Most of these organic solvents, whether pure or mixtures, have three or four carbon atoms. This is because solvent molecules interact between amino acid residues and their polar groups cannot emerge from the protein molecule towards the exterior unless their carbon chains are at least as long as that of the residues (Mossé, 1961). This is not a limitation though, since soaps (14–18 carbon atoms) and detergents such as dodecylsulfate (12 carbon atoms) are good solvents for zein in water (Foster, 1949).

One limitation on the amount of zein that can be dissolved in a solvent is the viscosity that can be handled by the mixing equipment (Pomes, 1971). Acetone, dioxane and dioxolane form excellent solvent mixtures with water for zein and produce solutions of far less viscosity than those with alcohol (Oshlack et al., 1994). Binary and ternary solvents tend to stabilize zein against gelation and denaturation, e.g. pyrilidene:water (1:1 v/v) is also a good solvent for zein, based on Carbon-13 NMR measurements (Augustine and Baianu, 1987).

3.2. Aqueous solvents

Solubility of zein in water can be increased by either acidic or alkaline deamidation or enzymatic modification. Acid or alkali treatments with HCl (pH < 1) or NaOH (pH > 12) are commonly used to convert of glutamine and asparagine amino acids to the acid or salt forms (Unger and Howland, 1961; Reiners et al., 1973; Payne and Tyrpin, 1990; Morawsky et al., 1996; Funatsu and Shibata, 1998). Alkaline solubility has been attributed to the phenolic hydroxyl group of the amino acid tyrosine. About 25% of the amino acid residues of zein contain an amide group and 10% of the residues in zein are proline (rich in amines/amides). Thus, zein is soluble, even in ammonia. However, such treatments consume large quantities of chemicals or cause significant degradation of the protein. Hydrolysis with alkaline earth hydroxides such as barium, calcium, strontium and lithium tend to degrade less protein and provide better properties of zein films (Loew, 1968). Van Blanton and Scallet (1980) treated zein with alkylene oxide to make water soluble zein.

Hydrolyzing the amide groups of glutamine and asparagine residues to carboxyl groups solubilizes zein at lower pH. Zein or deamidated zein peptides may also be esterified or reacted with fatty alcohol to form fatty acid esters or fatty acylated zein. Some modified esters exhibit an increased tolerance to hydrocarbons (Pomes, 1971). Zein forms a complex by sonication with the phosphatidic acid group of lecithin to form a water soluble zein-phosphatidate complex, which exhibits emulsifying properties under a wide range of pH (Kito, 1987; Utsumi and Kito, 1991). This complex can be digested with Pronase E to increase its emulsifying capacity, thereby increasing food uses. Similarly, phosphorylation with $POCl_3$ in the presence of essential amino acids results in an 11-fold improvement in digestibility (Matheis, 1991).

3.3. Enzymatic modification

Enzymatic hydrolysis is another means of increasing solubility of zein in aqueous solutions. Mannheim and Cheryan (1993) used dual phase sequential enzymatic modification with Alcalase in each phase and ultrafiltration to prepare water-soluble zein. The new form displayed improved functional properties including solubility, foaming and moisture adsorption. Attempts with other enzymes have met with limited success (Saito et al., 1988).

3.4. Gelation of zein during extraction

The choice of a solvent for zein is determined not only by its solubility but also its gelling characteristics. Zein gels easily and this phenomenon, which is quite well documented, has been referred to as 'a troublesome characteristic of zein' (Swallen, 1941b). The time required to gel depends on the solvent, the concentration of solvent (less water results in slower gelation), temperature (higher temperatures promote gelation), pH and mechanical factors promoting denatura-

tion of protein (e.g. agitation). Gelation could be a result of denaturation of zein and/or due to the presence of insoluble 'bodies' that act autocatalytically to precipitate or gel the zein (Dimitroglou, 1996). Other factors that affect zein stability include type of corn and method of steeping, drying conditions and time of storage, in case of zein made from corn gluten meal (Abe, 1989). Aqueous alcohol dispersions, containing $< 10\%$ zein, gel very slowly, but such low concentrations and their correspondingly low viscosities are not very useful industrially (Evans and Manley, 1943). Most applications of zein require 20–40% concentrations and such solutions will most likely gel in less than a day.

Primary solvents provide better protection against gelation than those containing water (Manley and Evans, 1942, 1943). Stability of zein in solution is important for successful commercial applications, and can be increased by inclusion of a third organic component (Evans and Manley, 1944). For example, inclusion of 5% formaldehyde in acetone–water or addition of rosin or shellac to alcohol–water solutions, or addition of stabilizers, such as propylene glycol (Coleman, 1944), substantially increased the resistance of the system to gelation. Other approaches to increasing stability of zein have included 'aging' of precipitates in hot (40–50°C) water (Baldoni, 1954), adding hydrochloric, boric or phosphoric acids (Coleman, 1942), and rapid cooling to temperatures below 10°C after alcohol extraction (Swallen, 1940). Similarly, addition of triethanolamine to alcohol–water systems markedly increased the transparency of films formed from the mixtures.

4. Methods of production of zein

4.1. General methods

The various methods used for manufacture of zein are summarized in Table 4. Many of these still remain at the laboratory level and have not reached commercial application. The major differences between the various processes are: (a) the raw material; (b) the solvent used for the extraction; (c) the method of purification of the zein, if any; and (d) the recovery method. The raw material is usually corn gluten meal (CGM). It is a coproduct of corn wet milling (Fig. 2) containing a minimum of 60% protein (dry basis). Almost all of the zein in the raw corn ends up in CGM, i.e. about one-half or more of the protein in CGM is zein (Table 1). CGM is used as animal feed in the US, which provides a low-cost raw material ($\approx$ US$120–240 per tonne). However, the quality of CGM varies widely, not only in its actual protein content, but also the manner in which the corn has been pretreated prior to separation of the CGM (e.g. the steeping and milling conditions) and in the final drying step, which is sometimes performed at very high temperatures. Recovery of zein from CGM decreases with an increase in drying temperature of CGM (Wu et al., 1997a). Lower recoveries and protein purities result in CGM subjected to freeze- and spray-drying compared to oven-drying.

Raw dry-milled corn may have some advantages as the starting material. The zein is in its native form, but since the zein content of the raw material is only $\approx 4\%$ (dry basis), the yield and concentration of zein in the extractant is low (Shukla and Cheryan, 2000), which could lead to higher recovery costs unless the methods used for solvent recovery and concentration of zein are more efficient and cost-effective (Cheryan, 1999). DDGS is a coproduct of dry grind ethanol production (Fig. 2), with a protein content of 27–30% (Table 1). It is also dried at high temperature and varies widely in quality and composition from plant to plant. It is an undesirable raw material for production of high-quality zein (Wu et al., 1981; Wolf and Lawton, 1997).

Most of the processes described in the literature use two solvents in succession: a polar solvent such as aqueous solutions of ethanol or isopropanol for extraction, and a nonpolar solvent such as hexane or benzene for removal of fats and color pigments.

Zein was manufactured on a large scale by Corn Products Corporation (CPC) from 1939 to 1967. At its peak, the market size was > 7000 tonnes per year. Since the 1970s, zein production has fallen below 500 tonnes per year. It is currently manufactured in US by Freeman Indus-

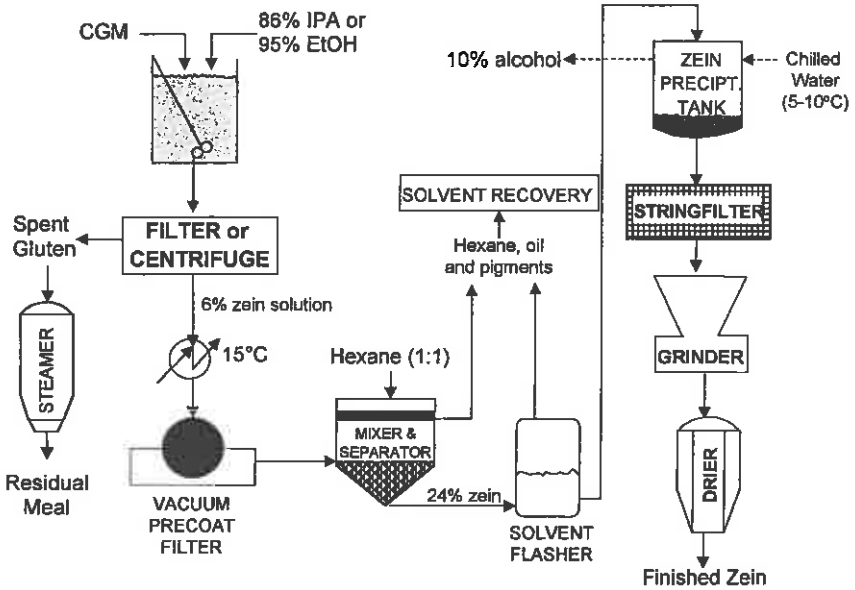

Fig. 4. CPC process for production of zein from corn gluten meal. Adapted from Swallen, (1938, 1941a,b).

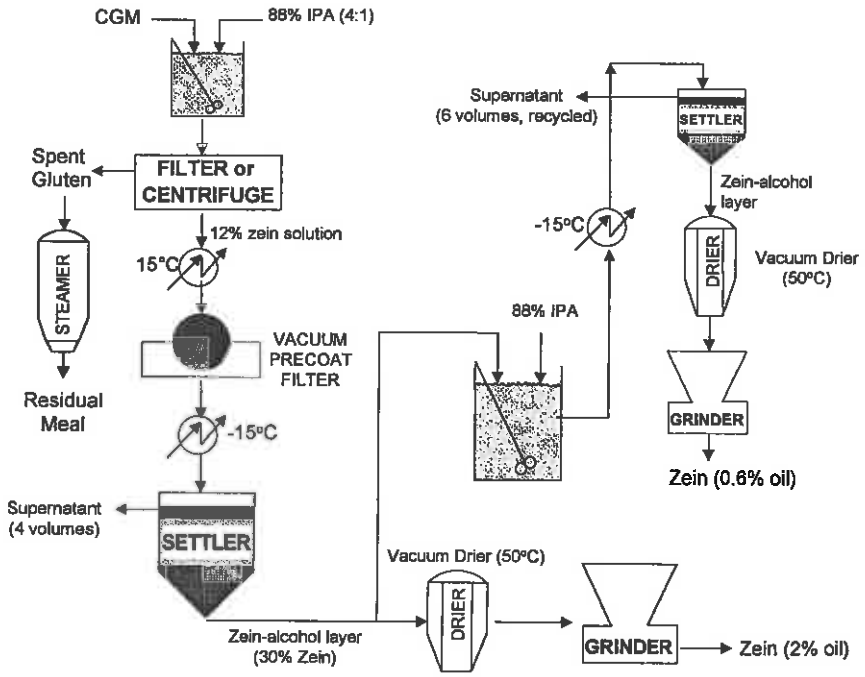

Fig. 5. Nutrilite process for production of zein from corn gluten meal. Adapted from Carter and Reck (1970).

tries, Tuckahoe, NY and in Japan by Showa Sangyo Corp., Kyoto. The commercial processes appear to follow one or both processes shown in Figs. 4 and 5. These process are quite similar and based on well known principles (Swallen and Haute, 1938; Swallen, 1938, 1941a, 1942, 1943; Reiners et al., 1973). In the CPC process (Fig. 4), CGM, dried or wet (Walsh et al., 1944), is contacted with hot 86–88% IPA or 93–95% ethanol at high pH and elevated temperature (50–60°C) in either a batch or continuous extractor for ≈30 min to 2 h. Although the addition of reducing agents has been mentioned in the literature (Tsai, 1980), it appears that no reducing agent was used in any industrial process.

The extract is filtered and/or centrifuged. The filtrate, containing zein (≈6% w/v) and impurities, is clarified by standing or vacuum filtration (Swallen, 1940) and then cooled. A non-solvent for zein (e.g. toluol, hexane or benzene) is then added to extract the non-protein impurities such as fats and color pigments. The zein is finally precipitated either in excess amounts of cold water or at low temperatures (−15 to −25°C). It is then vacuum dried and ground to yield a light yellow product.

The CPC process was modified over the years to include alkali treatment, where the pH was raised to 12 with NaOH and held for 30 min for deamidation of amino acid residues (Pomes, 1971). The pH was subsequently lowered with HCl and zein precipitated in cold water. This procedure improved the stability and gelation properties of zein (Reiners et al., 1973).

IPA is the preferred solvent for zein extraction because it is more efficient in terms of its capacity (higher zein concentration at the same solvent:solids ratio than with ethanol). In addition, the subsequent separation (e.g. hexane extraction for pigment and oil removal) is better because a sharper separation occurs between the zein layer and the hexane layer. Less IPA gets dissolved in the hexane layer resulting in lower distillation costs. The heat of vaporization of ethanol is considerably higher (204.5 cal/g) compared to IPA (159.35 cal/g) which leads to higher distillation costs with ethanol.

The major disadvantages of the CPC process were high operating costs due to the complex solvent recovery systems required (primarily distillation), low yield and high solvent losses during extraction (Manley and Evans, 1944; Morris et al., 1956). It was also plagued by frequent gelation of the zein due to variation in solution pH. The quality of the final product was quite variable. The more recent nutrilite process (Carter and Reck, 1970) does not use a nonpolar solvent to remove the oil, resulting in a zein containing 2% oil on a dry basis. The oil can be reduced by re-extracting with 88% IPA (Fig. 5). This process appears to control gelation problems better because low temperatures provide stability against denaturation of zein. However, significant costs are associated with large volumes of solvent and low temperature precipitation.

Morris et al. (1956) and Morris and Wilson (1959) used an interesting approach of using 28% IPA-6% lime to produce oil-free and pigment-free zein. Their process required large volumes of solvent (up to 20 washes) and high temperatures (75°C). Zein has also been extracted from corn using solvents such as 55% ethanol–45% NaOH, 70% ethanol at high pH and 55% ethanol, sometimes with sonication followed by water displacement or membrane concentration (Table 4). Oil and protein can be simultaneously extracted using aqueous ethanol-NaOH mixtures as the solvent (Cao et al., 1996, Chen and Hoff, 1987; Hojilla-Evangelista et al., 1992; Wu et al., 1997a).

Commercially available zein differs significantly from that prepared by direct solvent extraction of raw whole corn (Boundy et al., 1967). This is primarily because the commercial zein is manufactured from CGM which has been produced in a wet milling process where the first step is the steeping of corn in a solution of sulfur dioxide, which cleaves disulfide bonds between polypeptide chains of zein and decreases cystine content.

4.2. White zein

Many potential applications of zein have been hindered in part because of its yellow color. This is caused by xanthophylls, carotenoids, and other color pigments present in corn. They appear to be associated with the hydrophobic proteins such as zein, and thus, the color pigments are concentrated

in CGM and are co-extracted with the zein. These pigments tend to transfer their colors to the surfaces under the coating, which is undesirable in many applications. Numerous attempts have been made to manufacture color-free zein. Osborne (1891) cooled the extract to remove 'fatty matter' after zein extraction. Donard and Labbé (1903) first proposed using benzene, petroleum or amyl alcohol to remove oils and color pigments from zein. A second, more general process, involves treatment of impure zein after extraction from CGM with a non-solvent for zein such as toluol, benzol, ether or high strength (88–100%) ethyl or propyl alcohol (Carter and Reck, 1970; Pearce, 1941). A ternary phase mixture is formed and color-free zein is recovered by precipitating in large volumes of water. However, these solvents do not swell or penetrate the zein matrix sufficiently to remove all impurities. In addition, larger volumes of solvents have to be handled. Mason and Palmer (1934) used ethylene dichloride/ether to remove oil and color impurities. Oncley et al. (1949) treated white corn with petroleum ether at 25°C followed by extraction with IPA below 0°C and freeze drying. Their product was stable and colorless in solutions.

Table 5
Properties of zein[a]

Property	Characteristics
Bulking value, l/kg	0.805
Color	Light cream
Dielectric constant, at 500 V, 60 cycles, 25–90°C (molded discs)	4.9–5.0
Diffusion coefficient	3.7×10^{-14} m^2/s
Einstein viscosity coefficient	25
Glass transition temperature	165°C
Isoelectric point, pH	6.2 (varies between 5 and 9)
Molecular weight	35 000 (varies between 9.6 and 44 K)
Partial specific volume	0.771
Physical form	Amorphous powder
Sedimentation coefficient	1.5 s
Specific gravity, at 25°C	1.25
Thermal degradation point	320°C

[a] Data sources: Watson et al. (1936); Oncley et al. (1949); Russell and Tsao (1982); Magoshi et al. (1992).

Walsh et al. (1944) oxidized zein extracts with halogens or hypohalites of alkali or alkali earth metals, followed by bleaching with organic peroxides such as benzoyl, acetyl or urea peroxide. Although the products had low color, they could not be used for food and pharmaceutical applications due to the use of non-GRAS solvents. The associated recovery and distillation costs of the solvents are high. On the other hand, using low concentrations of IPA (28–33%) will reduce the relative rate of extraction of color pigments compared to zein (Morris et al., 1956; Morris and Wilson, 1959). However, the zein has to be washed with up to 20 vol to increase purity. Recently, Cook et al. (1993, 1996) used 95% ethanol followed by passage through carbon or ion-exchange resin to produce purified zein. However, as many as five washes with 100% ethanol are required. Takahashi et al. (1994) used absolute acetone and low temperatures (-10°C) to recover relatively white zein. Zein that is comparatively white in color can be produced from waxy corn due to its low pigment and xanthophyll content (Watson and Ramstad, 1987). Freeman Industries produces relatively white zein by using CGM made from waxy corn (Freeman, P.G., 1999, personal communication).

The relatively high cost of zein today can be partly explained by the large amounts of organic solvents used for extraction of the zein and (usually another solvent) for removal of lipid materials. In addition, energy-intensive processes (e.g. evaporation, distillation) are needed to remove the solvents. If raw corn is used as the starting material, the protein content in the extract is lower (Shukla and Cheryan, 2000) leading to even higher solvent-removal costs. One possible alternative is to use membrane technology (e.g. microfiltration, ultrafiltration) to simultaneously purify the zein, concentrate the solution and recycle the extracting solvent (Kampen, 1995; Cheryan, 1999). Membrane technology is an inherently low-energy process and low in operating cost, and its capital cost is comparable to traditional methods such as evaporation (Cheryan, 1998).

Table 6
Selected uses of zein

Use	Reference
Adhesives, binders	(Coleman, 1939, 1942 Coleman, 1944)
Biodegradable plastics	(Lai and Padua, 1997; Lai et al., 1997)
Chewing gum	(Campbell and Zibell, 1992; Zibell et al., 1992; Wolf et al., 1999)
Chewing gum (anticariogenic)	(Kruppa, 1984)
Coating (edible, moisture-resistant) for food products	(Glasser, 1983; Haralampu and Sands, 1991; Wasa and Takahsahi, 1998)
Cosmetic powders	(Avalle, 1998; Schlossman, 1986)
Delivery systems for acid sensitive drugs	(Mazer et al., 1992; Ting and Hsiao, 1999)
Electrophoretic coatings	(Korinko and Hunt, 1999)
Fat substitutes	(Akkaway et al., 1999)
Fibers	(Uy, 1996, 1997)
Fibers, dietary	(Freeman, 1995a,b; McArdle, 1995; Lamb and Seeds, 1998)
Fibers, textile	(Zhang et al., 1997; Cuq et al., 1999)
Hair fixative	(Morawsky et al., 1996)
High potency sweeteners	(Zibell, 1989)
Labels, varnishes	(Pomes, 1971; Reiners et al., 1973)
Long acting matrix tablet formulations	(Beatty and Boettner, 1984; Mazer et al., 1992; Oshlack et al., 1994)
Microspheres	(Mathiowitz et al., 1991)
Microencapsulated pesticides	(Redding, 1990)
Nutrient delivery system for ruminants	(Morikawa et al., 1999; Witt and Dew, 1999)
Paper surfaces, planographic printing plates, glossy magazine covers	(Paesschen and Prien, 1972; Trezza and Vergano, 1994)
Photo stabilization of abamectin	(Demachak, 1995; Demachak and Dybas, 1997)
Photographic films, photographic emulsions	(Wang et al., 1998)
Printing inks	(Leckley, 1951)
Reverse cationic floatation of ores	(Peres and Correa, 1996)
Reverse osmosis membranes	(Fisher and Hsiao, 1969)
Starch-based polymers	(Cole and Daumesnil, 1989; Jane and Spence, 1995; Takahashi et al., 1995, 1996; Parris et al., 1997; Wang, 1999)
Surgical closure of body organs and blood vessels	(Muxfeldt and Dahlke, 1981)
Taste masking of oral drugs	(Cuca et al., 1994; Meyer and Mazer, 1997)
Test strips, biological	(Riebel et al., 1987)
Wound dressing	(Errede et al., 1983; Fontinos, 1999)

5. Industrial and consumer applications of zein

Industrial properties of commercial zein are shown in Table 5. Zein can form tough, glossy, hydrophobic, greaseproof coatings that are resistant to microbial attack, with excellent flexibility and compressibility. This has been of interest to commercial processors since its discovery. Coatings of zein films appear to be one of its most promising applications. During the 1970s, almost 75% of the annual production of 500 tons per year was used in coating medical tablets (Reiners et al., 1973). In recent years, new applications of zein (Table 6) have emerged such as adhesives, laminated board and solid color printing (Wang et al., 1998), in composition corks, heat and moisture set inks (Leckley, 1951) and pigment binding (Pomes, 1971; Lower, 1999). Some other applications are discussed below.

5.1. Controlled release applications

To delay release of drugs until the tablet reaches the intestine to protect it from stomach acid and to provide a mechanism for constant release of drugs in the blood stream (Beatty and Boettner, 1984), drugs are usually incorporated into protein microspheres for delivery to the cells of reticuloendothelial system, mucosal membranes of mouth or the gastrointestinal tract (Mathiowitz et al., 1991). Such microspheres may also be used for the delayed release of pesticides, fertilizers and agents for environmental cleanup. Zein mixed with pheromones is used to encapsulate pesticides to exterminate

pests and to diminish the pesticide odor and provide a safe working environment for humans (Redding, 1990). Microparticles of zein can also be used as a fat substitute and to encapsulate selected dietary fat (Stark and Gross, 1990).

5.2. Coatings

Zein-based coatings have been used successfully in cosmetic products to prevent direct contact between the epidermis and the inorganic chemicals used in such products (Avalle, 1998; Schlossman, 1986). When mixed with a conductive polymer, it successfully provides electromagnetic interference (EMI) and electrostatic discharge (ESD) properties for electron device coatings (Rivas, 1999). Other applications include paper coatings for glossy magazine covers (Trezza and Vergano, 1994), in toilet cleansing blocks (Campbell and Ferrando, 1997), making dry-chemical test agents (Riebel et al., 1987), composite wound dressings (Errede et al., 1983), artificial bristles useful in making several types of commercial brushes (Lougovoy, 1932; McMeekin et al., 1950), as a support for electrophoretic deposition of brazing material in aircraft engines (Korinko and Hunt, 1999), in phonograph records to improve wear resistance and tone quality and for artificial jewellery (Martin, 1970).

5.3. Fibers

During the 1950s, zein-based textile fibers were sold under the brand name *Vicara* for clothing purposes (Seymour, 1966) and for stuffing furniture (Martin, 1970). Such fibers were produced by spinning alkaline zein solutions, coagulating them with acids and salts and curing them with formaldehyde (Croston et al., 1945). Obvious environmental implications associated with disposal of these chemicals made production expensive and difficult. Recently, the Dupont Company appears to have solved some of these issues and has patented a process for making cross-linked fibers from zein (Uy, 1996, 1997; Pelosi, 1997).

5.4. Biodegradable films and plastics

One of the most promising applications of zein appears to be for biodegradable films and plastics used for packaging. Worldwide demand for such products has been estimated to be 15000–250000 ton per year. Unplasticized zein films were too brittle for most applications (Parris and Coffin, 1997), but incorporating crosslinking agents (e.g. citric acid, formaldehyde, butanetetracarboxylic acid) increased tensile strength two to three times. Zein films can be modified and strengthened by incorporating highly stable silicate complexes into protein structures (Lee et al., 1998). They have been shown to have higher strength and lower gas permeability than unmodified films. The marketability of zein-based plastic films can be improved by incorporating food-grade anti-microbial compounds into the packaging film (Padgett et al., 1998). Extensive reviews on protein based edible films and coatings have been published by Gennadios et al. (1994) and Baker et al. (1994).

Two types of biodegradable plastics can be made from zein: destructured starch–zein composites and zein plasticized with fatty acids. Numerous publications in the 1990s have reported on such products. In the former case, mixtures of starch, zein and a crosslinking agent (e.g., aldehyde, epichlorohydrin) are compression-molded to produce water-resistant plastics (Jane and Spence, 1995). Such plastics reportedly undergo 60% biodegradation in 180 days (Spence et al., 1995) and have been used to make bottles, sheets, films, packaging materials, pipes, rods, laminates, sacks, bags and powders (Cole and Daumesnil, 1989). Parris et al. (1997) found that glycerine-plasticized zein films containing 1–8% starch had lower water vapor permeability values and were more water-resistant than unplasticized films.

Fatty acid-zein composites are produced by plasticizing zein with oleic and linoleic acids and precipitating in cold water (Lai et al., 1997; Lai and Padua, 1997; Padua et al., 1997). Such plastics show high ductility and tensile strength compared to other biopolymers.

5.5. Health applications

Zein, although deficient in essential amino acids, could have some nutraceutical or pharmaceutical value. Upon hydrolysis with the enzyme thermolysin, α-zein produces angiotensin-converting enzyme (ACE)-inhibitory peptides (Ariyoshi, 1993). Such hydrolysates can reduce blood pressure in hypotensive rats. Antioxidative activity of zein has been demonstrated by Wang et al. (1991a,b).

6. Conclusions

Zein has good potential in the specialty food, pharmaceutical and biodegradable plastic industries, but only if the cost of manufacture can be decreased. Numerous studies have been conducted since the early 1900s on different methods of manufacturing zein, but few of them appear to have been commercially successful. The pace of research in this area has increased again in the 1990s, with much of the attention focusing on reducing the number and amount of solvents used and/or removing the solvent and recycling it at low cost. With the growing realization of the uniqueness of zein as an industrial and specialty polymer, there will probably be more commercial ventures in the future.

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