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# Determinants for yield and quality of zein extracted from corn gluten meal

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# 1. GENERAL INTRODUCTION

Zein is the predominant protein found in maize comprising 52% of the whole kernel and 60% of the endosperm proteins (Wilson 1987). Zein, a prolamine, is soluble in aqueous alcohol and relatively insoluble in water. The protein has been used in a variety of food and nonfood applications due to its hydrophobicity and functional properties, such as film and fiber formation, thermoplasticity and adhesiveness. The price of zein, however, is quite high (over $20/kg), and thus, impedes its utilization. Currently, about 0.54 million kg (1.2 million lb) of zein per year is produced and used primarily as a coating for confections and pharmaceuticals (Shukla 1992). In order to increase industrial utilization of zein, developing processes that can reduce the cost of zein are essential.

Zein is isolated from corn gluten meal by extracting corn gluten meal at 60 °C with 88% aqueous isopropyl alcohol containing 0.25% NaOH (Carter and Reck 1973). The clarified extract is then chilled to -10 to -20 °C causing zein to precipitate. The supernatant is decanted and the lower layer is dried using a vacuum dryer. The yield of zein extracted using this process is estimated at only 22% of the gluten when approximately 65% protein is available. A significant increase in the yield of zein will be a major factor in reducing the price. Furthermore, as the requirement for corn syrups and fuel-grade ethanol increase in the United States, the amount of corn wet milled will also increase as will corn gluten meal, a co-product of wet milling. Approximately 1.2 billion bushels of corn was

processed by wet milling in 1993 and approximately 922 million kg (2,031 million lb) of corn gluten meal was produced (Andreas 1994). This overabundance of high protein feed (over 60% protein in corn gluten meal) (Wright 1987) could ultimately depress prices and decrease the profits of wet milling processors. By finding new markets for zein, more corn gluten meal could be used to help offset the potential overabundance of this co-product.

The process of producing zein from corn gluten meal, however, has not been critically examined in the public domain for many years, and research on the influence of corn gluten meal quality on zein extraction has not been published. This dissertation examines factors that influence yield and purity of zein extracted from corn gluten meal. The influences of maize hybrid and drying process on corn gluten meal quality and zein yield were examined. The investigation was carried out using both commercial and pilot-plant-produced corn gluten meal. These data will add important information to the current knowledge base to find ways to improve the yield of zein extracted from corn gluten meal and ultimately increase the utilization of the protein.

## Dissertation Organization

This dissertation consists of four papers which will be submitted to the journal of Cereal Chemistry. The first paper discusses the factors in commercially produced corn gluten meal that influence the yield and purity of the extracted zein. The

second paper reports on developing a process for the production of a relatively high protein content corn gluten meal using a pilot-plant-scale wet-milling facility. The third paper examines the effect of drying on zein extraction yield and purity, and zein composition. The final paper describes the influence of adding phosphate in the extraction solvent on the yield and purity of zein extracted from corn gluten meal. The four papers are preceded by a General Introduction and a Literature Review, and followed with General Conclusions.

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# 2. LITERATURE REVIEW

## Composition of the Corn Kernel

A corn kernel consists of the germ (the embryo), endosperm (the energy supply for the initial growth), hull (the protective coating), and tip cap.

The endosperm is the major part of the kernel and accounts for 81.9% of the total weight (db). It is composed primarily of starch 86.4% (db) and protein 9.4% (db), and contains very little oil (0.8%) (Earle et al. 1946). The prolamine zein is the predominant protein found in the endosperm accounting for 60% of the proteins. Glutelins are the next major class found in the endosperm accounting for 26% of the protein followed by albumins and globulins at 6% (Wilson 1987).

The germ, or embryo, accounts for about 11.9% of the dry weight of the corn kernel (Earle et al. 1946) and has a proximate composition of 34.5% oil, 18.8% protein, 10.1% ash, 10.8% sugar and 8.2 % starch (db). Unlike the endosperm, the major class of proteins are albumins and globulins, each at 30% respectively, followed by glutelins (23%) and a relatively low level of prolamins (5%) (Lasztity 1979).

The hull and tip cap account for 6.1% of the weight of the kernel. The major component in the hull and tip cap is fiber, however, they also contain small amounts of starch (7.3% and 5.3% for hull and tip cap, respectively), protein (3.7% and 9.1%) and oil (1.0% and 3.8%) (Earle et al. 1946).

# Zein

## Zein Nomenclature

Zein, the predominant storage protein found in the maize kernel, was initially designated as a prolamine by Osborne based on its solubility in relatively strong alcohol or dilute aqueous alkali (Osborne and Mendel 1914). The heterogeneous nature of zein was demonstrated by sodium dodecyl sulfate (SDS) gel electrophoresis and isoelectric focusing (Wilson et al. 1981; Righetti et al. 1977).

Several nomenclature schemes have been suggested by a number of researchers to identify the heterogeneous protein fractions found in zein. Wilson (1985) proposed a nomenclature system for zein polypeptides based on the apparent molecular weight when analyzed by SDS-PAGE using the following designators: reduced soluble protein (MW 27-31 kD), A-zein (MW 21-26 kD), B-zein (MW 18-24 kD), C-zein (MW 15-18 kD), and D-zein (MW 9-13.5 kD). Esen (1987) proposed a nomenclature system for the zeins based on solubility. He defined three distinct fractions and gave them each a Greek letter designator: a) α-zein, soluble in 50-95% (v/v) isopropyl alcohol which accounts for 75-85% of total zein with MW of 21-25 kD and 10 kD polypeptides; b) β-zein, soluble in 30-85% (v/v) isopropyl alcohol containing a reducing agent, includes MW 17-18 kD polypeptides and accounts for 10-15% of the total zein (the separation from α-zein is based on insolubility in 90% isopropyl alcohol); c) γ-zein, soluble in 0-80% isopropyl alcohol containing a reducing agent, accounts for 5-10% of the total zein and is made up of

MW 27 kD polypeptides (the separation from α- and β-zeins is based on solubility in 30% isopropyl alcohol with 30 mM sodium acetate, pH 6.0).

More recently, zein was divided into four classes based on differences in solubility, amino acid composition, electrophoretic and chromatographic characteristics, and immunological properties (Shewry and Tatham 1990; Wilson 1991). α-Zeins, corresponding to A and B zeins or Z19 and Z22, constitute 71-85% of the total zeins, and have a true MW of 24 and 27 kD based on cDNA sequences. β-Zeins, corresponding to C-zein or Z15, have a true MW of 17 kD, and account for 1-5% of the total zeins. γ-Zeins, reduced soluble proteins or Z27, have an true MW of 22 kD. The 18 kD polypeptides (Z16) which were in the β-zein class, now belong to the γ-zein class because the immunological characteristics is more similar to the γ-zeins (Esen 1987). γ-Zeins, including 18 kD polypeptides, account for 5-15% of the total zeins. δ-Zeins, corresponding to D-zein or Z10, account for the 1-5% of the total zeins and have a true MW 14 kD.

**Amino Acid Compositions of Zeins**

All of the zeins are devoid of lysine and tryptophan, and have relatively high amounts of proline and glutamine/glutamic acid. α-Zeins (both Z19 and Z22) have similar amino acid compositions. Glutamine (20%), leucine (19%), alanine (14%), proline (10%) and serine (7%) are the most prevalent amino acids in α-zeins (Wilson 1987). The majority of the glutamic residues (90%) are present as glutamine

(Righetti et al. 1977). α-Zeins also have very few sulfur-containing amino acids; cysteine and methionine account for only 1-2% of the amino acids (Shewry and Tatham 1990). β-Zeins, however, have a relatively high level of the sulfur-containing amino acids methionine and cysteine, 11% and 4%, respectively (Wilson 1987), as well as relatively high levels of tyrosine (8%) and glycine (9%). In addition to lysine and tryptophan, β-zeins are also deficient in phenylalanine and histidine, therefore, their pI values are in the acidic pH region (pH 3) (Righetti et al. 1977). The amino acid composition of γ-zeins is characterized by a very high proline (25%) and histidine (7%) contents resulting in γ-zeins having the most basic pI. γ-Zeins also contain relatively high levels of cysteine (6%), glycine (7%) and valine (7%). δ-Zeins also have a very high proportion of the sulfur-containing amino acids, methionine (22%) and cysteine (4%) (Shewry and Tatham 1990). δ-Zeins are deficient in arginine and, therefore, like β-zeins, have pI values in the acidic pH region (Righetti et al. 1977).

**α-Zein Structure**

The structure of α-zein has been studied for many years. Analysis by isoelectric focusing/SDS-PAGE indicates that α-zeins are a heterogeneous mixture of up to 15 proteins (Wall et al. 1984; Wilson 1985; and Wilson 1986). The complete primary structure of α-zein polypeptides deduced from cloned cDNA and genomic DNA shows that they are multigene family of 80-100 genes. Sequence

comparisons among polypeptides within α-zeins indicate homologies varying from 60-97% (Esen 1987). Z19 and Z22 consist of about 210 and 245 amino acid residues respectively. The sequences show unique N- and C-terminal domains of about 36-37 and 10 residues, respectively, separated by a repetitive domain consisting of blocks with an average length of 20 residues. The repeats are degenerate and rich in leucine and alanine. The size difference between Z19 and Z22 results from the insertion of an additional repeat unit in the C-terminal end of the protein. This insertion results in a total of 10 repeats in Z22, compared with 9 in Z19 (Shewry and Tatham 1990).

Electron micrographs of zeins indicate that α-zeins are rod-shaped molecules (Argos et al. 1982). The secondary structure, determined by optical rotatory dispersion and circular dichroism, has an α-helical content of approximately 50%, and the percentage of β-sheet structure is very low when α-zeins are dissolved in aqueous ethanol. Based on the apparent size, amino acid composition, α-zein sequences (deduced from cDNAs), and secondary structure determination, Argos and co-workers (1982) predicted a roughly cylindrical model for α-zeins. The model assumed that nine repeat peptides were folded back on one another in an antiparallel fashion forming a ring. Furthermore, two or three polar groups on helices can form hydrogen bonds with adjacent helices or other zein molecules.

Very little experimental evidence, however, supports the Argos' model, except that zeins in solution as asymmetric particles approximate prolate ellipsoids

or rods, with high axial rations. Tatham and his co-workers (1993) predicted the structure of α-zein based on the results of small angle x-ray scattering and viscometric analyses. They found that α-helical predictions were the strongest, occurring within repeat motifs in both Z19 and Z22 zeins. The strongest β-sheet predictions were located in the N-terminal region and β-reverse turns occurred predominantly in the C-terminal half. Within the Z19 zeins, two β-turns were predicted per repeat near the termini of repeats 6,7,8, and 9, whereas, within the Z22 zeins, two β-turns per repeat were for repeats 6, 8, 9, and 10. Therefore, the first prediction they gave was that the α-helix may be folded back on itself, either parallel or in half, forming a two-helix bundle. The second prediction is that the α-helix may be basically linear but distorted in some manner so as to make the apparent length of the molecule shorter. Since the structure is not very flexible, some parts of the zein molecules in this region may be reverse turns.

**β-, γ-, and δ-Zein Structures**

The primary polypeptide sequences of β-, γ- and δ-zeins show no sequence homology with the α-zein polypeptides (Shewry and Tatham 1990).

β-Zeins, encoded by two cDNAs and one gene, consist of 160 residues. The methionine residues are clustered, and 7 of 18 methionine residues occur between position 121 to 132 of the polypeptide, and another 7 of 18 methionine residues

occur between position 59 to 89 of the polypeptide. β-Zeins do not contain any repeated peptides, and no sequence similarity to the α-zeins. The secondary structure shows that β-zeins have little α-helical structure (33%), and relatively more β-sheet (55%), β-turn and random coil structure (Pedersen et al. 1986). No prediction of the complete structure has been hypothesized.

There are two types of γ-zeins, Z27 and Z16. γ-Zeins (Z27) contain 204 amino acid residues. Unique N- and C-terminal regions of 11 and 156 residues, respectively, flank a repetitive domain consisting of conserved hexapeptides with the sequence Pro-Pro-Pro-Val-His-Leu (Prat et al. 1985). The high hydrophilic nature of this domain may be responsible for its water solubility in the presence of reducing agents, and also for a rod-like conformation. At the C-terminal end, proline occurs at every second position between residues 70 to 91, and a cysteine rich region exists between residues 92 and 148. Another type of γ-zeins (Z16) has 164 residues. The repetitive domain consists of only two complete hexapeptides and two truncated hexapeptides of 3 and 5 residues. About 19-23% of α-helical and 11-34% of β-sheet structure are present in γ-zein polypeptides (Wu et al. 1983).

δ-Zeins consists of 129 residues with a central region containing 17 of 29 methionine residues, most as Met-Met doubles separated by 2-3 other residues. It does not contain any repeated sequence, and its conformation has not been studied (Shewry and Tatham 1990).

**Zein Properties**

Zein is devoid of the essential amino acids lysine and tryptophan, and is also low in threonine, valine and the sulfur-containing amino acids; but rich in proline, glutamine and asparagine. Due to the absence or relatively low levels of these essential amino acids, zein is not well balanced nutritionally for human and monogastric animal utilization.

The solubility of zein in water is very low; only 0.054 g/l water (Wahl 1934). Since it has a relatively low level of diamino acids and an abundance of dicarboxylic acids, zein is slightly acidic. The protein can be dissolved in a dilute aqueous alkali solution within a pH range of 11.3-12.7 or in a strong acidic solution (12 N HCl) (Ofelt and Evans 1949). Zein is also soluble in many organic solvents containing hydroxyl, carboxyl, and amino moieties in the proper ratio of polar and non-polar groups (Evans and Menley 1941; Menley and Evans 1943). Aqueous ethyl and isopropyl alcohol are the most common solvents for zein. Other organic solvents that have been used to solubilize zein include formic, acetic, and lactic acids, ethylene glycol, and pyridine. Zeins are also soluble in aqueous 6 M urea, however, α-zeins turn cloudy and precipitate when the concentration is lower than 5 M. β-Zeins, however, can remain in solution in aqueous urea of concentrations as low as 1 M, but eventually turn cloudy. γ-Zeins are soluble in urea, as well as in aqueous solutions without urea (Esen 1987).

Zeins are observed in isoelectric focusing (IEF) with 15 bands having pI's in the pH range 6-9, however, most of the bands are in the pH range 7-8. The β- and δ-zeins have pI's at an acidic pH of approximately 3 (Righetti et al. 1977), while the pI's of γ-zeins are in the basic pH range (pH 8-9) (Wilson et al. 1981).

Zeins are not glycoproteins because zein bands from IEF do not stain with periodic acid-fusion reagent (Righetti et al. 1977). Zeins exhibit only one lipoprotein component when the IEF bands are stained with Sudan black. This component is at pI 3, with MW 13.5 and 9.6 kD (β-zeins and δ-zeins), representing 3-5% of the total protein. This lipoprotein contains a carotenoid covalently bound to the polypeptide backbone and was found to be a component of the membrane that envelops the zein protein bodies in the polypeptide chain, since it is not released by alcohol, Triton X-100, or urea treatment.

Although zeins may be deamidated in alcohol solutions in the presence of a mineral acid at reflux temperatures (Pomes 1971), zeins are very resistant to deamidation in solutions with both acidic and alkaline pH (3.0, 8.7, and 11) at 50 °C for rather prolonged periods (8 and 22 hr) (Righetti et al. 1977).

Zein is very stable to heat in the dry form. It can be heated for several hours at 100 °C without any noticeable change in properties and does not decompose at temperatures as high as 200 °C (Swallen 1941). However, zein has the potential to become denatured (insoluble or gelling) in aqueous alcohol. An alcohol concentration of 90% (v/v) or higher is required to stabilize a zein solution at 22 °C;

below this concentration, the stability decreased rapidly. Under these conditions, the gelation time can decrease from 90 days in 90% ethanol to 10 days in 80% ethanol (Dimitroglou and Breene 1994). The gelation time also decreases as temperature increases, from more than 90 days at 6 °C storage to 5 days at 55 °C.

**Applications**

Since zeins do not have good nutritional value, and are expensive (>$20.0/kg), the major applications are limited to food coating and drug tabletting. This demand is estimated at approximately 540 thousand kg (1.2 million lb) of zein per year (Shukla 1992). However, zein can also be used in other food applications such as carriers and release agents for sweeteners (aspartame) and fat replacement using zein microparticulate. Recent research has also shown that zein tripeptides can decrease blood pressure because they inhibit angiotensin-converting enzymes, and the semiconducting property of zein can be especially valuable in developing edible microwave susceptors.

In addition to food utilization, zein is an excellent candidate for nonfood utilization due to its characteristic functional properties such as hydrophobicity, film and fiber formation. In the 1940's, zein was widely used as an adhesive for cork and wood, a paper coating for magazine covers and food containers, a raw material for plastics, films, fibers and laminated board (made from zein impregnated paper), and as a coating in solid color printing (Swallen 1941). As more low cost and better

performing chemicals were developed from petroleum, many uses of zein in non-food industries were replaced with petro-chemicals in most nonfood applications, however, the realization that the supply of petroleum is limited and because of environmental concerns may open a window of opportunity for zein in these applications.

## Zein Distribution in the Corn Endosperm

### Zein Synthesis and Protein Body Formation

Zein appears in the endosperm of the seed approximately 12 days after pollination, and continues to accumulate until maturity (approximately 50 days after pollination). The amount of alcohol soluble zein increases from 18% of the total nitrogen at 15 days to about 42% of the total nitrogen at 43 days after pollination (Murphy and Dalby 1971).

The fact that zein is located in protein bodies was discovered by Christianson et al. (1969) using microscopic examination of the zone sedimentation on a sucrose density centrifuge, tests of solubility in 70% ethanol, amino acid composition, and starch gel electrophoretic mobility. Zeins are synthesized by membrane bound polyribosomes and transferred into the lumen of the rough endoplasmic reticulum (RER) by cleaving the signal peptides, then assembled into protein bodies (Larkins and Hurkman 1978).

The distribution of various types of zeins, however, does not appear to be uniform throughout the endosperm. Combining immunolocalization techniques and light and electron microscopy, Lending and his coworkers (1988) observed that α-zeins are located in the center of protein bodies, while β- and γ-zeins are commonly found in the peripheral and central inclusions. They also observed that the outer cell layers of the endosperm have higher concentrations of β-, and γ-zeins, and the protein bodies within these cells are smaller and contain much less α-zein than those in internal regions of the endosperm (Lending and Larkins 1989). The differences in size (0.25-1.3 μm) and zein composition of protein bodies in the developing endosperm correlated with the stages of cell maturity. As cells matured, the protein body size increased, concomitant with an increase in the amount of α-zeins. Based on these results, Lending and Larkins (1989) proposed a model for zein deposition during protein body formation. Initial accretions within the RER consist of deposits of both β-, and γ-zeins, while containing little or no α-zeins. α-Zeins accumulate later and are observed within the β-, and γ-zeins. In the final stages of protein body maturation, α-zeins fill most of the core of the protein body and are surrounded by a thin layer of β- and γ-zeins.

**Zein in the Hard and Soft Endosperm**

In the endosperm, starch granules are embedded in a protein matrix accompanied by fine protein bodies. Protein bodies in the horny endosperm are

large and more numerous than in the floury endosperm (Wolf et al. 1969). Dombrink-Kurtzman and Bietz (1993) found that hard endosperm has more alcohol-soluble proteins than soft endosperm, and the fraction of α-zeins is an average of 3.3 times more in the hard than soft endosperm in normal endosperm genotypes. The amounts of γ-zeins are similar in both hard and soft endosperms, however, since the alcohol-soluble protein in soft endosperm is much less than in hard endosperm, the relative percentage of γ-zeins in soft endosperm is twice as much as in hard endosperm. The authors indicated that soft endosperm contains 'immature' protein bodies, which have more β- and γ-zeins, and hard endosperm contained more 'mature' protein bodies with an increased amount of α-zeins.

Pratt et al. (1995) tried to correlate α-zein content with kernel hardness by crossing three different hybrids of maize with low, high and extremely high hardness properties. They found that zein class and kernel density were not significantly correlated. The association of zein class with kernel hardness and density was highly genotype-specific, and there was no consistent relationship between individual or total zeins and kernel density across all populations.

## Zein Extraction

### Extractability of Zein

Zein was first isolated from whole white corn with 70% alcohol by Gorham in 1821 and was given the name 'zein' by him (Swallen 1941). Zein can be extracted

from corn gluten using 75-95% ethanol at 40-60 °C, then precipitated from the alcohol solution by adding salt or acetone, and purified by passing the solution through charcoal resulting in a nearly white product (Nykvist 1934). Zein can also be extracted from corn gluten using alkaline solutions. Wahl (1934) found that a 0.25% NaOH solution was the most suitable extracting solvent for zein. After adding acid (any acid had the same function), zein was precipitated from the aqueous solution. A pH of at least 11.5 was required to dissolve the zeins in an aqueous alkaline extract (Swallen 1941). Isopropyl alcohol (or other small chain alcohols, such as methanol, ethanol, and n-butanol) mixed with diethylene glycol, ethylene glycol, ethylene glycol monoethyl ether, ethylene glycol monomethyl ether, propylene glycol, or benzyl alcohol, were used to prepare zein solutions directly from gluten by Coleman (1944, 1945).

Although zein can be dispersed in many organic and mixed solvents, the more common solvent used for laboratory extraction from corn flour or CGM is 70-90% ethanol or 55-88% isopropyl alcohol. The flour or meal is first defatted with hexane or ethyl ether; then water- and salt-soluble proteins are removed with 0.5% NaCl before the process of zein extraction (Osborne and Mendel 1914).

Zein extraction yield was improved when 70% ethanol with 0.5% sodium acetate was used as the extraction solvent (Nagy et al. 1941). The extraction yield was further improved when a reducing agent [i.e., 2-mercaptoethanol (2-ME)] was added with 55% isopropyl alcohol (0.6% v/v) (Landry and Moureaux 1970). By

using these two improvements, both alcohol-soluble and cross-linked zeins were extracted together from corn meal. The concentration of 2-ME from 1 to 100 mM (0.0078-0.78% w/v) did not affect extraction yield at 60 °C, however, when the extraction was carried out at 25 °C, the extraction yield increased as the concentration of 2-ME increased from 1 to 100 mM (Tsai 1980).

Esen (1986) indicated that α-zeins were extractable from corn meal with 60% and 90% isopropyl alcohol. Only a limited amount of β-zeins was extracted with 60% isopropyl alcohol, and no detectable γ-zeins were extracted with either 60 or 90% isopropyl alcohol. All zeins can be completely extracted out by using 60% isopropyl alcohol plus 1% 2-ME solvent after three extractions. Ninety percent isopropyl alcohol extracted 75-80% of the total zeins which were composed of mainly α-zeins and a few of δ-zeins. Following 90% isopropyl alcohol extraction, the extracts with 60% isopropyl alcohol plus 1% 2-ME contained predominantly 40-45% β-zeins and 30-35% γ-zeins, and only minor α-zeins (Esen et al. 1985). Esen (1987) demonstrated that most of the α-zeins did not appear to be present as large oligomers and polymers, but as monomers, while all the γ-zeins and most of the β-zeins were oligomers or polymers which are insoluble in aqueous alcohol unless intermolecular disulfide linkages are reduced.

Based on the solubilities of α-, β-, and γ-zeins, a new extraction method was developed by Wallace and his co-workers (1990). The extraction involved solubilizing of the total endosperm proteins in an alkaline buffer containing 12.5 mM

sodium borate (pH10), 1% SDS and 2% 2-ME for 60 min at room temperature (rt). After centrifugation, the non-zein proteins in the supernatant were subsequently precipitated by the addition of ethanol to 70% of the final concentration. More than 98% of α-, β-, and γ-zeins were extracted from the endosperm meal, and more than 99% of zeins remained in the final alcohol supernatant.

Wilson (1991) reported that 55% isopropyl alcohol with 5% 2-ME plus 0.5% Na acetate (PMA) is an efficient solvent for total zein extraction. About 99% of the total α-zein, and over 90% of β-, and γ-zeins were extracted from endosperm meal by extracting twice using PMA; each extraction was processed for 2 hr (rt).

**Production Zein from Corn Gluten Meal**

Zein remained as a laboratory product until commercial production from yellow corn gluten began in 1939 (Pomes 1971). Zein is abundant in CGM, a by-product of the wet-milling process. CGM contains 60-70% proteins (db), of which zeins account for 60% of the total (db) (Watson and Yahl 1967).

Zein extracted from CGM has been studied since the 1930's (Swallen and Haute 1938; Swallen 1938; Horesi et al. 1941), however, the first commercial preparation process for zein extraction from CGM was implemented in 1942, based on a patent by Swallen et al. and the Corn Products Refining Company (1942). CGM was extracted with hot (60 °C) aqueous 85% (v/v) isopropyl alcohol. The extract, containing all of the oil in the CGM, xanthophyll pigments, and some water-

soluble materials, was mixed with hexane at 80-120% of its volume. About 97% of the oil, 90-95% of the xanthophyll pigment and most of the isopropyl alcohol remained with the hexane. After the hexane layer was separated, zeins were precipitated by injecting the extract into cold water, and the precipitated zein was spray dried with hot air. About 50% of the protein in the CGM, or 70% of the total zein content, could be obtained. A revamped process for zein extracted from CGM was built by the Corn Products Refining Company which yielded about 6.8 million kg (15 million lb) zein per year in 1957 (Forbath 1957). This process reportedly reduced process time, maintenance costs and reagent consumption, while increased yield by 20%, and simplified the operation.

Based on the fact that zein can be dissolved in a dilute alkaline solution (Morris et al. 1956), a process for recovering 'whole zeins' from corn gluten was developed by Morris and Wilson (1959). The product from this process had a different zein composition from previous commercially produced zeins which were obtained by 85% isopropyl alcohol extraction, because all zeins, not only α-zein, were extracted. Zeins were successfully extracted from CGM using an aqueous system containing 28-33% (w/w) isopropyl alcohol and at least 6% lime. The extraction was more efficient when the system was heated at a temperature ranging from 70 °C to the boiling point of the alcohol. The slurry was stirred for 15 min, centrifuged, and allowed to cool (to 30 °C) and settle. The supernatant liquor was decanted and fresh alcohol solvent was added for a subsequent extraction. After 10

extraction cycles, not only α-zeins, but almost all of the β- and γ-zeins were obtained. The total process used only one organic solvent (isopropyl alcohol) at a low concentration, therefore, the cost of solvent and solvent recovery was very low.

The current reported commercial method for zein production from CGM is an improved process based on Swallen's research. A single step of extraction is carried out with 88% (w/w) isopropyl alcohol containing 0.25% NaOH at 55-65 °C. The zein-alcohol extract is chilled at -10 to -20 °C to separate zeins from the solvent (Carter and Reck 1970). Repetition of the extraction with 88% isopropyl alcohol, followed by cooling and decanting cycles, are carried out to increase in zein purity. The process does not require solvent distillation which may cause denaturation of proteins; therefore, high quality zein can be obtained. Another advantage is that a second solvent is not required for oil and pigment removal. The operating costs, due to the solvent mixture recovery system required, are decreased, and the operation is reportedly more safe. However, low yields (20-24%) and variable zein quality (i.e. gelling properties) are still problem (Dimitroglou and Breene 1994). Today, only one company, Freeman Industries, Inc., Tuckahoe NY, produces zein using a process reportedly similar to the Carter and Reck patented process.

## Corn Wet-Milling Process

### Wet Milling General Procedure

The amount of corn wet milled in this country has increased in response to the increased demand for sweeteners and fuel-grade ethanol. About 1.2 billion bushels of corn were processed by the wet milling process in 1993. As a by-product of wet milling, the amount of CGM available has also increased. An estimated 922 million kg (2,031 million lb) CGM was produced in 1993 (Andreas 1994). Most of the CGM is used as a high-protein feed for poultry, and only a very small amount of CGM is used to produce zein.

The general wet milling process consists of steeping corn in an aqueous solution of 0.1-0.25% $SO_2$ at 48-52 °C for 24-50 hr. The diffusion of $SO_2$ into the endosperm ultimately releases starch from the protein matrix. The $SO_2$ also prevents the growth of putrefactive organisms and softens the corn kernel. About 7% of the total dry weight of corn is solubilized into the steep water, including minerals, vitamins, soluble proteins, and sugars. After steeping, the corn at 45% moisture is coarsely ground, and the germs are separated. A second milling grinds the starch and protein into a fine powder, and the hull and fiber (in larger piece sizes) are removed by a 200-mesh screen. Starch is separated from proteins (often referred to as gluten) using a centrifuge based on their different densities. Starch, the main product of wet milling, is used in food and non-food applications. Steepwater, germ, fiber, and gluten are dried and sold as by-products including:

steep water as a nutrient source in biochemical fermentations, germ to produce corn oil, the mixture of steepwater, defatted germ meal and fiber as gluten feed, and gluten as CGM (Watson et al. 1951; Anderson 1963; Eckhoff 1992).

**Pilot-Plant Wet-Milling Processes**

A pilot-plant wet-milling operation can be used for milling large quantities of corn for the purpose of generating information needed for plant scale up. A typical pilot-plant processing method and facilities for wet-milling were described by Anderson (1957). Four bushels of grain were steeped in a batch process using a conical bottom tank in which steepwater was drawn continuously from the bottom, passed through a heat exchanger and added back into the steeping tank. The steeped grain was then fed by a screw conveyor to an 8 inch (0.20 m) Bauer mill (single running disc grinder) for the first grinding. The germ was separated by floatation from the starch slurry, screened, and washed on a Rotex gyratory shaker with a 60-mesh screen. The underflow from the germ separator was dewatered on the shaker with a 200-mesh screen, and passed though either a buhr-stone mill (single runner type) or a Rietz disintegrator (a vertical attrition mill) for the second grinding. The material after the second grinding was passed over the Rotex shaker with a 26-mesh screen for the recovery of the coarse fiber. The underflow was then passed though the second shaker equipped with a 200-mesh screen to recover fine fiber. The remaining mill starch slurry can be separated into starch and gluten by

either tabling or centrifugation after adjusting the specific gravity of the slurry. The pilot plant starch table was 14 m (46 ft) long and 0.3 m (12 in) wide and had a pitch of 0.06 m (2.5 in) for its entire length (about 0.26°).

Rubens (1990) described a pilot-plant wet-milling which more closely parallels commercial milling operations. The process involves two grinding steps with germ removal after the first grind by hydrocyclones; starch/protein separation was achieved with hydroclones and a disc-type centrifuge. Three bushels of corn were steeped in the batch-type operation. Steepwater was continuously circulated over the corn, and temperature, pH, and $SO_2$ concentration were controlled. After steeping, the soften corn was milled in a Foos-type mill (Sprout Waldron) (single running disc grinder) at 900 rpm. The slurry was then adjusted with water to a specific gravity of 1.059 -1.066, and processed in a hydrocyclone (Dorr-Oliver, 0.06 m (3 in), type NZ Dorrclone) for germ recovery. After germ removal, the fiber was separated by grinding using a refiner equipped with fine bar plates and operated at 3,100 rpm, followed by fiber recovery using a vibrating about 1.22 m (48 in) screening device (Sweco) with a 230-mesh screen. Final separation of the starch and gluten is accomplished using hydroclones (Dorr-Oliver 10 mm Dorrclones) for thickening, primary separation and washing. The starch was then transferred to a 0.91 m (36 in) basket centrifuge with a fabric filter surface for dewatering, and dried in a flash dryer. The pilot plant process he described for dent corn gave a starch yield of 58.8% with 0.63% protein.

**Drying of Corn Gluten Meal**

Since high starch recovery is the primary goal of wet milling, and most CGM is used as poultry feed, there is less attention given to the quality of CGM. Commercially, gluten is thickened with a nozzle bowl centrifuge and dewatered by either a decanting centrifuge or a rotary drum filter (Blanchard 1992). The thickened gluten with 55-60% moisture is dried to 12% moisture by flash, rotary, or steam heated tubular dryers. Flash dryers process less material, have relatively short retention times (few seconds), and less thermal efficiency. Rotary dryers are relatively inexpensive and simple, but are cumbersome and difficult to control, so that scorching of gluten is common. Steam tubular dryers are more efficient than flash dryers and relatively inexpensive as well. Therefore, the steam tubular systems have been the dryers of choice in recent years. The drying temperature is typically less than 400 °C to avoid a dark colored product, burnt particles, and offensive odor and haze in the dryer exhaust (May 1987).

**Influence of Drying Conditions on Corn Proteins**

The severity of artificial drying may cause chemical and physical changes of wet-milled products. Extensive heat can not only decrease starch yield and purity, but can also decrease the amount of extractable proteins. McGuire and Earle (1958) found that the proteins extracted from corn using water, 5% salt solution, and 0.01N KOH solution were decreased significantly ($P<0.05$) with increased drying

temperature of the kernels in the range of 48.9 to 93.3 °C. The nitrogen content extracted with 60% ethanol at 79.4 °C had no effect on kernels that had been air dried or dried at 93.3 °C. There was also no indication that any critical damage had occurred at any particular corn kernel drying temperature. The soluble protein contents in the filtrate of the steepwater and ground steeped grain were decreased when the drying temperature of corn kernels increased from 60 to 93 °C (Watson and Hirata 1962). Wall et al. (1975) reported that a substantial decrease in salt-soluble proteins and a small decrease in alcohol soluble (70% ethanol) proteins occurred when whole corn was dried from 25 to 15% moisture at 143 °C. They concluded that extensive heat treatment of native whole corn denatures protein resulting in molecular aggregation through noncovalent hydrophobic interaction and covalent disulfide cross-links that contribute to protein insolubility. Weller et al. (1987) observed that severe decreases in ethanol (70%) soluble protein content occurred when the highest harvest moisture corn (30% moisture) were dried from 50 to 71 °C. The loss in solubility was most likely due to chain unfolding and the formation of new intermolecular disulfide bonds within the endosperm protein.

**Influence of CGM Processing on Zein Properties**

The influence of commercial processing on the properties of zein was studied by Boundy et al. (1967). Zein extracted directly from corn endosperm (lab zein) has a lower sulfur content than the zein obtained from CGM (commercial zein) due to

the $SO_2$ in the steeping process associating with zein to form S-sulfocysteine residues. No free sulfhydryl groups were present in any of the zein samples. All cysteine/cystine residues in lab-produced zein were in the disulfide form, but only 30% of the cysteine/cystine in the commercial zein was present in disulfide form, others were in the S-sulfocysteine form.

Neumann and his co-workers (1984) compared the proteins extracted from commercially-produced wet and dried CGM. They reported that the amount of alcohol soluble proteins was greater from CGM than from native corn due to the action of the $SO_2$ cleavage of disulfide bonds in the steepwater. Wet CGM contained more salt-soluble proteins than dried CGM, but the amount of alcohol soluble protein was only slightly higher in the wet CGM compared to dried CGM. Both CGM samples had the same amount of total sulfur-containing amino acids and S-sulfocysteine, but dried CGM had no cysteine compared to the 9.9% of cysteine found in the total cysteine/cystine content in the wet CGM. The absence of cysteine and large amount of cystine in the dried CGM must be due to the quantitative oxidation of sulfhydrals to the disulfide bond during heating. The S-sulfo linkage was stable at neutral and mildly acidic pH, and S-sulfocysteine was not degraded by the commercial drying.

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# 3. FACTORS AFFECTING YIELD AND COMPOSITION OF ZEIN EXTRACTED FROM COMMERCIAL CORN GLUTEN MEAL

A paper to be submitted to Cereal Chemistry

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## ABSTRACT

Twelve corn gluten meal samples obtained from six wet-milling plants were processed into zein. Zein was extracted using 88% aqueous isopropyl alcohol at pH 12.5, followed by chilling. Protein recovery ranged from 21.3 to 32.0%, and protein purity from 82.1 to 87.6%. Protein recovery increased as the protein purity increased, with a correlation coefficient of $r = 0.76$ ($p<0.01$). One of the major factors influencing extraction yield was protein composition; especially α-zein content which ranged from 53.4 to 64% of the total protein in the corn gluten meal samples. The intensity of red color of the corn gluten meal was negatively correlated with protein recovery and zein purity with correlation coefficients of $r = -0.66$ and -0.72 ($p<0.02$), respectively.

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## INTRODUCTION

Zein, the predominant storage protein found in maize, was initially designated as a prolamine by Osborne based on its solubility in aqueous alcohol solution and its high content of proline, glutamine and asparagine (Osborne and Mendel 1914). Zein is a heterogeneous protein separable into four classes based on solubility, amino acid composition, electrophoretic, and immunological properties (Shewry and Tatham 1990; Wilson 1991). The major group of proteins in zein, α-zeins (MW 19,000 and 22,000 D), account for about 71-85% of the total zein fraction. β-Zeins (MW 14,000 D) are 1-5% of the total zein fraction, and γ-zeins (MW 28,000 and 16,000 D) are 10-20% (Wilson 1991). δ-Zeins (MW 10,000 D) comprise the remaining 1-5%.

Corn gluten meal (CGM) is a valuable co-product of the wet-milling process and is used primarily as poultry feed because of its high xanthophyll and protein and low fiber contents. CGM contains 60-70% protein, and zein comprises about 60% of the total protein on a dry basis (db). Zein isolation from CGM has been studied since the 1930's. Nykvist (1934) reported that zein was extracted from CGM using a 75-95% ethanol solution at 40-60 °C, then precipitated from the alcohol solution by adding salt or acetone. Swallen and his coworkers studied every step and apparatus related to zein extraction (Swallen and Haute 1938; Swallen 1938; Horesi et al. 1941). In 1942, the first commercial process for zein extraction from CGM was implemented based on a patent by Swallen et al. and the Corn Products

Refining Company (Swallen and Haute 1942). CGM was extracted with hot (60 °C) aqueous 85% (v/v) isopropyl alcohol, then hexane was added to the clarified extract by removing the oil and pigments. After the hexane layer was removed, zeins were precipitated by adjusting the polarity with cold water, and then spray dried. The reported yield was 50% based on the weight of the protein content in the CGM. The process used of two solvents and was relatively expensive.

Based on the fact that zein can be dissolved in dilute alkali, Wahl (1934) extracted zein from CGM using a 0.25% NaOH solution. A pH of at least 11.5 with a total alkali content of about 1.2% NaOH (based on the weight of zein) is required (Swallen 1941). Morris and his co-workers (1956 and 1959) developed a process with an extraction solvent containing a low level of organic solvent and $Ca(OH)_2$. Whole zein (including α-, β-, and γ-zeins) was successfully extracted from CGM using an aqueous system containing 28-33% (w/w) isopropyl alcohol and at least 6% lime. The extraction was more effective when the system was heated to a temperature ranging from 70 °C to the boiling point of isopropyl alcohol. The slurry was stirred for 15 min, centrifuged, and allowed to cool and settle. The amount of total N extracted was about 75%. The supernatant liquor was then decanted and added to fresh alcohol solvent for subsequent extraction. After 10 extraction cycles, the zein contained only 2% of the oil, color bodies and other non-protein impurities. Because the extract did not have to be treated with hexane, the process eliminated

the cost of hexane in the defatting step that involved separation and recovery of mixed solvents.

The current reported commercial method for zein production from CGM is an improved process based on Swallen' s research (1941). A single extraction is carried out with 88% (w/w) isopropyl alcohol containing 0.25% NaOH at 55-65 °C. The extract is chilled at -10 to -20 °C to precipitate zeins from the solvent (Carter and Reck 1970). An increasing zein purity results from repetition of the process using 88% isopropyl alcohol followed by the cooling and decanting cycles. The process does not require separation of zein from the solvent by distillation or an additional extraction with a second solvent (e.g. acetone). Another advantage is that an additional solvent is not required for oil and pigment removal. The operating costs, due to the complex solvent mixture recovery system required, can be decreased, and the operation is reported to be more safe. However, the low yield (about 20-24% of initial CGM weight) and variable quality (e.g. protein content, color intensity, and gelling properties in alcohol solution) of extracted zeins are still problems (Dimitroglou and Breene 1994).

Since zein is an excellent candidate for food and non-food uses because of its unique properties, such as hydrophobicity and film and fiber formation, methods to improve production yield are extremely important to increase zein utilization. If the factors that influence the yield of zein were identified, yields might be significantly increased. The objectives of this research project were to: 1) measure

and compare the yields and purities of zein recovered from commercial CGM; and 2) identify the factors that influence zein recovery and quality.

## MATERIALS AND METHODS

### Corn Gluten Meal Samples

Twelve CGM samples were obtained from six different wet-milling plants in Iowa and Illinois.

### Extraction of Zein

CGMs were extracted in the lab based on the procedure of Carter and Reck (1970). CGM (30 g) was continuously stirred in a 400-ml beaker with 120 g of extracting solvent containing 88% (w/w) isopropyl alcohol and 12% (w/w) pH 12.5 aqueous NaOH at 60 °C for 1 hr (Fig. 1). The mixture was centrifuged for 15 min at 8,000 x g in a Beckman Model J2-21 centrifuge (Palo Alto, CA); the supernatant was decanted and filtered to remove the alcohol insoluble residue. About 50 ml of the extraction solvent was used to wash the residue. The filtered solution was chilled to -18 °C overnight using a refrigerated bath. The zeins formed a taffy-like bottom layer precipitate and the top clear solution was decanted. For purifying zeins, 120 g of 88% aqueous isopropyl alcohol was added to redissolve the zeins at room temperature (rt), followed again by chilling and cool centrifuging. The precipitated zein was dried in a vacuum oven at about 50 °C and 0.6-0.8 bar

pressure. The extraction process was replicated five times for each CGM sample.

Zein yield was calculated as the percentage of the extracted zein weight from the total CGM weight (db). Protein recovery was calculated as the percentage of the protein in the extracted zein from the protein of the CGM. Protein purity was defined as the protein content in the extracted zein.

**Proximate Analysis**

Moisture contents of the CGM samples were determined by the Karl Fischer method E203-75 (ASTM 1975) using a Karl Fischer automatic titrator (Fisher Scientific Model 392, Pittsburgh, PA). The crude fat content was measured using the Goldfisch apparatus (Laboratory Construction Co., Kansas City, MO) method 30-20 (AACC 1983). The nitrogen contents in CGM and zein samples were determined by Kjeldahl (Tecator, Sweden) analysis, method A-18 (CRA 1986), and the protein content was estimated by using the nitrogen conversion factor of 6.25.

**Color Measurement**

The HunterLab Labscan (Hunter, Fairfax, VA) was used to measure the color of the CGM samples. The **L** value indicates the lightness, 0 to 100 representing dark to light. The **a** value gives the degree of the red/green color, with a higher positive **a** value indicating more red. The **b** value indicates the degree of the yellow/blue color, with a higher positive **b** value indicating more yellow.

**Protein Fraction Isolation and HPLC Analysis**

The CGM samples (0.40 g) were extracted in 50-ml centrifuge tubes with 10 ml of 0.5 M NaCl solution. The mixture was shaken for 20 min (rt) at 130 rpm, and centrifuged at 15,000 x g for 15 min in a Beckman Model J2-21 centrifuge. The supernatant containing water and salt-soluble proteins was collected, and the extraction procedure was repeated one time. The precipitate was then extracted with 10 ml of 55% (v/v) isopropyl alcohol and 5% (v/v) 2-mercaptoethanol plus 0.5% (w/v) sodium acetate solvent (PMA). The mixture was shaken for 2 hr (rt) and centrifuged at 15,000 x g for 15 min. The supernatant containing total zeins was collected for high performance liquid chromatography (HPLC) analysis, and the residue was washed twice with 10 ml of PMA. The protein contents of the water- and salt-soluble fraction and PMA insoluble residue were determined by using the Kjeldahl method.

The reverse phase high performance liquid chromatography (RP-HPLC) system consisted of a Beckman Model 110A pump system (Fullerton, CA), an ISCO UV detector (214 nm) (Lincoln, NE), an AXXI-CHROM Model 710 microprocessor HPLC solvent programmer/system controller (Cole Scientific Inc., Los Angeles, CA), and a Shimadzu Model R3A integrator and printer (Kyoto, Japan). A Vydac (Hesperia, CA) $C_{18}$ column (25 cm x 4.6 mm, 5-μm particle size, 300 Å pore size) was used to analyze the α-, β-, and γ-zein contents in the samples (Dombrink-Kurtzman and Bietz 1993). A 20-μl sample (about 25 μg protein) was injected for

analysis. Solvent A [15% (v/v) acetonitrile (ACN) plus 0.1% (v/v) trifluoroacetic acid (TFA)] and solvent B [80% ACN plus 0.1% TFA] were used to make a non-linear gradient. The starting buffer was 38.4% ACN, increasing at 0.8125 %/min for 10 min, 0.093 %/min for 7 min, 0.609 %/min for 8 min, 0.186 %/min for 35 min, and 1.95 %/min for 3 min, ending at 64.4% ACN. The column was eluted at 56 °C and 1.0 ml/min solvent flow rate.

### Statistical Analysis

Analysis of variance was used to determine significant effects using the 5% significant level for least significant differences (LSD). Correlation coefficients were calculated by using the SAS correlation procedure.

## RESULTS AND DISCUSSION

### Proximate Composition and Color of the CGM

The CGM samples differed significantly in proximate composition, most notably in protein content and color (Table I). Protein contents of the CGM samples ranged from 61.5 to 74.0% (db). In regard to color, the samples looked obviously different. Lightness of the CGM samples was highly correlated with yellow color ($r = 0.95$ for **L** and **b** values at $p<0.0001$), and negatively correlated with the red color ($r = -0.54$ for **L** and **a** values at $p<0.1$). The differences in the proximate composition

and color may be caused by corn hybrid, corn treatment and storage, the wet-milling process, and CGM drying conditions.

**Zein Extraction Yield and Purity**

Zein yield ranged from 17.2 to 26.6%, while protein purity ranged from 82.1 to 87.6% (Table II). There were significant differences in zein yield and purity among the samples ($p<0.001$). Over 70% of the error in zein extraction yield and purity was contributed by the CGM samples, and the rest of the error was contributed from replication.

There was a linear trend between the protein recovery and zein purity (Fig. 2). The protein recovery increased significantly as protein purity increased with a correlation coefficient of 0.76 ($p<0.01$). The relationship of zein yield and protein purity had a similar linear trend as that of protein recovery. The zein yield positively correlated with the protein purity with a correlation coefficient $r = 0.61$ at the 0.05 significance level. Thus, when the yield of extracted zein is high, the extracted zein has a high protein content.

**Influence of Color on Zein Recovery and Purity**

The red color of the CGM influenced zein extraction yield and purity. The protein recovery decreased as the a-value of CGM increased. The correlation coefficient between protein recovery and a-value of the twelve samples was -0.66

(Fig. 3) having a significance level of $p<0.02$. The zein yield also decreased as the a-value of CGM increased ($r = -0.57$ at $p<0.1$). A -0.72 correlation coefficient ($p<0.01$) showed that protein purity was also significantly negatively correlated with red color (Fig. 4), which indicates that less protein or more impurities were extracted from CGM high in red color. Because the red color is likely intensified during drying of the CGM, a larger a-value may be an indication of chemical browning during more "severe" drying. High drying temperature can denature and aggregate zeins, and tightly bind zeins with other components present, thus decreasing protein recovery and protein purity.

Since the wet-milling plants that the CGM samples were collected from have different steeping and milling procedures, CGM drying systems (e.g. flash dryer or rotary dryer) and drying temperatures (May 1991), it is difficult to simply compare sample color and obtain a relationship between the drying of the CGM and the denaturation or aggregation of zein. However, the intensity of the red color may be used as an indirect parameter to measure the loss of zein extraction capability.

The moisture and fat contents in the CGM ranged from 5.94 -11.72 % and 0.91-2.35 % (Table 1), respectively, and did not influence the zein extraction yield or purity.

**Influence of the Zein Content of CGM on Zein Extraction Yield**

The protein fraction analysis measured the level of water- and salt-soluble, alcohol-soluble (plus reducing agent), and alcohol-insoluble proteins present in CGM. The differences in the levels of these protein fractions between CGM samples were significant (Table III). Alcohol-soluble proteins, zeins, ranged from 40 to 50% (db) of the total weight of the CGM and were 60-71% of the protein content in CGM. The water- and salt-soluble proteins were only 2-6% (db) of the weight of CGM. The alcohol-soluble and salt-soluble protein contents in CGMs were similar to results reported by Neumann et al. (1984).

Small amounts of β-zein (0.7-2.9%) and γ-zein (4.7-10.6%) were present in the CGM, but much higher contents of α-zein (86.1-93.8%) were presented in the CGM samples (Table IV). The ratio of α-zein to the protein content of CGM, calculated by dividing the amount of α-zeins by the protein content of the CGM, ranged from 53.4 to 64.0% among CGM samples.

The differences in the total extractable zein and α-zein contents among samples could be attributed to the corn hybrid (Wilson 1991; Dombrink-Kurtzman and Bietz 1993). The differences in the way that corn gluten was dried may also account for these difference because of protein denaturation.

Zeins can be dissolved in aqueous alcohol, and the solubilities are different according to their class. α-Zeins and δ-zeins can be easily extracted with 90% isopropyl alcohol. β-Zeins and γ-zeins cannot be extracted with 90% isopropyl

alcohol solution, but they can be extracted along with α-zeins using 60% isopropyl alcohol (Esen 1986). β-Zeins are methionine-rich and γ-zeins are proline-rich, and both (γ, β) occur as large homo or hetero oligomers and multimers linked through intermolecular disulfide bonds. Therefore, β- and γ-zeins can be extracted by a solvent with a reducing reagent, e.g., 2-mercaptoethanol. When using 88% isopropyl alcohol without a reducing reagent, as is the extraction solvent in our procedure, the extraction product should contain almost exclusive α-zeins and possible tiny amounts of δ-zeins. Therefore, total zein or total α-zein content of the CGM should be monitored to control zein extraction and obtain stable yields and qualities.

The protein content of CGM in our investigation was significantly correlated with total zein content ($r = 0.72$) and the total α-zein content ($r = 0.75$) at the 0.01 significance level. Zein yield was positively correlated with the protein content of CGM, and more highly correlated with the total α-zein content in the CGM. Both plots of zein yields to protein contents of CGMs and to the total α-zein contents in the CGMs showed that there was an increased linear trend in the data (Figs. 5 and 6). The relationship of zein extraction yields to total α-zein contents had a correlation coefficient of 0.55 ($p<0.1$), compared to 0.48 ($p<0.12$) between yields and the protein contents of CGMs. The relatively low correlation coefficient values in the yield to protein and α-zein content could be due to the small sample pool (only 12 samples) for the statistical analysis.

**The Relationships of Red Color and α-Zein Content With Zein Yield and Purity**

Three dimensional scatter graphs show the relationships of α-zein content and red color intensity in the CGM with zein extraction yield and protein purity. Zein yield was the highest at the highest α-zein content and the lowest red color intensity, and the lowest at the lowest α-zein content and the highest red color intensity in the CGM (Fig. 7). Both red color intensity and α-zein content in CGM function together and affect extraction yield. When a low yield was obtained from a relatively high red color CGM with high α-zein content, the red color may caused by overheating during drying. When a relative high yield was obtained from high red color CGM with high α-zein content, the red color may be caused by corn hybrid. Protein recovery had similar associations with α-zein content and red color intensity in CGM as the yield. Lower red color intensity in CGM gave the higher protein purity, and the protein purity also increased as the α-zein content increased (Fig. 8).

**Extraction Efficiency From the Commercial CGM**

Comparing the protein recovery value (Table II) with the ratio of α-zeins to the protein content of the CGM (Table IV), this extraction method only obtained approximately 35-50% of the extractable α-zeins in the CGM. The low extraction efficiency may be due to disulfide bonds present among zeins and other proteins because no reducing reagent was used during extraction. Some zeins form oligomers or polymers by cross-linking disulfide bonds which impede the extraction

with alcohol (Neumann 1984). The lower extraction efficiency may also be due to hydrophobic interactions between zeins and other proteins, or other compounds such as lipids, decreasing the solubility of zein in alcohol solution.

## CONCLUSIONS

Zein was extracted from 12 CGM samples with a high temperature (60 °C) solvent extraction process followed by chill separation. Zein yield and protein recovery were positively correlated with zein purity. As the total zein content, especially total α-zein content, increased in the CGM samples, zein yield and protein recovery also increased. High red color of the CGM had a negative effect on the zein extraction yield, protein recovery and zein purity. Both α-zein content and red color intensity function together and affect zein extraction yield and purity.

## ACKNOWLEDGMENT

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Table I
Proximate Compositions and Colors of Commercial Corn Gluten Meal Samples[a]

| CGM | Moisture (%) | Fat (% db) | Protein (% db) | Color | | |
|---|---|---|---|---|---|---|
| | | | | L | a | b |
| A | 7.19 h | 1.08 gh | 73.0 b | 55.86 b | 4.38 g | 23.37 ef |
| B | 8.78 e | 1.57 e | 68.7 f | 50.37 f | 6.48 e | 23.14 f |
| C | 5.94 j | 1.14 g | 66.8 g | 45.63 g | 7.37 bc | 20.90 g |
| D | 6.67 i | 1.68 de | 66.3 h | 52.22 e | 7.46 bc | 23.91 e |
| E | 10.10 b | 1.89 c | 66.2 h | 54.35 d | 7.04 d | 24.88 d |
| F | 9.54 c | 1.06 gh | 71.6 c | 55.42 cd | 7.62 b | 25.50 cd |
| G | 11.72 a | 2.35 a | 74.0 a | 56.95 b | 7.66 b | 26.29 b |
| H | 9.29 d | 1.71 d | 69.8 d | 50.44 f | 8.60 a | 23.44 ef |
| I | 7.51 g | 1.74 d | 65.5 i | 43.56 h | 8.52 a | 19.64 h |
| J | 8.05 f | 1.40 f | 69.1 e | 52.11 e | 7.69 b | 23.62 ef |
| K | 8.76 e | 2.08 b | 61.5 j | 64.11 a | 5.72 f | 27.75 a |
| L | 8.07 f | 0.91 i | 69.1 e | 56.84 bc | 7.12 cd | 25.90 bc |

[a] Means followed by different letters within the same column are significantly different ($p<0.05$).

Table II
Yields, Protein Purities and Protein Recoveries of Extracted Zein[a]

| CGM | Yield (%) | Protein Purity (%) | Protein Recovery (%) |
|---|---|---|---|
| A | 26.6 a | 87.6 a | 32.0 a |
| B | 24.1 b | 86.3 bc | 30.3 ab |
| C | 21.7 cd | 85.8 cd | 27.9 bc |
| D | 22.5 bc | 84.8 def | 28.9 b |
| E | 22.6 bc | 87.5 ab | 29.8 ab |
| F | 22.3 bc | 84.1 efg | 26.2 cd |
| G | 22.5 bc | 84.3 efg | 25.6 cd |
| H | 18.8 ef | 82.1 h | 22.1 ef |
| I | 17.2 f | 84.0 fg | 21.9 ef |
| J | 20.2 de | 83.1 gh | 24.3 de |
| K | 18.6 ef | 84.8 def | 25.6 cd |
| L | 17.3 f | 85.4 cde | 21.3 f |

[a] Means followed by different letters within the same column are significantly different ($p<0.05$).

Table III
Protein Compositions (%) of Corn Gluten Meal Samples[a]

| CGM | Albumins & Globulins | Zeins | Alcohol-Insoluble Proteins |
|---|---|---|---|
| A | 2.8 g | 49.8 a | 20.4 e |
| B | 3.4 f | 44.8 d | 20.5 de |
| C | 4.5 c | 41.8 e | 20.5 de |
| D | 4.2 cd | 39.8 g | 22.3 bc |
| E | 3.7 ef | 47.1 b | 15.4 g |
| F | 2.1 hi | 46.8 b | 22.7 b |
| G | 6.1 a | 47.0 b | 20.9 d |
| H | 2.5 gh | 45.4 c | 21.9 c |
| I | 1.9 i | 41.7 e | 21.9 c |
| J | 2.0 i | 41.5 ef | 25.6 a |
| K | 5.0 b | 41.1 f | 15.4 g |
| L | 4.0 de | 45.4 c | 19.7 f |

[a] Means followed by different letters within the same column are significantly different ($p<0.05$).

Table IV
Zein Contents of Corn Gluten Meal Samples

| CGM | α-Zeins in Total Zeins[a] (%) | β-Zeins in Total Zeins[a] (%) | γ-Zeins in Total Zeins[a] (%) | α-Zeins to the Proteins in CGM (%) |
|---|---|---|---|---|
| A | 93.8 a | 0.74 e | 4.67 e | 64.0 |
| B | 91.0 bc | 1.66 bc | 5.56 de | 59.3 |
| C | 89.4 cd | 1.03 de | 8.07 b | 55.9 |
| D | 89.0 cd | 2.18 b | 6.86 bcd | 53.4 |
| E | 88.5 de | 1.97 c | 7.20 bc | 63.0 |
| F | 87.6 de | 2.12 b | 7.71 b | 57.3 |
| G | 89.1 cd | 2.94 a | 5.49 de | 56.6 |
| H | 89.6 bcd | 2.07 b | 6.58 bcd | 58.3 |
| I | 86.1 e | 1.39 cd | 10.58 a | 54.8 |
| J | 92.0 ab | 1.35 cd | 5.44 de | 55.3 |
| K | 87.7 de | 1.81 bc | 8.04 b | 58.6 |
| L | 92.0 ab | 1.03 de | 5.76 cde | 60.4 |

[a] Means followed by different letters within the same column are significantly different ($p<0.05$).

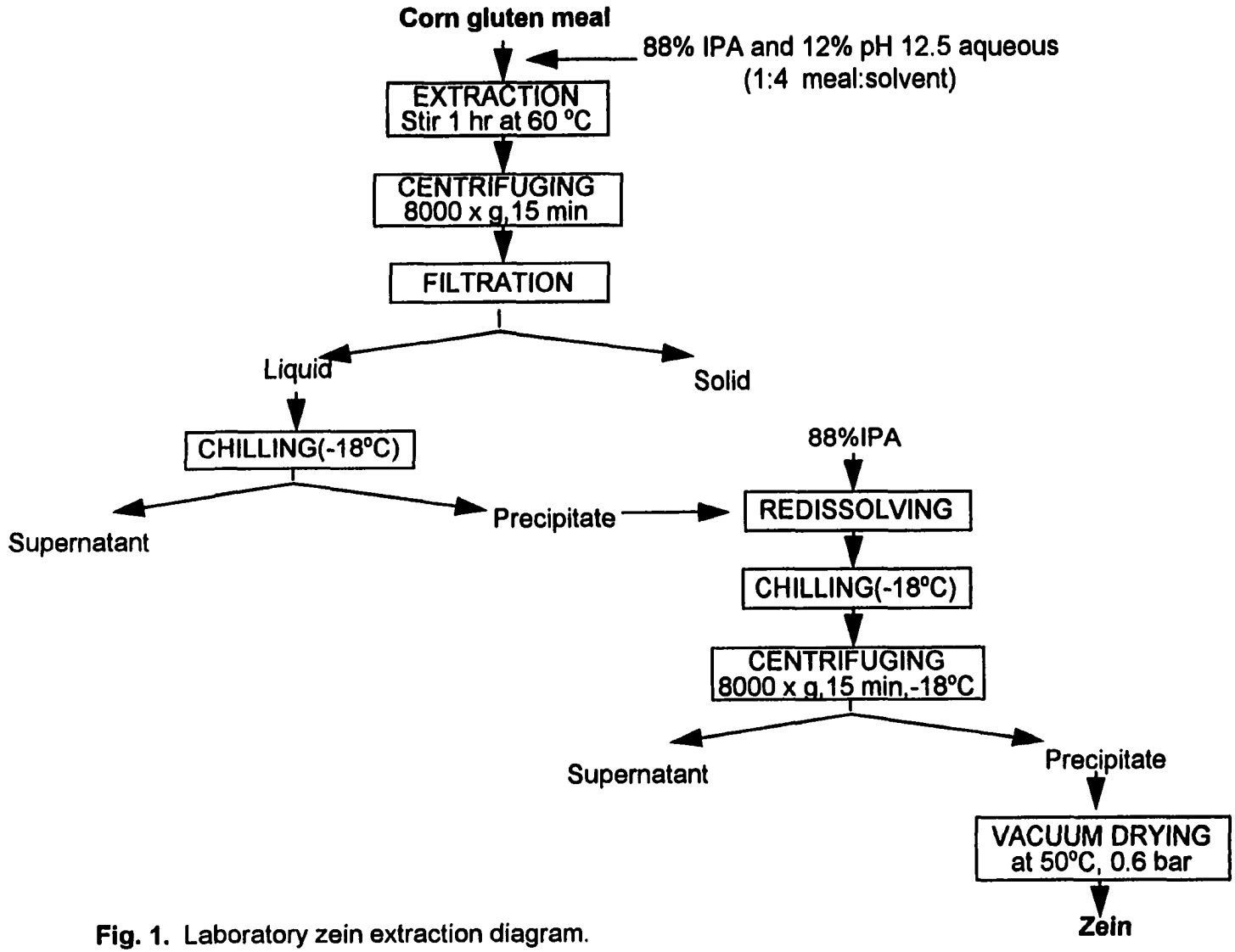


**Fig. 1.** Laboratory zein extraction diagram.

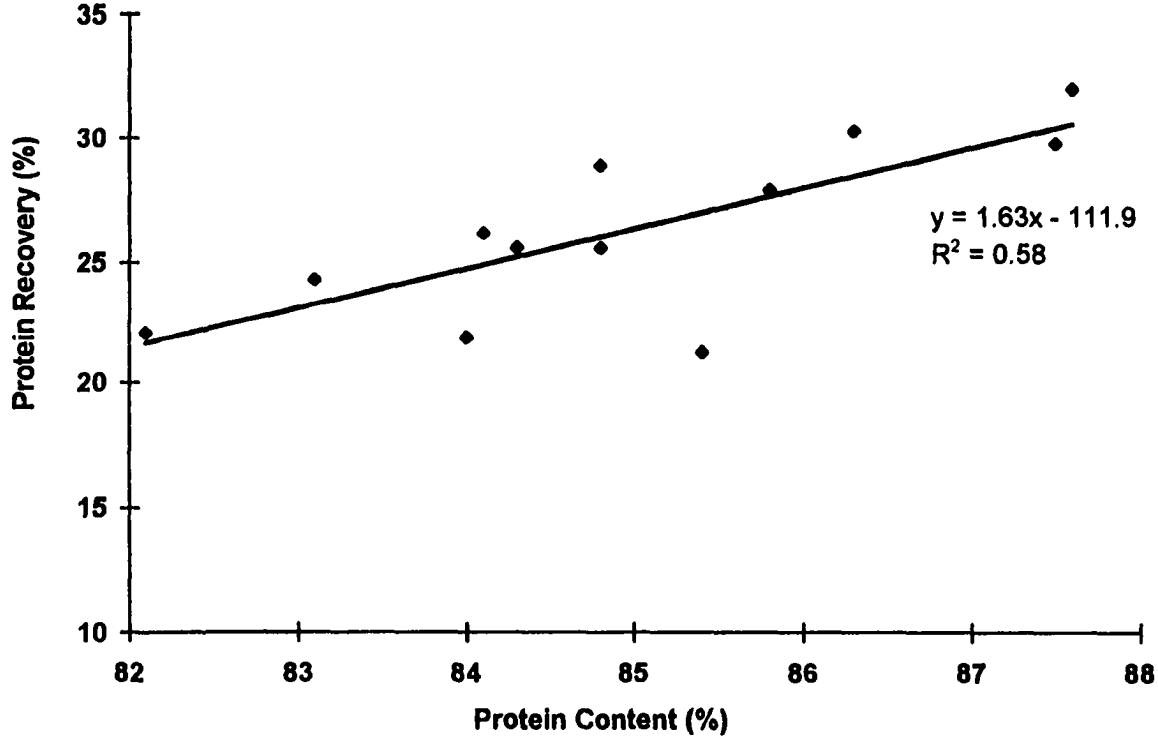


**Fig. 2.** Relationship between protein recovery and purity of extracted zein.

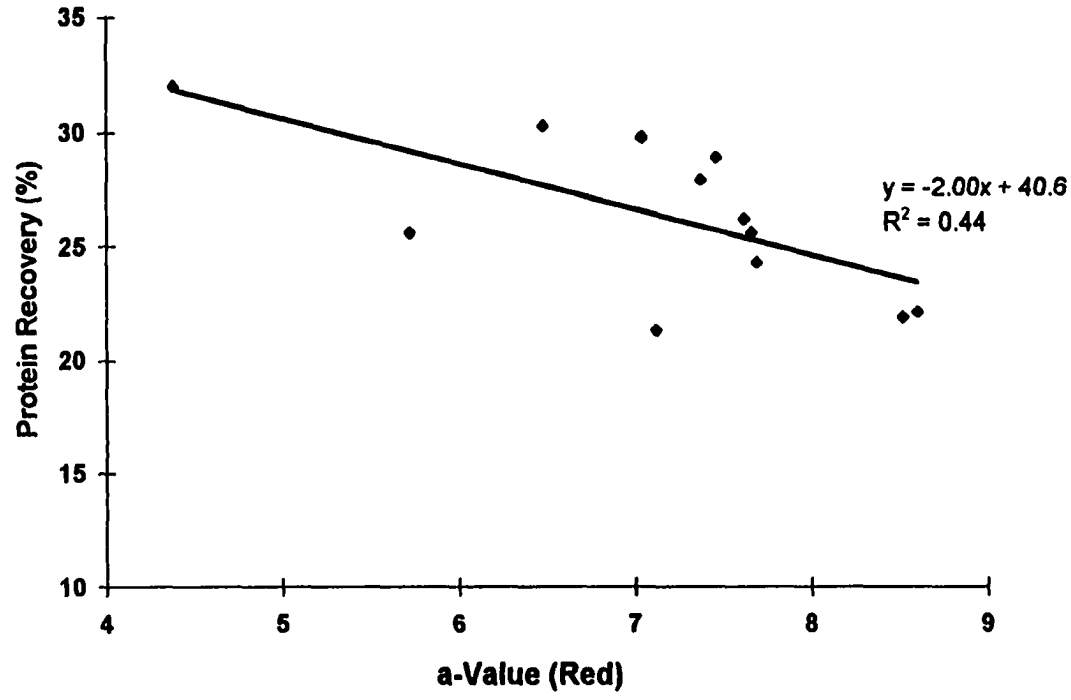


**Fig. 3.** Relationship between protein recovery and the red color of the corn gluten meal samples.

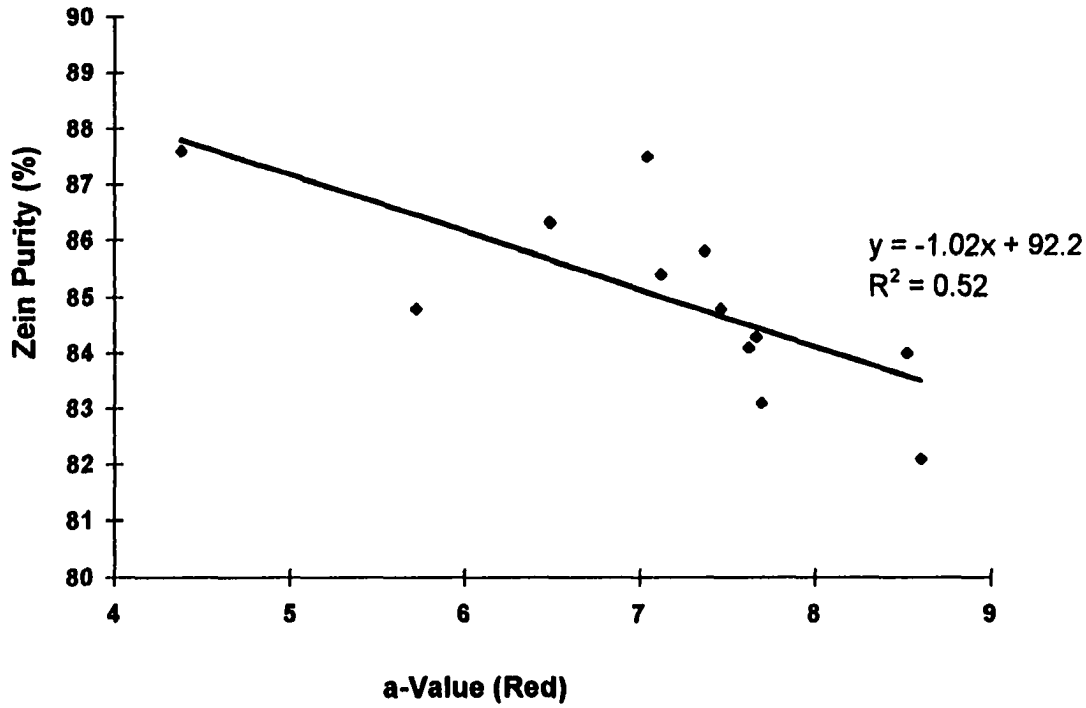


**Fig. 4.** Relationship between the zein extraction purity and the red color of the corn gluten meal samples.

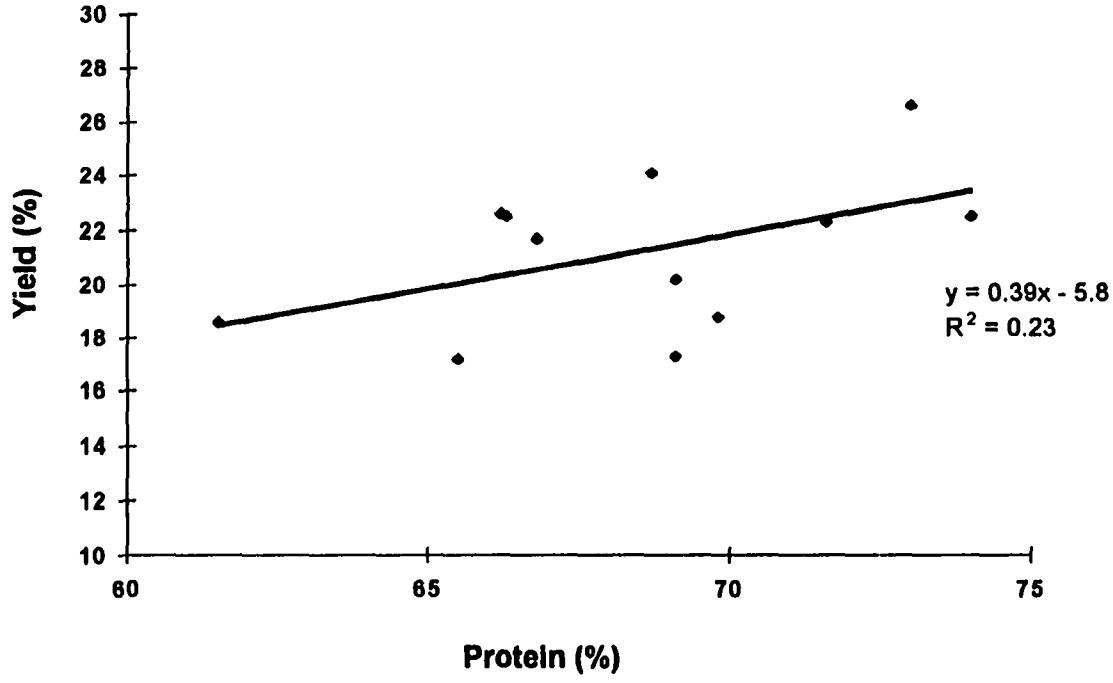


**Fig. 5.** Relationship between the zein extraction yield and the protein content of corn gluten meal samples.

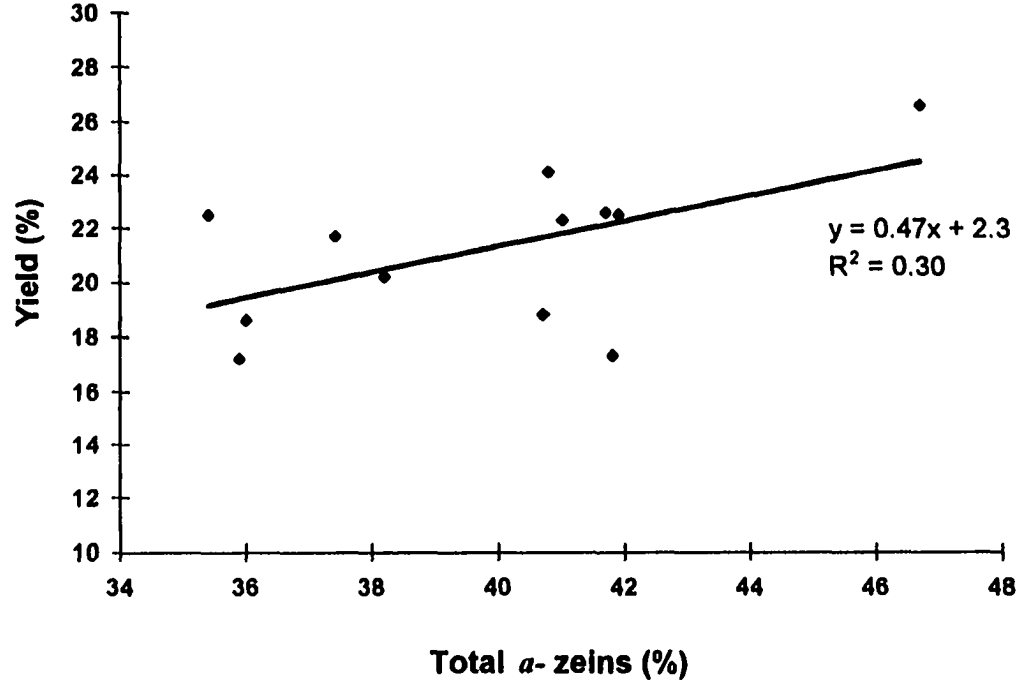


**Fig. 6.** Relationship between zein extraction yield and the total *a*-zein content in the corn gluten meal samples.

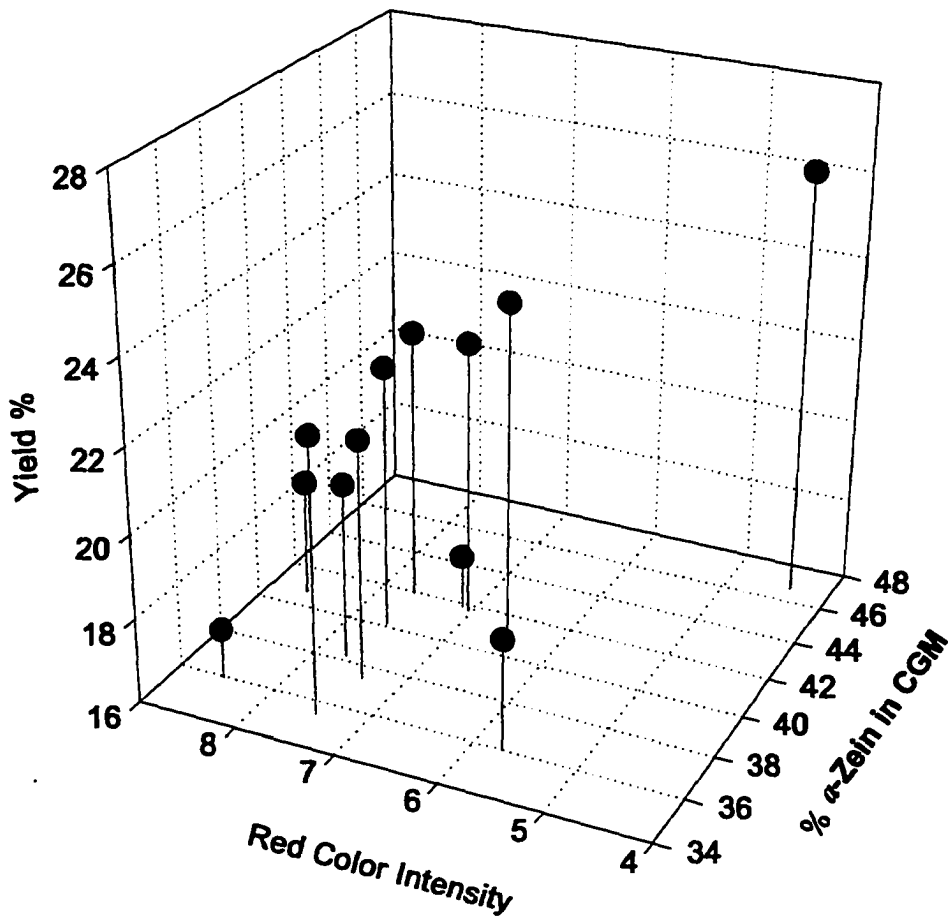


**Fig. 7.** Relationships between extraction yield and the total α-zein content and red color intensity of the corn gluten meal samples.

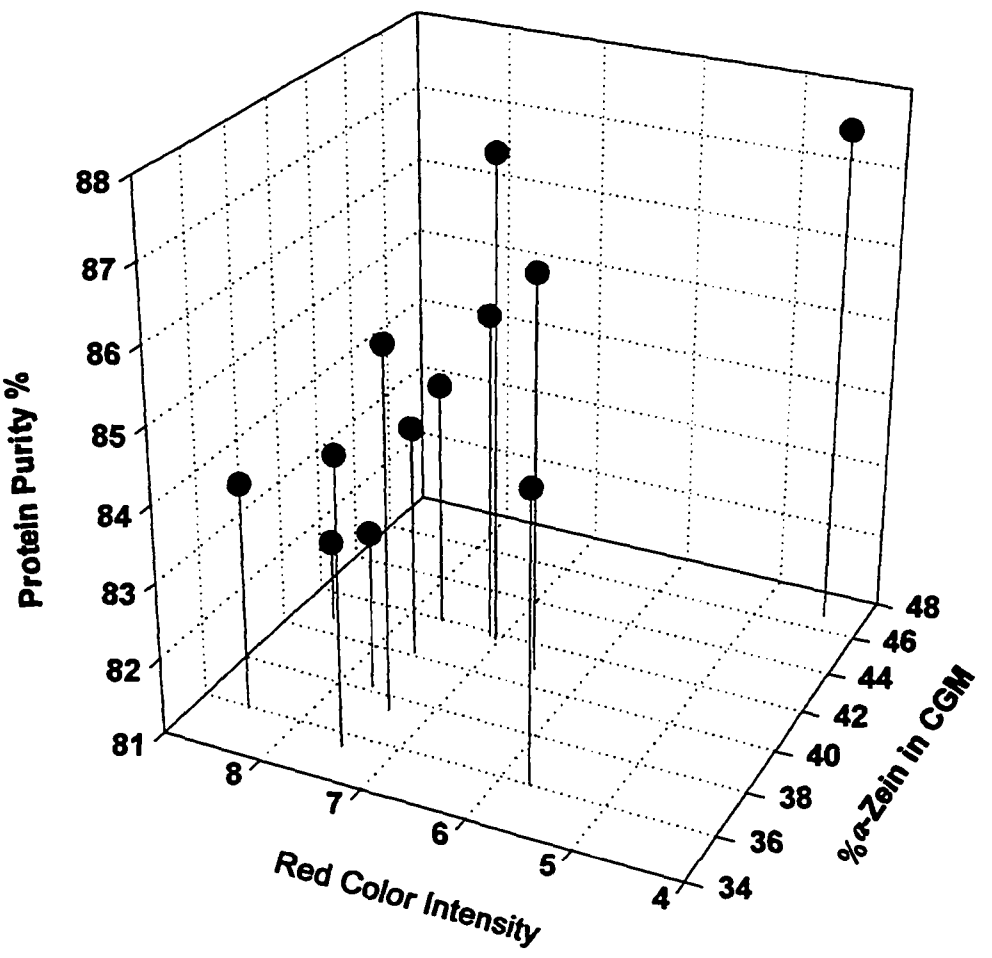


**Fig. 8.** Relationships between protein purity of extracted zein and the total α-zein content and red color intensity of the corn gluten meal samples.

# 4. NOTE ON A PILOT-PLANT WET-MILLING PROCESS FOR PRODUCING CORN GLUTEN MEAL

A paper to be submitted to Cereal Chemistry

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## ABSTRACT

Corn gluten meal with a relatively higher protein content was produced successfully in a pilot-plant wet-milling facility. Higher yield and protein content gluten was produced from the corn with higher protein content. Drying gluten with low heat produced a lighter colored corn gluten meal than high temperature dried gluten meal. Maize hybrid also affected the color of corn gluten meal.

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## INTRODUCTION

The amount of corn processed by the wet-milling industry has grown rapidly as the demand of sweeteners and fuel-grade ethanol has increased. About 1.2 billion bushels of corn was processed by wet milling in 1993 (Andreas 1994). As a co-product of wet milling, the amount corn gluten meal (CGM) produced has also increased. It was estimated that about 922 million kg (2,031 million lbs) of CGM was produced in 1993. Most of the CGM was used as a high-protein feed for poultry; only a very small amount of CGM was used to produce zein.

Both laboratory- and pilot-plant-scale wet-milling processes can be used to evaluate the wet-milling characteristics of corn, and the effect of processing techniques on product yield and quality (Watson et al. 1951; Anderson 1957, 1963; Eckhoff et al. 1993). A pilot-plant study is the process of choice when large samples of starch or other by-products are required for further study, or data is required to generate information needed for plant scale-up. The typical wet-milling pilot-plant process involves two grinding steps followed by starch/gluten separation with a starch table (Anderson 1957). Rubens (1990) described a pilot-plant wet-milling process which more closely parallels commercial milling operations. This process involves two grinding steps with germ removal after the first grind by hydroclones; starch/gluten separation is achieved with hydroclones and a disc-type centrifuge. For dent corn, Rubens' process had a starch yield of 58.8% with 0.63% protein, and a gluten yield of 7.6% with 53.8% protein.

Since high starch recovery is the primary goal of wet milling, and most of the CGM is used as a high protein feed for poultry, there is less attention on the milling process to improve the quality of protein in the CGM. However, maize genotype and the severity of artificial drying affect gluten yield and protein quality. MacMasters and his coworkers (1959) reported that the protein content in gluten decreased, and starch recovery decreased as the corn drying temperature increased from ambient temperature to 93.3 °C. The amounts of salt-soluble proteins and the proteins dissolved in 0.01N KOH solution significantly decreased when corn was dried at high temperature (93.3 or 143 °C) (McGuire and Earle 1958; Wall et al. 1975).

Commercially, gluten is thickened with a nozzle-bowl centrifuge, and dewatered by either a decanting centrifuge or a rotary drum filter. Gluten meal is then dried by flash, rotary, or steam heated tubular dryers (Blanchard 1992). Flash dryers process less material, have a relatively shorter dryer retention time (several seconds) and less thermal efficiency. Rotary dryers are relatively inexpensive and simple, but are cumbersome and difficult to control so that scorching of gluten is common. Steam tubular dryers are more efficient than flash dryers and less expensive. Therefore, the steam tubular systems have been the dryer of choice in recent years. Drying temperatures less than 400 °C are recommended to avoid a dark colored product, burnt particles, and offensive odor or haze in the dryer exhaust (May 1987).

Neumann et al. (1984) reported that wet CGM contained more salt-soluble proteins than does dried, but the amount of alcohol soluble proteins was only slightly different between wet and dried CGM obtained from a large commercial plant. Since drying CGM has been shown to be an important factor that influence CGM quality, the objectives of the research were: 1) to modify a pilot-plant wet-milling process to obtain CGM in relatively high yield and protein content; and 2) to evaluate the influence of hybrid and drying process on CGM color.

## MATERIALS AND METHODS

### Maize

Two varieties of yellow dent maize grown in 1994 were selected based on protein content and availability. One hybrid, Wilson D110, had 10.2% (db) protein, and the other hybrid, Pioneer 3394, had 8.6% (db). The harvested kernels were dried at ambient temperature in a bin with an air system to 13-14% moisture, and stored at 4.4 °C.

### Wet-Milling Procedure

The pilot-plant design and procedure were based on the process design of Anderson and Rubens (Anderson 1957; Rubens 1990), with the goal of obtaining gluten with a higher protein content than is usually obtained in laboratory milling, and similar to what is achieved in commercial production. A 265-L conical bottom

steeping tank (Process/Storage System, Model 70-gallon JOVC, Charlevoix, MI), with a hot water jacket system which controlled the temperature of the steepwater at 48-50 °C, was used as the steep tank. Twenty kilograms of corn kernels were steeped in a 50-L solution containing 0.2% sulfur dioxide and 0.44% lactic acid for 40 hr. Degermination was accomplished by first grinding in a Sprout-Bauer 0.35 m Dice Mill (Reliance Electric Co., Cleveland, OH) at 900 rpm, with a suitable plate gap based on kernel size, and a feed rate of 300 rpm. A continuous stream of distilled water at the rate of 5 L/min was supplied to prevent clogging and reduce heat built-up. The specific gravity of the ground corn was then adjusted to 1.04-1.05 to facilitate germ floatation. The germs were separated by hand using a wire mesh screen and washed through a 60-mesh screen with three 3-liter portions of water. The wash water was transferred back to the degerminated slurry and a second grinding was carried out using a Stephan grinder (Stephan Food Processing Technology, Germany) with 0.005 and 0.02 mm knife gaps at 3200 rpm speed. After the second grind, the fiber was separated using a Kason Vibroscreen separator (Kason Corporation, Model K30-1-SS, Linden, NJ) with a 0.76 m diameter, 200-mesh stainless steel screen. The separated fiber was washed with 150 L of fresh distilled water at a 5 L/min rate through the 200-mesh screen. The starch and gluten were separated from the remaining mill starch fraction by means of a starch table, 6.1 m long and 0.51 m wide with a slope of 0.54° (0.057 m pitch) for the entire length. The specific gravity of the mill starch slurry was adjusted to

1.04 using the fiber wash water and pumped onto the table at a rate of 1000 ml/min. The gluten fraction was collected in a bucket at the distal end of the table. About 35 L of distilled water was used to wash the tabled starch and collected as the wash water fraction. The gluten collected from the table overflow was stored in a cooler (5 °C) for future processing.

The fiber and germ fractions were dried in a forced-air oven for 48 hr at 50 °C. The starch was air dried on the table at room temperature with an air fan for 24 hr, and then dried in a forced-air oven for 48 hr at 50 °C.

**Gluten Treatment**

The gluten slurry was allowed to settle by storing at 5 °C overnight (about 15 hr), and concentrated to 60-80 L by siphoning off the supernatant. The majority (80% by volume) of the slurry was dewatered using a vacuum drum filter (Filtration Engineers, East Moline, IL) with a belt covering the filter surface (polypropylene, 3-8 μm retention) for the purpose of building up the cake. The resulting gluten cake contained 53-60% moisture, and was divided into three parts and dried in a forced-air oven at 50, 100, and 150 °C until the moisture content of gluten was less than 10%.

The remaining (20%) gluten slurry was allowed to settle again and concentrated to a level of 1.8-2.0 % solids by siphoning off the supernatant. One-half of the concentrated slurry was dried using a spray drier and the other half with

a freeze drier. Spray-drying was performed with a Yamato Pulvis Mini-Spray (Yamato Scientific Co., Model GA-31, Tokyo, Japan) with an inlet temperature of 120 °C, an outlet temperature of 60 °C, and 7.5-8 ml/min sample feed rate. Freeze drying was carried out using a Virtis Freeze Drier (Virtis Company, Inc., Model Ultra 35 SL, Gardener, NY). A 50.8 x 30.5 x 2.5 $cm^3$ stainless steel box filled one half deep with gluten slurry was prefrozen at -20.5 °C before it was placed into the freeze drier. Freeze drying was carried out at 80 millitorr vacuum and 25-27 °C shelf temperature for 72 hr.

The milling procedure and gluten drying processes were replicated three times for each hybrid. All of the dried CGM was stored at 4 °C until use.

**Sample Analysis**

The initial moisture, starch, protein and oil contents of maize kernels were determined by a GAC III fixed-filter, near-infrared reflectance (NIR) analyzer (Dickey-John Corp., Auburn, IL). The starch, protein and oil data were reported on a dry basis (db).

The wet milling fractions, dried germ, fiber, starch, steepwater, starch wash water, and the first concentrated gluten slurry, were analyzed for proximate composition. Moisture contents of the wet-milling fractions were determined by drying a 2.00-g sample in a convection oven for 3 hr at 130 °C (AACC 1983, method 44-15A). Crude fat was determined by using the Goldfisch method (AOAC 1984,

method 14-084 and 14-085). Protein contents were determined by measuring total nitrogen content using the Kjeldahl method and a protein conversion factor of 6.25 (CRA 1986, method A-18). The yields of wet milling fractions (starch, fiber, gluten, germ, steepwater solids, and wash water solids) were determined as the percentages of initial maize dry solids. The recovery of starch (or protein) was calculated as the ratio of the total weight of starch (or protein) recovered from wet milling to the total weight of starch (or protein) present in the corn.

The moisture content of the dried corn gluten meal was determined by the Karl Fischer method (ASTM 1975, method E203-75). The color of the CGM samples was measured with the HunterLab Labscan (Hunter, VA).

### Statistical Analysis

The general linear model and the test of least significant difference (LSD) at the 5% level were used to evaluate means.

## RESULTS AND DISCUSSION

### Yields and Recoveries of Wet-Milling Products

Table I shows the proximate analysis data of the two selected hybrids used to produce CGM. The Wilson D110 had higher protein and oil content, and lower starch content than did Pioneer 3394.

The starch yields of two hybrids were low (Table II), compared to the typical values of plant-scale (67.5%) (Johnson 1991) and lab-scale (59.2-65.1%) milling (Watson and Yahl 1967), but were similar to the pilot-plant results of Rubens (1990). Pioneer 3394 produced higher starch yield than did Wilson D110. This result was expected because of the high initial starch content in the Pioneer 3394 maize. The protein contents of the starch for both hybrids were less than 0.5%. Even though the protein contents were higher than 0.3%, typical values in commercial starch, the quality of starch produced in our procedure was better than Rubens (0.63%).

Wilson D110 maize produced significantly higher ($p<0.05$) gluten yield with a significantly higher ($p<0.05$) protein content than did Pioneer 3394. These results were also anticipated because Wilson D110 maize had a higher protein content. The gluten yields and protein contents obtained by our pilot-plant procedure were similar to Rubens' results, in spite of the fact that Rubens' procedure used a hydrocyclone compared our starch table procedure to separate gluten and starch. The yield of our gluten was lower than previous laboratory studies using tabling as the separation method for starch and gluten (6.9-7.9%), but the protein content of gluten was higher than that reported (40.8-46.0%) (Watson and Yahl 1967; Eckhoff et al. 1993).

Although the fiber yields of our process were close to the fiber yield of Rubens (1990), it was higher than the typical industrial values of 11.5% (Johnson 1991). This was primarily due to the presence of starch in the fiber fraction. The

germ yield of our procedure was low, because germs, especially broken germs, were not easily skimmed out by hand. The residue from the broken germs in the second grind is believed to have caused the relatively high oil content in the Pioneer 3394 gluten (11.0% db). The oil content in the gluten produced by an industrial wet-milling plant is 7% db (Johnson, 1991).

The steepwater yield of our process was lower than that of plant-scale (7.5%) (Johnson 1991) but higher than that of lab-scale (3.9-4.0%) wet-milling processes (Watson and Yahl 1967). Wilson D110 produced significantly higher steepwater yields than did Pioneer 3394. There was no significant difference in the protein contents of steepwater for both hybrids (34.5 and 34.7%); however, these results were lower than for plant-scale (46.0%) (Johnson 1991) and lab-scale (58.6-63.4%) (Watson and Yahl 1967) milling. The low protein loss in the steepwater may have contributed to the high protein content in the gluten. The wash water fraction was specifically produced for the production of a high quality starch and high protein content gluten. The yields of wash water were low, 0.4-0.6%, with a protein content of 7.7% for Pioneer 3394, and 14.5% for Wilson D110. Comparable data reported by Watson and Yahl (1967) had 6.7% yield and 13.4% of protein content for lab-scale experiments.

**Effect of Drying on CGM Color**

The colors of CGM dried with different drying methods were significantly different (Table III). As oven temperature increased, the **L** value (lightness) significantly decreased, and **a** value (positive **a** value = red color) significantly increased for both hybrids. From 50 to 100 °C, the **L** value decreased about 10 units, and **a** value increased over 2 units; while increasing the drying temperature from 100 to 150 °C, the **L** value decreased about 3.5 units for Wilson CGM and 1.7 units for Pioneer CGM, and **a** value increased by 0.7 and 0.4 units, respectively. The color change in CGM from 50 to 100 °C was much larger than from 100 to 150 °C. The **b** value (positive **b** value = yellow color) also varied among treatments, but the variation in the **b** value was less than for the other two parameters. Compared to the oven drying, the freeze- and spray-dried CGMs were lighter and had a lower intensity of red color, because the CGM was subjected to less heat during freeze- and spray-drying than in oven-drying. The spray-dried CGM was lighter and had lower red color than the freeze-dried CGM because it was subjected to less heat relative to freeze-drying. In spray-dryiny, the gluten was dried in only a few seconds at a temperature range of 120-60 °C, compared to 72 hr at 25-27 °C shelf temperature in the freeze dryer. The CGM from Pioneer 3394 was more red and yellow in color than the CGM of Wilson D110 because the hybrid Pioneer 3394 maize kernels were more red and yellow than the Wilson D110. The higher crude

fat content with attendant pigments might also play a role in the darker color of Pioneer 3394 CGM.

## CONCLUSIONS

The pilot-plant procedure successfully produced gluten with relatively high protein content compared to laboratory-scale milling. Maize hybrids with different initial protein content affect gluten yield and protein content of CGM. The color difference of CGM was contributed to the drying method and maize hybrid. As the temperature increases, the lightness of the CGM decreases and red color intensities increase.

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Table I
Proximate Compositions of Maize Hybrids

| Composition | Pioneer 3394 | Wilson D110 |
|---|---|---|
| Moisture (%) | 14.0 | 13.9 |
| Starch (% db) | 72.6 | 71.5 |
| Protein (% db) | 8.6 | 10.2 |
| Oil (% db) | 3.4 | 4.5 |

Table II
Yields, Protein and Oil Contents and Recoveries of Wet-Milled Products[a]

| Product | Pioneer 3394 | Wilson D110 | Rubens[b] |
|---|---|---|---|
| Starch | | | |
| Yield (% db) | 60.4 ± 0.7 | 57.9 ± 2.0 | 58.8 |
| Starch Recovery (%) | 82.8 | 80.5 | |
| Protein (% db) | 0.49 ± 0.10 | 0.46 ± 0.04 | 0.63 |
| Protein Recovery (%) | 3.5 | 2.6 | |
| Oil (% db) | 0.06 ± 0.02 | 0.06 ± 0.05 | |
| Gluten | | | |
| Yield (% db) | 6.0 ± 0.2 | 7.4 ± 0.2 | 7.6 |
| Protein (% db) | 49.8 ± 1.0 | 53.7 ± 1.8 | 53.8 |
| Protein Recovery (%) | 35.0 | 39.0 | |
| Oil (% db) | 11.0 ± 1.7 | 7.0 ± 0.8 | |
| Fiber | | | |
| Yield (% db) | 21.5 ± 3.2 | 18.6 ± 1.5 | 21.8 |
| Protein (% db) | 8.9 ± 0.2 | 10.8 ± 0.4 | |
| Protein Recovery (%) | 22.2 | 19.7 | |
| Oil (% db) | 3.0 ± 0.4 | 2.1 ± 0.4 | |
| Germ | | | |
| Yield (% db) | 5.9 ± 0.1 | 6.6 ± 0.4 | 10.5 |
| Protein (% db) | 12.6 ± 0.1 | 14.4 ± 0.4 | |
| Protein Recovery (%) | 8.6 | 9.3 | |
| Oil (% db) | 36.0 ± 1.4 | 47.0 ± 0.5 | |
| Steepwater | | | |
| Yield (% db) | 4.4 ± 0.07 | 5.3 ± 0.2 | 5.1 |
| Protein (% db) | 34.7 ± 0.4 | 34.5 ± 1.6 | |
| Protein Recovery (%) | 17.8 | 17.8 | |
| Wash Water | | | |
| Yield (% db) | 0.4 ± 0.09 | 0.6 ± 0.03 | |
| Protein (% db) | 7.7 ± 1.2 | 14.5 ± 5.6 | |
| Protein Recovery (%) | 0.36 | 0.80 | |
| Total Solids Recovery (%) | 98.6 ±3.37 | 96.3 ± 0.18 | |

[a] All data are the means of three millings.
[b] Rubens (1990).

Table III
Colors of Dried CGMs[a]

| CGM | Color[b] | | |
|---|---|---|---|
| | L | a | b |
| Wilson D110 | | | |
| Oven Drying, at 50 °C | 74.35 a | 2.32 a | 23.41 a |
| at 100 °C | 64.14 b | 4.74 b | 24.48 bc |
| at 150 °C | 60.65 c | 5.46 c | 24.70 bcd |
| Freeze Drying | 77.82 d | 1.88 d | 24.91 d |
| Spray Drying | 78.69 d | 1.15 e | 24.17 b |
| Pioneer 3394 | | | |
| Oven Drying, at 50 °C | 66.94 e | 6.40 f | 26.02 e |
| at 100 °C | 59.57 fc | 8.52 g | 25.48 f |
| at 150 °C | 57.87 g | 8.98 h | 25.46 f |
| Freeze Drying | 76.31 h | 4.00 i | 27.09 g |
| Spray Drying | 77.78 hd | 2.93 j | 27.19 g |

[a] Means of three millings.
[b] Data in the same column with different letter are significantly different at $p < 0.05$.

# 5. EFFECTS OF MAIZE HYBRID AND MEAL DRYING CONDITIONS ON YIELD AND QUALITY OF EXTRACTED ZEIN

A paper to be submitted to Cereal Chemistry

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## ABSTRACT

In this study, corn gluten meals (CGM) produced from two maize hybrids and subjected to five drying treatments were used to determine their effects on zein extraction. Zein extraction yields, protein recoveries and purities were higher in CGM from maize with the higher protein content. The yield and protein recovery of zein decreased as the drying temperature increased. Zein yield, protein recovery and purity were significantly lower in the CGMs subjected to freeze- and spray-drying than oven-drying. A relatively higher pH value in the CGM slurries, more α-zeins with less polarity, and more α-zeins with pI 7.3 were characteristics of freeze- and spray-dried CGMs. An explanation of these results based on the mechanisms of protein changes during drying is discussed.

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## INTRODUCTION

Zein has been commercially extracted from corn gluten meal (CGM), a co-product of wet milling, since the 1940's (Swallen 1942). The current commercial process, based on the patent of Carter and Reck (1970), uses a hot isopropyl alcohol base solution to extract zein followed by chilling the extract to separate the zein from the solvent. However, high variation in the yield and quality has plagued the processors. Although there have been numerous studies on the technology of zein extraction such as alternative extraction solvents, changes in temperature, extraction time, and ratio of solvent to CGM (Swallen 1941; Evans et al. 1945; and Russell and Tsao 1982), studies investigating how CGM quality affects zein extraction yield and quality have not been published.

Watson and Yahl (1967) showed that maize hybrid and severe artificial drying of the grain affect milling results, including CGM yield and quality. Extensive heat treatment may also cause chemical and physical changes to proteins and decrease their extractability. McGuire and Earle (1958) reported that the proteins extracted with water, 5% salt solution, and 0.01N KOH solution decreased significantly ($P<0.05$) when the drying temperature of the corn kernels increased from 48.9 to 93.3 °C. The nitrogen content of the extract obtained with 60% ethanol at 79.4 °C was no different from that of the air-dried sample and the sample dried at 93.3 °C. There was also no indication of critical damage occurring at any particular temperature (ambient to 93.3 °C) of corn drying. Watson and Hirata (1962)

observed that the soluble protein contents of steepwater filtrate and ground steeped grain decreased when the drying temperature of corn kernels increased from 60 to 93.3 °C. Wall and co-workers (1975) reported that a substantial decrease in salt-soluble proteins and a small decrease in alcohol-soluble proteins occurred when whole corn was dried from 25 to 15% moisture at 143 °C. They also observed decreased sulfhydral contents with increasing temperature. They indicated that an extensive heat treatment of native whole corn denatured protein and caused molecular aggregation through noncovalent hydrophobic interaction and covalent disulfide cross-links contributing to protein insolubility. Weller et al. (1987) observed a severe decrease of ethanol-soluble protein occurred when high harvest moisture corn (30% moisture) was dried from 50 to 71 °C. They hypothesized that the solubility loss was most likely due to chain unfolding and the formation of new intermolecular disulfide bonds within endosperm protein.

Neumann and his co-workers (1984) compared the proteins extracted from commercial wet and dried CGM. They found that the yields of alcohol-soluble proteins were higher from CGM than from native corn. They indicated that $SO_2$ added in the steepwater cleaved protein disulfide bonds of corn proteins during steeping, and increased zein extractability. Wet CGM contained more salt-soluble protein than did dried CGM, but the amount of alcohol-soluble protein was only slightly higher in wet than dried CGM. About 50% of cysteine-cystine in the commercial CGM was present in the cysteine or disulfide form; the remaining 50%

was in the S-sulfocysteine form which is stable at neutral and mildly acidic pH, and does not readily decompose during commercial drying. About 5-10% of the cysteine of the total cysteine-cystine content was present in the wet CGM, but no cysteine was found in commercial dried CGM. Therefore, they indicated that the absence of cysteine and large amount of cystine in the dried CGM must be due to the quantitative oxidation of sulfhydrals to disulfide bonds during heating.

The objectives of the present study were to: 1) determine the influence of drying temperature and drying method on the yield and purity of extracted zein; 2) investigate the effects of the maize hybrid on the zein yield and purity; and 3) evaluate the changes of proteins in the CGMs after wet milling and drying.

## MATERIALS AND METHODS

### Preparation of Corn Gluten Meal and Corn Endosperm Meal

CGM was produced from two maize hybrids, Pioneer 3394 and Wilson D110, by using a pilot-plant wet-milling process (Wu et al. 1995a). CGM was dried using three different methods, oven-, freeze- and spray-drying. The forced-air oven drying was carried out at 50, 100, and 150 °C. All of the CGMs were stored at 4 °C.

Corn endosperm meal was produced by first soaking corn kernels in deionized water at 50 °C overnight. After the pericarp and germ were removed by hand, the softened endosperm was ground by a mortar-pestle. The endosperm meal was dried at room temperature (rt) under the hood, passed through a 35-mesh

screen, and stored in a refrigerator. The moisture content of the endosperm meal was measured by the Karl Fischer (Fisher Scientific, Model 392, Pittsburgh, PA) method (ASTM 1975, method E203-75).

**Zein Extraction**

Zein was extracted from CGM using hot aqueous isopropanol extraction and low temperature separation (Carter and Reck 1970; Wu et al. 1995b), except that the ratio of CGM to solvent was changed from 1:4 to 1:6. Zein yield was calculated as the percentage of the initial CGM weight calculated on a dry basis (db). Protein recovery was calculated as the ratio of the protein content in the extracted zein to the protein content in the CGM (db). Protein purity was calculated as the protein content in the extracted zein; the Kjeldahl (Tecator, Sweden) method (CRA 1986, method A-18) was used to measure total nitrogen content, and the protein content was estimated based on total nitrogen content using the conversion factor 6.25.

**Separation of Protein Fractions**

CGM samples (0.40 g) and corn endosperm powder (2.0 g) were extracted with 10 ml and 20 ml, respectively, of 0.5 M NaCl solution for 20 min (rt). After centrifugation for 15 min at 15,000 x g in an Beckman Model J2-21 centrifuge (Palo Alto, CA), the supernatant was collected. The extraction was repeated once, and the supernatants were combined for protein analysis. The residues were extracted

with 10 ml (CGM) or 20 ml (corn endosperm powder) of 55% (v/v) isopropyl alcohol and 5% (v/v) 2-mercaptoethanol plus 0.5% (w/v) sodium acetate (PMA). The mixture was shaken for 2 hr (rt) at 130 rpm, and centrifuged at 15,000 x g for 10 min. The supernatant was collected. The aforementioned extraction with PMA was repeated once. The residue was then washed with 5 ml (CGM) or 10 ml (corn endosperm powder) PMA, and centrifuged. All of the extracted supernatants and wash solutions were collected and analyzed for protein contents. Protein was measured by the Kjeldahl and the protein conversion factor of 6.25.

**HPLC Analysis**

Samples for high performance liquid chromatography (HPLC) analysis were prepared by the extraction of corn endosperm powder (1.25 g) and CGM (0.30 g) with 5 ml PMA for 2 hr (rt). The supernatant was diluted 10 times with 55% (v/v) isopropyl alcohol and 5% (v/v) 2-mercaptoethanol (PM) solvent before injection. Zein (30 mg) was dissolved in 1 ml PMA, and diluted 10 times before injection.

The reverse phase HPLC analysis used an HP-1050 HPLC system with an automatic sample injector, gradient solvent delivery system, oven heater, a Diode-Array detector and a 486 computer with a Chemstation program. A Vydac (Hesperia, CA) $C_{18}$ column (25 cm x 4.6 mm, 5-μm particle size, 300 Å pore size) was used to analyze the samples (Dombrink-Kurtzman and Bietz, 1993). Zein separation was performed using nonlinear gradients of increasing acetonitrile (ACN)

in water with 0.1% (v/v) trifluoroacetic acid (TFA). The starting buffer was 30% ACN, increasing to 50% ACN at 20 min (at a rate of 1.00%/min), then to 56% ACN after 35 min (0.17%/min), ending at 64% ACN after 5 min (5.8%/min). The column was operated at 55 °C and a flowrate of 1.0 ml/min. The 20-µl sample (about 20-30 µg protein) was injected for analysis. The eluate was monitored at 214 and 280 nm on the Diode-Array detector.

**Isoelectric Focusing**

Isoelectric focusing (IEF) was performed using the model 111 mini IEF cell (Bio-Rad, Richmond, CA). An ultra-thin polyacrylamide gel (125x65x0.4 mm) was used to separate zeins. The gel contained 5% polyacrylamide, 1% ampholine in the pH range of 5-7, 1% ampholine in the 7-9 pH range, and 6M urea (Righetti et al., 1977). The samples were dissolved in a 6M urea buffer with a final concentration of 20 mg/ml (Wilson, 1984). The IEF was run for 90 min, and the gel was stained with Coomassie blue G-250 following the Bio-Rad instruction manual.

**Protein Deamidation and $NH_3$ Determination**

Deamidation of CGM protein was conducted in a 25x150 mm test tube with a rubber stopper. A glass tube that was inserted into the test tube at about 2 cm away from the bottom was connected to a high purity helium tank (99.99%), and another glass tube that passed through the rubber stopper was connected to a

collection tube. Before the reaction, the CGM sample (1.00 g) was thoroughly mixed with a preweighed amount of deionized distilled water to reach 30% (w/w) moisture content. The reaction tube was then heated in a bath at a constant temperature of 110 °C for 2.5 hr. Helium gas (100 ml/min) was passed through the reaction tube during heating, and carried released $NH_3$ to the collection tube containing 10 ml 1N HCl solution. The 5 ml aliquots of HCl was diluted to 10 ml with deionized distilled water before being analyzed for ammonia by an ammonia electrode (Orion Research Inc., Boston, MA). A reference curve was prepared using standard ammonium chloride solutions with a range of 10 ppm to $10^{-2}$ ppm. Deamidation was replicated three times for each CGM samples.

**pH of CGM Slurry**

The pH of the CGM slurry was determined with an Ionalyzer Model 501 (Orion Research Incorporated, Boston, MA) after stirring 10 g of CGM in 20 ml of deionized water for 10 min. The deionized water was boiled to remove $CO_2$ before the analysis.

**Statistical Analysis**

Statistical analysis was carried out using a randomized complete block design. Three millings were three blocks. CGM drying treatments were a factorial combination of hybrid and treatment (2x5). The general linear model and the test of

least significant difference (LSD) at 5% level were used to determine the significant difference between means.

## RESULTS AND DISCUSSION

### Influence of CGM Drying Temperature on Zein Extraction

Zein extraction yields from CGM dried in the forced-air oven at different temperatures ranged from 17.1-21.5% for Wilson D110 and 16.8-17.8% for Pioneer 3394 (Table I). The yield was significantly higher from the Wilson D110 CGM dried at 50 °C than those dried at 100 and 150 °C. The zein extraction yield of Pioneer 3394 also decreased as the oven drying temperature increased, although the changes were not significantly different at $p<0.05$. The fact that higher zein yield was obtained at 50 °C indicates that protein was less aggregated during drying.

Zein extraction yield was affected by the genotype of the maize. The yields of zein extracted from Wilson D110 was higher than from Pioneer 3394 at the same temperatures, although there were no significant differences between the yields extracted from the CGM dried at 150 °C. The results also show that there was an interaction between hybrids and drying temperature. High temperature treatment not only decreased zein extractability, but also decreased the difference caused by maize genotype.

Protein recoveries showed similar trends as did yield (Table II). Protein recovery from CGM dried at 50 °C was significantly higher than those dried at 100

and 150 °C for both hybrids. There were interactions between hybrids and oven drying treatments for protein recovery. The hybrid influence on protein recovery was significantly different at 50 °C, but not at higher drying temperatures.

Protein fraction analysis showed that hybrid means were significantly different in total protein, extractable albumin and globulin, and zein contents in oven-dried CGM (Table III). The oven-dried CGM of Wilson D110 had higher total protein, extractable albumin and globulin, and zein contents than those of Pioneer 3394. The higher original extractable zein content in the CGM can account for the higher extraction yield and protein recovery. There were no significant differences for the total protein, and albumin and globulin contents at different oven-drying temperature, however, the zein content of CGM dried at 150 °C was significantly lower than the CGM dried at 50 and 100 °C. The reason for the lower zein content in the CGM dried at 150 °C may be due to zein aggregation during drying, forming a large insoluble polymer which contributed to zein insolubility. Therefore, the extraction yields and protein recoveries were the lowest in the CGM dried at 150 °C. The interaction of hybrid and temperature may indicate that the zein aggregation in the high protein content CGM is more sensitive to temperature change than the zein in the low protein content CGM.

Protein purity of the extracted zein significantly decreased as the drying temperature increased from 50 to 150 °C ($p<0.05$) (Table IV), and the mean of 50 °C treatment exceeds the means of the 100 and 150 °C treatments by 3.1% and

5.2%, respectively. Wilson D110 had a significantly higher protein purity than that of Pioneer 3394 ($p<0.05$), and the mean difference between the hybrids was 8.1% in oven drying. The low purity may be explained by the presence of other alcohol-soluble compounds, such as oil, in the CGM. Since the CGM of Pioneer 3394 contained more oil than did the CGM of Wilson D110 (Wu et al. 1995a), this could explain why the protein purity values for Pioneer 3394 were low. Zein binding with other components, such as oil (Izzo and Ho 1989) or products of the Maillard reaction, at high temperature may be another reason.

HPLC analysis of α-, β-, and γ-zeins in corn endosperm meal and CGM indicated that wet milling decreased the β- and γ-zein contents. The β- and γ-zein contents of Wilson D110 endosperm meal were 3.34 and 19.73%, respectively, of the total zein content, and the values decreased to 0.83 and 5.67%, respectively, of the total zein content in the wet gluten. The β- and γ-zein contents of Pioneer 3394 also decreased from 5.49 and 21.25% in the endosperm meal to the 1.72 and 10.41%, respectively, in the wet gluten. Hybrid differences also affect α-, β-, and γ-zeins contents. Since the calculations of α-, β-, and γ-zeins were on a percentage basis, Wilson D110 had lower extractable β-, and γ-zein contents than Pioneer 3394; therefore, higher α-zein content than Pioneer 3394 (Table V). Drying temperature did not significantly affect the extractable β- and γ-zein contents, but the extractable α-zein content was significantly different in the CGM dried at 50 and 150 °C (Table V). Since only α-zeins were extracted from CGM with the extraction

procedure in this study, the relatively low total extractable α-zeins would produce low extraction yield and protein recovery.

In the ideal situation, the amount of extracted protein should equal the extractable α-zein content in the CGM, i.e. α-zein recovery equals 100%. The α-zein recoveries for Wilson D110 CGM were 51.3, 42.0, and 42.3% for the 50, 100 and 150 °C treatments, respectively (Table VI), and for the Pioneer 3394 CGM were 52.3, 49.5 and 50.5% for the 50, 100, and 150 °C treatments, respectively. The low α-zein recovery values may result from the formation of cross-linking disulfide bonds in some α-zeins during heating. The amount of $SO_2$ used in the wet milling steeping process should have cleaved the disulfide bonds among the zeins and converted them to cysteine and S-sulfocysteine residues. The S-sulfocysteine residues are stable in neutral and mildly acidic conditions without degradation under the commercial drying conditions (Neumann et al. 1984). However, the cysteine residues can reform disulfide bonds in the presence of oxygen during heating. Wall et al. (1975) observed a significant decrease in sulfhydral content in corn when the temperature increased from 15 to 143 °C, and Neumann et al. (1984) also reported that CGM proteins formed disulfide bonds during commercial drying. Therefore, as drying temperature increases, more disulfide bonds reform. The α-zeins with S-sulfocysteine residues can be extracted as monomers or oligomers by an alcohol aqueous, but cross-linked zeins cannot be easily extracted. Since Wilson D110 CGM contained more protein than Pioneer 3394 CGM, the same amount $SO_2$ or

steeping time may not have been sufficient to cleave all the disulfide bonds. Therefore, the α-zein recovery values of Wilson D110 CGM were lower than the Pioneer 3394. The major oxidation reaction of cysteine residues seems to occur in the temperature range 50 to 100 °C, because the α-zein recovery values decreased significantly in this temperature range.

**Influence of CGM Drying Method on Zein Extraction Yield**

Comparing drying methods for Wilson D110 CGM, the yield, protein recovery, and protein purity of zein extracted from freeze- and spray-dried CGMs had similar values that were not significantly different from the values of oven drying at 150 °C, but were much lower than those of the oven dried at 50 °C ($p<0.05$) (Table I, II, and IV). Zein extracted from Pioneer 3394 CGM had a similar trend, but extraction yields and protein recoveries from freeze- and spray-dried CGMs were much lower than those of all the oven drying treatments. Hybrid effects were significant ($p<0.05$) on yield, protein recovery, and protein purity in the freeze- and spray-drying treatments. Drying method influenced zein extraction more for Pioneer 3394 than for Wilson D110.

Total protein contents of the freeze- and spray-dried CGMs were slightly lower but not significantly different from those from oven-dried CGM (Table III). However, the content of extractable albumin and globulin was about 60% higher in the spray-dried, and approximately twice as high in the freeze-dried CGM compared

to oven-dried. One reason for the higher amounts of water- and salt-soluble proteins in the freeze- and spray-dried CGMs was the difference in the collection of the CGM prior to the drying. Gluten used for oven drying was dewatered using a vacuum drum filter, while the gluten used for freeze- and spray-drying treatments was concentrated by siphoning free liquid after setting. The solids content in the wet gluten for oven drying was 40-46%, but was only 1.8-2.0% in the gluten slurry used for freeze and spray drying. Therefore, the CGM dried from the gluten slurry contained more water-soluble compounds, including water- and salt-soluble peptides and proteins, sugars, organic acids, etc.. However, a more important reason for the high amount of water- and salt-soluble proteins may be that there was less oxidation of cysteine in the freeze- and spray-drying process, and less disulfide bond formation among proteins will markedly increase the extractability of the water- and salt-soluble proteins . The zein contents of the freeze- and spray-dried CGMs were not significantly different from oven drying at 50 and 100 °C.

The relative ratios of α-, β-, and γ-zeins in the freeze- and spray-dried CGMs were significantly different from those in oven-dried CGM (Table V). The β-, and γ-zein contents were higher in the freeze- and spray-dried CGMs than those in the oven-dried CGM. Because of the cysteine contents of the γ- and β-zeins (Shewry and Tatham 1990), γ- and β-zeins can form disulfide bonds easier as temperature increases compared to α-zeins. The less heat treated and shorter oxygen exposure time gave less opportunity for disulfide bond formation in β- and γ-zeins; therefore,

the freeze- and spray-dried CGMs contained relatively high β- and γ-zeins, and low α-zeins.

The α-zein recovery values for Wilson D110 CGM were 38.4 and 38.7% for the freeze- and spray-drying treatments, respectively (Table VI), and 28.2 and 30.4% for the freeze- and spray-drying treatments for Pioneer 3394. The α-zein recoveries of the freeze- and spray-drying treatments were much lower than that of the oven-drying treatment, even lower than that of 150 °C treatment; however, the extractable α-zein contents were not significantly lower in the freeze- and spray-dried CGMs than the oven-dried CGMs treated at 50 and 100 °C, and also significantly higher than that of 150 °C oven treatment (Table III).

Low yield, protein recovery, and α-zein recovery are theoretically the result of the unavailability of some α-zeins in the freeze- and spray-dried CGMs. Wall and coworkers (1984) indicated that noncovalent hydrophobic interactions and covalent disulfide bonds were two factors that denature protein and cause molecular aggregation in zein. The high extractable albumin and globulin amounts, and the relatively high extractable β- and γ-zein content in the freeze- and spray-dried CGMs demonstrated there was less cross-link formation among proteins. Therefore, the hydrophobic interactions between proteins may be a major reason for the unavailability of α-zeins in the freeze- and spray-dried CGMs. Since α-zein contain

about 20% glutamic acid/glutamine residues and 90% of this residue is present as glutamine (Righetti et al. 1977), α-zein deamidation during oven heating is possible.

Zhang et al. (1993a) measured the percentage of thermal deamidation in soy protein, casein, lysozyme and gliadin when the proteins were heated at 115°C for 2 hr in a water-limited environment. They reported that gliadin, not like other proteins, had a maximum deamidation (about 8%) at a moisture content of less than 10%, because it contains about 30% glutamine. The deamidation at acidic conditions (pH 3) was believed to be via a direct hydrolysis pathway, and the primary protein sequence did not affect the deamidation rate (Zhang et al. 1993b). Deamidation was accelarated by increased pH and the presence of anions, such as phosphate, bicarbonate (Shih 1990), chlorate, and sulfate (Shih and Kalmar 1987). The ammonia released from the amide could play an important role in the non-enzymatic browning reaction. More available amide in protein results in more maillard reaction (Izzo and Ho 1993).

Deamidation in acidic conditions removes amides from protein, generates acidic side chains, and increases the charge density on proteins. Protein conformation also changes during deamidation by increasing electrostatic repulsion and decreasing hydrogen bonding (Matsudomi et al. 1982). Changes of charge density and protein conformation led to protein unfolding and, thus, enhanced protein solubility (Matsudomi et al. 1981). Deamidation of zein increased solubility (Casella and Whitaker 1990). Since more heat was used in oven-dried CGMs, more

deamidation of protein could occur in the oven-dried CGMs; therefore, more zein in the oven-dried CGMs could dissolve in the extraction solvent and higher extraction yield and protein recovery were obtained from oven-dried CGMs than from freeze- and spray-dried CGM.

The data of ammonia released from deamidation of CGM protein proved our hypothesis (Table VIII). The fact that the highest amount of ammonia was released from freeze-dried CGM indicated that the lowest level of deamidation occurred during freeze drying. The $NH_3$/protein value obtained from spray-dried CGM was significantly lower than that from freeze-dried, but significantly higher than that of oven-dried. The lowest $NH_3$/protein value obtained from the oven-dried CGM samples at 50 °C also demonstrated that heat-treating CGM for a long time caused more deamidation of glutamine; there was almost four times more deamidation than the freeze-dried CGM. Both hybrids, Wilson D110 and Pioneer 3394, had a similar trend of deamidation for the different drying treatments.

The pH of the CGM slurry slightly increased as the oven temperature increased from 50 to 150 °C (Table VII). The change was greater in the Wilson D110 hybrid than the Pioneer 3394 hybrid. The pH values of freeze- and spray-dried CGM slurries were not significantly different, but significantly higher than the pH values of oven-dried CGM slurries. The difference of CGM slurry pH was caused by protein changes during drying. Disulfide bond formation will increase the slurry pH value, and deamidation of glutamine during the heat will decrease the

slurry pH value. Non-enzymatic browning not only decreases the availability of proteins, especially in non-zein proteins, but also decreases ammonia released from deamidation, thus decreasing the pH of the CGM slurry. These factors reduced the pHs of oven-dried CGM slurries to lower values than those of the freeze- and spray-dried slurries, as well as the high-temperature-dried CGM.

**Characteristics of Zein Extracted from CGMs Dried Differently**

The HPLC chromatograms revealed that wet milling and the zein extraction process do not alter the peak patterns of α-zeins. Hybrids have been identified to be the cause for differences in α-zein patterns (Figs.1 and 2). The chromatograms of zeins extracted from CGMs showed no major differences appeared in peak patterns among the drying methods (Figs. 3 and 4). However, the abundance of individual peaks from the oven-dried CGM compared to the freeze-dried CGM shows a peak height decrease at earlier retention times accompanied with an increase peak height in the later retention times. The phenomenon of α-zein peaks shift from earlier to the later retention time in the freeze- and spray-dried zeins is more distinct in the chromatograms of Pioneer 3394. Since reverse phase HPLC was used, the peaks eluting late are more hydrophobic than the early peaks. Therefore, HPLC showed that more hydrophobic α-zeins were extracted from less heat-treated CGMs.

The IEF pattern of zein showed there were no large differences between the oven-drying treatments; however, the distribution of zein bands from the freeze-drying treatment differed from those of the oven-drying treatments. There was a strong band at pI 7.3 in zein from the freeze-dried treatment, while the band pI 6.9 was relatively weak, when compared with the zein pattern from the oven-dried treatments for both hybrids (Figs. 5 and 6). Zein from the spray-dried treatment showed a similar band pattern as the sample from the freeze-dried treatment. A possible explanation for the stronger band pI 7.3 and weaker band pI 6.9 is deamidation of the glutamine in the acidic condition and heat treatment.

## CONCLUSIONS

Ten CGM samples (two hybrids x five drying treatments) were used to study the factors influencing zein extraction. Zein extraction yield, protein recovery, and protein purity were higher in the CGM obtained from maize high in protein content than from maize low in protein content. The yield, protein recovery, and protein purity increased as the oven drying temperature decreased. Protein aggregation by formation of disulfide bonds at high temperature likely occurred.

The explanation for the influence of different drying method on zein extraction is more complex. The freeze- and spray-dried CGM produced lower zein yields, protein recoveries, and protein purities than those obtained for the oven-dried CGMs. Besides the CGM collection and drying process being different, protein

change during drying was a major factor influencing the yield and protein recovery. The CGM treated with low heat (low temperature or short time) had relatively high pH values in the slurries, and more hydrophobic α-zeins and more α-zeins appeared at the pI 7.3 band rather than in the pI 6.9. These characteristics of α-zeins in the freeze- and spray-dried CGMs are due to less deamidation during drying. The aggregation of proteins with noncovalent interaction decreases the extractability of α-zeins, and could also have a major influence on zein extraction yield and purity.

## ACKNOWLEDGMENT

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Table I

Zein Extraction Yield (% of Initial Weight, db) of CGM Dried Under Different Conditions[a]

| Hybrid | Treatment | | | | |
|---|---|---|---|---|---|
| | Oven Drying | | | Freeze Drying | Spray Drying |
| | 50 °C | 100 °C | 150 °C | | |
| Wilson D110 | 21.5 aA | 18.2 bA | 17.1 bcA | 16.4 cA | 16.4 cA |
| Pioneer 3394 | 17.8 aB | 17.2 aB | 16.8 aA | 10.4 bB | 11.5 cB |

[a] Means of nine replications. Data in the same row with different lower case letters were significantly different at $p < 0.05$. Data in the same column with different capital letters were significantly different at $p < 0.05$.

Table II

Protein Recovery (%) of Zein Extracted from CGM Dried Under Different Conditions[a]

| Hybrid | Treatment | | | | |
|---|---|---|---|---|---|
| | Oven Drying | | | Freeze Drying | Spray Drying |
| | 50 °C | 100 °C | 150 °C | | |
| Wilson D110 | 32.1 aA | 26.0 bA | 24.1 bA | 24.4 bA | 24.8 bA |
| Pioneer 3394 | 27.5 aB | 25.7 bA | 24.2 bA | 14.2 cB | 15.7 cB |

[a] Means of nine replications. Data in the same row with different lower case letters were significantly different at $p < 0.05$. Data in the same column with different capital letters were significantly different at $p < 0.05$.

Table III

Protein Composition (db) in CGM Dried Under Different Conditions[a]

| Components | Treatment | | | | | Hybrid Means[b] |
|---|---|---|---|---|---|---|
| | Oven Drying | | | Freeze Drying | Spray Drying | |
| | 50 °C | 100 °C | 150 °C | | | |
| CGM Protein (%) | | | | | | |
| Wilson D110 | 56.6 ± 1.3 | 56.5 ± 1.2 | 56.5 ± 1.0 | 54.3 ± 1.1 | 53.9 ± 2.6 | 55.6 a |
| Pioneer 3394 | 49.1 ± 2.8 | 49.2 ± 3.3 | 49.1 ± 2.9 | 49.1 ± 2.0 | 48.6 ± 3.3 | 49.0 b |
| Treatment Means[c] | 52.8 a | 52.9 a | 52.8 a | 51.7 a | 51.3 a | |
| Extractable Alb+Glo (%) | | | | | | |
| Wilson D110 | 1.3 ± 0.2 | 1.2 ± 0.3 | 1.4 ± 0.2 | 2.3 ± 0.5 | 1.7 ± 0.4 | 1.6 a |
| Pioneer 3394 | 0.8 ± 0.2 | 0.8 ± 0.1 | 0.7 ± 0.2 | 1.9 ± 0.1 | 1.6 ± 0.6 | 1.2 b |
| Treatment Means[c] | 1.1 a | 1.0 a | 1.1 a | 2.1 b | 1.7 c | |
| Extractable Zein (%) | | | | | | |
| Wilson D110 | 37.8 ± 0.9 | 37.4 ± 0.9 | 34.5 ± 0.8 | 37.3 ± 1.0 | 37.4 ± 0.5 | 36.9 a |
| Pioneer 3394 | 29.5 ± 1.5 | 29.3 ± 1.4 | 26.9 ± 0.4 | 28.5 ± 0.8 | 28.9 ± 1.0 | 28.7 b |
| Treatment Means[c] | 33.7 a | 33.4 a | 30.7 b | 32.9 a | 33.3 a | |

[a] Means of six replications.
[b] Data in the same column with different letters were significantly different at $p < 0.05$.
[c] Data in the same row with different letters were significantly different at $p < 0.05$.

Table IV

Protein Purity (%) of Zein Extracted from CGM Dried Under Different Conditions[a]

| Hybrid | Treatment | | | | | Hybrid Means[b] |
|---|---|---|---|---|---|---|
| | Oven Drying | | | Freeze Drying | Spray Drying | |
| | 50 °C | 100 °C | 150 °C | | | |
| Wilson D110 | 84.4 | 80.5 | 79.5 | 80.4 | 81.5 | 81.3a |
| Pioneer 3394 | 76.0 | 73.7 | 70.6 | 66.7 | 66.1 | 70.6b |
| Treatment Means[c] | 80.2 a | 77.1 b | 75.0 c | 73.5 c | 73.8 c | |

[a] Means of nine replications.
[b] Data in the same column with different letters were significantly different at $p < 0.05$.
[c] Data in the same row with different letters were significantly different at $p < 0.05$.

Table V

Extractable Zein Composition (%) in CGM Dried Under Different Conditions[a]

| Components | Treatment | | | | | Hybrid Means[b] |
|---|---|---|---|---|---|---|
| | Oven Drying | | | Freeze Drying | Spray Drying | |
| | 50 °C | 100 °C | 150 °C | | | |
| α-Zein (%) | | | | | | |
| Wilson D110 | 93.6 ± 0.2 | 93.3 ± 0.2 | 93.2 ± 0.3 | 92.2 ± 0.3 | 92.3 ± 0.3 | 92.9 a |
| Pioneer 3394 | 87.7 ± 0.3 | 87.5 ± 0.2 | 87.3 ± 0.1 | 86.4 ± 0.1 | 86.6 ± 0.3 | 87.1 b |
| Treatment Means[c] | 90.6 a | 90.4 ab | 90.2 b | 89.3 c | 89.5 c | |
| β-Zein (%) | | | | | | |
| Wilson D110 | 1.07 ± 0.02 | 1.07 ± 0.01 | 1.12 ± 0.03 | 1.12 ± 0.03 | 1.18 ± 0.01 | 1.11 a |
| Pioneer 3394 | 2.49 ± 0.34 | 2.59 ± 0.26 | 2.65 ± 0.38 | 2.91 ± 0.31 | 2.77 ± 0.35 | 2.68 b |
| Treatment Means[c] | 1.78 a | 1.83 ab | 1.89 ab | 2.02 b | 1.98 ab | |
| γ-Zein (%) | | | | | | |
| Wilson D110 | 5.35 ± 0.21 | 5.66 ± 0.23 | 5.71 ± 0.27 | 6.64 ± 0.37 | 6.48 ± 0.34 | 5.97 a |
| Pioneer 3394 | 9.80 ± 0.13 | 9.96 ± 0.29 | 10.03 ±0.35 | 10.65 ± 0.34 | 10.61 ± 0.41 | 10.21 b |
| Treatment Means[c] | 7.58 a | 7.81 a | 7.87 a | 8.64 b | 8.55 b | |

[a] Means of six replications.
[b] Data in the same column with different letters were significantly different at $p < 0.05$.
[c] Data in the same row with different letters were significantly different at $p < 0.05$.

Table VI

α-Zein Recovery (%) in CGM Dried Under Different Conditions[a]

| Hybrid | Treatment | | | | |
|---|---|---|---|---|---|
| | Oven Drying | | | Freeze Drying | Spray Drying |
| | 50 °C | 100 °C | 150 °C | | |
| Wilson D110 | 51.3 | 42.0 | 42.3 | 38.4 | 38.7 |
| Pioneer 3394 | 52.3 | 40.5 | 50.5 | 28.2 | 30.4 |

[a] Means of three millings.

Table VII

pH of CGM slurry[a]

| Hybrid | Treatment | | | | |
|---|---|---|---|---|---|
| | Oven Drying | | | Freeze Drying | Spray Drying |
| | 50 °C | 100 °C | 150 °C | | |
| Wilson D110 | 4.08 a | 4.15 b | 4.19 c | 4.31 d | 4.37 d |
| Pioneer 3394 | 4.15 a | 4.20 b | 4.20 b | 4.29 c | 4.35 c |

[a] Means of three millings. Data in the same row with different letters were significantly different at $p < 0.05$.

Table VIII

Ammonia Released in Deamidation Process[a]

| CGM | NH3/Protein (mg/g) |
|---|---|
| Wilson D110 | |
| Oven drying, at 50 °C | 0.0031 e |
| at 100 °C | 0.0042 d |
| at 150 °C | 0.0041 d |
| Freeze Drying | 0.0157 a |
| Spray Drying | 0.0062 c |
| | |
| Poineer 3394 | |
| Oven drying, at 50 °C | 0.0036 d,e |
| at 100 °C | 0.0048 d |
| at 150 °C | 0.0048 d |
| Freeze Drying | 0.0125 b |
| Spray Drying | 0.0060 c |

[a] Means of three replications. Data in the same row with different letters were significantly different at $p < 0.05$.

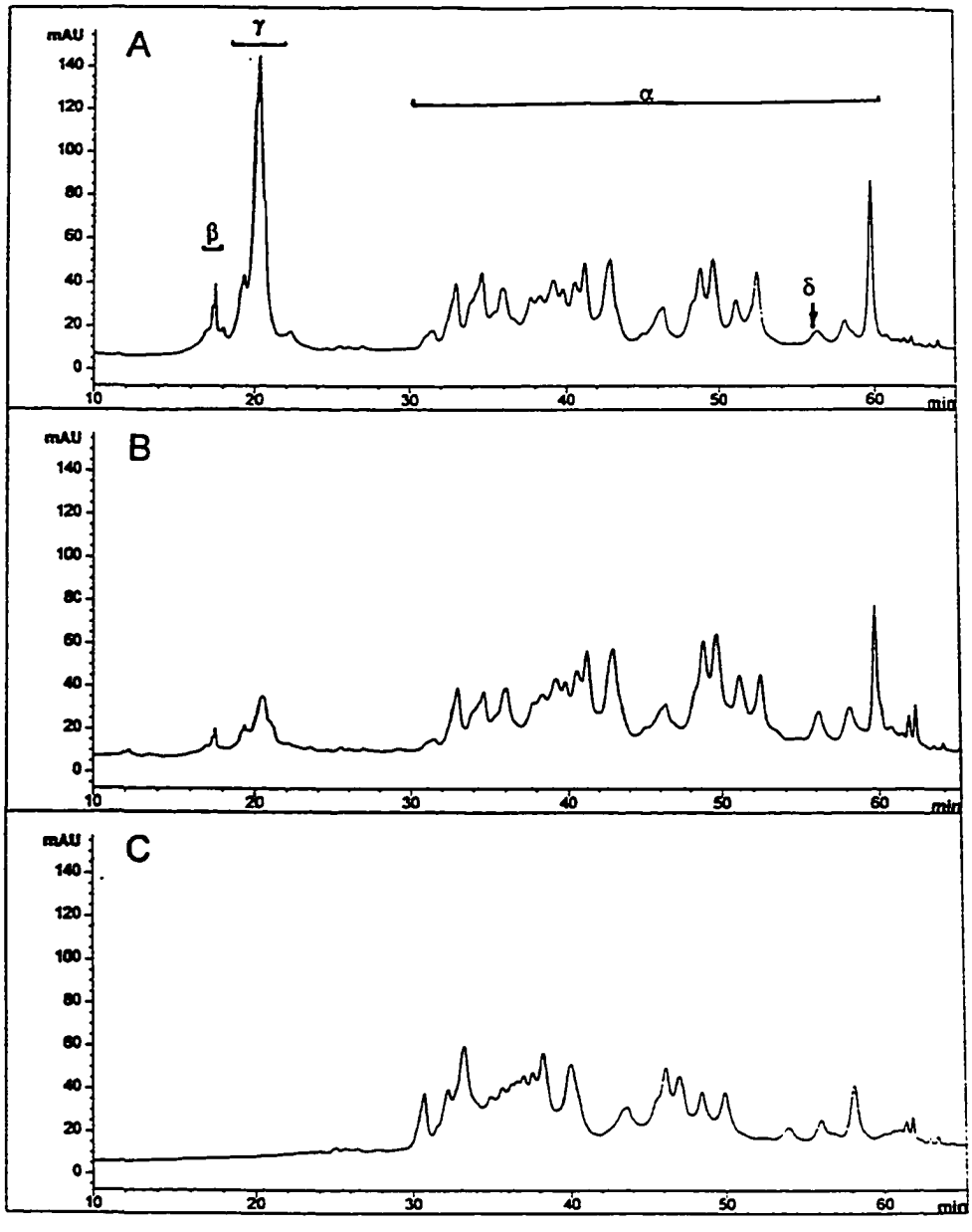


**Fig. 1.** RP-HPLC analysis of alcohol-soluble proteins from Wilson D110 corn (A), CGM (B), and extracted zein (C). Total zeins were extracted or dissolved with a solvent containing 55% (v/v) isopropyl alcohol with 5% (v/v) 2-mercaptoethanol plus 0.5% (w/v) NaAc. Zeins were separated using a $C_{18}$ column and a non-linear acetonitrile gradient from 30 to 64%. The proteins were monitored at 214 nm. The Greek letters, α, β, γ, and δ, refer to zein classes.

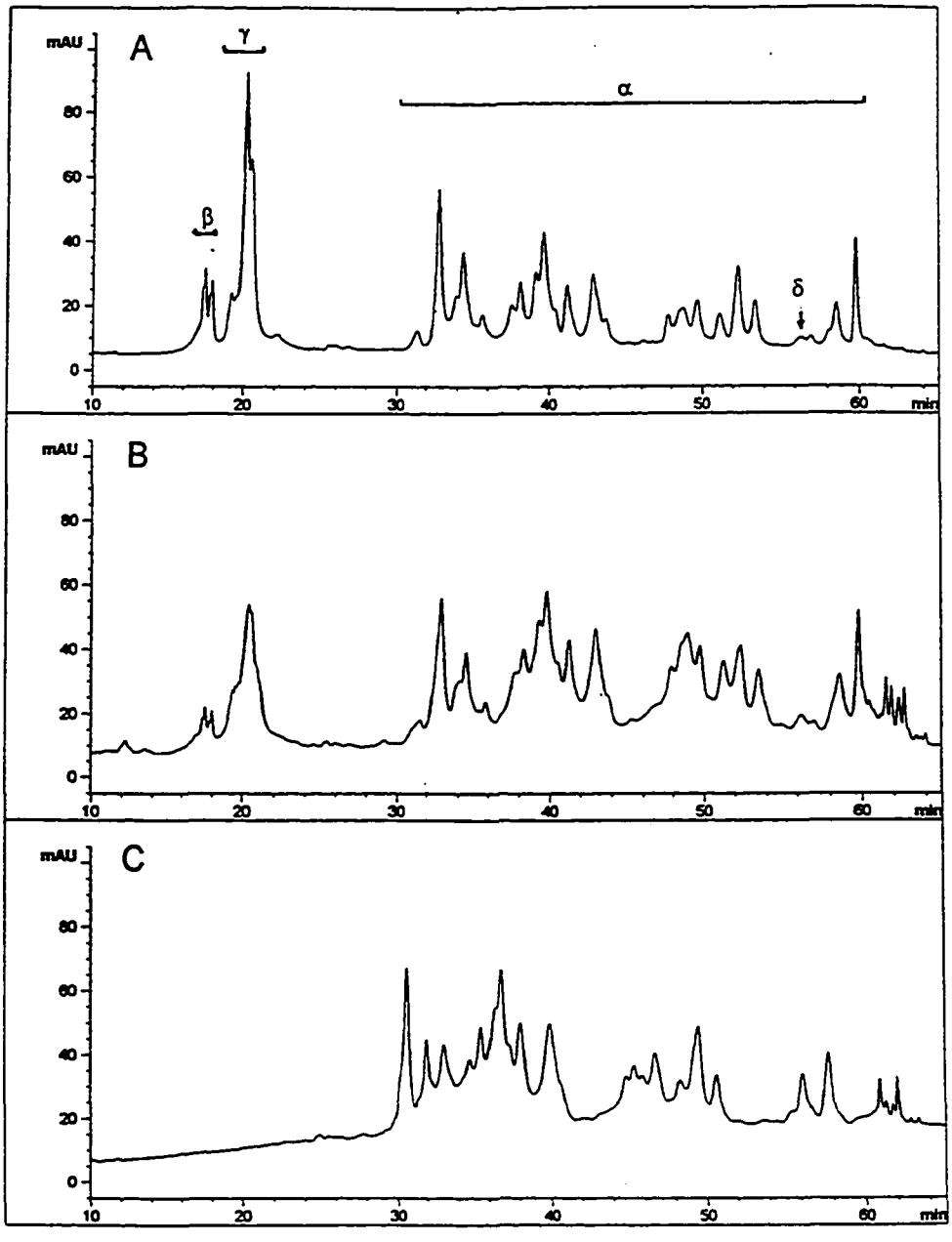


**Fig. 2.** RP-HPLC analysis of alcohol-soluble proteins from hybrid Pioneer 3394 corn (A), CGM (B), and extracted zein (C). Total zeins were extracted or dissolved in a solvent containing 55% (v/v) isopropyl alcohol with 5% (v/v) 2-mercaptoethanol plus 0.5% (w/v) NaAc. Zeins were separated using a $C_{18}$ column and a non-linear acetonitrile gradient from 30 to 64%. The proteins were monitored at 214 nm. The Greek letters, α, β, γ, and δ, refer to zein classes.

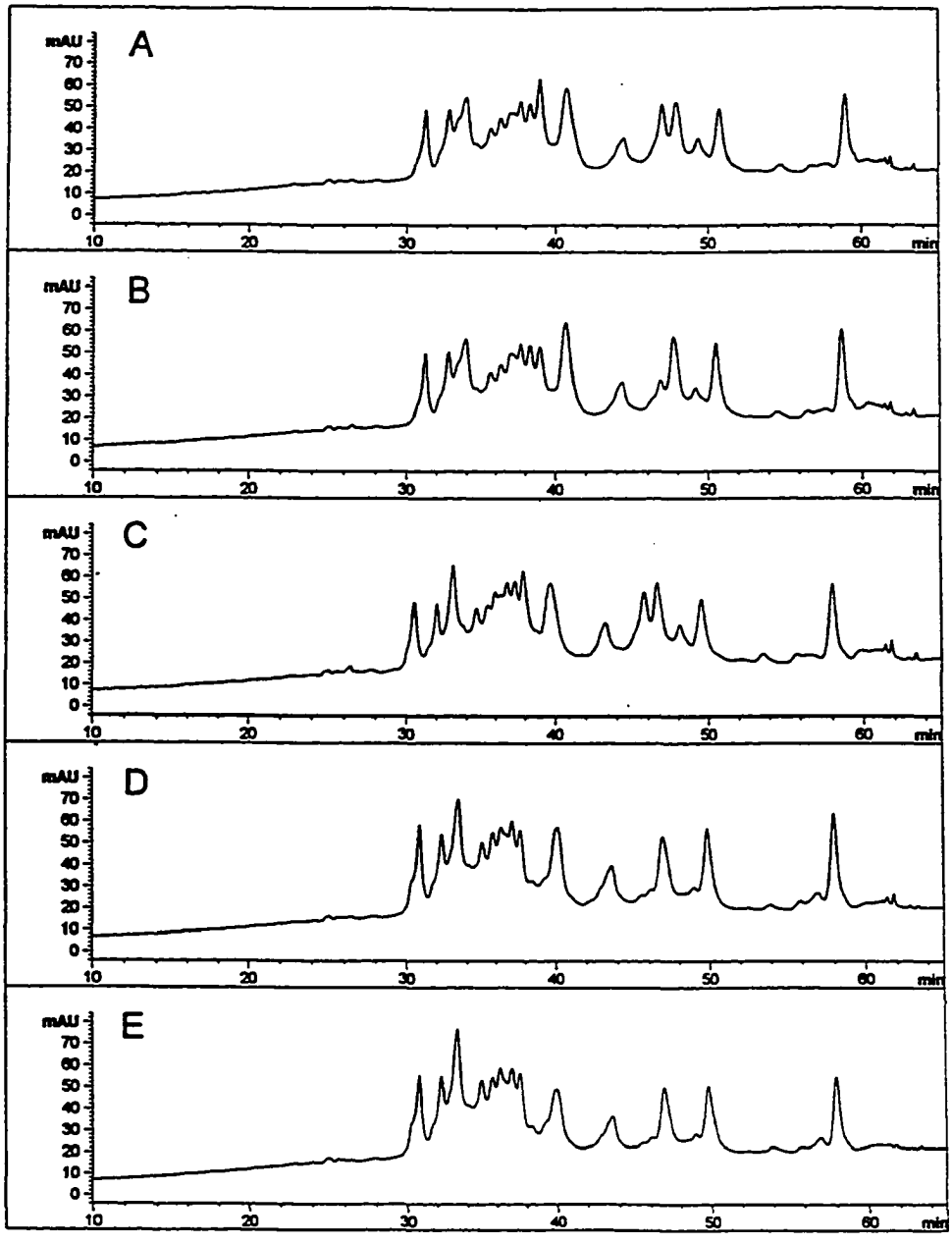


**Fig. 3.** RP-HPLC analysis of alcohol-soluble proteins from the zein extracted from hybrid Wilson D110 CGM dried at oven 150 °C (A); CGM dried at oven 100 °C (B); CGM dried at oven 50 °C (C); spray-dried CGM (D); and freeze-dried CGM (E). Zeins were dissolved in a solvent containing 55% (v/v) isopropyl alcohol with 5% (v/v) 2-mercaptoethanol. Zeins were separated on a $C_{18}$ column and using a non-linear acetonitrile gradient from 30 to 64%. The proteins were monitored at 214 nm.

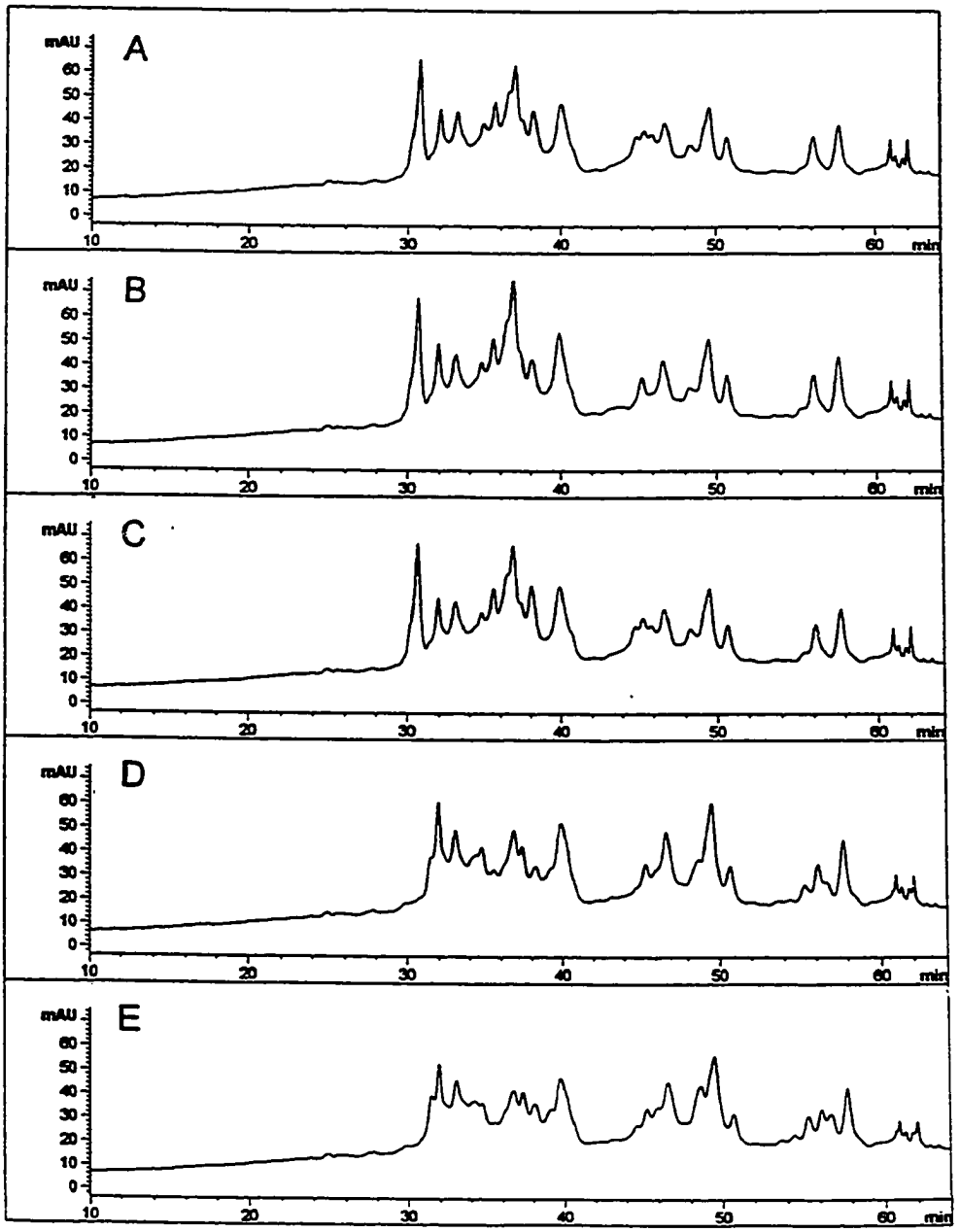


**Fig. 4.** RP-HPLC analysis of alcohol-soluble proteins from the zein extracted from hybrid Pioneer 3394 CGM dried at oven 150 °C (A); CGM dried at oven 100 °C (B); CGM dried at oven 50 °C (C); spray-dried CGM (D); and freeze-dried CGM (E). Zeins were dissolved in 55% (v/v) isopropyl alcohol with 5% (v/v) 2-mercaptoethanol. Zeins were separated on a $C_{18}$ column and using a non-linear acetonitrile gradient from 30 to 64%. The proteins were monitored at 214 nm.

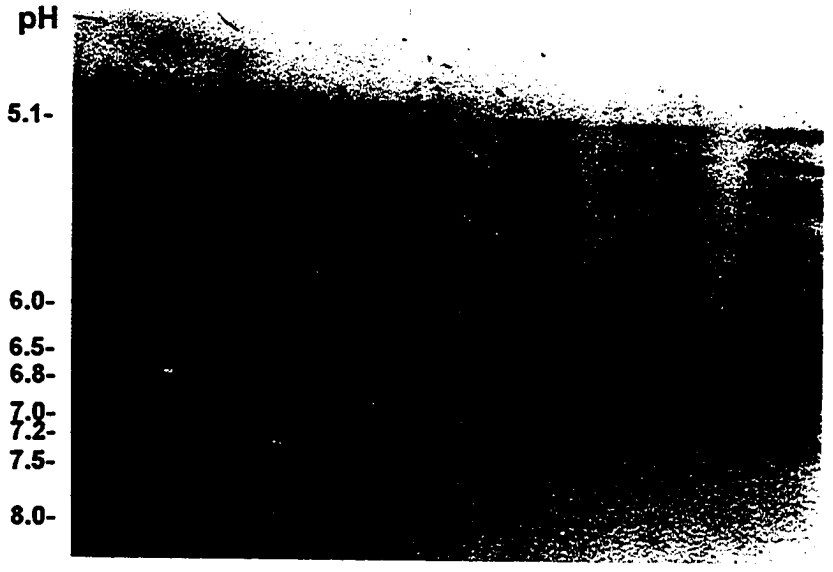


**Fig. 5.** Isoelectric focusing of hybrid Wilson D110 zeins in 5% polyacrylamide gel, 1% ampholine pH 5-7, 1% ampholine pH 7-9, and 6M urea. Lane 1, pI marker proteins; lanes 2 to 6 (respectively): zeins extracted from corn gluten meals dried in oven at 50 °C, in oven at 100 °C, in oven at 150 °C, in the freeze drier, and in the spray drier.

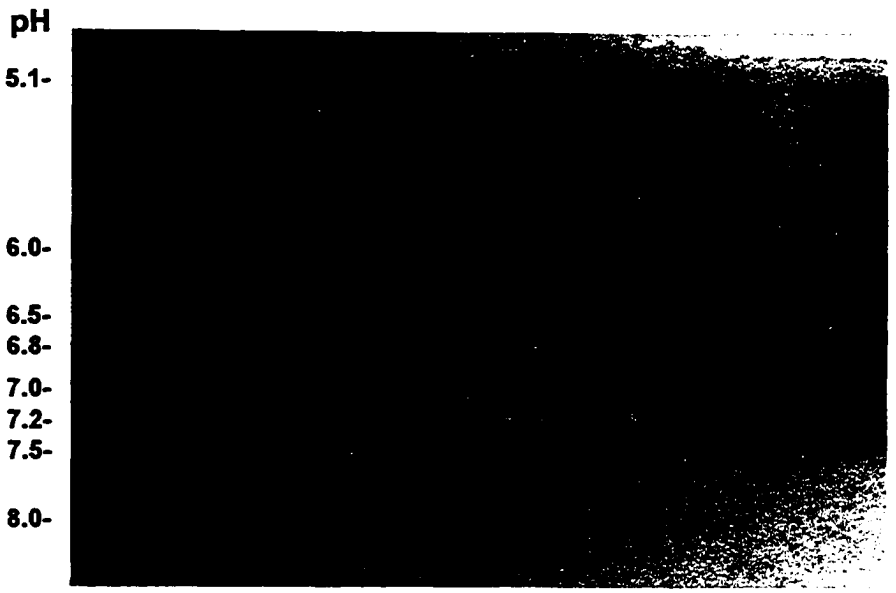


**Fig. 6.** Isoelectric focusing of hybrid Pioneer 3394 zeins in 5% polyacrylamide gel, 1% ampholine pH 5-7, 1% ampholine pH7-9, and 6M urea. Lane 1, pI marker proteins; lanes 2 to 6 (respectively): zeins extracted from corn gluten meals dried in oven at 50 °C, in oven at 100 °C, in oven at 150 °C, in the freeze drier, and in the spray drier.

# 6. INFLUENCE OF PHOSPHATE BUFFER ON YIELD AND RECOVERY OF ZEIN EXTRACTED FROM CORN GLUTEN MEAL

A paper to be submitted to Cereal Chemistry

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## INTRODUCTION

It was well known that zein cannot be dissolved in water, but can be dispersed in basic aqueous solutions. Wahl (1934) reported that 0.25% NaOH solution was the most suitable solvent for extracting zein from corn gluten meal (CGM). Swallen (1941) found that a pH of at least 11.5 with a total alkali content of about 1.2% NaOH (based on the weight of zein) is required to extract zein. A zein dispersion (10% w/v concentration) was made by dissolving zein in a pH range of 11.3 to 12.7 with 1.4-6.4% NaOH (based on the weight of zein) (Ofelt and Evans 1949). Any pH range that deviated from the 11.3-12.7 rapidly reduced the solubility of zein in aqueous solution. Zein has also been extracted with a solvent containing a low level of organic solvent (about 30% (w/w) of isopropyl alcohol) and 6 M

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$Ca(OH)_2$ (Morris et al. 1956, 1959). The extraction efficiency increased when the solvent was heated to temperatures ranging from 70 °C to the boiling point of isopropyl alcohol.

CGM, a valuable co-product of the wet-milling process, contains 60-70% protein, and zeins comprise about 65% of the total proteins (db) (Watson and Yahl, 1967). The current commercial method for zein production from CGM was developed by Carter and Reck (1970). A single extraction is carried out at 55-65 °C with 88% (w/w) isopropyl alcohol containing 0.25% NaOH. The zein alcohol extract is chilled at -10 to -20 °C to precipitate zein from the solvent. The process is simple, and protein quality is considered good because the process does not require the removal of solvent by distillation. Another advantage is that a second solvent is not required to remove the oil and pigments. The operating costs of a complex solvent mixture recovery system can be decreased, and the operation is reported to minimize explosion hazards. However, variable yields and protein qualities of extracted zeins are problems.

The $SO_2$ present in the steeps of the wet milling process cleaves the intermolecular disulfide bond and releases starch granules from the protein matrix (Blanchard 1992). In addition, the $SO_2$ also increases protein solubility in alcohol aqueous (Neumann et al. 1984), decreases the pH of solution, and functions as an anti-microbial agent. Therefore, the CGM obtained from the wet milling process is not neutral but acidic. The pH value of the CGM is dependent upon the $SO_2$

concentration during steeping, and the concentration of lactic acid produced. To maintain the extraction solution for zein in the optimum pH range of 11.3-12.7, a buffer solution was used. The phosphate buffer is one of the very few buffer solutions which can buffer the solution in the pH range of 12-13, and, furthermore, is an odorless food-grade reagent. Therefore, phosphate was selected to buffer the pH of the extraction solution in an attempt to increase the zein extraction yield.

## MATERIALS AND METHODS

### Corn Gluten Meal Samples

CGM samples (12) were obtained from six different wet-milling plants in Iowa and Illinois. The samples were collected and processed in December 1993 and March 1994.

### Extraction of Zein

The extraction procedure used was that described by Wu et al. (1995a). The extraction process was replicated three times for each sample and each of the following zein extraction conditions:

a. CGM with 88% (w/w) isopropyl alcohol and 12% water adjusted with NaOH to pH 11.5, 12.0, 12.5, 13.0, and 13.5. The extraction was carried out at 60 °C with a ratio of 1:4 CGM:solvent (w/w).

b. CGM with 88% (w/w) isopropyl alcohol and 12% phosphate buffer solution. The concentrations of phosphate in the buffer were 0, 0.001, 0.005, and 0.01M. The pH of the aqueous buffer was adjusted to 12.5 with 6M NaOH. Extraction was carried out at 60 °C at a ratio of 1:4 CGM:solvent (w/w).

c. CGM with 88% (w/w) isopropyl alcohol plus 12% 0.005M phosphate buffer (pH 12.5) at 50, 60, 70, and 80 °C. Extraction was carried out at a ratio of 1:4 CGM:solvent (w/w).

d. the ratios of 1:4, 1:6, and 1:8 (w/w) of CGM to the isopropyl alcohol-phosphate buffer (0.005M phosphate and pH12.5) were used to compare zein extraction efficiency. Extraction was carried out at 60 °C.

The extraction yield was calculated as the weight percentage of extracted zein from the total weight the CGM (db). Protein purity was the protein content of the extracted zein. Protein content was determined by using the 6.25 x total N content determined by Kjeldahl (Tecator, Sweden) analysis (CRA 1986, Method A-18). Protein recovery was calculated as the percentage of the protein in the extracted zein to the protein content of the CGM (db).

**pH Determination**

The pH value of CGM slurry was determined using the method of Wu et al. (1995b).

## RESULTS AND DISCUSSION

### Influence of CGM Slurry pH on Zein Recovery

The pHs of the twelve CGM slurries ranged from 3.7 to 4.4. Protein recovery was not linear, but polynomial, as the pH of CGM slurry increased (Fig. 1). The pH differences of the CGM slurries could be attributed to differences in wet milling and CGM production methods, such as steeping temperature and time, concentrations of $SO_2$ and lactic acid, and drying conditions. The protein conformation in CGM was probably changed by cleaving the disulfide bond with $SO_2$, oxidation with air to reform disulfide bond during heat, deamidation of glutamine, and aggregation by hydrophobic bond. The difference of the CGM slurry pH indirectly indicated the change of protein conformation. The CGM slurries with pH's lower than 3.9 and higher than 4.3 gave lower zein extraction yield and protein recovery.

### Influence of Extraction Solvent pH on Zein Extraction

The CGMs with the highest slurry pH (4.36) and the lowest slurry pH (3.77) were selected to study the influence of pH on zein extraction. The original protein and total α-zein contents in the two CGM samples were not big different (Table I). The optimum pH for zein extraction changed for the different CGM used. For CGM D, the yield and purity of zein extracted increased as the solvent pH increased from 11.5 to 12.5, and decreased as the solvent pH increased from 12.5 to 13.5 (Table II). However, for CGM I, the highest yield and protein recovery were obtained at the

extraction solvent pH of 13.0 instead of pH 12.5. The reason for this may be its low initial CGM slurry pH. Low CGM slurry pH required more base to neutralize acid and make the pH of extraction solution to 11.3-12.7 which is the optimum pH range for zein solubilization.

**Effect of Phosphate Buffer**

Low concentrations of phosphate markedly increased zein extraction yield. Since the solubility of the phosphate in isopropyl alcohol is relatively low, concentrations lower than 0.01 M of phosphate are suggested. The functions of the phosphate were to: 1) buffer the extraction solution pH to approximately 12.5; 2) facilitate separating extracted zeins from the solvent during chilling; and 3) accelerate deamidation of proteins during extraction and increase zein solubility (Shih 1990). The yields and protein recoveries of extracted zein in both CGM samples increased when 0.001M phosphate was added. The yield in CGM D sample increased 1.1 and 5.6% in CGM I compared to values obtained in the absence of 0.001M phosphate in the extraction solvent (pH 12.5) (Table II), and the protein recovery increased 1.2 and 7.1% for CGM D and I, respectively. The buffer efficiency was more significant in CGM I because of its low initial CGM slurry pH. As the phosphate concentration increased to 0.005 M, the extraction yield and protein recovery reached maxima, and then decreased when the concentration of

phosphate was 0.01M. Protein purity decreased as phosphate concentration increased.

The temperature data showed that when 0.005 M phosphate was added to the extraction solvent, extraction at 70 °C gave the highest yield (21%). When the temperature increased from 60 to 80 °C, the purity of extracted zein increased from 86.3 to 87.8%.

The extraction yield increased from 17.1 to 18.1% when the ratio of CGM to the solvent was changed from 1:4 to 1:6, and the yields were not different when the ratio of CGM to the solvent was changed from 1:6 to 1:8. The protein purities were 86.3, 86.7 and 87.7% for the 1:4, 1:6 and 1:8 ratios, respectively.

Based on the previous results, the optimum conditions of 88% isopropyl alcohol with 0.005M phosphate buffer (pH12.5), 1:6 CGM/solvent ratio, and 70 °C were used to extract zein from CGM D and I. For CGM D, the extraction yield was 21.0%, protein purity was 87.3%, and protein recovery was 27.7%. The ratio of total α-zein recovery (the extraction yield to the total α-zein content in Table I) was 59.3% compared to 42.9% without phosphate in the extraction solvent. For CGM I, the extraction yield was 20.1%, protein purity was 89.0%, and protein recovery was 27.3%. The ratio of total α-zein recovery was 56.0%, compared to 29.0% without phosphate.

Small amounts phosphate added to the extraction solvent can markedly increase zein extraction yield (38-52%). The phosphate functions as a buffer and

salting-out reagent, and may be a catalyst for protein deamidation. For both the high and low pH CGM samples, the solvent with a small amount phosphate added improve protein recovery. There were no significant differences in HPLC α-zein distribution, and IEF polyacrylamide gel from zein extracted with or without phosphate. Whether the zein extracted with phosphate has any difference in the functionality will need to be investigated.

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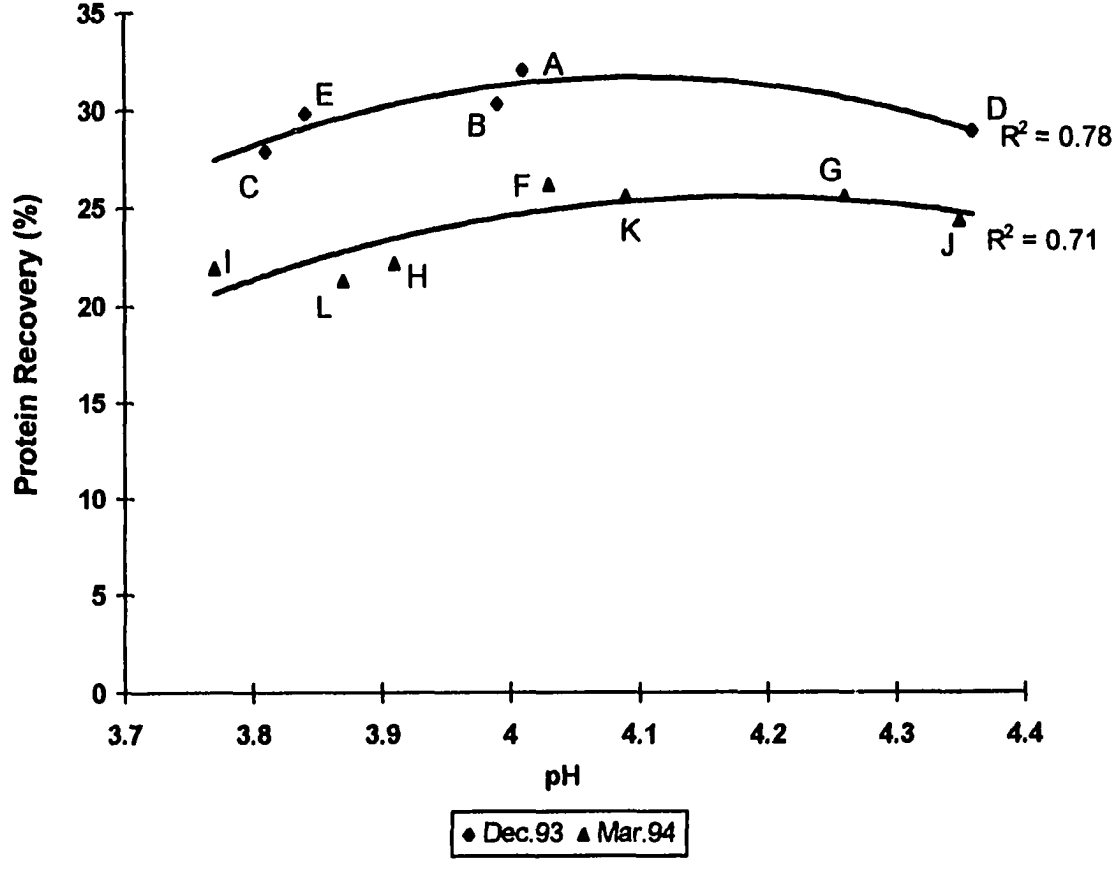


**Fig.1.** Protein recovery as affected by pH of the CGM slurry.

Table I

Moisture, pH, and Protein Contents of Corn Gluten Meals from Different Wet Mills[a]

| CGM | Moisture (%) | pH | Protein (% db) | Total α-zeins (% db) |
|---|---|---|---|---|
| D | 6.67 a | 4.36 a | 66.3 a | 35.4 a |
| I | 7.51 b | 3.77 b | 65.5 b | 35.9 a |

[a] Means followed by different letters within the same column are significantly different ($p<0.05$).

Table II

Effects of pH & Phosphate on Protein Yield, Purity and Recovery of Zein Extraction[a]

| pH | Conc.of Phosphate (M) | CGM D | | | CGM I | | |
|---|---|---|---|---|---|---|---|
| | | Yield (%) | Protein Purity (%) | Protein Recovery (%) | Yield (%) | Protein Purity (%) | Protein Recovery (%) |
| 11.5 | 0 | 10.4 d | 87.7 abc | 13.5 d | 7.7 d | 87.1 abc | 10.2 d |
| 12.0 | 0 | 13.1 c | 88.8 a | 17.5 c | 10.5 c | 87.5 abc | 14.0 c |
| 12.5 | 0 | 15.2 abc | 88.7 a | 20.4 abc | 10.4 c | 88.4 a | 14.1 c |
| 13.0 | 0 | 14.3 bc | 87.9 ab | 18.9 bc | 13.2 b | 88.2 ab | 17.8 b |
| 13.5 | 0 | 5.9 e | 86.5 bc | 7.7 e | 7.9 cd | 86.8 bc | 10.5 d |
| 12.5 | 0.001 | 16.3 ab | 88.2 ab | 21.6 ab | 16.0 a | 87.2 abc | 21.2 ab |
| 12.5 | 0.005 | 17.1 a | 86.3 bc | 22.3 a | 16.6 a | 86.5 c | 21.9 a |
| 12.5 | 0.01 | 14.5 bc | 85.7 c | 18.7 bc | 14.5 ab | 86.4 c | 19.2 ab |

[a] Means followed by different letters within the same column are significantly different ($p<0.05$).

# 7. GENERAL CONCLUSIONS

The investigation of factors that influence the yield and purity of zein extracted from commercial CGM samples revealed that protein recovery ranged from 21.3 to 32.0% and protein purity from 82.1 to 87.6%. Protein recovery increased as purity increased with a correlation coefficient 0.76 ($p<0.01$). One of the major factors influencing extraction yield was protein content, especially α-zein content which ranged from 53.4 to 64% of the total protein in the CGM samples. The intensity of red color of the CGM was negatively correlated with protein recovery and zein purity, with correlation coefficients of $r = -0.66$ and -0.72, respectively, at the 0.02 significant level.

The production of CGM from the wet-milling pilot-plant facility showed that high protein-containing gluten can be produced successfully; however, to obtain protein contents closer to commercial levels, gluten yield had to be sacrificed. The higher protein hybrid produced gluten in higher yield and with high protein content.

CGM drying temperature and method were major factors in determing yield and purity of zein extracted from the meal. Color differences in CGM were due to the method of drying, temperature, and the maize hybrid milled. Low temperature oven-dried CGM produced lighter colored CGM as did freeze and spray drying compared to CGM dried at higher temperatures. Zein extraction yield, protein recovery and protein purity were also higher in the CGM obtained from maize containing higher protein content. The yield and protein recovery of zein decreased

as the drying temperature increased. Protein aggregation at high temperature is believed to be the primary reason for the lower yields. Zein yield, protein recovery, and protein purity were also significantly lower from CGMs obtained from freeze- and spray-drying compared to oven-dried CGM. A relatively high pH value of the CGM slurries, more less polarity α-zeins, and more α-zeins with a pI 7.3 were the characteristics of the freeze- and spray-dried CGM. Possible changes in protein during drying are deamidation, oxidation of sulfhydral groups on protein, and some hydrophobic bond formation which could interfere with zein extraction yield.

Small amounts of phosphate added into the extraction solvent can markedly increase zein extraction yield (38-50%). For both high and low slurry pH CGMs, protein recovery values were increased when small amounts phosphate were added to the solvent. The zein extracted with phosphate showed no significant difference in α-zein distribution on the HPLC (high performance liquid chromatography) chromatogram and IEF (isoelectric focusing) gel.

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## APPENDIX. ANALYSIS OF VARIANCE TABLES

I. ANOVA Tables for the Sample Means of Zein Extracted from Cmmercial CGM.

Yield %

| Source | d.f. | SS | MS | F | P>F | % of Error Contribution |
|---|---|---|---|---|---|---|
| CGM | 11 | 453.9 | 43.27 | 18.776 | <0.0001 | 78 |
| Rep | 48 | 105.5 | 2.189 | | | 22 |
| Total | 59 | 559.4 | | | | |

Protein Purity %

| Source | d.f. | SS | MS | F | P>F | % of Error Contribution |
|---|---|---|---|---|---|---|
| CGM | 11 | 150.8 | 13.71 | 13.887 | <0.0001 | 72 |
| Rep | 48 | 47.4 | 0.987 | | | 28 |
| Total | 59 | 198.1 | | | | |

II. ANOVA Tables for the Randomized Block Design in the Influence of Maize Hybrids and Heat Treatments on Zein Extraction Experiments.

Yield %

| Source | d.f. | MS | F | P>F | (or Pr > \|T\|) |
|---|---|---|---|---|---|
| Milling | 2 | 3.7 | 9.8 | 0.0013 | |
| Hybrid | 1 | 76.9 | 205.22 | 0.0001 | |
| Treatment | 4 | 41.2 | 110.03 | 0.0001 | |
| F.S. vs Oven | 1 | | | | 0.0001 |
| F. vs S. | 1 | | | | 0.1653 |
| Hybrid * Trt | 4 | 8.9 | 23.79 | 0.0001 | |
| Error | 18 | 0.4 | | | |
| Total | 29 | | | | |

Protein Purity %

| Source | d.f. | MS | F | P>F | (or Pr > \|T\|) |
|---|---|---|---|---|---|
| Milling | 2 | 6 | 0.74 | 0.4917 | |
| Hybrid | 1 | 847 | 108.43 | 0.0001 | |
| Treatment | 4 | 46 | 5.92 | 0.0032 | |
| F.S. vs Oven | 1 | | | | 0.0019 |
| F. vs S. | 1 | | | | 0.8813 |
| Hybrid * Trt | 4 | 20 | 2.65 | 0.0670 | |
| Error | 18 | 8 | | | |
| Total | 29 | | | | |

Protein Recovery %

| Source | d.f. | MS | F | P>F | (or Pr > \|T\|) |
|---|---|---|---|---|---|
| Milling | 2 | 4.4 | 3.90 | 0.0391 | |
| Hybrid | 1 | 172.5 | 154.18 | 0.0001 | |
| Treatment | 4 | 110.5 | 98.79 | 0.0001 | |
| F.S. vs Oven | 1 | | | | 0.0001 |
| F. vs S. | 1 | | | | 0.1394 |
| Hybrid * Trt | 4 | 34.2 | 30.54 | 0.0001 | |
| Error | 18 | 1.1 | | | |
| Total | 29 | | | | |