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Two fraction extraction of α-zein from DDGS and its characterization

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ABSTRACT

Zein was recovered from corn distiller's dried grains with solubles (DDGS) by a modified method using 70% (w/w) aqueous 2-propanol (70-IPA) or 70% (v/v) aqueous ethanol (70-EtOH) solvents, and a commercial method using 88% (w/w) aqueous 2-propanol (88-IPA). Yield, purity, and film properties of the isolated zein were determined. The modified procedure extracted two fractions of zeins: a mostly α-zein fraction, and a mostly γ-zein fraction. The modified method increased α-zein yield from 4% to 14%. Enzyme cellulase pretreatment did not improve zein yield, but grinding did. The α-zein fraction showed electrophoretic bands at 40, 22, 19, and 10 kDa, corresponding to α-zein dimer, α_1-zein, α_2-zein, and δ-zein, respectively. The α-zein of DDGS retained its film forming capability. The α-zein film of unmodified DDGS was cloudy and rough, unlike the clear and smooth films of α-zeins isolated from corn gluten meal and enzyme-treated DDGS.

1. Introduction

Corn zein is comprised α, β, γ, and δ zeins based on zein's solubility in 2-propanol (Esen, 1987, 1990). The α- and δ-zein are found in the protein body core, while β- and γ-zein are on the periphery of the protein body (Thompson and Larkins, 1989; Mohammad and Esen, 1990), however, α- and δ-zein are the only zeins considered true prolamins. β- and γ-zein are considered glutelins based upon Osborne's solubility principles (Osborne, 1891), but were considered zeins because of their inclusion within the zein protein bodies.

Zein can be extracted from three different corn materials: dry-milled corn (DMC), CGM, and DDGS. The basic source material is DMC, which contains ~6.8% to 8.0% protein, of which 52% is considered zein proteins (Rausch et al., 2009). Some corn hybrids have dry-milled endosperm fractions with protein content as high as 18.7% (Wolf et al., 1975). Past extractions of zein from DMC were uneconomical because of the large amounts of solvent used and low extraction yields. CGM, co-product of corn wet-milling industry, is typically used to extract commercial zein. Most commercial extraction methods use CGM to extract zein because it contains 62–74% protein on dry basis (Wu et al., 1997). Zein can also be extracted from DDGS or distillers' dried grains (DDG). They are co-products of the dry-grind ethanol process with or without the addition of condensed wet stillage or solubles (Kwiatkowski et al., 2006).

The typical protein content of DDGS is 28–30%, which is higher than in DMC (Singh et al., 2002). However, yields of zein from DDGS have been low. Wu et al. (1981) found that the zein proteins in DDGS had poor solubility in aqueous alcohols, which was attributed to protein denaturation during distillation of dry-grind ethanol after fermentation and drying. Recently, crude zein was extracted from a co-product of whiskey production process, corn distillers' grains with solubles (CDGS), which is similar to the DDGS of the dry-grind ethanol process (Wolf and Lawton, 1997). Zein yields of 3.2–6.6% were reported but the extracted zein contained only 37–57% protein (Wolf and Lawton, 1997). Xu et al. (2007) extracted zein from defatted DDGS using 70% ethanol and 0.25% sodium sulfite at acidic pH and extracted about 44% of the protein with a solid product that was 90%. Acetic acid was utilized to dissolve zein in DDG (Selling and Woods, 2008) yielding the total protein of ~12% with the protein content of zein extracted at 20%. Some DDGS undergoes a high-heat drying process, which can affect extractability of zein from protein bodies (Batterman-Azcona and Hamaker, 1998). During corn-based bioethanol fermentation, the use of proteases to release free nitrogen for yeast may also be detrimental to zein protein integrity and quality (Bothast and Schlicher, 2005). High temperature drying condition of DDGS could also be a source of damage to the zein. The soluble fraction of DDGS contains low-molecular-weight compounds, which may hamper extraction, and would probably be better left out (Kim et al., 2008).

Currently, two companies in the United States have developed zein extraction procedure based upon the dry-grind ethanol process. The COPE (corn oil and protein extraction) method used by Prairie Gold Inc. (Bloomington, IL) obtained zein from a front-end extraction fraction (Cheryan, 2009). Not only zein, but also a high

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value corn oil is extracted during the process (Johnson and Lusas, 1983). The zein and corn oil with high xanthophylls are extracted simultaneously and separated by membrane technology (Cheryan, 2002). A major feature of the COPE method is to produce zein not altered during fermentation. The COPE process can also be modified to produce zein products with just α-zein or a combination of α-, β-, and γ-zeins.

The other new extraction procedure based on the dry-grind ethanol process was developed by POET Inc. (Sioux Falls, SD) using a no-cook BFRAC™ dry mill ethanol process to produce POET's Dakota Gold® HP™ DDG from which zein INVIZ ™ was extracted (POET, 2010). This back-end extracted zein has not possibly been altered with a steep step, which would reduce disulfide, but instead, has been passed through fermentation. One of the benefits of this method is that the extraction substrate has high protein content (~40%), and allows for higher extraction rates than dry-milled corn. The disadvantage is that to optimize extraction efficiency, a low titer solvent must be used and gives only one product containing α-, β-, and γ-zeins. Both zein products (COPE zein and INVIZ) are new and no commercial products are available to evaluate.

Recently, we modified the CGM zein extraction procedure of Carter and Reck (1970) (Fig. 1) (Anderson and Lamsal, 2011). The best solvent systems used for extraction were 70% (w/w) aqueous 2-propanol (70-IPA) and 70% (v/v) aqueous ethanol (70-EtOH). Both solvents recovered 45% zein from CGM, which was significantly higher than the 28% achieved by commercial procedure using 88% (w/w) aqueous 2-propanol (88-IPA) (Anderson and Lamsal, 2011). The objectives of the present study were to: (i) determine the efficacy of the modified method and solvents to extract zein from DDGS, (ii) evaluate the effect of DDGS pretreatments (grinding, hydrolytic enzyme) on zein extraction, and (iii) characterize the molecular and film forming properties of the zein extracted from DDGS.

2. Materials and methods

2.1. Materials

DDGS was obtained from Lincolnway Energy (Nevada, IA). For ground preparations, the DDGS was milled using a lab grinder (Nutrimill Grain Mill 760200). The CGM was obtained from Cargill Inc. (Eddyville, IA). Kobayashi zein DP was purchased from Kobayashi Perfumery Co. (Japan) and used as a benchmark zein containing 96.9% protein (N × 6.25) on dry weight basis. The extraction procedure for Kobayashi zein was not available through the company.

2.2. Composition analysis

The moisture content was determined by drying the samples in a convection oven at 130 °C for 3 h (Method 44-19, AACC, 2000). Crude free fat contents were extracted using hexane as solvent (Method 30-25, AACC, 2000) with the Goldfish apparatus using hexane as a solvent (Labconco Corp., Kansas City, MO, USA). Solids contents of the samples were determined by drying overnight in an oven at 103 °C (Dickey et al., 1997). The crude protein content of the DDGS and the extracted zein solids were determined using Dumas nitrogen combustion method (Method 992.23, AOAC International, 1998) in an Elementar Vario MAX CN analyzer (Elementar Analysesysteme GmbH, Hanau, Germany). The analyses were all completed in duplicate and values are given in moisture-free basis. The particle sizes of the ground and unground DDGS were measured using standard Taylor series sieves with mesh sizes of 12, 20, 30, 50, 100, and 200 in a RO-TAP sieve shaker.

2.3. α-Zein extraction

The zein was extracted from the DDGS using two different extraction procedures as summarized in Fig. 1. Method A used 88-IPA with sodium bisulfite (0.5%) and NaOH (0.25%), and Method B used either of two solvents, 70-IPA or 70-EtOH with sodium bisulfite (0.5%) and NaOH (0.25%). In Method A, zein fractions were extracted and cold precipitated promptly after extraction; the key difference in Method B was that the solvent concentrations were increased to either 95% (v/v) aqueous ethanol or 88-IPA to precipitate β- and γ-zeins leaving α-zein in solution. Then, the α-zein was cold precipitated. Both procedures utilized varying particle size and enzyme treatments.

The isolated α-zein is described using protein purity, zein yield, protein recovery, and α-zein extraction efficiency. Protein purity is the percent protein content of the isolated α-zein. Zein yield = % [(mass α-zein-rich solids)/(total mass of DDGS)] × 100. Protein recovery, % = [((protein purity, %/100) × (mass α-zein-rich solids))/(total protein in DDGS mass)] × 100. The α-zein extraction efficiency was the zein yield corrected for α-zein content of DDGS. α-Zein extraction efficiency = [((protein purity/100) × (mass α-zein-rich solids, %))/(mass of α-zein protein in DDGS)] × 100.

2.4. Enzyme-assisted extraction of α-zein

DDGS was pretreated with a mixture of enzymes cellulase and pectinase (0.4% Multifect GX GC and 0.1% Multifect pectinase FE, both from Genencor®) prior to extraction of α-zein using Methods A or B. A 0.5% (v/v) enzyme in a 0.1 M sodium acetate buffer, pH 4.0, was mixed with the DDGS at 1:4 DDGS-to-solution. The enzyme-added DDGS slurry was incubated, with stirring, at 50 °C, for 2 h. The slurry was then centrifuged at 8000 × *g* for 15 min (Beckman, Palo Alto, CA). After centrifugation, the solids were washed 3 times with 250 mL of distilled water to remove hydrolyzed sugars. Zein was then extracted following Method A or B, after adjusting for water content in solids.

2.5. Total zein in DDGS

The total zein protein in DDGS was determined based on the method of Wu et al. (1997), scaled-up for ease of extraction. Ten g of DDGS (db) was extracted first with 250 mL of 0.5 M NaCl to remove the salt- and water-soluble proteins. The slurry was stirred for 20 min at room temperature in a sealed 400-mL centrifuge tube, centrifuged at 8000 × *g* for 15 min and the supernatant was collected. The pellet extraction with salt was repeated one more time. The total zein of the remaining pellet was determined by extracting with 250 mL of 55% (v/v) aqueous 2-propanol and 5% (v/v) 2-mercaptoethanol with 0.5% (w/v) sodium acetate solvent (PMA). The contents were stirred for 2 h at room temperature, centrifuged at 8000 × *g* for 15 min and supernatant collected. The pellet was washed twice with 50 mL of PMA. The total zein and residual pellet were dried and protein contents were determined.

2.6. SDS-PAGE and densitometry

The protein molecular weights and relative amount of the extracted zeins were analyzed with sodium dodecyl sulfate-polyacryalmide gel electrophoresis (SDS-PAGE) and densitometry. Sample buffer was prepared with the composition of 125 mM Tris–HCl at pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.05% bromophenol blue. The α-zeins did not dissolve in buffer easily, so were dissolved in 70% ethanol first and then added to the sample buffer to produce 3 μg/μL upon loading. The samples were heated at 100 °C for 4 min in a water bath. The SDS-PAGE gels were