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Extraction of protein from distiller's grain

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ABSTRACT

We have investigated the feasibility of extracting the oil and protein from distiller's grain (DG) to obtain a higher-valued protein-rich product and a carbohydrate-rich residue better suited for conversion to fermentable sugars. Protein extractions based on aqueous ethanol, alkaline-ethanol, and aqueous enzyme treatments were compared. Three of the methods extracted a significant amount of the protein from dried, defatted DG (DDDG). The enzymatic extraction decreased the crude protein content in the solid phase for both milled and unmilled DDDG from 41% (dry weight) to approximately 10% (dry weight) protein in the residual solid; this corresponded to extraction of 90% of the protein in the original DDDG. The alkaline-ethanol extraction was similarly effective for milled but not unmilled DG. Simple extraction with alcohol was not as effective. Amino acid analysis of each protein extract was consistent with it consisting mainly of zeins. For the protease-assisted extractions, 95% the proteins were in the form of peptides smaller than 10 kDa.

1. Introduction

Production of byproduct distiller's grain (DG) has increased along with the number of dry grind ethanol production facilities. In the dry-grind ethanol process, DG is the solid residue recovered from the still bottoms following fermentation and distillation. It is dried to be sold as distiller's dried grain (DDG) or blended with the dried solubles in the bottoms and sold as distiller's dried grain plus solubles (DDGS). Currently most of the DG is being used as cattle feed in one of these forms, but oversupply and an increase in *E. coli* O157 (Hegeman, 2008) outbreaks fuel the search for alternative uses. DG contains protein, oil, and non-starch carbohydrates from the corn along with some portion of yeast material (Kim et al., 2008). An analysis of the composition of DG from the same source as used in this work showed 34.4% crude protein, 10.9% ether extractable fat, 52.7% carbohydrates, and 2.0% ash (Kim et al., 2008).

A significant effort is being made to obtain additional fermentable sugars from the carbohydrate (mainly cellulose) portion of the DG (Ladisch et al., 2008). Because of the complex nature of these carbohydrates, acid hydrolysis has advantages over enzymes in being able to hydrolyze multiple types of linkages, with solid acid catalysts favored for reduced degradation of the desired monosaccharide products (Bootsma et al., 2008). That route for obtaining the sugars is to extract them from the DG (or DDGS), leaving behind a protein-enriched DG. However, some proteins are also extracted and/or degraded leading to poisoning of the hydrolysis catalyst (Bootsma et al., 2008) and a less valuable feed (Perkis et al., 2008). The current work explores direct extraction of proteins with potentially greater value than DG, while leaving behind a carbohydrate residue with reduced fouling potential.

The proteins in DG are believed to be mainly zeins (Wolf and Lawton, 1997; Shukla and Cheryan, 2001). Zeins are alcohol soluble storage prolamins comprising 45–50% of the proteins found in the corn endosperm (Wang et al., 2005). Shukla and Cheryan (2001) have reviewed zein extraction from corn and corn gluten meal, the zein-containing fraction from corn wet-milling. Zeins have been characterized by size and the variants are denoted α, β, and γ (Esen, 1986). The β- and γ-zein differ from the α-zein in the number of disulfide linkages (Esen, 1986; Parris and Dickey, 2001) and have been characterized as disulfide-linked aggregates of components found in native zeins (Esen, 1986). The disulfide linkages change the solubility characteristics. The α-zein is soluble in 95% aqueous ethanol, whereas the β- and γ-zein are soluble in 60% aqueous ethanol but not in 95% aqueous ethanol (Esen, 1986; Parris and Dickey, 2001).

DG is in contact with alcohol at much lower concentrations during fuel ethanaol fermentation and recovery since commercial yeast are limited to 18% v/v EtOH (Ethanol Red product specification sheet, http://www.fermentis.com/FO/EN/08-Ethanol/20-10_product_eth.asp, accessed 9/9/08); thus, zeins are unlikely to have been extracted. The extraction of zeins from DG has been shown to be quite difficult. Extraction at 60% ethanol and 60 °C was only capable of extracting 1.5–3.9% of the crude zein in ethanol byproducts (Wolf and Lawton, 1997). Alkaline-ethanol extractants have shown better extraction of proteins from DG (Rosentrater et al., 2006). Here we have considered use of a protease to produce more soluble peptides

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or pretreatment with a reducing agent to break disulfide linkages present in the native zein or formed during process to eliminate insoluble aggregates.

Reducing agents have been used to break disulfide linkages in many cereal grains including corn (Jay et al., 2005). Sodium bisulfite at a concentration of 100 mM improved the digestibility of kafirins from sorghum (Hamaker et al., 1986). Kafirins are similar to zeins in that they are both alcohol-soluble storage prolamins (Salinas et al., 2006).

An alternative to breaking the disulfide linkages and aqueous alcohol extractions would be hydrolyzing the proteins. Proteases have been used to increase protein solubility (Rothberg and Axilrod, 1967). Methods have been developed using proteases to successfully extract proteins from soybeans (Jung et al., 2006) and corn germ (Moreau, 2004). Hydrolyzed proteins are attractive feed supplements because they have decreased antigenicity and are easier to digest (Ludlow, 2005). Up to 10% hydrolyzed DG has been used to replace soybean concentrate with no reduction in the weight gain of turkeys normally fed corn and soybean concentrate (Tucker et al., 2004). Hence, hydrolyzed DG could be used to replace the higher priced soybean concentrate.

A protein-rich extract of either intact zeins or hydrolysates for soy concentrate replacement offers potential economic benefit over selling it as part of DG. While higher in price than could be expected for the film market, zeins have a value of $19–$22/kg for use as specialty products (Lawton, 2003). Soy concentrate, for example ARCON®SM, is 70% protein and sells for $3.25/kg (per Chicago Sweetners, Chicago, IL) or $4.64/kg of protein. The market value of DDG is $170 a ton (May 2008, Commodities Specialty Co., Minneapolis, MN). For 32% protein content DDG, this corresponds to $0.58/kg of protein.

Extractions were performed here using aqueous ethanol, alkaline-ethanol, and aqueous enzyme solutions. Pretreatments of milling and reduction with sodium bisulfite (for the aqueous ethanol extractions) were also evaluated. The extractions were assessed on the basis of protein extracted, protein in the residual fiber fraction, and extracted protein size and amino acid composition.

2. Methods

2.1. Materials

All chemicals were from Fisher Scientific unless otherwise noted. DG was provided by Big River Resources, West Burlington, Iowa. The DG contained 67% moisture and was stored frozen (−20 °C) until use. The extractions were performed in 250 mL beakers using 150 mL of the solvents listed in Table 1 and 10 g of dried, defatted DG (DDDG). Solutions containing ethanol were prepared using absolute ethanol. The enzyme used was Protex 6L (an alkaline serine-endopeptidase, active range pH 7.0–10.0, 30–70°C) provided by Genencor International Inc., Rochester, NY.

2.2. Oil extraction

A hexane wash was used for both the proximate analysis of the DG and prior to any protein extraction step. The hexane-extractable oil content of the DG was determined by mass difference of the dry weights before and after a hexane wash. For the hexane wash, 50 g of DG was stirred with 250 mL hexane in an ice bath for 1 h. The DG and hexane were separated by centrifugation in 250 mL centrifuge bottles at 3000*g* for 15 min and 25 °C (Sovall RC-5B, Newton, CT) using a HS-4 Swinging Bucket rotor. The supernatant was decanted and the extraction was repeated. The remaining solids were dried at room temperature overnight.

Table 1
Solvents used for protein extraction from DDDG.

Type of Extraction	Solvent Used
Aqueous Ethanol	60% Ethanol
Aqueous Ethanol	95% Ethanol
Alkaline-Ethanol	45% Ethanol and 55% 1 M NaOH
Aqueous Enzyme	0.67% v/v Protex 6L in deionized water (pH 8.0)

2.3. Protein extraction with ethanol

Extractions were performed using 60% and 95% aqueous ethanol, and 45% ethanol in 1 M NaOH, at a solid:liquid ratio of 1:15. The solution was stirred at 520 rpm for 2–10 h at 50 °C in a water bath. Two hours became the standard time as no additional protein was extracted beyond that point. The solids and the extract were separated by centrifugation (25 °C, 20 min at 3000*g*). The solid fraction was analyzed for residual mass and crude protein content after drying for 6 h at 130 °C. Amount of nonprotein solids extracted was calculated by difference. Proteins were precipitated from the extract by pH adjustment to 7.0 using 5 M HCl. The precipitate was characterized by amino acid analysis, SDS–PAGE, and total N analysis.

2.4. Enzyme-aided extraction

A mixture of 10 g of DDDG, 1 mL Protex 6L and 150 mL deionized water was stirred at 520 rpm for 2 h at pH 8.0 in a 50 °C water bath. Conditions were patterned after previous use of the same enzyme (de Moura et al., 2008); lower enzyme levels required longer time. The pH was maintained at 8.0 with 2 N NaOH using an automatic titrator (Metrohm titrator, model STAT Titrino 718). The solids and extract were separated by centrifugation in 50 mL centrifuge bottles at 3000*g* for 20 min and 25 °C. The size of the proteins in the extract were analyzed using size exclusion chromatography (SEC) and the solid fraction was analyzed for crude protein content after drying for 6 h at 130 °C.

2.5. Disulfide bond reduction

A mixture of 10 g of DDDG and 150 mL of 100 mM $NaHSO_3$ was stirred at 520 rpm at 50 °C for 2 h. The solid and liquid fractions were separated by centrifugation (25 °C, 20 min at 3000*g*). The supernatant was decanted and the solid phase was extracted with 60% and 95% aqueous ethanol solutions.

2.6. Particle size distribution/size reduction

The volume- and surface-weighted mean diameters ($D_{4,3}$ and $D_{3,2}$) of the DDDG were measured using a laser light scattering particle size analyzer (Mastersizer 2000 S, Malvern Instruments, Ltd., Chicago, IL) before and after milling (Retsch mill with a 0.5 mm screen).

2.7. Protein content determination

The crude protein (N × 5.9) (Kim et al., 2008) of the solids was based on the nitrogen content determined by combustion analysis (Berner and Brown, 1994) (Rapid NIII combustion analyzer; Elementar Americas, Inc., Mt. Laurel, NJ).

2.8. Protein analysis of alkaline-ethanol extract

The alkaline-ethanol extract was pH adjusted to 7.0 using 5 M HCl (Ludlow, 2005). The pH adjustment caused precipitation of a fraction of the proteins from the extract. The precipitated proteins