HEMICELLULOSE FIBER GUM FROM DISTILLERS GRAIN: ISOLATION, STRUCTURE AND PROPERTIES

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Abstract

Isolation of hemicellulose from distillers dry grain with solubles (DDGS) was investigated. Hemicellulose fiber gum (HFG) is a mixture of hemicelluose, protein, ash and starch. It was extracted from a commercial DDGS by heating with or without alkali. Three extraction methods (water heating, alkaline heating and alkaline hydrogen peroxide heating) were evaluated. Yield of HFG and the recovery of hemicellulose were obtained. High heating temperature (100 and 120°C), alkali or hydrogen peroxide facilitated the release of hemicellulose from the cell wall matrix. However combining alkali with 2.5% H_2O_2 did not extract more hemicellulose out than did alkali alone. The highest hemicellulose recovery was 32% achieved by cooking at 120°C with 2% alkaline solution. Hemicellulose can function as an emulsifier in the oil-in-water emulsions, such as beverage, and potentially replace gum arabic. HFGs obtained by a series of extracting methods were applied in both the concentrated emulsion with the gum: oil: water ratio of 0.5:1: 8.5 and the diluted emulsion with the gum: oil: water ratio of 0.005: 0.01: 1. The emulsion stability was evaluated by turbidity and creaming test. HFG extracted by 2% NaOH solution at 120°C and HFG extracted by 2% NaOH and 2.5% H_2O_2 solution at 100°C showed the best emulsifying ability among 15 HFG samples.

DDGS was produced from corn, sorghum, wheat in the lab. HFGs extracted from sorghum and wheat DDGS were compared with that from corn DDGS. The composition of the three DDGS varied in protein, fat and non-starch carbohydrate contents. Sorghum and wheat DDGS contained higher levels of protein and lower levels of fat and non-starch carbohydrate than corn DDGS. HFG was extracted by 2% NaOH solution at 100°C for one hour and purified by 100% ethanol. The yield of HFG from corn, sorghum and wheat DDGS was 21.08, 11.07, 11.64% respectively, while the hemicellulose recovery was 30.95, 29.74, 22.71% respectively. The water extractable hemicelluloses from all three DDGS had similar ratios of arabinose to xylose.

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CHAPTER 1 - HEMICELLULOSE FIBER GUM FROM DISTILLERS GRAIN: ISOLATION, STRUCTURE, AND PROPERTIES

ABSTRACT AND KEYWORDS

Abstact Isolation of hemicellulose from a commercial distillers dry grain with solubles (DDGS) was investigated. Hemicellulose fiber gum (HFG) was a mixture of hemicelluose, protein, ash, starch. It was extracted from DDGS by heating with or without alkali. Three extraction methods (water heating, alkaline heating and alkaline hydrogen peroxide heating) were evaluated. Yield of HFG and recovery of hemicellulose were obtained for each method. High heating temperature (100 and 120°C), alkaline or hydrogen peroxide facilitated the release of hemicellulose from the cell wall matrix. However combining alkali with 2.5% H_2O_2 did not extract more hemicellulose out than did alkali alone. The highest hemicellulose recovery was 32% achieved by cooking at 120°C with 2% alkaline solution. After alkaline extraction, the insoluble residue was hydrolyzed by protease. However the hemicellulose yield did not significantly increase. This result indicated that protein-hemicellulose complex may not be fully responsible to the unextractability of hemicellulose. Hemicellulose can function as an emulsifier in the oil-in-water emulsions, such as beverage, and can potentially replace gum arabic. HFGs obtained by a series of extracting methods were applied in both concentrated emulsions with the gum: oil: water ratio of 0.5:1: 8.5 and diluted emulsions with the gum: oil: water ratio of 0.005: 0.01: 1. The emulsion stability was evaluated by the turbidity and creaming test. HFG extracted by 2% NaOH solution at 120°C and HFG extracted by 2% NaOH and 2.5% H_2O_2 solution at 100°C showed the best emulsifying ability among 15 HFG samples.

Keywords. Distillers dry grain with soluble, hemicellulose fiber gum, arabinoxylan, extraction, emulsifier, emulsion

INTRODUCTION

Ethanol production process consists of grinding, cooking, liquefication, saccharification, fermentation, and distillation (Rosentrater and Krishnan 2006; Rosentrater and Lehman 2008). The nonfermentable residues are separated out after distillation as whole stillage. They are centrifuged to remove water; the supernatant is then evaporated to produce condensed corn distillers soluble (CCDS) which is recombined with the precipitate, dried and sold as distillers dried grains with soluble (DDGS) (Rosentrater and Lehman 2008). Fermentation from one bushel of corn (56 lbs) yields 17.6 lbs ethanol, 17 lbs DDGS and 18.4 lbs carbon dioxide (Kelsall et al. 2003). Production of DDGS steeply increased with the surge of ethanol production in US, and the value of DDGS was depressed.

Composition of DDGS has been analyzed by many researchers. Dong et al. (1987) reported that there are 24.7% protein, 46.1% neutral detergent fiber (NDF), 11.0% lipid and 12.0% ash in corn DDGS. Spiehs et al. (2002) identified the composition and nutrient values of DDGS produced by 10 different ethanol plants in the Minnesota-South Dakota (MNSD) region, and determined the nutrient variability among these plants. There are 28.7~31.6% crude protein, 10.2~11.7% fat, 36.7~49.1% NDF, and 5.2~6.7% ash among ten DDGS samples. Belyea et al. (2004) investigated the relationship between composition of corn and composition of DDGS, and examined five years' crops and corresponding DDGSs. On average, the DDGS consists of 31.3% protein, 11.9% crude fat, 4.6% ash, 10.2% crude fiber, 17.2% acid detergent fiber (ADF) and 5.1% starch. Despite variations, non-starch carbohydrate is a major component in DDGS.

Due to high nutrition profile of DDGS, it is predominately used to replace portion of the traditional animal feed (Jacques 2003). Some researchers started studies of utilization of ethanol-manufacturing residues in food products (e.g. Rosentrater and Krishman 2006). However, when incorporated in foods, DDGS often has a negative impact on the flavor and color. Additionally, food containing DDGS has lower shelf

life due to fatty acids and pigments. Currently there is no commercial food product containing DDGS (Rosentrater and Krishnan 2006).

Corn bran has been studied to produce corn fiber gum (CFG) for decades (Wolf et al. 1955, Watson et al. 1959; Doner et al. 1997; Doner et al. 2000; Gaspar et al. 2007; Yadav et al. 2009). Hemicellulose (arabinoxylan) from CFG is proved to be the major component and contribute to the emulsifying property (Yadav et al. 2007a). Considering the similarity between corn bran and DDGS, we think it is feasible to extract hemicellulose fiber gum from DDGS and apply it as the emulsifier in the food system. Isolating hemicellulose fiber gum can potentially create additional needed value from DDGS.

The earliest patents on extracting hemicellulose from corn bran trace back to 1950's (Wolf et al. 1955; Rutenberg et al. 1957; Watson et al. 1959). Wolf et al. (1955) first introduced the alkaline extraction of hemicellulose. They discovered that corn hulls, hemicellulose-containing substances, could be treated with certain alkalis including sodium carbonate, sodium hydroxide, soda ash, potassium carbonate within the range of pH 9 to pH 13 to solubilize hemicellulose compounds, and render them available to water extraction. Wolf's method produced a crude hemicellulose potentially contaminated by salts, proteins and lipids. The liquid water-miscible organic acids (acetic, butyric, propionic, pentanoic acid) were employed to purify the crude hemicellulose (Schweiger 1973). Doner and his coworkers improved the hemicellulose isolation method (Doner et al. 1997, 1998, 2000, 2001b) through combining alkali with hydrogen peroxide. Hydrogen peroxide acting as an oxidant is added either after the heating process (Doner et al. 1997, 1998, 2001b; Yadav et al. 2007a) or during heating (Doner et al. 2000) to bleach the gum and increase the yield.

To extract corn fiber gum from corn bran, the raw material needs to be milled to decrease the particle size (pass through 20 mesh) (Doner et al. 1998; Yadav et al. 2007a; Gaspar et al. 2007), which allows greater surface area to be exposed during processing. α-amylase is recommended to remove the starch in the raw material (Doner et al. 1998; Yadav et al. 2007a; Gaspar et al. 2007). The alkaline hydrogen peroxide extraction of corn fiber is best conducted around pH 11.5 (Doner et al. 1998;

Yadav et al. 2007a). Hydrogen peroxide can be applied during heating at the hydrogen peroxide: corn fiber: water ratio of 0.1:1:20; and 0.4% sodium hydroxide and 0.37% calcium hydroxide solution are used (Doner et al. 2000). Hydrogen peroxide can also be applied after heating to bleach the extract at the hydrogen peroxide: corn fiber ratio of 1:10 (Doner et al. 1998; Yadav et al. 2007a). In studies by Doner et al. (1998, 2000), NaOH is more effective than $Ca(OH)_2$ to extract hemicellulose based on the yield of hemicellulose. The blend of $Ca(OH)_2$ and NaOH was also reported for the alkaline extraction (Yadav et al. 2007a & 2007b). There was no agreement on the effects of these two alkalis on hemicellulose extraction. Doner et al. (1997) also studied the influence of temperature of extraction, and showed that high temperature (60°C) benefited the extraction. However, in the further studies, boiling temperature is used (Doner et al. 2001a; Gaspar et al. 2005; Yadav et al. 2007a). Hemicellulose A precipitates under neutral condition; hemicellulose B is soluble under acid or neutral or base condition (BeMiller 2007; Muralikrishna et al. 2007). Based on this principle, by neutralizing the solution after alkaline treatment, hemicellulose A can be separated from hemicellulose B which is precipitated by adding ethanol (Doner et al. 1998; Gaspar et al. 2005; Yadav et al. 2007a). Oven-drying is used for recovering hemicellulose B and removing ethanol solvent (Doner et al. 1998; Gaspar et al. 2005; Yadav et al. 2007a).

Pure hemicellulose and corn fiber gum (including hemicellulose and other minor components) are documented as emulsifiers in the beverage or other emulsions (Ogasawara et al. 2004; McPherson et al. 2006; Yadav et al. 2007a, 2007b). Ogasawara et al. (2004) reported the application of water-soluble hemicellulose (0.2~0.6%) in acidic milk beverage to replace pectin and CMC as stabilizers. The water-soluble hemicellulose extracted from soybeans was able to effectively stabilize the acid milk up to 21 days at 10°C. McPherson et al. (2006) disclosed the application of hemicellulose hydrolyzate from corn hull. They applied the hemicellulose, hemicellulose hydrolyzate, and mixture of these two to emulsify and encapsulate essential oil flavorants (citrus oils), and found that the hemicellulose and hydrolyzate are more efficient than gum arabic which is a traditional emulsion stabilizer for

essential oil flavorants. Yadav et al. (2007a) studied the emulsifying properties of corn fiber gum samples, and concluded that corn fiber gum is generally superior or equal to the acacia gum in their experimental system, and very promising for beverage emulsion stabilization.

Hemicellulose is a group of polysaccharides extracted from plant cell walls by alkali (Ebringerova et al. 2000; Bemiller 2007; Mohnen et al. 2008). The composition and structural characteristics vary among plant species. Mohnen et al. (2008) concluded that most types of hemicellulose are arabinoxylan, xyloglucan, mixed-linkage glucans and mannans. For cereals, hemicellulose is composed of a linear or branched chain of xylopyranosyl units, and the backbone is attached with short side chains containing one to a few uints of L-arabinofuranosyl, D-galactopyranosyl, D-glucuronopyranosyl, and/or 4-O-methyl-D-glucuronopyranosyl units (BeMiller 2007). Methylation analysis of arabinoxylan isolated from corn bran indicates that the xylan backbone is highly branched with only 23% of unbranched xylose residues, and also highly substituted with oligomeric side chains with only 15% of unsubstituted xylose residues (Muralikrishna et al. 2007). Ebringerova et al. (2000) reported that the xylan backbone of arabinoxylan is highly branched with Xyl*p*-, Ara*f*-, and Gal*p*-mono-, di-, and trisaccharide side chains. In corn bran, arabinoxylan mainly exists in the pericarp and aleurone layer and comprises of 32.2% arabinose, 52.1% xylose, 8.7% galactose, 6.9% uronic acid and trace glucose (Ebringerova et al. 2000, Doner et al. 2001b). Some other reports suggested that corn arabinoxylan (corn fiber gum) contains 48~55% xylose, 35~40% arabionse, 5~7% galactose, 1~2% glucose and 3~5% glucuroinc acid (Singh et al. 2000; Doner et al. 2001b; Yadav et al. 2009).

Arabinoxylan plays an important role in the cell wall structure (Fincher et al. 1986; Saha 2003). In corn bran, due to the highly branched structure, arabinoxylan chains are bridged through ferulic acid dimmers, which induces the insolubility of arabinoxylan (Fincher et al. 1986; Saulnier et al. 1995a). Saulnier et al. (1995a) calculated that approximately 60 ferulic acid esters are bedded in one arabinoxylan molecule which is cross-linked through approximately 5 diferulic bond. Also because of the highly branched structure, hydrogen bonds between arabinoxylan and other cell

wall components do not likely exist (Saulnier et al. 1995b; Ebringerova et al. 2000; Muralikrishna et al. 2007). Besides the diferulic bond, some researchers proposed that protein-polysaccharide linkages might be the main reason of insolubility of maize bran arabinoxylan (Ebringerova et al. 1994; Saulnier et al. 1995b). In the study of cell wall polysaccharide interactions in maize bran (Saulnier et al. 1995b), the insoluble residue after alkaline extraction was further treated by sodium chloride, and more arabinoxylans were released when the protein-heteroxylan linkage was broken down. However the nature of protein-polysaccharide linkage was not disclosed (Saulnier et al. 1995b).

The protein components are attributed to the emulsifying potential of hemicellulose. Most food products are emulsion-based food either during the processing or as the final form, such as dairy items, beverage, sauces, salad cream, cake betters, etc. Essentially, emulsion is composed of two immiscible liquids with one of liquids dispersed in the other one as small size droplets (McClements 1998). Food emulsions are thermodynamically unstable systems and the two phases will eventually separate (McClements 1998). There are several physical mechanisms responsible to the instability: gravitational separation, flocculation, coalescence and Ostwald ripening (McClements 1998). The two immiscible liquids (such as oil and water) normally have a different density, and due to the gravitational force, dispersed droplets have a tendency to move upward or downward, which is referred to as creaming or sedimentation. Because of thermal energy and other forces acting on the droplets in emulsions, they are in continual motion and frequently collide with each other. If two droplets come together to form an aggregate but still retain their integrity, this phenomenon is named as flocculation; however if two droplets merge into one single larger droplet, this process is coalescence. When food product is conveyed to consumers as emulsion, the homogeneous appearance, in other words, the stability of the emulsion is quite crucial. The covalent protein-polysaccharide biopolymer (such as gum arabic and hemicellulose) is not only highly surface active due to the hydrophobic proteineous parts, but also highly solvated by the aqueous

medium due to the hydrophilic polysaccharide (Dickinson 1995 and Garti 2001). Therefore they could be potentially used as emulsifiers.

Traditionally DDGS is used in animal feed, however this market is already oversupplied in regions with a high density of ethanol production. Therefore, developing high value product from DDGS is of particular interest to the biofuel industry. The goal of this study is to increase the value derived from DDGS. Specifically, we extract hemicellulose fiber gum from DDGS. This hydrocolloid has higher value as a food and industrial ingredient and could potentially be used as emulsifiers. Increasing the value of DDGS will help increase the sustainable production of fuels from biomass, meet the pressing needs for the ethanol industry and offer a realistic opportunity to create additional needed value from the DDGS.

MATERIALS AND METHODS

Materials

DDGS was provided by MGP Ingredients Inc. (Atchison, KS) and ground to a 20 mesh particle size by a commercial blender (Dynamics Corporation of America, New Harford, CT). Gum arabic was supplied by TIC Gums (Belcamp, MD); sucrose and orange oil were purchased from Sigma Chemicals (St Louis, MO). Celite 577 (cat.22142) as the filter aid agent was purchased from Sigma Chemicals (St Louis, MO).

Total starch assay kit was purchased from Megazyme International Ireland Limited (Wicklow, Ireland). It includes thermostable α-amylase (3000 U/ml) and amyloglucosidase (200 U/ml). STARGEN 001 (a blend of α-amylase and glucoamlyase), GC 106 (acid fungal protease), and Protex 6L (alkaline protease) were obtained from Genencor (Kansas City, MO), and α-amylase (Liquozyme) from Novozymes (Franklinton, NC). STARGEN 001 had an activity ≥456 GSHU/g (granular starch hydrolyzing units). Activity of GC 106 was ≥1000 SAPU/g

(spectrophotometric acid protease units) and activity of Protex 6L was ≥580,000 DU/g. Liquozyme (α-amylase) had activity 240 KNU/g (kilo novo units).

Corn bran sample

Corn bran was obtained from Cargill Dry Corn Ingredients Inc. (Indianapolis, IN), and was ground to 20 mesh using a commercial blender (Dynamics Corporation of America, New Harford, CT). Destarching was carried out by adopting Doner's method (2000). Corn bran (100 g) was suspended in 360 ml water, and pH was adjusted to 6.5 by 1 M HCl. Liquozyme (α-amylase, 5 ml) was added and the slurry was brought to boil for 4 hours. After cooling down to 25°C, the fiber rich residue was removed by centrifugation. And the corn fiber was washed twice with water. The destarched corn bran was air dried in an oven at 40°C for overnight.

Extraction of hemicellulose fiber gum (HFG)

Water heating without alkali

Ground DDGS (60 g, dry weight) was mixed with 210 ml distilled water and 20 ml H_2O_2 solution (30% w/w) or 230 ml distilled water in a glass beaker. pH was adjusted to 7.0 by adding 10 ml 5 M NaOH solution. The mixture was heated at 80, 100 or 120°C in a Parr reactor (Parr Instrument, Moline, IL). After one hour of heating and continuous stirring, the slurry was cooled to 25°C and centrifuged (2500×g, 15 min) to remove the insoluble materials. For the sample without H_2O_2 added during heating, the slurry was treated with 20 ml H_2O_2 (30% w/w) at 25°C before centrifugation. The supernatant was decanted and collected for HFG recovery. The insoluble fraction was washed three times with 400 ml distilled water. The pH of supernatant was adjusted to 4.5. The supernatant was set overnight and centrifuged again (2500×g, 20 min) to further remove the insoluble fraction. After centrifugation, the supernatant was concentrated to 100 ml, and centrifuged at 8000×g for 15 min. A precoat Celite-577 filter plate was used to clarify the supernatant and the retentate was freeze-dried.

Water heating with alkali

Ground DDGS (60 g, dry weight) was mixed with 240 ml distilled water, and 2 meq/gram DDGS of alkali was dissolved in the slurry. NaOH or $Ca(OH)_2$ was applied as alkali sources. The recovery procedure was same as described in water heating extraction.

Alkali and hydrogen peroxide extraction

Ground DDGS (60 g, dry weight) was mixed with 235 ml distilled water, and 2 meq/gram DDGS of alkali was dissolved in the slurry. H_2O_2 solution (5 ml, 30% w/w) was added into the slurry before cooking. The heating process was conducted at 100°C for one hour with continuous stirring in the Parr bench-top stirred reactor (Moline, IL). The recovery procedure was same as the alkaline heating method. NaOH or $Ca(OH)_2$ was applied as alkali sources during heating.

Protease treatment of DDGS insoluble residue

One set of sequential extractions of HFG from DDGS and DDGS insoluble residue was carried out as the scheme shown in **Fig. 1-1**. First, DDGS was extracted by NaOH as previously described. The soluble fraction was isolated as HFG, and the insoluble residue was air-dried at 70°C to final moisture content < 2% and ground by the commercial blender (Dynamics Corporation of America, New Harford, CT). In the second extraction step, four approaches were applied on the DDGS insoluble residue. The insoluble residues were subjected to the dual-temperature (55 & 100°C) heated with four different extracting solutions. The extracted hemicellulose fiber gums were referred to S1, S2, S3 and S4. For treatment one, residue (15 g) was suspended in distilled water (60 ml) and sequentially extracted at 55°C for 3 hours and 100°C for 1 hour. For treatment two, residue (15 g) was suspended in distilled water (pH 8~9, 60 ml) containing protease (Protex 6L, 0.17% w/w) and incubated at 55°C for 3 hours and the slurry was cooked for another hour at 100°C. For treatment three, residue (15 g) was first extracted at 55°C for 3 hours, and then boiled in sodium hydroxide solution (pH12, 60 ml) for 1 hour. For treatment four, both protease (Protex 6L, 0.17% w/w) and sodium hydroxide were applied.

HFG purification

HFG (10 g) was dispersed in 100 ml distilled water and the pH was adjusted to 4.5 by 1 M HCl solution. Protease (GC106, 0.02% w/w) and the blend of α-amylase and amyloglucosidase (STARGEN 001, 0.02% w/w) were added in the solution. The enzymatic hydrolysis was carried out at 35°C overnight. The pH of the solution was adjusted to 8.0 by 1 M NaOH, and alkali protease (Protex 6L, 0.02% w/w) was added. The solution was incubated at 35°C overnight. The hydrolyzed solution was precipitated by 3 volumes of 100% ethanol. The yellowish rubbery sediment was centrifuged and air-dried at 40°C overnight to obtain the purified HFG.

Composition analysis

Protein was measured by nitrogen combustion (LECO FP-528, St. Joseph, MI) according to AOAC method 990.03. Crude fat, ash, and moisture content were determined by AOAC method 920.39, AOAC method 942.05, and AACC air oven method 44-19, respectively.

Starch content was determined by high performance anion-exchange chromatograpy (Dionex Corporation, Sunnyvale, CA) with pulsed amperometric detection (HPAEC-PAD) after hydrolyzing HFG and DDGS by Megzyme® Total Starch Assay Kit. HFG and DDGS (100 mg) were weighed into a screw-cap tube, dissolved in 2 ml of deionized water, and boiled for 5 min. Thermostable α-amylase (3 ml, diluted 1:30 in 100 mM sodium acetate buffer, pH 5.0) was added. The sample was incubated in a boiling water bath for 6 min. After adding 0.1 ml amyloglucosidase and 4.9 ml deionized water, the sample was incubated at 50°C for 30 min. After hydrolysis, the solution was boiled for 10 min to denature enzymes. Hydrolyzed HFG and DDGS was diluted 80 times with distilled water, filtered and injected into CarboPAC1 (Dionex Corporation, Sunnyvale, CA) column at 25°C. NaOH (150 mM) was employed as the eluent at a flow rate of 1 ml/min. Standard glucose solutions (0, 3, 4, 5 and 6 μg/ml) were injected and analyzed to obtain the standard curve. Quantitation was based on integrated peak area relative to the area of known quantity of standard glucose.

Glycerol was also analyzed by HPAEC-PAD. DDGS (100 mg) was weighed and dissolved in 10 ml deionized water. The liquid sample was thoroughly stirred for 1 hour and centrifuged to remove the insolubles. After filtered, the sample was injected into CarboPAC1 column at 25°C. NaOH (150 mM) was used as the eluent at a flow rate of 1 ml/min. Standard glycerol solutions (0, 10, 15, 20 and 25 μg/ml) were injected to obtain the standard curve.

Carbohydrate composition of hemicellulose in HFG

Sugar composition was determined by HPAEC after acid hydrolysis of HFG and DDGS. The sample preparation was according to the method by Doner et al (2001b). Sample (100 mg) was weighed into a screw-cap tube and mixed with 2 ml 12*N* H_2SO_4, vortexed periodically over 4 hours at room temperature. The solution was then diluted to 2*N* H_2SO_4 with distilled water and boiled for 1 hour. After cooling to 25°C, 2 g $BaCO_3$ was added to neutralize the solution, and removed by centrifugation. Hydrolyzed HFG and DDGS was diluted 60 times and injected into CarboPAC1 column at 25°C. NaOH (13 mM) was employed as the eluent at a flow rate of 1 ml/min. Standard sugar solutions (mixture of arabinose, galactose and xylose) were injected and analyzed to obtain the standard curve. Concentrations of all standards were 0, 10, 15, 20 and 25 μg/ml. Quantitation was based on integrated peak area relative to the area of known quantity of standard sugars.

Molecular weight (MW) distribution of HFG

MW distribution of hemicellulose was analyzed by gel permeation chromatography (GPC). HFG sample was dissolved in dimethyl sulfoxide (DMSO) (HPLC grade, Sigma, St. Louis, MO) at 0.2~0.5% concentration and filtered through a 2 μm filter and then injected by an autosampler into a PL-GPC 220 system (Polymer Laboratories Inc., Amherst, MA, USA) with three Phenogel columns (00H-0642-K0; 00H-0644-K0; 00H-0646-K0; Phenomenex Inc., Torrance, CA, USA), one guard column (03B-0290-K0, Phenomenex Inc., Torrance, CA, USA), and a differential refractive index detector. Eluenting solvent was DMSO containing 0.5 mM $NaNO_3$,

and flow rate was 0.8 mL/min. The column oven temperature was controlled at 80°C. Standard dextrans (American Polymer Standards Co., Mentor, OH, USA) with different MWs were used for MW calibration.

Emulsion properties of HFG

Emulsion preparation

Concentrated emulsion: The oil-in-water concentrated emulsions were prepared in duplicate with the following formula: orange oil 10% (w/w); HFG 5% (w/w); sodium benzoate 0.1% (w/w); citric acid 0.3% (w/w) and water to make up to 100% (w/w). Two-stage homogenization by using a laboratory bench top homogenizer (PRO Scientific Inc., Oxford, CT) was applied to prepare the emulsion: 15,000 rpm for 120 s; 20,000 rpm for 120 s.

Diluted emulsion: The homogenized concentrates (1.5 ml) were diluted to 150 ml with 10% (w/w) sucrose solution containing 0.1% (w/w) sodium benzoate and 0.3% (w/w) citric acid. After diluting, the solution was homogenized at 15,000 rpm for 30 s.

Turbidity

Diluted emulsions were kept in glass bottles sealed with caps and stored at 25°C. A blank emulsion was prepared with the same sucrose solution and 0.1% oil, but no emulsifier, and homogenized at 15,000 rpm for 30S. The absorbance of emulsion was determined by a transmission spectrometer (U-2010, HITACHI Instrument, Pleasanton, CA) at 650 nm against distilled water. By adapting the equation (T= 2.303AV/l), where A is the observed absorbance, V is the dilute factor and l is the pathlength of the cuvette, the absorbance was converted to turbidity (T) (Yadav et al. 2007a). The higher turbidity indicates the better emulsion stability (Yadav et al. 2007a).

Creaming test

Concentrated emulsion (10 ml) from the bottom of the emulsion was immediately transferred to a10 ml graduate cylinder after preparation. The cylinder was sealed and placed at 25°C. The volume of cream was recorded every day. The amount of cream and/or oil separation with time indicates the extent of emulsion breakdown or the degree of gravitational separation (McClements 1998).

Particle size distribution of emulsions

Particle size distribution of emulsions was measured using a laser scattering instrument (LA910, Horiba Inc., Irvine, CA). Both fresh emulsion and aged emulsion were measured. The emulsion was stirred before measurement to ensure the samples were homogeneous. Volumes of particles were calculated based on the assumption that all particles were spherical.

Statistical anaylsis

Macanova 4.12 (School of Statistics, University of Minnesota, Minneapolis, MN) was used to perform ANOVA and Tukey's honest significance difference (HSD) analysis. The level of significance was $P<0.05$ throughout the study.

RESULTS AND DISCUSSION

Characteristics of DDGS

The composition of DDGS was shown in **Table 1-1**. Hemicellulose was 23.88% as estimated by xylose, arabionse and galactose contents. In cereal crops, arabinoxylan is the major type of hemicellulose, which is composed of xylose, arabinose, galactose and small amount of glucose and glucuronic acid (Singh et al. 2000; Ebringerova et al. 2000; Doner et al. 2001b; BeMiller 2007; Yadav et al. 2009).

Cellulose was 20.36% (100% - percentages of protein, fat, ash, glycerol, starch and hemicellulose). Besides non-starch carbohydrate, there were five other main components: 28.60% protein, 11.09% fat, 7.71% starch, 4.12% ash and 4.24% glycerol (**Table 1-1**).

In order to characterize the water soluble fraction, DDGS was extracted by water at 25°C, and the water soluble fraction was recovered by centrifugation and freeze-drying. MW distribution of DDGS soluble was shown in **Fig. 1-2**. After washing at 25°C, two populations of carbohydrate were extracted out. One contained low MW carbohydrates (2~4 glucose units), and the other one was a group of larger carbohydrates with molecular weight from 10^3 to 10^6 g/mol. According to the HPAEC-PAD results of the free monosaccharide in DDGS soluble (not shown), there was only trace amount (≤0.01%) of glucose, arabinose, xylose and maltose. Yield, recovery and sugar composition of DDGS soluble were summarized in **Table 1-2**. By using acid hydrolysis adapted from the method described by Doner et al. (2001a), carbohydrates were degraded into single sugars. Hemicellulose estimated by the sum of arabinose, galactose and xylose was 10.52% of DDGS soluble, and 4.58% of hemicellulose in DDGS was recovered by washing. In addition, DDGS soluble fraction contained 23.56% glycerol which was a product of yeast fermentation (Kelsall et al. 2003).

Effect of the ratio of solid to water on the extraction

Five levels (5, 10, 15, 20 and 25%) of the solid content were applied in the alkaline heating extraction. S5, S10, S15, S20 and S25 represented the HFG extracted at 5, 10, 15, 20 and 25% solid contents, respectively (**Table 1-3**). Insoluble residues were also collected and analyzed. R5, R10, R15, R20 and R25 represented the insoluble residue after extracting HFG at 5, 10, 15, 20 and 25% solid contents, respectively. S15 had the highest yield of HFG and recovery of hemicellulose, and 45% of hemicellulose was extracted out. S25 had the lowest yield and recovery of

hemicellulose. However S25 contained the highest hemicellulose (28.96%) and generated most hemicellulose (1.89 g) from each batch. Considering the significant amount of water used in the extraction, increasing the solid content can reduce the cost of water and increase the efficiency. Therefore solid content of 25% was chosen for this study. From the composition of insoluble residues, the recovery of hemicellulose was 55~72%, which corresponded with the recovery of hemicellulose in HFG. Apparently 55~72% hemicellulose was not extracted out by the alkaline heating method. The ratio of xylose to arabinose (X/A) represents the branch degree of arabinoxylan (Andrewartha et al. 1979, Maes et al. 2002). The higher the ratio means the less arabinose attaching on the xylose backbone. Apparently, the arabinoxlyan existed in insoluble residue had higher ratio of X/A (1.84~2.16) comparing with those in HFG (1.48~1.67). In wheat flour and bran, the relationship of the solubility and X/A ratio of arabinoxylan were studied and established by Andrewartha et al (1979) and Maes et al (2002). As Maes et al (2002) reported, the X/A ratio was 2.63 in the cellulose rich residue which was obtained after the alkaline extraction of arabinoxylans from wheat bran. Conversely, in the alkaline extract fraction, the X/A ratio was 1.22. Similarly here, the higher X/A ratio in the insoluble residue indicated the existence of unextractable arabinoxylans.

Water heating extraction

The composition and yield of HFG and the hemicellulose recovery by water heating extraction were listed in **Table 1-4**. As heating temperature increased from 80 to 120°C, yield of gum and recovery of hemicellulose dramatically increased, especially in the presence of H_2O_2. Among three cooking temperatures, heating at 120°C rendered highest yield of HFG (30.53 or 18.59% with or without the addition of H_2O_2) (**Table 1-4**).

The effects of H_2O_2 on the extraction were demonstrated by comparing yield and hemicellulose recovery of HFG 1, 3, 5 to those of HFG 2, 4, 6. For HFG 2, HFG

4, and HFG 6, only 5~9% of the hemicellulose was extracted, and more protein was co-extracted out compared with HFG 1, 3, 5. More hemicellulose was extracted out when H_2O_2 was added. Especially for HFG 5, more than 30% of hemicellulose was extracted out with a higher yield of HFG. **Figure 1-3** showed the MW distribution of HFG5 and HFG11. Both of them appeared a sharp peak between 10^2 to 10^3 g/mol and a broad peak ranging from 10^3 to 10^6 g/mol. The soluble materials of DDGS also contained significant amount of small molecules (10^2~10^3 g/mol) (**Fig. 1-2**). After either H_2O_2 solution or alkaline solution extraction at 120°C, small molecules were isolated out from DDGS and became part of hemicellulose fiber gum. HFG5 extracted by H_2O_2 solution at 120°C did not consist of significant amount of polysaccharide with molecular weight from 10^4 to 10^6 g/mol but still had certain amount of smaller polysaccharide with molecular weight between 10^3 and 10^4 g/mol.

Except HFG5, water extraction only recovered 5~9% of hemicellulose and along with significant amount of impurities (protein, ash and starch) (**Table 1-5**). This result was consistent with the earlier reports which indicated that water failed to extract the hemicellulose from corn bran, due to that the cell wall matrix of protein, cellulose and lignin was not effectively decomposed (Doner et al. 1998; Hromadkova et al. 2008). Surprisingly when using hydrogen peroxide as an oxidizing reagent at high temperature (120°C), the recovery of hemicellulose dramatically increased to 32%. However **Fig. 1-3** showed that the peak between 10^5 and 10^6 g/mol disappeared for HFG5 compared with HFG11. It is possible that the combination of H_2O_2 and high temperature can effectively break the cell wall matrix and release hemicellulose, but at same time, partially degrade hemicellulose.

Alkali heating extraction

Alkali alone was used to help the release of hemicellulose from the cell wall matrix. **Table 1-4** summerized composition, yield of HFG and recovery of hemicellulose. Regardless the cooking temperature, HFG (7~12) extracted by alkali

had 24~32% of hemicellulose recovery compared to HFG (2, 4, 6) extracted only by water with 5~9% hemicellulose recovery. Ash content dramatically increased from 13~23% to 25~29% after adding alkali during cooking. The protein content of HFG extracted by alkali was slightly lower than those of HFG extracted by water. Starch content significantly decreased for alkali extracted HFG compared with water extracted HFG.

The same amount (2 meq/gDDGS) of NaOH or $Ca(OH)_2$ gave different initial pH values. NaOH gave the initial pH 12.11 to 12.26, and $Ca(OH)_2$ rendered the initial pH varied from 11.74 to 11.85. The type of alkali did not induce the significant difference in the yield of HFG and recovery of hemicellulose among samples of HFG7~HFG12. However, at 80°C, NaOH generated a higher hemicellulose content of HFG (30.71 vs. 26.19%) compared with $Ca(OH)_2$. These results did not totally agree with the previous values published by Doner et al (1998). They reported that under the same extracting procedure, NaOH resulted in a higher yield (40 vs. 21%) of corn fiber gum (CFG) extracted from the destarched corn bran than did $Ca(OH)_2$. In the present study, extracting with NaOH induced the higher protein content but the lower starch content as compared to the extraction with $Ca(OH)_2$.

Temperature influenced the alkaline extraction in the same way for the water extraction. At elevated cooking temperatures, the content and recovery of hemicellulose of HFG (7~12) increased for both NaOH and $Ca(OH)_2$ extraction. At 120 °C, 32% of hemicellulose (HFG11&12) was extracted out, but at 80°C, only 24% of hemicellulose was obtained after extraction. Increasing the heating temperature did not significantly influence the protein and starch content, but the ash content reduced from 29 to 25%.

Alkali and hydrogen peroxide extraction

Table 1-4 shows the composition and yield of HFG13~15 and the recovery of hemicellulose. HFG13, which was extracted by 2 meq/gDDGS of NaOH, had the highest hemicellulose content and the recovery of hemicellulose compared to HFG14 and 15. Reducing the concentration of NaOH from 2 meq/gDDGS to 1 meq/gDDGS

made the initial pH dropped from 12.01 to 9.21. Meanwhile the hemicellulose content significantly decreased from 24.70 to 12.43%, along with the decrease of gum yield and hemicellulose recovery. In a study by Hespell (1998), it was shown that the recovery of corn fiber xylans (hemicellulose from corn bran) decreased from 18.5% to 13.3% when reducing the KOH concentration from 2% to 0.56%. Hromadkova et al. (2008) used a series of NaOH concentration to extract phenolics-rich heteroxylans (hemicellulose) from wheat bran. The yield of hemicellulose was 8.5, 18.1 and 19.8%, when 0.5, 2 and 5% NaOH were used respectively. High pH or alkaline concentration favors the yield of HFG and the recovery of hemicellulose, no matter if H_2O_2 is used.

Comparison of three extraction methods

Alkali heating (HFG7~12) was the most efficient approach for the HFG extraction based on the hemicellulose content of HFG and the recovery of hemicellulose (**Table 1-4**). The alkali hydrogen peroxide method provided the slightly higher yield of HFG, however the recovery of hemicellulose was not as high as those by alkaline heating method. One possible reason is that certain amount of H_2O_2 was decomposed into H_2O and O_2 in the presence of alkali at high temperature. Only small amount of H_2O_2 contributed to the oxidizing action compared with the water heating or alkaline heating. In the other two methods, H_2O_2 was added to the slurry after cooking at 25°C. However it was believed that the oxidizing reagent (H_2O_2) will help to cleave the arabinoxylan diferulic linkage and cellulose-arabinoxylan bonds, and release more hemicellulose (Doner et al. 2001a; Gaspar et al. 2005). In the study of extracting corn fiber gum (CFG) from destarched corn bran (Doner et al. 2001a), after adding 10% H_2O_2 (based on destarched corn bran weight), CFG increased from 28 to 37%. In the study by Gaspar et al. (2005), there was a slight increase (2%) of the hemicellulose yield after adding 10% H_2O_2. The relation between CFG yield and the ratio of H_2O_2 to corn bran was determined by Doner et al. (2001a). When the ratio ranged from 0 to 0.2, the increase of the ratio led to the increase of yield, but between

0.2 and 1.0, the greater ratio did not increase the yield but slightly decreased it. The starting material in this study was DDGS containing protein, starch, lipid besides hemicellulose, but previous studies used destarched corn bran which was composed of little starch protein and lipids. The differences in the starting materials may be the reason for the different impact of H_2O_2 on the alkaline extraction of HFG.

The sugar compositions and hemicellulose contents of HFG, DDGS, destarched corn bran and corn fiber gum (CFG) are listed in **Table 1-5**. Apparently sugar profiles of different HFGs were similar regardless of the extraction methods applied, indicating that DDGS contained a highly homogeneous hemicellulose. Corn fiber gum (CFG) was extracted from destarched corn bran by sodium hydroxide cooking (100°C), same procedure as the alkaline extraction. Destarched corn bran consisted of 56% hemicellulose, and CFG contained 39% hemicellulose which was higher than the hemicellulose content of HFG9~12 (**Table 1-5**). However the yield of CFG was 31%, and the recovery of hemicellulose was 21% which was lower than those of HFG9 & 10. It showed that by 100°C alkaline extraction, hemicellulose was easier to extract out from DDGS than from destarched corn bran (**Table 1-5**).

Water extracted HFG consisted of low level of hemicellulose (9~13%), except HFG5 which was extracted by water and H_2O_2 at 120°C. HFG5 contained 25% hemicellulose. In contrast, alkali extracted HFG had a high hemicellulose content (26~34%), especially for those extracted at 100 and 120°C. As reported by Ebringerova et al. (1994) and Maes et al. (2002), the solubility of arabinoxylan was partially related to the arabinose substitution degree or xylose to arabionse ratio (X/A). **Table 1-5** showed the X/A value of HFG, DDGS, destarched corn bran and CFG. HFG1 & 2 had the significant lower X/A ratio and higher arabinose substitution degree, indicating that under the mild extraction (water extracting at 80°C), only the highly substituted arabinoxylan was extracted. At more severe extraction conditions (HFG7~14), the less substituted arabinoxylan was extracted.

MW distributions of HFG, CFG and gum arabic are shown in **Fig. 1-4**. Both gum arabic and CFG had only one peak between 10^4 and 10^6 g/mol; HFG had two peaks, one located between 100-1000 g/mol, and another broad peak from 10^3 to 10^6

g/mol. Gum arabic and CFG were composed of significant amount of polymers which are considered to contribute to the emulsion stability. However, HFG only consisted of lower level of polymers, but large portion of di-, trisaccharides.

Extractability of HFG

In the current study, by alkaline extraction at 120°C, 32% of hemicellulose was extracted out from DDGS (**Table 1-4**). Regarding to the extractability of hemicellulose (arabinoxylan) from corn bran or wheat bran fiber, a few studies were reported with various hemicellulose yields. Doner et al. (2001a) applied NaOH and hydrogen peroxide at 100°C and obtained 37% corn fiber gum (hemicellulose as the major component) from corn bran. With the similar approach, Yadav et al. (2007a) reported that 26% of corn fiber gum can be extracted out from dry milling corn bran. Hespell (1998) used the sequential potassium hydroxide extraction and achieved 27% of arabinoxylan recovery from corn bran. Millan et al. (2007) extracted the arabinoxylan from corn bran by mild alkali (0.5M NaOH) at 25°C for 24 hours, and obtained the arabinoxylan yield of 66.0%. The highest yield of hemicellulose from destarched corn bran was documented by Gaspar et al. (2007). After cooking at 120°C in the 2% alkaline solution under 2 bar pressure, 80% of hemicellulose was extracted out from the destarched corn bran (Gaspar et al. 2007).

The question is raised as to whether the cell wall structure limits the extractability of hemicellulose. Ferulic acid was postulated to form the ester cross-link between the arabinoxylan chains (Fincher et al. 1986; Saulnier et al. 1995a; Saha 2003). Saulnier et al. (1995b) suggested that both the diferulic and protein-arabinoxylan linkages cause the insolubility of the corn bran arabinoxylans.

Table 1-6 listed the sugar composition, the yield and the hemicellulose recovery of hemicellulose fiber gum extracted from DDGS insoluble residues (S1~S4). S1, as the blank control, contained highest hemicellulose (22%), but had lowest yield (10%) and lowest hemicellulose recovery (7%). Compared with the

sample extracted by alkali (S3), the higher hemicellulose content was obtained by protease extraction (S2). However, the hemicellulose recovery of gum S3 was two times higher than that of gum S2. Alkali and protease-alkali treatments (S3 vs. S4) did not appear to have significantly different effects on either gum yield or hemicellulose recovery. Apparently, alkali was more powerful to release hemicellulose from the cell wall matrix than protease. There was no significant difference between S1 and S2. If the extractability of hemicellulose was partially attributed to protein-arabinoxylan linkages (Saulnier et al. 1995b), more hemicellulose should release with the addition of protease. From our results, the protein-arabinoxylan linkage was not the main reason for the unextractable hemicellulose. So the ester covalent bond which could be partially de-esterified by alkaline between carbohydrates limits the extractability of hemicellulose from corn bran.

HFG purification

As shown in **Table 1-4**, each HFG had significant amounts of impurities including protein, ash and starch. From the MW distribution, only limited polysaccharides existed in HFG. Ethanol precipitation was introduced to isolate large MW polysaccharides (Skuratowicz 2006; Yadav et al. 2007a). As shown in **Table 1-7**, after ethanol precipitation, the purified gum contained 61.45% of hemicellulose, 21.08% of ash, 9.86% of glucose and 8.88% of protein. Apparently, protease and α-amylase hydrolysis helped to remove proteins and starch from HFG and increased the hemicellulose content. The low MW peak disappeared and the high molecular peak was increased (**Fig. 1-4**). The MW distribution of purified HFG was close to that of gum arabic and CFG. Polysaccharide of purified HFG had MW from 10^4 to 10^6 g/mol.

Emulsion stability

Stability of the emulsions prepared by HFG and gum arabic was evaluated by monitoring turbidity of diluted emulsions and conducting creaming test on

concentrated emulsions as described previously. Within 7 days, all emulsions showed varying degrees of instability. The higher turbidity was an indication of better emulsion stability. **Fig. 1-5** showed the turbidity of diluted emulsions right after preparation (zero day), 1 day and 7 days.

Right after preparation (**Fig. 1-5A**), emulsions stabilized by alkali extracted HFG 7~12 had higher turbidity than emulsions prepared by water extracted HFG 1~6. The alkaline hydrogen peroxide extracted HFG 13~15 seemed to have the similar emulsifying ability to HFG 7~12. Purified HFG also delivered a good emulsion with a high turbidity. Among all HFGs including the purified HFG, HFG11 provided the most homogeneous and stable emulsion right after preparation. HFG4 extracted by water without alkali or H_2O_2 failed to show any stabilizing and emulsifying capability, since the emulsion presented the same low turbidity as to the blank containing no emulsifiers. However, HFG1, 3, 5 and 6 delivered better emulsions with the turbidity two times higher than the blank.

During the storage, flocculation and coalescence occurred in each emulsion at various degrees. Emulsions prepared with gum arabic and purified HFG showed the best stability with turbidity dropping from 494-462 to 99-82. Despite the high turbidity (483) for fresh emulsion, HFG11 did not effectively maintain a stabilized emulsion over storage with turbidity dropping to 43. The emulsion prepared with HFG13 extracted by 2% NaOH and 2.5% H_2O_2 had the same turbidity with HFG11 emulsion. Surprisingly, neither HFG14 extracted by 2% $Ca(OH)_2$ and 2.5% H_2O_2 nor HFG15 extracted by 1% NaOH and 2.5% H_2O_2 gave the higher turbidity than other HFGs extracted by water or alkali alone. HFGs obtained by water extraction (**Fig. 1-5C**) appear to have the lowest turbidity, especially for HFG4 and 6 extracted only by water.

The various emulsifying ability of HFGs is attributed to the gum composition. From the composition (**Table 1-4**), it was obvious that HFG1~6 contained low hemicellulose but high protein and starch. Hemicellulose was considered as the component contributing to the emulsifying ability of HFG (Yadav et al. 2007a & 2007b). Therefore the lower hemicellulose content resulted in the inferior emulsifying

capacity. In contrast, HFG (7~15) and purified HFG containing more hemicellulose delivered better stabilized emulsions. HFG7, 9 and 11 functioned more effectively as emulsifiers than did HFG8, 10 and 12. HFG13~15 extracted by the alkaline hydrogen peroxide method contain significant less hemicellulose compared to HFG7~12 extracted by alkaline method. Therefore, emulsions prepared by HFG13~15 were less stable than those prepared by HFG7~12.

Table 1-4 showed that HFG7, 9 and 11 contained slightly more protein and hemicellulose, but less starch. It was proposed that certain protein, peptide or amino acid attached on the hemicellulose by either covalent or ionic bond (Yadav et al. 2009). As to gum arabic, it is widely known that the polypeptide chain attaching to the carbohydrate adsorbs preferentially onto the surface of the oil droplets and carbohydrate blocks dissolve in water to inhibit flocculation and coalescence through electrostatic and steric repulsions (Akiyama et al. 1984, Garti et al. 2001). Based on the protein content of HFG listed in **Table 1-4**, it was possible that HFG extracted by NaOH had more protein bonded on the hemicellulose than HFG extracted by $Ca(OH)_2$. Therefore, even though there were similar amounts of hemicellulose in HFG7 vs. HFG8, HFG9 vs. HFG10 and HFG11 vs. HFG12 (**Table 1-4**), HFG7, 9 and 11 can stabilize the emulsion more effectively than HFG8, 10 and 12 (**Fig. 1-5**).

HFG can be grouped into two categories based on the creaming test (**Fig. 1-6**). For HFG1~6 extracted by water, after one day storage, the volume of cream reached to the highest value and did not continuously increase over six days, except that there was gradual increase at the first three days for HFG5. HFG4 had an extremely low cream volume, but according to the observation of the emulsion right after preparation, only partial orange oil was integrated into the oil-in- water emulsion, and the rest oil was immediately coalesced and floated above the emulsion. Besides HFG4 emulsion, HFG5 had the lowest cream volume and HFG6 had the highest (**Fig. 1-6A**). The trend in **Fig. 1-6A** indicated that water extracted HFG could only stabilize the emulsion over short period (one day), and HFG contained higher amount of hemicellulose (HFG5) was able to stabilize the emulsion and postpone the cream occurring over a longer period (3 days).

The second category was alkali extracted HFGs (**Fig. 1-6B** and **Fig. 1-6C**). Alkaline extracted HFGs seemed to stabilize emulsions more effectively and delay the cream occurring during storage. Even though, at second day, all emulsions including gum arabic and purified HFG had a cream layer, the volume of the cream was gradually increased rather than reaching the highest at second day. According to the creaming test (**Fig. 1-6B**), purified gum was the most efficient emulsifier and even better than gum arabic, reflected by the lowest cream volume in aged emulsion.

Gum arabic and purified HFG had higher molecular weight ranging from 10^4 to 10^6 g/mol (**Fig. 1-4**), and conversely HFG showed a bimodal distribution including the low molecular weight (10^2~10^3 g/mol) fraction and the higher molecular weight (10^4~10^6 g/mol) fraction. The correlation between emulsion stability and average molecular weight was reported by Dickinson et al. (1995) in gum arabic and Yadav et al. (2009) in corn fiber gum. It was suggested that the gum with high molecular weight and high protein content tends to be superior emulsifiers compared to the gums with lower MW and low protein content.

Particle size distribution of emulsions

The particle size distribution of fresh emulsions stabilized by HFG or gum arabic had one peak ranging from 1 to 10 μm (**Fig. 1-7**). On the other hand, the emulsion prepared with purified gum showed a bimodal particle size distribution (**Fig. 1-7B**). Besides the bigger particles (1~10 μm), there was another peak indicating a group of smaller particles (0.1~1 μm) in purified HFG emulsion.

For the aged emulsions, particle size increased (**Fig. 1-7**). For HFG (**Fig. 1-7A)**, it was illustrated that bigger particles (10~100 μm) occurred and the frequency% of small particles (1~10 μm) was decreased after storage. However emulsions stabilized by purified HFG or gum arabic did not have this dramatic change within the short period storage (**Fig. 1-7B&C**). However, the small particle (0.1~1 μm) peak disappeared in the aged purified HFG emulsion (**Fig. 1-7B**).

The changes of particle size distribution indicated that during storage, due to aggregation, flocculation and coalescence, large particles formed (McClements,

1998). Because of gravitational force and lighter density, large oil particles gradually move upward, form cream layer and induce breakage of emulsions. The occurrence of large particles indicates unstable emulsion. In aged emulsions, the presence of less large particles indicates more stable emulsions. In conclusion, both purified HFG and gum arabic have a superior emulsifying ability over unpurified HFG, which agrees with the turbidity and creaming test results.

CONCLUSIONS

High temperature and alkaline could increase the yield of HFG. Water extraction did not effectively generate a high yield of hemicellulose, unless the hydrogen peroxide was used at 120 °C. Alkaline extraction could deliver a high yield of HFG and hemicellulose. HFG was a mixture containing ash, starch, protein and hemicellulose. HFG showed a promising ability to stabilize both the concentrated and diluted emulsions. The purified HFG possessed a great ability as emulsifier when compared with gum arabic.

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Table 1-1 Composition[a] of distillers dry grain with solubles (DDGS) (weight %)

(N=2)

Protein	Fat	Ash	Glycerol	Starch	Hemicellulose	Cellulose[b]
28.6±1.65	11.09±0.04	4.12±0.00	4.24±0.09	7.71±0.53	23.88±1.51	20.36

[a]Based on DDGS dry weight.

[b]Cellulose was calculated as 100% - the sum of other components.

Table 1-2 The sugar composition[a] and yield[b] of soluble materials in distillers dry grain with solubles (DDGS) (weight %) (N=2)

Arabinose	Galactose	Xylose	Glucose	Hemi cellulose[c]	Yield	Recovery[d] of hemicellulose
3.36±0.12	1.60±0.14	5.56±0.22	19.94±2.55	10.52	10.40±0.43	4.58

[a]Based on the dry weight of solubles from DDGS
[b]Based on the DDGS dry weight
[c]Hemicellulose was calculated as 100% - the sum of arabinose, galactose and xylose.
[d]Recovery was calculated as the weight percentage of hemicellulose in the DDGS soluble based on the hemicellulose originally existing in DDGS.

Table 1-3 The yield, recovery of hemicellulose and sugar composition of hemicellulose fiber gum (HFG) and insoluble residues obtained from different initial solid contents after alkaline extraction (weight %) (N=2)*

	Arabinose (A) %	Galactose %	Xylose(X) %	Hemicellulose %	X/A**	Yield %	Recovery of hemicellulose %
Extracted HFG							
S5	8.06bc±0.61	2.16b±0.16	11.92c±0.82	22.14d	1.48a	37.76de	35.01f
S10	8.33abc±0.09	2.39ab±0.18	13.50bc±0.25	24.22bcd	1.62ab	35.97de	36.48ef
S15	9.53ab±0.30	2.86ab±0.16	15.89ab±0.56	28.28abc	1.67ab	38.30d	45.36de
S20	8.85abc±0.28	2.45ab±0.13	13.35bc±0.29	24.65bcd	1.51c	31.63ef	32.65ef
S25	10.42a±0.17	2.86ab±0.09	15.68ab±0.69	28.96ab	1.50c	26.08f	31.62f
Insoluble Residues							
R5	7.93bc±0.60	3.28b±0.01	16.09ab±0.11	27.30abcd	2.03ab	63.54a	72.64a
R10	7.36bc±0.10	2.60ab±0.24	13.54bc±0.85	23.50cd	1.84ab	60.59ab	59.63bc
R15	7.18c±0.81	2.86ab±0.26	15.54ab±0.02	25.58bcd	2.16a	56.14bc	60.14abc
R20	7.09c±0.13	2.49ab±0.36	13.08bc±1.05	22.66d	1.84ab	58.22abc	55.25cd
R25	9.15abc±0.11	3.38a±0.10	18.74a±0.47	31.27a	2.05ab	53.49c	70.04ab

*Mean values followed by the same letter are not significantly different at $P < 0.05$.

** X/A represents the ratio of xylose to arabinose

Table 1-4 Effect of extracting conditions on the yield and composition of hemicellulose fiber gum (HFG) and the recovery of hemicellulose (weight %) (N=2)*

ID	Temp. (°C)	Heating with H_2O_2 (%w/wDDGS)	Alkaline meq/gDDGS	Protein	Fat	Ash	Starch	Hemi-Cellulose	Yield	Recovery of hemicellulose
1	80	10	0	15.16±0.01	0.22±0.01	21.72±0.01	6.56±0.01	9.77e	14.92±0.69	6.10e
2	80	0	0	11.23±0.03	0.15±0.01	23.49±0.06	6.62±0.01	9.51e	11.67±0.43	4.64e
3	100	10	0	20.53±0.06	0.20±0.01	17.94±0.04	11.12±0.04	13.18e	16.45±0.38	9.07de
4	100	0	0	11.83±0.01	0.18±0.02	21.42±0.15	7.32±0.23	9.77e	16.56±0.40	6.77de
5	120	10	0	26.73±0.04	0.19±0.01	13.83±0.05	11.82±0.19	25.39cd	30.53±2.42	32.43a
6	120	0	0	14.26±0.00	0.19±0.01	20.34±0.03	7.36±0.56	9.42e	18.59±2.34	7.33de
7	80	0	2NaOH	12.81±0.00	0.18±0.02	29.27±0.23	3.17±0.00	30.71ab	18.59±1.90	24.04bc
8	80	0	$2Ca(OH)_2$	12.03±0.02	0.25±0.03	29.42±0.00	4.41±0.27	26.19bc	21.97±1.28	24.08bc
9	100	0	2NaOH	13.73±0.02	0.18±0.00	27.79±0.04	3.03±0.06	31.74a	21.07±1.91	27.99ab
10	100	0	$2Ca(OH)_2$	12.09±0.01	0.19±0.01	27.45±0.04	3.86±0.27	32.46a	22.09±1.99	30.01a
11	120	0	2NaOH	16.63±0.03	0.13±0.01	25.73±0.23	2.86±0.05	33.76a	21.94±1.38	31.02a
12	120	0	$2Ca(OH)_2$	13.14±0.00	0.14±0.00	27.23±0.15	3.77±0.39	32.73a	23.82±1.67	32.64a
13	100	2.5	2NaOH	15.48±0.04	0.01±0.01	27.95±0.13	5.22±0.38	24.70cd	24.15±1.15	24.98bc
14	100	2.5	$2Ca(OH)_2$	15.06±0.01	0.02±0.01	23.41±0.13	6.73±0.14	20.19d	26.85±0.51	22.70c
15	100	2.5	1NaOH	15.32±0.03	0.01±0.01	30.35±0.00	7.65±0.55	12.43e	21.37±1.21	11.26d

*Mean values followed by the same letter are not significantly different at $P < 0.05$.

Table 1-5 The sugar composition and hemicellulose content of hemicellulose fiber gum (HFG), distillers dry grain with soluble (DDGS), destarched corn fiber and corn fiber gum (CFG) (weight %) (N=2)*

	Arabinose %	Galactose %	Xylose %	Hemicellulose %	X/A ratio
1	4.24 ±0.62	0.35 ±0.17	5.17 ±0.72	9.76h	1.22b
2	3.92±0.31	0.78± 0.06	4.81 ±0.44	9.51h	1.23b
3	5.45 ±0.45	0.63± 0.08	7.10 ±0.53	13.18h	1.30ab
4	4.00 ±0.30	0.81 ±0.02	4.96 ±0.50	9.77h	1.24ab
5	9.93 ±1.09	1.28 ±0.09	14.17 ±0.18	25.38efg	1.43ab
6	3.43 ±0.37	1.14 ±0.31	4.85 ±0.63	9.42h	1.41ab
7	11.99 ±0.12	1.15 ±0.07	17.75 ±1.02	30.89cde	1.48ab
8	10.82 ±0.35	0.97 ±0.04	14.40 ±0.23	26.19def	1.33ab
9	12.98 ±0.34	1.09 ±0.19	17.67 ±1.01	31.74cd	1.36ab
10	13.38 ±0.20	1.28 ±0.07	17.79 ±0.07	32.45c	1.33ab
11	13.90 ±0.34	1.36 ±0.12	18.51 ±0.14	33.77bc	1.33ab
12	13.54 ±0.44	1.48 ±0.35	17.71 ±1.05	32.73c	1.31ab
13	9.03±0.70	2.59±0.00	13.07±0.12	24.69fg	1.45ab
14	7.54±0.29	2.57±0.07	10.08±0.30	20.19g	1.34ab
15	4.85±0.01	1.66±0.57	5.93±0.35	12.44h	1.22b
DDGS	9.07±0.59	0.85±0.10	13.96±0.82	23.88fg	1.54ab
Destarched corn bran**	20.63±0.11	4.70±0.17	31.28±0.91	56.61a	1.51ab
CFG***	13.70±0.57	3.52±0.56	21.76±0.98	38.98b	1.59a

*Mean values followed by the same letter are not significantly different at $P < 0.05$.

**Corn bran was destarched according to a method by Doner et al. (2000).

***CFG was the corn fiber gum from bran.

Table 1-6 The sugar composition and yield of hemicellulose fiber gums (S1, S2, S3 and S4) extracted by the protease, NaOH or protease combined with NaOH solutioins (weight %)(N=2)*

Sample	Arabionse (A)%	Galactose %	Xylose (X)%	Hemicellulose %	Yield** %	Recovery*** of hemicellulose %	X/A
S1	8.68±0.33	1.89±0.00	11.66±0.04	22.23a	10.59b	7.53b	1.34
S2	5.36±0.04	1.09±0.13	10.27±0.01	16.72b	15.55b	8.31b	1.91
S3	5.42±0.14	1.19±0.07	7.98±0.20	14.59c	34.97a	16.32a	1.47
S4	5.76±0.12	1.15±0.02	8.68±0.23	15.59c	39.93a	19.91a	1.50

* Mean values followed by the same letter are not significantly different at $P < 0.05$.

**Based on the dry weight of residue

***The recoveries of hemicellulose of S1~S4 are based on the hemicellulose content of residue.

Table 1-7 Composition* of purified hemicellulose fiber gum (HFG) (weight %) (N=2)

Protein	Ash	Glucose	Hemicellulose
8.66±0.08	21.08±0.22	9.86±0.40	61.45±1.11

*Based on dry weight of purified HFG

Figure 1-1 Scheme of sequential extraction of hemicellulose fiber gum (HFG) from distillers dry grain with solubles (DDGS) and DDGS insoluble residue

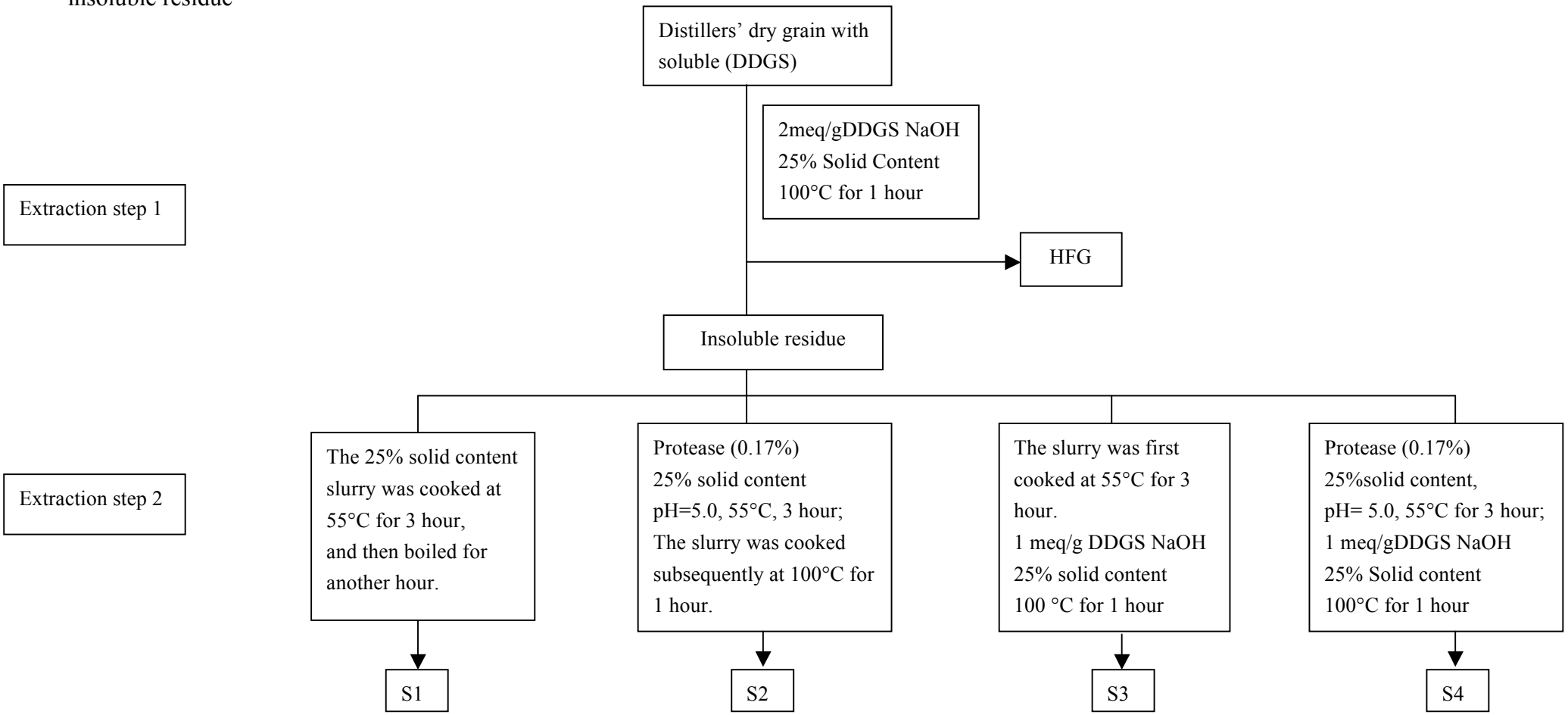


Figure 1-2 Molecular weight distribution of the water soluble fraction of distillers dry grain with solubles (DDGS) as determined by gel permeation chromatograph

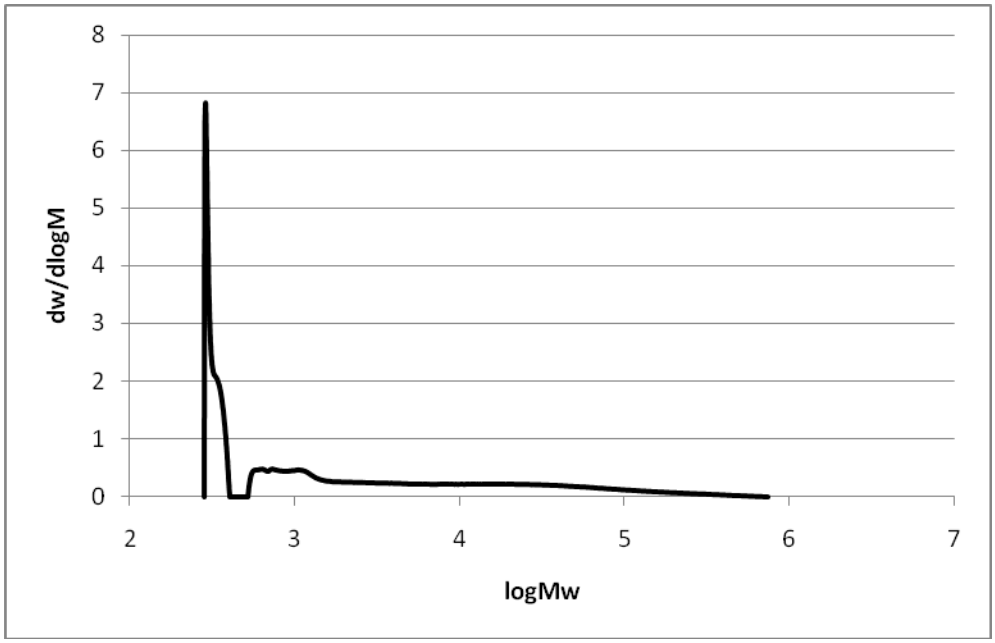


Figure 1-3 Molecular weight distribution of hemicellulose fiber gum (HFG5) extracted by water and H_2O_2 solution at 120°C and hemicellulose fiber gum (HFG11) extracted by sodium hydroxide solution at 120°C as determined by gel permeation chromatograph. HFG5 (solid line), HFG11(dashed line)

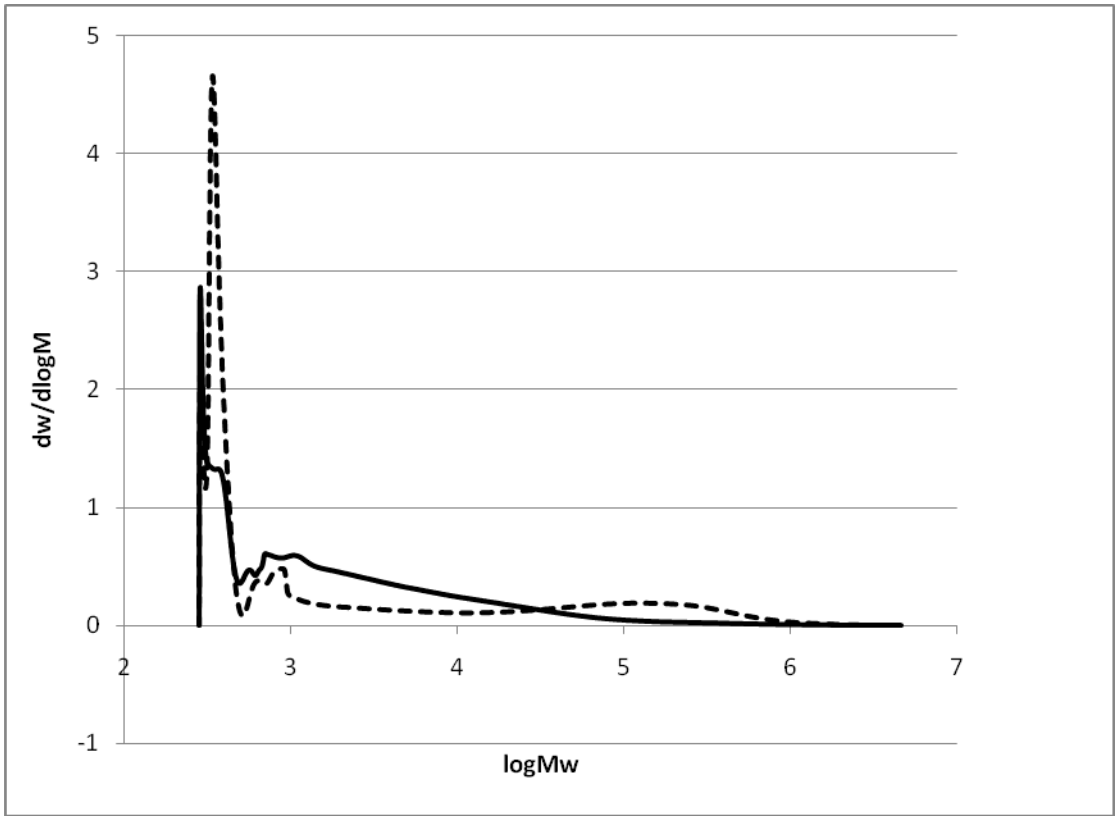


Figure 1-4 Molecular weight distribution of hemicellulose fiber gum (HFG) extracted by 2% NaOH solution at 100℃, purified HFG by ethanol precipitation, commercial gum arabic and corn fiber gum (CFG) extracted from destarched corn bran by 2% NaOH solution at 100℃. HFG (solid line), purified HFG (dashed line), CFG (dotted line), gum arabic (dashed and dotted line)

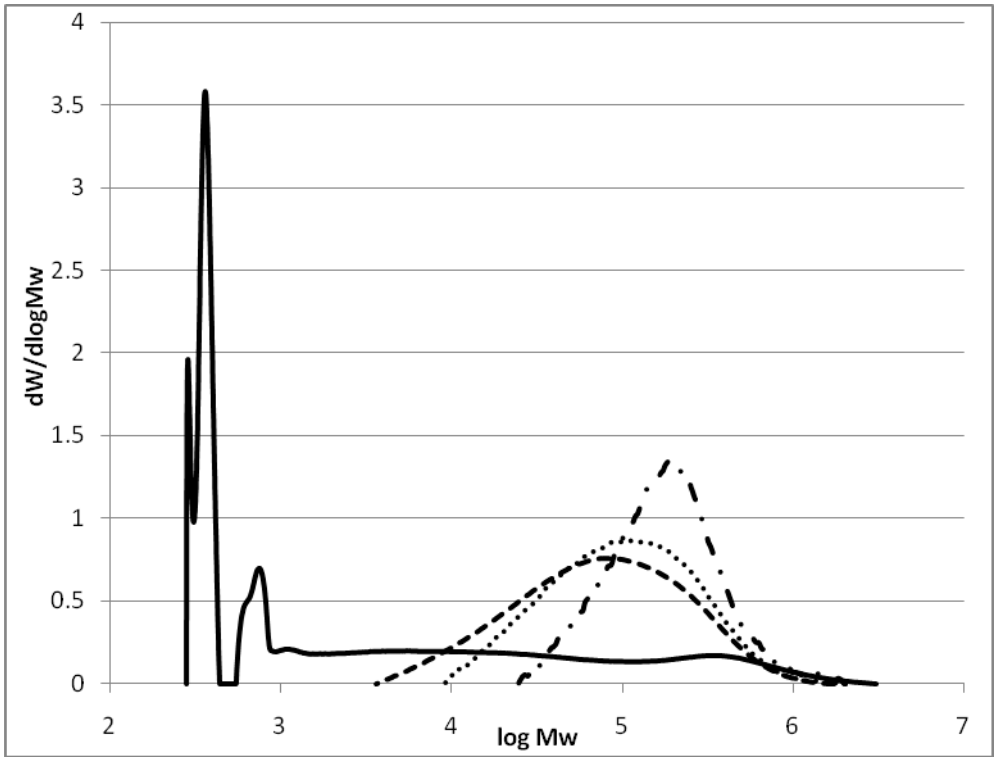
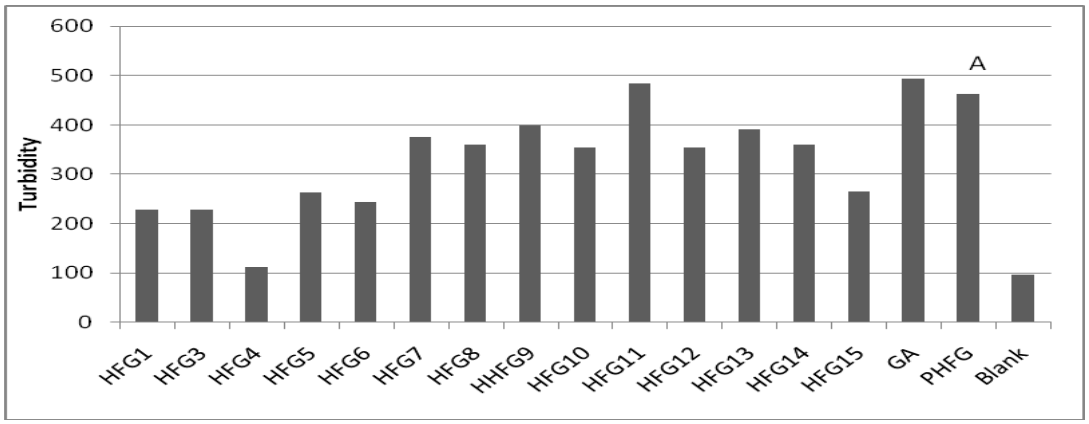
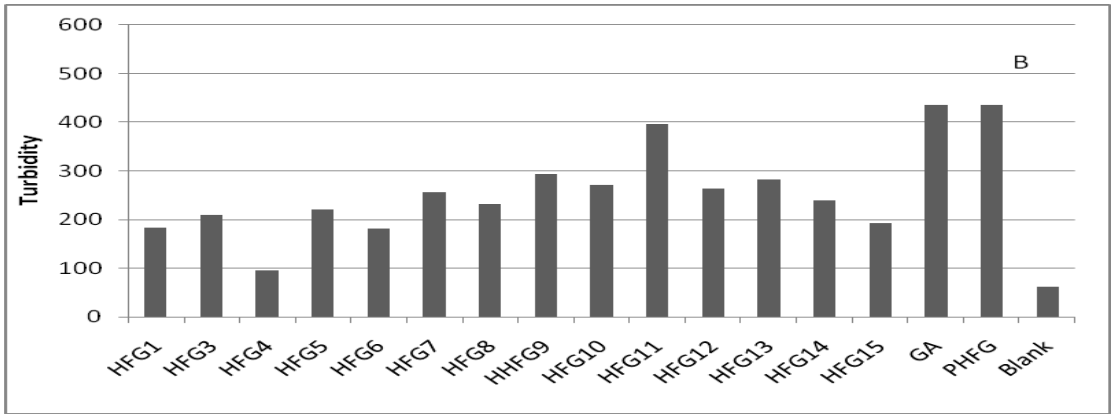


Figure1- 5 Turbidity of diluted oil-in-water emulsions (oil: gum: water = 0.01: 0.005: 1) at 25°C right after preparation (A), after 1 day storage (B) and after 7 days storage (C) PHFG = purified HFG, GA = gum arabic, and Blank only contains equivalent amount of orange oil but no gum.





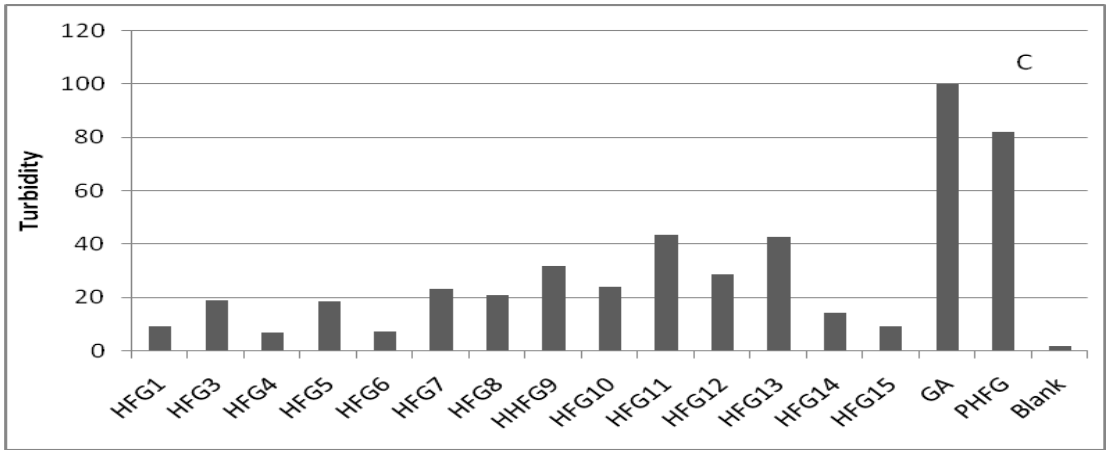
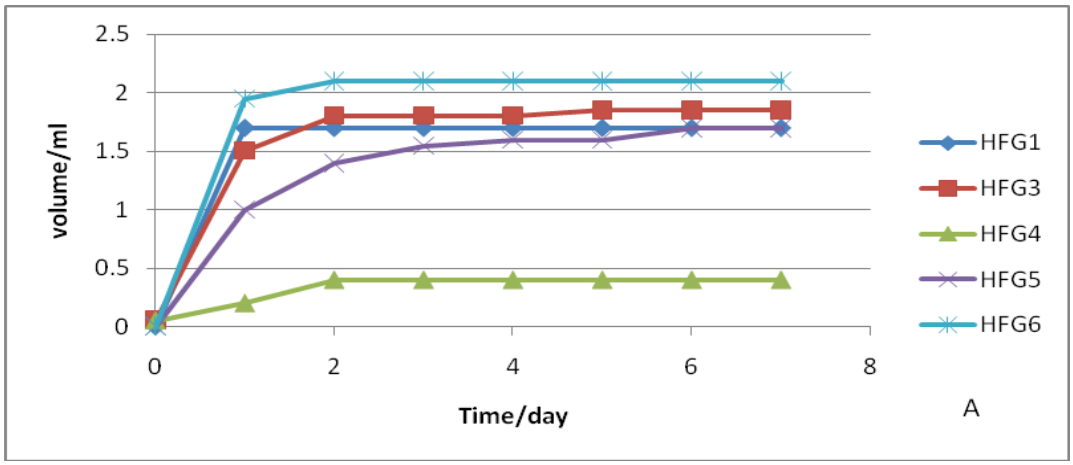
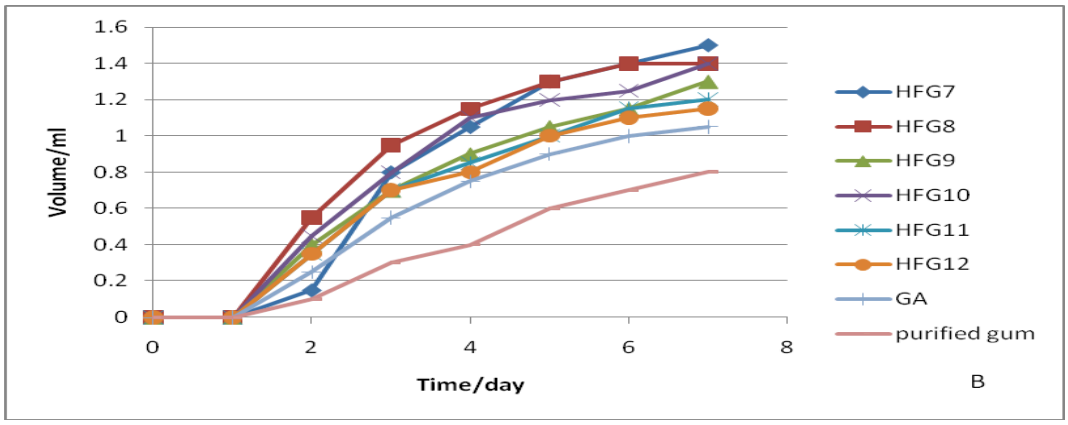


Figure1- 6 Volume of cream (ml) of oil-in-water concentrated emulsions (oil: gum: water = 0.1: 0.05: 8.5) at 25°C within 7 days. (A) hemicellulose fiber gum (HFG) extracted by water heating; (B) HFG extracted by alkaline heating; (C) HFG extracted by alkaline hydrogen peroxide heating (The detailed information of HFGs is listed in Table 1- 4.





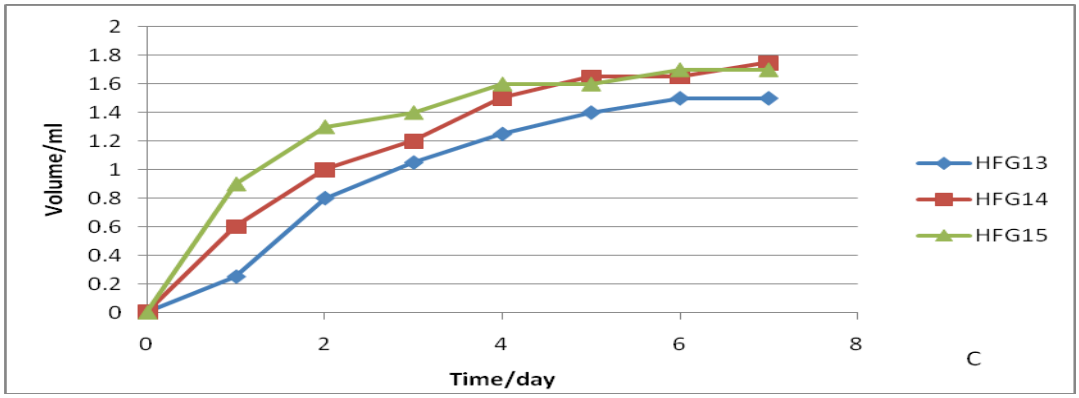
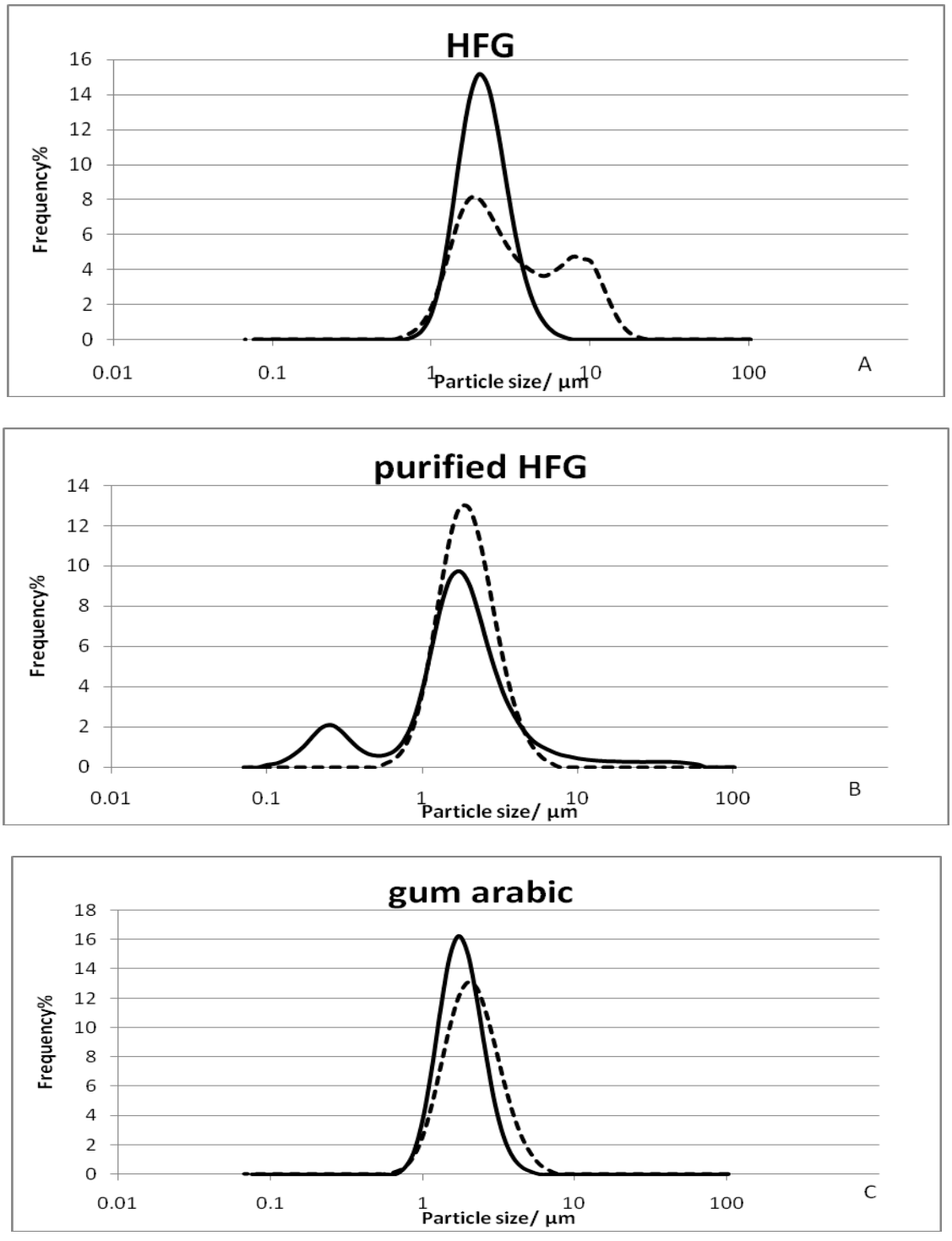


Figure1- 7 Particle size distributions of fresh and aged emulsions stabilized by hemicellulose fiber gum (A) extracted by 2% NaOH solution at 100°C, purified HFG (B) and commercial gum arabic (C) as determined by laser scattering particle size distribution analyzer. Fresh emulsion (solid line), aged emulsion (dashed line).



CHAPTER 2 - HEMICELLULOSE FIBER GUM FROM WHEAT, SORGHUM AND CORN DISTILLERS GRAINS: ISOLATION AND STRUCTURAL CHARACTERIZATION

ABSTRACT AND KEYWORDS

Abstract: Lab-made corn, sorghum and wheat DDGS were utilized to produce hemicellulose fiber gum (HFG). The composition of three DDGS varied in protein, fat and non-starch carbohydrate contents. Sorghum and wheat DDGS contained higher levels of protein and lower levels of fat and non-starch carbohydrate than corn DDGS. HFG was extracted by 2% NaOH solution at 100°C for one hour and purified by ethanol. The yield of HFG from corn, sorghum and wheat DDGS was 21.08, 11.07, 11.64%, respectively, while the hemicellulose recovery was 30.95, 29.74, 22.71%, respectively. The water extractable hemicelluloses from all three DDGS had similar ratios of xylose to arabinose.

Keywords: Distillers dry grain with solubles, Hemicellulose fiber gum, Extraction, Hemicellulose

INTRODUCTION

Growing demand in energy has led to a rapid expansion of the ethanol industry in U.S. Currently corn primarily accounts for 95% feedstock for the ethanol production (Wang et al. 2008). However, there is ongoing research to explore and investigate the possibilities and potentials of other grains including sorghum and wheat for ethanol production (Wu et al. 2006; Taylor et al. 2006; Wang et al. 2007; Wang et al. 2008; Zhao et al. 2009). Studies (Wang et al. 2008, Zhao et al. 2009) show that sorghum and certain wheat genotypes (waxy wheat) are comparable to corn with respect to ethanol yield.

Expansion of the ethanol production from cereal grains has led to a large amount of dried distillers grain with soluble (DDGS). There are roughly 180 completed ethanol production facilities in the U.S. with the capacity to produce 9.0 billion gallons of ethanol and 273 million tons of distillers grain (www.ethanolrfa.org). In addition, there are nearly 20 more facilities under construction. Markets for DDGS are oversupplied in regions with a high density of ethanol production. It is estimated that only 60 million tons of DDGS is consumed through animal feed (Dooley 2008). Researchers have worked to increase the corn bran value by extracting hemicellulose (Doner et al. 1997, Yadav et al. 2007). Successful extraction of hemicellulose from DDGS could help increase the value of DDGS.

Composition of DDGS has been analyzed by a number of researchers. Dong et al. (1987) reported that there are 24.7% protein, 46.1% neutral detergent fiber (NDF), 11.0% lipid and 12.0% ash in corn DDGS. Spiehs et al. (2002) documented that corn DDGS contained 28.7~31.6% crude protein, 10.2~11.7% fat, 36.7~49.1% neutral detergent fiber (NDF), and 5.2~6.7% ash. In sorghum DDGS, there are 45.3% protein, 12.3% fat, 2.1% ash , 11.6% fiber and 5.7% starch (Wu et al. 1984). Therefore, DDGS could be a promising resource for non-starch carbohydrate.

The molecular structure of hemicellulose varies in different plants (Muraliksrishna et al. 2007; Mohnen et al. 2008). In cereal, hemicellulose is composed of a linear or branched chain of xylopyranosyl units, and the backbone is attached with short side chains containing one to a few units of L-arabinofuranosyl, D-galactopyranosyl, D-glucuronopyranosyl, and/or 4-O-methyl-D-glucuronopyranosyl units (BeMiller 2007). Corn bran hemicellulose has a highly branched structure according to methylation analysis (Muraliksrishna et al. 2007). It is reported that xylan

backbone contains only 23% unbranched xylose residues, and also highly substituted with oligomeric side chains with only 15% of unsubstituted xylose residues (Muraliksrishna et al. 2007). Brillouet et al. (1982) disclosed the structure of heteroxylan which was major fraction of hemicellulose from wheat bran and precipitated by 60~70% ethanol. Heteroxylan from wheat bran consisted of 50% unsubstituted and 50% mono- or di- substituted xylose was, which was less branched compared with corn bran hemicellulose (Brillouet et al. 1982).

In this study, alkaline extraction method adapting from Yadav et al. (2007) was applied to isolate hemicellulose fiber gum (HFG) from lab-made corn, sorghum and wheat DDGS. The objective of this study was to compare compositions of different sources of DDGS (corn, sorghum and wheat) and compositions and structure characteristics of HFG extracted from different sources of DDGS.

MATERIALS AND METHODS

Materials

The *Saccharomyces cerevisiae* strain ATCC 24860 was used for fermentation. Wheat, corn, sorghum grains were purchased from the warehouse of Farmer's Coop (Manhattan, KS) and ground by using a Magic plus mill set at level IV (Pleasant Hill Grain Inc., Hampton, Nebraska). DDGS of wheat, sorghum and corn was produced from ground grains as described by Wu et al. (2006) and was ground to a 20 mesh particle size by a commercial blender (Dynamics Corporation of America, New Harford, CT).

Total starch assay kit was purchased from Megazyme International Ireland Limited (Wicklow, Ireland). It includes thermostable α-amylase (3000 U/ml) and amyloglucosidase (200 U/ml). STARGEN 001 (blend of α-amylase and glucoamlyase), GC 106 (acid fungal protease), and Protex 6L (alkaline protease) were obtained from Genencor (Kansas City, MO), and α-amylase (Liquozyme) from Novozymes (Franklinton, NC). STARGEN 001 had activity ≥456 GSHU/g (granular starch hydrolyzing units). Activity of GC 106 was ≥1000 SAPU/g (spectrophotometric acid protease units) and activity of Protex 6L was ≥580, 000 DU/g. Liquozyme (α-amylase) had activity 240 KNU/g (kilo novo units).

Celite 577 (cat.22142) as filter aid agent was purchased from Sigma Chemicals (St. Louis, MO).

Preparation of distillers grain

Each grain (1,300 g) was suspended with 4000 ml of tap water in a 3 gallon, steam-heated kettle. Liquozyme (α-amylase, 800 μl) was added into the slurry. The temperature was gradually raised to 95°C. The liquefaction process continued for 1 hour at 95°C. The liquefied mash was transferred into a 5 L fermentation tank of the Bioflow fermentor. The mash was cooled to 30°C, and the pH was adjusted to 4.20 with 6 M H_2SO_4. Yeast extract (12 g), KH_2PO_4 (4 g), glucoamylase (4 ml) and activated yeast (2 g) were blended into the mash to start the saccharification and fermentation. After 72 hours incubation at 30°C, the finished beer (~5000 g) was boiled in the steam-heated kettle. The residuals were then dried in flat baking pans in an air-forced oven for 48 hours at 50°C. Same procedure was applied for all three grains.

Analytical methods

Protein was measured by nitrogen combustion (LECO FP-528, St. Joseph, MI) according to AOAC method 990.03. Crude fat, ash, and moisture content were determined by AOAC method 920.39, AOAC method 942.05, and AACC air oven method 44-19, respectively.

Starch content was determined by high performance anion-exchange chromatograpy (Dionex Corporation, Sunnyvale, CA) with pulsed amperometric detection (HPAEC-PAD) after hydrolyzing HFG and DDGS by Megzyme® Total Starch Assay Kit. HFG and DDGS (100 mg) were weighed into a screw-cap tube, dissolved in 2 ml of deionized water, and boiled for 5 min. Thermostable α-amylase (3 ml, diluted 1:30 in 100 mM sodium acetate buffer, pH 5.0) was added. The sample was incubated in a boiling water bath for 6 min. After adding 0.1 ml amylogluconsidase and 4.9 ml deionized water, the sample was incubated at 50°C for 30 min. After hydrolysis, the solution was boiled for 10 min to denature and precipitate enzymes. Hydrolyzed HFG and DDGS was diluted 80 times and injected into CarboPAC1 (Dionex Corporation, Sunnyvale, CA) column at 25°C. NaOH (150 mM) was employed as the eluent at a flow rate of 1 ml/min. Standard glucose solutions (0, 3, 4, 5 and 6 μg/ml) were injected and analyzed to obtain the standard curve. Quantitation was based on integrated peak area relative to the area of known quantity of standard glucose.

Hemicellulose fiber gum (HFG) extraction

Ground DDGS (100 g, dry weight) was mixed with 400 ml distilled water, and 8 g NaOH was dissolved in the slurry. The mixture was boiled in the water bath for one hour with continuous agitation. After one hour boiling and continuous stirring, the slurry was bleached by 20 ml H_2O_2 (30% w/w) at 25°C for 1 hour and centrifuged (2500×g, 15 min) to remove the insoluble materials. The supernatant was decanted and collected for HFG recovery. The insoluble fraction was washed three times with 600 ml distilled water. The pH of supernatant was adjusted to 4.5 by 1 M HCl and was subjected to the two-step enzymatic hydrolysis. In step one, the enzymatic hydrolysis was carried out at 35°C for overnight, by using protease (GC106, 0.02% w/w) and the blend of amylase and amyloglucosidase (STARGEN 001, 0.02% w/w) at pH 4.5. In step two, the pH of the solution was adjusted to 8.0 by 1 M NaOH, and alkaline protease (Protex 6L, 0.02% w/w) was added. The solution was incubated at 35°C for overnight. Subsequently the cloudy hydrolyzate was clarified by a precoat Celite-577 filter plate. The transparent solution was concentrated to 500 ml and blended with 3 volumes of absolute ethanol. The yellowish rubbery sediment was separated by centrifugation and air dried at 40°C. Same procedure was applied for all three DDGS.

Sugar profile of HFG

Sugar composition was determined by HPAEC after acid hydrolysis of the HFG and DDGS. The sample preparation was according to a method by Doner et al. (2001). Sample (100 mg) was weighed into a screw-cap tube and mixed with 2 ml 12*N* H_2SO_4, vortexed periodically over 4 hours at room temperature. The solution was then diluted to 2*N* H_2SO_4 with distilled water and boiled for 1 hour. After cooling to 25°C, 2 g $BaCO_3$ was added to neutralize the solution, and removed by centrifugation. Hydrolyzed HFG and DDGS was diluted 60 times and injected into CarboPAC1 column at 25°C. NaOH (13 mM) was employed as the eluent at a flow rate of 1 ml/min. Standard sugar solutions (mixture of arabinose, galactose and xylose) were injected and analyzed to obtain the standard curve. Concentrations of all standards were 0, 10, 15, 20 and 25 μg/ml. Quantitation was based on integrated peak area relative to the area of known quantity of standard sugars.

Molecular weight (MW) distribution of HFG

The hemicellulose was analyzed by gel permeation chromatography (GPC) to determine MW distribution. HFG sample was dissolved in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO) at 0.4% (W/V). GPC analysis was performed using PL-GPC 220 Integrated GPC/SEC fully automated system (Polymer Laboratory, MA). The system is equipped with a differential refractive index (DRI) detector and three Phynogel 00H-0646-KO, 00H-0644-KO, 00H-0642-KO columns (Phenomenex, Torrance, CA) connected in a series. The mobile phase in the column was DMSO with 5 mM $NaNO_3$. The flow rate was 0.8 ml/min, while the column oven temperature was controlled at 80°C. The electronic outputs of the DRI detectors were collected by GPC software (version. 3.0, Polymer Laboratories, A Varian, Inc. Company). Peaks were assigned using DRI chromatograms. DRI signals were used to determine the molecule weight of sample. amples were injected into GPC using an autosampler.

Statistical anaylsis

Macanova 4.12 (School of Statistics, University of Minnesota, Minneapolis, MN) was used to perform ANOVA and Tukey's honest significance difference (HSD) analysis. The level of significance was $P<0.05$ throughout the study.

RESULTS AND DISCUSSION

Characteristics of DDGS

The compositions of the three DDGS samples are listed in **Table 2-1**. Hemicellulose content was highest for corn DDGS (28.03%) and lowest for sorghum DDGS (11.47%). Corn DDGS contained the lowest protein (29.65%) and the highest fat (13.83%); while wheat DDGS contained the highest protein (43.38%) and the lowest fat (3.60%). Regarding to the non-starch carbohydrate, corn DDGS had the highest hemicellulose content (28.03%) and sorghum DDGS consisted of lowest hemicellulose (11.47%). Composition of DDGS differed based on grain used.

Characteristics of hemicellulose fiber gum (HFG)

According to previous study (E and Shi 2010), the combination of boiling temperature and NaOH (2%) delivered high HFG yield and high hemicellulose recovery. Therefore, this method was used in the current study. The yield of HFG and hemicelluose recovery were listed in **Table 2-2**. Corn DDGS rendered the highest HFG yield (21.08%) and hemicellulose recovery (30.95%); while sorgum and wheat DDGS only generated half the HFG yield as compared with corn DDGS. However, the recovery of hemicellulose for sorghum HFG was not significantly different compared to that of corn HFG (29.74 vs. 30.95%). It is reported that arabioxylan chains are bridged through ferulic acid dimmers, which partially limits the extractability of arabinoxylan (hemicellulose) (Saulnier et al. 1995a). Alkali is believed to cleave the diferulic bond and increase the hemicellulose extractability (Saulnier et al. 1995b, Hromadkova et al. 2008). Hemicellulose with high substitution degree was prone to be more soluble (Maes et al. 2002). The reason that corn and sorghum DDGS had higher hemicellulose recovery was postulated that after de-esterification, due to highly branched structure, corn and sorghum hemicelluloses were more soluble than wheat hemicellulose.

After enzymatic hydrolysis and ethanol precipitation, the final gum still contained large amount of impurities (**Table 2-3**). HFG contained 22~38% ash as well as 8~16% protein. Besides these impurities, there was small amount of glucose, especially for wheat HFG (0.3%). The sugar profile of each HFG was included in **Table 2-4**. The ratios of xylose to arabinose of three HFGs were not significantly different, indicating that water extractable hemicelluloses from corn, sorghum and wheat DDGS had similar structure properties. The ratios of xylose to arabinose were 1.06~ 1.22, suggesting that water extractable hemicelluloses were highly branched.

The MW distributions of three HFGs are shown in **Fig. 2-1**. Carbohydrates in the purified HFGs were predominately polymers with MW ranging from 10^3 to 10^6 g/mol. Hemicellulose from corn HFG appeared to have more high MW molecules than sorghum and wheat HFG. Interestingly, the peak of wheat HFG displayed a tri-modal distribution with two shoulders at 10^3 and 10^6 g/mol, and one peak at 10^5 g/mol.

CONCLUSIONS

DDGS made from wheat, sorghum and corn had different protein, fat and non-starch carbohydrate contents. Corn DDGS had the highest level of hemicellulose. Starch residue in DDGS from all three grains ranged from 2.85~4.86% (db). The yield% of hemicellulose extracted from various types of grains was in the order of corn> sorghum> wheat DDGS. The final HFG extracted from DDGS from all grain types had high amounts of protein and ash contents. Future work will be focused on obtaining more purified hemicellulose and also in identifying the bonds between protein and hemicellulose.

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Table 2-1 Composition* of three distillers dry grain with solubles (DDGS) (weight %) (N=2)**

	Protein	Fat	Ash	Starch	Hemicellulose***	Cellulose****
Corn DDGS	29.65c±0.07	13.83a±0.10	6.38a±0.14	4.86a±0.15	28.03a±0.56	17.25
Sorghum DDGS	37.37b±0.86	10.13b±0.19	6.34a±0.02	2.85b±0.25	11.47c±0.49	31.84
Wheat DDGS	43.38a±0.29	3.60c±0.06	6.18a±0.07	4.04ab±0.28	19.61b±0.15	23.19

*Based on DDGS dry weight
** Mean values followed by the same letter are not significantly different at $P < 0.05$.
*** Hemicellulose = Arabinose+galactose+xylose
****Cellulose was calculated as 100% - the sum of other components.

Table 2-2 Yield* of hemicellulose fiber gum (HFG) and recovery** of hemicellulose (weight %) (N=2)***

	Yield of HFG %	Recovery of hemicellulose %
Corn HFG	21.08a±1.10	30.95a±0.75
Sorghum HFG	11.07b±0.32	29.74a±1.08
Wheat HFG	11.64b±0.23	22.71b±0.37

*Based on the dry DDGS
**Based on the dry weight of hemicellulose in DDGS
*** Mean values followed by the same letter are not significantly different at $P < 0.05$.

Table 2-3 Composition* of hemicellulose fiber gum (HFG) (weight %) (N=2)**

	Protein %	Ash %	Glucose %	Hemicellulose %
Corn HFG	8.37c±0.01	36.86b±0.05	3.79b±0.23	41.15a±1.00
Sorghum HFG	10.11b±0.01	38.19a±0.19	5.83a±0.10	30.80b±1.12
Wheat HFG	16.34a±0.01	22.16c±0.15	0.30c±0.02	38.24a±0.63

*Based on HFG dry weight

** Mean values followed by the same letter are not significantly different at $P < 0.05$.

Table 2-4 Sugar profile* of hemicellulose fiber gum (HFG) (weight %) (N=2)**

	Arabinose %	Xylose %	Galactose %	X/A***
Corn HFG	16.87a ±0.59	20.63a±0.35	3.65a±0.07	1.22a±0.01
Sorghum HFG	12.95b±0.84	14.10c±0.15	3.75a±0.14	1.09a±0.05
Wheat HFG	16.75ab±0.50	17.72b±0.36	3.77a±0.24	1.06a±0.01

*Based on HFG dry weight

**Mean values followed by the same letter are not significantly different at $P < 0.05$.

***X/A represents the ratio of xylose and arabinose.

Figure 2-1 The molecular weight distribution of hemicellulose fiber gum (HFGs) from corn, sorghum and wheat DDGS as determined by gel permeation chromatography. Wheat HFG (solid line), corn HFG (dotted line), and sorghum HFG (dashed line).