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ORIGINAL PAPER

Emulsifying properties of an arabinoxylan–protein gum from distillers' grains and the co-production of animal feed

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Abstract In order to improve corn ethanol profitability and energy efficiency, a natural gum consisting of primarily arabinoxylan and crude protein (CP) was extracted from distillers' grains (DG), a major byproduct from the dry grind corn ethanol production. DG was fractionated into an alkali-soluble gum fraction and an alkali-insoluble residue fraction by extracting with 1–5 % NaOH at 25–75 °C for 1–5 h. The extraction conditions, which significantly affect the yields and compositions of DG gum and residue, were statistically modeled to optimize yields and compositions. DG gum had 8–22 % CP, which could all be reduced to about 8 % by purification with bentonite clay. The isolated gums (purified and unpurified) were made into emulsifying agents, whereas residues were characterized as animal feed. The results demonstrated that the purification process affects the emulsifying properties of the DG gum-derived emulsifying agents. In parallel, the DG residue was found to have increased fiber digestibility and metabolizable energy compared to the original DG. An economic analysis determined that concurrent productions and utilizations of DG gum and DG residue could improve the cost and energy balance of the current corn ethanol process.

Keywords Alkaline extraction · Animal feed · Distillers' grains · Economic analysis · Emulsifying property · Gum

Abbreviations

ADF	Acid detergent fiber
ADICP	Acid detergent insoluble crude protein
CP	Crude protein
DG	Distillers' grains
ME	Metabolizable energy
NDF	Neutral detergent fiber
NDFD	Neutral detergent fiber digestibility

Introduction

Bioethanol is a potential substitute for fossil fuels and its use in the transportation sector has become a

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strategy for the United States to reduce its energy dependence on imported petroleum and greenhouse gases (GHG) emission. Leading the world in bioethanol production, the annual output of the United States was approximately 56 billion liters in 2012 using corn grain as the chief feedstock (RFA 2013). However, the rising of corn price has reduced the profits and increased ethanol cost of manufacture (Brown and Brown 2012). Additionally, the energy balance to produce corn ethanol (the ratio of biofuel energy output to the fossil energy input) is about 1.3 (Shapouri et al. 2003), which is much lower than sugarcane ethanol (~8.0), biodiesel (~3) and cellulosic ethanol (~2–36) (Brown and Brown 2012). However, this ratio can be improved by considering byproducts' energy credits (Shapouri et al. 2003). Consequently, if extra high value products with low energy input, are produced from corn ethanol biorefinery system, the corn ethanol profitability and energy efficiency will be greatly enhanced.

Distillers' grains (DG) are major byproducts from dry grind corn ethanol production. The DG product has roughly one-third of its weight being crude protein (CP), and has abundant levels of fat, cellulose and hemicelluloses (Kim et al. 2010). DG is primarily used as low-value animal feed for protein source and its high fiber content limits its utilization by non-ruminants (Lamsal et al. 2012). Thus, converting this polysaccharide portion of DG into high-valued bioproducts while still retaining the CP for animal feed utilization would be an effective way of valorizing corn ethanol byproducts.

Extensive studies have been carried out valorizing DG by converting the carbohydrates into ethanol or furfural (Dien et al. 2008; Kim et al. 2008; Tucker et al. 2004; Xiang and Runge 2014). However, as these processes typically require costly and severe chemical, biochemical, and/or mechanical treatments, their economy and fossil energy usage is less favorable. On the other hand, extracting the polymeric form of polysaccharides from DG for material use could be an alternative way to valorize DG, but few studies have considered this (Xiang et al. 2014), possibly because as such large amounts of DG have only recently become widely available due to ethanol production boost (Shapouri et al. 2003).

The most widely used method to fractionate hemicelluloses from plant biomass is using alkali or alkaline peroxide (Sun et al. 2002). The major hemicelluloses extracted from corn biomass are highly branched arabinoxylans (Ebringerová and Heinze 2000). Extensive studies have described the potential use of corn hemicelluloses for various types of polymeric materials such as films and emulsifying agents (Woo 2001; Whistler 1993). Unlike those relatively pure arabinoxylans directly extracted from corn bran or corn kernels, the hemicellulosic gum extracted from DG, however, could be considerably heterogeneous. The DG contains relatively high amounts of CP, which could be co-extracted with hemicelluloses and may affect the properties and processabilities of the DG gum. Methods to remove protein contamination in hemicellulosic materials were extensively studied (Izydorczyk and Biliaderis 1995) and one of the effective ways is using clay (Izydorczyk et al. 1991). However, CP may not necessarily be a contamination for the extracted DG gum materials, as it has been reported that protein can increase the corn fiber gum's emulsifying properties (Yadav et al. 2007).

We recently reported a study fractionating DG into an alkaline-soluble hemicellulose-rich DG gum and an alkaline-insoluble protein-rich DG residue, and examined concurrent utilizations of DG gum as paper coatings and DG residues as animal feed (Xiang et al. 2014). In this study, we have expanded upon the previous work and conducted additional studies to evaluate the effects of different alkali extraction conditions on the yield and composition of the DG gum and residues. Additionally, the animal feed characterization of DG residue has been more thoroughly evaluated. We also proposed a novel utilization of the isolated gum as emulsifying agents and investigated the effects of purification on its emulsifying properties. Based on our approach to valorize DG, a new process was considered and its economic feasibility examined.

Experimental section

Fractionation of DG into DG gum and DG residue

Distiller's grains (DG) samples were obtained dried from Didion Milling Inc. (Cambria, WI, USA). In order to create statistical models including temperature, alkaline concentration and reaction time as variables to predict the gum and residue yields and

qualities, two sets of screening experiments with factorial matrices were designed. The first set of the experiments included a full 2^2 factorial matrix with constant reaction time of 3 h (temperature: 25 and 75 °C, alkaline: 1 and 5 %) as well as one central point (50 °C, 3 % and 3 h). The second set was designed to include the reaction time into the model and it had a 2^2 full factorial matrix with constant reaction temperature as 50 °C (alkaline: 1 and 5 %, reaction time: 1 and 5 h) including one central point with the same conditions as in the first matrix. Duplicate runs were performed for each combination and triplicate runs for the central point. Based on the experimental conditions described by the matrices, for each run, the DG and NaOH solution (1:20 solid to liquid weight ratio; 1:10 and 1:6 ratios were also tested for condition 50 °C, 3 % and 3 h) were mixed, and maintained at constant temperatures by stirring on a magnetic stirring hot plate for a desired reaction time. The mixture was then separated into alkaline soluble and insoluble fractions through centrifugation. The collected alkaline insoluble fraction or residue (DG residue) was washed with water and freeze-dried for further animal feed characterization. The alkali-soluble part was adjusted to pH 5.5 by 6 M HCl, concentrated to approximately one-third of its original volume on a rotary evaporator at reduced pressure, and slowly poured into a three-time volume of 95 % ethanol with constant stirring. The precipitated solid was separated and washed with 95 % ethanol through filter paper to give an alkali-soluble gum fraction (DG gum). Several conditions were investigated for DG gum purification process to remove CP. After the alkali-soluble fractions were adjusted to pH 5.5, bentonite clay (~20 g/L) was added. The solution was stirred at 500 rpm for 1 h, and then centrifuged and filtered to remove the clay. The clay free solution then was used for the concentration and ethanol precipitation steps.

Structural analysis of DG gum

Samples being determined for neutral sugar contents were hydrolyzed according to the procedure described previously (Min et al. 2014) and the hydrolysate was then run through a ICS-3000 Ion Chromatography (IC) System (Dionex, Sunnyvale, CA, USA) according to the method described by Xiang et al. (2014).

Crude protein content was determined by a combustion method according to AOAC official method 990.03 using a FlashEA 1112 Series CN analyzer (Thermo Electron, Waltham, MA, USA).

Molecular weights of DG gum were determined by gel permeation chromatography (GPC). DG gum samples were acetylated in a DMSO/*N*-methylimidazole/acetic anhydride system, and the acetylated DG gum samples were subjected to GPC analysis on a Shimadzu LC-20A LC system equipped with a refractive index (RI) detector (RID-M20A, Shimadzu, Columbia, MD, USA) using the conditions previously described (Xiang et al. 2014; Tobimatsu et al. 2013). The data acquisition and computation used Shimadzu LCsolution version 1.25 software.

FT-IR spectra of DG gum were collected on a Spectrum 100 FT-IR spectrometer (PerkinElmer, Waltham, MA, USA) equipped with a universal ATR sampling accessory. The spectrum was acquired in the range of 650–4,000 cm^{-1} with a total of eight scans.

DG gum emulsifying properties

DG gum samples or a gum arabic sample (MP Biomedicals, Santa Ana, CA, USA) were suspended (5.0 wt%) in an aqueous solution containing 10.0 wt% sucrose, 0.1 wt% sodium benzoate and 0.3 wt% citric acid, and the suspension was then put into a sonicator for 2 h to dissolve and ensure a homogeneous solution. The gum solution was then diluted 50 times into a 0.1 wt% gum solution and 0.2 wt% orange oil (Now Foods, Bloomingdale, IL, USA) was added. The gum and oil mixture were then mixed with high shear in a Waring 700 s Blender for 2 min. The final composition of the gum/oil emulsion was 0.1 wt% gum, 0.2 wt% orange oil, 10 wt% sucrose, 0.1 wt% sodium benzoate and 0.3 wt% citric acid. The surface-area-average diameter (droplet size, d_{32}) and zeta potential of the emulsion was measured right after the emulsions were prepared by a particle size analyzer (90Plus, Brookhaven, Long Island, NY, USA) according to the procedure described by Sun and Gunasekaran (2009). The turbidity of the emulsion was determined by a UV–Vis spectrophotometer (UV-mini-1240, Shimadzu, Columbia, MD, USA) following the procedure described by Yadav et al. (2007). The turbidity of the diluted gum solution before adding oil was also measured in order to calculate a

relative turbidity for the gum/oil emulsion to exclude the effects of original cloudiness of the DG gum solutions.

Animal feed characterization of DG residue

The ash content was determined according to the NREL LAP (NREL/TP-510-42622) using an Isotemp Muffle Furnace (Fisher Scientific). Crude fat content was determined by using hexane to extract the sample in a Soxhlet apparatus for 24 h according to AOAC official method 2003.06. CP content was determined by AOAC official method 990.03. Soluble CP was evaluated based on the procedure described by Krishnamoorthy et al. (1983).

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined according to AOAC official method 973.18 and AOAC 2002.04. Acid detergent insoluble crude protein (ADICP) was determined by measuring the CP content in the ADF portion. In vitro true dry matter digestibility (IVTDMD) analysis was performed using raw DG as a control, and with DG residue to determine its feasibility to be considered as an animal feed. IVTDMD measurement was done following the procedure described by Goering and Van Soest (1970) except that the incubation time was 30 h. NDF digestibility (NDFD) was calculated based on NDF and IVTDMD as (Goeser and Combs 2009).

$$\%NDFD = \left(1 - \frac{100 - IVTDMD}{NDF}\right) \times 100$$

Model and economic analysis

Statistical models and analysis of variance were generated by JMP Pro 10 (SAS). Process mass and energy flows were estimated by Aspen Plus V7.3 (AspenTech). Optimum economic model was created and calculated by Matlab R2010a (MathWorks).

Results and discussion

Fractionation of DG into DG gum and DG residue

The chemical compositions and animal feed characterizations (Tables 1, 2) of the raw DG material used in this study were consistent with previous studies (Kim et al. 2010; Robinson et al. 2008). Similar to our previous studies (Xiang et al. 2014), the fractionated DG gum, in general, had significantly enriched levels of arabinan (15–19 %) and xylan (19–27 %) with highly reduced CP content (8–22 %), while the fractionated DG residue were overall consisted of abundant levels of cellulosic fibers (ADF) and CP depending on the conditions (Tables 1, 2 and Online Resource 1).

Yields and chemical compositions of the fractionated DG gum and DG residue were much dependent on the alkaline gum extraction conditions (Tables 1, 2 and Online Resource 1). To better understand the effects of the reaction conditions on gum extraction and to predict the theoretical optimum condition, multiple regression models at 95 % confidence interval were generated to fit the yields and compositional data of DG gum and DG residue within the range of the experimental matrices (temperature: 25–75 °C, NaOH conc.: 1–5 %, and reaction time: 1–5 h). The complete analysis of variance models may be seen in Online Resource 2, with regression models shown as Eq. (1) through (4) in Online Resource 3.

Based on the models, 3-D graphs at constant reaction time (3 h) and constant reaction temperature (50 °C) were generated (Figs. 1, 2). Overall, the extraction severity had a positive relationship with the yield and of DG gum, although the effect of extraction time on both DG gum yield and polysaccharide/CP ratio appeared to be less significant compared to those of extraction temperature and NaOH concentration. Our model predicted that DG gum could be obtained in the maximum yield of ~30 % by an extraction at ~60 °C, with 3 % NaOH for 5 h. The polysaccharide/CP ratio of DG gum had its maximum towards the high end of reaction severity; gums extracted at 75 °C with 5 % NaOH for 3 h had the highest carbohydrate content (~62 %) with the lowest CP level (~8 %) in this study.

Compared to DG gum, the yields and CP contents of DG residue varied more under the different extraction conditions. As the yield of DG gum increased with increased extraction severity, the yield of DG residue accordingly decreased. However, whereas CP levels in DG gum decreased with increased extraction severity, CP contents of DG residue also appear to decrease considerably, suggesting that severe alkaline conditions degraded the

Table 1 Yields and chemical compositions of unpurified and purified DG gum from different alkaline extraction conditions [mean ± standard error (SE)]

Temp.°C	NaOH %	Time (h)	Total yield %	CP %	Ara %	Gal %	Glu %	Xyl %	Man %	Total sugar %
Raw DG			–	37.4 ± 1.0	6.8 ± 0.1	2.5 ± 0.0	13.4 ± 0.2	9.8 ± 0.2	1.4 ± 0.0	33.9 ± 0.3
Unpurified										
25	1.0	3	11.7	16.4 ± 1.4	15.3 ± 0.2	3.5 ± 0.0	8.0 ± 0.2	19.3 ± 0.3	1.6 ± 0.0	47.7 ± 0.3
25	5.0	3	25.7	17.2 ± 1.9	15.6 ± 0.2	3.8 ± 0.0	13.0 ± 0.2	21.5 ± 0.4	1.5 ± 0.0	55.3 ± 0.9
75	1.0	3	24.6	14.0 ± 0.0	17.4 ± 0.3	4.5 ± 0.1	6.4 ± 0.1	23.1 ± 0.4	1.5 ± 0.0	52.9 ± 0.9
75	5.0	3	24.3	7.8 ± 0.1	19.3 ± 0.0	4.8 ± 0.0	9.3 ± 0.1	26.6 ± 0.2	1.5 ± 0.1	61.5 ± 0.5
50	3.0	3	27.6	17.9 ± 0.1	16.2 ± 0.1	3.8 ± 0.3	10.3 ± 0.2	21.2 ± 0.8	1.5 ± 0.1	53.0 ± 1.3
50	1.0	1	18.2	17.9 ± 0.5	16.0 ± 0.5	3.0 ± 0.0	6.4 ± 0.1	19.3 ± 0.5	1.3 ± 0.3	46.1 ± 1.3
50	1.0	5	23.8	21.8 ± 0.5	16.1 ± 0.3	3.2 ± 0.0	5.9 ± 0.0	20.0 ± 0.2	1.3 ± 0.1	46.6 ± 0.6
50	5.0	1	27.4	17.6 ± 0.2	16.2 ± 0.3	3.4 ± 0.1	10.9 ± 0.1	20.8 ± 0.4	1.4 ± 0.0	52.7 ± 0.8
50	5.0	5	26.9	16.9 ± 0.1	17.6 ± 0.2	3.7 ± 0.0	11.1 ± 0.1	22.0 ± 0.3	1.4 ± 0.0	55.8 ± 0.5
Purified										
75	1.0	3	17.5	8.9 ± 0.2	19.6 ± 0.2	4.4 ± 0.0	6.7 ± 0.0	27.6 ± 0.2	2.3 ± 0.0	60.7 ± 0.4
75	5.0	3	21.3	7.7 ± 0.0	20.9 ± 0.3	4.7 ± 0.0	5.8 ± 0.1	27.9 ± 0.6	2.2 ± 0.1	61.4 ± 1.0
50	1.0	5	14.6	8.3 ± 0.3	20.0 ± 0.1	4.4 ± 0.0	6.2 ± 0.0	26.5 ± 0.1	2.3 ± 0.0	59.5 ± 0.2
50	5.0	5	18.7	7.4 ± 0.0	20.4 ± 0.3	4.5 ± 0.1	9.2 ± 0.1	27.1 ± 0.4	2.0 ± 0.1	63.1 ± 0.8

Table 2 Animal feed characterizations and metabolizable energies of DG residues from different alkali extraction conditions (mean ± SE)

Conditions	Raw DG	50–3.0–3	25–1.0–3	25–5.0–3	75–1.0–3	75–5.0–3
Yield (% of raw DDG)	–	30.0	60.7	35.8	23.1	13.3
Ash (% of DM)	3.7 ± 0.0	4.7 ± 0.2	2.9 ± 0.0	3.2 ± 0.1	1.0 ± 0.1	0.6 ± 0.0
Fat (% of DM)	10.7 ± 0.0	2.3 ± 0.1	1.9 ± 0.1	2.1 ± 0.3	2.5 ± 0.3	0.4 ± 0.0
Crude protein (% of DM)	37.4 ± 1.0	12.7 ± 0.2	47.5 ± 0.5	39.4 ± 0.2	12.0 ± 0.3	3.4 ± 0.2
Soluble CP (% of CP)	12.8 ± 1.0	9.6 ± 0.3	6.0 ± 0.8	4.2 ± 0.2	17.7 ± 1.5	43.6 ± 1.8
ADICP (% of CP)	12.1 ± 0.6	16.6 ± 1.4	5.8 ± 0.7	6.9 ± 1.7	10.8 ± 0.9	25.7 ± 0.6
NDF (% of DM)	36.6 ± 0.9	65.4 ± 0.1	54.7 ± 2.4	74.3 ± 1.0	74.3 ± 1.6	83.0 ± 2.3
NDFD30 (% of NDF)	41.4 ± 1.0	92.3 ± 0.2	81.8 ± 2.8	86.5 ± 1.6	90.4 ± 0.8	88.5 ± 0.1
ADF (% of DM)	14.9 ± 0.2	40.1 ± 0.3	29.8 ± 1.1	38.0 ± 0.9	46.3 ± 0.6	58.2 ± 0.8
Lignin (% of DM)	1.4 ± 0.1	2.7 ± 0.0	–	–	–	–
ME (MJ/kg DM)	12.89	14.52	13.62	13.72	15.00	14.27

E.g. 50–1.0–5 means the gum was extracted at 50 °C, 1.0 % and 5 h

protein into soluble amino acids and/or small peptides (Saulnier et al. 1995).

Purification of DG gum

Gums from four selected conditions with highest (50–1.0–5, 21.8 %), lowest (75–5.0–3, 7.8 %) and medium (50–5.0–5, 16.9 % and 75–1.0–3, 14.0 %) CP contents were purified using bentonite clay (Izydorczyk et al. 1991). As can be seen from Table 1, CP contents of purified gums from the selected conditions were all decreased to about 8 %, whereas the total sugar contents were increased up to ~60 % (arabinan and xylan contents, ~20 and ~27 %), leading to similar chemical compositions for all selected purified DG gums. In addition, FT-IR analysis of the DG gum films determined that the peaks from N–H bending in amides at 1,547 cm^{-1} (Jagadeesh

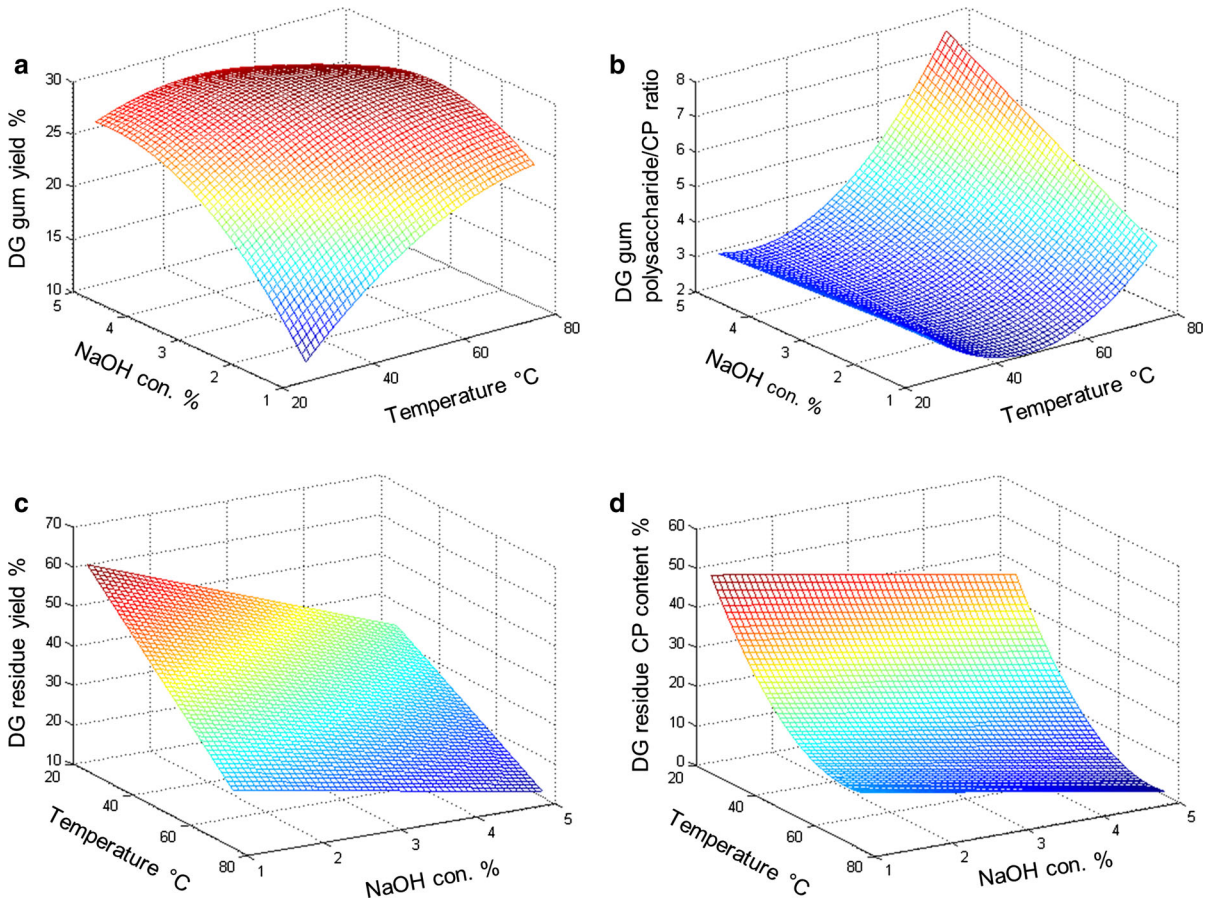

Fig. 1 The effects of temperature and NaOH concentration from the statistical models at constant reaction time of 3 h on **a** DG gum yield, **b** DG gum polysaccharide/CP ratio, **c** DG residue yield, and **d** DG residue CP content

et al. 2011) vanished with the clay purification process (Online Resource 4). These results indicate the effective removal of CP by bentonite clay. The 8 % CP from all the selected purified gums that were not removed by bentonite clay might be referred to structural proteins such as hydroxyproline-rich glycoprotein that are linked through alkali resistant glycosidic bonds to hemicelluloses (Saulnier et al. 1995; Yadav et al. 2007), or a portion possibly even the subunits of a newly confirmed hemicelluloses-protein complex in plants (Tan et al. 2013).

Molecular weight distributions of DG gum

The molecular weights of some selected DG gum samples were determined by GPC (Table 3 and Online Resource 5). The averaged molecular weight (M_w and M_n) and the polydispersity index (PDI, M_w/M_n) of acetylated DG gum samples were in the range of the values reported for other isolated hemicelluloses or gums (Yadav et al. 2009 and Xiang et al. 2014). The averaged molecular weights of DG gum increased as the extraction temperature and alkaline concentration increased, indicating that high molecular mass portions of DG hemicelluloses were more effectively extracted under severe alkaline conditions (Saulnier et al. 1995). After purification with bentonite clay, the M_w and M_n of DG gum did not vary, but PDI decreased considerably. Thus, the clay treatment was effective to produce gums composed of arabinoxylans with narrower molecular mass distributions. Given that the purified gum of 75–5.0–3 had little CP removed but showed a decreased PDI, however, the clay might somewhat remove some short chain polysaccharides or fatty acids together with CP (Asselman and Garnier 2000).

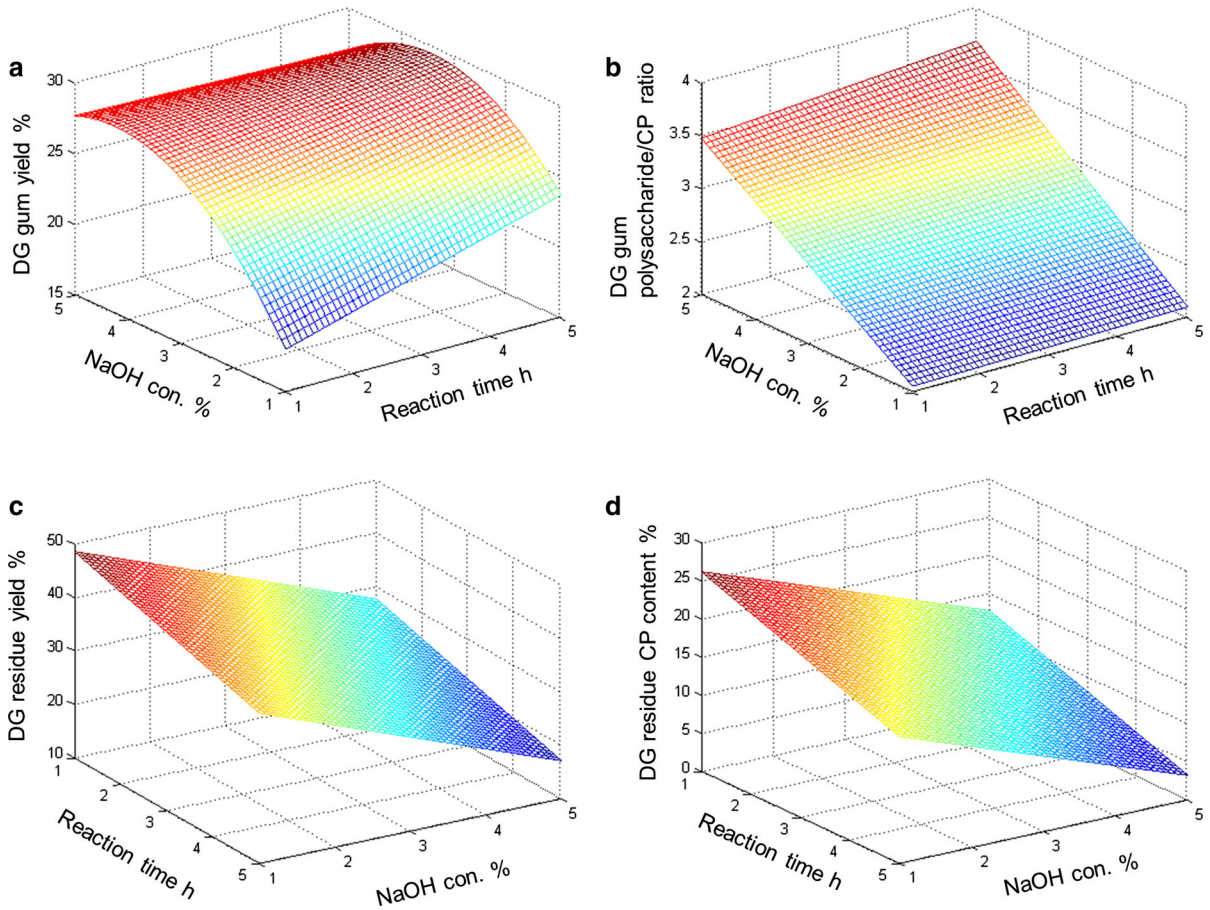

Fig. 2 The effects of reaction time and NaOH concentration from the statistical models at constant reaction temperature of 50 °C on **a** DG gum yield, **b** DG gum purity, **c** DG residue yield, and **d** DG residue CP content

DG gum emulsifying properties

The surface-area-average particle size and zeta potential profiles for emulsions without emulsifying agents (control) and emulsions aided with gum arabic and with DG gum from selected conditions (purified and unpurified) were measured and shown in Fig. 3. Gum arabic is a common emulsifying agent used in beverage making industry and thus was used to compare with DG gum. Similar to gum arabic, DG gum were able to effectively reduce the droplet size in the oil–water emulsion showing potential emulsifying properties (Fig. 3a). Among emulsions with unpurified DG gum, sample 75–5.0–3 with the lowest CP content had the smallest droplet size of about 570 nm, while sample 50–5.0–5 with the highest CP content had the largest droplet size of about 930 nm. However, comparing within the same alkali conditions, emulsions with unpurified DG gum had smaller droplet size than with purified DG gum demonstrating a more homogeneous emulsion. For zeta potential (Fig. 3b), all emulsions aided with DG gum had significant lower zeta potential (−6 to −12 mV) than gum arabic (−23.8 mV) showing possible higher tendency for the oil droplets to aggregate, creating less stable emulsions.

For the emulsion stabilities as described by the changes in turbidity over time (Yadav et al. 2007), when fresh prepared (Fig. 4a), most of the emulsions have similar or higher relative turbidities than the control sample except samples 75–1.0–3 and 50–1.0–5. After 1 and 7 days (Fig. 4b, c), the turbidity of the control sample quickly dropped from ∼110 to ∼20 cm^{-1} and to below 5 cm^{-1} suggesting

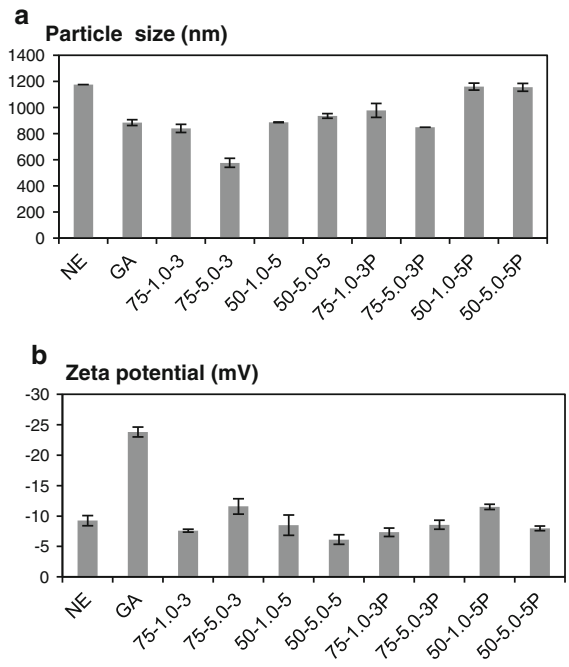

Fig. 3 **a** Particle size and **b** zeta potential (with standard error bars) for oil–water emulsions without emulsifying agents (*NE*), with gum arabic (*GA*) and unpurified and purified DG gum as emulsifying agents (e.g. 50–1.0–5 means the gum was extracted at 50 °C, 1.0 % and 5 h; letter P means purified DG gum)

Table 3 Molecular weights of unpurified and bentonite clay purified DG gum (mean ± SE)

Gum samples	Molecular weights		
	M_n (kDa)	M_w (kDa)	M_w/M_n
Unpurified			
75–1.0–3 (14.0)	62.9 ± 0.8	492 ± 18.9	7.8 ± 0.4
75–5.0–3 (7.8)	81.3 ± 0.4	527 ± 10.4	6.5 ± 0.2
50–1.0–5 (21.8)	56.4 ± 0.8	411 ± 19.2	7.3 ± 0.2
50–5.0–5 (16.9)	44.3 ± 0.9	443 ± 16.4	10.0 ± 0.2
Purified			
75–1.0–3 (8.9)	72.5 ± 5.3	482 ± 35.9	6.6 ± 0.0
75–5.0–3 (7.7)	140 ± 18.5	610 ± 8.8	4.4 ± 0.5
50–1.0–5 (8.3)	64.2 ± 3.2	376 ± 2.7	5.9 ± 0.3
50–5.0–5 (7.4)	67.1 ± 4.7	391 ± 5.1	5.9 ± 0.3

E.g. 50–1.0–5 (21.8) means the gum was extracted at 50 °C, 1.0 % and 5 h, and the gum contains 21.8 wt% CP

significant oil droplet aggregation, whereas all the other samples maintained relatively higher turbidities indicating the effectiveness of DG gum and gum arabic to stabilize the oil–water emulsion. The

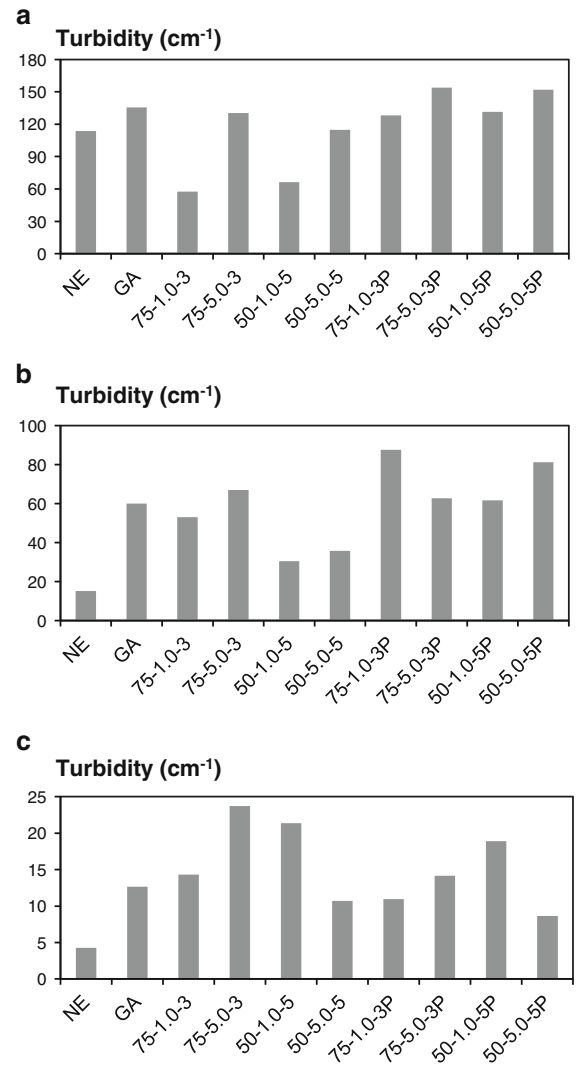

Fig. 4 Relative turbidities of oil–water emulsions **a** right after preparation, **b** after 1 day of preparation, and **c** after 7 days of preparation (NE, no emulsifying agents; GA, gum arabic as emulsifying agents; for DG gum as emulsifying agents, 50–1.0–5 means the gum was extracted at 50 °C, 1.0 % and 5 h and letter P means purified DG gum)

comparable emulsion stabilities between DG gum and gum arabic emulsions contradicted the prediction from zeta-potential data (will further explain later in this section). Comparing within the same alkali conditions, after a short time of preparation, the purified gum emulsions had higher turbidities than unpurified gum emulsions. However, after 7 days, the turbidities of the unpurified gum emulsions were overall higher than the purified gum emulsions showing better emulsifying stability.

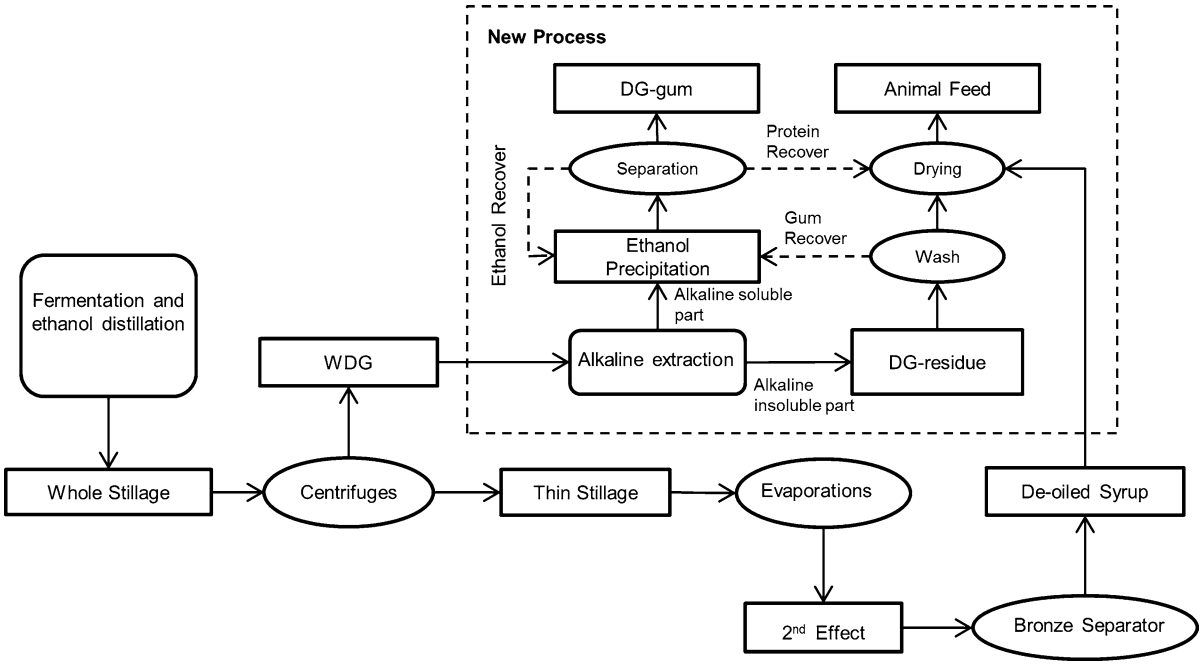

Fig. 5 DG gum extraction process as a new process is designed to be inserted into the typical dry grind corn ethanol plant biorefinery system to extract more values out of DG

The DG gums without purification were overall showing potential emulsifying properties by comparing with gum arabic. The structures of gum arabic and DG gum are mainly polysaccharides-protein complex that the former one contains arabinogalactan-protein complex, while the latter one contains arabinoxylan–protein complex (Islam et al. 1997; Saulnier et al. 1995). As for oil–water emulsions aided with gum arabic, the protein with its hydrophobicity was proved to be anchored to the oil droplet, which helps the stable penetration of hydrophilic polysaccharides into the aqueous phase preventing the oil droplets aggregation by steric effects (Randall et al. 1988). The role of protein in aiding the emulsifying process for gum arabic might also be applied to DG gum, as suggested by the results that unpurified DG gum had better emulsion forming properties (smaller particle size) and emulsion stabilities than purified DG gum. Additionally, the higher zeta-potential of emulsions aided with gum arabic than DG gum might be explained by the different electrical charges of their polysaccharides groups, in which gum arabic had much higher uronic acid content (~20 wt%, Randall et al. 1988) than DG gum (~5 wt%, Xiang et al. 2014). However, polysaccharide steric effect instead of charge effect was found to hold the major responsibility for protein-anchored oil droplet repulsion (Randall et al. 1988), which could explain the fact that the low zeta-potential of DG gum emulsions did not affect their stabilities.

DG residue animal feed characterization

To effectively utilize all fractions of DG, the DG residue after gum extraction also needs to be used, and thus was characterized for its potential as animal feed. Animal feed provides the animal with energy and thus investigating its metabolizable energy (ME) level is a basis to evaluate animal feed value. The ME of animal feed is mainly calculated from the digestion of fat, CP, non-fiber carbohydrate and digestible portion of fibrous carbohydrate (Robinson et al. 2004; Weiss et al. 1992). Recent advances in animal feed science have developed several relationships to determine the energy level of feed by considering the contribution of each nutritional component (NRC 2001; Robinson et al. 2004). In this study, the equation developed by Robinson et al. (2004) was used to estimate the ME level of DG and DG residue, and the same approach was applied to DG before by Robinson et al. (2008).

The calculation mainly involved several individual components including: (1) NDF, which represents the fibrous carbohydrates including cellulose, hemicelluloses, and lignin; (2) NDF digestibility (NDFD), which determines the digestible portion of NDF (Goering and Van Soest 1970; Van Soest et al. 1991); (3) ADF, representing the least digestible fibrous portion mainly consisting of cellulose and lignin; and (4) ADICP, which is the protein fraction remaining in ADF and indigestible to the animal.

The individual nutritional components and calculated ME for selected DG residues are determined and shown in Table 2. Compared to raw DG, the DG residue overall had decreased crude fat content, enriched fiber content, increased fiber digestibility, and slightly higher levels of metabolized energy (ME). NDF, ADF and NDFD of DG residue all increased compared to raw DG. With only minimal amount of lignin in DG, the increase of ADF indicated the increase of cellulose content in DG residue and was the main reason for the increase of NDF according to the data (Table 2). The increase of NDFD suggested the fiber portion of DG residue was more digestible by cows, which could be the result of increase in the accessibility of the recalcitrant cellulose from the alkaline treatment conditions (Hendriks and Zeeman 2009). Among different alkali extraction conditions, NDF and ADF had higher values at severer conditions, while the values of NDFD vary only slightly that even the mildest condition (25–1.0–3) could elevate the NDFD from 40 % of raw DG to 80 %. The final calculated ME of DG residue increased about 7–16 % compared to raw DG suggesting DG residue held more energy per unit weight than raw DG. Additionally, as already discussed in the earlier section, CP content of DG residue increased as the reaction severity decreases. Consequently, at less severe alkali conditions, DG residue displays improved yield, energy and nutritional value as well as significantly enhanced fiber digestibility suggesting its potential animal feed utilization.

Economic analysis

The DG gum's commercial implementation as a potential film or emulsifying agents depends greatly on the economics of the extraction process. Unfortunately, the optimum yields or qualities of DG gum and DG residue do not share similar conditions. Consequently, an economic model incorporating DG gum and DG residue yields as well as the variable costs such as energy cost, raw material costs and others was developed in order to optimize the conditions and evaluate the economics of the process. The energy cost was calculated by Aspen Plus and the Aspen model process flow chart can be seen in Online Resource 6, of which a simplified version can be seen in Fig. 5. As shown in the chart, ethanol was recycled after gum precipitation, and the ethanol soluble protein was recovered into animal feed as proposed by Xiang et al. (2014), and some extra alkaline soluble portion (for gum precipitation) was collected by washing the DG residue. For the model, although the solid/liquid ratio from the experiment matrix (Table 1) was 1:20, experiments with reduced ratios (1:6 and 1:10) were conducted and shown similar yields of DG gum and DG residue compared to ratio 1:20 (Online Resource 7). Thus lower ratios could be used. Additionally, in an industrial process, materials will be recycled. Some other considerations for the model were also taken into account and the assumptions as the basis for the model were summarized:

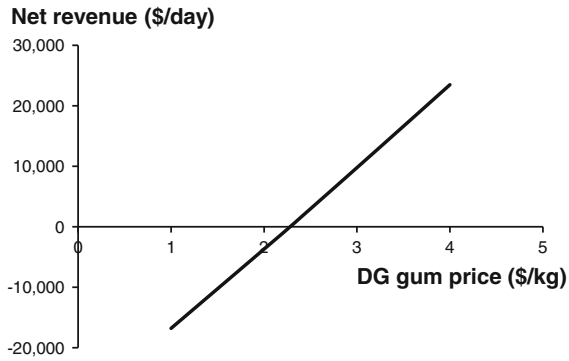

Fig. 6 The net revenues of DG gum producing process at different DG gum prices as calculated by the economic model

1. The model was based on a corn ethanol plant with WDG yield of 150,000 kg/day.
2. Costs for machine, time, and labor were not taken into account.
3. The model is only valid for the alkali extraction conditions in this study (temperature: 25–75 °C, alkali concentration: 1–5 % and reaction time: 1–5 h).
4. Solid to liquid ratio for alkali extraction is assumed to be 1:6 and the better mixing of industrial reactor would give similar yield results compared to yields from the experiments.

Table 4 Economic model calculation summary of optimum net revenue from DG gum extraction process with corresponding yield, condition and individual material and energy costs

Items	Values	Sources
Basis of WDG (MC = 69.1 %)	150,000 kg/day	Assumption (1)
Price of hydrochloric acid	0.2 US$/kg	Alibaba (2014)
Price of sodium hydroxide	0.4 US$/kg	CMAI (2011)
Price of ethanol	0.75 US$/kg	Nasdaq (2014a)
Price of DDG for feed (MC = 10.4 %)	0.25 US$/kg	Hofstrand and Johanns (2014)
Price of gum	3.0 US$/kg	Assumption (6)
Price of electricity	0.062 US$/kWh	EIA (2014)
Price of natural gas	4.2×10^{-6} US$/kJ	Nasdaq (2014b)
Model calculated optimum alkali extraction condition giving the best economy		
Temperature in alkali extraction	69 °C	Model optimization
Alkaline concentration	2.0 %	Model optimization
Reaction time in dilute acid extraction	5 h	Model optimization
Yields at optimum condition		
Yield of DG gum	29.5 %	Equation (1)
Yield of DG residue + recovered protein	53.8 %	Equation (3)
Cost and revenue when gum price is 3.0 $/kg and at optimum condition		
1. Cost of thermal energy (using natural gas)	7,324 US$/day	Usage estimated by Aspen
2. Cost of electricity and water	658 US$/day	Usage estimated by Aspen
3. Cost of raw materials (excluding WDG)	17,235 US$/day	
4. Revenue of selling DDG solely as feed	12,932 US$/day	Value of DDG for feed = 0.25 $/kg
5. DG gum revenue	41,020 US$/day	
6. Animal feed revenue	6,958 US$/day	
Net revenue gained	9,829 US$/day	5 + 6 − 1 − 2 − 3 − 4
New value of DDG	0.44 US$/kg	(5 + 6 − 1 − 2 − 3)/DDG basis

5. DG gum price was assumed to be 3 $/kg for the base case but was varied to determine its effect (Fig. 6). DG gum price was assumed the same for gums from different conditions; animal feed price was assumed the same for DG residue from different conditions.

As shown in Table 4 for the model calculation, the cost of raw materials, including mostly ethanol for precipitation, far exceeded other cost of productions. Additionally, unlike producing furfural from DG (Xiang and Runge 2014), animal feed production in this study did not have a significant impact on the net revenue. Thus, further improvement of the process should focus on increasing the yield of gum production and reducing the ethanol usage. The model predicted the highest revenue was 9,829 US$/day more than marketing DDG solely as animal feed amounting to an additional annual revenue of more than 3.5 million US$ for a corn ethanol plant with capacity of 150,000 kg WDG/day. The value of DDG increased from 0.25 US$/kg for animal feed to 0.44 US$/kg for combined gum and feed production. However, this calculation is based on the gum price of 3 US$/kg (a typical price for gum arabic). Since the gum price is not certain, a graph was generated to display the net revenue for different gum prices (Fig. 6), as shown from which, the gum price had to be at least 2.3 US$/kg in order to make profits.

Conclusions

The gum extracted by NaOH solution from DG contained about 50–60 % polysaccharides, mainly arabinoxylans, and 10–20 % CP. Alkaline extraction conditions (alkali concentration, temperature and

reaction time) were shown to have significant effects on DG gum and DG residue yields and chemical compositions. High polysaccharide/CP ratio of DG gum was created at a high reaction severity, while high yield and CP content of DG residue appeared at a low severity. Bentonite clay was effective in removing CP from DG gum, but it was only able to lower the CP content to about 8 %.

Distillers' grains gum had unique emulsifying properties potential for practical applications, which were affected by the purification process. Unpurified DG gum was superior to the purified gum with better emulsifying properties. Additionally, DG residue had higher fiber content, fiber digestibility and ME level than raw DG. However, the CP content, an important nutrition in animal feed, depended greatly on extraction conditions. From the economic analysis, the DG gum production process is highly economically feasible and would make the corn ethanol industry more profitable.

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References

Asselman T, Garnier G (2000) Adsorption of model wood polymers and colloids on bentonites. Colloids Surf A 168:175–182

Brown RC, Brown TR (2012) Why are we producing biofuels. Brownia LLC, Ames

Dien BS, Ximenes EA, O'Bryan PJ, Moniruzzaman M, Li XL, Balan V, Dale B, Cotta MA (2008) Enzyme characterization for hydrolysis of AFEX and liquid hot-water pretreated distillers' grains and their conversion to ethanol. Bioresour Technol 99:5216–5225

Ebringerová A, Heinze T (2000) Xylan and xylan derivatives—biopolymers with valuable properties, 1. Naturally occurring xylans structures, procedures and properties. Macromol Rapid Commun 21:542–556

Goering HK, Van Soest PJ (1970) Forage fiber analyses (apparatus, reagents, procedures, and some applications). In: ARS/USDA Handbook No. 379. Superintendent of Documents, US Government Printing Office, Washington, DC

Goeser J, Combs D (2009) An alternative method to assess 24-h ruminal in vitro neutral detergent fiber digestibility. J Dairy Sci 92:3833–3841

Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100:10–18

Islam AM, Philips GO, Sljivo A, Snowden MJ, Williams PA (1997) A review of recent developments on the regulatory, structural and functional aspects of gum Arabic. Food Hydrocoll 11:493–505

Izydorczyk M, Biliaderis CG, Bushuk W (1991) Comparison of the structure and composition of water-soluble pentosans from different wheat-varieties. Cereal Chem 68:139–144

Izydorczyk MS, Biliaderis CG (1995) Cereal arabinoxylans: advances in structure and physicochemical properties. Carbohydr Polym 28:33–48

Jagadeesh D, Reddy DJP, Rajulu AV (2011) Preparation and properties of biodegradable films from wheat protein isolate. J Poly Environ 19:248–253

Krishnamoorthy U, Sniffen CJ, Stern MD, Van Soest PJ (1983) Evaluation of a mathematical model of rumen digestion and an in vitro simulation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuff. Br J Nutr 50:555–568

Kim Y, Hendrickson R, Mosier NS, Ladisch MR, Bals B, Balan V, Dale BE (2008) Enzyme hydrolysis and ethanol fermentation of liquid hot water and AFEX pretreated distillers' grains at high-solids loadings. Bioresour Technol 99:5206–5215

Kim Y, Hendrickson R, Mosier NS, Ladisch MR, Bals B, Balan V, Dale BE, Dien BS, Cotta MA (2010) Effect of compositional variability of distillers' grains on cellulosic ethanol production. Bioresour Technol 101:5385–5393

Lamsal BP, Pathirapong P, Rakshit S (2012) Microbial growth and modification of corn distillers dried grains with solubles during fermentation. Ind Crop Prod 37:553–559

Min DY, Li QZ, Chiang V, Jameel H, Chang HM, Lucia L (2014) The influence of lignin–carbohydrate complexes on the cellulase-mediated saccharification I: transgenic black cottonwood (western balsam poplar, California poplar) *P-trichocarpa* including the xylan down-regulated and the lignin down-regulated lines. Fuel 119:207–213

National Research Council (NRC) (2001) Nutrient requirements of dairy cattle, 7th edn. National Academy Press, Washington

Randall RC, Phillips GO, Williams PA (1988) The role of the proteinaceous component on the emulsifying properties of gum arabic. Food Hydrocoll 2:131–140

Renewable Fuel Association (RFA) (2013) Battling for the Barrel: 2013 Ethanol Industry Outlook

Robinson PH, Givens DI, Getachew G (2004) Evaluation of NRC, UC Davis and ADAS approaches to estimate the metabolizable energy values of feeds at maintenance energy intake from equations utilizing chemical assays and in vitro determinations. Anim Feed Sci Technol 114:75–90

Robinson PH, Karges K, Gibson ML (2008) Nutritional evaluation of four co-product feedstuffs from the motor fuel ethanol distillation industry in the Midwestern USA. Anim Feed Sci Technol 146:345–352

Shapouri H, Duffield JA, Wang M (2003) The energy balance of corn ethanol revisited. Trans ASAE 46:959–968

Saulnier L, Marot C, Chanliaud E, Thibault JF (1995) Cell-wall polysaccharide interactions in maize bran. Carbohydr Polym 26:279–287

Sun CH, Gunasekaran S (2009) Effects of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum. Food Hydrocoll 23:165–174

Sun R, Sun XF, Liu GQ, Fowler P, Tomkinson J (2002) Structural and physicochemical characterization of hemicelluloses isolated by alkaline peroxide from barley straw. Poly Int 51:117–124

Tan L, Eberhard S, Pattathil S, Warder C, Glushka J, Yuan CH, Hao ZY, Zhu X, Avci U, Miller JS, Baldwin D, Pham C, Orlando R, Darvill A, Hahn MG, Kieliszewski MJ, Mohnen D (2013) An *arabidopsis* cell wall proteoglycan consists of pectin and arabinoxylan covalently linked to an arabinogalactan protein. Plant Cell 25:270–287

Tobimatsu Y, Chen F, Nakashima J, Escamilla-Trevino L, Jackson L, Dixon R, Ralph J (2013) Coexistence but independent biosynthesis of catechyl and guaiacyl/syringyl lignin polymers in seed coats. Plant Cell 25:2587–2600

Tucker MP, Nagle NJ, Jennings EW, Ibsen KN, Aden A, Nguyen QA, Kim KH, Noll SL (2004) Conversion of distiller's grain into fuel alcohol and a higher-value animal feed by dilute-acid pretreatment. Appl Microbiol Biotechnol 113:1139–1159

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597

Weiss W, Conrad H, Stpierre N (1992) A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim Feed Sci Technol 39:95–110

Whistler RL (1993) Hemicelluloses. In: Whistler RL, BeMiller JN (eds) Industrial gums, polysaccharides and their derivatives, 3rd edn. Academic Press, San Diego, pp 295–308

Woo DH (2001) Stabilization of the emulsion prepared with dietary fiber from corn hull. Food Sci Technol 10:348–353

Xiang Z, Runge T (2014) Co-production of feed and furfural from dried distillers' grains to improve corn ethanol profitability. Ind Crop Prod 55:207–216

Xiang Z, Watson J, Tobimatsu Y, Runge T (2014) Film-forming polymers from distillers' grains: structural and material properties. Ind Crop Prod 59:282–289

Yadav MP, Johnston DB, Hicks KB (2009) Corn fiber gum: new structure/function relationships for this potential beverage flavor stabilizer. Food Hydrocoll 23:1488–1493

Yadav MP, Johnston DB, Hotchkiss AT, Hicks KB (2007) Corn fiber gum: a potential gum arabic replacer for beverage flavor emulsification. Food Hydrocoll 21:1022–1030

Web references

Alibaba (2014) http://www.alibaba.com/showroom/hydrochloric-acid-price.html. Accessed 26 May 2014

CMAI (2011) http://www.cmaiglobal.com/marketing/samples/cmr_summary.pdf. Accessed 26 May 2014

Energy Information Administration (EIA) (2014) http://www.eia.gov/electricity/monthly. Accessed 26 May 2014

Hofstrand D, Johanns A (2014) Ethanol, corn and DDGS prices at production facility by state. In: agricultural marketing resource center. http://www.agmrc.org/renewable_energy/ethanol/ethanol-prices-trends-and-markets/. Accessed 26 May 2014

Nasdaq (2014a) http://www.nasdaq.com/markets/ethanol.aspx?timeframe=1y. Accessed 26 May 2014

Nasdaq (2014b) http://www.nasdaq.com/markets/natural-gas.aspx?timeframe=1y. Accessed 26 May 2014