Utilization of selected biorenewable resources: solubilization of lignocellulosics and conjugation of soybean oil

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Utilization of selected biorenewable resources: solubilization of lignocellulosics and conjugation of soybean oil.

by

Reed E. Oshel

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Co-Majors: Chemistry; Biorenewable Resources and Technology

Program of Study Committee:
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Iowa State University
Ames, Iowa
2007

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ABSTRACT

In recent years, concern has risen over the use of fossil fuels due to their contribution to global warming, and to our dependence on imports of petroleum from nations that could pose a threat to national security. As a result, it has become increasingly important to develop technologies to replace fossil fuel based products with biorenewable alternatives. In this thesis nearly quantitative solubilization of lignocellulosic materials using phosphite esters has been realized, and is presented as a potential pretreatment for production of fermentable sugars for use in manufacturing commodity chemicals, specifically ethanol. Water solubilization of lignocellulosics using phosphite esters will enhance digestibility by disrupting the lignocellulose structure, changing cellulose morphology, and cleaving some glycosidic bonds. In a second project, soybean oil, which contains un-conjugated polyunsaturated fatty acid esters, is isomerized into oil containing conjugated polyunsaturates. The process is carried out under photochemical conditions using iodine as a catalyst in a hexanes solution to achieve 99% conjugation. The resulting conjugated soybean oil is demonstrated to have enhanced drying properties for use in alkyd resins.
CHAPTER 1. UTILIZATION OF SELECTED BIORENEWABLE RESOURCES OVERVIEW

1.1 Introduction

Biorenewable resources are defined as organic materials of recent biological origin, and therefore exclude all petroleum and coal derived products. The utilization of these materials in applications currently using fossil fuel derived materials is important in environmental, economic and political aspects. Processing fossil fuels and their eventual breakdown releases CO₂ into the atmosphere that would not otherwise be involved in normal environmental cycles, and elevated CO₂ levels can be linked to global warming (Brown, 2003). A decrease in demand for materials derived from petroleum would lighten the need for crude oil imports. If biorenewable resources are utilized instead petroleum based products, there is an opportunity to create more domestic jobs and new products through the production and processing of those biorenewable materials. In fact, the greatest potential in this area lies in rural communities which are in need of stronger economies (Brown, 2003). Consequently, increased domestic production and decreased, or leveled off, imports of crude oil would boost the national economy and decrease dependency on foreign oil. A decreased dependency on foreign oil would limit the influence of oil producing, politically turbulent nations on the United States of America.

Recognizing these important impacts of using biorenewable resources, the Department of Energy in 1998 issued the vision statement titled, Plant/Crop-Based Renewable Resources 2020: A Vision to Enhance U.S. Economic Security Through Renewable Plant/Crop-Based Resource Use. In that statement it was stated that, “The vision is to provide continued economic growth, healthy standards of living, and strong national
security through the development of plant/crop-based renewable resources that are a viable alternative to the current dependence on nonrenewable, diminishing fossil fuels.”

Furthermore, the goal is to increase plant-derived materials to meet 10% of chemical feedstock demand by 2020. In order to meet this goal, the Department of Energy followed up their vision statement with “The Technology Roadmap for Plant/Crop-Based Renewable Resources 2020” which identified four barriers: Plant Science, Production, Processing, and Utilization to achieving the goals set by the vision statement. Both the vision and the roadmap documents invite readers from industry, academia, and government to take part and contribute to realizing that vision by overcoming the barriers. We accepted this invitation in the two categories of processing and utilization, and have chosen lignocellulosics and soybean oil as resources to explore for industrial applications.

1.1.1 Solubilization of Lignocellulosics Strategy

Lignocellulosic materials represent the most abundant renewable resource. They are comprised of a complex structure of cellulose, hemi-cellulose, and lignin. They are also a virtually untapped industrial source of fermentable sugars. Pretreatment of lignocellulosics for production of ethanol currently accounts for as much as 33% of the operating costs (Brown, 2003). Development of new processing techniques would allow for more economical ethanol or other value-added chemical production through fermentation of sugars obtained from the cellulose and hemi-cellulose portions of lignocellulosic materials. It would also be desirable if, in the processing, we would be able to produce a value-added co-product from the un-fermentable portions including lignin. A suitable pretreatment would allow a wide variety of plant material, including herbaceous material and woody biomass from both softwood and hardwood, to be utilized as ethanol feedstocks.
Phosphorylation of cellulose can greatly change its chemical properties. The goal of using phosphite esters as a pretreatment for lignocellulosics processing is to derivatize components of lignocellulosic materials to interrupt intermolecular bonding (mostly H-bonding), in addition to acting as a traditional solvent. The derivatized material including the cellulose portion should be subsequently water-soluble in order to be compatible with biological downstream processing such as enzymatic saccharification or fermentation. Since cost and environmental friendliness are of great concern in an application intended for large scale production, efforts aimed at recovery and recycling of the phosphite ester or to produce a value-added co-product will also be discussed.

1.1.2 Soybean oil Conjugation Strategy

Soybean oil (SBO) has been widely used in alkyd resins for inks and paints in the past as what is classified as a semi-drying oil. Drying oils like linseed oil have a higher number of polyunsaturated fatty acids (e.g. linolenic, 18:3, cis-9, cis-12, and cis-15) in their triglyceride structure (i.e. more C=C bonds per fatty acid chain). Fast-drying oils, like tung oil, dry even faster than linseed oil, not because they have more C=C bonds, but because the C=C bonds in their triglyceride structure are conjugated (e.g. eleosteric acid, 18:3, trans-9, trans-11, and cis-13) (Gooch, 2002). The C=C bonds in SBO are naturally unconjugated in the form of linoleic acid (18:2, cis-9, cis-12) or linolenic acid (see above), which means pairs of C=C bonds are separated by a methylene CH₂ group. In order to enhance the reactivity of SBO and upgrade its drying ability, a chemical transformation of its fatty acids to either more unsaturated or conjugated fatty acids seems to be required. Herein we focus on the conjugation of SBO to enhance its drying properties. In devising a method to achieve this isomerization to conjugated C=C bonds, it is important for industrial feasibility that the
catalyst used be environmentally friendly, efficiently removed from the final product, and either cheap or recyclable. In addition, the conjugation should not disrupt the triglyceride backbone of the SBO, and afford improved drying properties over linseed oil.

1.2 Criteria Review

Efforts in developing the technologies described above will be discussed first and foremost in terms of chemical analysis and transformations since the chemistry relating to biorenewable materials is the focus of the program of study culminating in this thesis. However, results will ultimately be viewed in terms of impact on industrial compatibility/feasibility and on the environment. Because the focus of these efforts is to advance the use of biorenewable materials as replacements for fossil fuel derived ones, any use of fossil fuel materials will be avoided if possible. If materials are used that are not derivable from biorenewable resources, recycling will be emphasized/discussed. Considering the stage of development of the technologies we have investigated, no detailed economic or cost benefit analysis will be reported at this time except for general reference to current starting material cost and/or current processing economy.
CHAPTER 2. SOLUBILIZATION OF LIGNOCELLULOSICS VIA PHOSPHITE ESTERS AND AQEOUS PHOSPHATE SOLUTIONS

(submitted to Bioresource Technology, see Vitae below)

2.1. Introduction

Methods for the degradation of cellulosic materials to oligosaccharides and sugar alcohols aimed at facilitating ethanol production, continue to be the subject of wide and intense interest. Such methods include cellulose treatment with enzymes [mainly cellulases and hemicellulases (Demain et al., 2005; Fan et al., 1987; Zhang and Lynd, 2004)], mineral acids (Mok et al., 1992), bases (Ishida et al., 2005), supercritical water (Sasaki et al., 2000), hot water in the presence of a strongly acidic cation exchange resin (Kim et al., 2003; Ladisch et al., 2003), hot water solutions of lanthanide salts (Sakaki et al., 2002), and, more recently, platinum or ruthenium-supported catalysts that accomplish conversion to sugars (Fukuoka and Dhepe, 2006).

Approaches to simple disruption of the hydrogen bonds in cellulose have also been described, as for example, hot water treatment (Kobayashi et al., 2005), pH controlled hot water treatment (Mosier, 2005), extrusion/explosion processing of ammonia-impregnated fibers (AFEX) (Dale et al., 1998, 1999, 2005; Liu and Shi, 2005), steam explosion (Sun et al., 2005; Josefsson et al., 2002; Jain et al., 1999; Wu et al., 1998, 1999), ultrasound treatment (Yang et al., 2004), and dissolution in ionic liquids (Zhu et al., 2006). The use of mixtures of electron-donor solvents with nitrogen oxides, lithium chloride, triethylamine oxide, methylmorpholine oxide, trifluoroacetic acid, orthophosphoric acid, and aqueous solutions of zinc chloride for dissolving cellulose, has been reviewed (Grinshpan, 1998).
In addition to dissolution of cellulosic materials in some of the aforementioned media, some chemical derivatization can and probably does occur, as in the cases of trifluoroacetic and orthophosphoric acids to form trifluoroacetate and phosphate esters, respectively. Dissolving cellulose in an acid anhydride can lead to regioselectively functionalized polymers (El Seoud and Heinze, 2005), and regioselective esterification and etherification of glucose has been demonstrated to influence the processing and use of these products (Burkart et al., 1996). The synthesis of cellulose sulfonates (e.g., tosylates and mesylates) provides polymers with interesting properties as well as intermediates to new cellulosic products (Siegmund and Klemm, 2002). Fatty acid esters of cellulose lead to novel bioplastics and films (Song et al., 2002; Satge et al., 2004). Such esters also open new synthetic possibilities for introducing functional groups into cellulose providing pathways to cellulose esters and ethers and their derivatives, as well as biologically active molecules covalently bound to cellulose (Bojanic et al., 1998). The reaction kinetics of the production of cellulose ethers (e.g., methyl, hydroxyethylmethyl and hydroxyethyl) have also been reviewed (Doenges, 1990).

As a percentage of the approximately 89% dry matter in Distillers Dry Grains and Solubles (DDGS) obtained from Big River Resources, LLC, Burlington, IA, cellulose and starch (polyglucoses) comprise ca 16 and 5%, respectively, and the hemicelluloses (polypentoses) xylan, and arabinan comprise a total of about 13.5% (Hendrickson et al., 2007). None of these polysaccharides have appreciable solubility in water, and so it is desirable to develop reasonably mild methods for degrading and/or derivatizing these materials in such a way as to solubilize them in water, since water is the solvent of choice for the commercial production of ethanol by enzymatic means. Thus water solubilization of these polysaccharides and heteropolysaccharides facilitate access to them by cellulases and
fermentation enzymes. A recent review (Moiser et al., 2005) describes desired traits in a pretreatment, including its effect on biomass surface area, cellulose crystallinity, and hemicellulose and lignin processability. A review of current pretreatment technologies is also given (Moiser et al., 2005). A coordinated effort to develop leading pretreatment technologies was also reported earlier in this journal (Wyman et al. 2005).

Phosphitylation has been developed in recent years as a technique for derivatizing carbohydrates, nucleosides and nucleotides (Dabkowski and Michalski, 2004, 2005; Laneman, 2005; Ahmadiben and Parang, 2005; Oka et al., 2003; Parang et al., 2001), although this technique has been known longer for simple alcohols (Dabkowski et al., 2005; Watanabe et al., 1990). In our efforts to solubilize cellulosic materials with phosphite esters, we have taken advantage of this well known reaction which is an equilibrium transesterification process in which a new phosphite ester is formed with the release of a new alcohol.

Our efforts described in this chapter are focused on developing a novel method for pretreating DDGS which renders the water-soluble product suitable for digestion in animal feed and/or to enzymatic hydrolysis to enzymatically fermentable sugars for ethanol production after minimal neutralization with base. Transesterification can be driven by the

$$\text{P} \quad {\begin{array}{c} \text{OEt} \\ \text{OEt} \\ \text{OEt} \end{array}} \quad + \quad \text{HOR} \quad \leftrightarrow \quad (\text{EtO})_2\text{POR} \quad + \quad \text{EtOH} \quad \text{(Reaction 1)}$$

$$\text{P} \quad {\begin{array}{c} \text{OR} \\ \text{OR} \end{array}} \quad + \quad \text{HO-Cellulose} \quad \leftrightarrow \quad (\text{RO})_2\text{PO-Cellulose} \quad + \quad \text{ROH} \quad \text{(Reaction 2)}$$

R = H, alkyl, or aryl

**Figure 1.** Reactions of phosphite esters with alcohol or carbohydrate hydroxyl
release of a more volatile alcohol as in Reaction 1 in Figure 1 wherein R is larger than an ethyl group. It has been observed in our laboratories that phosphite esters are also capable of dissolving cellulosic materials to varying degrees (depending on the source) via conversion of at least some of the hydroxyl groups to phosphite ester groups in a phosphitylation reaction (e.g., reaction 2, Figure 1) the details of which are incompletely understood.

2.2 Methods and Materials

2.2.1 Materials

Hot Water Treated Distillers Dry Grains and Solubles (HWT-DDGS) was provided by Professor Rick Hendrickson of Purdue University. This material had been prepared by heating light stillage from Big River Resources, LLC to 160 °C for 20 minutes. Samples of HWT-DDGS were centrifuged and the solids were separated from the liquid solubles. The recovered water-insoluble solids were then dried under vacuum at 45 °C until free flowing dry solids were obtained. Before treatment with phosphites, the dried HWT-DDGS was ground to 0.5 mm. AFEX DDGS was provided by Professor Bruce Dale of Purdue University. Lignin was obtained from Westvaco as Indulin AT, a kraft pine lignin polymer.

Some of the phosphorus compounds utilized in this study (namely, 1-6) are shown in Figure 2. 2,6,7-trioxa-1-phosphabicyclo[2.2.1]heptane (1) was prepared according to a published procedure (Denny, et al., 1973). 4-methyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane (2) and 4-ethyl-2,6,7-trioxa-1-phosphabicyclo[2.2.2]octane (3) in Figure 2 were also prepared as reported earlier (Wadsworth and Emmons, 1962). Phosphite 3 was also a kind gift from Rhodia. 2,6,7-Trioxa-1-phosphabicyclo(2.2.2)octane-4-methanol (4) was prepared according to a literature method (Pike et al., 2001) as were 1,3,2-dioxaphosphorinan-2-one (5) and 5,5-Dimethyl-1,3,2-dioxaphosphorinan-2-one (6) (Figure 2) (Maffei and Buono, 2003). Phosphorous acid (99%), (MeO)₃P (99+%), (EtO)₃P (98%), (iPrO)₃P (95%), (PhO)₃P
(97%), and (EtO)₂P(O)H (94%) were all purchased from Aldrich Chemical Company and were used as delivered.

2.2.2 Sample procedures for thermal reactions

2.2.2.1 Treatment of HWT-DDGS with 3

HWT-DDGS (250 mg), 3 (7.14 g) and a small octagonal magnetic stir bar were charged to a 15 mL Ace Glass 150 psi pressure tube. The tube was sealed with a Teflon cap and submersed up to its seal in a 100 °C oil bath in order to ensure even heating of the tube and its contents and to avoid sublimation of 3 to cooler parts of the tube. The mixture was stirred for 24 hrs and then it was allowed to cool to room temperature whereupon 10 mL of methanol was added. After all the phosphite and derivatized HWT-DDGS were allowed to equilibrate with the methanol over night, the solids were filtered and weighed (Figure 3).

Figure 2. Phosphite esters 1-4, phosphonates 5 and 6, and potential products 7 and 8.
2.2.2.2 Treatment of HWT-DDGS with 3/water

This reaction was carried out as described in the preceding paragraph except that 1 mL (1.25 eq.) of water was also charged to the pressure tube after adding 3. However, no submersion of the tube to its seal was required because sublimation of 3 does not occur in the presence of water.

2.2.2.3 Treatment of HWT-DDGS with all other phosphites/phosphonates with and without water

These treatments were performed as described in section 2.2.1 using 250 mg of HWT DDGS and 44 mmol of the appropriate phosphorus compound. Water (1.25 eq) was added to the phosphorus compound unless otherwise stated.

2.2.3 Sample procedure for microwave reactions

HWT-DDGS (1 g), 3 (28.6 g) and a magnetic stir bar, were charged to an 80 mL reaction vessel and the vessel was placed in a CEM Discover microwave reactor. The conditions employed were 300W, 130 °C, a maximum pressure of 230 psi, and a 6 hr reaction time.

**Figure 3.** Phosphite treatment.
After the reaction vessel had cooled and was removed from the reactor, its contents were treated in the same manner as described in section 2.2.1.

2.2.4 \textit{NMR spectroscopy}

All NMR spectroscopy was conducted on a Varian VXR-400MHz NMR instrument. Samples were prepared in either MeOH or H$_2$O to avoid relatively slow H-D exchange with the P-H hydrogen leading to observation of unwanted P-D couplings which would needlessly complicate the $^{31}$P NMR spectrum. CDCl$_3$ was used as the external reference.

2.3. \textit{Results and Discussion}

2.3.1. \textit{Solubility Studies with phosphite esters}

A survey of the series of commercially available acyclic phosphite esters P(OMe)$_3$, P(OEt)$_3$, P(Oi-Pr)$_3$ and P(OPh)$_3$ revealed poor solvent properties at temperatures in excess of 100 °C for HWT-DDGS (and for other cellulosic materials as well) even after prolonged treatment. With the bicyclic phosphite esters 1 – 4 (Figure 2), however, we encountered more encouraging results. The decision to evaluate these bicyclic esters originated from the observation by David G. Hendricker (a graduate student in this lab at the time) over 40 years ago that a boiling stick (bonded balsa wood) in a beaker containing a reaction mixture for the

\begin{table}
\centering
\begin{tabular}{|c|c|c|l|}
\hline
Entry & Phosphite & Pretreatment & Solubility observations \\
\hline
1 & 2 & AFEX & Partial with small decrease in solute mass; slight tint to the phosphite solution \\
2 & 3 & AFEX & Partial with small decrease in solute mass, but greater than for Entry 1 \\
3 & 2 & HWT & Mostly soluble; significant decrease in solute mass; distinct coloration of the phosphite solution \\
4 & 3 & HWT & Mostly soluble; even greater decrease in solute mass than for Entry 3; distinct coloration of the phosphite solution \\
\hline
\end{tabular}
\caption{Screening of pretreated DDGS solubility in 2 and 3.\textsuperscript{a}}
\end{table}

\textsuperscript{a}3.5\% (w/w) of DDGS (35 mg) in 2 or 3 (1 g) was employed. Each sample was heated at
synthesis of 3 appeared to disintegrate/dissolve.

Although the reaction of 1 with HWT-DDGS produced no appreciable solubilization and results for 4 revealed poor solubility in the same reaction, both 2 and 3 proved interesting. Compounds 1 and 4 are not commercially available but 2 and 3 are. The nature of our initial screening experiments with phosphites 2 and 3 deserves comment at this point. Because these phosphites are viscous liquids at their melting points, thus making filtration difficult at best, we first made qualitative visual estimates of how much HWT-DDGS appeared to be solubilized after pretreatment with these phosphite esters. For that purpose we used a large mass ratio of phosphite to HWT-DDGS in a centrifuge tube having a conical bottom in order to enable visual estimation of how much undissolved material remained in the molten phosphite (see Table 1). When HWT-DDGS solubilization occurs, the phosphite changes from clear to yellowish. The solubility in Entry 2 in Table 1 was estimated at approximately 25% reduction in solids by centrifugation of the hot reaction mixture after stirring for 24 hours at 80 °C, and the solubility in Entry 4 was estimated at 50% by the same method. From these tests it was determined that 3 was the better medium for solubilizing the HWT-DDGS which exhibited somewhat greater solubility than the AFEX pretreated material.

In order to gain a more quantitative estimate of the solubility of the HWT-DDGS in 3, the reaction in Entry 4 of Table 1 was repeated except that stirring was continued for 48 instead of 24 hours. Because the supernatant from the reaction mixture was soluble in methanol, the reaction mixture was extracted with methanol followed by filtration of the remaining solids. The percent solubility was then calculated from equation 1. The solubility of HWT-DDGS
\[ \frac{(\text{Initial HWT-DDGS Mass} - \text{Remaining DDGS Mass})}{\text{Initial HWT-DDGS Mass}} \times 100 \] (equation 1)

in 3 was found to be only 19% (Table 2, Entry 1). It should be noted that equation 1 does underestimate the amount of HWT-DDGS that solubilized, however, since the elemental phosphorus content increases from 0.47% in the HWT-DDGS to 2.28% in the solid material remaining after reaction of the HWT-DDGS with 3. In addition, it was observed in larger

**Table 2. Optimization of HWT-DDGS solubilization protocol.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Phosphate</th>
<th>Lignocellulose solute</th>
<th>Temp/Energy source</th>
<th>% Mass change in solute$^g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>HWT-DDGS$^b$</td>
<td>100 °C</td>
<td>-19%</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>HWT-DDGS$^b$</td>
<td>μλ</td>
<td>-10%</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>HWT-DDGS$^b$</td>
<td>100 °C</td>
<td>+15%</td>
</tr>
<tr>
<td>4</td>
<td>3/H$_2$O$^c$</td>
<td>HWT-DDGS$^b$</td>
<td>100 °C</td>
<td>-92%$^b$</td>
</tr>
<tr>
<td>5</td>
<td>3/H$_2$O$^c$</td>
<td>HWT-DDGS$^b$</td>
<td>80 °C</td>
<td>-86%</td>
</tr>
<tr>
<td>6</td>
<td>3/H$_2$O$^c$</td>
<td>HWT-DDGS$^b$</td>
<td>150 °C</td>
<td>-99%</td>
</tr>
<tr>
<td>7</td>
<td>3/H$_2$O$^c$</td>
<td>HWT-DDGS$^b$</td>
<td>μλ</td>
<td>-74%$^d$</td>
</tr>
<tr>
<td>8</td>
<td>4/H$_2$O$^c$</td>
<td>HWT-DDGS$^b$</td>
<td>100 °C</td>
<td>+750%</td>
</tr>
<tr>
<td>9</td>
<td>3/H$_2$O$^c$</td>
<td>AFEX$^b$</td>
<td>100 °C</td>
<td>-62%</td>
</tr>
<tr>
<td>10</td>
<td>3/H$_2$O$^c$</td>
<td>DG$^e$</td>
<td>150 °C</td>
<td>-99%</td>
</tr>
<tr>
<td>11</td>
<td>3/H$_2$O$^f$</td>
<td>HWT-DDGS$^b$</td>
<td>150 °C</td>
<td>-99%</td>
</tr>
</tbody>
</table>

$^a$ 3.5% (w/w) of lignocellulose solute (250 mg) in phosphite (7.14 g) was employed. Samples were heated at 100°C for 24 hours.  
$^b$  Ground to 0.5 mm particle size.  
$^c$ 7.14 g phosphite/1 mL (1.25 eq) H$_2$O  
$^d$ Phosphite solution turned black and the $^{31}$P NMR spectrum showed formation of (O)HP(OH)$_2$.  
$^e$ Obtained from Big River Resources, LLC and used without further treatment; water content ca 41%.  
$^f$ 1:1 mol ratio of phosphite to water pre-reacted at 100°C prior to addition of lignocellulosic material.  
$^g$ Calculated as the difference in mass of insoluble portions of the solute divided by the original mass of the solute. See text for explanation of the signs.  
$^h$ Avg. of multiple trials ranging from 86-97%.
scale reactions (ca 1 g of HWT-DDGS and 28.6 g of phosphite) that some precipitation occurred upon methanol extraction. This precipitate, which is presumed to be partially derivatized HWT-DDGS, would add mass to the insolubles and thus further skew the results obtained with equation 1. It should be emphasized here that, to the extent that reactions are occurring between 2 and 3 with cellulosic OH groups, these phosphites are behaving as reactive derivatizing solvents as well as conventional solvating solvents.

2.3.2 Solubility Studies with 3 in the presence of water

Having determined 3 to be the better solubilizing agent, we shifted our focus to developing a standard, optimized treatment. We first looked at using an alternate energy source (Table 2, Entry 2), but microwave irradiation did not afford higher solubility. Presuming that an extra hydroxyl group on the phosphite might afford greater solubility owing to increased disruption of hydrogen bonding among the cellulose chains, 4 was investigated as a solubilizing agent. However, 4 revealed a "positive insolubility" (and hence the positive coefficient for the HWT-DDGS mass change in Entry 3 of Table 2. This result is likely to be due to a derivatization and/or adsorption phenomenon involving surfaces of the solids.

The HWT-DDGS we used contains approximately 8-10% moisture, which may in part account for its better solubility behavior in 3 than the AFEX-DDGS (see above). Thus we were delighted to observe that the addition of 1 mL (0.056 mol, 1.25 eq) of water to 7.14 g (0.044 mol, 1.00 eq) of 3 followed by heating to 100 ºC resulted in what appeared to be nearly complete dissolution of the 250 mg of HWT-DDGS (Table 2, Entry 4). Addition of methanol to the reaction mixture followed by filtration showed that 92% of the HWT-DDGS had dissolved, leaving a very small amount of an almost colorless insoluble material (HWT-
DDGS and/or derivatized HWT-DDGS) which was weighed. Upon evaporation of the methanol from the filtrate, a viscous yellow residue remained. A 200 mg sample of this residue readily dissolved in 2 mL of water at room temperature. The maximum solubility of this product residue in water has yet to be determined. Decreasing the reaction temperature lowered the solubility (Table 2, Entry 5), while increasing the temperature to 150 °C improved the solubility to nearly quantitative (Table 2, Entry 6). Again, microwave irradiation failed to improve solubility, and the use of 4 with water added to it increased the mass of the insoluble material (Table 2, Entries 7 and 8, respectively). Using 3/H2O was also effective for solubilizing AFEX and DDG that had not been treated with hot water (Table 2, Entries 9 and 10, respectively). The result with DDG is significant in that no other pretreatment is necessary when using 3/H2O.

On the basis of the foregoing results, it is likely that at least partial hydrolysis of 3 (Bertrand et al.) occurs to produce an active reagent(s) (Scheme 1) for solubilizing cellulose

**Scheme 1. Hydrolysis of 3.**

- $\text{H}_2\text{O}\rightarrow \text{3}$
- $\text{H}_2\text{O}\rightarrow \text{9}$
- $\text{H}_2\text{O}\rightarrow \text{10}$
- $\text{H}_2\text{O}\rightarrow \text{11}$
- $\text{H}_2\text{O}\rightarrow \text{12}$
and starch. (The nature of the reaction involving lignin is not clear yet although we have shown that a methylphenyl-ether linkage is probably not cleaved since 1,4-dimethoxybenzene is unreactive with 3 under our conditions.) When 3 is hydrolized before adding the HWT-DDGS, the solvent properties are the same as when water is combined with 3 and HWT-DDGS at the same time (Table 2, Entry11). This indicates that hydrolysis of 3 probably occurs faster than its solubilizing action. 31P NMR spectroscopic studies demonstrate that in the presence of water, hydrolysis of 3 (δ 94 ppm) produces peaks ranging from 12 - 4 ppm, which is in the phosphonate [O=PH(OR)2] region of 0 – 20 ppm (Tebby, 1991). On the other hand, a mixture of methanol and 3 displayed no peaks in the phosphonate region (even after heating to 150 °C) and only the 94 ppm peak corresponding to 3 was still present.

Since only 1.25 equivalents of water were added to the reaction (Table 2, Entry10 and 11) we suspect that most of the phosphite 3 was hydrolyzed to the phosphonate 9 (Scheme 1) and was not completely hydrolyzed to phosphorous acid and trimethylolpropane (11 and 12, respectively, in Scheme 1). This point is of some importance because these results strongly suggest that the phosphite is not merely a source of acid for the well known acid-catalyzed hydrolysis of polycarbohydrates.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Phosphite (g)</th>
<th>H2O or MeOH (mL)</th>
<th>% Solubleb</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.14</td>
<td>0.00</td>
<td>19%</td>
</tr>
<tr>
<td>2</td>
<td>7.14</td>
<td>1.00</td>
<td>99%</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>0.07</td>
<td>86%</td>
</tr>
<tr>
<td>4</td>
<td>7.14</td>
<td>0.08</td>
<td>37%</td>
</tr>
<tr>
<td>5</td>
<td>0.93</td>
<td>1.00</td>
<td>89%c</td>
</tr>
<tr>
<td>6</td>
<td>4.50</td>
<td>1.00</td>
<td>100%d</td>
</tr>
<tr>
<td>7</td>
<td>7.14</td>
<td>1.76 MeOH</td>
<td>36%</td>
</tr>
</tbody>
</table>

*aAll treatments were performed with 250 mg HWT-DDGS heated at 150 °C for 48 h. bDetermined from (weight of insoluble material after methanol extraction)/(initial weight of lignocellulosic material)x100%. c10 mol% 3 based on water; reaction mixture turned black. d50 mol% 3 based on water.
To further investigate the role of water, we conducted a series of experiments in which the amount of water and phosphite were varied (Table 3), and we also employed pure anhydrous phosphorous acid (Table 4, Entry 11) in this study. It is important to note that solubility is poor when no or only a catalytic amount of water is added (Entries 1 and 4, respectively). Entry 3 shows that a substantial quantity of HWT-DDGS material can be solubilized in the 3/H2O mixture since the solution formed is 43% w/w in HWT-DDGS based on the weight of phosphite 3. When excess water is used (Entry 5, Table 3) the result is similar to when pure phosphorous acid is employed (Table 4, Entry 11). Thus the solubility appears to be good but the solution turns black, indicating possibly undesired decomposition.

One of the more promising results is shown in Entry 6 of Table 2 where 3 is used in only 50 mol% relative to water, and yet almost complete solubility is observed. This suggests that less phosphite 3 may be necessary while not sacrificing solubility appreciably. This, however, raises the question of whether 10 (the secondary hydrolysis product of 3) may also be a good or even better solubilizing agent. In two solubility experiments with a 1:1 weight ratio of HWT-DDGS:3, wherein 1 eq of water was added in one experiment and 2 eq in the other trial, no significant difference in solubility was observed. These results imply that the dihydrolized species 10 in Scheme 1 was equivalent in solubilizing action as was 9. An approximately equimolar amount of methanol had a measurable effect on the solubility of HWT-DDGS using 3 (Table 3, Entry 7) as opposed to the presence of no added co-solvent (Table 3, Entry 1). The origin of this effect is presently unclear.

Since adding water improved the lignocellulose solubilizing ability of 3, we returned to our study of acyclic phosphites that had earlier revealed poor solubilizing properties by
themselves, in order to investigate the effectiveness of adding water to them. We also wished to examine several phosphonates that resemble compound 5. Such compounds could prove to be solubilizing solvents that could rival 3/H2O. The results summarized in Entries 1-4 of Table 4 demonstrate that solubilities were indeed improved by in the presence of water, but the solubilities realized were inferior to those achieved with 3/H2O. The good to excellent solubilities observed with the cyclic phosphonates (Table 4, Entries 7-10) supports our hypothesis that one or more hydrolyzed products of 3 are important in providing high solubilities of lignocellulosic material.

It was observed that the acyclic candidate diethylphosphonate (Table 4, Entry 5) did not provide HWT-DDGS solubility as great as a mixture of triethyl phosphite and 1.25 eq of water (Entry 2) although these solubilizing systems might have been expected to be approximately equivalent solvent systems according to our original hypothesis. However, the hydrolysis of triethyl phosphite also produces ethyl alcohol which was shown to enhance solubilization in the presence of diethylphosphonate (see Entries 5 and 6). On the other hand, however, addition of 1 eq of ethanol to 2,2-dimethyl-1,3-propane phosphonate (Entry 9) showed no effect on solubility. It is apparent that these observations comprise a complex phenomenon requiring additional study to understand the role of ethanol in the solubilization using triethyl phosphite. The possibility that the superior solubilization properties of 3/H2O may be attributed to the hydroxyl group present on a molecule of 9 (monohydrolized 3) should also be investigated. To confirm that hydrolyzed phosphites 9 and 10 are not merely sources for phosphorous acid, we employed commercially available phosphorous acid (11,
99% pure crystalline solid) as a solubilizing agent for HWT-DDGS and also for lignin (Entry 11, Table 4, and Entry 9, Table 5, respectively). As seen in these entries, phosphorous acid was quite effective in solubilizing HWT-DDGS and it even partially dissolved lignin. However, in both cases, the reactions produced black solutions and black residues. Although this result is not surprising in the case of the lignin experiment (since the lignin used was

Table 4. Screening of phosphites and phosphonates with HWT-DDGS.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Entry</th>
<th>Phosphite</th>
<th>H\textsubscript{2}O or EtOH</th>
<th>% Soluble\textsuperscript{b}</th>
</tr>
</thead>
</table>
| 1     | \[
\text{MeO}^\text{O} \text{O}^\text{Me} \text{P}^\text{O} \text{Me} \text{O}^\text{Et} \text{O}^\text{Et}
\] | 1.25 eq | 86% |
| 2     | \[
\text{EtO}^\text{O} \text{O}^\text{Et} \text{P}^\text{O} \text{Et} \text{O}^\text{Et}
\] | 1.25 eq | 73% |
| 3     | \[
\text{iPrO}^\text{O} \text{O}^\text{iPr} \text{P}^\text{O} \text{iPr} \text{O}^\text{iPr}
\] | 1.25 eq | 46% |
| 4     | \[
\text{PhO}^\text{O} \text{O}^\text{Ph} \text{P}^\text{O} \text{Ph} \text{O}^\text{Ph}
\] | 1.25 eq | 31% |
| 5     | \[
\text{EtO}^\text{O} \text{O}^\text{Et} \text{P}^\text{O} \text{Et} \text{O}^\text{Et}
\] | 0.00 eq | 36% |
| 6     | \[
\text{EtO}^\text{O} \text{O}^\text{Et} \text{P}^\text{O} \text{Et} \text{O}^\text{Et}
\] | 1.00 eq EtOH | 63% |
| 7     | \[
\text{HO}^\text{O} \text{O}^\text{OH} \text{P}^\text{O} \text{OH} \text{O}^\text{OH}
\] | 0.00 eq | 74% |
| 8     | \[
\text{HO}^\text{O} \text{O}^\text{OH} \text{P}^\text{O} \text{OH} \text{O}^\text{OH}
\] | 0.00 eq | 77% |
| 9     | \[
\text{HO}^\text{O} \text{O}^\text{OH} \text{P}^\text{O} \text{OH} \text{O}^\text{OH}
\] | 1.00 eq EtOH | 77% |
| 10    | \[
\text{HO}^\text{O} \text{O}^\text{OH} \text{P}^\text{O} \text{OH} \text{O}^\text{OH}
\] | 0.00 eq | 90%\textsuperscript{c} |
| 11    | \[
\text{HO}^\text{O} \text{O}^\text{OH} \text{P}^\text{O} \text{OH} \text{O}^\text{OH}
\] | 0.00 eq | 86%\textsuperscript{d} |
| 12    | \[
\text{MeO}^\text{O} \text{O}^\text{Me} \text{P}^\text{O} \text{Me} \text{O}^\text{Me}
\] | 0.00 eq | 27% |
| 13    | \[
\text{MeO}^\text{O} \text{O}^\text{Me} \text{P}^\text{O} \text{Me} \text{O}^\text{Me}
\] | 1.00 eq | 61% |

\textsuperscript{a}All treatments were performed with 250 mg HWT-DDGS heated at 150 °C for 48 Hrs. \textsuperscript{b}Determined from: (weight of insoluble material after methanol treatment)/(initial weight of lignocellulosic material)\times100\%. \textsuperscript{c}80 °C. \textsuperscript{d}Solids and solution blackened.

\textsuperscript{a}All treatments were performed with 250 mg HWT-DDGS heated at 150 °C for 48 Hrs. \textsuperscript{b}Determined from: (weight of insoluble material after methanol treatment)/(initial weight of lignocellulosic material)\times100\%. \textsuperscript{c}80 °C. \textsuperscript{d}Solids and solution blackened.
black-brown) blackening of the tan HWT-DDGS sample is likely due to undesirable decomposition. The alkyl phosphonate ethyldiethylphosphonate in Entry 12 of Table 4 solubilized only 27% of the HWT-DDGS sample, while the same experiment carried out with the addition of 1.00 equiv of water produced 61% solubility (Entry 13) implying that the relatively small solubility observed in Entry 12 might be due to hydrolysis of one of the –OMe ester groups of the alkyl phosphonate by moisture in the HWT-DDGS and/or in the alkyl phosphonate.

It is interesting to note that our observation of 99% solubility of HWT-DDGS in 3/H$_2$O is over 60% more than the total 34.5% carbohydrate analysis (Hendrickson et al., 2007) indicating substantial solubilization of other constituents by 3/H$_2$O. Since spectroscopic analysis of the complex mixture we produce is extremely difficult, we studied some model systems in an effort to elucidate some of the chemistry that was occurring in the solubilization process. To insure that we were indeed solubilizing the cellulose content of

<table>
<thead>
<tr>
<th>Entry</th>
<th>Component</th>
<th>Phosphite</th>
<th>Water</th>
<th>Solubility$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cellulose$^c$</td>
<td>3</td>
<td>1.25 eq</td>
<td>99%</td>
</tr>
<tr>
<td>2</td>
<td>Cotton</td>
<td>3</td>
<td>1.25 eq</td>
<td>97%</td>
</tr>
<tr>
<td>3</td>
<td>Cellobiose$^d$</td>
<td>3</td>
<td>1.25 eq</td>
<td>99%$^i$</td>
</tr>
<tr>
<td>4</td>
<td>Me-Cellobiose$^e$</td>
<td>3</td>
<td>1.25 eq</td>
<td>99%$^i$</td>
</tr>
<tr>
<td>5</td>
<td>Xylan$^f$</td>
<td>3</td>
<td>1.25 eq</td>
<td>99%</td>
</tr>
<tr>
<td>6</td>
<td>Lignin$^g$</td>
<td>3</td>
<td>1.25 eq</td>
<td>99%</td>
</tr>
<tr>
<td>7</td>
<td>Zein$^h$</td>
<td>3</td>
<td>1.25 eq</td>
<td>99%</td>
</tr>
<tr>
<td>8</td>
<td>Corn Oil$^i$</td>
<td>3$^k$</td>
<td>1.25 eq</td>
<td>31%</td>
</tr>
<tr>
<td>9</td>
<td>Lignin$^g$</td>
<td>11</td>
<td>0.00 eq</td>
<td>15%$^l$</td>
</tr>
</tbody>
</table>

Table 5. DDGS model component solubility.$^a$

$^a$All treatments were performed with 250 mg of component and 44 mmol of phosphite heated at 150 °C for 48 hrs. $^b$Determined from: (weight of insoluble material after methanol treatment)/(initial weight of lignocellulosic material)x100%. $^c$Chromatography grade from Aldrich. $^d$D-(+)-cellobiose from Aldrich. $^e$Permethylated according to Mendonca and Laine, Carbohydr Res. 2005, 2055-59. $^f$From oat spelts (Aldrich). $^g$Indulin AT kraft pine lignin from Westvaco. $^h$From Aldrich. $^i$Hy-vee salad grade corn oil. $^j$100 °C. $^k$One g of corn oil:1 g of 3. $^l$Reaction mixture turned black.
HWT-DDGS, we examined cellulose, cotton and cellobiose as substrates. As expected, all gave very good solubilities (Table 5, Entries 1-3). Permethylolation of the cellobiose did not effect the solubility (Entry 4), suggesting perhaps that glycosidic bond breaking was occurring, further evidence for which will be discussed below. We would not expect selectivity for certain carbohydrates in our process, and this is illustrated by the almost complete dissolution of xylan, another carbohydrate component of HWT-DDGS (Entry 5). As a percentage of the ca 89% dry mass content of DDGS, low molecular weight phenolics make up about 6-7%. Although these materials are not useful for conversion to ethanol, we showed that pine lignin is also highly soluble in 3/H₂O (Table 5, Entry 6) and interestingly, the material remaining after methanol extraction and evaporation also was water-soluble. It should be emphasized again that, because of the possibility of reactions involving transesterification with cellulosic OH groups, these phosphites are poised to behave as reactive solvents as well as conventional solvating solvents (see also later). The excellent solubility of Zein seen in Entry 7 of Table 5 implies that the protein content of the DDGS is undoubtedly also solubilized. This observation may be important in considering separations downstream. However, any protein phosphitylation that may occur would have to be analyzed for compatibility with livestock if such materials were to be used as a high protein feed additive.

Corn oil (Entry 8 in Table 5) was only 31% soluble in an equal mass of 3/H₂O, and interestingly, the un-dissolved corn oil appeared to be decolorized. Since filtration was not a viable option, methanol was added to dissolve polar materials in the reaction mixture, leaving the remaining oil as a separate phase which was then extracted into hexanes. Separation of the hexanes from the methanol solution phase followed by evaporation under reduced
pressure of the hexanes extract left a colorless oil containing no phosphorus detectable by $^{31}$P NMR spectroscopy. A $^1$H NMR spectrum of this oil indicated that the glycerol backbone of the oil had undergone a reaction. It is conceivable that the mildly acidic hydrolysis product 9 (Scheme 1) facilitates hydrolysis of the triglyceride with the excess 0.25 eq of water to form diglyceride plus free fatty acid. Such hydrolysis could be acid-catalyzed via the phosphite tautomer of 9 depicted on the left side of reaction 3 in Figure 4. Indeed, an FT-IR spectrum of the colorless oil revealed an –OH peak that could be attributed to the presence of diglyceride. The free fatty acids liberated during hydrolysis of triglyceride to diglyceride should be soluble in methanol, and this process could account for the 31% solubility of the oil in the 3/H$_2$O medium, assuming that the refined corn oil we used is composed of ca 99% triglycerides (Moreau and Hicks, 2004). Methanol was removed from the MeOH-soluble portion of the reaction mixture leaving a viscous yellowish oil that was analyzed by $^{31}$P NMR and $^1$H NMR spectroscopies. The $^{31}$P NMR spectrum showed phosphorus signals attributable to unreacted 9 and 10, and also multiple minor peaks due to unidentified phosphorus species in the 0-20 ppm range. This range could be associated with various phosphitylated components of the corn oil. The $^1$H NMR spectrum corroborated the $^{31}$P NMR spectroscopic results by displaying minor unidentified peaks and high intensity peaks due to the presence of 9 and 10. The fact that there were no phosphorus peaks in the colorless oil extracted into hexanes implies that a value-added usable corn oil co-product might be obtained using our treatment protocol.

It is likely that when a large excess of phosphite 3/H$_2$O is employed to achieve 99% HWT-DDGS solubility that the oil content will be completely hydrolyzed and therefore soluble in methanol. However, in cases such as Entry 3 of Table 3, where lower
phosphite:HWT-DDGS ratios are used, the lower solubility encountered could be due, at least in part, to insoluble oil present in the HWT-DDGS. This may be promising since lower mass of phosphite usage is desired from an economical standpoint. Thus if lower biomass solubility is primarily due to an increase in insoluble materials that do not contain an economically significant source of fermentable sugars, the excess phosphite required to obtain 99% biomass solubility would not be necessary to obtain economically viable yields of fermentable sugars as well as potentially value-added feed product.

An additional potential contributing factor to the solubility of the HWT-DDGS is contained in Scheme 1. In this scheme is illustrated the formation of a donor-solvent/additive mixture somewhat analogous to such mixtures referred to in the introduction in which electron-donor solvents (e.g., DMSO) with the additives trifluoroacetic or orthophosphoric acid have been used to dissolve cellulose (Grinshpan, 1998). In our solubilizing solvent system \( \frac{3}{H_2O} \), each reactant and product in Scheme 1 is an electron pair donor solvent component since each possesses at least three oxygens, each of which bears two lone pairs of electrons. Moreover, 3 features a phosphorus lone pair and the same is true for the tautomers of 9 – 11 because of the well-known tautomeric equilibrium generalized for 9 – 11 in reaction 3 of Figure 4. In this equilibrium, the phosphorus atom in the tautomer on the right has an electron lone pair while the one on the left does not. It should be noted, however, that this equilibrium does lie far to the left.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lingocellulosic Solute</th>
<th>% Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cornstover(^b)</td>
<td>80%</td>
</tr>
<tr>
<td>2</td>
<td>Kenaf(^b)</td>
<td>78%</td>
</tr>
<tr>
<td>3</td>
<td>Pine(^b)</td>
<td>99%</td>
</tr>
<tr>
<td>4</td>
<td>Poplar(^b)</td>
<td>98%</td>
</tr>
<tr>
<td>5</td>
<td>Newsprint(^c)</td>
<td>78%</td>
</tr>
</tbody>
</table>

\(^a\) All reactions conducted with 250mg of lignocellulosic solute, 7.14 g of 3, and 1 ml of water, heated to 150°C for 24 hrs. \(^b\) Dried and ground to 0.5 mm. \(^c\) Shredded with a cross cutting commercial paper shredder.
Our success in solubilizing DDGS, prompted us to explore the versatility of our treatment with other lignocellulosic materials. To gauge the flexibility in feedstock for our process we examined herbaceous material, woody biomass from both hardwood and softwood, and a manufactured cellulosic material. Table 6 shows that successful solubility was expanded to include herbaceous materials (Entries 1-2), softwood (Entry 3), hardwood (Entry 4), and a paper product (Entry 5). All exhibited high solubilities that would be an advantage in an industrial setting. As a versatile method of solubilizing lignocellulosic materials, this pretreatment would be very adaptable to local feedstocks, and would allow for more universal processing plant design. Moreover, the feedstock range, implies that a single processing plant could easily adapt to handling seasonal feedstocks, or a switch to a different crop during a given year because of drought or disease effects on the normally processed crop. Mixing of feedstocks should also be possible.

2.3.3 Spectroscopic Studies

2.3.3.1. Reactions of DDGS with 3

A $^{31}$P NMR spectrum (in CD$_3$OD) of the solid residue remaining after evaporation of the methanol extract in the treatment of HWT-DDGS with 3 showed a single peak at 94 ppm corresponding to 3 (in CD$_3$OD or CD$_3$Cl). In addition, there were minor peaks between 0-20 ppm, the region in which monoalkyl and dialkyl phosphites typically appear (Tebby, 1991). This is consistent with the presence of phosphitylated HWT-DDGS as will be further discussed in the next section. If the methanol had caused significant transesterification of 3 (or of phosphitylated HWT-DDGS) P(OCH$_3$)$_3$ would have been formed, with the appearance of a corresponding $^{31}$P NMR peak at 140 ppm. However, no peaks were observed in this region.
2.3.3.2. Reactions of DDGS with 3 in the presence of water

$^{31}$P NMR spectra of product mixtures resulting from treatment of HWT-DDGS with 3 compared with those from the reaction of 3 with 1.00 eq. of water added, and those resulting from treatment with a mixture of 3 and 1.25 equivalents of water, were all complex and quite similar in appearance. However, the latter two reaction mixtures displayed (as expected) no peak at 94 ppm corresponding to 3. Because of similarities among the phosphorus O-alkyl substituents in the hydrolysis products of 3, and in the products of transesterification reactions of 9 with cellulosic OH groups, it was difficult to distinguish which $^{31}$P signals corresponded to phosphite species 9a, a’, b, b’ resulting from the conformer/isomer

Figure 4. Tautomer, isomer and conformation changes in hydrogen phosphonates
equilibria (Mosbo and Verkade, 1973) shown in reactions 4 and 5 in Figure 4, which $^{31}$P resonance was due to 10, and which peaks represented phosphite groups on derivatized HWT-DDGS depicted as 7 in Figure 1. We thus synthesized 5 and 6 (Figure 2) as model compounds for the first hydrolysis intermediate 9 in Scheme 1. A $^{31}$P spectrum of pure 5 in deuterated methanol reveals part of the reason for the complexity of $^{31}$P NMR spectra of 3/H$_2$O/cellulosic material mixtures. First, an approximately 5.4 ppm difference in chemical shift is observed between the two phosphorus resonances (12.4 and 7.0 ppm in CH$_3$OH) assignable to the presence of the two conformers of 5 shown in reaction 6 in Figure 4. These conformers exist in an equilibrium which is slow on the NMR time scale in CH$_3$OH but is either fast in CDCl$_3$, or else this solvent greatly favors one of the conformers wherein its resonance appears as a single peak at 4.1 ppm. Secondly, deuterium/hydrogen exchange equilibria between the OD deuterium in CD$_3$OD and the P-H bonds in 5, 9a, a’, b, and b’ and presumably 7, cause additional $^{31}$P NMR spectral features to appear owing to P-D spin-spin coupling. Thus the nuclear spin of $^2$D is 1 and each $^{31}$P NMR resonance of 5 appears as a triplet of three equally spaced lines of equal intensity $[^1J(^{31}P-^2D)] \approx 106$ Hz. $^{31}$P NMR spectra of pure 5 in MeOH with a CDCl$_3$ lock inserted showed a simple spectrum of only two peaks (12.4 ppm and 7.0 ppm) corresponding to the two conformers shown in reaction 6, since P-D spin-spin coupling was necessarily absent. Thirdly, as noted above, the $^{31}$P NMR spectrum of 9 (the initial hydrolysis product of 3) gains additional complexity owing to the presence of two conformations in a slow equilibrium with each other for each isomer of 9 (namely, 9a,a’ and 9b,b’) as shown in reactions 4 and 5 of Figure 4.
The aforementioned issues also complicated the CD₃OD-solution $^{31}$P NMR spectra of the viscous product left after evaporation of the methanol extract of 99% HWT-DDGS solubilized in excess 3/1.25 equiv water. In order to enhance the intensity of the phosphitylated HWT-DDGS resonances in the $^{31}$P NMR spectra, 1.0 g of this material was reacted with 1.0 g of 3 and 0.14 g (1.25 equiv) of water with the aim of reacting all of the phosphite using an excess of HWT-DDGS. About half of the HWT-DDGS was solubilized (0.59 g) not only suggesting that all the phosphite that could be utilized to solubilized the HWT-DDGS had indeed been utilized under our conditions, but also indicating that at least a 50 w/w% solution of HWT-DDGS in an approximately equimolar mixture of phosphite 3 and water could be achieved. The latter result portends well for potential economical industrial processing using our pretreatment protocol (provided that phosphite can be economically recovered for recycling—see below). A $^{31}$P NMR spectrum in CH₃OH of the resulting product revealed phosphorus signals from 0-20 ppm, but no peak at 94 ppm for 3. There were multiple peaks (0-20 ppm) as would be expected for such a mixture. Some of these peaks were new, some corresponded to unreacted 9, but none could be unambiguously identified as a single derivative. It is clear that considerable additional work is required with components separated from this complex solution of DDGS in order to determine which ones are being derivatized and which are merely being solubilized. However, our efforts on model substrates mentioned above may help us solve some of these spectral problems in the near future.

In an attempt to shed further light on the solubilization process of HWT-DDGS by our 3/H₂O protocol, we reacted D-(+)-permethylated cellobiose with 5 (which functioned as a solvent comparable to 3/water for HWT-DDGS) in a 1:1 molar ratio. The virtual absence
of hydroxyl groups in 5 (owing to the very minor concentration of the second tautomer) and the unreactivity of 3/H$_2$O with ether linkages restricts any reaction under our conditions to cleavage of the glycosidic bond of D-(+)-permethylated cellobiose. In addition to $^{31}$P NMR peaks characteristic of unreacted 5 (12.4 and 7.0 ppm) two additional $^{31}$P NMR peaks appeared; a major resonance at 11.2 ppm and a minor one at 7.9 ppm. The presence of the major resonance is consistent with glycosidic bond cleavage, since the D-(+)-permethylated cellobiose was determined via FTIR spectral analysis to contain no significant amount of

**Figure 5.** Plausible mechanism for phosphonate cleavage of glycosidic bonds in cellulose by either a stepwise (A) or a concerted (B) reaction.
free –OH groups or water. The minor peak $^{31}$P NMR at 7.9 ppm is thought perhaps to be due to reaction of 5 with free –OH impurities owing to incomplete methylation of cellobiose in concentrations sufficiently low to be undetectable by FTIR spectroscopy.

The $^{31}$P NMR spectra of the reaction product of HWT-DDGS with 3/H$_2$O mixtures (which presumably produced 9) also exhibited peaks in the 11 ppm region consistent with glycosidic bond cleavage. Because the glycosidic linkage is quite robust, we inferred that there might be evidence in the $^{31}$P NMR spectra of the esterification product stemming from reaction of POH/[P(O)H] tautomeric groups in 5 and in 9 with one or more different cellulose OH groups in the DDGS to give 8. As stated earlier, however, $^{31}$P NMR chemical shifts between 75-162 ppm typical for such phosphite esters (Tebby, 1991) were not observed. We then conjectured that glycosidic bond cleavage arose via acid-catalysis by phosphorous acid formed via hydrolysis in DDGS/3/H$_2$O reactions. Such hydrolysis can also be ruled out, however, at least in the reaction of 5 with D-(+)
permethylated cellobiose mentioned above, since there are peaks in the $^{31}$P NMR spectra of the reaction products (4 - 13 ppm) which are consistent with the formation of new dialkyl phosphonate species (-2.5 to +22.5 ppm, Tebby, 1991). Moreover, no water is present in the reaction, which would be required for hydrolysis of the D-(+)
permethylated cellobiose. On the basis of these arguments, we believe that glycosidic linkages are cleaved, and a plausible mechanism is shown in Figure 5. Nucleophilic attack on the phosphorus by the glycosidic oxygen lone pair [analogous to acetate oxygen attack of a dialkylphosphonate (Luz and Silver, 1962)] facilitates cleavage of the glycosidic bond to produce a six-membered ring carbocation of one glucose unit that is known to occur during acid-catalyzed hydrolysis (Xiang et al., 2003). Subsequent equilibrium reactions eventually allow the ring of the cyclic dialkyl phosphonate
to open and the resulting oxyanion could then attack the cyclic carbocation to give a dialkylphosphite bonded to two glucose acetals as shown in Figure 5. It is also conceivable that a four-membered ring intermediate is formed as shown in B of Figure 5, despite increased steric congestion that could be involved.

2.3.4. A rationale

Our original goal was to develop a phosphitylating solvent/reagent for efficiently disrupting the robust hydrogen bonding occurring between strands of native cellulose polymers, thus causing solubilization. Phosphitylating agents 3 and 4 were envisioned to accomplish this objective by reacting with cellulose to form one new OH group as in reaction 7 by employing 3, or two new OH groups as in reaction 8 with the use of 4. The postulate was that one or two OH groups in the different chemical environments provided by the their new more random locations might better hydrogen bond with water than with cellulose hydroxyl groups. With our discovery that 3 is a better solvent/reagent than 4, along with the observation that the presence of water greatly improves the solubility of DDGS in 3, the possibility that phosphorous acid (11) would function as an efficient derivatizing agent.

**Figure 6.** Originally hypothesized reactions of 3 and 4 with cellulose which are apparently not major derivatization pathways according to $^{31}$P NMR
existed. This seems to be suggested by the results in Entry 11 of Table 4 and Entry 9 of Table 5.

Although phosphorous acid may play a partial role, the observation that 3 appears to be a better solvent for DDGS than 2 or 4 indicates that the bicyclic phosphite does not simply serve as a source of phosphorous acid in the presence of water. Supportive of this view is the apparent decomposition observed with phosphorous acid (Entry 5 of Table 3, Entry 11 of Table 4, and Entry 9 of Table 5) compared with the results obtained with a mixture of 3/H₂O. It is also interesting that an analogous mixture of 4/H₂O did not provide good solubility despite the presence of the extra OH group which was originally postulated to enhance the ability of 4 to hydrogen bond with water or methanol. In fact, the use of 4 led to a significant increase in the mass of the insoluble portion of the HWT-DDGS, presumably because of derivatization.

We believe that 9 (which is formed first via hydrolysis of 3 in Scheme 1) acts as an active phosphitylating/solubilizing agent. If this is true, it may not be necessary to completely remove the water from the light stillage. Indeed we have observed that un-pretreated Distillers Grains (DG) which has a significant moisture content, showed good solubility (Table 2, Entry 10).

2.4. Solvent/reactant recovery

2.4.1. Reactions of DDGS with 3

Compound 3 is an easily sublimable solid. After evaporation of the methanol extract of the reaction mixture produced by heating DDGS in 3, approximately 67% of 3 was recovered (Figure 3). Recovery of additional 3 should be possible since the $^{31}$P NMR
spectrum of the residue remaining after sublimation displayed a prominent peak for 3. We have also experienced some success in recovering unreacted 3 by ether extraction (Figure 3). However, the ether layer also contained other phosphorus-containing species as indicated by $^{31}$P NMR spectroscopy, and their identities are presently unclear. In addition, the ether-insoluble portion also revealed the presence of a significant amount of 3 via $^{31}$P NMR spectroscopy.

2.4.2. Reactions of DDGS with 3 in the presence of water

As noted earlier in this paper, a reaction mixture of 3 and water upon heating produces EtC(CH$_2$OH)$_3$ and (O)HP(OH)$_2$ (11) in an equilibrium reaction. Phosphite 3 can be partially regenerated by elimination of water upon heating in the presence of a catalytic amount of triethylamine. Initial efforts at recovering 3 by distillation/sublimation from the aforementioned product mixture were difficult because 3 seems to co-distill with water, giving a distillate consisting of a mixture of 3 and hydrolysis products 9 and 10. However, this does not pose a major concern since the distillate could be recycled. In addition, aqueous solutions of the reaction products produced from reactions of 3 with DDGS, as in Entry 6 of Table 2, have a pH of 1 and have been determined via $^{31}$P NMR spectroscopy to contain phosphorous acid as the only phosphorous species present. This indicates that the phosphorous acid could be recycled and be recombined with trimethylolpropane to regenerate 3, or 9 and 10 (i.e., the reverse of Scheme 1), provided that isolation techniques would be sufficiently cost effective and capable of obtaining the phosphorous acid in sufficient purity from fermentation stillage. Alternatively, phosphite salts could be precipitated and sold as fertilizer to alleviate some of the cost of not recycling the phosphite.
Further work aimed at answering the many questions remaining, such as phosphite recoverability and biological compatibility, are underway. It is also conceivable that the poorer solubilization properties of acyclic phosphites relative to 3 could be offset by their lower cost. It is clear that downstream testing with different enzymatic saccharification protocols with subsequent or simultaneous fermentation will be necessary to determine the optimum solubilizing system by weighing solubility performance, sugar yield, phosphite recoverability demands/limits, costs associated with materials and energy required. It may even be the case that some systems that provide poorer solubility using lower temperatures or employing a phosphite other than 3 would be more compatible with downstream processing into ethanol and/or other products, based on potential selectivity in the compounds that may be solubilized/derivatized.
CHAPTER 3. CONJUGATION OF SOYBEAN OIL

3.1 Introduction

Polyunsaturated fatty acids, by definition, contain more than one C=C bond (Gunstone et al., 1994). These fatty acids can be either conjugated or unconjugated as shown in Figure 7. It is known that the presence of conjugated C=C bonds increases the reactivity of the fatty acids and the triglycerides in which they appear (O’Brien, 2004; Gooch, 2002). The primary markets for polyunsaturated triglycerides are in paints and other surface coatings where they function as reactive diluents. Reactive diluents in this case are solvents that can dilute a surface coating material to proper consistency and then polymerize when exposed to air, thus allowing the coating to dry.

Figure 7. Photochemical isomerization of soybean oil (SBO)

The process of converting an unconjugated fatty acid to a conjugated one is referred to as isomerization or conjugation. The triglyceride structure of SBO is made of saturated, monounsaturated and polyunsaturated fatty acid esters. SBO contains 51% linoleic esters (18:2, 18 carbons, 2 C=C bonds) and 7% linolenic esters (18:3) both of which are naturally unconjugated polyunsaturated fatty acid esters. It also contains 14% saturated fatty acid
esters and 23% oleic (18:1) esters. In addition, the C=C bonds in linoleic and linolenic acid esters are naturally cis.

Triglycerides containing conjugated diene fatty acid esters can be obtained by the dehydration of castor oil. Triglycerides containing conjugated triene fatty acids are found in tung oil (Martens, 1961). Both of these conjugated oils are used in alkyd resins to speed drying times and to increase surface coating toughness, mar resistance, and imperviousness to water and alkali (Gooch, 2002). In addition, Swern et al. reported in 1948 that conjugated fatty acids, such as α-eleostearate found in tung oil, undergo rapid autoxidation via addition of peroxide radicals to the C=C bond system. The result is a greater number of C-C bonds, and C-O-C ether linkages in the drying products of conjugated oils than in the drying products of unconjugated oils where autoxidation occurs by abstraction of a hydrogen from the methylene between the C=C bonds followed by isomerization and subsequent addition of a molecule of oxygen to form a conjugated peroxide drying intermediate (Swern et al., 1948; Tallman et al., 2004; Szori et al., 2007). This difference in the drying mechanism may account for the faster drying times and more robust film properties of dried conjugated oils. Because of difficulties in producing castor and tung oil crops, however, the amounts of these oils are limited. Furthermore, prices are higher for these oils than for other vegetable oils, such as SBO.

Processes for converting the unconjugated C=C bonds in SBO to conjugated C=C bonds include heating the oil to high temperatures with excess caustic in a high-boiling glycol or in water at high temperatures in a pressure vessel. Base-catalyzed conjugation of separated C=C bonds has also been accomplished in other alkali media and with potassium t-butoxide (Bagby and Carlson, 1989). These methods essentially split the fatty acids from the glycerol with which they are normally bonded, and hence require subsequent re-esterification of the fatty acids with glycerol to obtain a drying oil. Typically, only 30-50% conjugation is
obtained with these methods. Furthermore, in both processes, cleavage of the ester bonds to the glycerol moiety is a significant and undesirable side reaction.

Nickel catalysts have been used to isomerize the C=C bonds in SBO directly, although only 30-34% conjugation was obtained, even at high temperatures (Markely, 1951). Several iron and chromium compounds that isomerize C=C bonds have also been developed, but these processes also require high temperatures (Markely, 1951). Furthermore, these iron and chromium compounds are not catalysts but are consumed in the reactions. Two acid catalysts, thionyl chloride and toluenesulfonic acid, are also known to catalyze the isomerization of fatty oils, methyl linoleate (ML) and related esters, but again, they are very inefficient, resulting in less than about 50% conjugation (Bagby and Carlson, 1989).

A number of heterogeneous and homogeneous catalyst systems of transition metal complexes have also been reported for the conjugation of ML and certain vegetable oils. For example, platinum (DeJarlais and Gast, 1971; Hsu et al., 1989; Bailar and Itatani, 1967), nickel (Bailar and Itatani, 1967), rhodium (Basu and Kasar, 1986; Singer and Stein, 1972; Basu et al., 1985), ruthenium (Ucciani, 1981, 1982; Deshpande et al., 1985; Mukesh et al., 1985; Basu et al., 1985, 1986; Mukesh et al., 1985(2), 1988, 1989; Cecchi et al., 1982, 1984; Ucciani et al., 1983; Deshpande et al., 1985; Krompiec et al., 1997, 1998; Sleeter, 1996), bimetallic nickel-ruthenium (Mukesh et al., 1988), chromium (Tucker and Riley, 1985; Fraken, 1970) and iron complexes (Frankel and Metlin, 1967) are known to catalyze such reactions. However, dimerization, polymerization and hydrogenation are troublesome side reactions, and in some instances separation of the catalyst at the end of the reaction is not feasible. Other investigations with ruthenium carbonyl cluster complexes give conversions of ML in excess of 80% at elevated temperatures (Frankel and Metlin, 1967). Ruthenium alkoxides have also been used as catalysts, but the high temperatures required lead to large amounts of polymers and monoenes (Narasimhan et al., 1989). More recently, a ruthenium(0) complex containing naphthalene and cyclooctadiene was found to isomerize
ML in acetonitrile (Pertici et al., 1999). However, the processes employing transition metal catalysts are not only energy-intensive, but such metals are known to be toxic to the environment. Other methods using rhodium metal complexes have been found to conjugate SBO relatively efficiently as homogeneous catalysts in solution (Larock et al., 2001). However, this approach has been deemed expensive because no cost-effective method currently exists to recover this expensive and toxic metal (Bagby and Carlson, 1989).

As a result of the problems with the above methods, attempts have been made to conjugate C=C bonds using photochemical methods (Gangidi and Proctor, 2004; Seki et al., 1991; Canaguier et al., 1986). However, yields from these methods have not exceeded 80%.

Before discussing our improved approach, a brief discussion of the mechanisms that have been put forth for the iodine-catalyzed photoconjugation of methylene-interrupted C=C bonds will be given. Two such mechanisms have been proposed. Both mechanisms depend on the homolytic dissociation of iodine to two iodine radicals. Mechanism A in Figure 8 is initiated by an iodine radical abstracting a proton to produce a bi-allylic radical that is stabilized by distribution of the radical electron over the five carbons involved in the π-system (Egger and Benson, 1966; Tallman et al., 2004; Szori et al., 2007). The bi-allylic radical moves via resonance to the end of the diene system forming the thermodynamically favored trans C=C bond in the conjugated system. Finally, a hydrogen atom is picked up from the easily homolytically dissociated HI molecule generated earlier, to terminate the process and regenerate the iodine radical catalyst. Mechanism B differs from A in that instead of abstracting a methylene proton, the iodine radical adds to one of the C=C bonds forming a bridged iodo radical intermediate. This intermediate allows formation of a di-iodo species after reacting with an iodine molecule, a well-known mechanism for cis–trans isomerization (Skell and Pavlis, 1964). Such isomerization may be required prior to conjugation (Dai et al., 1994). The di-iodo species is not stable and subsequently decomposes releasing HI and forming the more stable conjugated trans C=C configuration.
The organoiodide iodine atom is removed by exchange with HI terminating the process and generating a molecule of iodine which homolytically dissociates with exposure to light starting the process over.

We now present our approach to the photochemical conjugation of plant oils with iodine as a catalyst. This approach avoids the use of toxic metals and high temperatures, and produces virtually quantitative yields of conjugated SBO (CSBO). We also discuss the the drying properties of the CSBO and an iodine recovery process that uses a biorenewable resource.

**Figure 8. Iodine catalyzed conjugation**
3.2 Methods and Materials

3.2.1 Materials and Apparatus

RBD (refined, bleached, deodorized) soybean oil was kindly provided by Cargill and West Central Cooperative. HPLC grade hexanes and Iodine were purchased form Aldrich and used as received. Potato, waxy maize, and soluble potato starch (Robyt et al., 1996) was kindly supplied by Prof. Robyt at the Iowa State University, Department of Biochemistry, Biophysics and Molecular Biology. GELP75 and NOVA6600 were soluble starches kindly supplied by National Starch. Lintner’s starch was purchased as Fisher Soluble Starch from Fisher Scientific and was used as received.

The reaction apparatus was constructed from a metal trashcan for secondary containment and air circulation, with a large (26 L) glass bath inserted. The glass bath was wrapped with reflective steel, and the PVC lid was sealed using Permatex Ultra Black hi-temp RTV silicone liquid gasket. This setup was designed to mimic an industrial glass coated steel reactor. Four reflux condensers were attached through which -10°C cooling fluid was passed using an Isotemp bath circulator. The chilled condensers prevented escape of hexanes or iodine. The reactor was also fitted with an argon inlet, a mechanical stirrer, and a port for

Figure 9. Conjugation reactor
sampling. The light fixture consisted of a standard overhead projector bulb (500W with a maximum intensity at 520 nm) in a socket that was lowered into a glass tube extending from the top of the reactor down into the center (Figure 9). The light fixture was positioned so that the light bulb was approximately in the center of the reaction mixture, and the stirring propeller was positioned to be even with the lamp.

3.2.2 Iodine catalyzed photoconjugation

The reactor described in section 4.2.1 above was charged with 2.3 L of soybean oil and 21.7 L of hexanes while purging with argon. The argon flow was decreased to a few bubbles per second, and the lamp was turned on to heat the soybean oil/hexanes solution to reflux temperature. After reflux had been reached, the lamp was turned off, and a solution of 4.25 g of iodine in 2 L of hexanes was added to the reaction through the sampling port again while purging with argon. The lamp was then turned back on, and the reaction was conducted with external air flow for cooling to maintain mild refluxing temperature. An argon atmosphere was maintained throughout the reaction using a flow of approximately one bubble argon every two seconds.

3.2.3 Removal of iodine

3.2.3.1 Using Charcoal

Iodine was removed from the reaction mixture with a 27:1 weight ratio of Calgon activated charcoal : iodine with mechanical stirring for 12 hrs. The charcoal and adsorbed iodine was then removed by filtration through a bed of diatomaceous earth (to catch fine particulates) on top of Whatman 1 filter paper.

3.2.3.2 Using Starch

Iodine was removed from the reaction mixture with a 17:1 weight ratio of potato starch : iodine. The potato starch was wetted with a 0.5:1 weight ratio of distilled water : starch before addition to the reaction mixture containing iodine. After mechanical stirring for 12 hrs the starch iodine complex was filtered from the reaction mixture with Whatman 1 filter paper.

3.2.4 Conjugated soybean oil fractionation.

Fractionation was conducted using a bench top centrifuge in a cooling box constructed of Styrofoam insulation board fitted with a cooling coil. The CSBO samples
were first frozen in 50 mL centrifuge tubes. Then they were centrifuged at 1000 rpm in the box as they warmed to the set temperature (8-17°C).

3.2.5 **BK Drying tests.**

The BK Drying test is the ASTM method for screening oils for their drying properties. The advantage of BK drying tests are that they are inexpensive and can be automated. A BK drier consists of a plate holding slides that have had an application of uniformly thin coatings of oil containing drying additives (solidified fractions were melted to apply a coating to the slides). During the test, a needle is dragged across the slide and the pattern made by the needle is used to classify the stages of drying (Figure 10). However, due to influences of humidity and temperature, an internal control must always be run as a standard for comparison. Our BK drying tests were provided through collaboration with Dr. John Massingill, Director of the Texas State University-San Marcos, Institute for Environmental and Industrial Science, Center for Coatings and Biobased Technology. According to standard operating procedures oxalate driers were added in the following proportions relative to the weight of oil: cobalt, 0.09%; zirconium, 0.3% and calcium, 0.2%.

**Figure 10. Stages of BK Drying test**

<table>
<thead>
<tr>
<th>Coating film on glass panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1: Set-to-touch Time</td>
</tr>
<tr>
<td>Stage 2: Tack-free Time</td>
</tr>
<tr>
<td>Stage 3: Dry-hard time</td>
</tr>
<tr>
<td>Stage 4: Dry through Time</td>
</tr>
</tbody>
</table>

Direction of needle motion

Adapted from BK drying test description provided by Dr. Massingill, Texas State University-San Marcos.
3.2.6. Calculation of percent conversion.

The percent conversion of unconjugated C=C bonds to conjugated ones was calculated from $^1$H NMR spectra obtained on oil samples in CDCl$_3$ on a Varian VXR-400MHz NMR instrument. $^1$H NMR spectra were acquired for the starting SBO and were integrated using the peaks at 3.15 and 3.30 ppm corresponding to the glycerol backbone as a standard, since they remain intact during the reaction. The percent change in the integration of the peak at 2.7 ppm, which corresponds to the CH$_2$ in between two unconjugated C=C bonds, was calculated using Equation 2.

\[
\frac{\text{(integ. of 2.7 ppm peak of SBO) – (integ. of 2.7 ppm peak of CSBO)}}{\text{(integ. of 2.7 ppm peak of SBO)}} \times 100\% \tag{Equation 2}
\]

3.3 Results and discussion

3.3.1 Polymerization

Over the course of the development of this technology, many samples had been generated. Over time, some samples were observed to form solids. It was first believed that these solids stemmed from polymerization of the oils as would be expected in a drying oil. However, polymerization of soybean oil during drying is due to the autoxidation process that would not occur in samples kept under inert atmosphere. If polymerization due to autoxidation were occurring, the number of olefinic protons would decrease due to breaking of C=C bonds in order to form new C-O or C-C bonds (Gooch, 2002). Analysis of the $^1$H NMR spectra taken of solid portions from an aged conjugated soybean oil sample dissolved in CDCl$_3$, revealed no change in the ratio of olefinic protons to aliphatic protons. In fact, even solids forming in a sample that was left open to air for 24 hrs showed no change in the ratio of olefinic protons to aliphatic protons in the $^1$H MNR spectra. This led us to believe that the soybean oil was not polymerizing but solidifying, and that the solidification
happened at a faster rate than autoxidation. Further investigation of this phenomenon is discussed in the following section.

Comparing $^1$H NMR spectra of the un-conjugated soybean oil starting material and the conjugated oil product after irradiating the soybean oil in hexanes solution in the presence of iodine as a catalyst, we found that there was no change in the ratio of olefinic protons to aliphatic protons even after long reaction times at reflux temperatures. This implies that even at elevated temperature in the presence of iodine as a free radical initiator, polymerization does not occur. The low reactivity of CSBO to free radical polymerization is known in the literature. Thus attempts to make novel polymeric materials out of CSBO were unsuccessful unless co-polymerized with more active olefins (Valverde and Larock, 2005).

### 3.3.2 Separation

In light of the evidence suggesting that the oil was separating at room temperature (discussed in the previous section), we decided to test this hypothesis by centrifuging samples in a cooled centrifuge. Centrifuging 50 mL 99% conjugated soybean oil, which was first frozen at 0°C, at 1000 rpm while warming from 0°C to 17°C over about 6 hrs gave solidified and liquid portions of approximately equal proportions. Varying the temperature of separation from 17°C down to 8°C afforded a liquid to solid ratio of 20:80. The separation is related to composition. It is known that saturated fatty acids have higher melting points than unsaturated ones, unsaturated fatty acids with trans C=C bonds have higher melting points than those with cis C=C bonds, conjugated unsaturated fatty acids have higher melting points than their unconjugated analogues, and that single fatty acid differences in triglycerides change their melting properties (Bailey, 1950). To support our hypothesis that fatty acid composition is responsible for melting point differences and drying properties, isomer analysis of the conjugated oil including that for conjugated linoleic acid (CLA) isomers, was obtained through collaboration with Dr. Kenneth Doll at the USDA NCAUR. As is seen in Table 8, the solidified portions on average had higher concentrations of trans-
trans conjugated C=C bonds, and higher concentrations of saturated fatty acids. Both of these factors would lead to increased melting points of the corresponding triglycerides. It should be mentioned that at room temperature the solid fraction is considerably liquified and at slightly elevated temperature the solid fractions are completely liquifed.

### 3.3.3 Drying properties

The results of BK drying tests conducted for CSBO samples fractionated under various conditions are summarized in Table 7. It is interesting that, at least in the case of the 098 samples, both the liquid and the solid separated at low temperature showed better drying properties than the original unseparated sample. It was expected that the solidified fraction would have poorer drying properties due to suspected higher concentration of saturated fatty acids. Both of these factors would lead to increased melting points of the corresponding triglycerides. It should be mentioned that at room temperature the solid fraction is considerably liquified and at slightly elevated temperature the solid fractions are completely liquified.

<table>
<thead>
<tr>
<th>Sample (L/S)</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1</th>
<th>C18:2</th>
<th>CLA 9C11T</th>
<th>CLA 10T12C</th>
<th>CLA Trans-Trans</th>
</tr>
</thead>
<tbody>
<tr>
<td>098 L (50/50)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.8 ± 0.1</td>
<td>3.3 ± 0.1</td>
<td>3.8 ± 0.9</td>
<td>24.5 ± 1.0</td>
</tr>
<tr>
<td>098 S (50/50)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.8 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>30.5 ± 0.5</td>
</tr>
<tr>
<td>122 L (50/50)</td>
<td>13.1 ± 1.0</td>
<td>6.7 ± 1.0</td>
<td>30.2 ± 1.1</td>
<td>2.9 ± 0.8</td>
<td>8.1 ± 0.5</td>
<td>4.7 ± 0.7</td>
<td>34.4 ± 0.8</td>
</tr>
<tr>
<td>122 S (50/50)</td>
<td>15.3 ± 0.4</td>
<td>8.0 ± 0.9</td>
<td>26.9 ± 0.5</td>
<td>2.6 ± 0.4</td>
<td>6.9 ± 0.03</td>
<td>4.0 ± 0.2</td>
<td>36.4 ± 0.4</td>
</tr>
<tr>
<td>122 L (20/80)</td>
<td>12.0 ± 0.2</td>
<td>6.2 ± 0.1</td>
<td>33.0 ± 0.7</td>
<td>2.9 ± 0.1</td>
<td>8.2 ± 0.4</td>
<td>4.9 ± 0.2</td>
<td>32.9 ± 0.1</td>
</tr>
<tr>
<td>122 S (20/80)</td>
<td>14.8 ± 0.6</td>
<td>8.3 ± 0.4</td>
<td>27.6 ± 0.8</td>
<td>2.4 ± 0.3</td>
<td>6.9 ± 0.2</td>
<td>3.8 ± 0.4</td>
<td>36.3 ± 0.1</td>
</tr>
</tbody>
</table>

*These percentages are relative to raw linseed oil (LO) *b The BK drier did not catch this stage for LO *b The BK drier did not catch this stage for the conjugated oil sample *c The two Cargill samples represent different 5 gallon shipments of alkali refined, bleached and deodorized soy bean oils *d Unseparated conjugated oil *e Unsolidified 50% of the oil left after repeated cycles of cooling in a refrigerator/centrifugation *f Solidified 50% of the oil after repeated cycles of cooling in a refrigerator/centrifugation *g Unsolidified 90% obtained from 098 unsep’d by centrifuging while cooling from RT to –4°C h Solidified 10% obtained from 098 unsep’d by centrifuging while cooling from RT to –4°C *b Unsolidified 50% obtained from 098 unsep’d by centrifuging while warming from 0°C to 8°C j Solidified 50% obtained from 098 unsep’d by centrifuging while warming from 0°C to 8°C k Unsolidified 20% of the oil left after centrifuging while warming from 0°C to 8°C. *l Solidified 80% of the oil left after centrifuging while warming from 0°C to 8°C. *m Unsolidified 50% of the oil left after centrifuging while warming from 1°C to 17°C. *n Solidified 50% of the oil left after centrifuging while warming from 1°C to 17°C.
acids in the solidified triglycerides (see later). Unfortunately, due to the failure to observe a
dry through time for linseed oil in Entries 6-10, further comparisons between separated and
unseparated oils cannot be made at this time. Nonetheless, the BK drying results show that
the conjugated SBO produced by our approach has potential for good drying properties, thus
meriting advancement of these oils to more conclusive (albeit expensive) alkyd resin tests in
the future.

In order to examine the phenomenon of unseparated CSBO having poorer drying
properties than the separated fractions, we coated 3 glass slides, each with one of the CSBO
samples from Entries 1-3 in Table 7. It was observed that a coating made of the low
temperature-separated solid fraction solidified on the glass slide in about 24 hrs and took a
little over 5 days to completely dry under ambient conditions. A coating made from the
liquid fraction stayed liquid for over 48 hrs and also took about 5 days to completely dry.
The coating from the unseparated CSBO showed the appearance of small particles of
solidified oil dispersed throughout the coating after approximately 24 hrs and took almost 12
days to completely dry. Based on this observation we thought that there might be two
different factors responsible for the better drying performance of the solid and liquid
fractions compared with the unseparated CSBO. We hypothesized that the solid fraction may
benefit from closer proximity to other functional groups during the drying process due to a
semi-crystalline structure formed upon solidification because of its higher saturated fatty acid
content. By contrast, the liquid fraction might contain a lower concentration of saturated
fatty acids, which would mean there are more C=C bonds to react during drying. Because
the solidification occurred faster than drying under these ambient conditions the result would
be an heterogeneous drying medium.
The two different factors that aid drying in the separated fractions would impede drying in the mixed oil owing to the molecular reorganization difficulty imposed by the dilution effect on each factor. An SEM image (courtesy of Prof. Lin, in our department) was obtained of the fully dried coating from the unseparated CSBO (Figure 11). The raised portions correspond to the solidified regions and appear to be somewhat ordered in contrast to the unsolidified portions in the “valleys”, which appear much less ordered. The SEM image reveals the heterogeneous nature of the coating made from unseparated CSBO. Although this evidence supports our hypothesis, much could yet be learned by synthesizing pure triglycerides with known saturated and conjugated fatty acid contents and studying their drying properties. Such a study was not in the scope of this project.

Table 7. 24 hr BK dry times

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sample</th>
<th>Set-to-touch(^a)</th>
<th>Tack free(^a)</th>
<th>Dry hard(^a)</th>
<th>Dry through(^a)</th>
<th>Cargill Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>098 unsep’d(^d)</td>
<td>34% faster</td>
<td>4% faster</td>
<td></td>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>2</td>
<td>098 liquid(^e)</td>
<td>25% faster</td>
<td></td>
<td>37% faster</td>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>3</td>
<td>098 solid(^f)</td>
<td></td>
<td>Just as fast</td>
<td></td>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>4</td>
<td>111 liquid(^g)</td>
<td>15% faster</td>
<td>4% faster</td>
<td></td>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>5</td>
<td>111 solid(^h)</td>
<td>53% faster</td>
<td>13% slower</td>
<td></td>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>6</td>
<td>121 liquid(^i)</td>
<td>Just as fast</td>
<td>27% slower</td>
<td>29% slower</td>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>7</td>
<td>121 solid(^j)</td>
<td>Just as fast</td>
<td>36% slower</td>
<td>29% slower</td>
<td></td>
<td>Sample 1</td>
</tr>
<tr>
<td>8</td>
<td>122 unsep’d(^d)</td>
<td>8% slower</td>
<td>1% faster</td>
<td>20% slower</td>
<td></td>
<td>Sample 2</td>
</tr>
<tr>
<td>9</td>
<td>122 liquid(^k)</td>
<td>8% slower</td>
<td>14% slower</td>
<td>33% slower</td>
<td></td>
<td>Sample 2</td>
</tr>
<tr>
<td>10</td>
<td>122 solid(^l)</td>
<td></td>
<td>Just as fast</td>
<td>27% slower</td>
<td></td>
<td>Sample 2</td>
</tr>
<tr>
<td>11</td>
<td>122 liquid(^m)</td>
<td>44% slower</td>
<td>50% slower</td>
<td>55% slower</td>
<td>4% faster</td>
<td>Sample 2</td>
</tr>
<tr>
<td>12</td>
<td>122 solid(^n)</td>
<td>44% slower</td>
<td>50% slower</td>
<td>55% slower</td>
<td>9% faster</td>
<td>Sample 2</td>
</tr>
</tbody>
</table>

\(^a\) These percentages are relative to raw linseed oil (LO).  \(^b\) The BK drier did not catch this stage for LO.  \(^bb\) The BK drier did not catch this stage for the conjugated oil sample.  \(^c\) The two Cargill samples represent different 5 gallon shipments of alkali refined, bleached and deodorized soy bean oils.  \(^d\) Unseparated conjugated oil.  \(^e\) Unsolidified 50% of the oil left after repeated cycles of cooling in a refrigerator/centrifugation.  \(^f\) Solidified 50% of the oil left after repeated cycles of cooling in a refrigerator/centrifugation.  \(^g\) Unsolidified 90% obtained from 098 unsep’d by centrifuging while cooling from RT to –4°C.  \(^h\) Solidified 10% obtained from 098 unsep’d by centrifuging while cooling from RT to –4oC.  \(^i\) Unsolidified 50% obtained from 098 unsep’d by centrifuging while warming from 0°C to 8°C.  \(^j\) Solidified 50% obtained from 098 unsep’d by centrifuging while warming from 0°C to 8°C.  \(^k\) Unsolidified 20% of the oil left after centrifuging while warming from 0°C to 8°C.  \(^l\) Solidified 80% of the oil left after centrifuging while warming from 0°C to 8°C.  \(^m\) Unsolidified 50% of the oil left after centrifuging while warming from 1°C to 17°C.  \(^n\) Solidified 50% of the oil left after centrifuging while warming from 1°C to 17°C.
3.3.3 Iodine removal

3.3.3.1 Charcoal

Using excess iodine/hexanes solution and measuring by UV the concentration of iodine before and after charcoal removal, it was determined that a 27:1 weight ratio of charcoal to iodine was needed to efficiently remove the iodine from the reaction mixture. Charcoal was effective for removing the iodine from the reaction mixture but had some drawbacks. Starting with granulated charcoal, we stirred the reaction mixture overnight to make sure complete removal was achieved. While stirring, the granules of charcoal would collide and tend to break apart leaving a very fine charcoal powder which required a bed of Celite to facilitate filtration. However, this may be avoided industrially by passing the reaction mixture through a packed column of charcoal of sufficient size instead of stirring. The other disadvantage became apparent when trying to recycle the charcoal and iodine. Thus, attempts to remove the iodine from the charcoal including extraction with toluene, and sublimation were only marginally successful. The best method was soxhlet extraction of the
iodine form the charcoal using hexanes. Still, this method removed less than 50% of the iodine absorbed. There is precedence for this observation in the literature. Bahatia et al. describe reversible and irreversible absorption of iodine in the charcoal due to pore blockage. As expected, reuse of the charcoal after soxhlet extraction was not nearly as effective as fresh charcoal. Polymer bound Ph₃P was also effective for removal of iodine from hexanes, but again attempts to regenerate the Ph₃P and recover the iodine were unsuccessful. Toluene extraction, sublimation, and soxhlet extraction with hexanes all afforded negligible recovery of iodine.

3.3.3.2 Starch

In iodometry, starch is used as an indicator for iodine. In that process starch shows the presence of iodine by forming a blue starch/iodine complex. With this in mind we thought starch might be an effective way to remove iodine in the form of a starch-iodine complex. An initial experiment using dry potato starch was unsuccessful in removing any of the iodine from an iodine-hexanes solution, and no blue starch-iodine complex was observed (Table 9, Entries 1 and 4). However, in iodometry an aqueous solution of starch is used as an indicator; not just starch by itself. There is some debate on the role of water here since the structure of the starch iodine complex is not completely clear at this time. What is known is that the starch-iodine complex consists of linear polyiodine compounds of some form trapped inside the helix formed by the α 1-4 linked glucose units in starch (Teitelbaum et al., 1980; Saenger, 1984; Calabrese and Kahn, 2000; Minik et al., 1991). Some (Rendleman, 2003; Teitelbaum et al., 1980; Calabrese and Kahn, 2000) believe the role of water is to permit the hydrolytic disproportionation of some iodine forming an I⁻ ion necessary to form polyiodide species and therefore the starch-iodine complex. Others believe that water is necessary to facilitate a starch conformation capable of accepting the iodine molecules to form the complex (Smith and Smith, 1969; Rundle and French, 1943). Using a slurry of 10% potato
starch in water, complete removal of iodine was achieved (Entries 2 and 5). Later it was
determined that only 50 wt% of water to starch was needed to achieve complete removal of
starch from hexanes (Entries 3 and 6). This “wetted” starch has an advantage over an
aqueous slurry in that it can easily be filtered from the reaction mixture. The soluble starches
in Entries 7-9 were easily gelatinized either by addition of water before iodine adsorption
(Entries 8 and 9), or upon heating while attempting to release iodine from the complex. In
both cases gelatinization seemed to be unfavorable for transport of iodine in and out of the
starch-iodine complex.

Table 9 summarizes the performance of different starches in terms of both iodine
absorption and subsequent removal from the starch iodine complex. In the case of the wetted
starch, it appears that removal of iodine is not achieved because of water evaporation. It has
been suggested by Choudhury in 1947 that liberation of iodine from the starch iodine
complex is driven by replacement of iodine with water molecules. Our experiments seem to
support that suggestion since water is necessary to afford liberation of the iodine from the
starch iodine complex, and increased water content aids in release of iodine (Entry 2 and 5 of
Table 9). Given the advantage of filtration for “wetted” starch, and that using Lintner’s
starch adds an additional step involving acid treatment to achieve “lintnerization”, current
efforts are focused on using wetted potato starch to remove iodine from the reaction mixture,

<table>
<thead>
<tr>
<th>Entry</th>
<th>Starch</th>
<th>Water (g)</th>
<th>Adsorption</th>
<th>Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lintner’s</td>
<td>0</td>
<td>None</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Lintner’s</td>
<td>50</td>
<td>Complete</td>
<td>Complete</td>
</tr>
<tr>
<td>3</td>
<td>Lintner’s</td>
<td>2.5</td>
<td>Complete</td>
<td>Marginal</td>
</tr>
<tr>
<td>4</td>
<td>Potato</td>
<td>0</td>
<td>None</td>
<td>N/A</td>
</tr>
<tr>
<td>5</td>
<td>Potato</td>
<td>50</td>
<td>Complete</td>
<td>Complete</td>
</tr>
<tr>
<td>6</td>
<td>Potato</td>
<td>2.5</td>
<td>Complete</td>
<td>Marginal</td>
</tr>
<tr>
<td>7</td>
<td>Robty Potato</td>
<td>50</td>
<td>Complete</td>
<td>Partial</td>
</tr>
<tr>
<td>8</td>
<td>GELP75</td>
<td>50</td>
<td>incomplete</td>
<td>N/A</td>
</tr>
<tr>
<td>9</td>
<td>NOVA6600</td>
<td>50</td>
<td>Incomplete</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 9. Starch adsorption and removal of iodine

---

a All experiments conducted using 50 mL of 163 ppm iodine in hexanes solution to
mimic conjugation reaction iodine concentrations b Distilled water c based on
visual disappearance of purple iodine color in hexanes d based on disappearance
of blue color of starch-iodine complex e Fisher Sci. soluble starch f Robty et al.
1996 g Water soluble starches obtained from National Starch
followed by addition of water to make aqueous slurries for liberation of iodine from the iodine-starch complex. It should be noted that while iodine has been removed from the starch solution, it has not been recovered due to the small laboratory scale on which we have been operating. Iodine adsorbed by a 10% starch aqueous slurry was observed to be driven back into hexanes during one recovery attempt, and iodine was also observed as small crystals in a chilled receiving flask when it was co-distilled with water, in which iodine is sparingly soluble. While recycling is not yet optimized on the laboratory scale, starch has shown the potential to be an effective means of recycling iodine, and has the advantage of also being a biorenewable resource.
CHAPTER 4. SUMMARY AND CONCLUSIONS

4.1 Utilization

The two areas of utilization applicable to the biomass feedstocks selected are commodity chemical production, including ethanol production and fast drying alkyd resins for used in paints and coatings. The ethanol industry is reported to reach an annual production of 10.9 billion gal. in 2009 and the paints and coatings industry reported an annual production of 1.3 billion gal. in 2004. Due to the large size of these industries the efforts described herein have potential to have a large impact on the usage of biorenewables, if they are developed to the point were they are competitive with current technology. In this thesis, progress towards industrially applicable technologies have been made, and the potential shown herein provides compelling evidence for taking further steps in developing these technologies.

4.1.1 Lignocellulosics Solubilization

Phosphite esters have proven to be versatile solvent/pretreatment system for solubilizing lignocellulosic materials. The most effective phosphite, 3, is made from relatively cheap starting materials (trimethylolpropane and triethylphosphite or phosphorous acid), and even though trimethylolpropane is not currently made from biorenewable resources, it has potential to be made from a biorenewable resource since there are ongoing efforts in developing biorenewable 1-butanol which is its synthetic precursor. While phosphorous acid or triethylphosphite are not made from renewable resources they are made from abundant minerals and either water or ethanol, respectively. Obviously it will be advantageous to recycle these materials if economically feasible. Hydrolysis of phosphite/DDGS derivatives at the end of the treatment in aqueous solutions at room temperature or mild heating not underivatizes material for downstream processing, but provides options for recycling. If trimethylolpropane and phosphorous acid can be sufficiently purified from waste water streams, heating could drive off water and regenerate
the active species 9 and 10. While it is difficult to drive the regeneration all the way back to 3, it is not necessary and perhaps not preferable to do so since the toxicity of 3 is much higher than 9 or 10.

Now that we have developed a method to solubilize lignocellulosic material, the next step is to move forward with saccharification and fermentation studies which are currently underway. Promising aspects of this technology are that: 1) solubilizing the material definitely changes the morphology of the cellulose to be much less crystalline which should enhance digestibility in biological saccharification 2) hydrolysis of glycosidic bridging phosphite moieties should yield shorter cellulose chains, again enhancing saccharification, and 3) options for tuning the process for maximum production of sugars and potentially value-added side streams exist by varying the temperature and phosphite concentration, and the use of unrecycled phosphite in fertilizers.

4.1.2 SBO conjugation

Photochemical isomerization using a projector lamp as a light source and iodine as a catalyst in a hexanes solution, has been shown to achieve nearly quantitative conversion of SBO to CSBO. Iodine is capable of catalyzing this reaction at very low catalyst loadings. While iodine itself is not biorenewable, it is readily produced industrially from abundant mineral deposits, salt brines, and salt wells, and it is also obtainable from some kelp. In addition to iodine having a fairly low cost, evidence has been shown that iodine may be recyclable using starch as a recovery agent. Starch is biorenewable and is recyclable in our protocol. While hexanes is a product of the petroleum industry, it is currently used to extract SBO from soybeans on an industrial scale. In that industrial setting systems have been designed and used that provide greater than 99% recyclability of hexanes. Because there are no expensive and toxic metals used in this process it should be an economical and environmentally friendly technology.
BK drying tests have suggested that after fractionation of the oil, the drying properties of CSBO exceed even that of linseed oil. This would move SBO up from a semi-drying oil classification to a fast-drying oil in competition with higher-cost plant oils such as linseed oil and tung oil in fast drying alkyd resin applications. In addition, technologies are being developed to use plant oils in water-based paints, and CSBO may aid in this technology by being as an economical fast-drying oil. Further advances in this area are necessary to displace water-based paints that use petroleum derived materials for film formation, and to move away from solvents traditionally used in alkyd resins that are usually petroleum derived. The progress presented herein merits the next step in testing the CSBO produced in alkyd resin formations. While the iodine recovery and separation aspects of the process are still being refined, alkyd resin tests will give valuable information relating to the coatings formed by CSBO. Such tests will provide information on color retention, mar resistance, and imperviousness to water and alkali, as well as more industrially relevant drying times.

4.2 Criteria Review

In the introduction it was stated that the goal of the research presented here was to develop processing techniques that would move forward the use of biorenewable resources in industrial products as viable alternatives to petroleum products currently in use. The research presented has focused on biorenewable materials that would be alternatives in the commodity chemical (including transportation fuel such as ethanol) and paint and coatings industries. Solubilization of lignocellulosic materials was investigated with the goal of improving the production of ethanol. The ethanol industry has grown rapidly because of ethanol’s viability as an alternative to pure gasoline in the form of 10% and 85% ethanol/gasoline blends. Lignocellulosic materials have not yet been utilized by the ethanol industry except on a demonstration scale due to the high cost and low efficiency of lignocellulosic pretreatment. In addition, the only co-product from the dry-milling corn-to-ethanol process is DDGS, which has limited use in animal feed (although technologies are being developed to produce
other co-products or other uses for DDGS). The methods described in this research for processing lignocellulosic materials show promise in providing cheap and effective means of producing fermentable sugars for use in not only ethanol production, but other chemicals (e.g. butanol, propanol, lactic acid, and xylitol) that can be produced by fermentation processes. While the phosphite ester method described herein has potential for recycling, any phosphorous acid in the side streams represents potential value added co-products. Further development of this technology could increase the ability of ethanol to meet fuel demands, since cellulose is more abundant than starch, which is the current feedstock used for ethanol production.

Soybean oil has been used in the paint and coatings industry in alkyd resins and inks. However, in fast drying applications it is passed over in favor of faster drying oils such as linseed and tung oil. These oils are in shorter supply, making them more expensive, and they are often imported to meet domestic demand. The use of alkyd resins has declined in recent years due to the advance of water-borne latex paints that use petroleum products. Alkyd resins still have performance advantages that have maintained their market share, however. A faster drying SBO, in the form of CSBO, may increase alkyd resin use by providing a cheaper high-performance alkyd resin, and they might also enhance recent technologies using water-borne plant oil-based paints (USB, 2005).

The petroleum industry has become well developed over the past 45 years, and it has taken diminishing supplies and political and environmental pressures to drive efforts in replacing an increasing fraction of its usage. The goal to meet 10% of the chemical feedstock demand using biorenewable resources by the year 2020 is a reasonable one, and the research results reported here represent steps towards that goal.
APPENDIX A. SUPPORTING MATERIAL FOR SOLUBILIZATION OF LIGNOCELLULOSICS

### Gross Matter Composition

<table>
<thead>
<tr>
<th>Material</th>
<th>% Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture Content</td>
<td>11.2%</td>
</tr>
<tr>
<td>Dry Matter Content</td>
<td>88.8%</td>
</tr>
</tbody>
</table>

### Dry Matter Composition (Polymeric Analysis)

<table>
<thead>
<tr>
<th>Component</th>
<th>% Dry Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Extractables</td>
<td></td>
</tr>
<tr>
<td>Ethanol Extractables</td>
<td>10.7%</td>
</tr>
<tr>
<td>Glucan (total)</td>
<td>20.9%</td>
</tr>
<tr>
<td>Cellulose</td>
<td>16.0%</td>
</tr>
<tr>
<td>Starch</td>
<td>5.2%</td>
</tr>
<tr>
<td>Xylan</td>
<td>8.2%</td>
</tr>
<tr>
<td>Arabinan</td>
<td>5.3%</td>
</tr>
<tr>
<td>Protein</td>
<td>26.4%</td>
</tr>
<tr>
<td>Ash</td>
<td></td>
</tr>
<tr>
<td>Total Dry Matter</td>
<td>71.5%</td>
</tr>
</tbody>
</table>

Provided by Rick Hendrickson et. al. at Purdue University
Graphical depiction of phosphite treatment of DDGS on 250g Scale

DDGS + 1/1.25eq water

150°C → 24 hrs

MeOH

Filter off insolubles

Evaporate off MeOH

H₂O
$^{31}$P NMR of pure 1,3-propanephosphite (5), both conformers present
$^{31}$P NMR of pure 1,3-propanephosphite (5) reacted with Me-Cellobiose for 24 Hrs at 100°C.
$^{31}$P NMR of trimethylol propane phosphite (3)/1.25eq H$_2$O reacted with DDGS for 24 Hrs at 150°C.
APPENDIX B. SUPPORTING MATERIAL FOR SOYBEAN OIL CONJUGATION
$^1$H NMR of Cargill RBD Soybean oil, after conjugation using I$_2$ and light at reflux temperature for 9 hrs


Gangidi, R.R., Proctor, A., 2004 Photochemical production of conjugated linoleic acid from soybean oil. Lipids, 39, 6, 577-582.


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I am indebted to many people that have helped me on a journey that is ending with this thesis and would like to take this opportunity to thank them. First and foremost I am grateful to my wife Abbey and my family who have been supportive and patient with me through frustrations, and have rejoiced with me in successes. My journey would not have been possible without the guidance and support of Prof. John G. Verkade. He has imparted wisdom to me for the past three years that I will not soon forget in the areas of chemistry, industry and life. I was lucky to have lab mates throughout my tenure here that have been helpful and supportive through advice and many favors. I am grateful to Iowa State University of Science and Technology under Contract No. W-7405-ENG-82 with the U.S. Department of Energy for financial support in the area of lignocellulosics solubilization through the Midwest Consortium for Biobased Products and Bioenergy, and the United Soybean Board for financial support in the area of soybean oil conjugation. Most of all, I thank God for bringing me to Iowa State University, leading me to develop unforgettable relationships, and creating the world for me to explore.
VITAE

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