Glucose Levulinates as Bioplasticizers

WENXIANG XUAN
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Student: Wenxiang Xuan
Supervisor: Minna Hakkarainen

Master of Science Thesis
KTH School of Science and Engineering
Department of Fiber and Polymer technology
Division of Polymer technology
SE-100 44 STOCKHOLM
Abstract

Glucose, as the most plentiful sugar in nature, is a renewable resource and possesses excellent record in health safety. Levulinic acid is a platform chemical which plays an important role in biomass transformation and reactive intermediates. Both glucose and levulinic acid can be produced by biomass conversion with green processing technologies.

Due to the rising needs for bio-based, eco-friendly and non-toxic plasticizers, glucose levulinates as bio-plasticizers were synthesized from glucose and levulinic acid, by utilizing microwave radiation or conventional condensation reaction (direct-heating method). Acid number for the reaction liquor was measured by acid-base titration to follow the decrease of acid groups due to the reaction and the trend in the acid number within reaction time displayed the process of esterification and possible sensitivity of the reaction rate to reaction scale. It showed that microwave radiation had superior ability in enhancing reaction speed but it was also more sensitive to reaction scale and generated more diverse products than the direct-heating method. Besides, the process of reaction and formation of ester bonds was followed and confirmed by FTIR.

The achieved levulinate products were extracted by 2-propanol and ethyl acetate. The practices showed several serious problems in 2-propanol extraction, including high dosage required for NaCl and solvent and difficulties in purification. The ethyl acetate proved to be a suitable solvent for this study and the extracted products from the Con-24hrs and Micro-3/4/5/6/7hrs were characterized by $^1$H NMR, $^{13}$C NMR and LDI-MS. The results from spectrum suggested the presence of GL$_\alpha$ and GL$_\eta$ type of levulinates. That means the glucose levulinates were successfully synthesized although the dehydration side reaction of glucose was inevitable leading to the generation of glucosidic bonds. In addition, BG (mixture of glucose and glycosidic levulinates) was evaluated by solution casting of starch and PVC. In order to minimize the microbial contaminations in solution casting of starch, a modified method was raised and applied. The results showed that 40% BG had good miscibility with starch and the conclusion was further proved by DSC measurements, while the BG performed poor miscibility with PVC.

Keywords:

Glucose esters
Levulinic acid
Plasticizer
Starch
Poly (vinyl chloride)
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1 Introduction

Plasticizer and bio-plasticizer

The development and use of plasticizers has long history and started far before the booming of modern chemical industry. The most common plasticizer at ancient time is water. The early applications include pottery and painting creation. People used water to mix clay to form specific shape and dispersed colorful minerals as pigments for the purpose of painting. For example, the earliest known clay figurines of fertility images of women found in Europe were from 24000 BC and the hand imprints in Lascaux Caves, France, were drawn in 30000 BC [1]. Nowadays, those old methods are still widely applied in art field. However, the term ‘plasticizer’ in modern society is more referred to the additives used in plastic industry. Plasticizers are expected to possess properties such as decreasing the glass transition temperature of the polymer, making material more flexible and modifying rheological properties (eg. viscosity) etc. Therefore, various types of plasticizers other than water are needed and have been designed later and synthesized. In 2008, around 5.6 millions of tons of plasticizers were consumed worldwide. Europe and China contributed 19.6 % and 33.9 % of the total value respectively. When it comes to the details, the majority of plasticizers are used in the manufacture of polyvinylchloride (PVC). Phthalates are the most important group of PVC plasticizer and they constituted 88% of total use of plasticizers all over the world [2]. Nevertheless, the health and environmental issues of plasticizers are increasing concerns. For instance, children may put toys in their mouths and their skin directly contacts the migrated plasticizers. If the plasticizers are not safe enough, then there exists harm for health. Therefore, the use of some phthalates has been restricted in EU in the application of children’s toys since 1999. Furthermore, when the toys are dumped in environment, the plasticizers are leaking into surroundings more and more with increasing time. The environmental impact of plasticizers can be negative and toxic for creatures. Thus, it is essential to develop non-toxic, eco-friendly and bio-degradable plasticizers.

The definitions of bio-plasticizer can be diverse although generally they are considered as plasticizers produced from renewable materials. Due to this property, they may be bio-degradable and green as well. Citrates and glucose esters are two typical bio-plasticizers which have reliable records in safety and non-toxicity with excellent plasticizing performances. For instance, acetyl tributyl citrate (ATBC), as one commercialized plasticizer, can be used as main plasticizer in PVC for food packing and medical products [3]. But it is prone to migrate into protein liquids (eg. milk) [4] and the diffusion coefficient and migration extent of ATBC are sensitive to the fat content of the food in package [5]. Thus, the resistance to migration is included in plasticizer evaluation items. As an example, glucose hexanoate esters demonstrated good miscibility with PVC and showed better mechanical properties and migration resistance as compared to glucose pentaacetate and sucrose octaacetate blends [6]. Meanwhile glucose hexanoate esters displayed the largest improvements in strain at break when used as plasticizers in polylactide films with additional benefit of low migration tendency [7].

Glucose and levulinic acid (LeA)

Glucose is one of the most plentiful naturally-occurring substances, both in free form and combined with water or other sundry molecules. It appears in fruits, honey, blood and so on, even in urine. Due to its intrinsic formation by photosynthesis and its chemically reactive functional groups (five hydroxyl groups and aldehyde structure), therefore, it is naturally chosen as the main building block in cellulose and hemicelluloses which are widely spread in biomass and contribute to the formation of plant tissues. Glucose is also the repeating unit of starch and the industry can therefore produce glucose from biomass and starch (from potato and corn) by degradation or chemical treatment, such as acid-catalyzed hydrolysis or by enzyme-catalyzed hydrolysis. Glucose is classified as aldohexose and it is usually presented in the form of two cyclic isomers (α and β-D-glucopyranose, six-atom ring, α form is shown in Figure 1) in aqueous solution. Open-line structures (aldehyde-D-glucose) and D-glucofuranose (α and β, five-atom
ring) are less common. The phase diagram of glucose-water system has been investigated and proved that 
\( \beta \)-D-glucose solution is the dominant and stable form when temperature is above 115 \(^\circ\)C [8].

![Figure 1](image1.jpg)  
*Figure 1* The absolute stereochemistry structure of \( \alpha \)-D-glucose (from SciFinder)

![Figure 2](image2.jpg)  
*Figure 2* The structure of levulinic acid (from SciFinder)

Levulinic acid (shown in Figure 2) can be produced by cellulose transformation. Some pathways for the 
production of LeA from cellulose include hydrothermal decomposition reaction of cellulose catalyzed by 
ionic liquids [9], utilizing the synergistic effect of microwave radiation and acid catalysis [10] and treating 
microcrystalline cellulose in pure water with cellulase-mimetic solid acid catalyst [11]. Besides cellulose, 
other carbohydrates have been promoted as new biomass resources. Xylose and furfural can both be 
converted into LeA by acid catalyst in dimethoxymethane/methanol [12]. Another successful case is 
presented with the help of mesoporous niobium-containing oxides [13]. Agarose derived from seaweed 
can also be converted under microwave radiation [14]. More interestingly, agriculture waste - wheat straw 
is converted to LeA at atmospheric pressure by continuous extraction of the reactive system with 
organic solvent [15]. LeA is called a green platform chemical due to the fact that it is the product from 
biomass transformation and it is also an important intermediate for further production of numerous 
value-added chemicals. Attempts of making a full product chain have been made and here is an 
encouraging and successful example. LeA was produced from glucose and oil palm fronds and the 
obtained LeA got directly converted to ethyl levulinate through esterification reaction. Both reactions 
were driven by acidic ionic liquid catalyst [16].

**Synthesis of levulnates**

Levulinates, eg. alkyl levulinites, can be synthesized through esterification process with the presence of 
catalyst. The following catalysts have been reported: micro-mesoporous H/BEA zeolite derivatives[17], 
nano-sized TiO\(_2\) [18], modified H-ZSM-5 (micro/meso-HZ-5) [19], sulfated Si- doped ZrO\(_2\) solid acid 
catalysts with enlarged surface areas [20], aluminum-substituted MCM-41 (Al-MCM-41) [21], a set of 
acidic ion- exchange resins [22]. In addition, direct-conversion of biomass into levulinites has been 
investigated. Ethyl levulinate was gained from glucose by using ethanol as a medium with extremely low 
sulfuric acid concentration [23]. Single-step acid-catalyzed solvolysis of cellulolic biomass can produce 
alkyl levulinites (butyl-, pentyl-, and hexyl levulinites) as well [24]. Carbohydrates can be directly 
converted into levulinate by potassium phosphotungstate [25] and heteropolyanion-based ionic liquids 
[26]. When it comes to green chemistry, microwave radiation shows great potential. The speed-up reaction 
rate, less usages of solvent and high efficiency of heating are appealing for the chemists to cooperate 
microwave radiation to realize faster synthesis rate. For example, the esterification of levulinic acid to alkyl 
levulinites has been successfully displayed with the presence of different metal salt catalysts under
microwave [27]. The preparation of ethyl levulinate from levulinic acid and ethanol is reported by using microwave heating and sulfuric acid as catalysts [28].

**Purpose of study**

The study will focus on the microwave-assisted synthesis of glucose levulimates from glucose and levulinic acids and the characterizations of products which have potentials to be used as bio-plasticizers. Both synthesis and processing details will be investigated. The plasticizing effect will be evaluated by DSC measurements on solution casted films. The conventional condensation reaction (direct-heating method) will be used as benchmark.
2 Experimental

2.1 Chemicals

The α-D-Glucose (anhydrous, 96%), diethyl carbonate (99%), levulinic acid (≥97%), poly (vinyl chloride) (Product Number: 389323), sulfuric acid (≥95%), sodium carbonate anhydrous (>99.5%), sodium chlorite (80% RT) were purchased from Sigma-Aldrich Chemie GmbH. Chloroform (HPLC grade), dichloromethane (HPLC grade), ethyl acetate (analytical reagent grade) and tetrahydrofuran (HPLC grade) were from Fisher Scientific. Acetone (GPR Rectapur), diethyl ether (GPR Rectapur), ethanol (96%, GPR Rectapur), glycerol (GPR Rectapur) and 2-propanol (GPR Rectapur) were supplied by VWR. Anhydrous magnesium sulfate (>98%), methanol (hypergrade for LC-MS), sodium chloride (GR for analysis), sodium hydroxide (GR for analysis), sodium hydrogen carbonate (GR for analysis) and water (hypergrade for LC-MS) were bought from Merck KGaA. Potassium carbonate (>99%, for analysis, anhydrous) was from Acros Organics. Tapioca starch was supplied by Ibu Tani, cap anak no.1 (Bogor, Indonesia). Phenolphthalein was from coating division, KTH (unknown purity, no label). Eldorado® corn oil was from Axfood AB. Yes Original dish detergent was from P&G (containing 15 - 30 % sodium C12-14 alkyl sulfate, sodium laureth sulfate, sodium lauryl sulphate, sodium C12-14 pareth-3 sulfate and 5 - 15 % lauramine oxide and PEG-8 propylene glycol ether) [29]. All chemicals above were used as received. GPR Rectapur is equal to chemically pure (CP). GR for analysis represents analytical reagent (AR).

The molecular weight of PVC was determined by SEC results in author’s previous project. The number average molecular weight is 64600 ± 1300 g/mol and weight-average molecular weight is 137500 ± 700 g/mol. Dispersity equals 2.1.

2.2 Direct Synthesis of Glucose Levulinate by Conventional Condensation Reaction

Glucose and levulinic acid were heated in a 100 mL three-neck round-bottom flask for 8 and 24 hours at 140 °C under nitrogen (N2 pressure: 1 - 1.6 Bar). The molar ratio of glucose and levulinic acid was 1:4. A blank comparison experiment with pure glucose was conducted as well with identical reaction conditions. The reaction apparatus is shown in Figure 3 and the information about samples is described in Table 1.

![Figure 3 Apparatus for direct heating Synthesis](image)
Table 1 Information of samples synthesized by direct-heating Synthesis

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Reaction Time &amp; Batch</th>
<th>Extraction Solvent</th>
<th>Glu Input (g)</th>
<th>LeA Input (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con-8hrs</td>
<td>24 hours. Batch 1</td>
<td>2-propanol</td>
<td>49,87</td>
<td>128,60</td>
</tr>
<tr>
<td>Con-24hrs-1</td>
<td>24 hours. Batch 1</td>
<td>2-propanol</td>
<td>22,58</td>
<td>58,08</td>
</tr>
<tr>
<td>Con-24hrs-2</td>
<td>24 hours. LeA batch 2</td>
<td>Ethyl acetate</td>
<td>22,52</td>
<td>58,06</td>
</tr>
<tr>
<td>Blank</td>
<td>8 hours.</td>
<td>Ethyl acetate</td>
<td>37,4635</td>
<td>0,00</td>
</tr>
<tr>
<td>Con-BG</td>
<td>Precipitates in extraction of Con-24hrs-2 with ethyl acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con-Sediment</td>
<td>Precipitates in extraction of Con-24hrs-BG with ethyl acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.3 Microwave-assisted Synthesis of Glucose Levulinic Acid

MILESTONE FlexiWAVE MA186 was used as synthesis platform. Glucose and levulinic acid were placed in a 500 mL three-neck round-bottom flask for 100 minutes, 180 minutes and 3-7 hours at 140 °C under nitrogen (N2 pressure: around 1 Bar). The molar ratio of glucose and levulinic acid was 1:4. The microwave heating programme contained two stages.

Stage 1: 10 minutes to heat up to 140 °C with 60 % stirring and external ventilation.

Stage 2: Keep temperature at 140 °C for 3 or 7 hours with 60 % stirring and external ventilation.

The reaction apparatus is shown in Figure 4 and the information about samples is described in Table 2.

Figure 4 Apparatus for microwave-assisted synthesis

Table 2 Information of samples synthesized by direct-heating Synthesis

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Reaction Time &amp; Batch</th>
<th>Extraction Solvent</th>
<th>Glu Input (g)</th>
<th>LeA Input (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-100</td>
<td>100 minutes</td>
<td>2-propanol</td>
<td>63,75</td>
<td>162,59</td>
</tr>
<tr>
<td>Micro-180</td>
<td>3 hours. LeA batch 1</td>
<td>2-propanol</td>
<td>67,61</td>
<td>174,16</td>
</tr>
<tr>
<td>Micro-7hrs</td>
<td>7 hours. LeA batch 2</td>
<td>Dichloromethane</td>
<td>112,60</td>
<td>291,32</td>
</tr>
<tr>
<td>Micro-Xhrs</td>
<td>X = 3,4,5,6,7. LeA batch 2</td>
<td>Ethyl acetate</td>
<td>112,60</td>
<td>291,32</td>
</tr>
<tr>
<td>BG</td>
<td>Precipitates in extraction of Micro-7hrs with dichloromethane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sediment</td>
<td>Precipitates in extraction of BG with ethyl acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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2.4 Extraction Method

The extraction process with 2-propanol, dichloromethane and ethyl acetate were studied.

**Extraction with 2-propanol**

Target: Con-8hrs, Con-24hrs-1, Micro-100, Micro-180.

The reaction liquor was neutralized by NaHCO$_3$ (s) and saturated NaHCO$_3$ (aq.). The solution was then extracted with 2-propanol, equal volume to water phase was used. Saturated NaCl (aq.) was added into the funnel to enhance the separation. The propanol phase was collected and later treated by anhydrous magnesium sulfate and filtered under vacuum for 4 cycles. The filtrate was transferred to rotary evaporator to remove the propanol and further dried in vacuum oven for at least 24 hours.

**Extraction with dichloromethane**

Target: Micro-7hrs

The reaction liquor was first dissolved in deionized water and then neutralized by 30 % (w/w) potassium carbonate untill no bubbles were generated. Next, the mixture was extracted with dichloromethane in separation funnel. The funnel was gently rotated. The black bottom phase was collected and isolated by rotary evaporator and was further dried in vacuum oven for at least 24 hours.

**First Extraction with ethyl acetate**

Target: Micro-3hrs, Micro-4hrs, Micro-5hrs, Micro-6hrs, Micro-7hrs, Con-24hrs-2.

Reaction liquor was first dissolved in deionized water and then neutralized by 30 % (w/w) potassium carbonate until no bubbles were generated. Then, the dilution was extracted with ethyl acetate in separation funnel. Only for the sample Micro-7hrs, saturated NaCl (aq.) was added into the funnel which created a new brown middle layer. The funnel was gently rotated and let stand still for phase separation. The upper ethyl acetate phase and black bottom precipitates were collected. The ethyl acetate phase was rinsed with deionized water one time to lower salt concentration. The solvent in the black bottom precipitates and ethyl acetate phase were separated by rotary evaporator. The substances left after evaporation were further dried in vacuum oven for at least 24 hours.

**Second extraction of black precipitates with ethyl acetate**

Target: Con-BG, Micro-BG.

The black precipitates with first diluted with deionized water and then extracted by ethyl acetate and left to phase separation for overnight. The water phase was dried in 120 °C oven. Ethyl acetate phase and sediment (black bottom phase in second extraction) were driven by rotary evaporator and all the substances left were further dried in vacuum oven for 96 hours.
2.5 Understanding of Reaction System

2.5.1. Compatibility with Sulfuric Acid

A compatibility test was designed to check the possibility of cooperation with concentrated sulfuric acid as catalyst for reaction system. Concentrated sulfuric acid is a traditional catalyst in esterification reaction. Both pre-dispersion and post-adding methods were tested. The two methods are listed below.

Pre-dispersion method

20.01 g LeA and 0.82 g 98 % H₂SO₄ were mixed first, then small amount of glucose was added at R.T. The mixture was heated in round-bottom flask to 140 °C.

Post-adding method

46.33 g glucose and 116.19 levulinic acid were added first, followed by addition of 4.22 g 98 % H₂SO₄. The mixture was heated in round-bottom flask at for 5 min at 140 °C.

2.5.2. Reaction Rate - Acid Number Measurement

The acid number (AN) is a value in milligrams that is defined as the weight of NaOH needed to neutralize a gram of reaction liquor. The value is usually determined by acid-base titration. The AN versus time curve can establish a profile of reaction rate and here the decrease of AN proves the consumption of LeA in the esterification reaction. Thus, the acid number also offers the degree of esterification (%) which is calculated as:

\[
\text{Degree of esterification (\%)} = 1 - \frac{AN_{\text{reaction liquor}}}{AN_{\text{raw materials}}}
\]

Around 0.3 g reaction liquor was dissolved in 20 - 30 mL deionized water homogeneously and then neutralized by 0.1 M NaOH with stirring. The phenolphthalein was used as indicator. The terminal of titration was reached when the pink color of solution could last for more than 15 seconds. Solo titration was conducted on Con-24hrs-1 and triplicate titration was applied to the rest.

2.5.3. Presence of Ester Linkage - Fourier Transform Infrared Spectrometry (FTIR)

Glucose contains a primary hydroxyl group at C6 position and secondary hydroxyl groups at C1 - C4 positions plus one -C-O-C- group in the sugar ring. Due to the strong intermolecular hydrogen bonding effect, levulinic acids usually exist as dimers, especially considering the high concentration in this case. The hydroxyl groups in Glu, carboxyl groups in LeA, and ester bonds in synthesized esters could be seen in FTIR spectra. Excluding the possible volatile loss of LeA, the carbonyl groups are supposed to be constant before and after reaction. The decreasing concentration of carboxyl groups in LeA (3300 - 2500 cm⁻¹) and increasing concentration of ester bonds are expected within rising reaction time. Additionally, the characteristic peak of carbonyl groups varies in ester (1750 - 1725 cm⁻¹), ketone (1725 - 1705 cm⁻¹) and carboxylic group (1740 - 1700 cm⁻¹). Those shifts can be used to identify the source of carbonyl groups. Moreover, the asymmetric stretching vibration in ester bond is 1275 - 1185 cm⁻¹ and 1160 - 1050 cm⁻¹ for symmetric stretching vibration [30].

The formation of ester linkages in reaction liquor were followed by Perkin-Elmer Spectrum 2000 FTIR spectrometer (Norwalk, CT) equipped with a single reflection attenuated total reflectance (ATR) accessory (golden gate) from Graseby Specac (Kent, UK). Each sample was scanned 16 times with a wavenumber region of 4000 cm⁻¹ - 600 cm⁻¹.

-10-
2.6 Product Analysis

2.6.1. Solubility Behavior

Con-24hrs-1 was used to study the solubility behavior during extraction of glucose levulimates. Around 0.2 g Con-8h-1 was placed in a 10 mL vial and neutralized by saturated NaHCO₃ (aq). Then saturated NaCl (aq) and organic solvent were added into the vial and shaken for 5 seconds. Finally, the solution was left to separate for 24 hours.

2.6.2. ¹H NMR & ¹³C NMR

The NMR spectra of glucose and levulonic acid have significant differences in the peak shifts and therefore, it is possible to easily identify the local structures in glucose levulimates by finger printing the characteristic peaks. NMR measurements were completed by Bruker Avance III HD 400NMR instrument at room temperature. The NMR was equipped with BBFO probe, Z-gradient and automated tuning and matching device. D₂O and CDCl₃ were used as solvent.

2.6.3. Laser Desorption Ionization - Mass Spectrometry (LDI - MS)

The extracted/dissolved products from various phases were dissolved in methanol/water solution (v/v = 1:1) with a concentration of around 1 mg/mL. No matrix was applied. The pipette was used to transfer 1 µL sample dilution to the metal plate precisely. The analysis was performed on Bruker Ultra Flex time-of-flight (TOF) mass spectrometer (Bruker Daltonic, Bremen, Germany) in a positive mode. The calibrated mass-to-charge (m/z) ratio range was 500-1600 and 1600-3500 with a reflector voltage of 26.3 kV and an accelerated voltage of 25 kV.

2.7 Plasticizing Performance Evaluation on Starch and PVC

2.7.1. Solution Casting for Starch

Polymer films were obtained by solvent casting method in petri dishes (diameter = 137 mm). All petri dishes were flushed with 75% (v/v) ethanol in advance for the purpose of disinfection. Two casting methods below were tried and the modified one succeeded.

Original method

Around 10 g starch or its blend was dispersed in 100 ml ultrapure water and was heated at 80 °C with high speed stirring. After 30 min gelatinization, the solution was poured into petri dishes with cover and dried at room temperature for 7 days and another 2 days in a 25 °C vacuum drying chamber.

Modified method

Around 10 g starch or its blend (20% and 40% BG or glycerol) was dissolved in 150 ml boiled deionized water at 80 - 85 °C with high speed stirring. When the gelation was reached and the solution turned hazy, the solution was poured into petri dishes when still hot. As for PVC, 2 g PVC or its blend (20% and 40% BG) was dissolved in 50 mL THF at 40 °C with stirring. When the solution had cooled down to room temperature, it was poured into petri dishes. The petri dishes with solution were stored in fume hood for 3 days at room temperature to evaporate solvent (no cover for starch-containing films), followed by 2 days in 25 °C vacuum drying chamber.
2.7.2. Solution Casting for PVC

Around 2 g PVC or its blends (20% and 40% BG) BG were prepared for PVC films. The BG part was dissolved in acetone first and then the PVC was dissolved in 60 ml THF at 40 °C with stirring. After 30 minutes, the acetone was injected into the THF solution by syringe with fierce stirring. The mixture was poured into petri dishes with cover and dried at room temperature for 2 days.

2.7.3. Differential Scanning Calorimetry (DSC)

The glass transition temperature of the films was measured by Mettler Toledo DSC 820 Module. Triplicate samples of every film were analyzed. The samples were first heated from 25 °C to 100 °C and then kept at 100 °C for 2 minutes, after which the films were cooled down to -100 °C and kept there for 3 minutes, followed by the second heating scan to 200 °C. Both heating and cooling rate were 10 °C per minute. Measurements were done under nitrogen atmosphere and only the second heating scan was used to determine the glass transition temperature.

2.8 Emulsion Stability Evaluation

Around 1 mL corn oil and 7 mL 5% BG (aq.) or Yes Original was placed in 10 mL vials. The two vials were horizontally placed on IKA® KS 130 BASIC orbital shaker to shake for 10 minutes at 640 rpm. Then the vials were left for 24 hours.
3 Results and Discussion

The synthesis, processing, products and performance are the four critical parts in the development of new functional materials.

In this study, three topics were covered to develop and understand the synthesis reaction: compatibility with acidic catalyst (H₂SO₄), reaction rate and ester bonds formation (FTIR). The processing section mainly focused on the extraction phenomenons. The synthesized levulinites were characterized by ¹H NMR, ¹³C NMR and LDI-MS to determine the local structures and molecular weight. As for performance evaluation, the plasticizing effect of BG, which is the main products in Micro-7hrs, was evaluated by solution casting with starch and PVC as polymer matrices.

3.1 Understanding of Reaction System

3.1.1. Compatibility with Sulfuric Acid

![Figure 5 Appearance of liquor with the presence of H₂SO₄.](image)

In general, glucose is very sensitive to the presence of concentrated H₂SO₄ which may cause oxidation and dehydration reactions on glucose. However, sulfuric acid is a common catalyst in esterification reaction. In some situations, sulfuric acid exists as impurities in rough glucose or LeA if they are produced from biomass transformation through acid-catalyzed hydrolysis. If the concentrated H₂SO₄ can be applied as a catalyst for glucose/LeA system, then the processing procedures can be coherent and the final yield and reaction rate will both increase a lot. All those parameters would be extremely meaningful to green production. Therefore, the compatibility of the system with sulfuric acid was studied with two different feeding sequences, pre-dispersed or post-added.

The pre-dispersion test showed that LeA could be mixed with 98 % H₂SO₄ homogeneously and the dehydration reaction speed was quite slow at R.T. Very light yellow color could be observed. However, when the reactants were heated, the liquor turned black quickly. The post-adding test showed that the concentrated H₂SO₄ could form clumps in the mixture of LeA and Glu. The dehydration reaction caused by concentrated H₂SO₄ happened even at R.T. and speed up dramatically under heating. Eventually, dark brown-black liquor containing carbon residues was obtained (seen in Figure 5).

Thus, concentrated H₂SO₄ is not compatible with the reaction system and could not be used as a catalyst in this case.
3.1.2. Reaction Rate - Acid Number Measurement

**Figure 6** Acid number vs. Reaction time curves (0 - 24 hours). They give comparisons between conventional and microwave synthesis by comparing the development of acid number during 0-24 hours.

**Figure 7** Acid number vs. Reaction time curves (0 - 8 hours)
Figure 8 The effect of scale-up in microwave synthesis. Batches of 241 g and 404 g were compared.

Esterification reaction is a reversible equilibrium process and the reaction speed is controlled by concentration and structure of reactants and products, reaction time, temperature, type of catalyst, solvent and etc. Due to the variation of reaction parameters in every batch, it is necessary to describe the extent of reaction in reaction liquor by a fast and comparable way. Since the degree of esterification was expected to be varied by reaction time, a curve of reaction extent versus reaction time was established. In order to do so, the concept of acid number (AN) was introduced and it was determined by acid-base titration. The AN can be converted into degree of esterification.

The overall curves illustrated that the microwave radiation exhibited higher efficiency in accelerating reaction speed, compared with conventional condensation reaction by direct heating. In addition, the real reaction pathways in synthesis were somewhat different for the two methods, according to the shape of the curves.

Figure 6 clearly demonstrated that microwave indeed accelerated the speed of reaction dramatically, regardless the differences in scale. In direct-heating synthesis, the degree of esterification after 3 hours for Con-24hrs-1 is 12.2 % and that for Micro-100 is 51.4 %. In Con-8hrs, the degree of esterification after 7 hours for direct-heating synthesis is 19.4 ± 0.2 % and that for Micro-7hrs is 51.6 ± 0.1 %.

Furthermore, the curve shape altered in those two methods. The curve of direct-heating synthesis follows a decreasing reaction rate with increasing time. The shape of full curve shows some similarity with logarithmic function (seen in Figure 7). However, the full curve of microwave-assisted synthesis seems to be more linear. The differences in the shape of AN vs. time curve may suggest that microwave radiation involved other reaction mechanisms. Besides, no wide gap of esterification degree between the two methods was found in the first 30 minutes of reaction time in which temperature had already been 140 °C for at least 10 minutes. The microwave radiation was expected to have more significant impact on yield than direct-heating. Given the facts above, the influences of steric hindrance and reactive points of glucose need to be considered if probably the kinetics affects reaction more than the thermodynamics during the early stage of esterification.

Nevertheless, the microwave-assisted method appeared to be more sensitive to reaction scale than direct-heating. As shown in Figure 8, Micro-7hrs (404 g) is 1.67 times of Micro-100 (242 g) in scale, but it took 2.3 times of reaction time to reach similar degree of esterification. Similar situation had not been found in direct-heating synthesis, though the scale of Con-8hrs (180 g) is 2.25 times of Con-24hrs-1 (80 g).
3.1.3. Presence of Ester Linkage – Fourier Transform Infrared Spectrometry

![FTIR spectrum](image)

**Figure 9** The FTIR spectrum of micro-Xhrs (X=1, 2, 3, 4, 5, 6, 7)

![O-H str. in LeA, C=O str.](image)

**Figure 10** The characteristic peaks of functional groups in micro-Xhrs (X=1, 2, 3, 4, 5, 6, 7)

The curve of AN versus time confirmed the consumption of LeA, but the formation of ester bonds need to be confirmed and followed by FTIR. All the used reactants and expected products contain IR-active functional groups which are detectable and measurable in FTIR test. Every new ester bond consumes one hydroxyl group and one carboxyl group. Thus, the increasing transmittance of carboxyl groups as a falling trend in transmittance of ester groups in FTIR spectra supports the rising degree of esterification with longer reaction time, which was displayed in Figure 9 & 10.

Figure 9 presents that the reactant liquors possessed similar FTIR spectrum with various intensity of characteristic peaks, even in finger print region. That implies a high probability that the main dominant reaction path was identical within increasing time. On the other hand, the obtained products shared similar structural units and functional groups. More details are illustrated in Figure 10, illustrating the gradient changes in transmittance of 7 curves in 3300 - 2500 cm\(^{-1}\), 1750 - 1700 cm\(^{-1}\) and 1300 - 1100 cm\(^{-1}\), corresponding to carboxyl group stretching vibration, carbonyl group stretching vibration and \(-\text{C-O-C-}\) stretching vibration respectively. The rising of transmittance in wavenumber region of 3300 - 2500 cm\(^{-1}\)
and falling of transmittance at 1738 cm⁻¹ prove the emerging formation of ester linkages within increasing reaction time, because the hydroxyl groups in glucose and LeA are consumed and meanwhile more ester groups are generated. Higher degree of esterification, therefore, can be expected. The absorption at 1206 cm⁻¹ and 1159 - 1153 cm⁻¹ can be contributed to the asymmetric and symmetric stretching vibration of –C-O-C- bonds. However, except esters, the glycosidic bonds can also contribute to the absorption in that region. The later LDI-MS characterizations for obtained products imply the presence of glycosidic bonds. Hence, those two peaks are not reliable enough to confirm the formation of ester bonds. The absorption at 1706 - 1701 cm⁻¹ is given by acetyl groups in LeA and they should keep constant before and after reaction if they are not involved in the reactions. However, due to the chain length is altered, the maximum absorption peak may shift little bit with various degree of substitution.

3.2 Extraction Process

The selection of neutralizer

![During neutralization vs Neutralized](image)

*Figure 11 The appearance of the dilution during and after neutralization (by 30% K₂CO₃)*

Due to the high viscosity and high level of unreacted LeA in reaction liquor, choosing an appropriate neutralizer can simplify the post treatments with less attention for processing. High concentration base usually has excellent efficiency but is very easy to over-neutralize the liquor and which can cause hydrolysis of esters if not well handled. Low concentration of base is safer but the volume of solution may expand too much and consequently it requires higher usage of organic solvent in extraction and longer processing time. Hence, the neutralizing process with NaHCO₃ (s), saturated NaHCO₃ (aq.), 1 M Na₂CO₃ (aq.) and 30% K₂CO₃ (aq.) were studied.

Table 3 below shows the solubility of chemicals in water at ambient temperature and potassium is more basic than sodium. As seen in Figure 11, foams and clumps were generated during neutralization with 30% K₂CO₃. Similarly, more severe foaming and volume expanding phenomenon was observed in neutralization with solid NaHCO₃ powder. Due to the extremely low water content in high-viscosity reaction liquor, the NaHCO₃ powder required high shearing and the longest processing time to get well dispersed and hydrated to complete neutralization. The decreased reaction speed also occurred in neutralizations with saturated NaHCO₃ (aq.) and 1 M Na₂CO₃ (aq.). However, due to the lowest OH⁻ concentration in saturated NaHCO₃ (aq.), the required volume of basic dilution was the biggest, which led to higher dosage of extraction solvent.

Considering the final results, 30% K₂CO₃ (aq.) is recommended as neutralizer.

Table 3 The water solubility data of neutralizers as provided by MSDS
<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Solubility in water (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO₃ (s)</td>
<td>50 g/l</td>
</tr>
<tr>
<td>Na₂CO₃ (s)</td>
<td>217 g/l at 20 °C; 2.07 mol/Kg at 293.15 K [31]</td>
</tr>
<tr>
<td>K₂CO₃ (s)</td>
<td>138 g/l at 20 °C; 112 g/100 mL at 20 °C [32]</td>
</tr>
</tbody>
</table>

**The selection of solvent**

![Figure 12 The salting out phenomenon of GLs 24hrs](image)

**Table 4 The selection results of extraction solvent**

<table>
<thead>
<tr>
<th>Solvent Name</th>
<th>Vapor Pressure (at 20,0 °C)</th>
<th>Boiling Point</th>
<th>Dielectric Constant (at 20,0 °C)</th>
<th>Emulsifing Effect</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform</td>
<td>213,3 hPa</td>
<td>60.5 - 61.5 °C</td>
<td>4.8</td>
<td>Yes</td>
<td>Succeed</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>470,9 hPa</td>
<td>39.8 °C</td>
<td>9.1</td>
<td>Maintain 3 layers for 2 days</td>
<td>Succeed</td>
</tr>
<tr>
<td>Diethyl carbonate</td>
<td>13 hPa (23.8 °C)</td>
<td>125 - 126 °C</td>
<td>3.1</td>
<td>No</td>
<td>Fail</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>97,3 hPa</td>
<td>76.5 - 77.5 °C</td>
<td>6 at 25,0 °C</td>
<td>Slightly</td>
<td>Succeed</td>
</tr>
<tr>
<td>Cyclohexane</td>
<td>102,7 hPa</td>
<td>80.7 °C</td>
<td>2 at -7 °C</td>
<td>No</td>
<td>Fail</td>
</tr>
<tr>
<td>Ethyl ether</td>
<td>563 hPa</td>
<td>34,6 °C</td>
<td>4,3</td>
<td>No</td>
<td>Fail</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>43,2 hPa</td>
<td>82,2 °C</td>
<td>18,3</td>
<td>No</td>
<td>Soluble</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
<td>-</td>
<td>24,5</td>
<td>No</td>
<td>Soluble</td>
</tr>
<tr>
<td>Acetone</td>
<td>-</td>
<td>-</td>
<td>20,7</td>
<td>No</td>
<td>Soluble</td>
</tr>
<tr>
<td>Water</td>
<td>23,4 hpa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Note: 1 hPa = 100 Pa

The ideal solvent in this study should be low toxic, high efficiency (excellent ability to dissolve synthesized products), cheap and easy to be evaporated. The vapor pressure of solvent determines the evaporation speed and boiling point provides the limit operation temperature for rotary evaporator. Dielectric constant
offers general idea of polarity of substances which has dominant effect on the solubility of targeting products. Taking those factors into consideration, several common solvents in the lab have been tested. The selection was performed on Con-24hrs-1.

The Figure 12 showed three typical evaluation results in extraction: emulsion system, two-phase system and three-phase system. The emulsion system refers to the phenomenon that the synthesized substances have good affinity to solvent and have ability to lower interface tension, leading to the formation of emulsion with shearing. But after several days, it turned to be three-phase system, which contains precipitates, solvent phase and water phase. Because emulsion is a thermodynamically unstable system and it always ends with phase separation. The two-phase system (solvent phase and water phase) was only achieved when 2-propanol was applied. In addition, homogeneous solution was obtained by ethanol and acetone, which made the further purification difficult.

Hence, 2-propanol was first selected and tried on Con-8hrs, Con-24hrs-1, Micro-100 and Micro-180 because two-phase system is more economic for processing. However, dichloromethane was used as an alternative solvent on Micro-7hrs after the failure in 2-propanol extraction (more details discussed in next part). Regardless of all those attempts above, ethyl acetate was finally chosen as the solvent for BG and Con-24hrs-2.

**Extraction with 2-propanol**

![Propanol extraction on Con-24hrs-1](image1)

![The color of extracted 2-propanol phase](image2)

Several serious drawbacks appeared during extraction with 2-propanol and therefore this extraction method was not used on other samples.

Firstly, the extraction process required high dosage of 2-propanol and NaCl. The water and propanol still dissolved each other to a high degree even though they were phase separated. When the 2-propanol phase was treated by rotary evaporator, the presence of water was found in cold trap according to the growth of ice crystals and the 2-propanol could not be removed at temperature higher than 80 °C and reduced pressure. Hereafter, the extracted phase was treated by anhydrous magnesium sulfate and filtered under vacuum. Secondly, over 50 % volume of organic phase was lost or bonded to MgSO₄ in dewatering process, although the filtrate containing 2-propanol and synthesized products were achieved. Then the filtrate was transferred to rotary evaporator again to remove the 2-propanol. Nevertheless, it still failed to evaporate 2-propanol even though the dewatering step mentioned above was repeated for 4 times.
Perhaps the salt residue from NaCl (aq) and synthesized substances organized together to give very strong interaction with water and 2-propanol which resulted in huge difficulties in rotary evaporation.

Figure 13 provided the image of the two-phase system after 2-propanol extraction on Con-24hrs-1. The color of the extracted 2-propanol phase is supposed to be brown (by microwave) or black (by direct heating) as shown in Figure 14. The source of yellow color in Mico-180 and Con-8hrs was from chemical bleaching by sodium chloride, which was mistakenly read as sodium chloride and used and consequently ruined those two samples. Despite many efforts, the problems connected with 2-propanol extraction could not be solved.

The precipitates in extraction with dichloromethane or ethyl acetate

![Image](image1)

**Figure 15** The formation of three-phase system from the Con-24hrs-2 in first extraction

![Image](image2)

**Figure 16** The formation of three-phase system from the Con-24hrs-2 in second extraction

The formation of the three-phase system was the most surprising phenomenon in this study which only occurred in the case of Micro-7hrs and conventional condensation method. The precipitates from the first time extraction could also be extracted for the second time with ethyl acetate and a new three-phase system emerged again.

In Figure 15, the formation of the three-phase system was shown for the Con-24hrs-2 extraction with ethyl acetate and no saturated NaCl (aq) was needed. As for the Micro-7hrs, extracted with dichloromethane, it also formed three-phase system without the aid of saturated NaCl (aq), but additional salt solution could speed up the separation. Meanwhile there was no precipitate found in the Micro-3hrs, Micro-4hrs, Micro-5hrs and Micro-6hrs systems with or without the help of saturated NaCl (aq), which provided an important clue about the precipitates, which thus only exists in samples after longer reaction time. In other words, the precipitates may have higher substitution of LeA in structure due to longer reaction time.

Further investigations of the precipitates were conducted. As soon as the precipitates were isolated, they were extracted for the second time (Figure 16), which means the precipitates were diluted and extracted with ethyl acetate again. Three phases were obtained one more time. The colored water phase and organic phase suggested the precipitates in Figure 15 were a mixture and both water-soluble and hydrophobic components.
3.3 Product Analysis from Distilled Fractions

Figure 17 The distilled fractions from the microwave synthesis

Figure 18 The 1H NMR spectra of the distilled products and LeA (D₂O, Normalized by the largest peak).

Figure 19 The FTIR spectra of the distilled products and LeA.

The distilled fractions were found in ventilation tube (see Figure 17) and were later characterized by 1H NMR and FTIR and compared with pure LeA. The fractions were identified as levulinic acid.

The 1H NMR spectra (Figure 18) of the distilled fractions shared almost the same chemical shifts with pure LeA (both two batches). At the same time, the FTIR spectra of fractions had same pattern with pure LeA, including the fingerprint region (Figure 19).

Additionally, the test also proved that the two batches of levulinic acid were chemically identical according to the same performances in 1H NMR and FTIR spectrum.
3.4 Product Analysis of Con-24hrs-2

The substances in water phase and organic phase from the first extraction of Con-24hrs-2 were characterized by $^1$H NMR, $^{13}$C NMR and LDI-MS. The precipitates from the first extraction were further treated by second extraction with ethyl acetate. The substances in water phase, organic phase and the precipitates were also characterized by the techniques mentioned before. Information about the structure and molecular weight of the synthesized products were achieved. The results from solvent phase represented the successful synthesis of glucose levulinates and glycosidic levulinates with various molecular weights. The byproduct - glucose dimer - had been synthesized as well, though it was not detected in the blank experiment. However, the products in water phase were unable to be identified due to the possible screening effect by high concentration of LeA residue in the water phase.

Solvent Extracts

![Image of NMR spectra]

**Figure 20** The $^1$H NMR spectra of the extracted products from the Con-24hrs-2 (CDCl$_3$, Normalized by the largest peak)
The NMR peaks of the compounds from ethyl acetate phase are displayed in Figure 20 & 21. Due to the poor solubility of glucose in CDCl₃, it is hard to see the characteristic peaks of glucose on spectra. But, the shift of carbonyl group in the ¹³C NMR spectra could prove the formation of ester bonds that connect to glucose. For example, the carbonyl carbon in ethyl acetate displays a peak at 170.96 ppm while the carbonyl carbon in LeA has a peak at 178.70 ppm in Figure 21. Despite the presence of solvent residue, the components in the first and the secondary extractions could be confirmed as levulinates since they both have carbonyl carbon shift and possess full characteristic peaks of LeA. Viewing the ¹H NMR and ¹³C NMR spectrum together, the components from the first and second extraction and the sediment seem to share very similar structures.
Figure 24 The LDI-MS spectra of the Con-sediment

Table 5 The LDI-MS peaks of solvent extracts and sediment

<table>
<thead>
<tr>
<th>m/z</th>
<th>381</th>
<th>480</th>
<th>596</th>
<th>740</th>
<th>838</th>
<th>954</th>
<th>970</th>
<th>1098</th>
<th>1158</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st. solvent extracts</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>2nd. solvent extracts</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Sediment</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
</tbody>
</table>

Figure 22 - 24 show the LDI-MS spectrum of the synthesized products. The peaks detected in the spectrum can correspond to glycosides, sodium adducts and potassium adducts (K₂CO₃ was used as neutralizer) of glucose esters. Very similar products were observed in the first and second extracts, which means for some reason the glucose esters remained in Con-BG. The peaks seen in Table 5 were assigned as follows: 381 = G₂-K⁺, 480 = G₂L₄-K⁺, 596 = GL₄-Na⁺, 740 = G₃L₂-K⁺, 838 = GL₃-K⁺, 954 = G₂L₆-Na⁺, 970 = G₂L₆-K⁺, 1098 = unknown. 1158 = unknown.

The dehydration reaction between the glucose also happened and thus some glycosides could be found in the spectra. Still, G₂L₄ and GL₄ were the main components in the ethyl acetate phase, while the dimer of glucose and its esters with very low substitution degree were more dominant in the sediment. That explained their poor solubility in ethyl acetate due to the hydrophilic property of glycosidic linkages.

-24-
Water Extracts

**Figure 25** The $^1$H NMR spectra of the water-extracted products from the Con-24hrs-2 ($D_2O$, Normalized by the largest peak)

**Figure 26** The $^{13}$C NMR spectra of the water-extracted products from the Con-24hrs-2 ($D_2O$, Normalized by the largest peak)
The glucose peak at 95.92 ppm belongs to β-C1 and the peak at 92.11 corresponds to α-C1, as shown in Figure 25. Strangely, no sugar structures are linked to water-soluble components according to Figure 25 and Figure 26. Considering the high amount of LeA in the water phase, one possible reason to explain the disappearing of ring structure is that the possible products may be screened by very intensive peaks from LeA.

![Figure 27 The LDI-MS spectra of the first water extracts from the Con-24hrs-2](image)

![Figure 28 The LDI-MS spectra of the second water extracts from the Con-24hrs-2](image)

**Table 6** The LDI-MS peaks of the water extracts from the Con-24hrs-2

<table>
<thead>
<tr>
<th>m/z</th>
<th>389</th>
<th>397</th>
<th>479</th>
<th>495</th>
<th>513</th>
<th>657</th>
<th>755</th>
<th>872</th>
<th>918</th>
<th>1016</th>
<th>1114</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st. water extracts</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>2nd. water extracts</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
</tbody>
</table>

The peaks detected in water phase do not fit any estimated molecular weight with G.L type, including its sodium or potassium adducts. However, higher molecular weight substances can be released from Con-BG into water though the exact chemical structures are unclear, comparing Figure 27 with Figure 28.

**Blank experiment**

![Figure 29 The 1H NMR spectra of the possible products (left: D$_2$O, Normalized by the largest peak; right: D$_2$O, Normalized by peak range 4.65 - 4.50 ppm)](image)
Figure 30 The $^{13}$C NMR spectra of the possible products ($D_2O$, Normalized by the largest peak)

Figure 31 The LDI-MS spectra of possible products from the blank experiment (left) and background noise (right)

Nothing new was found in NMR spectrum (Figure 29 & 30), only tiny amount of glucose residue. No obvious targeting molecule or its sodium/potassium adducts could be seen in LDI-MS spectra (Figure 31). In addition, glucose does not show any peak in LDI-MS.
3.5 Product Analysis of Micro-Xhrs (X=3, 4, 5, 6, 7)

The substances in organic phase from Micro-Xhrs and BG and the precipitates from BG were characterized by $^1$H NMR, $^{13}$C NMR and LDI-MS. Information about structure and molecular weights of synthesized products were achieved. The results from solvent phase suggest the successful synthesis of glucose levulimates and glycosidic levulinates with various molecular weights. The byproduct - glucose dimer - had been formed as well. But, BG contains more diverse levulinates with higher molecular weight than the low-yield solvent extracts of Micro-3/4/5/6/7hrs.

**Solvent Extracts**

![Graph showing NMR spectra](image)

*Figure 32* The $^1$H NMR spectra of the extracted products from the Micro-3/4/5/6/7hrs (CDCl$_3$, Normalized by the largest peak)*
Figure 33 The $^1$H NMR spectra of the extracted products from the Micro-3/4/5/6/7 hrs (CDCl$_3$, Normalized by the largest peak)

One likely pattern can be seen in Figure 32 & 33 for microwave samples with increasing reaction time from 3 hours to 7 hours. The peaks of region 175 - 60 ppm in $^1$H NMR most likely belong to glucose. The local structures in organic compounds can be similar according to NMR spectrum, though the BG seems little bit different to others. Figure 34 below shows the color of products from ethyl acetate phase of Micro-6hrs and Micro-7hrs.

Figure 34 The appearance of the products Micro-6hrs and Micro-7hrs

Unfortunately, the yield of the ethyl acetate phase is very low, as seen in Table 4. Only several hundred milligrams of the products could be extracted from 12 - 15 grams of untreated reaction liquor of the Micro-3/4/5/6/7hrs.

Table 4 The yield of Micro-3/4/5/6/7hrs

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Micro-3hrs</th>
<th>Micro-4hrs</th>
<th>Micro-5hrs</th>
<th>Micro-6hrs</th>
<th>Micro-7hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield / mg</td>
<td>139,6</td>
<td>123,9</td>
<td>244,9</td>
<td>476,0</td>
<td>841,9</td>
</tr>
</tbody>
</table>
Figure 35 The LDI-MS spectra of the products in the solvent extracts from Micro-3hrs

Figure 36 The LDI-MS spectra of the products in the solvent extracts from Micro-4hrs

Figure 37 The LDI-MS spectra of the products in the solvent extracts from Micro-5hrs

Figure 38 The LDI-MS spectra of the products in the solvent extracts from Micro-6hrs

Figure 39 The LDI-MS spectra of the products in the solvent extracts from Micro-7hrs
Table 7 The LDI-MS peaks of solvent extracts from Micro-Xhrs (X=3,4,5,6,7)

<table>
<thead>
<tr>
<th>m/z</th>
<th>382</th>
<th>398</th>
<th>480</th>
<th>497</th>
<th>498</th>
<th>524</th>
<th>552</th>
<th>597</th>
<th>613</th>
<th>625</th>
<th>742</th>
<th>840</th>
<th>874</th>
<th>1003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro-3hrs</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
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</tr>
<tr>
<td>Micro-4hrs</td>
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<td>*</td>
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<td>*</td>
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<tr>
<td>Micro-5hrs</td>
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<td>*</td>
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</tr>
<tr>
<td>Micro-6hrs</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<td>*</td>
<td>*</td>
<td>*</td>
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</tr>
</tbody>
</table>

Generally speaking, the relative intensity and molecular weight of the esters increased with increasing reaction time. The dehydration reaction between glucose happened in microwave environment as well since glycosides were detected. The peaks seen in Table 7 are assigned as follows: 382 = G2-K⁺, 398 = GL₂-K⁺, 480 = G3L₁-K⁺, 497/498 = GL₃-Na⁺, 597 = GL₄-Na⁺, 613 = GL₄-K⁺, 625 = G3L₁-Na⁺, 524, 552, 742, 840, 874, 1003 = unknown.

**Components in BG**

![Graph of components in BG]

**Figure 40** The ¹H NMR spectra of the extracted products from the BG (CDCl₃, Normalized by the largest peak)
Figure 41 The $^{13}$C NMR spectra of the extracted products from the BG (CDCl$_3$, Normalized by the largest peak)

The sediment (the precipitates in the extraction of BG) contained ethyl acetate residue and LeA structures according to the characteristic NMR peaks in Figure 40 and Figure 41. The extracted part (ethyl acetate phase) of BG showed clear LeA structure and probable glucose trace which displays rough and low-intensity peaks at the region 6.5 - 3.5 ppm in $^1$H NMR spectra (Figure 40).

Figure 42 The LDI-MS spectra of untreated BG
Table 8 The LDI-MS peaks of BG and its solvent extracts and sediment

<table>
<thead>
<tr>
<th>m/z</th>
<th>382</th>
<th>480</th>
<th>597</th>
<th>613</th>
<th>625</th>
<th>642</th>
<th>741</th>
<th>757</th>
<th>839</th>
<th>885</th>
<th>903</th>
<th>956</th>
<th>984</th>
<th>1001</th>
<th>1018</th>
<th>1116</th>
<th>1244</th>
<th>1262</th>
<th>1361</th>
<th>1377</th>
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<tr>
<td>BG</td>
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<tr>
<td>Solvent extracts</td>
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</tbody>
</table>

Obviously, more high molecular weight products were synthesized by microwave radiation as compared to the direct-heating method. The ester and glycoside combinations seem very similar in the solvent extracts and in the sediment, though it is somewhat confusing that they existed in both phases of the extraction. Comparing Figure 42 with Figure 43 and 44, one drawback of LDI-MS in this case appeared. Without the help of matrix, not all molecules could be ionized and got detected. Several peaks shown in the extracts and sediment disappeared in Figure 43. The peaks seen in Table 8 are assigned as follows: 382 = G₂-K⁺, 480 = G₂L₁-K⁺, 597 = GL₁-Na⁺, 613 = GL₂-K⁺, 625 = G₃L₁-Na⁺, 642 = G₃L₁-K⁺, 741 = G₃L₂-K⁺, 757 = G₃L₂-K⁺, 885 = G₃L₂-Na⁺, 903 = G₃L₂-K⁺, 984 = G₃L₃-Na⁺, 1018 = G₅L₅-Na⁺, 1116 = G₅L₁-Na⁺, 1244 = G₅L₄-Na⁺. 839, 956, 1001, 1262, 1361, 1377 = unknown.
3.6 Emulsion Stability Test

![Figure 45](image.png) The appearance of the emulsions after 24 hours (left: BG, right: Yes Original)

Inspired by the three-phase phenomenon in the extraction, the surface activity of the BG was of interest. Therefore, a simple test was carried out to check the emulsion stabilizing performance of BG though the results were not promising.

After 24 hours, the oil emulsified with BG was coalesced too much so that pure oil phase was formed and it floated on top of the emulsion phase. While the “Yes Original” sample could still maintain the bubbles (red circle) and had more thick emulsion layer with whiter color, which suggested the oil droplets were smaller and more stable.

Thus, it is very likely that the BG has poor performance in lowering surface tension between corn oil and water and in stabilizing the emulsion.
3.7 Solution Casting of Starch and PVC

To evaluate the miscibility and plasticization efficiency of the glucose levulinates, they were solution casted to films with starch or PVC. The BG with usage level of 40% showed good miscibility with starch while BG was not compatible with PVC since phase separation was observed.

The firstly prepared solution-casted starch films were let to dry with cover for 7 days at room temperature before final drying in vacuum chamber. However, films prepared by this process suffered from microbial contaminations and thereafter a modified casting method was set up. The new modified method allowed the production of films with acceptable smooth surface within shorter time and less microbial contaminations. The time needed for preparing films was minimized from 9 days to 4 days.

**Original method**

![Figure 46 The microbial infection on the films in batch 2 (left-20% glycerol, right-40% glycerol)](image)

![Figure 47 The starch films with 40% BG](image)

Severe microbial infections can be found in all three batches of prepared starch films. Figure 46 reveals the contaminated situation in the second batch. Several precautions were applied to minimize the microbial infection, including:

1. Use boiled ultrapure water
2. Disinfect petri dishes before use with 75% (V/V) ethanol
3. Introduce as little water as possible in casting

Unfortunately, these methods did not work so well. Though the high level of glycerol can withdraw more contaminations, but this property comes from the decreasing activity of water. High level of glycerol decreases the activity of water and less free water is thus available for the growth of microorganism. In other words, the contaminations are inhibited. Another big disadvantage of standard method is the fact that the obtained film has very little area that is suitable for morphology observations, such as SEM (see Figure 47). Inspired by one attempt with open-dried method, better films had been achieved with modified method.
Modified method

Figure 48 The casted starch films from the modified method

In the modified method, period for solution casting was minimized from 9 days to 4 days. Smooth surface could be achieved locally (Figure 48). The dried films were smashed before measurements (eg. DSC) to minimize the thickness distribution. To sum up, the modified method was a fast and suitable method for starch solution casting.

Figure 49 The casted PVC films with 20% and 40% BG

Figure 49 showed the poor miscibility of BG with PVC. The yellowish patterns were caused by the phase separation of BG from PVC. Thus, no further DSC measurements were necessary.
3.8 Thermal Properties of Glucose Levulinates

![Graph showing thermal properties of glucose levulinates.](image)

**Figure 50** The DSC curves of the starch films and the plasticized blend films

The glass transition temperatures of pure starch and its blend films were measured. Figure 50 suggested BG at usage level of 40% had fairly good miscibility with starch, though it was not as good as glycerol.

The reported glass transition temperature of starch is around 100 °C while it is almost impossible to see the glass transition temperature in Figure 49. Moreover, the final plasticizing effect of 40% BG need to be confirmed by the mechanical test on its film, but the films with glycerol can be revealed from petri dishes and the films with BG are more like rigid and brittle pieces. Therefore, the suitable mechanical test for two films may be different since tensile test, as an example, is a better characterization technique for glycerol film than BG film.
4 Conclusions & Future work

Comparison of the conventional direct-heating method and microwave-assisted reaction for synthesis of glucose levulinates showed that microwave radiation has huge advantages in fastening the reaction process. Both GL$_2$-type glucose levulinates and GJL$_2$-type glycosidic levulinates were successfully synthesized by microwave-assisted reaction and conventional condensation reaction, although the synthesized products from microwave-assisted reaction were more diverse and with higher molecular weight. Besides, the side reactions of dehydration of glucose are inevitable in both methods and the yield of highly hydrophobic esters is low as well. Furthermore, acid number measurement was shown to be powerful tool to check the extent of reaction in real time with acceptable errors.

The obtained levulinates showed some surfactant-like properties, such as salt-sensitivity and affinity to both water and solvent. The various product combinations made the extraction processes little bit different, because the affinity to water/solvent for the different products differs and showed to be a problem for further isolation. Three-phase separation system occurred after the direct-heating method. However, it was not an issue for microwave-assisted synthesis if right organic solvent is used and without use of salt solution.

A modified method for solution casting on starch was developed to against microbial contaminations that happened in original method a lot and it was proved to be feasible and efficient in the end. The preparation period was shortened to 4 days and the casted films possessed smooth region locally. However, the BG demonstrated very poor miscibility with PVC and the following DSC results illustrated that 40% BG starch had excellent miscibility with starch.

Due to the limited scope of this project, increasing the yield and selectivity on products are of great importance for future works. The characterization techniques and isolation method for diverse product packages also need to be improved and established, then the structure-property relationships will be more concrete and clear.
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## Appendix I - Collection of LDI - MS Spectrum

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The first extraction
The secondary extraction
Con-sediment
2. Product Analysis of Con-24hrs-2 - Water Extracts

The first extraction
The secondary extraction
3. Blank experiment

Possible products
Background noise
4. Product Analysis of Micro-Xhrs (X=3,4,5,6,7) - Solvent Extracts

Micro-3hrs
Micro-5hrs

[Graph showing a spectrum with various peaks and labels]
Micro-7hrs
5. Components in BG

Untreated BG
Solvent extracts
7 Appendix II - Datum of AN Measurements

Appendix II - Datum of AN Measurement.xlsx