

# Microwave Pyrolysis to Produce Levoglucosenone (LGO) and (S)- $\gamma$ -Hydroxymethyl- $\alpha,\beta$ -Butenolide (HBO) from Plant Feedstocks

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## Abstract

In our efforts to synthesize a library of modified nucleosides and oligonucleotides, variety and large quantity of the synthons and building blocks are required. To meet these needs, developing a simple and lower cost synthetic method is critical. Conventional fabrications of the key intermediates (S)- $\gamma$ -hydroxymethyl- $\alpha,\beta$ -butenolide (HBO) and its procures levoglucosenone (LGO) drawn our attention, particularly from diversity of carbohydrates resources. Certainly, these two  $\alpha,\beta$ -unsaturated lactone, D-ribonolactone can be obtained either from multistep synthesis, or from natural resources in fashion of pyrolysis. Nevertheless, both approach costs are relatively high because of multistep reactions, energy consumption and unsatisfied pyrolytic efficiency, difficulty of purification, as well as lower yield. Unlike traditional pyrolysis harsh conditions, this report provides a microwave (MW) method that implemented in two similar conditions, firstly, in microwave reactor pyrolysis taken place in the presence of PEG200 and acids under vacuum distillation. The process was completed rapidly, giving LGO as a major fraction. Secondly, Using PEG400 as the medium, two steps heating given LGO even better. Consequently, LGO and HBO can be obtained from cellulose, glucose, granulated sugar, soluble starch, and caster sugar, while LG can be directly synthesized from corn starch. Acidic catalysts are phosphoric acid and toluene sulfonic acid, both showed identical results. Accordingly, this method provides high proficiency, novel, facile, environmental friend, and economic approach to obtain these valuable chiral precursors for numerous bioactive compounds, pharmaceutical ingredients and materials constructions.

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## Keywords

Pyrolysis, Microwave, Carbohydrate, LGO, HBO

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### 1. Introduction

Carbohydrates are found in most agricultural products and plant mass. Cellulose, hemicellulose, starch, sugars, cotton fiber, paper, straw, corn stalks, etc. are all agricultural products or crude products, and even plant waste, providing abundant bioenergy and material resources [1]. These natural substances are not only our food and nutrition sources, but also renewable and sustainable bio-feedstocks [2]. It is apparent that their value can be heightened if properly processed to meet the needs of specific applications. Furthermore, these materials can be pyrolysis in the absence of oxygen to obtain valuable products, typically including LGO, 5-hydroxymethylfurfural (HMF), levoglucosan (LG), and 5-hydroxymethylfurfural, among others. However, obtaining purer, high-yield outcomes from these diverse materials remains a significant challenge.

In recent decades, rapid pyrolysis of phosphate-impregnated cellulose feedstocks has yielded bio-oils rich in 1,6-dehydrated sugars, suitable as high-value-added feedstocks for organic synthesis [3]. Moreover, 1,6-dehydrated sugars possess high functionality and conformational rigidity, serving as chiral building blocks for the preparation of biologically significant and structurally diverse products [4]. In most cases, dehydrated sugars, bio-oil fractions, are likely to be the primary products, such as LGO, a high-value platform compound used in the synthesis of pharmaceuticals as well as the production of biodegradable and biocompatible polymers because of easy to polymerize.

A search of past highly relevant and authoritative literature and patents provides detailed laboratory procedures. The use of the media, such as polyethylene glycol (PEG), sulfolane [5], soybean oil [6], and ionic liquids (IL's) [7], in presence of catalysts, like sulfuric acid, was not only because of their high boiling points, but also they facilitate heat transferring in the mixture despite their higher viscosities. These acclaimed techniques provide appreciated mechanistic insights into a variety of acid-catalyzed reactions, including phosphoric acid, sulfuric acid, boric acid, solid acids, and acidic resins, even Cr(III)-containing leather [8], and offered solutions for different catalytic systems to specific products and feedstocks [9].

We employed microwave pyrolysis to study the complete pyrolysis of glucose and cellulose and some other carbohydrate heterogenic substances as the primary starting materials to create the desired key intermediate that was then converted into chiral precursor HBO [10] that required a multistep synthesizing in the past [11].

Typically, the critical process conditions for pyrolysis require precise temperature control, generally operating within the range of 300°C to 370°C. Applying a

high vacuum or inert gas protection during the pyrolysis process to remove and collect the fractions out of the high-temperature reaction region would be necessary, because it prevents decomposition further and improves yield [10]. This process suffers from drawbacks such as high energy consumption, autoclave devices, complex equipment requirements, and most commonly gives poor yield, low purity, difficult separation and purification, as well as high cost.

Numerous studies have shown that this pyrolysis process generally follows a depolymerization pathway at 200°C - 500°C, where cellulose/hemicellulose chains break down into smaller sugar units (e.g., L-glucan). When the temperature rises to 250°C - 350°C, a dehydration reaction occurs, losing water molecules to form furans (e.g., from C6 sugars to furfural from C5 sugars). When the temperature reaches 350°C or higher, a pyrolysis reaction occurs, ultimately breaking down into light oxygen-containing compounds (acetic acid, acetone) and gases, while simultaneously forming char, biochar, and tar. At temperatures ranging from 300°C to 400°C, dehydrated sugars and furans are primarily generated, while at temperatures above 500°C, gases and biochar are mainly produced. Rapid pyrolysis is beneficial for increasing the yield of bio-oil, while slow pyrolysis produces only biochar.

If solvent involves, dehydration of cellulose to levoglucosenone should be in polar aprotic system. Moreover, soybean oil with less polar has been also used as the reaction medium to synthesize LGO via the pyrolysis of cellulose [11]. Currently, no reported pyrolysis method has been found to directly prepare HBO, but treating LGO with peroxides and lipases to convert it into HBO is a highly efficient method. First, Bayer-Villiger oxidation is performed to generate intermediate, followed by acid hydrolysis to generate 4-hydroxymethylbutyrolactone. The reaction is carried out in ethyl acetate solution, and the reaction system contains Cal-B lipase, hydrogen peroxide, and water, with the pKa value controlled at 7.5 [12].

An MW assisted cellulose pyrolysis to prepare LGO was reported to improve pyrolysis selectivity. In the presence of readily available clay, and MW heating can be carried out at lower temperatures (165°C - 220°C) than conventional pyrolysis [13]. Unfortunately, this MW method uses specific clay and is a completely solid-phase reaction lacking stirring. Although the yield was increased to 12% (w/w), it was difficult to scale up to practical applications due to the solventless heterogeneous reaction. To improve the specificity and yield of the reaction, we realized that rapid and uniform heating are the key factors affecting the reaction process and then developed a superior microwave method in this report.

To date, all methods have produced more than ten pyrolysis products. Even those with LGO or LG as the main components include formic acid, furan, acetone, glycolaldehyde, acetic acid, acetylacetone, 2-furfural, 3-furanethanol, 2-furanethanol, 5-methylfurfural, 2-hydroxy-3-methyl-2-cyclopenten-1-one, levoglucanone, and 5-hydroxymethylfurfural less or more. Obtaining these products efficiently is costly, has low conversion rates, and poor purity, severely limiting large-scale production. The generation of these multiple fractions also makes subse-

quent separation and purification more difficult. Therefore, from both quality and quantity perspectives, there is an urgent need to develop new technologies or methods.

## 2. Methods

Microwave radiation replaced conventional heating sources. Vacuum ensures aerobic need, and removing water, and distilling products instantly. Feedstocks are low cost, renewable and sustainable.

### 2.1. Chemicals and Solvents

All chemicals and solvents, such as toluene sulfonic acid (p-TSA), 85% phosphoric acid, cellulose, glucose, cotton were purchased from ChemicalBook. Other feedstocks were obtained from grocery markets, while wheat straw, wood and grass were from farm fields.

### 2.2. Instruments

Shimadzu LCMS-2050 is a compact single quadrupole mass spectrometer. It has Heated Dual Ion Source (DUIS), combining Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) and LabSolutions software. UV detection undergoes at 210 nm and 254 nm. Shimadzu GCMS-QP2020 single quadrupole mass spectrometer.

### 2.3. Microwave Reactor

CEM, The Discover<sup>®</sup> 2.0 is the premier microwave reactor; Power: 0 - 300 W, intelligent control; Temperature range: up to 300°C; Pressure range: up to 20 bar (~290 psi).

### 2.4. Analysis

HPLC reverse phase column is from Shimadzu, Shim-pack Scepter C18-120/3 μm, 4.6 × 150 mm. Testing condition: Mobile phase is acetonitrile /water gradient from 5/95 to 80/20 in 35 minutes at room. Injection amount is 5.0 μL.

Thin layer chromatography (TLC) was carried out on pre-coated silica gel 60 F254 thin layer on glass from ChemicalBook.

NMR: <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded on Varian VNMR 400 MHz spectrometer (Varian, Inc., Palo Alto, CA, USA).

## 3. Experimental

### 3.1. General Procedure of PEG Mediated PA. Method

To 125 mL one neck-round bottom reaction flask with a stirring bar added 6.0 g feedstock, followed by suspended into 15 mL 85% phosphoric acid and 20 mL PEG400 (b.p. > 250°C). The reaction flask was charged onto the microwave reactor with reducing pressure distillation apparatus. The reaction conditions were set at 30 W power, 150°C temperature for regular distillation till no water moisture

condensing, the collecting flask was changed by another one, temperature was set at 220°C. vacuum was about 14.5 psi to distill until no more fraction out, it took about 30 minutes.

### 3.2. General Procedure of Rapid Pyrolysis P-TSA Method

3.0 g of the raw material was added to a 125 mL microwave-safe single-necked round-bottom reaction flask equipped with an electron stirrer. Then add 0.3 - 0.5 g p-TSA, or 2% - 19% organic sulfonic acid (2% - 19% of the raw material mass), 20 mL of 8 - 10 times the mass of PEG400 (boiling point > 250°C). Place the reaction flask in a microwave reactor equipped with a vacuum distillation device. Set the reaction conditions to 30 - 60 W power and 150°C temperature and perform conventional distillation until no water vapor condenses. After replacing the collection flask, set the temperature to 220°C and the vacuum level to approximately 14.5 psi, and continue direct vacuum distillation until no distillate is precipitated. The entire process takes approximately 30 minutes.

### 3.3. LGO Conversion

To the prepared crude product LGO above was added 5 mL 30% hydrogen peroxide, 20 mL methanol, and 5 mL 0.1 M HCl. The mixture was refluxed till LGO was completely converted to HBO, monitoring by LC/MS. Sodium sulfite solid was then added to adjust the pH of the system to neutral, simultaneously quenching excess hydrogen peroxide. After concentration, the solution was purified by flash column chromatography using a gradient elution with ethyl acetate and methanol as the eluent phases. The volume concentration of ethyl acetate decreased from 100% to 40%, while the volume concentration of methanol increased accordingly from 0% to 60%. The target fraction was collected and concentrated to obtain pure HBO.

### 3.4. Structural Assignment

$[\alpha]_D^{20} -112.0$  (c 0.01, CHCl<sub>3</sub>). [(Lit.12 -114.5 (c 0.1, CHCl<sub>3</sub>)] <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δH 3.25 (s, 1H, H6), 3.79 (dd, 1H, J = 12 and 3.6 Hz, H5a), 3.99 (d, 1H, J = 12 Hz, H5b), 5.17 (m, 1H, H4), 6.20 (dd, 1H, J = 5.7 and 1.8 Hz, H<sub>2</sub>), 7.53 (dd, 1H, J = 5.7 and 1.5 Hz, H<sub>3</sub>). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δC 62.2 (t, C1), 84.3 (d, C4), 122.8 (d, C2), 154.0 (d, C3), 173.5 (s, C1) ESI: m/z [M + H]<sup>+</sup> called for C<sub>5</sub>H<sub>6</sub>O<sub>3</sub>: 114.0395, found: 114.98.

## 4. Results and Discussion

### 4.1. Developing the Novel Technique

As we realized, from earlier techniques, the biggest challenges to prepare the titled chiral valued compounds from agricultural products and biomasses are searching for an approach that must be more active catalysts; heating reaction mixture rapidly; setting the temperature as low as possible; making thermos transfer more evenly; pyrolyzing specifically; separating effortlessly; enlargeable quantitatively,

and solvents, ultimately to pursue more efficient, economical and ecological friendly approach. This MW method provides an appropriate solution to meet these needs, which has never been reported. LGO then HBO produced directly from these agricultural masses' pyrolysis described above assists us for further synthesizing nucleotide building blocks.

We take the advantages of MW reaction that can make the thermos transfer almost at the same time from interior and exterior of the mixture, the desired temperature level is able to reach rapidly. It offers a suite of significant compensation for chemical reactions, particularly for energy intensive processes of pyrolysis. The basis reason for these advantages lies in the fundamental difference in how heat is generated: volumetric and selective versus conventional conductive/convective heating.

In contrast to conventional heating, in an MW reactor, the energy interacts directly with the molecules of the material itself. This interaction, primarily through two mechanisms, dipole polarization and ionic conduction, causes molecules to align and collide with each other billions of times per second, generating heat instantaneously and throughout the entire volume of the materials.

Nevertheless, microwave reactors are not just a "faster heater". They represent a fundamentally different form of energy transfer that enables faster, more efficient, and more selective chemical processing. For pyrolysis, this translates to transformative potential to produce higher-quality bio-oils and chars with greater energy efficiency and better process control, making it a highly promising technology for sustainable waste valorization.

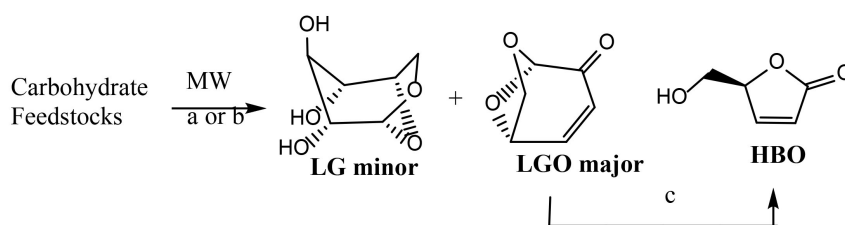
In common manner, pyrolysis is the thermal decomposition of materials at elevated temperatures in an inert atmosphere, the switch from conventional to MW is a game change. However, a challenge still exposes things such as stirring or mixing almost unbearable because of either high heterogeneity or viscosity of the reaction mixtures.

Volumetric and Rapid Heating impacts on pyrolysis, because that conventional way suffers from large temperature gradients, leading to uneven decomposition, while microwave heating ensures the entire biomass or waste feedstock heats uniformly and rapidly. Compacting by constant vacuum, this results in a more consistent product distribution and suppresses secondary cracking of the primary vapors. Therefore, selective or specific products may result.

On the other hand, MW also enhanced Quality of Bio-Oil, because the rapid, uniform heating of microwaves favors the cracking of large biomass molecules into volatile vapors quickly, minimizing their residence time at high temperature. This reduces secondary decomposition reactions, leading to a bio-oil with higher quality, lower viscosity and better stability. For this purpose, we adopted vacuum from beginning to end rather than inert gas atmosphere protection to ensure almost all energy is deposited directly into the feedstock. This leads to exceptional energy efficiency compared to heating a massive furnace, and the products are collected soon as created.

## 4.2. Comparison of Producing Approaches

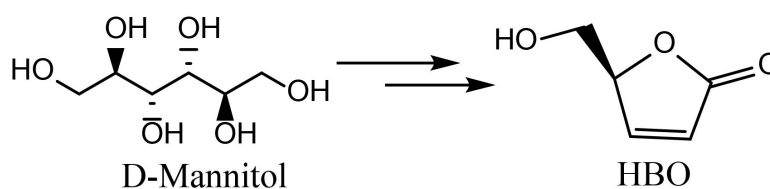
To better optimize the preparation process, we considered undergoing solvents or medium in the reaction mixture, PEG with 85% phosphoric acid (PA) with the substances were suspend initially, and kept stirring as vigorously as possible, although the mixture is heterogenies, evenly heating efficiency was much better, no concern of bumping under vacuum. The temperature was set at 220°C providing predominately the desired product LGO that was distilled out via vacuum, from which further step was needed to convert into HBO as **Scheme 1**. It certainly is that PEG and PA could react to each other partially to form PEG-phosphoric ester under such circumstances, which was observed by adding alcohol in our experiments, so does p-TSA.



a. PA, or p-TSA, PEG200, vacuum, MW; b. PA, or p-TSA, PEG400, vacuum, MW; c. 0.5 M HCl, MeOH, 30% H<sub>2</sub>O<sub>2</sub>, refluxing.

**Scheme 1.** Directly and LGO mediated preparation of HBO.

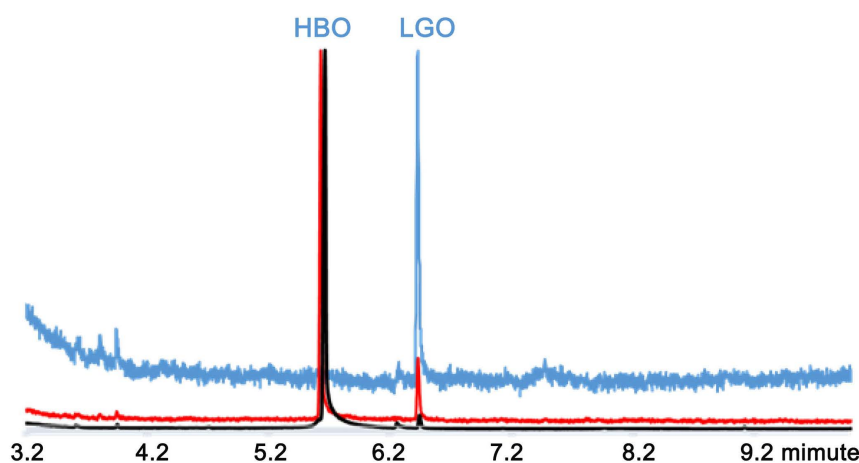
HBO was also obtained in a synthetic way in our lab following the reported method [14] in four steps from D-(+)-mannitol, as described in **Scheme 2**, which was a conventional and economical approach. Even though the overall yield is about 40%, it is adoptable in terms of larger scale preparation before a more efficient method emerges.



**Scheme 2.** Full synthesis of HBO.

Nevertheless, this full synthetic method could be no longer competitive with this developed MW method. Here LGO has been efficiently converted into optically pure HBO using a two-step in-situ involving Baeyer-Villiger oxidation and a lipase catalyzed acid hydrolysis. Baeyer-Villiger oxidation forms an intermediate format, followed by an acid hydrolysis step to give 4-hydroxymethyl butanolide [15]. To simplify this procedure, we struggled just treat LGO by H<sub>2</sub>O<sub>2</sub> under acidic conditions in methanol, the process was monitored by GC/MS, shown in **Scheme 3**, red is during the Baeyer-Villiger oxidation.

### 4.3. Analysis



**Scheme 3.** GC monitoring the reaction from LGO to HBO.

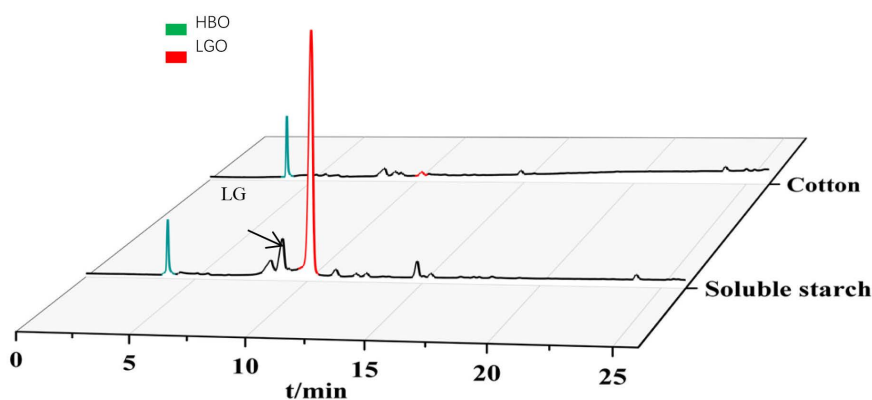
To our insight, catalyst effects, PA. and p-TSA for example, can remove LG and HMF and a few other derivatives dramatically, not so specific. In the past, some feedstocks need to be pretreated, the usual way was acid washing to remove alkali metals, reducing char and increasing favorable outcomes. While, in our trails, char formed inevitably in the reacted residue, none of feedstocks being pretreated, but some of them offered almost single fraction of LGO accordingly, particularly the p-TSA. When using approaches as previously reported pyrolysis, the procession would be agonized under punitive conditions and are not suitable for large-scale preparation or commercialization.

Carbohydrates are relatively stable chemically under ambient conditions, which predominantly rely on hydrogen bonds intramolecular and intermolecular. Pyrolysis undergoes at acetic, higher temperature and aerobic condition. The mechanism has been studied extensively [16].

In the practices, we found often that the crude products always aggregated or oligomerized soon as basified to pH 7.5 - 9.0 and formed various structures. We hypothesize that the interactions of any type of carbohydrates with sulfonic acid results in the change of the primary system of hydrogen bonds of single sugars and the polymeric saccharides, and the esterification. Combed the above-mentioned factors have a pronounced effect on the thermolysis of cellulosic materials, namely, on the ratio of the main reactions such as depolymerization and dehydration, and, correspondingly, on the composition of volatile products. Our insight into this favorable consequence of LGO is a thermodynamic result under the developed condition other than a new mechanism.

Regarding HBO, previous research had never directly obtained this specific product just via pyrolyzing, particularly for those structural subunits from a wide range of natural resources possess polysaccharides, therefore, to prepare large quantities of LGO even HBO, cellulose has been stared as an ideal feedstock for the reasons of economy and higher composition of glucose units traditionally. To

expand the range of feedstocks that may compose the same basic unit and can convert to same or/and compulsory products via pyrolysis, as shown in **Table 1**, we treated all these biomasses in same manner either in the present of PA. or p-TSA. As desired, among these biomasses, glucose, sugar, starch, cellulose yielded LGO predominately with little amount of HBO, while p-TSA system provided even better, each of them offers crude LGO yielding from 5% to 60% (W/W) approximately, in which, even cotton given a good fraction of HBO, its quantity is too less to undergo next reaction. We investigated the MW reaction in the present of PA. and p-TSA in PEG200/400 under MW, the volatile fractions contain both products, LGO and HBO, from which LGO is obviously preparable by this way, shown as **Scheme 4**.



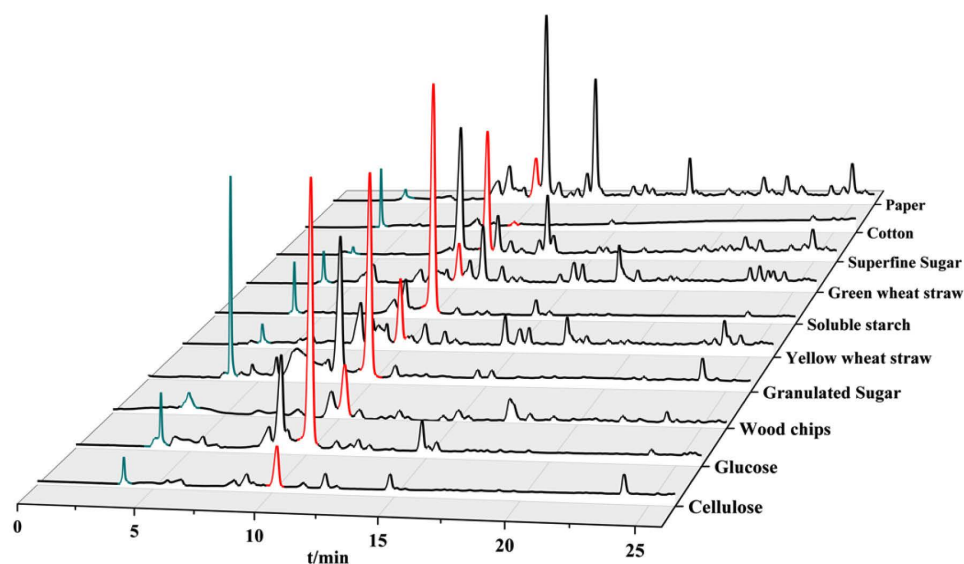
**Scheme 4.** LC PEG200 system: crude LGO and HBO from soluble starch and cotton.

Mobile phase: acetonitrile/water 0.01% - 62% in 34 minutes; Column: Shimadzu, Shim-pack Scepter C18-120/3  $\mu\text{m}$ ,  $4.6 \times 150$  mm.

The pyrolysis depends on temperature, microwave power and pressure, allowing the catalytic activity toward secondary decomposition of levoglucosan (LG) into LGO and continually to HBO.

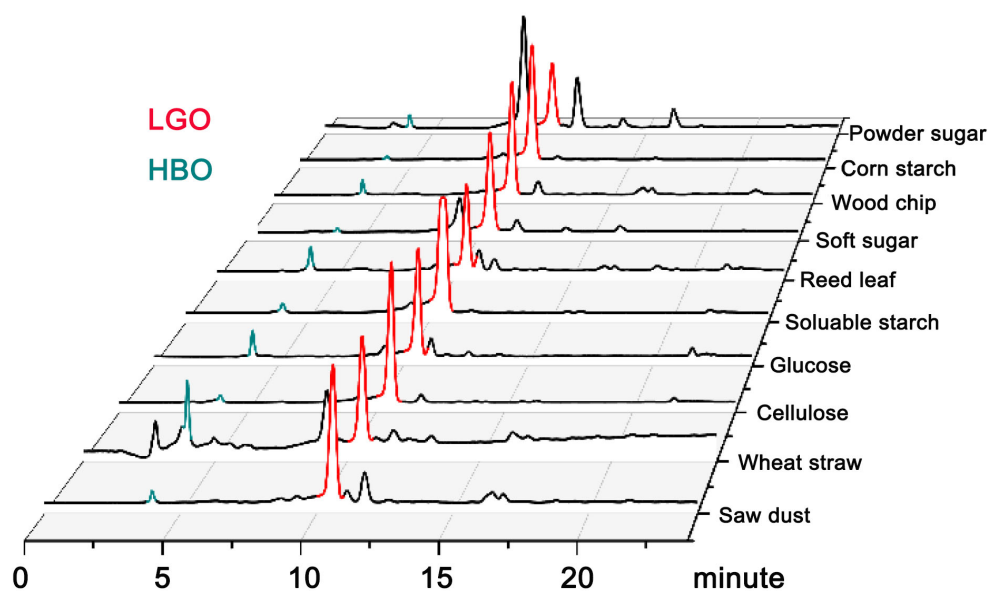
In contrast, the degree of development by dehydration reactions leading to the formation of LGO depends on chemical components of these biomasses, crystalline carbohydrates and the accessibility for the acidic catalyst, and mediated mixture. The ordered carbohydrates favor the formation of LGO whereas celluloses with a higher amount of less ordered regions show also parallel formation of LG as in **Scheme 5**.

As mentioned above, the key takeaway is that carbohydrate pyrolysis can transform low-value biomass into high-demand chemicals and fuels. Optimizing temperature, catalysts, and feedstock are critical to maximizing economic returns. Integrated biorefineries could combine pyrolysis with fermentation, converting LGO to ethanol for example, for higher profitability. In our effort, we eventually know which of the feedstocks can create LG, LGO and HBO individually for the first time, which can be practicable of fabrication, a few of them created less, shown as **Scheme 6** and **Table 1**.



1. HBO, 2. LGO.

**Scheme 5.** LC results from PEG + PA. system.



$R_{tLGO} = 10.67$  min.  $R_{tHBO} = 3.88$  min.

**Scheme 6.** LC results from PEG + p-TSA system.

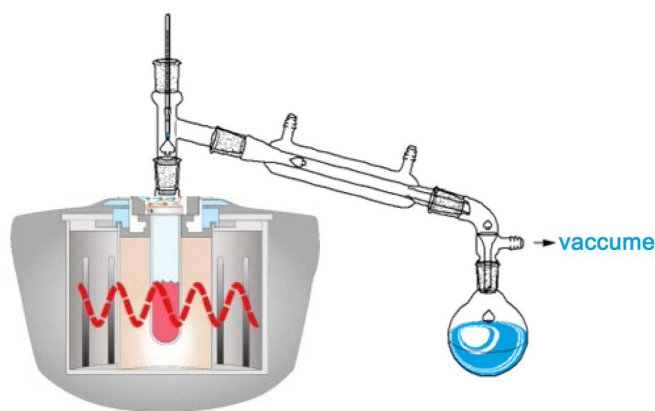
**Table 1.** PEG200 + PA. system: LGO approximal proportion from feedstocks.

	Cellulose	Glucose	Soluble Starch	Soft Sugar	Wood Chips	Powder Sugar	Corn Starch	Wheat Straw	Saw Dust	Reed Leaf
<b>LGO (proportion)</b>	80%	59%	79%	59%	67%	15%	83%	21%	55%	38%
<b>Crude Fraction/Row Material (w/w)*</b>	1.46/3.0	1.88/3.0	1.22/3.0	1.47/3.0	0.22/1.0	1.92/3.0	1.66/3.0	0.67/1.0	0.24/1.0	0.11/1.0

\*Total fractions mass versus raw material mass (g/g).

## 5. Conclusion

Microwave pyrolysis of agricultural feedstocks with high efficiency and specificities have been approved, shown as **Scheme 7**. It provides a new moderate method to pyrolyze agricultural mass into high valuable products and implies that if the condition is apropos, the producing specificity could be manipulated to meet the specific demand. Higher boiling point solvents promote the reaction system to be flowable, energy and mass to better transporting. PEG, p-TSA and PA play mediates, and catalysts create desired results efficiently, which paved a simple, economical and greened way to produce higher value of chiral procurers or synthons LGO and HBO, from which many valuable compounds can be obtained via further synthesizing. This technique substantially reduces reaction time, energy demand, and improves product conversion rate, selectivity, process control and safety. It provides a novel, facile, one-step, higher conversion from cheap crops mass into distinctly “green” materials.



**Scheme 7.** Microwave reaction apparatus.

## Author Contributions

Hengguang Li, Canglue Cheng conceived the project and designed the experiments. Weiqiang Yang, Yuansheng Huang, Siva Prakash performed the experiments. Yucheng Zheng, Zhinan Cai, Wei Song, and Wei Wang implemented analysis and data collection.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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## Abbreviations

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1. LGO	levoglucosenone
2. HBO	(S)- $\gamma$ -hydroxymethyl- $\alpha,\beta$ -butenolide
3. $\alpha$	Alpha
4. $\beta$	Beta
5. MW	Microwave
6. PEG200	Polyethylene glycol 200
7. HMF	5-hydroxymethylfurfural
8. LG	levoglucosan
9. PEG	Polyethylene glycol
10. IL's	Ionic liquids
11. Cr (III)	Chromium (III)
12. pKa	Negative logarithm of the acid dissociation constant
13. PA	Phosphoric acid
14. p-TSA	para toluene sulfonic acid
15. GC/MS	Gas chromatography-mass spectrometry
16. LCMS	Liquid chromatography-mass spectrometry
17. DUIS	Dual Ion Source
18. ESI	Electrospray Ionization
19. APCI	Atmospheric Pressure Chemical Ionization
20. nm	nanometers
21. mm	millimeters
22. $\mu$ L	microlitre
23. psi	Pounds per square inch
24. TLC	Thin Layer Chromatography
25. MHz	Megahertz
26. ml	Millilitre
27. g	gram
28. W	Watt
29. $^{\circ}$ C	Degree celsius
30. b.p	Boiling point
31. %	percentage
32. >	Greater than
33. s	singlet
34. d	doublet
35. t	triplet
36. m	multiplet
37. J	Coupling constant
38. Hz	Hertz

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