Canadian Journal of Chemistry
Revue canadienne de chimie

Conversion of *Symphytum officinale* L. and *Panicum virgatum* L. Plant Extracts to 5-Hydroxymethylfurfural Catalysed by Metal Chlorides in Ionic Liquids

Journal:	*Canadian Journal of Chemistry*
Manuscript ID	cjc-2017-0663.R1
Manuscript Type:	Article
Date Submitted by the Author:	09-Mar-2018
Complete List of Authors:	Martel, Alexandrine; Laurentian University, Chemistry and Biochemistry Montaut, Sabine; Laurentian University Ulibarri, Gerardo; Laurentian University Spiers, Graeme; Laurentian University, School of the Environment, Department of Earth Sciences and Department of Biology
Is the invited manuscript for consideration in a Special Issue?:	N/A
Keyword:	5-hydroxymethyfurfural, catalysis, comfrey, pre-treatment, switchgrass

Conversion of *Symphytum officinale* L. and *Panicum virgatum* L. Plant Extracts to 5-Hydroxymethylfurfural Catalysed by Metal Chlorides in Ionic Liquids

Alexandrine L. Martel[a], Sabine Montaut[a,]*, Gerardo Ulíbarri[a,]*, Graeme A. Spiers[b]

[a]*Department of Chemistry & Biochemistry, Biomolecular Sciences Programme, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON, P3E 2C6, Canada*

[b]*School of the Environment, Department of Earth Sciences and Department of Biology, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON, P3E 2C6, Canada*

*Corresponding author. Tel.:+1 705 675 1151 ext. 2185; Fax: +1 705 675 4844 (S. Montaut).

E-mail addresses: smontaut@laurentian.ca (S. Montaut), gulibarri@laurentian.ca (G. Ulíbarri).

Conversion of *Symphytum officinale* L. and *Panicum virgatum* L. Plant Extracts to 5-Hydroxymethylfurfural Catalysed by Metal Chlorides in Ionic Liquids

Alexandrine L. Martel[a], Sabine Montaut[a,]*, Gerardo Ulíbarri[a,]*, Graeme A. Spiers[b]

Abstract

The present work examined the potential for two plants grown on Canadian soil, *Symphytum officinale* L. (common comfrey) and *Panicum virgatum* L. (switchgrass) to produce 5-hydroxymethylfurfural using metal chloride catalysis in two ionic liquids, 1-butyl-3-methylimidazolium chloride or 1-ethyl-3-methylimidazolium chloride. Furthermore, two pre-treatments, namely the dilute sulfuric acid treatment and the methanol extraction, were studied as a way to improve sugar availability and increase 5-hydroxymethylfurfural yields compared to untreated biomass. The 0.5 M H_2SO_4 hydrolysis under autoclave conditions produced sugar rich extracts containing 230±23 mg of sugars per g of hydrolysed biomass for comfrey and 425±13 mg of sugars per g of hydrolysed biomass for switchgrass. The methanol extraction produced extracts high in simple sugars with concentration of 300±60 mg of sugars per g of dry extract for comfrey and 202±16 mg of sugars per g of dry extract for switchgrass. The yield of 5-hydroxymethylfurfural was improved from less than 1% using untreated biomass to 6.04% and 18.0% using dry methanol extracts of comfrey and switchgrass, respectively. These yields, although small, are important as they show for the first time that a methanol extract could enhance the metal chloride catalysis in ionic liquids for 5-hydroxymethylfurfural production from biomass.

Keywords

5-hydroxymethyfurfural, catalysis, comfrey, pre-treatment, switchgrass

Résumé

Le travail présent a examiné le potentiel de deux plantes poussant sur le sol canadien, *Symphytum officinale* L. (consoude commune) et *Panicum virgatum* L. (panic érigé) pour produire le 5-hydroxyméthylfurfural en utilisant la catalyse aux chlorures de métaux dans deux liquides ioniques, le chlorure de 1-butyl-3-méthylimidazolium ou le chlorure de 1-éthyl-3-méthylimidazolium. De plus, deux pré-traitements, soit le traitement à l'acide sulfurique dilué et l'extraction au méthanol, ont été étudiés comme moyens pour améliorer la disponibilité des sucres et augmenter les rendements en 5-hydroxyméthylfurfural par rapport à la biomasse non traitée. L'hydrolyse avec H_2SO_4 0.5 M dans des conditions d'autoclave a produit des extraits riches en sucres contenant 230±23 mg de sucres par g de biomasse hydrolysée pour la consoude et 425±13 mg de sucres par g de biomasse hydrolysée pour le panic érigé. L'extraction au méthanol a produit des extraits riches en sucres simples avec une concentration de 300±60 mg de sucres par g d'extrait sec de consoude et 202±16 mg de sucres par g d'extrait sec de panic érigé. Le rendement en 5-hydroxyméthylfurfural a été amélioré de moins de 1% en utilisant la biomasse non traitée à 6.04% et 18.0% en utilisant des extraits méthanoliques secs de consoude et panic érigé, respectivement. Ces rendements, bien que petits, sont importants car ils montrent pour la première fois qu'un extrait méthanolique peut améliorer la catalyse aux chlorures de métaux dans des liquides ioniques pour la production de 5-hydroxyméthylfurfural à partir de biomasse.

Mots-Clés

5-hydroxyméthyfurfural, catalyse, consoude, pré-traitement, panic érigé

Introduction

Lignocellulosic materials such as hardwood, softwood, agricultural residues, and other plant materials offer the most abundant renewable carbon source on the planet.[1] Plant biomass can be used to synthesize the molecule 5-hydroxymethylfurfural (HMF), a derivative of furan containing an aldehyde and an alcohol functional group which can be synthesized from glucose, and subsequently transformed to 2,5-dimethylfuran (DMF) by hydrogenation.[2,3] The energy content of DMF (31.5 MJ/L) is closer to the energy content of gasoline (35 MJ/L), and better than the energy content of EtOH (23 MJ/L).[2] DMF also has a higher boiling point (92-94°C) compared to EtOH (78°C), making it an important molecule for usage as a biofuel.[2]

Methods for transforming carbohydrates and cellulosic materials to HMF using ionic liquid mixtures and metal halide catalysts have been studied within the last decade.[2,4-26] Yields of HMF produced from glucose or fructose generally vary from around 50% up to nearly 100%.[4-6,10,12,14-17,19,26] Although glucose and fructose offer easy feedstocks to produce HMF, most sugars are locked away in lignocellulosic plants. The use of ionic liquids as solvents to dissolve biomass opens the door to using plant feedstocks directly for the conversion to HMF.

When using cellulose or biomass, yields are generally lower compared to the ones obtained from glucose or fructose. For example, from cellulose, a yield of 37.7% has been obtained using dual catalysts $CuCl_2$ and $CrCl_3$ in 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl) after 10 min at 140°C.[8] A yield of 62% of HMF was obtained using 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) and $CrCl_3$ after 2 min under microwave irradiation (MI).[10,19] Other solvent mixtures have also been used, such as a DMSO and [BMIM]Cl mixture with $AlCl_3$, which produced a 54.9% yield after 9 h at 150°C, or a *N,N*-dimethylacetamide containing 10 wt% LiCl (DMA-LiCl) and [EMIM]Cl mixture with $CrCl_2$ and HCl as catalysts, which produced a 54% yield after 2 h at 140°C.[2,20]

The plant biomass commonly reported in the literature primarily includes those high in starch and simple sugars such as corn stover, corn stalk, rice straw, wheat straw, yams, girasol tubers, potato tubers, acorns, and chicory roots, with yields of HMF approaching 50%.[2,5,9,11,19,23-25] Using an untreated biomass feedstock, the highest yield of HMF of 61.4% was found using wheat straw, dissolved in [BMIM]Cl with LiCl and $CrCl_3$ after 15 min at 160°C.[19] Using materials higher in cellulose usually results in a decrease of the HMF yield. Pine wood, for example, was transformed with a yield of 52% in [BMIM]Cl with $CrCl_3$•$6H_2O$ after 3 min at 100°C under MI.[25] Poplar wood, on the other side, was transformed to HMF with a yield of 26% in a H_2O and tetrahydrofuran (THF) mixture with $AlCl_3$•$6H_2O$ after 30 min at 180°C under MI.[22] Plants from the Poaceae family have also been used to produce HMF, with a yield of 21% being obtained from switchgrass in a H_2O and THF mixture with $AlCl_3$•$6H_2O$ after 30 min at 180°C under MI.[22] Additionally, bamboo fiber gave a 52.2% HMF yield using a H_2O, THF and NaCl mixture with NH_2SO_3H after 40 min at 180°C under MI.[18]

Pre-treatments of the plant materials are often used prior to the production of biofuels with the goals of removing hemicellulose and lignin, reducing cellulose crystallinity, and increasing cellulose porosity.[27] The main goal of the pre-treatment is therefore to improve sugar availability.[27] Very few studies have reported the use of a pre-treatment in combination with the production of HMF from plant biomass.[2,9,13,23,24] This is mainly due to crops high in simple sugars and starch not requiring pre-treatments. In one study, ammonia fiber explosion (AFEX) treated corn stover produced the same yield of HMF of 16% compared to untreated corn stover in a DMA-LiCl and [EMIM]Cl mixture with $CrCl_2$ after 6 h at 140°C.[2] Other studies showed the transformations of diluted HCl extract of girasol tubers, potato tubers, acorns, and chicory roots to HMF with yields between 50-59% in 1-octyl-3-methylimidazolium chloride ([OMIM]Cl) using different metal halide catalysts.[9,23,24] However, those studies did not compare untreated and treated material for the production of HMF. An HMF yield of 79%, the highest recorded yield for lignocellulosic biomass, was also obtained from the transformation of recovered biomass of wood chips after a diluted NaOH pre-treatment using $CrCl_3 \cdot 6H_2O$ in [BMIM]Cl at 120°C for 2 h.[13] In comparison, the yield obtained from untreated wood chips was 41%.[13] In the same study, a yield of 76% of HMF was also obtained from diluted NaOH pre-treated rice straw.[13] These studies show that both the extract and the recovered biomass after treatment may be useful in increasing HMF yields from lignocellulosic biomass. However, few treatments and feedstocks have been covered in the literature, and the comparison between untreated and treated feedstocks for the production of HMF is still lacking.

In the present study, we aimed to solve the issues related to using edible crops (such as soil erosion, depletion of food resources, and use of pesticides and herbicide) by using two non-edible crops, *Symphytum officinale* L. (common comfrey, family Boraginaceae), and *Panicum virgatum* L. (switchgrass, family Poaceae). Even if comfrey and switchgrass must currently be cultivated and are not considered to be bio-waste materials, the two plants were chosen on the basis that they produce a large amount of biomass and they can be cultivated in Canada. Both plants are perennials and can also be harvested each year following winter without the need to sow a new harvest. Switchgrass has previously been cultivated in currently unusable mine landfills.[28] Both plants were chosen purposefully for the bioremediation of mining sites as both comfrey and switchgrass show metal accumulating properties.[29-32] The large amount of plant biomass produced after bioremediation can then be used for biofuel production, which was further explored in the present study.

Common comfrey has never been explored in the literature as a feedstock for HMF production, while switchgrass has previously been studied for HMF production.[21,22] The comparison of the two plants will indicate the suitability of comfrey for biofuel production. The dilute acid pre-treatment and the MeOH extraction were studied to produce plant extracts rich in sugars which were used for HMF production. Acid extracts from plants have previously shown to be transformed into HMF in high yields.[9,23,24] To our knowledge, MeOH extracts have never been explored in the literature for the production of HMF. Prior to converting the extracts to HMF, sugars were quantified using two spectrophotometric assay, the phenol/H_2SO_4 spectrophotometric assay for total sugars and the 3,5-dinitrosalicylic acid (3,5-DNS) spectrophotometric assay for reducing sugars.[33,34] HMF was synthesized from glucose as a control to test the suitability of the reaction conditions, which included using different metal chloride catalysts in two ionic liquids, 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) or 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl). Untreated plant biomass, as well as the H_2SO_4 and methanol dried plant extracts of comfrey and switchgrass, were then used as biomass feedstocks for the conversion to HMF. It is the first time that this methodology for HMF production was applied to comfrey and switchgrass.

Experimental

Materials

The following chemicals were purchased from Sigma-Aldrich (St-Louis, US): 1-butyl-3-methylimidazolium chloride (≥95%), 1-ethyl-3-methylimidazolium chloride (≥95%), 3,5-dinitrosalicylic acid, activated charcoal (untreated powder, 100-400 mesh), $CuCl_2$ (≥97%), $CrCl_3•6H_2O$ (≥98%), H_2SO_4 (95-98%), NaOH (≥98%), and sodium potassium tartrate. The following chemicals were purchased from Fisher Scientific (Unionville, Canada): 5-hydroxymethylfurfural (≥98%), CH_2Cl_2 (≥99.9%), EtOAc (99.9%), glucose (anhydrous), MeOH

(≥99.9%), and phenol (≥99%). $CrCl_2$ (anhydrous, 99.9%) was purchased from Strem Chemicals (Newburyport, US).

Plant material

Symphytum officinale L. (common comfrey) was obtained from Ritchers Herbs (Goodwood, Canada). *Panicum virgatum* L. (switchgrass) seeds of the "Sunburst" cultivar were obtained from Ernst Seeds (Meadville, US). The switchgrass was cultivated as part of a study by Smith to determine the feasibility of growing the plant on low sulphur mine tailing with an approximately one meter compost cover (manufactured by GroBark).[28] Both plants were harvested at maturity in Sudbury (Ontario, Canada) in 2014 (comfrey) and 2015 (switchgrass), and were kept frozen until use. Prior to using, plants were air dried avoiding direct sunlight, and ground to a fine powder using a Magic Bullet® blender.

Pre-treatment protocols

Protocol A: the soluble sugars in the plants were quantified by extracting plant biomass with MeOH. Up to 5% (wt/v) of comfrey or switchgrass were incubated in MeOH at 40°C for 24 h. Samples were filtered to obtain the extracts.

Protocol B: the diluted acid treatment was performed using comfrey and switchgrass (5% wt/v) placed in a solution of 0.5 M H_2SO_4. Autoclave was conducted for 30 min at 121°C and 117-138 kPa, in a covered Erlenmeyer flask. Samples were filtered, and the filtrates were neutralized to a pH of 7 using 2 M NaOH.

Total and reducing sugar quantifications

The amounts of total and reducing sugars found in the recovered MeOH extracts and the H_2SO_4 extracts were quantified. The phenol/H_2SO_4 spectrophotometric assay, outlined by DuBois et al., was used to quantify the amount of total sugars in the solutions.[33]

The 3,5-DNS spectrophotometric assay was used to quantify the reducing sugars using the protocol outlined by Wood et al.[34] For both assays, glucose standards were used to build the standard curves. The Excel program (2013) was then used to plot the graphs, and calculate the amount of sugars in the solutions. Each standard curve was reproduced a minimum of three times.

Organic synthesis of HMF from glucose

As a control, transformation of HMF from glucose was performed prior to using plants as the substrate. The transformation of glucose followed the reaction protocol outlined by Abou-Yousef et al. for the conversion of cellulose to HMF, with minor modifications.[8] A 10 wt% substrate loading of glucose was put in either [BMIM]Cl or [EMIM]Cl at 140°C for 30 min under magnetic stirring, with different catalyst loading of $CuCl_2/CrCl_3 \bullet 6H_2O$, or $CuCl_2/CrCl_2$ mixtures, including a 3 wt% loading and a 3 mol% loading.

Organic synthesis of HMF from untreated and treated biomass

Both untreated and treated comfrey and switchgrass were used as a feedstock for the conversion to HMF. Prior to being used for the production of HMF, both types of extracts were dried using a rotary evaporator. A modified protocol of the reactions outlined from Abou-Yousef et al. was used for the conversion of the plant feedstocks to HMF, with the dissolution step being modified so that the plant material was added directly to [BMIM]Cl or [EMIM]Cl without the catalysts.[8] Comfrey or switchgrass were loaded at 10 wt% of the reaction mixture, and dissolved for 30 min at 120°C with magnetic stirring. Following the dissolution step, 3 wt% of $CuCl_2$ and 3 wt% of $CrCl_3 \bullet 6H_2O$ were then added to each mixture based on the amount of substrate, and the catalytic step was performed at 140°C for 30 min.

For the treated comfrey and switchgrass, including the MeOH and the 0.5 M H_2SO_4 dried extracts, substrate loading was maintained at 10 wt% of the reaction mixture. Reaction conditions were the same, however a catalytic step of 15 min and the catalyst combination of $CrCl_2/CuCl_2$ were also tested for the conversion of the MeOH extracts.

Reaction work-up

After completion of the reactions, each solution was diluted with distilled H_2O to reduce viscosity, and hot solutions were filtered. Fractions of 10 mL of HPLC grade EtOAc were used to extract the mixture until no HMF could be seen on thin layer chromatography (TLC) migrated with 10% $MeOH/CH_2Cl_2$ under UV light at a wavelength of 254 nm. MeOH extract samples were filtered over activated charcoal, and washed with EtOAc (3×10 mL) to remove the pigments. EtOAc extracts were subsequently concentrated by drying the extract, and re-dissolving in minimal EtOAc for GC-MS. All samples were filtered through a 0.45 µm filter (Canadian Life Sciences, Peterborough, Canada) prior to injection in the GC-MS.

5-Hydroxymethylfurfural quantification and identification

To quantify HMF in the reactions, the EtOAc extracts were injected into a Finnigan TRACE GC Ultra connected to a Finnigan Polaris Q MS detector using electron ionization (EI) onto a TR-5MS SQC column (L 30 m, ID 0.25 mm, film 0.25 µm) (Thermo Scientific, Waltham, US). Samples were injected using the TriPlus As auto injector (Thermo Scientific, Waltham, US) at a volume of 1.0 µL. The ion source was set at 200°C. Damping gas flow was set at 0.3 mL/min. He Carrier gas (Praxair, Sudbury, Canada) was used at a constant flow rate of 1.5 mL/min. The initial oven temperature was set at 50°C and held for 0.50 min, followed by an increased temperature slope of 15.0°C/min up to a final temperature of 250°C which was held for 1.00 min. The injector was set at 200°C with a split ratio of 40.

The standard curve was prepared using an HMF standard. The XCaliber program was used to plot the standard curve and for the quantification of HMF in the samples. The presence of HMF in the samples was identified both by retention time, and by mass spectra with the NIST

Mass Spectral Library (version 2.0). The main signals in the MS spectrum of HMF are seen at a *m/z* of 127 (M+1 peak), 126 (M_+ peak), 109 (lost of the OH group), 97 (lost of the CHO group), 95 (lost of the CH_2OH group), 81 (lost of formic acid) and 69 (lost of glyoxal). Yields of HMF were reported in mol% of HMF according to the sugar content of the extracts, or the amount of glucose used in the reaction (see supplementary material for calculations of the HMF yields).

Results and discussion

Total and reducing sugars in the MeOH plant extracts

Total sugar concentration in the comfrey MeOH extract was 300±60 mg of sugars/g of dry extract (30.0±6.0% of sugars in the dry extract) or 47.0±13.1 mg of sugars/g of dry biomass (DW) (4.70±1.31% of sugars in the DW) (Fig. 1). For reducing sugars, 264±106 mg of sugars/g of dry extract (26.4±10.6% of sugars in the dry extract) or 38.8±2.8 mg of sugars/g of DW (3.88±0.28% of sugars in the DW) were obtained. The percentage of comfrey biomass extracted in the MeOH was 19.6±11.1% (Fig. 1).

Total sugar concentration in the switchgrass MeOH extract was 202±16 mg of sugars/g of dry extract (20.2±1.6% of sugars in the dry extract) or 34.7±4.8 mg of sugars/g of DW (3.47±0.48% of sugars in the DW) (Fig. 1). Reducing sugar concentration was 91.9±1.6 mg of sugars/g of dry extract (9.19±0.16% of sugars in the dry extract) or 11.9±0.3 mg of sugars/g of DW (1.19±0.03% of sugars in the DW). For switchgrass, 17.7±2.3% of the biomass was dissolved in MeOH (Fig. 1).

Overall, total sugars in the MeOH extracts were lower for switchgrass (20.2±1.6% of sugars in the dry extract) than for comfrey (30.0±6.0% of sugars in the dry extract), indicating a lower amount of soluble sugars in switchgrass (Fig. 1).

The dissolution percentages of both plants (nearly 20%) (Fig.1) reflect the amount of cellulose (22.4±0.2% for comfrey and 40.1±1.7% for switchgrass), hemicellulose (9.6±0.7% for comfrey and 30.3±0.9% for switchgrass) and lignin (6.9±1.1% for comfrey and 7.2±0.3% for switchgrass) contained in each plant, as the MeOH extraction is known for only extracting the soluble portion of the sugars in the plant biomass.[35-37] The large variability in the biomass extracted from comfrey (19.6±11.1%) compared to switchgrass (17.7±2.3%) can be explained by comfrey having a lower amount of cellulose, hemicellulose and lignin (total of 38.9%) compared to switchgrass (total of 77.6%), leaving a larger percentage of comfrey being made of other components such as soluble sugars, proteins, lipids and organic acids.[37] The larger fraction of those components, some of which are soluble in MeOH, can explain the variation seen in the percentage of biomass extracted (19.6±11.1%) (Fig. 1). A wide variability in the amount of sugars found in the comfrey extract can also be seen (30.0±6.0% of sugars in the dry extract), which is likely due to the wide variability in the percentage of biomass extracted. However, the amount of soluble sugars in the dry biomass show smaller variability (4.70±1.31% of sugars in the DW) (Fig. 1), indicating that the variability is likely due to the solubility of the biomass in the MeOH, as opposed to the amount of soluble sugars found in comfrey.

On an industrial scale, the low dissolution of both plants in MeOH would not be economically viable unless the recovered materials could be used, or recycled. However, the MeOH extraction did produce an extract high in sugars, even if only a small fraction of the biomass was dissolved in the solvent.

Total and reducing sugars in the H_2SO_4 treated plants

The 0.5 M H_2SO_4 treated comfrey yielded a total sugar concentration of 230±29 mg of sugars/g of hydrolysed biomass (23.0±2.9% of sugars in the hydrolysed biomass) or 130±18 mg of sugars/g of DW (13.0±1.8% of sugars in the DW) (Fig. 2). Reducing sugar concentration was 256±13 mg of sugars/g of hydrolysed biomass (25.6±1.3% of sugars in the hydrolysed biomass) or 147±11 mg of sugars/g of DW (14.7±1.1% of sugars in the DW). Dissolution of the comfrey biomass using the 0.5 M H_2SO_4 treatment was 57.2±3.2% (Fig. 2).

Total sugar concentration in the acid treated switchgrass was 425±13 mg of sugars/g of hydrolysed biomass (42.5±1.3% of sugars in the hydrolysed biomass) or 189±3.7 mg of sugars/g of DW (18.9±3.7% of sugars in the DW) (Fig. 2). Reducing sugar concentration was 474±120 mg of sugars/g of hydrolysed biomass (47.4±12.0% of sugars in the hydrolysed biomass) or 204±3.2 mg of sugars/g of DW (20.4±0.32% of sugars in the DW). Dissolution of the switchgrass biomass using the 0.5 M H_2SO_4 treatment was 44.2±7.2% (Fig. 2).

For both plants, the amount of total and reducing sugars were similar indicating that all sugars in the plant extracts were reducing. Acid hydrolysis has previously been shown to be more suitable for degrading high amounts of cellulose, as well as converting xylan into xylose.[38-40] The quantities obtained therefore reflect the amount of cellulose found in each plant, with switchgrass containing 40.1±1.7% of cellulose, which is nearly double the 22.4±0.2% of cellulose found in comfrey.[37] This observation explains the switchgrass yield of nearly double the amount of sugars per weight of hydrolysed biomass (42.5±1.3% of sugars in the hydrolysed biomass) compared to comfrey (23.0±2.9% of sugars in the hydrolysed biomass) under the same conditions (Fig. 2). Concentration of sugars in mg/g of DW was also higher for switchgrass. However, dissolution of switchgrass in the acid was slightly less (44.2±7.2%) compared to the dissolution of comfrey (57.2±3.2%) (Fig. 2).

The acid treatment is more suitable than the MeOH treatment as it utilises a larger amount of the sugars found in the plant due to a larger amount of the biomass being dissolved in the acid. Use of an acid treatment may therefore be more viable on an industrial scale as it allows access to a portion of the sugars found in both the cellulose and the hemicellulose fractions of the plant biomass.

HMF production from glucose

Using glucose as a substrate for HMF conversion tested the suitability of the reaction system, including the type of catalyst and solvents, and the reaction time. The highest yield of HMF (based on the amount of glucose used) of 50.0% was obtained using [BMIM]Cl with the catalyst mixture $CrCl_3•6H_2O/CuCl_2$, at 3 mol% loading of each metal chloride (Table 1, entry 1). Using [EMIM]Cl instead of [BMIM]Cl tended to decrease the yield of HMF obtained (Table 1, entries 2 and 6). Using a catalyst loading of 3 wt% instead of 3 mol% also decreased the yield of HMF to 24.5% in [BMIM]Cl and 24.4% in [EMIM]Cl when using the $CrCl_3•6H_2O/CuCl_2$ mixture (Table 1, entries 3 and 4). However, due to the smaller amount of sugars found in the plant biomass, using a 3 wt% catalyst loading is more suitable for plant substrate. The use of a $CuCl_2/CrCl_2$ mixture did not seemingly change the yield of the reaction, with a 48.7% yield of HMF in [BMIM]Cl and a 31.0% yield of HMF in [EMIM]Cl (Table 1, entries 5 and 6).

The conditions tested for our reactions were similar to reactions found in the literature for the conversion of glucose or cellulose to HMF.[8,14] Abou-Yousef et al. obtained a yield of 37.7% of HMF from cellulose after 10 min at 140°C using [EMIM]Cl and 3 wt% of $CrCl_3$ and $CuCl_2$.[8] Peleteiro et al. obtained a yield of 65.0% from glucose after 2 h at 120°C using [BMIM]Cl and 25 mol% of $CrCl_3•6H_2O$.[14] The protocol used in the present study is therefore comparable to the current literature, and can further be applied for the transformation of plant biomass to HMF.

HMF production from untreated plant biomass

After a 30 min dissolution step at 120°C, and a 30 min catalytic step at 140°C, dissolution in [EMIM]Cl for untreated comfrey and switchgrass was better than dissolution in

[BMIM]Cl under the same conditions, with dissolutions of 49% for comfrey and 54% for switchgrass in [EMIM]Cl compared to dissolution efficiencies of 27% for comfrey and 30% for switchgrass in [BMIM]Cl. Using the catalyst combination $CrCl_3{\bullet}6H_2O/CuCl_2$ at a concentration of 3 wt% based on the amount of dry biomass used, detectable yields of HMF below 1% were obtained. These low yields could be due to the poor dissolution, as well as the type of feedstock used, which could be unsuitable. Different by-products are also possibly being formed, such as furfural (produced from the dehydration of pentoses such as xylose), or furylhydroxymethyl ketone, as seen in a study by Abou-Yousef et al.[8] However, those products were not detected by GC-MS or by TLC in the present study.

HMF production from sulfuric acid treated plant biomass

HMF production using the dried 0.5 M H_2SO_4 plant extracts for comfrey and switchgrass was studied (Table 2). In [BMIM]Cl, 100% of the acid extracts were soluble for both comfrey and switchgrass (Table 2, entries 1 and 2), while 92% and 88% of the extract for comfrey and switchgrass, respectively, were dissolved in [EMIM]Cl (Table 2, entries 3 and 4). The highest yield for the transformation of the dry comfrey H_2SO_4 extract was 2.31% in [EMIM]Cl (Table 2, entry 3), and the highest yield for the transformation of the dry switchgrass H_2SO_4 extract was 1.14% in [BMIM]Cl (Table 2, entry 2).

The sugars obtained in the H_2SO_4 extracts are possibly more complex than glucose, and therefore, are not as easily converted to HMF. Furthermore, prior to the reaction, the acid extracts were neutralized using NaOH, with a large quantity of Na_2SO_4 being produced as a by-product. Salts such as LiCl are sometimes used in reactions to produce HMF, but few studies have focused on utilizing other salts in the reactions.[2,19,41] To our knowledge, the effect of

Na_2SO_4 on the reaction has not been studied, and could potentially hinder the production of HMF.

HMF production from methanol treated plant biomass

The MeOH extracts were filtered through activated charcoal and directly injected in the GC-MS as a control. No HMF was present in the extracts prior to the reactions. Table 3 reports the yield of HMF obtained using the dry MeOH extract under different reaction conditions. Overall, the yield of HMF was improved from <1% using the untreated biomass, up to 6.04% for comfrey (Table 3, entry 1), and 18.0% for switchgrass (Table 3, entry 7), with the conditions used for production of HMF differing depending on the nature of the feedstock. The [BMIM]Cl solvent was more appropriate to transform comfrey biomass, while [EMIM]Cl showed better HMF conversion for switchgrass. Dissolution of the biomass in the ionic liquid was also superior using extracted biomass, with dissolution ranging from 43 to 71% (Table 3). A 3 mol% catalyst loading was also tested, with the obtained yields being below 1%, which confirmed that a 3 wt% catalyst loading is more suitable for plant feedstocks. Although yields are still modest, they are the first confirmed yields of HMF produced from comfrey and switchgrass using ionic liquids and metal halide catalysis. Most importantly, the use of pre-treatments have been confirmed to increase the amount of HMF produced compared to the untreated biomass.

The maximal yield obtained from switchgrass or comfrey is not expected to surpass the yields obtained from glucose due to the complexity of the plant material. Direct comparison between the conversion of extracts and the conversion of glucose also remains difficult due to the differences in optimum reaction conditions. However, when comparing the conversion using the same reaction conditions ([EMIM]Cl and a 3 wt% catalyst loading), the yield of 18.0%

obtained from treated switchgrass (Table 3, entry 7) approaches the yield of 24.4% obtained from glucose (Table 1, entry 4).

Comparison to the literature for the conversion of comfrey and switchgrass to HMF is also difficult since, to our knowledge, no plant MeOH extract has been tested for the production of HMF. However, our studies seem to rival the yields previously obtained from the conversion of switchgrass where HMF was produced in a H_2O/THF mixture with $AlCl_3•6H_2O$ in a yield of 21%.[22] HMF was also produced in a yield of 4.5% from switchgrass when placed in a solution of 1% H_2SO_4 for 2 min.[21] The best yield produced from comfrey is modest (6.04%), but it is also the first confirmed amount of HMF produced from this feedstock (Table 3, entry 1).

Conclusions

We have demonstrated that sugars can be extracted from comfrey and switchgrass using different pre-treatments, including MeOH treatment and acid hydrolysis. Acceptable yields of extractable sugars were shown for both plants. Soluble sugars made up 4.70±1.31% of the DW of comfrey, and 3.47±0.48% of the DW for switchgrass. Using a 0.5 M H_2SO_4 treatment, extraction of a total of 13.0±1.8% of sugars in the DW of comfrey, and 18.9±0.37% of sugars in the DW of switchgrass was possible.

Extracts rich in sugars were produced from the MeOH extraction, with concentrations of 300±60 mg of total sugars/g of dry extract for comfrey, and 202±16 mg of total sugars/g of dry extracts for switchgrass. H_2SO_4 extracts contained 230±29 mg of total sugars/g of hydrolysed biomass of comfrey, and 425±13 mg of total sugars/g of hydrolysed biomass of switchgrass. The best yield of HMF was obtained using the MeOH extracted soluble sugars found in each plant. Maximum yields of HMF were 6.04% for comfrey and 18.0% for switchgrass. These yields remain lower than the maximal yields obtained from transforming glucose to HMF in

[BMIM]Cl (50.0%) or [EMIM]Cl (31.0%), but are much higher than the yields obtained from untreated biomass (<1%).

The yields obtained from treated biomass, even if they are low, are important as they support the idea that biomass extracts are more suitable for HMF production. It is therefore possible that the yields of HMF obtained from biomass currently reported in the literature could be increased using pre-treatments. Pre-treatments also allow the use of more complex feedstocks as opposed to the starch and simple sugar rich edible plants currently used. The exploration of biomass treatments will therefore be important in the further development of an industry for biofuel production from complex biomass, in which obtaining small yields of HMF using complex biomass is a critical early step.

Acknowledgments

The authors would like to acknowledge the funds provided by the Center for Excellence in Mining Innovation (CEMI, Sudbury, Ontario, Canada), and the funds provided by Laurentian University through the Graduate Teaching Assistantship and the Laurentian University Summer Fellowship presented to Alexandrine L. Martel.

References

(1) da Costa Lopes, A. M.; João, K. G.; Morais, A. R. C; Bogel-Łukasik, E.; Bogel-Łukasik, R. *Sustain. Chem. Process* **2013**, 1, 1.

(2) Binder, J. B.; Raines, R. T. *J. Am. Chem. Soc.* **2009**, 131, 1979. doi: 10.1021/ja808537j.

(3) Guan, J.; Cao, Q.; Guo, X.; Mu, X. *Comp. Theor. Chem.* **2011**, 963, 453. doi.org/10.1016/j.comptc.2010.11.012.

(4) Chun, J.; Lee, J.; Yi, Y.; Hong, S.; Chung, C. *Starch/Stärke* **2010**, 62, 326. doi: 10.1002/star.201000012.

(5) Despax-Machefel, S. Développement de méthodologies de synthèse d'hydroxyméthylfurfural à partir de biomasse lignocellulosique. Doctoral dissertation, Université de Reims Champagne-Ardenne, Reims, France, 2013.

(6) Eminov, S.; Wilton-Ely, J. D. E. T.; Hallett, J. P. *ACS Sustainable Chem. Eng.* **2014**, 2, 978. doi: 10.1021/sc400553q.

(7) Fachri, B. A.; Abdilla, R. M.; Rasrendra, C. B.; Heeres, H. J. *Chem. Eng. Res. Des.* **2016**, 109, 65. doi.org/10.1016/j.cherd.2016.01.002.

(8) Abou-Yousef, H.; Hassan, E. B.; Steele, P. *J. Fuel Chem. Tech. (Ranliao Huaxue Xuebao)* **2013**, 41, 214. doi.org/10.1016/S1872-5813(13)60013-4.

(9) Lee, J. -W.; Ha, M. -G.; Yi, Y. -B.; Chung, C. -H. *Carbohydr. Res.* **2011**, 346, 177. doi.org/10.1016/j.carres.2010.11.009.

(10) Li, C.; Zhang, Z.; Zhao, Z. K. *Tetrahedron Lett.* **2009**, 50, 5403. doi.org/10.1016/j.tetlet.2009.07.053.

(11) Li, F.; Shi, G.; Wang, G.; Guo, T.; Lei, X. *Res. Chem. Intermed.* **2016**, 42, 6757. doi: 10.1007/s11164-016-2496-9.

(12) Liu, J.; Tang, Y.; Wu, K.; Bi, C.; Cui, Q. *Carbohydr. Res.* **2012**, 350, 20. doi.org/10.1016/j.carres.2011.12.006.

(13) Van Nguyen, C.; Lewis, D.; Chen, W. -H.; Huang, H. -W.; ALOthman, Z. A.; Yamauchi, Y.; Wu, K. C. W. *Catal. Today* **2016**, 278, 344. doi.org/10.1016/j.carres.2011.12.006.

(14) Peleteiro, S.; Garrote, G.; Santos, V.; Parajo, J. C. *Afinidad LXXI* **2014**, 567, 202. http://www.raco.cat/index.php/afinidad/article/view/281135.

(15) Ren, Q.; Huang, Y.; Wang, F.; Gao, J.; Xu, J. *Bioresources* **2013**, 8, 1563. http://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_08_2_1563_Ren_Conversion_Glucose/1987.

(16) Shi, J.; Liu, W.; Wang, N.; Yang, W.; Wang, H. *Catal. Lett.* **2013**, 144, 252. doi: 10.1007/s10562-013-1148-6.

(17) Song, J.; Zhang, B.; Shi, J.; Fan, H.; Ma, J.; Yang, Y.; Han, B. *RSC Adv.* **2013**, 3, 20085. doi:10.1039/C3RA43934H.

(18) Sun, J.; Yuan, X.; Shen, Y.; Yi, Y.; Wang, B.; Xu, F.; Sun, R. *Ind. Crops Prod.* **2015**, 70, 266. doi.org/10.1016/j.indcrop.2015.03.044.

(19) Wang, P.; Yu, H.; Zhan, S.; Wang, S. *Bioresour. Technol.* **2011**, 102, 4179. doi.org/10.1016/j.biortech.2010.12.073.

(20) Xiao, S.; Liu, B.; Wang, Y.; Fang, Z.; Zhang, Z. *Bioresour. Technol.* **2014**, 151, 361. doi.org/10.1016/j.biortech.2013.10.095.

(21) Yan, L.; Greenwood, A. A.; Hossain, A.; Yang, B. *RSC Adv.* **2014**, 4, 23492. doi:10.1039/C4RA01631A.

(22) Yang, Y.; Hu, C.; Abu-Omar, M.M. *Green Chem.* **2012**, 14, 509. doi:10.1039/C1GC15972K.

(23) Yi, Y. -B.; Lee, J. -W.; Hong, S. -S.; Choi, Y. -H.; Chung, C. -H. *J. Ind. Eng. Chem.* **2011**, 17, 6. doi.org/10.1016/j.jiec.2010.12.017.

(24) Yi, Y. -B.; Lee, J. -W.; Choi, Y. -H.; Park, S. -M.; Chung, C. -H. *Biomass Bioenergy* **2012**, 39, 484. doi.org/10.1016/j.biombioe.2012.01.011.

(25) Zhang, Z.; Zhao, Z. K. *Bioresour. Technol.* **2010**, 101, 1111. doi:10.1016/j.biortech.2009.09.010.

(26) Zhao, H.; Holladay, J. E.; Brown, H.; Zhang, Z. C. *Science* **2007**, 316, 1597. doi: 10.1126/science.1141199.

(27) Kumar, P.; Barrett, D.M.; Delwiche, M. J.; Stroeve, P. *Ind. Eng. Chem. Res.* **2009**, 48, 3713. doi: 10.1021/ie801542g.

(28) Smith, S. The feasibility of using a municipal compost cover over Cu-Ni tailings as a growth medium for biofuel crops. Master's dissertation, Laurentian University, Sudbury, ON, 2012.

(29) Chin, L.; Leung, D. W. M.; Taylor, H. H. *Chemosphere* **2009**, 76, 711. doi.org/10.1016/j.chemosphere.2009.04.059.

(30) Chin, L.; Leung, D. W. M.; Taylor, H. H. *Chem. Ecol.* **2009**, 25, 397. doi.org/10.1080/02757540903325088.

(31) Reed, R. L.; Sanderson, M. A.; Allen, V. G.; Zartman, R. E. *Commun. Soil Sci. Plant Anal.* **2002**, 33, 1187. doi.org/10.1081/CSS-120003881.

(32) Reed, R. L.; Sanderson, M. A.; Allen, V. G.; Matches, A. G. *Commun. Soil Sci. Plant Anal.* **2008**, 30, 2655. doi.org/10.1080/00103629909370403.

(33) DuBois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Anal. Chem.* **1956**, 28, 350. doi: 10.1021/ac60111a017.

(34) Wood, I. P.; Elliston, A.; Ryden, P.; Bancroft, I.; Roberts, I. N.; Waldron, K. W. *Biomass Bioenergy* **2012**, 44, 117. doi.org/10.1016/j.biombioe.2012.05.003.

(35) Chow, P. S.; Landhäusser, S. M. *Tree Physiol.* **2004**, 24, 1129. doi.org/10.1093/treephys/24.10.112.9.

(36) Blunden, C. A.; Wilson, M. F. *Anal. Biochem.* **1985**, 151, 403. doi.org/10.1016/0003-2697(85)90195-2.

(37) Godin, B.; Ghysel, F.; Agneessens, R.; Schmit, T.; Gofflot, S.; Lamaudiere, S.; Sinnaeve, G.; Goffart, J. P.; Gerin, P. A.; Stilmant, D.; Delcarte, J. *Biotechnol. Agron. Soc. Environ.* **2010**, 14, 549.

(38) Esteghlalian, A.; Hashimoto, A. G.; Fenske, J. J.; Penner, M. H. *Bioresour. Technol.* **1997**, 59, 129. doi.org/10.1016/S0960-8524(97)81606-9.

(39) McMillan, J. D. Pretreatment of lignocellulosic biomass. In *Enzymatic conversion of biomass for fuels production*; Himmel, M. E.; Baker, J. O.; Overend, R. P., Eds.; American Chemical Society: Washington, DC, 1994; pp. 292-324.

(40) Hinman, N. D.; Schell, D. J.; Riley, C. J.; Bergeron, P. W.; Walter, P. J. *Appl. Biochem. Biotechnol.* **1992**, 34/35, 639. doi.org/10.1007/BF02920584.

(41) Wang, C.; Fu, L.; Tong, X.; Yang, Q.; Zhang, W. *Carbohydr. Res.* **2012**, 347, 182. doi.org/10.1016/j.carres.2011.11.013.

Fig. 1. Comparison of total sugars obtained after a MeOH extraction of comfrey and switchgrass (n=4 for comfrey, n=3 for switchgrass).

Fig. 2. Comparison of total sugars obtained after a 0.5 M H_2SO_4 treatment after a 30 min autoclave for comfrey and switchgrass (n=4 for comfrey, n=3 for switchgrass).

Table 1. HMF production from glucose with a reaction time of 30 min at 140°C with a 10 wt% substrate loading.

Entry	Solvent	Catalyst 1	Catalyst 2	HMF Yield (%)
1	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	50.0
2	[EMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	31.0
3	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	24.5
4	[EMIM]Cl	$CrCl_3$•$6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	24.4
5	[BMIM]Cl	$CrCl_2$ (3 mol%)	$CuCl_2$ (3 mol%)	48.7
6	[EMIM]Cl	$CrCl_2$ (3 mol%)	$CuCl_2$ (3 mol%)	31.0

Table 2. HMF production from the dry 0.5 M H_2SO_4 extracts for comfrey and switchgrass. Substrate loading was 10 wt% of the reaction mixture. Catalyst mixture was $CrCl_3$•$6H_2O/CuCl_2$ at 3 wt% of the substrate. Dissolution step was 30 min at 120°C and catalytic step was 30 min at 140°C.

Entry	Dry 0.5 M H_2SO_4 Extract	Solvent	% dissolved biomass	Yield of HMF (%)
1	Comfrey	[BMIM]Cl	100	1.74
2	Switchgrass	[BMIM]Cl	100	1.14
3	Comfrey	[EMIM]Cl	92	2.31
4	Switchgrass	[EMIM]Cl	88	<1

Table 3. HMF production from the dry MeOH extract of comfrey and switchgrass. Substrate loading was 10 wt% of the reaction mixture. Catalyst loading was maintained at a 3 wt% loading based on the amount of extract. Dissolution step was maintained at 120°C for 30 min and catalytic step was maintained at 140°C.

Entry	Dry MeOH extract	Solvent	Catalysts	Catalytic step (min)	% dissolved biomass	Yield of HMF (%)
1	Comfrey	[BMIM]Cl	$CrCl_3$•$6H_2O$/$CuCl_2$	15	57	6.04
2	Comfrey	[BMIM]Cl	$CrCl_2$/$CuCl_2$	15	71	4.93
3	Comfrey	[BMIM]Cl	$CrCl_3$•$6H_2O$/$CuCl_2$	30	61	3.17
4	Comfrey	[EMIM]Cl	$CrCl_3$•$6H_2O$/$CuCl_2$	30	66	2.73
5	Switchgrass	[BMIM]Cl	$CrCl_3$•$6H_2O$/$CuCl_2$	15	43	<1
6	Switchgrass	[BMIM]Cl	$CrCl_3$•$6H_2O$/$CuCl_2$	30	60	2.00
7	Switchgrass	[EMIM]Cl	$CrCl_3$•$6H_2O$/$CuCl_2$	30	52	18.0
8	Switchgrass	[EMIM]Cl	$CrCl_2$/$CuCl_2$	30	48	14.1

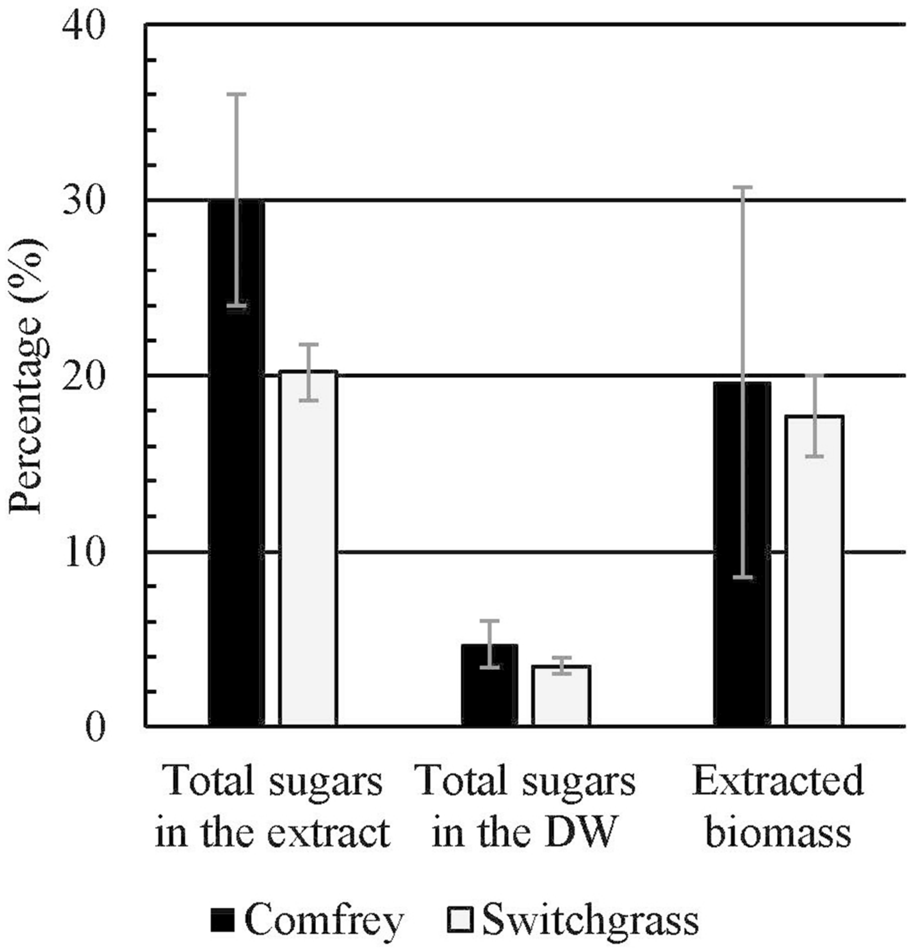

Fig. 1. Comparison of total sugars obtained after a MeOH extraction of comfrey and switchgrass (n=4 for comfrey, n=3 for switchgrass).

85x85mm (300 x 300 DPI)

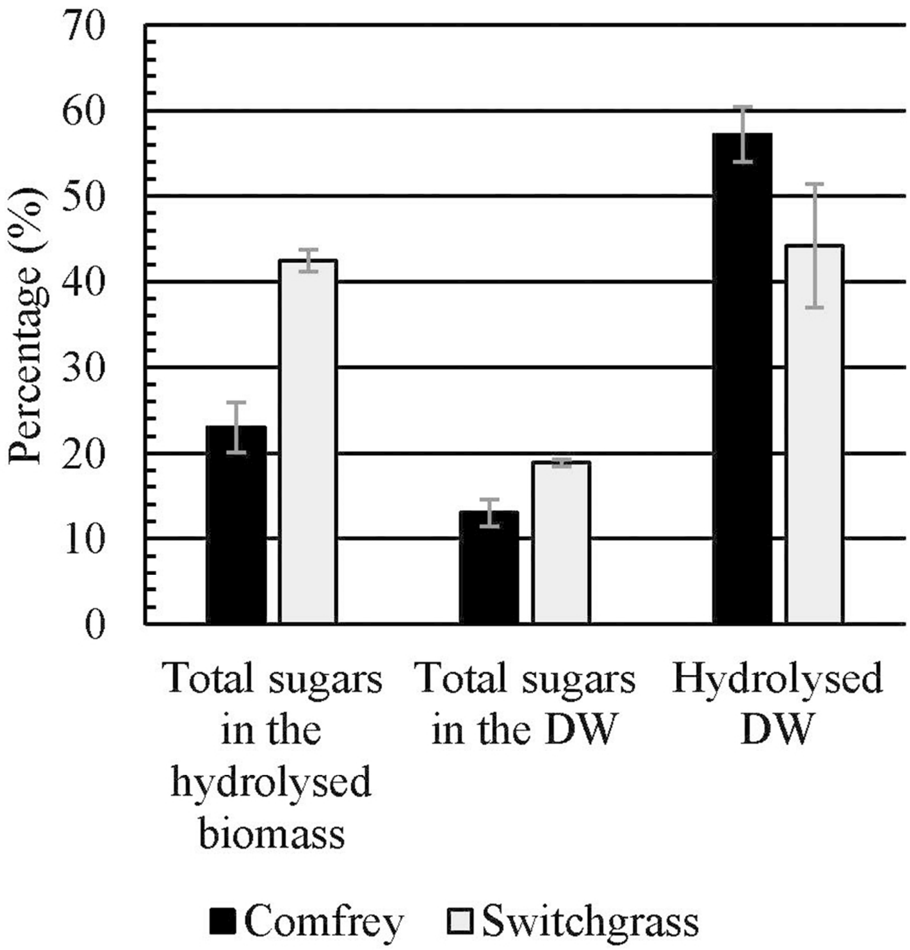

Fig. 2. Comparison of total sugars obtained after a 0.5 M H2SO4 treatment after a 30 min autoclave for comfrey and switchgrass (n=4 for comfrey, n=3 for switchgrass).

85x85mm (300 x 300 DPI)