INVESTIGATION OF POTENTIAL INHIBITORS FROM SWITCHGRASS IN BIOREFINERY

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Introduction

The inhibition of cellulolytic enzymes in the saccharification of carbohydrates into monosaccharides to produce ethanol in the biorefinery is broad-based. The causes of inhibition include substrate and product inhibition, mass transfer resistance, particle size effects and non-productive enzyme binding with lignin. Furthermore, phenolic molecules produced by the plants also inhibit enzyme hydrolysis at low concentrations (Ladisch et al, 1980). This presentation focuses on the quantification of the total phenolic compounds in conjunction with HPLC chromatograms of three switchgrass varieties to create a calibration/prediction models that could be used to rapidly determine the amount of phenolics compounds that are present in a biomass.

Experimental

- Three varieties (Alamo, (AL), EG1101 (EG1) and EG1102 (EG2) were collected from three farms totaling 80 hectares.
- Switchgrass samples were dried at 40 ± 5 ºC in a dry kiln for 72 hours.
- Samples were size reduced into 2 mm particle size
- Extraction with water and ethanol using Dionex ASE 350 extractor
- Filtration of extractives
- Total phenolics equivalent using Folin-Ciocalteau Reagent and gallic acid as reference phenolic compound.
- Perkin Elmer Flexar HPLC-PDA to acquire chromatograms at 370 nm wavelength
- IBM SPSS 20
- Multivariate data analysis using Unscrambler 9.0

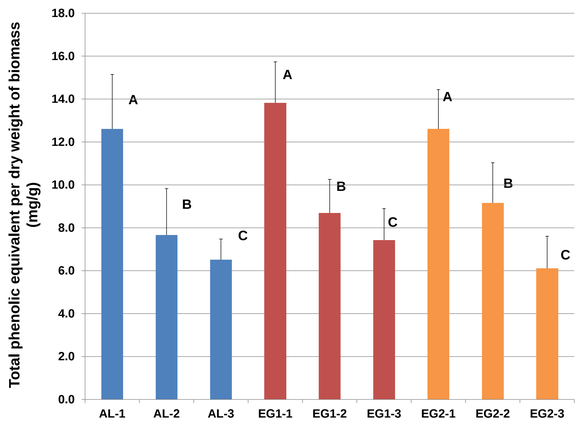

Figure 1: Bar graphs showing total phenolic equivalent in three varieties of switchgrass harvested at vegetative, transition and reproductive stages

Results and Discussion

Total phenolics equivalent in switchgrass ranged from 5.32-15.14 mg/g for Alamo, 4.05-15.95 mg/g for EG1101 and 4.25-14.33 mg/g for EG1102. The highest amount of total phenolics equivalent at vegetative stages for each variety was attributed to a high rate of production of phenolic compounds during the growth period of switchgrass at the early growth stage and lowest at reproductive stage.

Total phenolics equivalent of switchgrass showed no significant difference at each growth stage between varieties. However, there was significant difference between each growth stage within varieties (Figure 1). Alamo, EG1101 and EG1102 showed no variability between samples at different locations and vegetative stages.

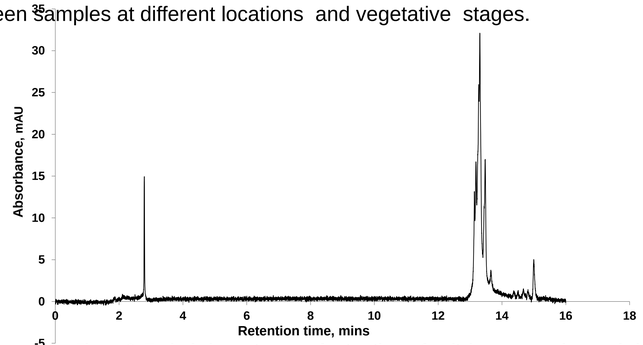

Figure 2: Typical chromatogram of extractives of switchgrass sample sampled at vegetative, transition and reproductive stages.

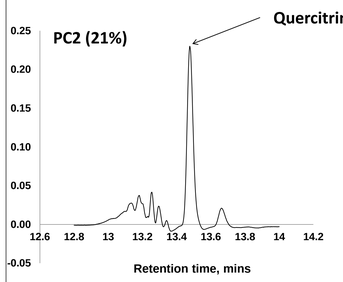

Figure 3: Graph PC2 using chromatogram of switchgrass extractives collected at 370 nm.

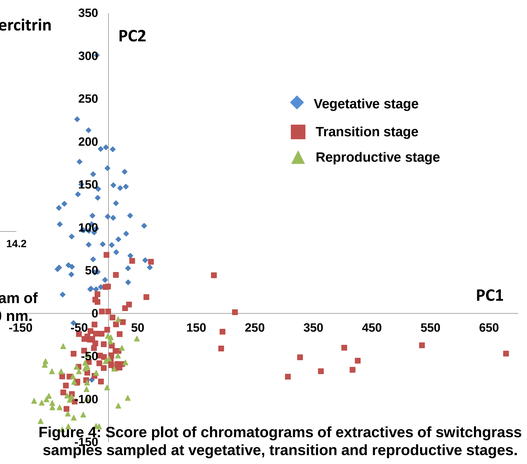

Figure 4: Score plot of chromatograms of extractives of switchgrass samples sampled at vegetative, transition and reproductive stages.

Figure 4 shows clusters of samples from vegetative, transition and reproductive stages along the PC2 axis. The loading plot shows a prominent peak, quercitrin at retention time of 13.49 minutes (Figure 3). Quercitrin is therefore one of the major components of the extractives that accounts for the discrimination of the switchgrass samples sampled at different growth stages.

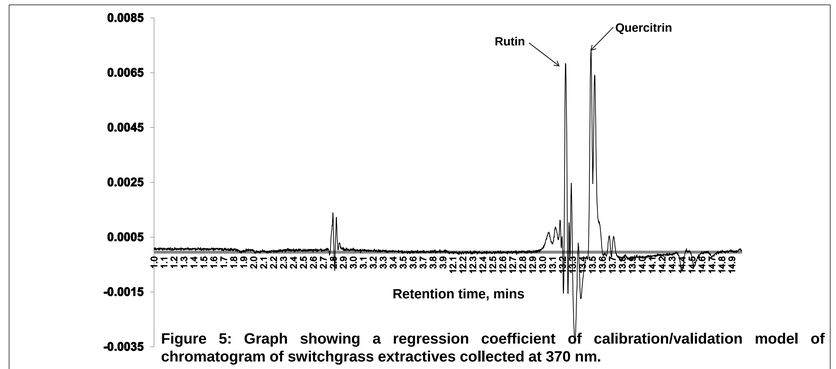

Figure 5: Graph showing a regression coefficient of calibration/validation model of chromatogram of switchgrass extractives collected at 370 nm.

Figure 5 shows the regression of coefficient of samples from vegetative, transition and reproductive stages with two identified prominent peaks rutin and quercitrin. These compounds are phenolic compounds.

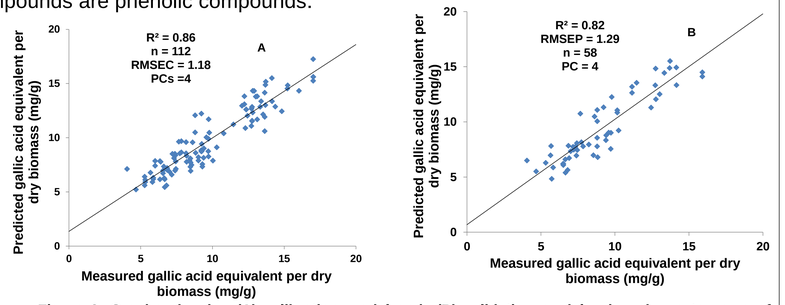

Figure 6: Graphs showing (A) calibration model and (B) validation model using chromatograms of switchgrass extractives collected at 370 nm.

The performance evaluation of the predictive model was based on the R^2 and RMSEP values.. Furthermore, the R^2 value of 0.824 is considered good for prediction (Figure 6).

Conclusions

There was no variability of total phenolics equivalent between the varieties as well as impact of location where the samples were harvested. Principal component analysis of HPLC chromatograms of switchgrass extractives showed that PC 2 (21%) accounted for the discrimination between the switchgrass varieties at three different growth stages. Furthermore, partial least squares regression model gave calibration and validation models with R^2 values 0.86 and 0.82 respectively with good predictive ability. With two important parameters including R^2 and RMSEP of a good and reliable predictive model for switchgrass extractives using chromatograms to assess the potential inhibitors, results of this work suggest that potential inhibitors from switchgrass can be predicted in the biorefinery using chemometrics.

Acknowledgement

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