Comparison of Carbohydrate Composition in Lignocellulosic Biomass by High Performance Liquid Chromatography and Gas Chromatography Analysis

Ji Young Jung, Si Young Ha, and Jae-Kyung Yang *

The carbohydrate composition (glucose, xylose, mannose, galactose, and arabinose) of lignocellulosic biomass *Liriodendron tulipifera*, *Populus nigra* × *Populus maximowiczii*, *Populus alba* × *Populus glandulosa*, *Populus euramericana*, *Salix alba*, *Quercus variabilis*, *Robinia pseudoacacia*, *Zelkova serrata*, *Abies holophylla*, *Pinus rigida*, rice straw, and peanut hull was investigated based on high-performance liquid chromatography (HPLC) and gas chromatography (GC) analyses derived from ASTM and NREL methods. The glucose content was higher in HPLC than in GC analysis, and the xylose, mannose, galactose, and arabinose contents were higher in GC than in HPLC analysis. The difference in carbohydrate composition was noticeable in the glucose, mannose, and arabinose contents of *Abies holophylla* and *Pinus rigida*, and this was affected by the species. A decision tree, as a data mining and artificial intelligence method, is a reliable and simple variable selection tool. This technique was used for carbohydrate analysis classification. Accordingly, 432 monosaccharide content reading data and analysis methods were used for model checking. It was found that arabinose was the most important splitting variable in carbohydrate analysis, and other monosaccharides did not influence the assay decision. However, the selection of a determination method for each sample should be considered comprehensively in future studies.

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Keywords: Lignocellulosic biomass; Carbohydrate analysis; High performance liquid chromatography; Gas chromatography; Decision tree

Contact information: Division of Environmental Forest Science/Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, 52828, Republic of Korea;
** Corresponding author: jkyang@gnu.ac.kr*

INTRODUCTION

Lignocellulosic biomass has become an alternative source for producing chemicals and fuels because it is renewable and can reduce greenhouse gas emissions by replacing petroleum sources (Binder and Raines 2009). The major components of lignocellulosic biomass are cellulose, hemicellulose, and lignin. The composition of lignocellulosic biomass varies according to the type, location, maturity, and climate conditions. On average it consists of approximately 15 to 30% of hemicellulose, 40 to 60% of cellulose, and 10 to 25% of lignin (Zhang *et al.* 2012; Wang *et al.* 2017). As its interest as a biorefinery source grows, analyzing the chemical composition of lignocellulosic biomass becomes more important and an accurate compositional analysis is needed to evaluate the conversion yields and process economics.

In the past, gravimetric methods have been utilized to measure cellulose,

hemicellulose, and lignin content. Recently, the American Society for Testing and Materials (ASTM) and the National Renewable Energy Laboratory (NREL) analysis methods have been used to analyze the chemical composition of lignocellulosic biomass. Among chemical analysis methods, lignin and minor components (extractive, ash, protein, among others) undergo similar analysis procedures; however, there are differences in the carbohydrate analysis procedures (Sluiter *et al*. 2010). Chromatography analyses, such as those described in ASTM and NREL methods, require acid hydrolysis of the carbohydrate to yield a mixed solution of sugar monomers, such as glucose, xylose, galactose, arabinose, and mannose. The solution is filtered, and the sugars are chromatographically separated and quantified. Two-stage sulfuric acid hydrolysis is most commonly used to fractionate biomass for both gravimetric and instrumental analyses. In general, this method is accepted as the standard for hydrolysis (Ritter *et al.* 1932). In this procedure, wood meal is treated with 72% H_2SO_4 at 30 °C for 2 h to depolymerize the carbohydrates. The recombined sugar monomers are further hydrolyzed in 3% H_2SO_4 at 121 °C for 1 h (Ritter *et al*. 1932; ASTM E1821-96 (2011); Sluiter *et al*. 2008a).

The individual sugars are separated by high-performance liquid chromatography (HPLC) or gas chromatography (GC). HPLC is currently the most efficient method for the routine separation and quantification of the five wood sugars (glucose, xylose, galactose, mannose, arabinose). In this case, no derivatization is necessary, and separation is achieved using water as the eluent. However, depending on the analysis method, quantitative results may be overestimated or underestimated (Mantovani *et al*., 2017; Pettersen 1984). GC analysis utilizes the alditol acetate derivatization. Sugar analysis by GC may be useful for specialized problems; however, the derivatization steps make it a time-consuming method for routine work. GC technology has the potential to become one of the recommended methods for monosaccharide composition analysis, despite its few drawbacks (Ruiz-Matute *et al*., 2011; Wolfrum and Sluiter 2009; Dan *et al*. 2021).

Depending on the characteristics of the analytical methods, instruments, lignocellulosic biomass, and technique used by the technician, there may be differences observed in the composition and content of carbohydrates analyzed. Therefore, users should make appropriate selections by considering various factors (Xu *et al*. 2013). In this study, short rotation crops, hardwood species, softwood species, and agriculture residue were selected as the materials for analysis. Carbohydrate composition was analyzed by HPLC and GC analyses, and then the differences in values between the analysis methods were compared. Using decision tree statistical analysis, factors influencing the choice of assay were determined.

EXPERIMENTAL

Materials

Short rotation crops (*Liriodendron tulipifera*, *Populus nigra* × *Populus maximowiczii*, *Populus alba* × *Populus glandulosa*, *Populus euramericana*, and *Salix alba*), hardwood species (*Quercus variabilis*, *Robinia pseudoacacia*, and *Zelkova serrata*), softwood species (*Abies holophylla*, *Pinus rigida*), and agriculture residue (rice straw and peanut hull) were used as raw materials. The raw material was air-dried to a moisture content of less than 10%, milled with a milling machine, and sieved to retain particles of 40- to 80-mesh size.

Compositional Analysis of Materials

Minor component analysis

The ethanol extractives and ash contents were determined using the method described by Sluiter *et al*. (2008b,c). Between 1 and 5 g (dry basis) of the Wiley milled raw material was extracted with 95% ethanol in a Soxhlet extraction apparatus (Misung Scientific Co. Ltd., Yangju, Republic of Korea) for a minimum of 24 h. The extracted material was filtered with a medium-porosity glass filtering crucible, air-dried overnight at ambient temperature, and stored. The extractives were separated from ethanol using a rotary vacuum evaporator (Buchi, Flawil, Switzerland). The ash of the raw material was obtained from raw material calcination at 550 °C for 3 h and weighed.

Lignin analysis

The total lignin content was determined using the method described by Sluiter *et al*. (2008a). Approximately 0.3 g of extractive-free raw material was hydrolyzed with 3 mL of 72% H_2SO_4 for 2 h at 30 °C. After completing the first hydrolysis step, the acid was diluted to 4% by adding 84 mL of distilled water, and the second hydrolysis step was completed in an autoclave at approximately 121 °C for 1 h. After completion of the autoclave cycle, the sample was cooled to 40 °C and the mixture was filtered. The solid residue remaining after acid hydrolysis is an acid-insoluble lignin. The acid-soluble lignin content in the hydrolysates was also quantified and was determined by measuring the absorbance at 205 nm using a UV-visible spectrophotometer (HITACHI U-3000, Tokyo, Japan). The filtrate was collected and used as a stock sample for carbohydrate analysis.

Carbohydrate analysis - HPLC

The carbohydrate composition was determined according Sluiter *et al*. (2008a). The carbohydrate content of lignocellulosic biomass was measured after a two-step acid hydrolysis procedure (lignin analysis) to fractionate the fiber. The hydrolysate was then analyzed for sugar content by HPLC using a Waters 2695 liquid chromatograph (Waters Corporation, Milford, MA, USA) with a refractive index detector. In the sugar content analysis using the Aminex HPX-87P column, the acid hydrolysate was neutralized by adding $CaCO_3$ and filtered with a Minisart syringe filter before injection into the HPLC system. Monomer sugars (glucose, xylose, mannose, galactose, and arabinose) were separated with an Aminex HPX-87P column (300 × 7.8 mm, Bio Rad, Hercules, CA, USA) at 80 to 85 °C using HPLC-grade water as the mobile phase at a flow rate of 0.6 mL/min and detected with a refractive index detector at 40 °C.

Carbohydrate analysis - GC

The carbohydrate composition of the raw samples was tested in parallel using GC according to ASTM E1821-96 (2011). This method describes a procedure for derivatizing monomers to their respective alditol acetates, and the sugar is determined after hydrolysis with sulfuric acid (lignin analysis). Conversion into alditol acetate sugar composition was analyzed using GC (YL6100 GC, Young Lin Ins. Co., Ltd., Anyang, Republic of Korea) equipped with a DB-225 capillary column (15 m long with an inner diameter of 0.25 mm, and film thickness of 0.25 mm, Agilent Technologies, Santa Clara, CA, USA). Each sample (2 μL) was injected *via* a split injector (200 °C, split ratio of 30:1) into a DB-225 capillary column (Agilent Technologies, Santa Clara, CA, USA).

The column temperature program was set as follows: an initial column temperature of 190 °C was held for 1 min before ramping at 10 °C per min up to 220 °C, where it was kept steady for 10 minutes with a total run time of 14 min. The carrier gas was nitrogen (flow rate of 40 cm/s), and the detector condition was 70 eV. The standard curve for each monosaccharide, using *myo*-inositol as the internal standard, was determined by plotting the concentration ratio against its area ratio.

Statistical Analysis

Statistical analysis was carried out with the SAS statistical software (SAS Institute Inc., version 9.4, Cary, NC, USA) using the analysis of variance test and comparing data mean using Duncan's multiple comparison range test for determining significant differences between the means.

The authors performed a decision tree to examine the analytical method that affected the carbohydrate content of the lignocellulosic biomass. A decision tree was presented using Weka 3.8 (The University of Waikato, New Zealand). The classifier selected was the J48 algorithm, and decisions were based on the main analytical method affecting the carbohydrate content of lignocellulosic biomass. The data used for the decision tree were 216 monosaccharide data by HPLC analysis, 216 monosaccharide data by GC analysis, and 432 monosaccharide data readings.

RESULTS AND DISCUSSION

Minor Component Content of Lignocellulosic Biomass

The extractive is easily soluble in water or organic solvents. Non-structural material as an extract must be removed from biomass prior to analysis to prevent interference with later analytical steps.

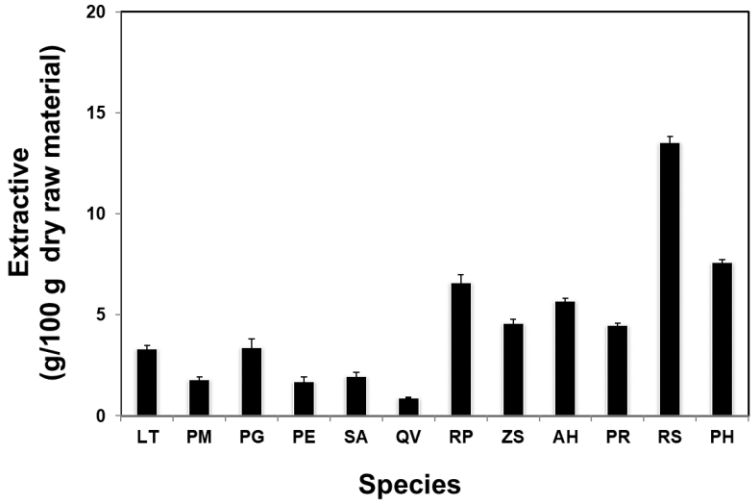

Fig. 1. Extractive content of different species in lignocellulosic biomass. Abbreviations: LT (*L. tulipifera*), PM (*P. nigra* x *P. maximowiczii*), PG (*P. alba* x *P. glandulosa*), PE (*P. euramericana*), SA (*S. alba*), QV (*Q. variabilis*), RP (*R. pseudoacacia*), ZS (*Z. serrata*), AH (*A. holophylla*), PR (*P. rigida*), RS (Rice straw), and PH (Peanut hull).

The extractive contents of *L. tulipifera*, *P. nigra* × *P. maximowiczii*, *P. alba* × *P. glandulosa*, *P. euramericana*, *S. alba*, *Q. variabilis*, *R. pseudoacacia*, *Z. serrata*, *A. holophylla*, *P. rigida*, rice straw, and peanut hull are shown in Fig. 1. The extracts of *L. tulipifera*, *P. nigra* × *P. maximowiczii*, *P. alba* × *P. glandulosa*, *P. euramericana*, *S. alba*, *Q. variabilis*, *R. pseudoacacia*, *Z. serrata*, *A. holophylla*, *P. rigida*, rice straw, and peanut hull were 3.3, 1.8, 3.4, 1.7, 2.0, 0.9, 6.6, 4.6, 5.7, 4.5, 13.5, and 7.6%, respectively. The extractive contents of lignocellulosic biomass were approximately 5%, and the highest extract content was 13.5% in rice straw. Nonstructural materials in biomass, which are easily extracted with water or solvents, may contribute significantly, up to 30% or more, to the mass closure and will interfere with the subsequent characterization of carbohydrates and lignin. Herbaceous biomass tends to contain more nonstructural materials than woody biomass (Sluiter *et al*. 2010).

Ash is an inorganic material bound to the physical structure of the biomass. The ash content in biomass is a measure of mineral and other inorganic matter. Wood-based ash is a non-combustible compound containing various elements such as calcium, potassium, magnesium, manganese, and silicon. The ash content is known to be about 1%. The wood-based ash measured in *L. tulipifera*, *P. nigra* × *P. maximowiczii*, *P. alba* × *P. glandulosa*, *P. euramericana*, *S. alba*, *Q. variabilis*, *R. pseudoacacia*, *Z. serrata*, *A. holophylla*, and *P. rigida* ranged from 0.2% to 1.8% (Fig. 2). In addition, the ash content was the highest in rice straw at 13.5%. The ash content of rice straw found in this study was in agreement with the content of 10 to 17% previously reported (Kargbo *et al.* 2009). In addition to significantly contributing to total mass closure, inorganic materials may interfere with acid hydrolysis.

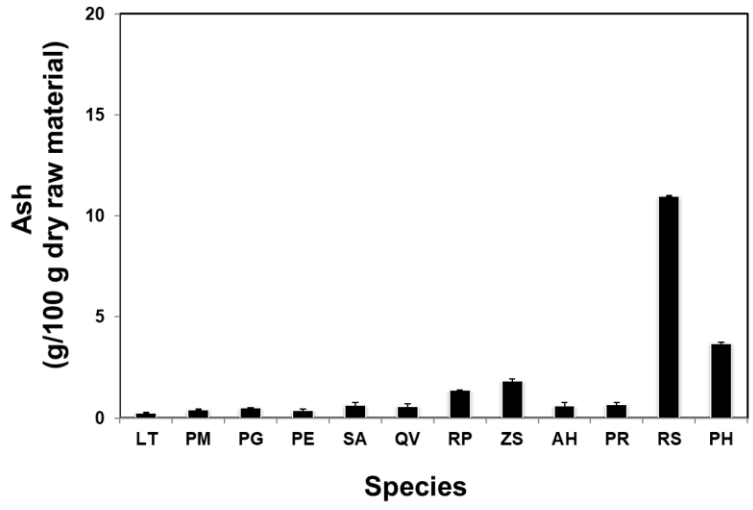

Fig. 2. Ash content of different species in lignocellulosic biomass. Abbreviations: LT (*L. tulipifera*), PM (*P. nigra* x *P. maximowiczii*), PG (*P. alba* x *P. glandulosa*), PE (*P. euramericana*), SA (*S. alba*), QV (*Q. variabilis*), RP (*R. pseudoacacia*), ZS (*Z. serrata*), AH (*A. holophylla*), PR (*P. rigida*), RS (Rice straw), and PH (Peanut hull)

Lignin Content of Lignocellulosic Biomass

Lignin is classified into acid-insoluble lignin and acid-soluble lignin. In general, lignin is acid-insoluble with a high-molecular weight. In this study, the lignin content was the sum of the acid-insoluble lignin and acid-soluble lignin and was expressed as the component excluding the mineral content (Sluiter *et al*. 2010).

The lignin content of *L. tulipifera*, *P. nigra* × *P. maximowiczii*, *P. alba* × *P. glandulosa*, *P. euramericana*, *S. alba*, *Q. variabilis*, *R. pseudoacacia*, and *Z. serrata* were 18.3 to approximately 23.6% (Fig. 3). The lignin contents were 34.6% and 35.3% in *A. holophylla* and *P. rigida*, respectively, indicating a high content in softwood species. The low lignin content of the rice straw was due to the ash content (Fig. 2b).

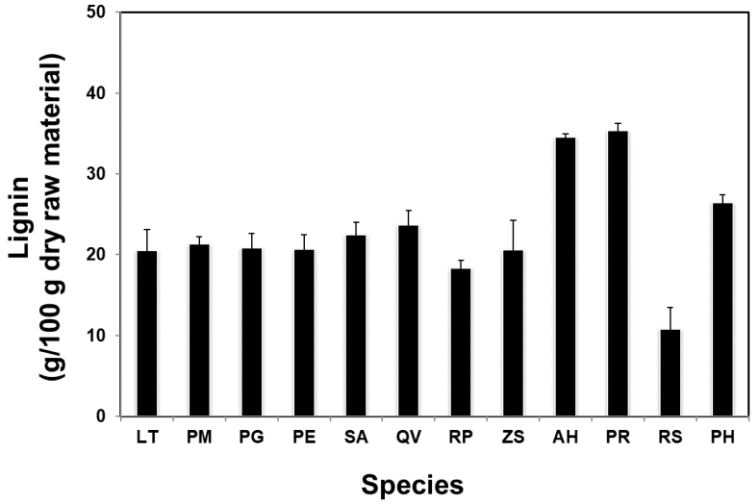

Fig. 3. Lignin content of different species in lignocellulosic biomass; Abbreviations: LT (*L. tulipifera*), PM (*P. nigra* x *P. maximowiczii*), PG (*P. alba* x *P. glandulosa*), PE (*P. euramericana*), SA (*S. alba*), QV (*Q. variabilis*), RP (*R. pseudoacacia*), ZS (*Z. serrata*), AH (*A. holophylla*), PR (*P. rigida*), RS (Rice straw), and PH (Peanut hull).

Comparison of Carbohydrates Composition in Lignocellulosic Biomass by HPLC and GC Analysis

Carbohydrate content

Figures 4(a)–(f) compare the data for glucose, xylose, mannose, galactose, arabinose, and total carbohydrate content, respectively, measured by HPLC and GC according to the NREL and ASTM analysis methods. Structural carbohydrates are analyzed as monomers, and it is not known the polymer types that originate each monomeric sugar. As the starch content of most seed-free herbaceous or woody feedstocks is low, the measured glucose is assumed to originate from cellulose (Sluiter *et al.* 2010).

The results of the glucose content of *L. tulipifera, P. nigra* × *P. maximowiczii, P. alba* × *P. glandulosa, P. euramericana, S. alba, Q. variabilis, R. pseudoacacia, Z. serrata, A. holophylla, P. rigida*, rice straw, and peanut hull based on the HPLC and GC analyses are shown in Fig. 4a. The glucose content of the analyzed lignocellulosic biomass ranged from 30.1 to 47.1%, and it was generally high in woody biomass, such as short rotation crops, hardwood, and softwood. In addition, a higher glucose content was measured by HPLC than by GC analysis.

The xylose content of *L. tulipifera,* P. nigra × *P. maximowiczii*, *P. alba* × *P. glandulosa, P. euramericana, S. alba, Q. variabilis, R. pseudoacacia, Z. serrata, A. holophylla, P. rigida*, rice straw, and peanut hull ranged from 6.7 to 18.7% and from 8.5 to 18.9% according to HPLC and GC analysis results, respectively (Fig. 4b). The xylose content was generally low in softwood species. Moreover, GC analysis indicated a higher xylose content compared to that provided by the HPLC analysis, except for *P. alba* × *P.*

glandulosa and rice straw.

The mannose contents measured by HPLC in woody crops (*L. tulipifera, P. nigra* × *P. maximowiczii, P. alba* × *P. glandulosa, P. euramericana, S. alba, Q. variabilis, R. pseudoacacia, Z. serrata, A. holophylla, and P. rigida*) and agricultural residue (rice straw and peanut hull) ranged from 1.1% to 2.1% and from 0.4 to 0.5%, respectively (Fig. 4c). In contrast, these contents measured by GC in woody crops ranged from 1.3 to 5.5% and 0.7 to 0.9%, respectively. By comparing these results, it is observed that the GC analysis indicated a higher mannose content than HPLC analysis in all species.

The galactose content differed according to species and analysis method. *L. tulipifera, P. nigra* × *P. maximowiczii, S. alba* showed high galactose contents by the HPLC method, whereas *P. alba* × *P. glandulosa, P. euramericana, Q. variabilis, R. pseudoacacia, Z. serrata, A. holophylla, P. rigida*, rice straw and peanut hull showed high galactose contents by the analytical method. (Fig. 4d).

The arabinose content of the analyzed lignocellulosic biomass ranged from 0.1 to 0.3% and from 0.6 to 3.1% by HPLC and GC analyses (Fig. 4e). In all species, GC analysis indicated a higher arabinose content compared to that by HPLC analysis, which may be because the GC analysis is more sensitive for trace contents. No significant differences were observed for carbohydrate content results, but a difference in the composition of each monosaccharide according to the analysis method was confirmed. In particular, trace amounts of simple sugars such as mannose and arabinose were considerably affected by the analysis method.

The composition and proportion of polymers in lignocellulosic biomass differ from species to species, even within a single plant, with age and growth stage, and between samples harvested from different parts of the same tree (Pérez *et al*. 2002). In addition, due to the complexity of polysaccharides, the complete release of polysaccharides is hindered; moreover, as there is lack of intrinsic fluorescent or chromophoric moieties, there may be differences depending on the analysis method (Irick *et al.* 1988; Liu *et al*. 2021).

Differences in value

The contents of each monosaccharide (glucose, xylose, mannose, galactose, and arabinose) measured by HPLC and GC analysis were compared, and the differences in values are shown in Fig. 4. According to the analysis, the difference value of glucose, xylose, mannose, galactose, arabinose, and carbohydrates was in the range of 1.6 to 4.2, 0.2 to 1.9, 0.2 to 4.4, 0.1 to 2.1, 0.6 to 2.8, and 0.3 to 6.5, respectively. In lignocellulosic biomass, both the chromatographic techniques yielded similar results for glucose. The difference in carbohydrate content according to the analysis was due to the hemicellulose sugar content. From the results obtained, it can be concluded that the results for xylose, mannose, galactose, and arabinose were influenced largely by the analytical method used. The determination of glucose was less affected in nearly all the samples analyzed. Similarly, it has been reported that arabinose, mannose, and galactose results are more influenced by the analytical method (Villanueva--Suárez *et al.* 2003). Mannose, galactose, and arabinose were the minor components in all the analyzed samples. They were difficult to analyze because of the low proportion in which they appeared. The difference in carbohydrate composition showed a large difference in the content of glucose, mannose, and arabinose of *A. holophylla* and *P. rigida*, which are softwood species, and this is affected by the species.

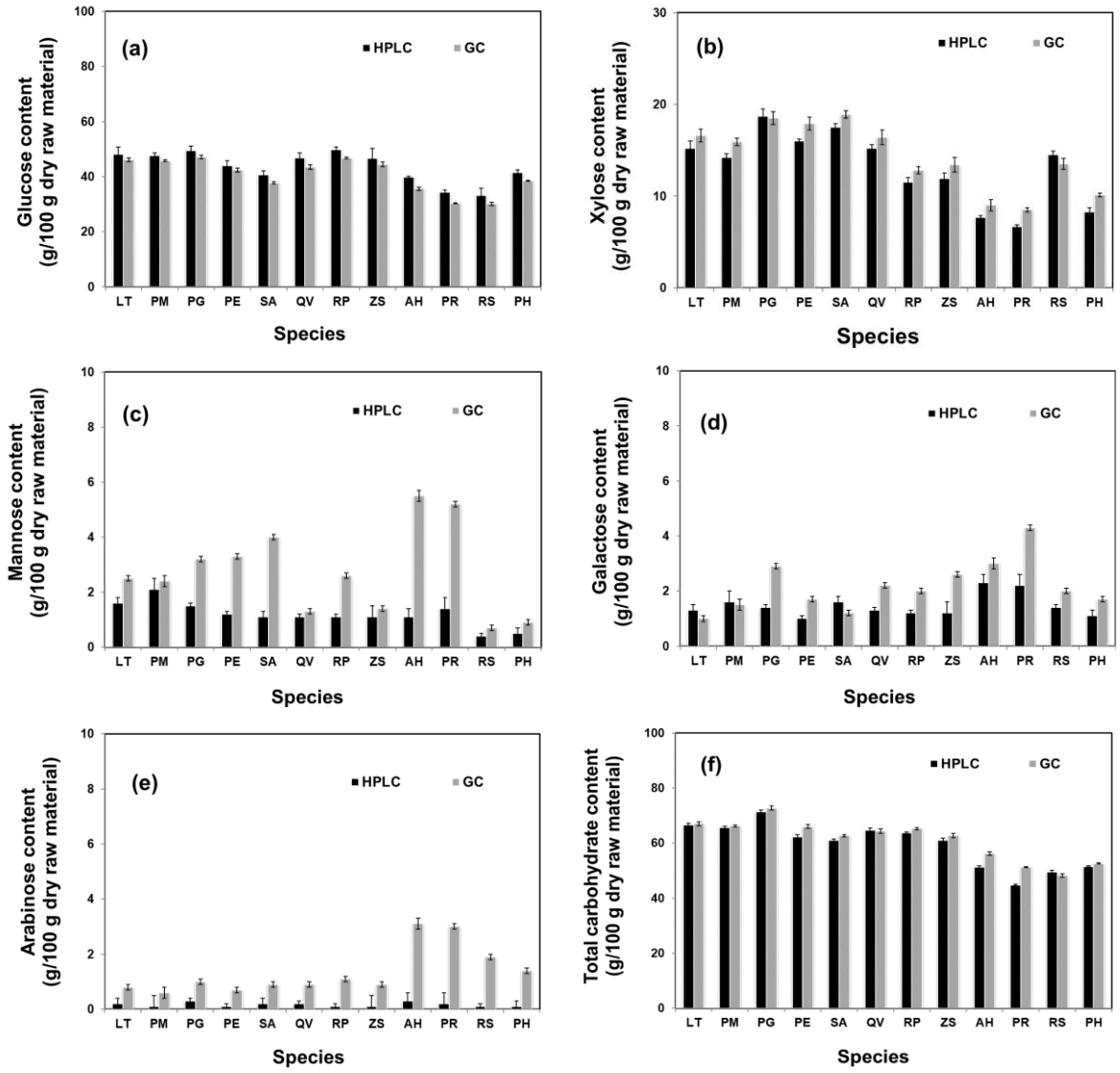

Fig. 4. Comparing GC and HPLC analysis of carbohydrate composition: (a) glucose, (b) xylose, (c) mannose, (d) mannose, (e) arabinose, and (f) total carbohydrate; Abbreviations: LT (*L. tulipifera*), PM (*P. nigra* × *P. maximowiczii*), PG (*P. alba* × *P. glandulosa*), PE (*P. euramericana*), SA (*S. alba*), QV (*Q. variabilis*), RP (*R. pseudoacacia*), ZS (*Z. serrata*), AH (*A. holophylla*), PR (*P. rigida*), RS (Rice straw), and PH (Peanut hull)

Decision Tree for the Prediction of Carbohydrate Analysis

Decision tree modeling is a reliable and simple variable selection tool, and it provides clear results from sophisticated data that allow its simple application (Bawah and Ussiph, 2018). Pourahmad *et al.* 2011; Heydari *et al.* 2012). Each node in the decision tree is determined by considering the highest information gain for all variables. If the variable gives a clear end product, the branch of this variable is terminated, and the target value is assigned to it. Overall, decision trees can help decide on a suitable method for carbohydrate analysis.

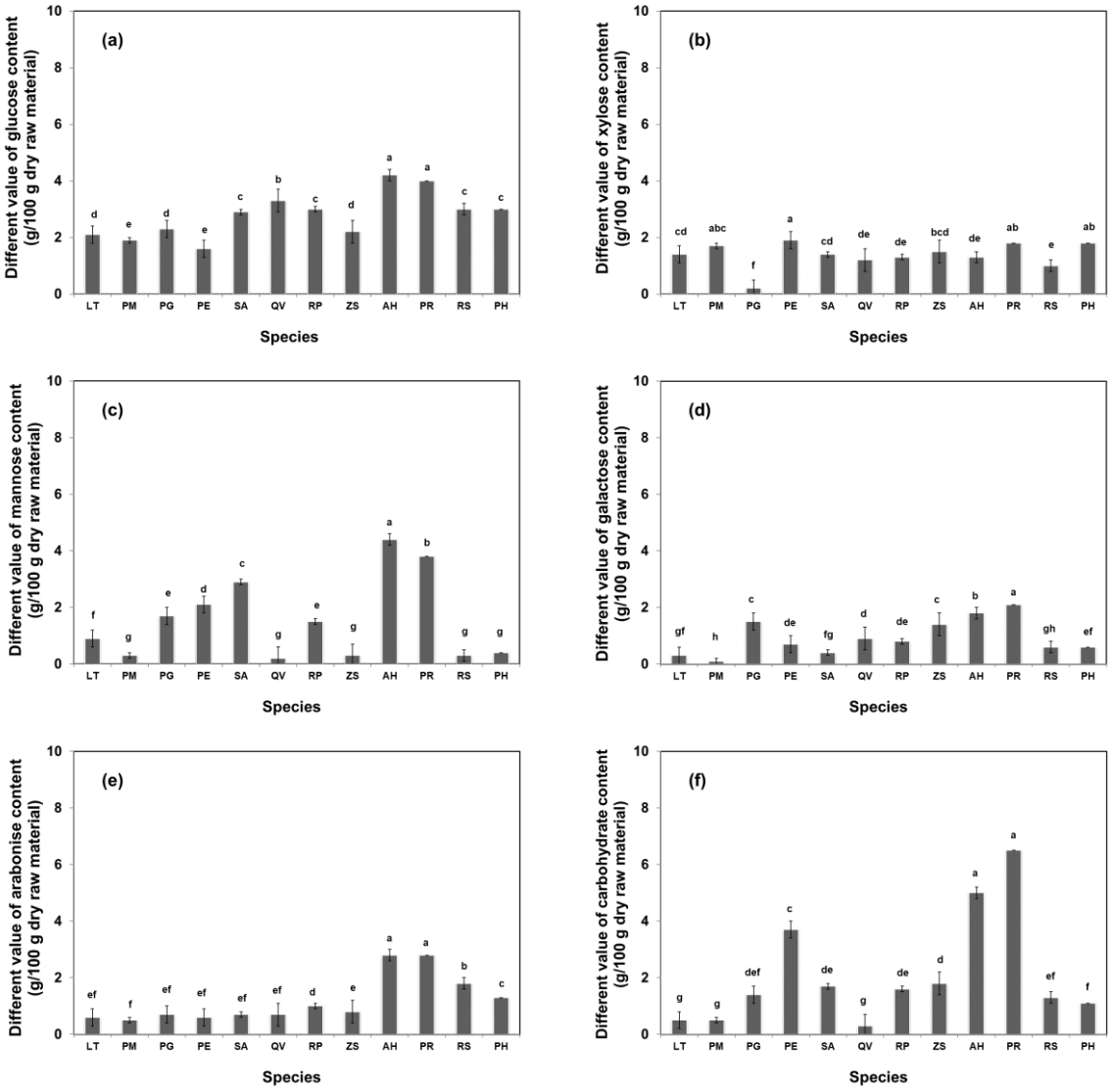

Fig. 5. Comparison of different values according to analysis methods in carbohydrate composition: (a) glucose, (b) xylose, (c) mannose, (d) mannose, (e) arabinose, and (f) total carbohydrate; Abbreviations: LT (*L. tulipifera*), PM (*P. nigra* × *P. maximowiczii*), PG (*P. alba* × *P. glandulosa*), PE (*P. euramericana*), SA (*S. alba*), QV (*Q. variabilis*), RP (*R. pseudoacacia*), ZS (*Z. serrata*), AH (*A. holophylla*), PR (*P. rigida*), RS (Rice straw), and PH (Peanut hull)

Accordingly, 432 monosaccharide content (glucose, xylose, mannose, galactose, and arabinose) reading data and analysis method were used for model checking (Fig. 6). The classification for other monosaccharides (glucose, xylose, mannose, and galactose) was not found using the decision tree model. The split divided the analysis method based on the arabinose content in their properties. Arabinose was the most important splitting variable in the carbohydrate analysis. In previous studies, lateral chains containing arabinose have been reported as the more sensible components to acid treatment, and there may be differences in accurate measurement depending on the selection of the analysis method (Theander and Westerlund 1986). In this analysis, a minor amount of sugar influenced the determination of the method. However, the selection of a determination method for each sample should be considered comprehensively in future studies.

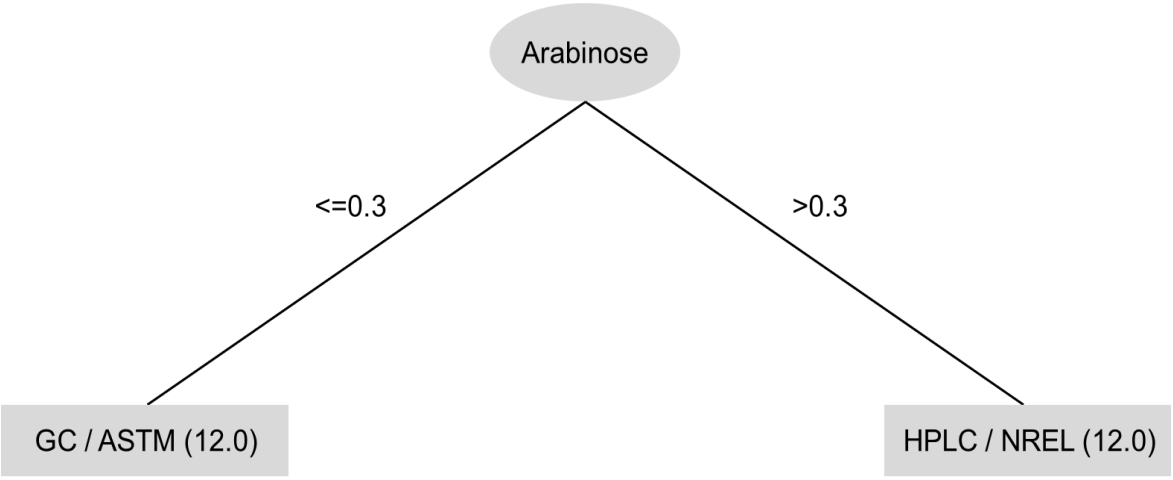

Fig. 6. Decision tree for the prediction of the analytical method for carbohydrate classification of lignocellulosic biomass. The predicted values are presented in the rectangles and ellipses. Numbers under the ellipses and rectangles are variances

CONCLUSIONS

In this study, analytical methods were compared to obtain reliable data for determining the carbohydrate composition of lignocellulosic biomass. The contents of carbohydrates, such as glucose, xylose, mannose, galactose, and arabinose, derived from both methods (HPLC and GC), were compared. To evaluate the difference between the mean values for each component, different values were calculated. In addition, factors influencing the choice of analysis were determined based on the decision tree statistical analysis.

1. The two major components of lignocellulosic biomass, glucose and xylose, showed similar results from both methods, whereas small amounts of mannose, galactose, and arabinose were significantly different. The cellulose (glucose) content was higher in HPLC analysis than in GC analysis, and hemicellulosic sugar contents (xylose, mannose, galactose, and arabinose) were higher in GC analysis than in HPLC analysis. There was a large difference in carbohydrate composition in the content of glucose, mannose, and arabinose of *Abies holophylla* and *Pinus rigida*, which are softwood species; thus, this parameter is affected by the species.
2. The classification for other sugars (glucose, xylose, mannose, and galactose) was not found using the results of the decision tree model. Arabinose was the most important splitting variable in the carbohydrate analysis.
3. For analysis in industries such as bioenergy, an analysis method that can be performed quickly and simply should be selected; however, in the medical and pharmaceutical fields, where trace components must be accurately measured, an analytical method that is more sensitive is desirable.

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APPENDIX

Table S1 compares the HPLC and the GC analysis methods generally used for carbohydrate analysis of lignocellulosic biomass. The HPLC analysis method and the GC analysis method have the same conversion of carbohydrates to monosaccharides through acid hydrolysis; however, there are significant differences in the pre-treatment method for instrumental analysis. The GC analysis method does not require neutralization of the sample; however, it requires an internal standard and an acetylation step of approximately 3 h compared to the HPLC analysis method. A relatively long pretreatment time was required. However, when analyzed by HPLC analysis, the analysis time was 35 min, and when analyzed by the GC analysis method, the analysis time was 14 min; thus, the HPLC analysis method required a relatively longer analysis time than the GC analysis method.

Table S1. Comparison of HPLC and GC Analysis Methods for Carbohydrate Content of Lignocellulosic Biomass

	HPLC	GC
Analysis Method	Sluiter *et al*. 2008a	ASTM E1821-96 (2011)
Acid Hydrolysis	O	O
Internal Standard	X	O
Neutralization	O	X
Acetylation	X	O
Injection Volume	10 to approx. 50 μL	2 to approx. 10 μL
Column	Aminex HPX-87P	DB-225 capillary
Column Temperature	80 to 85 °C	190 to 220 °C
Run Time	35 min	14 min