Cellulose
DOI 10.1007/s10570-015-0656-5

ORIGINAL PAPER

Lignin in plant biomasses: comparative metrological assessment of the detergent fiber and the insoluble dietary fiber methods

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Received: 21 October 2014 / Accepted: 13 May 2015
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Abstract The detergent fiber and the insoluble dietary fiber methods were compared to quantitate lignin in commelinid and non-commelinid magnoliophyta biomasses. This comparison was based on the precision of these methods and on the correlation between these methods. The present study showed that the insoluble dietary fiber method was more reliable to quantitate lignin because of its higher precision and smaller bias, as compared to the detergent fiber method. Nevertheless, the less tedious and resource consuming detergent fiber method can reliably be used to predict the results of the insoluble dietary fiber method with the correction factors determined in this paper. These correction factors of commelinid biomasses are distinctive of those of non-commelinid magnoliophyta biomasses. The lignin content should be corrected for protein-like compounds, otherwise lignin is significantly overestimated. Owing to these correction factors, the biofuel (e.g. cellulosic ethanol and biomethanation production), bio-based chemicals and feed sectors can use the detergent fiber method to rapidly and reliably estimate the available amounts of lignin of plant biomasses and rank them according to their suitability to be converted based on their lignin content.

Keywords Fibers · Van Soest · Klason · Correlation · Bioconversion

Abbreviations

ADF	Acid detergent fiber
ADL	Acid detergent lignin
asl	Above sea level
Cel.	Cellulose
CP	Corrected for protein-like compounds
DM	Dry matter
Hem.	Hemicelluloses
Lig.	Lignin
MRE	Mean standard residual error
NDF	Neutral detergent fiber
RPD	Ratio of the standard deviation to MRE
RSD	Relative standard deviation
RSDi	Intermediate precision RSD
RSDr	Repeatability RSD
SAH	Sulfuric acid hydrolysis
SD	Standard deviation
UCP	Uncorrected for protein-like compounds

Electronic supplementary material The online version of this article (doi:10.1007/s10570-015-0656-5) contains supplementary material, which is available to authorized users.

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Published online: 29 May 2015

UV Ultraviolet
VST Van Soest

Introduction

Lignin is the most important source of natural phenolic compounds (Carpita and McCann 2000). It is a complex three-dimensional phenylpropane macromolecule found in the cell walls of vascular plants. The core lignin is formed by the radical polymerization of three types of phenylpropane units: sinapyl, coniferyl and *p*-coumaryl alcohols (Sarkar et al. 2009; Frei 2013). Some phenolic acids such as ferulic and hydroxycinnamic acids can also be part of the lignin polymer and bind it to cell wall carbohydrates (hemicelluloses and pectins) (Ralph 2010). Lignin stiffens up the cell walls to provide to the plant structural and mechanical rigidity, and protect it from biotic and abiotic stresses (Carpita and McCann 2000). Therefore, lignin also forms an important barrier that prevents the enzymatic hydrolysis of cell wall carbohydrates (cellulose and hemicelluloses) in the rumen and in bioconversion processes such as cellulosic ethanol and biomethanation production. Enhanced rates of hydrolysis of plant biomasses require pretreatments to improve the enzyme accessibility to the cell wall polysaccharides (Frei 2013; Hayes 2013). Nevertheless, the key to have a cost-effective cellulosic ethanol biorefinery is also to recover the valuable lignin fraction. Lignin has a high heating value. It has been estimated that in a cellulosic ethanol biorefinery approximately 40 % of the lignin residue can be used to cover the energy needs of the process and the remaining 60 % of the lignin residue can be used to produce biofuels and bio-based chemicals (Hayes 2013). The quantitation of lignin is tedious because of its complex structure and bonds with the other cell wall components. Various methods have been developed for this purpose but each of them has its advantages and disadvantages (Hatfield and Fukushima 2005; Frei 2013). The detergent fiber (Van Soest 1973) and the insoluble dietary fiber (Theander et al. 1995) methods quantitate lignin gravimetrically after the extractions of non-structural compounds, hemicelluloses and cellulose. They are the most commonly used in the above context (Frei 2013).

The detergent fiber method fractionates the cell wall compounds into three residues: neutral detergent fibers (NDF containing mainly cellulose, hemicelluloses and lignin), acid detergent fibers (ADF containing mainly cellulose and lignin) and acid detergent lignin (ADL containing mainly lignin). The detergent fiber method estimates lignin as ADL but also cellulose as ADF–ADL and hemicelluloses as NDF–ADF (Godin et al. 2010). The neutral detergent fiber residue is obtained after extracting the biomass sample for 1 h with boiling aqueous neutral detergent of Van Soest (Van Soest and Wine 1967). The acid detergent fiber residue results from extracting the biomass sample for 1 h with boiling aqueous acid detergent containing 0.5 mol/L of sulfuric acid. The acid detergent lignin residue is obtained after an extraction by 12.2 mol/L of sulfuric acid at room temperature for 3 h from the acid detergent fiber residue (Van Soest 1973). The acid detergent lignin residue is considered as an underestimation of the lignin content because of the solubilization of some non-lignin phenolic compounds (ferulic and hydroxycinnamic acids) and of some lignin phenolic compounds (preferentially the sinapyl lignin) (Hatfield et al. 1994; Lowry et al. 1994; Hintz and Mertens 1996; Goff et al. 2012). This solubilization loss of some of the lignin phenolic compounds is induced by traces of the acid detergent (cetyltrimethylammonium bromide) in the acid detergent fiber residue during the extraction by 12.2 mol/L sulfuric acid (Hatfield et al. 1994; Lowry et al. 1994). The contamination of the acid detergent fiber residue by pectins can also be an issue in biomass samples with high pectins content. To avoid it as much as possible, an extraction with the neutral detergent extraction can be done prior to the acid detergent extraction (Mertens 2003; Cassida et al. 2007; Godin et al. 2011b).

The insoluble dietary fiber method begins with the extraction of the non-structural compounds. They are solubilized by one or more solvent (e.g. water, ethanol, neutral detergent, ether and/or hexane) extractions and/or enzymatic hydrolysis (e.g. amylase, amyloglucosidase and/or protease) of the biomass sample. The resulting residue is submitted to a two stage sulfuric acid hydrolysis with stage 1: solubilization 12.2 mol/L sulfuric acid at 30 °C for 1 h; and stage 2: hydrolysis 0.419 mol/L sulfuric acid at 121 °C for 1 h or 2 h. The liquid fraction is used for the quantitation of the released monosaccharides by chromatography. The

sum of these monosaccharides provides the content of the structural carbohydrates (cellulose and hemicelluloses). The insoluble residue corresponds to the insoluble Klason lignin (Theander et al. 1995; Godin et al. 2014). The insoluble Klason lignin is considered as an overestimation of the lignin content because it has a substantial higher content of protein-like compounds (Hatfield et al. 1994; Reeves 1997). During the acid hydrolysis, some non-lignin phenolic compounds (ferulic and hydroxycinnamic acids) are released (Hatfield et al. 1994; Goff et al. 2012). This fraction is called the acid soluble Klason lignin. It is measured by UV spectrophotometric absorption by (Hatfield and Fukushima 2005): the extinction coefficient of the acid soluble lignin which is specific to each type of biomass; the UV absorption at 320 nm to be at a specific wavelength of these soluble phenolic compounds without the inference of carbohydrate monomers (high UV absorption from 190 to 205 nm range), and of furfural and hydroxymethylfurfural (high UV absorption at 280 nm). These last molecules are the degradation products of carbohydrates under acid conditions.

The detergent fiber method is more commonly practiced, cheaper and faster but it is considered to substantially underestimate lignin compared to the insoluble dietary fiber method which substantially overestimates lignin (Theander and Westerlund 1993; Hatfield et al. 1994; Jung et al. 1997; Hindrichsen et al. 2006; Goff et al. 2012). The reasons for the underestimation by the detergent fiber method and the overestimation by the insoluble dietary method for the lignin quantitation are summarized in Table 1.

It is necessary to reliably quantitate lignin to improve the estimation of the available lignin resources and to enhance the assessment of its negative impacts on the enzymatic hydrolysis of the cell wall carbohydrates (cellulose and hemicelluloses) in the rumen and in bioconversion processes such as cellulosic ethanol and biomethanation production (Frei 2013; Hayes 2013). The aim of the present study is to assess which of the detergent fiber and insoluble dietary fiber methods give the most reliable result for the lignin content (corrected for protein-like compounds). Their precision was determined by their uncertainty (interval where the unknown true value can be observed with a confidence level of 68 %) and precision profile (interval where the unknown true value can be found with a confidence level of 95 %). Their bias was assessed by the mass balance of the neutral detergent fiber residue because it is a well standardized residue containing mainly cellulose, hemicelluloses and lignin, and minimal contents of non-structural carbohydrates (such as starch), pectins and organic nitrogenous compounds (such as proteins, protein-like compounds and tannins-proteins complexes) (Godin et al. 2011a, b). This study presents the first use of the precision profile for the cellulose, hemicelluloses and lignin content of the detergent fiber method and the lignin content of the insoluble dietary fiber method. The precision for the cellulose and hemicelluloses of the insoluble dietary fiber method was already determined for various plant biomasses by this mean (Godin et al. 2011a). They were consequently not assessed in the present paper.

Predicting the lignin content directly from the values of the relatively simple detergent fiber method would offer an interesting alternative to the more tedious and resource consuming insoluble dietary fiber method. Therefore, another purpose of this paper is to evaluate the reliability of such a prediction. This assessment was already realized for cellulose and hemicelluloses (Godin et al. 2014). It was consequently not assessed in the present paper.

Table 1 The causes of the bias in the lignin quantitation by the detergent fiber and insoluble dietary fiber methods

Acid detergent lignin	Underestimation of lignin	Solubilization of some non-lignin phenolic compounds (ferulic and hydroxycinnamic acids)	Hatfield et al. (1994); Lowry et al. (1994); Hintz and Mertens (1996); Goff et al. (2012)
		Solubilization of some lignin phenolic compounds (preferentially the sinapyl lignin)	Hatfield et al. (1994); Lowry et al. (1994)
Insoluble Klason lignin	Overestimation of lignin	Presence of protein-like compounds	Hatfield et al. (1994); Reeves (1997)

The investigated biomasses were commelinid (e.g. corn, fescue, miscanthus) and non-commelinid magnoliophyta (e.g. hemp, oak) plants. These types of biomasses were selected because they are the most commonly used in the above context.

Materials and methods

Biomass material

The analyzed samples of commelinid biomasses consisted of bamboo (*Phyllostachys vivax* Siebold and Zucc), cocksfoot (*Dactylis glomerata* L.), "cocksfoot-alfalfa" mixture (*Dactylis glomerata* L.–*Medicago sativa* L.), fiber corn (*Zea mays* L.), fiber sorghum [*Sorghum bicolor* (L.), Moench], immature rye (*Secale cereale* L.), immature spelt [*Triticum aestivum* L. ssp. *spelta* (L.) Thell.], miscanthus giganteus (*Miscanthus* × *giganteus* J.M. Greef and Deuter ex Hodk. and Renvoize), spelt grains [*Triticum aestivum* L. ssp. *spelta* (L.) Thell.], spelt straw [*Triticum aestivum* L. ssp. *spelta* (L.) Thell.], switchgrass (*Panicum virgatum* L.) and tall fescue (*Festuca arundinacea* Schreb.).

The analyzed samples of non-commelinid magnoliophyta consisted of aspen wood (*Populus* sp.), bean leaves and stalks (*Phaseolus vulgaris* L.), beech wood (*Fagus sylvatica* L.), bramble leaves and stalks (*Rubus fruticosus* L.), carrot leaves and stalks (*Daucus carota* L.), hemp (*Cannabis sativa* L.), Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decr.), Jerusalem artichoke leaves and stalks (*Helianthus tuberosus* L.), lupine leaves and stalks (*Lupinus albus* L.), nettle (*Urtica dioica* L.), oak wood (*Quercus* sp.), rapeseed straw (*Brassica napus* L.), reed (*Phragmites australis* (Cav.) Trin. ex Steud), sugar beet leaves and stalks (*Beta vulgaris* L.), sunflower leaves and stalks (*Helianthus annuus* L.), tagetes (*Tagetes patula* L.), tulip tree wood (*Liriodendron tulipifera* L.), willow wood (*Salix* sp.) and yucca leaves (*Yucca gloriosa* L.).

These samples came from crop trials performed in 2007, 2008, 2009, 2010 and/or 2011 at Libramont (Belgium) [498 m above sea level (asl); average annual temperature: 8.6 °C; average annual precipitation: 1260 mm; 49°55′N, 05°24′E] and at Gembloux (Belgium) (161 m asl; average annual temperature: 9.8 °C; average annual precipitation: 856 mm; 50°33′N, 04°43′E). A plot between 9 and 24 m^2 of the whole above ground biomass was harvested at 10 cm from the ground (with a Haldrup M-65 harvester) or manually harvested and then chopped (particle size 1–2 cm) for each biomass sample.

For each biomass, two subsamples of 750 g of the harvested crop trial plot were dried immediately after the harvest at 60 °C in forced circulation air oven for 72 h. After the drying process, the subsamples were milled first with a 4 mm screen BOA hammer mill (Waterleau, Herent, Belgium) and then with a 1 mm screen Cyclotec cyclone mill (FOSS, Hillerød, Denmark). For the storage of the samples, airtight bags were used. They were kept at room temperature and were protected from light in a dark box.

A subset of 6 biomasses (fiber corn, tall fescue, fiber hemp, miscanthus giganteus, aspen wood and pine wood) was used for the estimation of the precision (repeatability, intermediate precision and uncertainty) of the detergent fiber and insoluble dietary fiber methods. They were selected with the aim of covering a wide cellulose, hemicelluloses and lignin concentration range.

Chemical reagents and assays

All chemicals were of analytical grade or equivalent. Duplicate aliquots were measured on the 145 biomass samples, except for the estimation of the precision of the detergent fiber and insoluble dietary fiber methods. The information about this last assessment is detailed in the section corresponding to this point.

Detergent fiber method

The detergent fiber assay was based on the Van Soest (VST) method (Van Soest and Wine 1967; Van Soest 1973). Briefly, the neutral detergent fiber residue (NDF: weight of the neutral detergent fiber residue corrected for mineral components) was determined by the use of extraction 1: 0.1 mmol/L phosphate buffer at pH 7 for 15 min at 90 °C and the addition of an analytical thermostable α-amylase (Megazyme, Wicklow, Ireland) for samples which contained starch; and extraction 2: Van Soest neutral detergent at 100 °C for 1 h and the addition of sodium sulfite. The Van Soest neutral detergent solution is buffered at pH 7. It is composed of sodium lauryl sulfate, disodium ethylene-diaminetetraacetic dihydrate, disodium borate

decahydrate, disodium hydrogen phosphate anhydrous and 2-ethoxy-ethanol (Van Soest and Wine 1967).

The acid detergent fiber residue (ADF: weight of the acid detergent fiber residue corrected for mineral components) was determined by a two successive extractions. The first extraction is done with the Van Soest neutral detergent, as described above, without the addition of sodium to the Van Soest neutral detergent. Then, it is followed by the extraction with the Van Soest acid detergent at 100 °C for 1 h. The Van Soest neutral detergent solution is composed of cetyltrimethylammonium bromide and sulfuric acid 0.5 mol/L (Van Soest 1973). The acid detergent lignin residue (ADL: weight of the acid detergent lignin residue corrected for mineral components) was determined from the acid detergent fiber residue by extracting it by sulfuric acid 12.2 mol/L at room temperature for 3 h (Van Soest 1973). Sodium sulfite is added to the Van Soest neutral detergent extraction and is not added to the Van Soest neutral detergent extraction prior to the ADF extractions because it is a standard recommendation (Hintz and Mertens 1996). This addition of sodium sulfite generally improves the reproducibility, filtration step and standardization of the organic nitrogenous compounds solubilization (such as proteins, protein-like compounds and tannins-proteins complexes) for the Van Soest neutral detergent extraction. However, it doesn't enable to improve those aspects for the Van Soest acid detergent extraction (Hintz and Mertens 1996). Nevertheless, it also induces the loss of some non-lignin phenolic compounds (such as tannins, tannins-proteins complexes, and ferulic and hydroxycinnamic acids), it may induce the loss of some other not well identified compounds during the ADF extraction as well as during the NDF extraction (Hintz and Mertens 1996; Goff et al. 2012). The cellulose VST, hemicelluloses VST and lignin VST contents are calculated as ADF–ADL, NDF–ADF, and ADL, respectively (Godin et al. 2011a). The term "detergent fiber method" will be used to refer to data generated by the Van Soest method (NDF, ADF, ADL and their combinations).

Insoluble dietary fiber method

The insoluble dietary fiber assay was based on the sulfuric acid hydrolysis (SAH) method of Godin et al. (2011a, 2014). Briefly, the samples were first fractionated by Van Soest neutral detergent extractions, as described above. The Van Soest neutral detergent is used because it enables for the further analyses to have a well standardized residue containing mainly cellulose, hemicelluloses and lignin, and minimal contents of non-structural carbohydrates (such as starch), pectins and organic nitrogenous compounds (such as proteins, protein-like compounds and tannins-proteins complexes). The xylan, arabinan, galactan, mannan, total glucan and insoluble Klason lignin contents of the neutral detergent fiber residue left after these extractions were determined by a two stage sulfuric acid hydrolysis method, with stage 1: solubilization by 12.2 mol/L sulfuric acid at 30 °C for 1 h; and stage 2: hydrolysis by 0.419 mol/L sulfuric acid at 121 °C for 2 h. The released monosaccharides were analyzed by liquid chromatography. The hemicellulosic glucan content was determined by the same sulfuric acid hydrolysis method except that the cellulose solubilization step (incubation with 12.2 mol/L sulfuric acid at 30 °C for 1 h) was omitted. The insoluble residue of the two stage sulfuric acid hydrolysis corresponds to the insoluble Klason lignin content (corrected for mineral components). This residue was dried at 103 °C overnight. The cellulose SAH (cellulosic glucan; i.e. D-glucose of cellulose under its polymeric form) content was calculated as the difference between the total glucan and the hemicellulosic glucan contents. The hemicelluloses SAH content was calculated as the sum of the xylan, arabinan, galactan, mannan and hemicellulosic glucan contents. The monosaccharidic components (D-xylose, L-arabinose, D-glucose, D-mannose and D-galactose) of hemicelluloses are expressed under their polymeric form (xylan, arabinan, hemicellulosic glucan, mannan and galactan). The term "insoluble dietary fiber method" will be used to refer to data generated by the sulfuric acid hydrolysis method (cellulose SAH, hemicelluloses SAH, insoluble Klason lignin and their combinations).

Protein-like compounds in the lignin residues

The nitrogen content of the lignin residues was based on the nitrogen combustion (Dumas) method (Bicsak 1993) where at final stage only nitrogen was measured under its gaseous N_2 form. The protein-like compounds content of the lignin residues were quantitated by using 4.6 as the correction factor of nitrogen to protein-like compounds, as recommended for plant

biomasses (Hames et al. 2008; Templeton et al. 2009; Sluiter and Wolfrum 2013).

Precision assessment

The precision (repeatability, intermediate precision, uncertainty and precision profile) assessment of the detergent fiber and insoluble dietary fiber methods was based on applying ISO 5725 method. This assessment was used to determine the dispersion of the results for each component. It was assessed for cellulose VST, hemicelluloses VST, lignin VST and insoluble Klason lignin. The precision for cellulose SAH and hemicelluloses SAH was already assessed with this method by Godin et al. (2011a).

The estimation of the uncertainty can be summarized as follows (Godin et al. 2011a):

- Step 1: k concentration levels were selected in order to have a wide concentration range. Six biomass samples (fiber corn, tall fescue, miscanthus giganteus, fiber hemp, aspen wood and pine wood) were selected for their distinctive contents of cellulose, hemicelluloses and lignin.
- Step 2: The experimental design was made of 5 series ($p = 5$), 3 replicates ($n = 3$) per series and of 6 concentration levels ($k = 6$).
- Step 3: For each k concentration level the mean ($\bar{x}_k$), the repeatability RSD (RSDr), the intermediate precision RSD (RSDi), the expansion factor of the variability of the mean (ks), the relative uncertainty, the relative expanded uncertainty (with a coverage factor according to ISO 5725; ke $= 2$) were determined.
- Step 4: Interpretation and conclusions on the uncertainty of the method.

Detailed information about the methodology of this uncertainty determination can be found in Godin et al. (2011a).

The precision profile determination can be summarized as follows (Godin et al. 2011a):

- Step 1: The relative acceptance limits (noted $\pm$ λ) of each component were arbitrarily defined based on previous studies (Theander et al. 1995; Mertens 2002; Möller 2009) and on control charts of our laboratory. The arbitrarily defined limits are ± 5 % for cellulose VST, $\pm$ 10 % for hemicelluloses VST, $\pm$ 15 % for lignin VST and $\pm$ 10 % for insoluble Klason lignin.
- Step 2: k concentration levels were selected in order to have a wide concentration range. Six biomass samples (fiber corn, tall fescue, miscanthus giganteus, fiber hemp, aspen wood and pine wood) were selected for their distinctive contents of cellulose, hemicelluloses and lignin.
- Step 3: For each k concentration level the mean ($\bar{x}_k$), the repeatability, the intermediate precision and the expansion factor of the variability of the mean (ks) were determined to calculate the β expectation at 95 % tolerance limits.
- Step 4: For each k concentration level, the absolute β expectation at 95 % tolerance limits (TL), the absolute acceptance limits and the relative β expectation at 95 % tolerance limits were determined.
- Step 5: The precision profile for each k concentration level was built with the β expectation at 95 % tolerance limits of the observed values and the acceptance limits.
- Step 6: Interpretation and conclusions on the precision profile of the method.

Detailed information about the methodology of this precision profile determination can be found in Godin et al. (2011a).

General statistical assessment

The dataset was divided into two phylogenetic subsets, the commelinid (bamboo, cocksfoot, fiber corn, fiber sorghum, immature rye, miscanthus giganteus, reed, spelt, switchgrass and tall fescue) and the non-commelinid magnoliophyta (the other analyzed biomasses such as hemp, Jerusalem artichoke leaves and stalks, lupine leaves and stalks, nettle and aspen wood) biomasses, because their cell wall components are known to be significantly different (Carpita and McCann 2000; Godin et al. 2013b, 2014).

The linear orthogonal models (Deming regression with a variance error ratio of 1.4) of the lignin content (corrected for protein-like compounds and mineral components) between the detergent fiber and the insoluble dietary fiber methods were performed using JMP 11 (SAS Institute, Cary, USA). These linear orthogonal models were used to determine the

Table 2 Composition of plant biomasses analyzed by the detergent fiber and insoluble dietary fiber methods

	Commelinid			Non-commelinid magnoliophyta		Pinophyta
	Fiber corn	Tall fescue	Miscanthus giganteus	Fiber hemp	Aspen wood	Pine wood
Detergent fiber method						
Cellulose VS	20.1 ± 0.3	31.8 ± 0.3	51.1 ± 0.6	51.4 ± 1.2	64.0 ± 1.4	50.2 ± 0.9
Hemicelluloses VS	17.8 ± 0.8	26.8 ± 0.8	25.5 ± 1.0	13.3 ± 1.0	12.1 ± 1.1	11.6 ± 0.8
Lignin VST CP	2.00 ± 0.20	2.15 ± 0.22	10.6 ± 1.0	7.67 ± 0.51	15.1 ± 1.4	26.3 ± 1.0
Insoluble dietary fiber method						
Cellulose SAH*	18.2 ± 0.2	27.7 ± 0.8	46.9 ± 1.2	43.4 ± 2.0	51.4 ± 0.4	43.0 ± 1.4
Hemicelluloses SAH*	16.5 ± 0.2	23.3 ± 0.2	26.5 ± 1.0	17.3 ± 0.2	19.8 ± 0.4	23.7 ± 1.0
Insoluble Klason lignin CP	3.39 ± 0.22	7.35 ± 0.37	16.6 ± 1.0	12.3 ± 0.6	22.1 ± 1.5	28.8 ± 1.6

Mean values (g/100 g DM) and their expanded uncertainty of the precision estimation

SAH sulfuric acid hydrolysis; *CP* corrected for protein-like compounds; *VST* Van Soest

* Uncertainty based on the duplicate aliquot analysis

relationship between these methods. The linear least square models of the lignin content between the lignin residues corrected (CP) and uncorrected (UCP) of their protein-like compounds content were performed using JMP. These linear least square models were used to assess the prediction of the lignin residues corrected (CP) of their protein-like compounds content by the lignin residues uncorrected (UCP) of their protein-like compounds content. To evaluate the reliability of the linear models, the following parameters were determined: the coefficient of determination (R^2); the mean standard residual error (MRE); the ratio of the standard deviation to MRE (RPD). The following guideline was suggested for these parameters by Malley et al. (2005): excellent, $R^2 \geq 0.95$ and RPD ≥ 4.0; successful, $R^2 \geq 0.90$ and RPD ≥ 3.0; moderately successful, $R^2 \geq 0.80$ and RPD ≥ 2.3; moderately useful (for semi-quantitative screening purpose), $R^2 \geq 0.70$ and RPD ≥ 1.8. These parameters have been determined by Malley et al. (2005) for linear least square models. In the present study, they were also used for linear orthogonal models.

The mean comparison tests (with $\alpha = 0.05$) were based on the Student's *t* test. The data were paired for the comparison between the detergent fiber and the insoluble dietary fiber methods. The Student's *t* tests were performed using JMP 11 (SAS Institute, Cary, USA). For the mean comparison tests, the error bars correspond to the confidence interval of the mean. This statistical test was used to assess if there was a significant difference between these methods.

Results and discussions

Precision of the detergent fiber and insoluble dietary fiber methods

The selected commelinid (fiber corn, tall fescue and miscanthus giganteus) and non-commelinid magnoliophyta (fiber hemp and aspen wood) and pinophyta (pine wood) biomasses were analyzed for their content in cellulose, hemicelluloses and lignin. Table 2 summarizes the composition obtained for these phylogenetic types of biomasses by the detergent fiber and insoluble dietary fiber methods. These analyzed commelinid magnoliophyta biomasses generally have lower cellulose and lignin contents, and higher hemicelluloses content with both methods, as compared to these analyzed non-commelinid magnoliophyta and pinophyta biomasses (Table 2). Such differences are consistent with those of the cell walls of these different phylogenetic types of biomasses (Carpita and McCann 2000) and with those previously described in the literature (Godin et al. 2013a, b, c, 2014). For the biomasses of the analyzed phylogenetic types, the cellulose content is overestimated, and the hemicelluloses and lignin content are generally underestimated with the detergent fiber method, as compared to the insoluble dietary fiber method; except in the case of hemicelluloses in commelinid biomasses (Table 2). Such differences are consistent with those previously described in the literature (Hindrichsen et al. 2006; Godin et al. 2011a, 2014). They can be explained by the following facts: (1) for cellulose and

hemicelluloses quantitation, the insoluble dietary fiber method is based on their specific monosaccharidic contents while the detergent fiber method is affected by the contamination of undesired components present in the residues (Hintz and Mertens 1996; Cassida et al. 2007; Godin et al. 2011a, 2014); (2) the presence of some proteins, protein-like compounds, tannins, hemicelluloses and/or pectins in the acid detergent fiber residue which affects substantially more the non-commelinid magnoliophyta and pinophyta biomasses, as compared to the commelinid biomasses (Morrison 1980; Hintz and Mertens 1996; Cassida et al. 2007); (3) the absence of some lignin phenolic compounds (preferentially the sinapyl lignin) in the acid detergent lignin residue that is due to traces of the acid detergent (cetyltrimethylammonium bromide) (Hatfield et al. 1994; Lowry et al. 1994; Hintz and Mertens 1996; Goff et al. 2012); (4) the poorer organic nitrogenous compounds (such as proteins, protein-like compounds and tannins-proteins complexes) solubilization of the insoluble dietary fiber method, as compared to the detergent fiber method (Hatfield et al. 1994; Hintz and Mertens 1996; Reeves 1997).

The precision (repeatability, intermediate precision and uncertainty) of the detergent fiber and insoluble dietary fiber methods for cellulose, hemicelluloses and lignin quantitation of the six selected plant biomasses (concentration levels) are shown in Table 3, and in Fig. 1. The precision was assessed first based on the RSDr, RSDi and relative uncertainty (Table 3) for the analyzed components. For the precision of the cellulose VST, the highest values of the RSDr, RSDi and relative uncertainty were of 1.0 % for the RSDr, of 1.1 % for the RSDi and of 1.2 % for the relative uncertainty (Table 3). These precision values are approximately 2–3 times better, as compared to the precision of the cellulose SAH (Godin et al. 2011a). For the precision of the hemicelluloses VST, the highest values of the RSDr, RSDi and relative uncertainty were of 3.9 % for the RSDr, of 4.3 % for the RSDi and of 4.5 % for the relative uncertainty (Table 3). These precision values tend to be slightly less performant than the precision of the hemicelluloses SAH (Godin et al. 2011a). For the precision of the lignin VST, the highest values of the RSDr, RSDi and relative uncertainty were of 4.9 % for the RSDr, of 5.0 % for the RSDi and of 5.1 % for the relative uncertainty; whereas the insoluble Klason lignin has a better precision with the RSDr, RSDi and relative uncertainty values of 3.1 % for the RSDr, of 3.3 % for the RSDi and of 3.5 % for the relative uncertainty (Table 3). The higher precision of cellulose VST, as compared to hemicelluloses VST and also the other components, can be explained by the fact that estimation of cellulose VST (ADF–ADL) is based on the difference between two variables (ADF and ADL) measured on the same sub-samples (measurement in sequence; variables are dependent); while the estimation of hemicelluloses VST (NDF–ADF) is based on the difference between two variables (ADF and NDF) measured on different sub-samples (measurement in parallel; variables are independent). The relative reproducibility (RSDr) and relative repeatability (RSDi) tend generally to be similar for the assessed gravimetric quantitations. This means that this reproducibility mainly depends on the variability within a day (series) and not a lot on the variability between days (series). Therefore, the duplicate aliquot analysis of lignin for these gravimetric quantitations could be done on the same day (series) in the future.

Secondly, the precision profile of the analyzed components was used to compare them more globally (Fig. 1). All the precision profiles were acceptable. Indeed, the β-expectation tolerance limits of each profile fall within the acceptance limits. These acceptance limits were defined arbitrarily, as described in the materials and methods part. Therefore, at least 95 % of the results are expected to fall within the acceptance limits. Based on the precision profiles, it appears that the quantitation of the cellulose VST has the highest precision level (at least 95 % of the results are expected at ±5 % of the expected value) (Fig. 1), as previously explained. It is followed by the cellulose SAH (Godin et al. 2011a), hemicelluloses SAH (Godin et al. 2011a), hemicelluloses VST (Fig. 1) and the insoluble Klason lignin (Fig. 1) which have the same slightly lower precision level (at least 95 % of the results are expected at ±10 % of the expected value). The quantitation of lignin VST has the lowest precision level (at least 95 % of the results are expected at ±15 % of the expected value) (Fig. 1). It is due to the two lowest levels of concentration which are also the lowest levels of concentration of all the assessed data. For these two levels of concentration, the RSDr and RSDi have high values. It can be explained by the RSDr and RSDi that increase with decreasing level of concentrations. It is known as the

Table 3 Precision (repeatability, intermediate precision and uncertainty) of the detergent fiber and insoluble dietary fiber methods for cellulose, hemicelluloses and lignin corrected for protein-like compounds quantitation, based on 15 experimental results for each value

	Commelinid				Non-commelinid magnoliophyta		Pinophyta
	Fiber corn	Tall fescue	Miscanthus giganteus	Fiber sorghum	Fiber hemp	Aspen wood	Pine wood
Cel.							
Detergent fiber method							
Repeatability RSD (RSDr), %	0.78	0.60	0.37	/	0.66	1.0	0.90
Intermediate precision RSD (RSDi) (%)	0.78	0.60	0.44	/	1.1	1.1	0.90
Relative uncertainty (%)	0.80	0.62	0.46	/	1.2	1.1	0.93
Insoluble dietary fiber method							
Repeatability RSD (RSDr) (%)	/	1.9*	/	2.0*	2.1*	/	/
Intermediate precision RSD (RSDi) (%)	/	3.1*	/	3.1*	2.5*	/	/
Relative uncertainty (%)	/	3.3*	/	3.3*	2.6*	/	/
Hem.							
Detergent fiber method							
Repeatability RSD (RSDr) (%)	2.3	1.1	1.3	/	3.6	3.9	3.4
Intermediate precision RSD (RSDi) (%)	2.3	1.8	1.4	/	3.7	4.3	3.4
Relative uncertainty (%)	2.4	1.9	1.4	/	3.8	4.5	3.5
Insoluble dietary fiber method							
Repeatability RSD (RSDr) (%)	/	2.7*	/	1.8*	1.6*	/	/
Intermediate precision RSD (RSDi) (%)	/	2.7*	/	2.6*	1.6*	/	/
Relative uncertainty (%)	/	2.8*	/	2.8*	1.7*	/	/
Lig.							
Detergent fiber method							
Repeatability RSD (RSDr) (%)	3.8	3.5	4.9	/	3.2	4.4	1.9
Intermediate precision RSD (RSDi) (%)	4.8	4.4	5.0	/	3.2	4.4	1.9
Relative uncertainty (%)	5.1	4.6	5.1	/	3.3	4.6	1.9
Insoluble dietary fiber method							
Repeatability RSD (RSDr) (%)	3.1	2.2	2.4	/	1.0	2.6	2.3
Intermediate precision RSD (RSDi) (%)	3.1	2.9	2.4	/	2.3	3.3	2.6
Relative uncertainty (%)	3.2	3.1	2.5	/	2.5	3.5	2.7

Cel. cellulose; *Hem.* hemicelluloses; *Lig.* lignin

* Values from Godin et al. (2011a)

Horwitz curve (Boyer et al. 1985; Godin et al. 2011a). For the precision assessment, the conclusion is that the detergent fiber and insoluble dietary fiber methods have generally a similar level of precision (at least 95 % of the results are expected at ±10 % of the expected value), except that the precision of cellulose VST and lignin VST are slightly higher and lower, respectively.

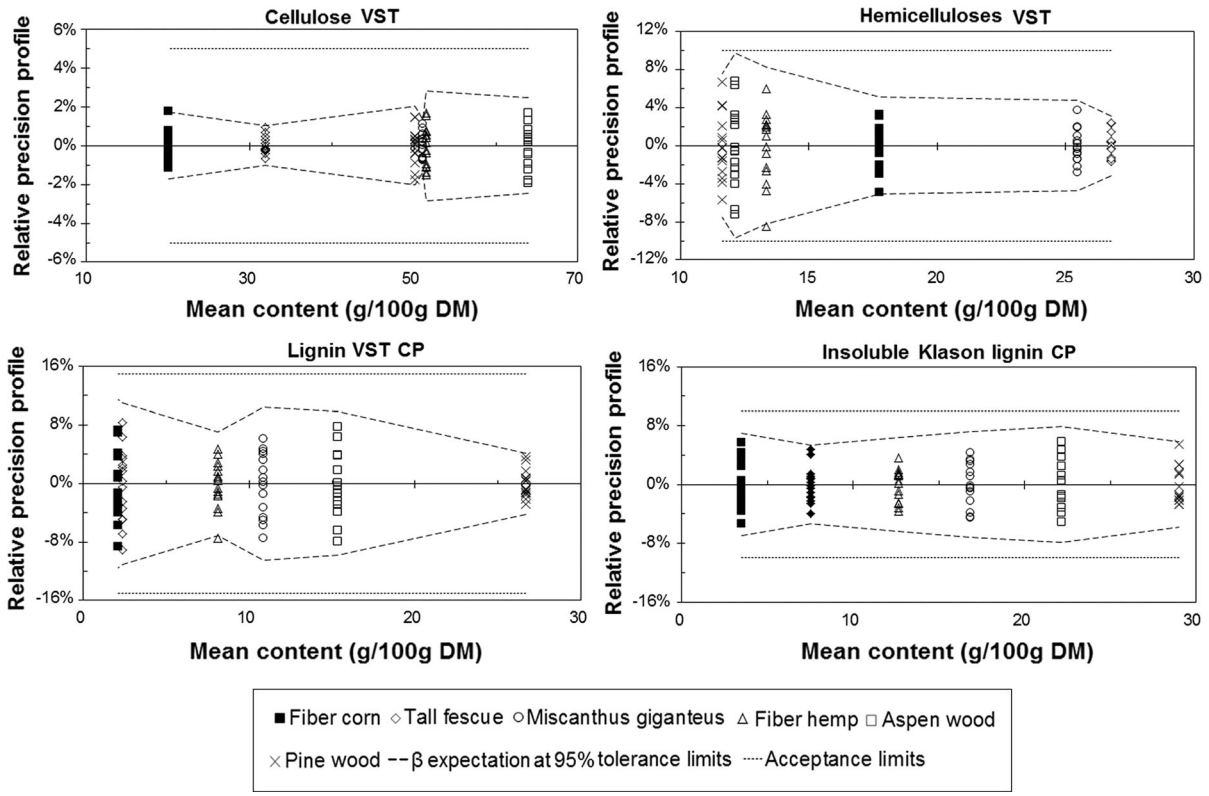

Fig. 1 Relative precision profile of the detergent fiber method for cellulose VST, hemicelluloses VST and lignin VST corrected for protein-like compounds (CP), and of the insoluble dietary fiber method for insoluble Klason lignin corrected for protein-like compounds (CP), based on 90 experimental results (15 analyses * 6 biomasses) for each component. *VST* Van Soest

Correlation between the lignin quantitation of the detergent fiber and insoluble dietary fiber methods

The linear orthogonal model of the lignin quantitation (corrected for protein-like compounds) between the detergent fiber and the insoluble dietary fiber methods are shown in Fig. 2. Table 4 summarizes the lignin quantitations by these methods. The analyzed samples were commelinid (bamboo, cocksfoot, fiber corn, fiber sorghum, hemp, immature rye, miscanthus giganteus, reed, spelt, switchgrass and tall fescue) and non-commelinid magnoliophyta (the other analyzed biomasses such as hemp, Jerusalem artichoke leaves and stalks, lupine leaves and stalks, nettle and aspen wood) biomasses. The bias of the lignin quantitation between these two methods and types of biomasses can be estimated by the slope of these linear model lines. These slopes show that the detergent fiber method substantially underestimates the lignin content for both types of biomasses, as compared to the insoluble dietary fiber method (Fig. 2). For both type of biomasses, the difference for the lignin content between these two methods is statistically significant (p value < 0.001). This underestimation for the detergent fiber method can be explained by the absence of some lignin phenolic compounds in the acid detergent lignin residue. These lignin phenolic compounds (preferentially the sinapyl lignin) are solubilized due to trace of acid detergent (cetyltrimethylammonium bromide) in the acid detergent fiber residue during the extraction by 12.2 mol/L sulfuric acid (Hatfield et al. 1994; Lowry et al. 1994). These slopes of the linear model lines also show that the underestimation of the lignin content by the detergent fiber method is higher in commelinid biomasses, as compared to non-commelinid magnoliophyta biomasses (Fig. 2). For both methods, the difference for the lignin content between these two types of biomasses is statically significant (p value < 0.001). This underestimation for the

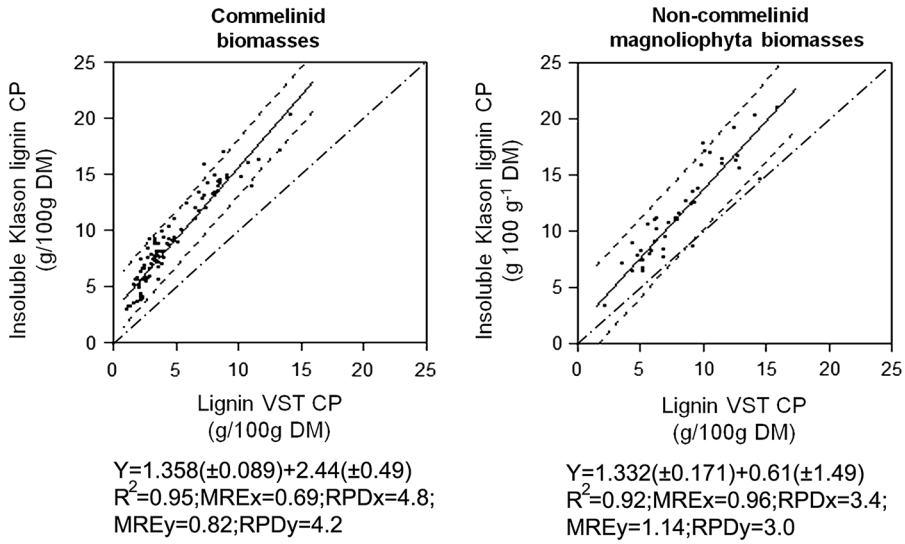

Fig. 2 Relationship between the detergent fiber and the insoluble dietary fiber methods for the content of lignin corrected for protein-like compounds (CP) in commelinid (n = 101) (*left*) and in non-commelinid magnoliophyta (n = 44) (*right*) biomasses. The *black dashed-dotted line* is the line of equality (y = x). The *black plain line* is the linear orthogonal model line. The *black lines with smaller dashes* in each plot are the 95 % confidence lines of the linear orthogonal model line. The *numbers in the brackets* of the linear orthogonal model equation correspond to the confidence interval of the mean. *VST* Van Soest

Table 4 Lignin contents for the linear models between the detergent fiber and insoluble dietary fiber methods for commelinid (n = 101) and non-commelinid magnoliophyta (n = 44) biomasses

	Mean ± SD (g/100 g DM)	Minimum (g/100 g DM)	Maximum (g/100 g DM)
Commelinid biomasses			
Lignin VST CP	4.78 ± 2.90	1.09	14.20
Lignin VST UCP	5.04 ± 2.94	1.13	14.71
Insoluble Klason lignin CP	8.94 ± 3.91	2.84	20.10
Insoluble Klason lignin UCP	9.19 ± 3.95	3.03	20.59
Non-commelinid magnoliophyta biomasses			
Lignin VST CP	8.17 ± 3.24	2.14	15.93
Lignin VST UCP	8.76 ± 3.20	2.35	16.19
Insoluble Klason lignin CP	11.49 ± 4.28	3.17	20.81
Insoluble Klason lignin UCP	11.97 ± 4.16	3.42	20.91

CP corrected for protein-like compounds; *UCP* uncorrected for protein-like compounds; *VST* Van Soest

commelinid biomasses can be explained by the solubilization of more non-lignin phenolic compounds (ferulic and hydroxycinnamic acids) during the acid hydrolysis (Hatfield et al. 1994; Goff et al. 2012). This solubilization has a stronger impact on the cell walls of commelinid biomasses because of the higher content for these compounds, as compared to non-commelinid magnoliophyta biomasses (Hatfield et al. 1994; Lowry et al. 1994; Hintz and Mertens 1996; Goff et al. 2012).

Another significant difference between the linear model lines of these two types of biomasses is the intercept. It is significantly different of zero for commelinid biomasses (p value < 0.001) and is not significantly different of zero for non-commelinid magnoliophyta biomasses (p value > 0.05) (Fig. 2). This can be explained by the difference of the cell walls of these two different phylogenetic types of biomasses. Commelinid biomasses have a higher content of non-

lignin phenolic, as compared to non-commelinid magnoliophyta biomasses. Therefore the impact of the solubilization of these compounds during acid hydrolysis is more important for cell walls of commelinid biomasses (Hatfield et al. 1994; Lowry et al. 1994; Hintz and Mertens 1996; Goff et al. 2012). The linear orthogonal models of the lignin quantitation (corrected for protein-like compounds) of commelinid and of non-commelinid magnoliophyta biomasses between the two assessed methods have good prediction performances owing to their good R^2 and RPD (values of $R^2 \geq 0.90$ and RPD ≥ 3.0) (Fig. 2). Therefore, these relationships are reliable enough to predict quantitatively the values of the more tedious and time consuming insoluble dietary fiber method by the relatively simple detergent fiber method. These relationships between the detergent fiber method and the insoluble dietary fiber method can be used in addition to those determined for cellulose and hemicelluloses by Godin et al. (2014).

Protein-like compounds in the lignin residues for the detergent fiber and insoluble dietary fiber methods

The protein-like compounds content of lignin in the lignin residue and in the whole dry matter are shown in Fig. 3. The lignin residue of non-commelinid magnoliophyta biomasses has substantial higher content of protein-like compounds, as compared to commelinid biomasses. For both methods, the difference between these two types of biomasses is statistically significant (p value < 0.05 when it is expressed relatively to lignin residue; p value < 0.001 when it is expressed relatively to the whole biomass). The higher protein-like compounds content of lignin in non-commelinid magnoliophyta biomasses can be explained by their higher contents of cell wall proteins and tannins (which form more tannins-proteins complexes), as compared to commelinid magnoliophyta biomasses (Reeves 1997). For both types of biomasses, the lignin residue of the detergent fiber method has a significantly (p value < 0.001) higher protein-like compounds content compared to the insoluble dietary fiber method (Fig. 3). It can be explained by the lower level of lignin concentration in the case of the detergent fiber method, as compared to the insoluble dietary fiber method. Indeed, the protein-like compounds content of the lignin residue is similar for both methods when it is expressed relatively to the whole biomass (Fig. 3). For both type of biomasses, the difference for this protein-like compounds content of the lignin residue between these two methods is not statistically significant (p value ≥ 0.05). This means that, for each type of biomasses, the protein-like compounds content of the lignin residue is quite constant, and is independent of the plant species and of the both used methods. Therefore, the residual protein-like compounds must be cell wall proteins which are strongly associated to lignin. However, the nitrogen of the lignin residue is probably not all coming from true proteins. It is considered that there are other potential sources of nitrogen such as tannins-proteins complexes, Maillards reaction artifacts, proteins artifacts, nucleobases artifacts and nitrogen from lignin (Melillo et al. 1982; Hintz and Mertens 1996). This quite constant nitrogen

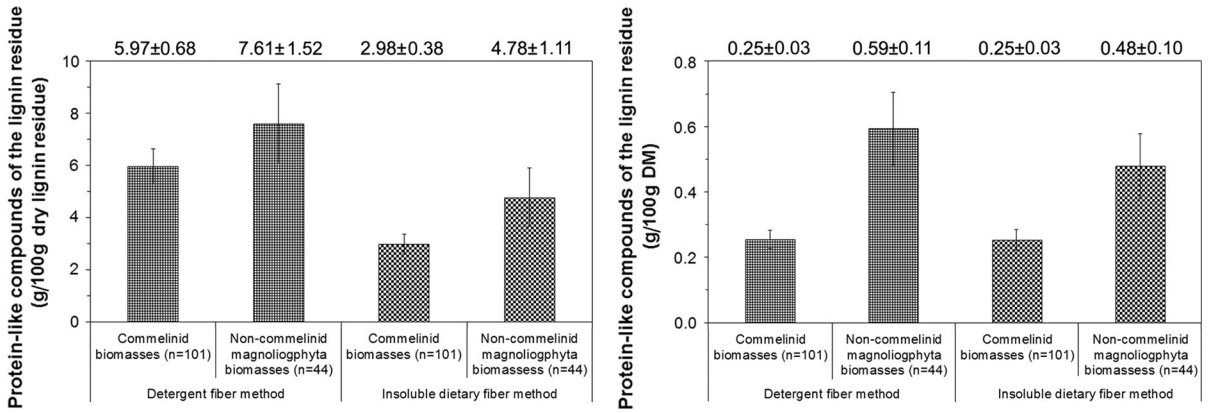

Fig. 3 Protein-like compounds content (mean ± confidence interval of the mean) of lignin in the lignin residue (*left*) and in the whole dry matter (*right*). *Error bars* correspond to confidence interval of the mean, n to the number of analyzed samples

content of the lignin residue for both methods and the high organic nitrogenous compounds (such as proteins, protein-like compounds and tannins-proteins complexes) solubilization of our version of the insoluble dietary fiber method can be explained by the use of the Van Soest neutral detergent in both methods. This enables for the further analyses to a well standardized residue containing mainly cellulose, hemicelluloses and lignin and minimal contents of non-structural carbohydrates (such as starch), pectins and organic nitrogenous compounds (such as proteins, protein-like compounds and tannins-proteins complexes) (Godin et al. 2011a, b). Indeed, the use of Van Soest neutral detergent enables the solubilization of an extra amount of these organic nitrogenous compounds (Hintz and Mertens 1996; Goff et al. 2012). Further, the Van Soest neutral detergent is not usually used in the insoluble dietary fiber method. Therefore, the lignin residue of the insoluble dietary fiber method has typically a higher content of protein-like compounds compared to the one of the detergent fiber method (Hatfield et al. 1994).

The lignin residue has to be corrected by its protein-like compounds content because it is statistically significant (p value < 0.001) for each method and each type of biomass. It would be interesting to find a way to correct for the protein-like compounds content of the lignin residue without having to quantitate it. This would make the quantitation of lignin (corrected for protein-like compounds) less tedious and time-consuming. There are significant correlations between the total protein-like compounds content and the protein-like compounds content of the lignin residue (data not shown). However, these correlations are not high enough to build reliable prediction models (the coefficients of correlation are generally around 0.6) (data not shown). This can be explained by the fact that probably not all the nitrogen of the lignin residue is coming from true proteins, as previously mentioned. Nevertheless, the lignin content corrected for protein-like compounds can be predicted quantitatively by the lignin content uncorrected for protein-like compounds (Table 5). In spite of the wide diversity of biomasses tested, the relationships between these two parameters have excellent prediction performances owing to their excellent R^2 and RPD (values of $R^2 \geq 0.95$ and of RPD ≥ 4.0) (Table 5). Therefore, they can be used to easily and rapidly estimate quantitatively lignin corrected for protein-like compounds of a sample based on its value without the protein-like compounds correction.

Comparison of the bias of lignin quantitation between the detergent fiber and insoluble dietary fiber methods

A reliable estimation of the lignin content requires the smallest possible bias and the highest precision. The precision of the lignin quantitation by the detergent fiber and insoluble dietary fiber methods has already been determined. It has shown that the insoluble dietary fiber method quantitates lignin with more precision. The bias is normally assessed from the quantitation of the pure compound. There is no native lignin standard for such a quantitation because of its complexity and imbrication in the cell walls. Thus, the bias of the lignin quantitation has been evaluated by the mass balance of the neutral detergent fiber residue because it is a well standardized residue containing mainly cellulose, hemicelluloses and lignin, and minimal contents of non-structural carbohydrates (such as starch), pectins and organic nitrogenous compounds (such as proteins, protein-like compounds and tannins-proteins complexes) (Godin et al. 2011a, b). This mass balance corresponds to the sum of the neutral detergent fiber residue's cellulose SAH, hemicelluloses SAH and lignin (corrected for protein-like compounds) contents (Fig. 4). The cellulose SAH and hemicelluloses SAH of the insoluble dietary fiber method have been chosen to estimate the contents of cellulose and hemicelluloses because the insoluble dietary fiber method estimates these components more reliably, as compared to the detergent fiber method (Wolfrum et al. 2009; Godin et al. 2011a, 2014).This mass balance of the neutral detergent fiber residue shows that the insoluble dietary fiber method has a higher balance (nearer to 100 %) and is thus less reliable for the lignin quantitation, as compared to the detergent fiber method (Fig. 4). The difference between the mass balance of these two methods is statistically significant (p value < 0.001) for each type of biomass. It can be explained by the absence of some lignin phenolic compounds in the acid detergent lignin residue. These lignin phenolic compounds (preferentially the sinapyl lignin) are solubilized due to trace of acid detergent (cetyltrimethylammonium bromide) in the acid detergent fiber residue during the extraction

Table 5 Linear least square models between lignin corrected for protein-like compounds (CP) and uncorrected for protein-like compounds (UCP) of the detergent fiber and the insoluble dietary fiber methods in commelinid (n = 101) and non-commelinid magnoliophyta (n = 44) biomasses

Y (g/100 g DM)	X (g/100 g DM)	Slope ± Conf	Intercept ± Conf	R^2	MRE	RPD
Commelinid biomasses						
Lignin VST	Lignin VST	0.985 ± 0.009	–0.18 ± 0.05	0.998	0.13	22
CP	UCP					
Insoluble Klason lignin	Insoluble Klason lignin	0.990 ± 0.008	−0.16 ± 0.08	0.998	0.16	24
CP	UCP					
Non-commelinid magnoliophyta biomasses						
Lignin VST	Lignin VST	1.007 ± 0.036	−0.65 ± 0.34	0.987	0.38	9
CP	UCP					
Insoluble Klason lignin	Insoluble Klason lignin	1.027 ± 0.023	−0.80 ± 0.30	0.995	0.32	13
CP	UCP					

Conf Confidence interval of the mean; *CP* corrected for protein-like compounds; *UCP* uncorrected for protein-like compounds; *VST* Van Soest

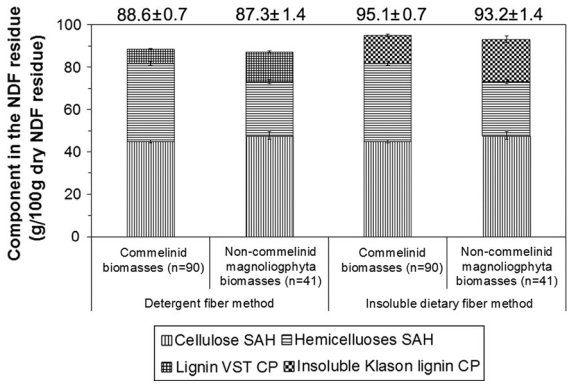

Fig. 4 Mass balance (mean ± confidence interval of the mean) of neutral detergent fiber residue. Error bars correspond to confidence interval of the mean, n to the number of analyzed samples. *CP* Corrected for protein-like compounds. *VST* Van Soest

by 12.2 mol/L sulfuric acid (Hatfield et al. 1994; Lowry et al. 1994). The non-commelinid magnoliophyta biomasses tend to have a lower balance of the neutral detergent fiber residue (further below 100 %), as compared to the commelinid biomasses (Fig. 4). This difference of mass balance is statistically significant (p value < 0.01) for the insoluble dietary fiber method but not for the detergent fiber method (p value ≥ 0.05). This can be explained by the smaller difference between these mass balances and the lower precision of the lignin quantitation of the detergent fiber method compared to the insoluble dietary fiber method. The lower mass balance of non-commelinid magnoliophyta biomasses can be explained by their higher contents of cell wall proteins and tannins (which form more tannins-proteins complexes), as compared to commelinid magnoliophyta biomasses. The unknown fraction of the neutral detergent fiber mass balance contains most probably some organic nitrogenous compounds (such as proteins, protein-like compounds and tannins-proteins complexes), acetyl groups of structural carbohydrates and some pectins which affect substantially more the non-commelinid magnoliophyta biomasses compared to commelinid magnoliophyta biomasses (Hintz and Mertens 1996; Carpita and McCann 2000; Cassida et al. 2007). For the use of the mass balance, the neutral detergent fiber residue can also be used to evaluate if the lignin quantitation is not excessively overestimated. An excessive overestimation of the lignin content would correspond to a mass balance higher than 100 %. For this use of this mass balance, the quantitation of cellulose and hemicelluloses must be reliable, and the unknown fraction of the neutral detergent fiber residue (100-cellulose SAH-hemicelluloses SAH-lignin) must not be too high.

Conclusion

Lignin needs to be quantitated reliably to improve the estimation of the available lignin resources and to enhance the assessment of its negative impacts on the enzymatic hydrolysis of the cell wall carbohydrates in

the rumen and in bioconversion processes such as cellulosic ethanol and biomethanation production. The present study showed that the insoluble dietary fiber method was more reliable to quantitate lignin because of its higher precision and smaller bias, as compared to the detergent fiber method. This bias was assessed by the mass balance of the neutral detergent fiber residue. This mass balance showed that the bias for lignin of the insoluble dietary fiber method was smaller owing to: (1) the lignin residue corrected for protein-like compounds which is most probably not only made of true proteins; (2) the Van Soest acid detergent (cetyltrimethylammonium bromide) not used during the diluted sulfuric acid hydrolysis step; (3) the Van Soest neutral detergent used to get a well standardized residue containing mainly cellulose, hemicelluloses and lignin, and minimal contents of non-structural carbohydrates (such as starch), pectins and organic nitrogenous compounds (such as proteins, protein-like compounds and tannins-proteins complexes). Nevertheless, the less tedious and resource consuming detergent fiber method can reliably be used to predict the results of the insoluble dietary fiber method with the correction factors determined in this paper (Fig. 2). These correction factors of commelinid biomasses are distinctive of those of non-commelinid magnoliophyta biomasses. The lignin content corrected for protein-like compounds can be predicted quantitatively by the lignin content uncorrected for protein-like compounds (Table 5). The lignin content should be corrected for protein-like compounds, otherwise lignin is significantly overestimated. Owing to these correction factors, the biofuel (e.g. cellulosic ethanol and biomethanation production), bio-based chemicals and feed sectors can use the detergent fiber method to rapidly and reliably estimate the available amounts of lignin of plant biomasses and rank them according to their suitability to be converted based on their lignin content.

Supporting information

Agronomic details about the mechanically harvested biomasses, the manually harvested biomasses and the biomasses used for the correlation assessment are shown in Tables S1 to S3 of the supporting information. Additional information about the precision estimation can be found in Tables S4 to S7 of the supporting information.

Acknowledgments This research was funded by the Walloon Agricultural Research Center (CRA-W) with the support of the Belgian Science Policy. The authors are grateful to the technicians of the BIOETHA2 project for their technical support.

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