Quantitative method applicable for various biomass species to determine their chemical composition

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Abstract

A quantitative method applicable for various biomass species to determine their chemical constituents was explored. The widely used wood analytical method was found to be not entirely applicable to different biomass species. It was then demonstrated that by incorporating protein and starch determinations, by ash-correcting the Klason lignin and holocellulose and also by protein-correcting Klason lignin and holocellulose of high protein content species, reliable summative results that enable comparison between different types of biomass materials were achieved. Thus, an analytical method with starch and protein determinations as well as ash and protein corrections was proposed for quantitative assay of chemical composition of various biomass species.

Keywords: quantitative method, chemical composition, *Phyllostachys heterocycla*, *Oryza sativa*, *Zea mays*, *Elaeis guineensis*.

1. Introduction

Although several efforts [1-3] were accomplished in the past to settle a universal method to determine the chemical components of biomass species, evidence with reasonable explanation to support the choice of a method or the addition of a given step in one procedure has never been provided. As wood is quantitatively predominant among biomass species [4], the method to determine its chemical composition is well established [5-7]. However, wood analytical procedure is not necessarily adequate for other kinds of biomass resources, especially for the herbaceous ones such as wheat straw and rice husk mostly used as feed and forage. Their chemical characterization is usually made, based on crop analysis for animal feeding, and terms and methods used to characterize herbaceous plants [8-10] differ from those to characterize wood [5].

Therefore, in order to determine biomass chemical composition on the same basis, an analytical method applicable to various biomass species has been studied in this work. For that purpose, widely used wood analytical method was discussed in its advantages and disadvantages, and a revised new analytical procedure applicable for different biomass species was established.

2. Materials and methods

Based on taxonomical classification of the vascular plants, six selected biomass species were analyzed on their morphological parts as exposed in Table 1 which shows their taxonomical classification, age, parts studied, sampling location and time, storage condition before delivery to the laboratory and condition during delivery in accordance with the biomass sample definition checklist recommended by Barton [11].

Upon arrival in the laboratory, the samples were air-dried, milled with Wiley mill (1029-C, Yoshida Seikakusho Co., Ltd.), and sieved to retain particles of 150-500 μm, 35-100 mesh according to ASTM E11-01 [12] in size. They were kept at room temperature, with 10-30 % humidity before analyses. These samples were oven-dried and the traditional wood analytical method [6] illustrated in Fig. 1 was firstly applied to characterize their chemical composition.

The oven-dried samples were incinerated at 600 ˚C [6] for 4h to determine ash content on the non-extracted samples. The non-extracted samples were then Soxhlet-extracted [13] with acetone until the solvent was clear of any color to determine the content of extractives. For these extractives-free samples, Klason and acid-soluble lignins were determined by using 72 % sulfuric acid through a modified Klason method [14]. In brief, 15 ml of 72 % H_2SO_4 was added to 1 g of extractives-free sample and left to react at room temperature for 2 h. Then, the mixture was diluted to 3 % H_2SO_4, autoclaved at 121˚C for 30 min and filtered to obtain Klason lignin gravimetrically and acid-soluble lignin from UV absorbance at 205nm, using absorptivity value of 110 $lg^{-1}.cm^{-1}$. Holocellulose was quantified with sodium chlorite treatment according to the procedure of Wise *et al.* [15] as adapted by Timell [16]. Subsequently, α-cellulose content was determined by extraction with 17.5 % aqueous sodium hydroxide of the holocellulose powder [6] and hemicellulose content was evaluated by difference between holocellulose and α-cellulose contents.

In the proposed analytical method illustrated in Fig.3, ash and extractives were determined in the same way as in wood analytical method. From the extractives-free samples, holocellulose content determined by the modified Wise method [16] was ash-

corrected and the numbers of chlorination treatments were 3 times for hardwood, 4 times for softwood and limited to 5 times or less for other biomass species. If the sample is still keeping a pronounced color, residual lignin correction was also performed on the holocellulose. Cellulose content as α-cellulose was determined in the same way as in wood analytical procedure [6] and hemicellulose content was obtained by subtracting the corrected cellulose content from the corrected holocellulose content. Klason lignin content obtained by sulfuric acid treatment of extractives-free samples [14] was also ash-corrected. For samples with high protein content, protein correction was additionally performed to the Klason lignin and holocellulose. Acid-soluble lignin content was also measured by UV-VIS spectroscopy at 205 nm [14]. Additional methods for starch and protein determinations from extractives-free samples were, respectively, made by perchloric acid method [17] and Kjeldahl nitrogen method by using a nitrogen factor of 6.25 [18].

3. Results and discussion

Table 2 shows the results obtained by applying the traditional wood analytical method in Fig.1 to the samples to quantify their chemical composition [6]. The use of its analytical method seems to be satisfactory for the summative results of Japanese cedar and Japanese beech, respectively, 998 and 999 g/kg in total yield. Noteworthy are the low summative yields for corn leaves (926 g/kg) and corn cob (923 g/kg). Given that wood species are mainly composed of cellulose, hemicellulose, lignin, extractives and inorganic constituents, their analytical method concentrates only in the determination of those constituents. Nonetheless, other species seem possible to contain additional components, for example, protein and starch etc. Therefore, the lower total yield resulted

in corn leaves and corn cob might be due to such constituents not measured but having quite important percentages.

A further important result in Table 2 is the extremely high total summative yields for bamboo, rice straw and rice husk to be 1138, 1179 and 1263 g/kg, respectively. Some authors reported similar results in their summative analyses [19,20], indicating an overestimation of the chemical constituents of the biomass species. The question then arises as to which components are overestimated. Bennett [21] and Angles *et al.* [22] discovered that inorganics mainly remained in the residual fraction when organic material was dissolved and separation was made.

Table 3 shows holocellulose and lignin contents of various biomass species as determined by traditional wood analytical method. For the obtained crude holocellulose and crude Klason lignin, contaminating inorganics were studied. Consequently, Ash-1 and Ash-2 were respectively resulted as incinerated inorganics. This finding on contamination is in good agreement with the above-mentioned previous studies [21,22]. Protein was also found to contaminate Klason lignin and holocellulose for corn leaves in which high protein was contained. The corrected holocellulose content is shown in Holocellulose (1) by subtracting Ash-1 and Protein-1 from crude holocellulose, while the corrected Klason lignin content was determined by subtracting Ash-2 and Protein-2 from the crude Klason lignin.

One is tempted to consider that ash is contaminated extensively only in high ash content samples, but even in low ash content ones like Japanese cedar, inorganic constituents still remained in holocellulose. The value in Ash-1 for Japanese cedar in

Table 3 to be 22 g/kg is even higher than the ash content of the original sample in Table 2 to be 3 g/kg. This fact may suggest that the treatment contaminated the samples with inorganics.

In order to define the inorganic contaminants, energy-dispersive X-ray (EDX) analysis [23] was applied to the ashes obtained from holocellulose and Klason lignin as compared to the ashes of the original samples. Fig. 2 shows the relative comparisons of the EDX spectra on the ashes for original sample of rice straw, its holocellulose and Klason lignin. It shows that Si is the main inorganic element in Klason lignin and holocellulose, representing most likely the insoluble inorganics from the sample itself, whereas Na and Cl were additionally found in holocellulose apparently from the reagent sodium chlorite. Although the washing procedure was accomplished according to the reference method [16], Na and Cl still remained in the holocellulose due most likely to the filtration step and the particles size of the sample. Therefore, ash correction may be necessary for both holocellulose and Klason lignin.

It has been widely demonstrated that chlorite delignification for holocellulose determination engenders a partial removal of the holocellulose [24]. Wood analytical method [6] recommended 4 times chlorinations for softwood and 3 times for hardwood. On the other hand, several literatures claimed that chlorination should be performed until the sample becomes whitish [25,26]. For an adequate determination, the number of chlorinations should be enough to remove lignin but not too extended to avoid a loss of sugars. Since biomass species are so heterogeneous, their delignification manner also differs from one species to another. For example, for softwood and hardwood, white residue could actually be recovered after, respectively, 4 and 3 times chlorinations.

Among monocotyledonous species, 3 times chlorinations were enough to obtain white residue in some samples as for corn leaves. However, for rice husk and bamboo, up to 8 and 9 times chlorinations were required to get whitish residue. Particularly for bamboo, 9 times chlorinations corresponded to 552 g/kg of holocellulose, whereas 4 and 5 times chlorinations, after which the sample was still yellowish and beige, yielded, respectively, 737 and 719 g/kg of holocellulose. Obviously, the extended chlorinations resulted in a loss of sugars. Therefore, for holocellulose determination of any biomass species, it is recommended not to exceed 5 times chlorinations. If the sample still has some color, it is necessary to determine lignin content in holocellulose and subtract it from the holocellulose content.

The aim of biomass extraction is to remove extractives in the sample that might interfere with further analyses. The mostly used solvents to extract biomass samples are ethanol-benzene mixture recommended by Tappi [13] or acetone [27], and lately ethanol and water in a two-step extraction [28]. It is reported that hot water removes starch as well as low molecular weight carbohydrates [29]. Acetone is, on the other hand, known to remove a quite wide range of components which might interfere with the measurements such as chlorophyll, likely to interfere with protein determination since it contains nitrogen. However, in opposition to hot water, starch and protein have low solubility in acetone. The determination of those components from extractives-free samples, avoiding interference with the extractives is, thus, possible.

In order to determine whether extractives represent a major reason of the excessive summative results, the extracted and non-extracted samples were compared for their ash contents. Consequently, for Japanese cedar, there was no ash in extractives,

whereas for the other samples, ash in extractives varied from 1 to a maximum of 7 g/kg of the original oven-dried biomass. Those values represent the inorganics counted twice as ash and extractives. Such an amount is not so significant that a correction can be ignored.

At this stage, it is, therefore, clear that some steps in wood analytical method are overlapping. Inorganics contribute to an overestimation of Klason lignin and holocellulose. For samples with high protein content, a fraction of the protein also contaminates the Klason lignin and the holocellulose. Hence, the traditional wood analytical method is not entirely applicable to various biomass species. Performing ash corrections to holocellulose and Klason lignin as well as protein corrections to Klason lignin of samples with high protein content are, thus, needed in order to get accurate quantitative values.

4. Proposed analytical method applicable to various biomass species

Through the advantages and disadvantages of the analytical method of wood, a revised analytical procedure is suggested in Fig. 3 which represents the proposed new analytical method applicable to various biomass species to quantify their chemical composition.

For starch determination, perchloric acid treatment followed by colorimetric determination [17] was compared to enzymatic hydrolysis [30] using Megazyme kit, followed by HPLC determination of glucose. The results were comparable for the 2 methods (data not shown here). It implied that both methods are suitable for starch content determination of biomass. However, since the perchloric acid one is less

laborious and allows a rapid determination of starch in a large number of samples, it is considered more convenient for biomass analysis. Starch determination of the samples was, thus, performed according to the perchloric acid method [17].

As reported in Table 4, the application of the proposed analytical method to the selected samples enables to get very satisfactory results. All the summative mass closures were between 950 and 1000 g/kg. It implies that the majority of the biomass constituents were quantified and no major components have been overlooked. The interferences between the different steps and the double counting of the materials were, thus, minimized. Summative results lower than 850 g/kg are quite rare when the proposed method is used but if it is the case, it might be necessary to determine other constituents such as lipid which might be present in some biomass in a relative amount according to the species and the part of the plant studied.

5. Conclusion

Through these lines of evidence, it can be concluded that the traditional wood analytical method is applicable to wood but not entirely applicable to other biomass species. Ash correction should be performed on holocellulose and Klason lignin. In addition, protein correction should also be performed, particularly for samples with high protein content. Protein and starch analyses should be included in the procedure, in addition to lignin, holocellulose, ash and extractives determinations. The proposed new analytical method with protein and starch analyses as shown in Fig.3 enables the majority of the biomass constituents to be quantified with satisfactory summative results. Thus, the

newly proposed analytical method allows to accomplish a direct comparison among various biomass species of their chemical constituents as shown in Table 4.

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Table 1. Taxonomical classification of the biomass species and their details.

Classification	Species	Age (years)	Parts studied	Sampling location	Sampling time	Storage condition before delivery to the laboratory (Temp; humidity)	Sample condition during delivery	Remarks
Gymnosperm	Japanese cedar (*Cryptomeria japonica*)	50	Trunk	Kyoto, Japan (35˚08'N, 135˚66'E)	12 / 2000	0-10 ˚C; 50-60 %	Air-dried, thinned	
Angiosperm								
Dicotyledon	Japanese beech (*Fagus crenata*)	45	Trunk	Kyoto, Japan (35˚22'N, 135˚82'E)	11 / 2000	0-10 ˚C; 50-60 %	Air-dried, thinned	
Monocotyledon	Bamboo (*Phyllostachys heterocycla* f. *pubescens*)	5	Culm	Kyoto, Japan (35˚01'N, 135˚46'E)	06 / 2008	10-20˚C; 60-70 %	Undried, cut into pieces	
	Rice (*Oryza sativa* var. *Japonica*)	0.5	Straw, Husk	Aichi, Japan (35°23'N, 136°87'E)	10 / 2007	20-40 ˚C; 70-85 %	Air-dried, husk separated from straw	Husk was detached from grain using a mechanical paddy de-husker
	Corn (*Zea mays* cv. Yumeno-corn)	0.4	Leaves, Cob	Aomori, Japan (40°49'N, 140°45'E)	09 / 2008	10-20 ˚C; 60-70 %	Undried, cob together with grains	Corn cob was separated from grains using a knife
	Oil palm (*Elaeis guineensis* Jacq.)	25	Trunk	Johor Bahru, Malaysia (1°46'N, 103°75'E)	03 / 2006	25-35 ˚C; 70-85 %	Air-dried, cut into blocks	

Table 2. Chemical composition of various biomass species determined by the traditional wood analytical method (g/kg).

Biomass	Holocellulose	Cellulose[a]	Hemicellulose[b]	Lignin		Extractives	Ash	Total
				Klason	Acid-soluble			
Japanese cedar	628	383	245	330	3	34	3	998
Japanese beech	732	439	293	212	30	19	6	999
Bamboo	737	394	343	333	18	38	12	1138
Rice								
Straw	666	345	321	317	18	45	133	1179
Husk	707	360	347	362	13	13	168	1263
Corn								
Leaves	579	341	238	170	20	47	110	926
Cob	681	277	404	154	29	27	32	923
Oil palm								
Trunk	600	306	294	247	39	36	41	963

[a] Cellulose = α-Cellulose

[b] Hemicellulose = Holocellulose – (α - Cellulose)

Table 3. Holocellulose and lignin of various biomass species as determined by traditional wood analytical method (g/kg).

Biomass	Holocellulose					Lignin			
	Crude holocellulose	Lignin-1	Ash - 1	Protein-1	Holocellulose (1)	Crude Klason	Ash - 2	Protein-2	Klason lignin
Japanese cedar	628	-	22	-	606	330	1	-	329
Japanese beech	732	-	9	-	723	212	2	-	210
Bamboo	737	23	9	-	705	333	140	-	193
Rice									
Straw	666	-	103	-	563	317	133	-	184
Husk	707	22	152	-	533	362	134	-	228
Corn									
Leaves	579	-	27[a]	40	512	170	13[a]	31	126
Cob	681	-	14	-	667	154	3	-	151
Oil palm									
Trunk	600	-	10	-	590	247	4	-	243

[a] Nitrogen-free ash which was corrected for nitrogen contained in protein

Table 4. Chemical composition of various biomass species as determined by the proposed new analytical method (g/kg).

Biomass	Holocellulose	Cellulose[a]	Hemicellulose[b]	Lignin		Extractives	Protein	Starch	Ash	Total
				Klason	Acid-soluble					
Japanese Cedar	606	379	227	328	3	34	5	1	3	980
Japanese Beech	723	439	284	210	30	19	6	5	6	999
Bamboo	705	394	311	193	18	38	13	11	12	990
Rice										
Straw	563	345	218	184	18	45	47	9	133	999
Husk	533	360	173	228	13	13	16	2	168	973
Corn										
Leaves	513	341	172	126	20	47	181	2	110	999
Cob	667	277	390	151	29	27	56	21	32	983
Oil palm										
Trunk	590	306	284	243	39	36	6	29	41	984

[a] Cellulose = α-Cellulose

[b] Hemicellulose = Holocellulose - α-Cellulose

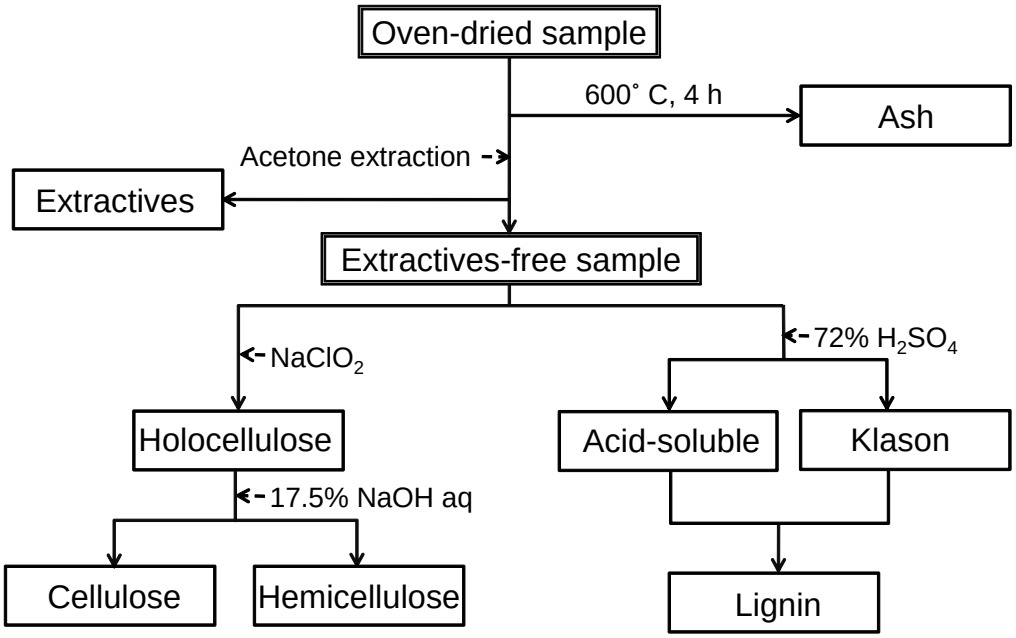

Fig. 1 Traditional wood analytical method to quantify its chemical composition [6].

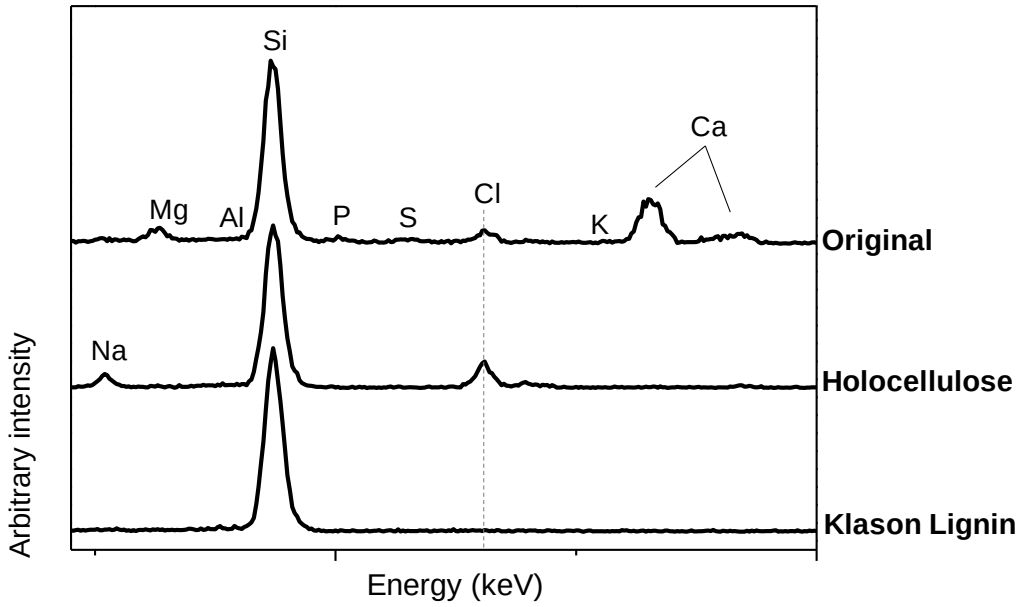

Fig. 2 Relative comparisons of the energy-dispersive X-ray spectra on the ashes for original sample of rice straw, its holocellulose and Klason lignin as determined by EDX analysis [23].

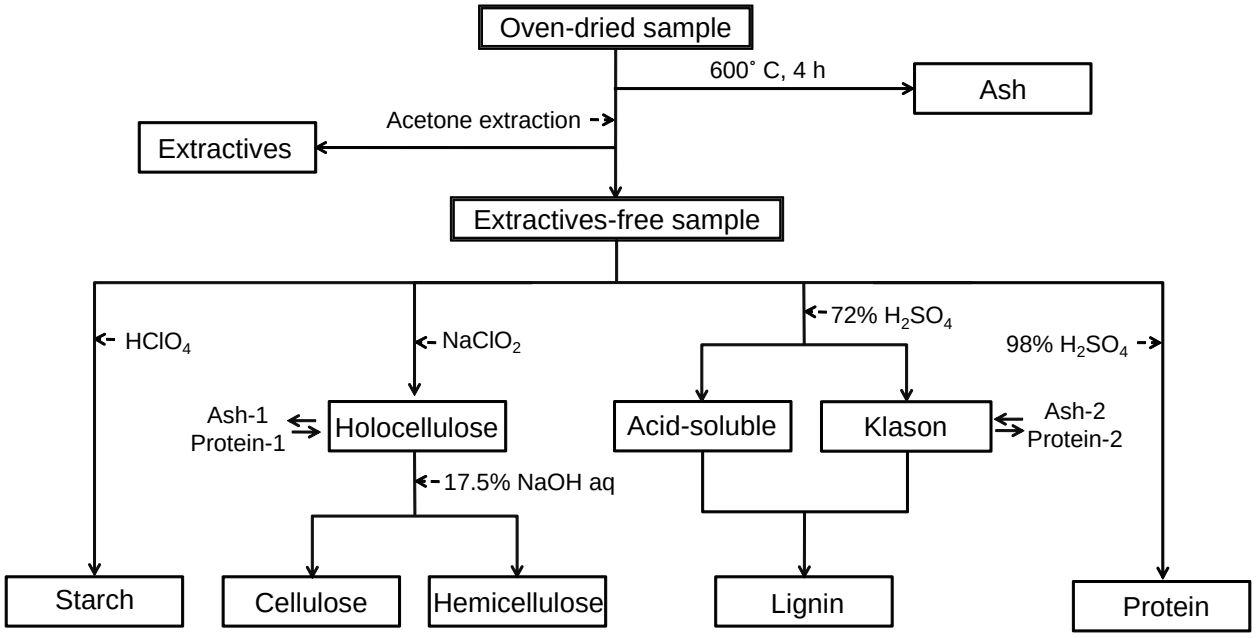

Fig. 3. Proposed new analytical method applicable to various biomass species to quantify their chemical composition.