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Article

Chemical Profiles of Wood Components of Poplar Clones for Their Energy Utilization

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Abstract: Selected and tested poplar clones are very suitable biomass resources for various applications such as biofuels, the pulp and paper industry as well as chemicals production. In this study, we determined the content of lignin, cellulose, holocellulose, and extractives, syringyl to guaiacyl (S/G) ratio in lignin, and also calculated higher heating values (HHV) among eight examined clones of *Populus* grown on three different experimental sites. The highest lignin content for all the examined sites was determined in 'I-214' and 'Baka 5' clones, whereas the highest content of extractives was found in 'Villafranca' and 'Baka 5' clones. The highest S/G ratio for all the examined sites was determined in 'Villafranca' and 'Agathe F' clones. The chemical profiles of main wood components, extractives, and the S/G ratio in lignin were also influenced by both the experimental site and the clone × site interaction. Higher heating values, derived from calculations based on the contents of lignin and extractives (or lignin only), were in close agreement with the previously published data. The highest heating values were found for 'Baka 5' and 'I-214' clones. The optimal method of poplar biomass utilization can be chosen on basis of the lignocellulosics chemical composition and the S/G ratio in lignin.

Keywords: cellulose; extractives; higher heating value; holocellulose; lignin; S/G ratio; *Populus*

1. Introduction

The development of human civilization over the past few decades is connected to an increase in energy consumption. New technologies have decreased specific energy consumption, but modern society and its rising living standards have raised demands for the total amount of energy required. Fossil fuels, which are the main source of energy at present, must be replaced by the other sources in the future, due to both their limited amounts and negative impact on the environment. Nuclear energy utilization is dangerous and following the recent problems in Fukushima (Japan), some countries have decided to limit or to stop energy production in nuclear power plants. Sasaki *et al.* [1] concerns the demand of replacing nuclear energy production in Japan with energy production from wood biomass. According to Ragauskas *et al.* [2] "Before we freeze in the dark, we must prepare to make the transition from non-renewable carbon resources to renewable bioresources". Besides other alternative energy sources (solar, wind, water, geothermal, *etc.*) biomass is a prospective resource of energy.

Biomass can be converted into many different forms of energy including heat, electricity, and liquid transportation fuels. It can also be used as feedstock for chemicals and pulp production [3–8]. Several different technologies exist for the conversion of lignocellulose to fuels, and the choice of technology can have substantial implications for both the environmental and agronomic aspects of biofuel production. Perhaps the simplest technology is to burn biomass to produce steam that can be used to generate electricity. Due, however, to a number of disadvantages with this process, some more sophisticated methods are of interest to both scientists and technologists (e.g., pyrolysis, hydrolysis, microbial and chemical conversions). While the combustion of biomass is by far the most direct and technically the easiest process, the overall efficiency of generating heat from biomass energy is low. Gasification, though, has many advantages over combustion. It can utilize low-value feedstocks and convert them not only into electricity, but also into transportation fuels. Selection of suitable plant species for renewable energy and products acquisition is very important from a technical, economic, environmental, and ethical point of view. Some plants store chemical energy mainly as mono- and oligosaccharides or as starch (sugar cane, corn, wheat) which are predominantly used for food. Others are non-food plants (trees, grasses, aquatic plants, e.g., duckweed) or agricultural residues (e.g., corn stover), and their suitability for conversion into bioenergy (especially bioethanol) depends on their chemical composition and structure [9–11].

With increasing government, academic, and industrial research efforts being put toward the production of biofuels and biomaterials from lignocellulosic feedstock, a few species have emerged as front-runners in this field [5]. Different applications such as the production of secondary biofuels or combustion for energy production require different wood properties. Various taxa of the genus *Populus* are woody plants well suited as renewable biomass resources (together with willows, pines, eucalypts, acacias, *etc.*). Compared with other hardwoods, the fast-growing poplars possess many advantages such as rapid growth, better adaptability, easier breeding, high economic efficiency, and a broad range of applications. In the production of bioethanol, lignin is the main limiting factor because it limits the accessibility of cellulose microfibrils to enzymatic depolymerization. Genetic engineering has developed poplars with a modified wood composition that makes them more suitable for the production of bioethanol. The modified wood contains less lignin and more cellulose [12–15].

The proportion of cellulose, hemicelluloses, and lignin in a biomass feedstock is an important criterion when determining both its suitability as an economically viable feedstock and also when making a decision concerning the optimum pathway for its conversion [5]. The higher heating values (HHV) for extractives, lignin, and holocellulose are consistent with their carbon content [16]. Lignin has a higher heating value in comparison with saccharides, and extractives have the highest heating values. Several authors have found a linear relationship between the HHV and the carbon content of the fuels [17,18]. For combustion, in general, there are more suitable woods of the species with higher amounts of lignin and extractives. The objective of the study was to determine the variation in chemical properties among eight examined clones of *Populus* grown on three different experimental sites from the viewpoint of their energy utilization.

2. Results and Discussion

2.1. The Content of Wood Components

The results showed that there were considerable differences in the content of main wood components (lignin, cellulose, hemicelluloses) as well as in extractives among the examined poplar clones (Tables 1–3).

On the experimental site at Sliepkovce, the highest percentages of lignin content were found in 'Baka 5', followed by 'I-214', while the clone 'Villafranca' had the lowest percentage of lignin content (Table 1). On the sites at Selice (Table 2) and Gabčíkovo (Table 3), 'Villafranca' again had the lowest content of lignin, whereas 'I-214' had the highest content of this biopolymer.

Table 1. The content of main wood components and extractives present in the examined clones of *Populus* grown on the Sliepkovce experimental site. Data represent percentages of oven dry weight (odw) (mean ± SD).

Clone	Lignin (%)	Cellulose (%)	Holocellulose (%)	Extractives (%)
'Agathe F'	22.15 ± 0.20 c	43.92 ± 0.26 d	82.91 ± 0.19 ab	1.73 ± 0.11 bc
'BL Constanzo'	22.28 ± 0.20 c	43.45 ± 0.18 e	82.13 ± 0.35 c	1.67 ± 0.17 bc
'I-214'	23.11 ± 0.15 b	43.09 ± 0.22 f	81.70 ± 0.21 d	1.69 ± 0.15 bc
'NE-367'	19.82 ± 0.17 e	45.94 ± 0.25 b	83.27 ± 0.25 a	1.78 ± 0.17 b
'Pannonia'	20.79 ± 0.21 d	45.73 ± 0.16 b	81.72 ± 0.26 d	1.49 ± 0.13 cd
'Robusta'	20.91 ± 0.17 d	46.91 ± 0.26 a	82.26 ± 0.29 c	1.36 ± 0.19 d
'Villafranca'	17.68 ± 0.22 f	44.80 ± 0.20 c	82.79 ± 0.22 b	3.75 ± 0.25 a
'Baka 5'	23.66 ± 0.23 a	44.72 ± 0.30 c	82.80 ± 0.26 b	1.62 ± 0.14 bc

Notes: Mean values followed by the same letters, a–f within columns across clones, are not significantly different at $P < 0.05$ (Duncan's multiple range test).

Table 2. The content of main wood components and extractives present in the examined clones of *Populus* grown on the Selice experimental site. Data represent percentages of oven dry weight (odw) (mean ± SD).

Clone	Lignin (%)	Cellulose (%)	Holocellulose (%)	Extractives (%)
'Agathe F'	20.09 ± 0.21 e	45.30 ± 0.21 b	83.97 ± 0.27 b	1.65 ± 0.16 c
'BL Constanzo'	21.85 ± 0.17 b	43.73 ± 0.22 d	83.38 ± 0.23 c	1.66 ± 0.17 c
'I-214'	22.99 ± 0.25 a	42.23 ± 0.24 e	83.37 ± 0.29 c	1.74 ± 0.18 c
'NE-367'	20.83 ± 0.18 cd	46.09 ± 0.18 a	83.75 ± 0.24 b	1.57 ± 0.19 c
'Pannonia'	21.13 ± 0.31 c	44.89 ± 0.27 c	82.98 ± 0.22 d	1.74 ± 0.20 c
'Robusta'	20.54 ± 0.24 d	44.89 ± 0.21 c	84.77 ± 0.25 a	2.16 ± 0.19 b
'Villafranca'	18.84 ± 0.20 f	43.70 ± 0.26 d	80.83 ± 0.22 f	5.36 ± 0.25 a
'Baka 5'	21.54 ± 0.17 b	44.74 ± 0.24 c	82.28 ± 0.22 e	2.05 ± 0.19 b

Notes: Mean values followed by the same letters, a–f within columns across clones, are not significantly different at $P < 0.05$ (Duncan's multiple range test).

Table 3. The content of main wood components and extractives present in the examined clones of *Populus* grown on the Gabčíkovo experimental site. Data represent percentages of oven dry weight (odw) (mean ± SD).

Clone	Lignin (%)	Cellulose (%)	Holocellulose (%)	Extractives (%)
'Agathe F'	18.89 ± 0.23 e	47.55 ± 0.16 a	84.49 ± 0.28 a	1.54 ± 0.10 e
'BL Constanzo'	21.38 ± 0.24 b	43.73 ± 0.22 fg	81.62 ± 0.32 cd	1.62 ± 0.15 e
'I-214'	22.04 ± 0.20 a	43.53 ± 0.27 g	82.98 ± 0.39 b	2.01 ± 0.14 c
'NE-367'	19.70 ± 0.25 d	46.44 ± 0.25 b	81.76 ± 0.25 c	1.86 ± 0.17 cd
'Pannonia'	20.97 ± 0.22 c	45.34 ± 0.13 c	81.14 ± 0.27 d	1.93 ± 0.15 cd
'Robusta'	21.21 ± 0.21 bc	44.21 ± 0.24 e	81.75 ± 0.36 c	1.71 ± 0.11 de
'Villafranca'	17.70 ± 0.21 f	44.79 ± 0.28 d	81.62 ± 0.27 cd	4.95 ± 0.19 a
'Baka 5'	21.86 ± 0.22 a	44.04 ± 0.13 ef	79.12 ± 0.20 e	3.82 ± 0.19 b

Notes: Mean values followed by the same letters, a–g within columns across clones, are not significantly different at $P < 0.05$ (Duncan's multiple range test).

The influence of both the experimental site ($F_{2,72} = 122.61$, $P < 0.001$) and the clone × site interaction ($F_{14,72} = 51.35$, $P < 0.001$) on lignin content was significant. The highest percentage of lignin content was found in poplars grown at Sliepkovce, whereas the lowest percentage was in poplars grown at Gabčíkovo. Taken together, 'I-214' was a clone with an average stability for lignin content and the highest mean for this trait in all three examined sites. The mean content of lignin for 'I-214' was found to change depending upon how the overall mean of lignin content changed on a particular site for all the examined clones. In this way 'Baka 5' resembled 'I-214'. On the other hand, 'Villafranca' had the lowest percentage of lignin content at Sliepkovce, where we determined the highest overall mean of lignin content for the examined clones.

The amounts of lignin among poplar clones were markedly different. They varied in a range from 17.68% ('Villafranca') to 23.66% ('Baka 5'). Similar results among different poplar clones were also determined by other authors: 21%–29% [5], 15.7%–27.9% [19], 17.7%–21.1% [20], 15.4%–23.4% [21], 19.9%–23.8% [22]. At all sites the lowest values were recorded for the clone 'Villafranca', which shows that this raw material is suitable for utilization at pulp production and for hydrolysis treatment.

Clones with high amounts of lignin are mainly suitable for direct combustion. From the aspect of calorific value, the most suitable clones are 'I-214' (22.04%–23.11% of lignin content) and 'Baka 5' (21.54%–23.66% of lignin content).

With regard to the total content of polysaccharides (*i.e.*, holocellulose), the highest percentages were found in 'Agathe F' grown at Sliepkovce (Table 1) and at Gabčíkovo (Table 3). On the site at Selice (Table 2), the highest content of holocellulose was determined from the 'Robusta' wood samples. 'Baka 5' was a clone with the lowest mean content of holocellulose for the three examined sites. The influence of both the experimental site ($F_{2,72}$ = 189.19, P < 0.001) and the clone × site interaction ($F_{14,72}$ = 58.80, P < 0.001) on the content of holocellulose was again significant.

Holocellulose yields varied in amounts from 79.12% ('Baka 5') to 84.77% ('Robusta'). The total polysaccharidic content primarily depends on the delignification method. Isolated specimens contained some amount of lignin, which influenced the summary wood analysis and its value is thus slightly higher than 100%. It is, thereby, relatively difficult to compare published results without consideration of the determination method, e.g., Telmo and Lousada [23] determined holocellulose amounts in poplar wood to be only 65.2% at the high amount of Klason lignin (31.7%). According to Balatinecz and Kretschmann [24] chemical composition of poplar wood is characterized by high polysaccharide content (approximately 80% holocellulose, made up of 50% cellulose and 30% hemicelluloses) and low lignin content (about 20% or less). Fengel and Wegener [25] summarized the results of several authors and presented the following data of various poplar clones: hollocellulose 78.4%–80.3%, cellulose 42.7%–51.0%, hemicelluloses 21.2%–31.7%, pentosanes 15.9%–19.9%, lignin 17.6%–20.9%, extractives 3.3%–3.8%, ash 0.4%. Our results are in good accordance with the reported values, and they confirm the suitability of the examined poplar clones for energy utilization.

The highest content of cellulose was found in 'Agathe F' grown at Gabčíkovo (Table 3), followed by 'Robusta' grown at Sliepkovce (Table 1) and 'NE-367' grown at Selice (Table 2). 'I-214' had the lowest content of cellulose for each of the three examined sites. The influence of both the experimental site ($F_{2,72}$ = 42.78, P < 0.001) and the clone × site interaction ($F_{14,72}$ = 66.91, P < 0.001) on the content of cellulose was significant.

The amounts of cellulose varied from 42.23% ('I-214') to 47.55% ('Agathe F'). Other authors have determined the following amounts of cellulose in poplar clones: 42%–49% [5], 45.82%–56.84% [26]. These values correspond with our results, the higher values reported by Klasnja *et al.* [26] are due to the method of cellulose determination. The authors used the method of Kürschner and Hoffer [27], which in comparison with the Seifert method [28] gives a higher result because of the higher amounts of hemicelluloses and lignin in the isolated cellulose [29–31]. The highest average yields of cellulose were determined in clone 'NE-367'.

'Villafranca' had the highest content of extractives for each of the three examined sites (Tables 1–3). On the site at Sliepkovce, 'Robusta' had the lowest content of extractives (Table 1), whereas 'Agathe F' and 'BL Constanzo' were among the clones having the lowest percentages of extractives on the sites at Selice (Table 2) and at Gabčíkovo (Table 3). With respect to this examined trait, 'I-214' was an average clone which exceeded the content of 2% only on the site at Gabčíkovo (Table 3). The influence of both the experimental site ($F_{2,72}$ = 82.18, P < 0.001) and the clone × site interaction ($F_{14,72}$ = 32.99, P < 0.001) on the content of extractives was significant. The highest percentages of extractives were found in poplars grown at Gabčíkovo, whereas the lowest percentages were from poplars grown at Sliepkovce.

The amounts of extractives in wood are usually relatively low. Despite this, they can markedly influence the properties of both woody feedstock and woody products. From Tables 1–3 it follows that among the examined poplar clones the amount of extractive matters varied in a range of 1.5%–2.0% (except for the clone 'Villafranca' which had an amount of extractives from 3.75% to 5.36%). These results are in accordance with the reports in the literature: 1.4%–2.7% [5], 3.1% [23], 3.4%–3.6% [32].

2.2. Lignin Monomer Composition

The contents of nitrobenzene oxidation products are presented in the tables 4–6 for the three examined sites. The main constituents of lignin in the examined clones of *Populus* are syringyl (S) subunits. Less abundant are guaiacyl (G) subunits, whereas the representation of *p*-hydroxyphenyl (H) subunits is in a very low percentage. The S/G ratio was significantly influenced by the clone ($F_{7,72}$ = 112.46, P < 0.001), site ($F_{2,72}$ = 90.24, P < 0.001), and their mutual interaction ($F_{14,72}$ = 51.64, P < 0.001). The highest S/G ratio was found in poplars grown at Selice, whereas the lowest ratio was from poplars grown at Gabčíkovo 'Villafranca' had the highest S/G ratio on the site at Sliepkovce (Table 4), 'Baka 5' had the highest ratio on the site at Selice (Table 5), and 'Agathe F' had the highest ratio on the site at Gabčíkovo (Table 6), respectively. Taken together, 'Villafranca' was a clone with the highest mean of S/G ratio for the three examined sites, whereas the lowest overall mean of this ratio was determined in 'I-214'. With respect to this trait, the behavior of 'I-214' was completely different from the overall means of all the other examined clones.

The nitrobenzene oxidation method has been widely used to characterize lignin structures. The main oxidation products are phenolic aldehydes (*p*-hydroxybenzaldehyde, vanillin, and syringaldehyde) as well as phenolic carboxylic acids (*p*-hydroxybenzoic, vanillic and syringic acids). These products originate from the corresponding phenyl-propane units and their α(β)-O-4 alkyl-arylethers due to oxidation of lignin in the alkaline environment. Therefore, the S/G ratio provides information on the relative amounts of the uncondensed guaiacyl- and syringylpropane units comprising the original lignin. In addition, the yield of these aldehydes reflects the degree of condensation of the lignin, because these uncondensed structures are cleaved by the nitrobenzene oxidation leaving condensed lignins as the residue. Recalcitrance to saccharification is a major limitation when converting lignocellulosic biomass to ethanol. Recalcitrance to both acid pretreatment and enzymatic digestion is directly proportional to lignin content [33]. In addition to lignin content, the S/G ratio can also significantly affect sugar release during biomass hydrolysis. Guaiacyl and syringyl aromatic subunits determine the type and number of crosslinks, as well as the reactivity of the lignin (S-rich lignin forms predominantly fewer cross-linking structures than G-rich lignin). Our results showed the S/G ratio among the examined poplar hybrids varied within a range of 1.65 ('I-214') to 2.77 ('Baka 5'), which correlates well with the data reported for poplars: 1.3–2.2 [5], 1.0–3.0 [19], 1.02–1.68 [21], 1.8–2.3 [34]. The apparent differences among these reports might arise from various methods of S/G ratio determination.

Table 4. The results of nitrobenzene oxidation (%) for the examined clones of *Populus* grown on the Sliepkovce experimental site. Data represent means ± SD.

Clone	*p*-HBAc [a]	*p*-HBA [a]	Sac [a]	SYR [a]	VAc [a]	VAN [a]	Total yield on wood	S/G [b]
'Agathe F'	0.89 ± 0.02 bc	0.04 ± 0.01 b	0.87 ± 0.10 a	8.92 ± 0.05 a	0.39 ± 0.02 b	3.72 ± 0.03 bc	14.83 ± 0.10 a	2.38 ± 0.02 c
'BL Constanzo'	0.91 ± 0.18 bc	0.06 ± 0.01 a	0.87 ± 0.06 a	8.11 ± 0.40 bcd	0.39 ± 0.03 b	3.58 ± 0.13 cd	13.92 ± 0.77 ab	2.27 ± 0.04 de
'I-214'	0.51 ± 0.07 d	0.05 ± 0.02 ab	0.83 ± 0.06 a	8.56 ± 0.37 ab	0.37 ± 0.07 b	3.93 ± 0.13 ab	14.08 ± 0.38 ab	2.19 ± 0.05 e
'NE 367'	1.05 ± 0.23 b	0.06 ± 0.02 ab	0.81 ± 0.10 a	7.63 ± 0.57 de	0.36 ± 0.03 bc	3.34 ± 0.19 de	13.29 ± 1.10 b	2.28 ± 0.05 de
'Pannonia'	0.24 ± 0.14 e	0.05 ± 0.02 ab	0.59 ± 0.17 b	7.85 ± 0.56 cde	0.22 ± 0.05 d	3.12 ± 0.42 e	12.06 ± 1.04 c	2.54 ± 0.15 b
'Robusta'	0.78 ± 0.15 c	0.06 ± 0.01 ab	0.92 ± 0.01 a	8.36 ± 0.12 bc	0.41 ± 0.01 ab	3.60 ± 0.09 cd	14.11 ± 0.17 ab	2.32 ± 0.04 cd
'Villafranca'	0.58 ± 0.10 d	0.06 ± 0.01 ab	0.80 ± 0.03 a	7.40 ± 0.31 e	0.31 ± 0.02 c	2.79 ± 0.13 f	11.91 ± 0.59 c	2.64 ± 0.03 a
'Baka 5'	1.26 ± 0.14 a	0.06 ± 0.01 a	0.89 ± 0.02 a	8.09 ± 0.04 bcd	0.45 ± 0.01 a	4.05 ± 0.03 a	14.80 ± 0.20 a	2.00 ± 0.01 f

Notes: Mean values followed by the same letters, a–f within columns across clones, are not significantly different at $P < 0.05$ (Duncan's multiple range test);
[a] *p*-HBAc, *p*-hydroxybenzoic acid; *p*-HBA, *p*-hydroxybenzaldehyde; SAc, syringic acid; SYR, syringaldehyde; VAc, vanillic acid; VAN, vanillin;
[b] S/G = (SAc + SYR)/(VAc + VAN).

Table 5. The results of nitrobenzene oxidation (%) for the examined clones of *Populus* grown on the Selice experimental site. Data represent means ± SD.

Clone	*p*-HBAc [a]	*p*-HBA [a]	SAc [a]	SYR [a]	VAc [a]	VAN [a]	Total yield on wood	S/G [b]
'Agathe F'	0.89 ± 0.02 bc	0.04 ± 0.01 bc	0.88 ± 0.04 bc	7.96 ± 0.24 bc	0.34 ± 0.02 b	3.05 ± 0.10 c	13.16 ± 0.37 bc	2.61 ± 0.01 b
'BL Constanzo''	1.06 ± 0.07 b	0.05 ± 0.01 b	0.92 ± 0.02 bc	8.06 ± 0.30 bc	0.42 ± 0.01 a	3.56 ± 0.13 b	14.06 ± 0.51 ab	2.26 ± 0.01 cd
'I-214'	0.89 ± 0.08 bc	0.04 ± 0.01 bc	0.91 ± 0.04 bc	5.57 ± 0.58 d	0.44 ± 0.02 a	3.54 ± 0.57 b	11.38 ± 0.95 d	1.65 ± 0.24 e
'NE 367'	0.55 ± 0.42 d	0.04 ± 0.01 bc	0.90 ± 0.10 bc	8.29 ± 0.07 ab	0.33 ± 0.06 b	3.26 ± 0.07 bc	13.38 ± 0.61 bc	2.56 ± 0.08 b
'Pannonia'	0.99 ± 0.04 b	0.03 ± 0.01 c	1.03 ± 0.03 a	8.55 ± 0.13 a	0.42 ± 0.02 a	3.59 ± 0.03 b	14.60 ± 0.17 a	2.39 ± 0.02 c
'Robusta'	1.51 ± 0.07 a	0.04 ± 0.01 bc	0.88 ± 0.14 bc	8.42 ± 0.18 ab	0.36 ± 0.04 b	3.94 ± 0.10 a	14.48 ± 1.10 a	2.17 ± 0.01 d
'Villafranca'	0.68 ± 0.17 cd	0.08 ± 0.01 a	0.84 ± 0.02 c	7.64 ± 0.16 c	0.34 ± 0.01 b	2.93 ± 0.08 c	12.51 ± 0.32 c	2.59 ± 0.03 b
'Baka 5'	1.03 ± 0.16 b	0.04 ± 0.01 bc	0.97 ± 0.02 ab	8.57 ± 0.41 a	0.36 ± 0.02 b	3.09 ± 0.11 c	14.06 ± 0.66 ab	2.77 ± 0.05 a

Notes: Mean values followed by the same letters, a–e within columns across clones, are not significantly different at $P < 0.05$ (Duncan's multiple range test);
[a] *p*-HBAc, *p*-hydroxybenzoic acid; *p*-HBA, *p*-hydroxybenzaldehyde; SAc, syringic acid; SYR, syringaldehyde; VAc, vanillic acid; VAN, vanillin;
[b] S/G = (SAc + SYR)/(VAc + VAN).

Table 6. The results of nitrobenzene oxidation (%) for the examined clones of *Populus* grown on the Gabčíkovo experimental site. Data represent means ± SD.

Clone	*p*-HBAc[a]	*p*-HBA[a]	SAc[a]	SYR[a]	VAc[a]	VAN[a]	Total yield on wood	S/G[b]
'Agathe F'	0.51 ± 0.11 bc	0.03 ± 0.01 bc	0.45 ± 0.04 b	5.57 ± 0.41 b	0.19 ± 0.04 c	2.20 ± 0.18 f	8.66 ± 0.41 b	2.52 ± 0.01 a
'BL Constanzo'	0.51 ± 0.02 bc	0.02 ± 0.01 bc	0.49 ± 0.07 ab	5.58 ± 0.49 b	0.24 ± 0.05 bc	2.74 ± 0.25 bc	9.58 ± 0.85 b	2.05 ± 0.02 e
'I-214'	0.59 ± 0.06 ab	0.02 ± 0.01 c	0.55 ± 0.02 ab	5.98 ± 0.22 ab	0.25 ± 0.01 b	3.15 ± 0.13 a	10.53 ± 0.34 a	1.92 ± 0.01 f
'NE 367'	0.57 ± 0.05 ab	0.02 ± 0.01 bc	0.45 ± 0.03 b	5.38 ± 0.26 b	0.22 ± 0.01 bc	2.52 ± 0.16 cde	9.15 ± 0.46 b	2.13 ± 0.04 d
'Pannonia'	0.56 ± 0.04 ab	0.03 ± 0.01 b	0.54 ± 0.03 ab	6.47 ± 0.30 a	0.25 ± 0.02 b	2.99 ± 0.14 ab	10.84 ± 0.49 a	2.17 ± 0.01 d
'Robusta'	0.50 ± 0.03 bc	0.03 ± 0.01 bc	0.59 ± 0.12 a	5.65 ± 0.34 b	0.25 ± 0.04 b	2.42 ± 0.10 def	9.42 ± 0.32 b	2.34 ± 0.04 c
'Villafranca'	0.42 ± 0.06 c	0.03 ± 0.01 b	0.47 ± 0.09 b	5.50 ± 0.72 b	0.19 ± 0.02 c	2.25 ± 0.35 ef	8.86 ± 1.24 b	2.45 ± 0.01 b
'Baka 5'	0.65 ± 0.13 a	0.07 ± 0.01 a	0.45 ± 0.02 b	4.65 ± 0.09 c	0.35 ± 0.02 a	2.60 ± 0.02 cd	8.61 ± 0.16 b	1.73 ± 0.02 g

Notes: Mean values followed by the same letters, a–g within columns across clones, are not significantly different at $P < 0.05$ (Duncan's multiple range test); [a] *p*-HBAc, *p*-hydroxybenzoic acid; *p*-HBA, *p*-hydroxybenzaldehyde; SAc, syringic acid; SYR, syringaldehyde; VAc, vanillic acid; VAN, vanillin; [b] S/G = (SAc + SYR)/(VAc + VAN).

Both the lignin content and the S/G ratio significantly affect the yield of sugars through hydrolysis. The saccharide release rate depends primarily on the saccharide type and the method of hydrolysis or pretreatment. According to Studer *et al.* [19] a strong negative correlation between sugar release and lignin content was found only for pretreated samples with an S/G ratio <2.0. For higher S/G ratios, sugar release was generally higher, and the negative influence of lignin was less pronounced. When examined separately, it was found that only glucose release correlated with lignin content and S/G ratio in this manner, whereas, the release of xylose was dependent on the S/G ratio alone [19]. Thus, with respect to the above mentioned, it may be stated that the 'Villafranca' clone is most suitable for hydrolysis treatment, as it demonstrated the lowest lignin content at all experimental sites (17.68%–18.84%) together with a high S/G ratio (2.45–2.64).

2.3. Lignin Content—S/G Ratio Relationship and Higher Heating Values

We tested the correlation between lignin content and S/G ratio for each experimental site. The closest negative relationship was found for the site at Sliepkovce ($r = -0.79$, $P < 0.001$), where the S/G ratio was dependent on the lignin content according to the equation $y = 4.129 - 0.085x$ ($R^2 = 0.62$). A similar close relationship was also found for the site at Gabčíkovo ($r = -0.78$, $P < 0.001$, $y = 4.927 - 0.135x$, $R^2 = 0.61$). However, this relationship had a lower correlation coefficient for the site at Selice ($r = -0.60$, $P < 0.001$, $y = 6.010 - 0.173x$, $R^2 = 0.36$). Taken together, the relationship was significant for the data representing all the experimental sites ($r = -0.55$, $P < 0.001$, $y = 4.418 - 0.102x$, $R^2 = 0.31$).

The results of some authors showed a decrease in lignin content with increasing S/G ratio among poplars [21,34]. However, no correlation was observed between the two variables of poplars grown for 2 years in the field or greenhouse [20]. Bose *et al.* [21] discovered that the lignin content for 13 poplars harvested from two different sites decreased from approximately 28% for an S/G of 1.0 to approximately 16.5% for an S/G of 1.68 ($y = 42.886 - 15.131x$, $R^2 = 0.8463$). The correlation was closer ($y = 42.366 - 15.123x$, $R^2 = 0.932$) in samples from a different site suggesting a dependency on geographic location. Our results confirmed the negative correlation between S/G ratio and lignin content determined in the genera *Populus* [21,35] and *Eucalyptus* [36,37]. In addition, we also showed that the closeness of correlation depends on the experimental site. In addition, we calculated HHV based on Equations 1–3 (see Part 3, Experimental Section). Significantly higher values were observed when using the equation according to White [16] (overall mean of HHV = 19.60 ± 0.10 kJ g^{-1}), followed by the equation according to Demirbas [38] (overall mean of HHV = 18.84 ± 0.14 kJ g^{-1}). The lowest values were observed when using the equation according to Telmo and Lousada [23] (overall mean of HHV = 17.20 ± 0.16 kJ g^{-1}). 'Baka 5', 'I-214', and 'BL Constanzo' had the highest heating values regardless of the method of calculation. On the other hand, 'NE-367', 'Agathe F', and 'Villafranca' were among the clones with the lowest values (Table 7).

Table 7. Higher heating values (kJ g^{-1}) for the examined clones of *Populus* as calculated from Equations 1–3. Data represent means ± SD.

Clone	Equation 1 [16]	Equation 2 [38]	Equation 3 [23]
'Agathe F'	19.52 ± 0.13 b	18.79 ± 0.15 cd	17.06 ± 0.22 c
'BL Constanzo'	19.63 ± 0.04 ab	18.92 ± 0.04 abc	17.24 ± 0.06 abc
'I-214'	19.71 ± 0.03 a	19.00 ± 0.06 a	17.37 ± 0.05 ab
'NE-367'	19.51 ± 0.03 b	18.77 ± 0.06 d	17.04 ± 0.06 c
'Pannonia'	19.57 ± 0.03 b	18.85 ± 0.02 bcd	17.14 ± 0.05 c
'Robusta'	19.57 ± 0.03 b	18.84 ± 0.03 bcd	17.14 ± 0.04 c
'Villafranca'	19.55 ± 0.10 b	18.59 ± 0.06 e	17.19 ± 0.18 bc
'Baka 5'	19.73 ± 0.08 a	18.97 ± 0.10 ab	17.42 ± 0.14 a

Notes: Mean values followed by the same letters, a–e within columns across clones, are not significantly different at $P < 0.05$ (Duncan's multiple range test).

The results calculated according to Equations 1 and 3 agree well with the published data for poplar wood: 19.38 kJ g^{-1} [5], 19.65 kJ g^{-1} [16], 19.3 kJ g^{-1} [22], 17.340–18.743 kJ g^{-1} [26], 18.60–19.27 kJ g^{-1} [39]. Klasnja *et al.* [40] determined that calorific value depends on the age of the poplar clone. For clone 'I-214', the highest heating values were determined in 2-year-old cuttings (more than 24 kJ g^{-1}), and in 12-year-old plants HHV were about 19 kJ g^{-1}. Lower HHV calculated in accordance with Equation 3 can be explained by the fact that authors [23] analysed specimens with a high content of extractive matters (7.01%–9.60%), which could influence the coefficients in Equation 3. Comparisons of the published data obtained by calorimetry and calculated HHV, according to Equations 1–3 and based on the chemical composition of poplar clones, allow us to state that these calculations can be used to determine the calorific value of poplar clones.

3. Experimental Section

3.1. Plant Material, Study Sites and Sampling

Eight certified *Populus* clones of the 'tested' category (EC Directive 105/1999/EC) were used in the study: six clones of *P.* × *euramericana* (Dode) Guinier hybrids ('Agathe F', 'BL Constanzo', 'I-214', 'NE-367', 'Pannonia', and 'Robusta'), a single clone of *P. alba* (L.) ('Villafranca'), and a single clone of *P. nigra* (L.) ('Baka 5'). Secondary poplar plantations were established in three different experimental sites comprised mainly of mixed oak–elm–ash floodplain forests. These study sites were located in Slovakia at Sliepkovce (48°40'N, 21°57'E, the soil is Eutric Fluvisol with a pH-H_2O of 7.29), at Selice (48°3'N, 17°57'E, the soil is Calcaric Fluvisol with a pH-H_2O of 7.60), and at Gabčíkovo (47°53'N, 17°35'E, the soil is Calcaric Fluvisol with a pH-H_2O of 7.75). The planted trees were spaced at a distance of 3 m × 3 m. In June 2009, four ramets per clone and site, at least 16 years of age, were nondestructively sampled by the removal of microcores at breast height, *i.e.*, 1.3 m from the tree base. The bark was removed and the biomass was stored at −18 °C prior to chemical analyses.

3.2. Chemical Analyses

Separated wood was mechanically disintegrated to sawdust, and the fraction of the size 0.5–1.0 mm was extracted in the Soxhlet apparatus with a mixture of ethanol and toluene (2:1 v/v). Extractives were determined according to the ASTM standard procedure D1107–96. Measurements were performed on four replicates per clone. Lignin content was determined according to the ASTM standard procedure D1106–96. Measurements were performed on four replicates per clone. Holocellulose content was determined using the method of Wise *et al.* [41] and cellulose content by the Seifert method [29]. Measurements were performed on four replicates per clone. Data were presented as the percentages of oven-dry weight (odw) per unextracted wood.

Nitrobenzene oxidation was carried out using 2 M NaOH in 10 mL stainless steel vessels at a temperature of 180 °C for 2 h, and oxidation products were analyzed by the isocratic high-performance liquid chromatography at conditions as follows: LiChrospher 100 RP-18, 5 µm, 4 × 100 mm ID column (Merck KGaA, Darmstadt, Germany), mobile phase water:methanol:acetic acid (850:150:1), flow rate of 1.0 mL min^{-1}, column temperature of 35 °C, detection via diode array detector in the 210–360 nm region. The quantities of nitrobenzene oxidation products were determined using the method of direct calibration [42]. Measurements were performed on four replicates per clone. An S/G ratio was calculated by the formula: S/G = (syringyl aldehyde + syringic acid) / (vanillin + vanillic acid).

3.3. The Higher Heating Value Calculation

HHV is an important trait in wood utilization for energy. It can be determined either by the use of a bomb calorimeter or by using calculations in accordance with the lignin and extractives content of the specimen. The HHV in our study was calculated using the equations of White [16] [Equation (1)], Demirbas [38] [Equation (2)], and Telmo and Lousada [23] [Equation (3)]:

$$\text{HHV (Btu lb}^{-1}) = 7{,}696 + 32.0(\text{L}) + 28.4(\text{E}) \quad (1)$$

$$\text{HHV (kJ g}^{-1}) = 0.0893(\text{L}) + 16.9742 \quad (2)$$

$$\text{HHV (kJ g}^{-1}) = 14.3377 + 0.1228(\text{L}) + 0.1353(\text{E}) \quad (3)$$

where HHV is higher heating value of unextracted wood, L is Klason lignin content (% odw, extractives-free wood), and E is the content of extractives (% odw).

3.4. Statistical Analysis

Data were normally distributed and thus subjected to one-way analysis of variance (ANOVA). Duncan's multiple range tests were used for pairwise comparisons of means. The effects of the experimental site and the clone × site interaction were tested by two-way ANOVA.

4. Conclusions

Among the examined poplar clones, the chemical profiles of the main wood components, extractives, and S/G ratio in lignin were influenced both by the experimental site and the clone × site interaction. The most suitable clones for energy utilization by combustion are 'Baka 5' and 'I-214'.

They have the highest heating values due to their high content of lignin and extractives. Our results confirmed the negative correlation between S/G ratio and lignin content. The 'Villafranca' clone is most suitable for hydrolysis treatment because it has the lowest lignin content at all experimental sites together with a high S/G ratio. The optimal method of poplar biomass utilization can be thus chosen on basis of the lignocellulosics chemical composition and the S/G ratio in lignin.

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References

1. Sasaki, N.; Owari, T.; Putz, F.E. Time to substitute wood bioenergy for nuclear power in Japan. *Energies* **2011**, *4*, 1051–1057.
2. Ragauskas, A.J.; Williams, C.K.; Davison, B.H; Britovsek, G.; Cairney, J.; Eckert, C.A.; Frederick, W.J.; Hallett, J.P.; Leak, D.J.; Liotta, C.L.; *et al.* The path forward for biofuels and biomaterials. *Science* **2006**, *311*, 484–489.
3. Dinus, R.J. Genetic modification of short rotation poplar biomass feedstock for efficient conversion to ethanol. Oak Ridge National Laboratory Website. Available online: http://www.ornl.gov/~webworks/cpr/v823/rpt/108278.pdf (accessed on 30 July 2012).
4. Dinus, R.J. Genetic improvement of poplar feedstock quality for ethanol production. *Appl. Biochem. Biotechnol.* **2001**, *91–93*, 23–34.
5. Sannigrahi, P.; Ragauskas, A.J.; Tuskan, G.A. Poplar as a feedstock for biofuels: A review of compositional characteristics. *Biofuels Bioprod. Bioref.* **2010**, *4*, 209–226.
6. Lucia, L.A.; Argyropoulos, D.S.; Adamopoulos, L; Gaspa, A.R. Chemicals and energy from biomass. *Can. J. Chem.* **2006**, *84*, 960–970.
7. Ragauskas, A.J.; Nagy, M.; Kim, D.H.; Eckert, C.A.; Hallett, J.P.; Liotta, C.L. From wood to fuels. Integrating biofuels and pulp production. *Ind. Biotechnol*. **2006**, *2*, 55–65.
8. Hamaguchi, M.; Cardoso, M.; Vakkilainen, E. Alternative technologies for biofuels production in kraft pulp mills—Potential and prospects. *Energies* **2012**, *5*, 2288–2309.
9. Carroll, A.; Somerville, C. Cellulosic biofules. *Annu. Rev. Plant Biol.* **2009**, *60*,165–182.
10. Kumar, A.; Jones, D.D.; Hanna, M.A. Thermochemical biomass gasification: A review of the current status of the technology. *Energies* **2009**, *2*, 556–581.
11. Chen, Q.; Jin, Y.; Zhang, G.; Fang, Y.; Xiao, Y.; Zhao, H. Improving production of bioethanol from Duckweed (*Landoltia punctata*) by pectinase pretreatment. *Energies* **2012**, *5*, 3019–3032.
12. Chen, Z.H.; Mao, Q.S. Characteristics and papermaking properties of the triploid of *Populus tomentosa*. *China Pulp Pap. Ind.* **2000**, *21*, 17–20.

13. Leplé, J.C.; Dauwe, R.; Morreel, K.; Storme, V.; Lapierre, C.; Pollet, B.; Naumann, A.; Kang, K.; Kim, H.; Ruel, K.; *et al.* Downregulation of cinnamoyl-coenzyme a reductase in poplar: Multiple-level phenotyping reveals effects on cell wall polymer metabolism and structure. *Plant Cell* **2007**, *19*, 3669–3691.
14. Yang, G.; Lucia, L.A.; Chen, J.; Cao, X.; Liu, Y. Effects of enzyme pretreatment on the beatability of fast-growing poplar APMP pulp. *BioResources* **2011**, *6*, 2568–2580.
15. Zhou, G.; Taylor, G.; Polle, A. FTIR-ATR-based prediction and modelling of lignin and energy contents reveals independent intraspecific variation of these traits in bioenergy poplars. *Plant Methods* **2011**, *7*, doi:10.1186/1746-4811-7-9.
16. White, R.H. Effect of lignin content and extractives on the higher heating value of wood. *Wood Fiber Sci.* **1987**, *19*, 446–452.
17. Susott, R.A.; DeGroot, W.F.; Shafizadeh, F. Heat content of natural fuels. *J. Fire Flammabil.* **1975**, *6*, 311–325.
18. Demirbas, A. *Biorefineries*: *For Biomass Upgrading Facilities*, 2nd ed.; Springer: London, UK, 2010; pp. 46–47.
19. Studer, M.H.; DeMartini, J.D.; Davis, M.F.; Sykes, R.W.; Davison, B.; Keller, M.; Tuskan, G.A.; Wyman, C.E. Lignin content in natural *Populus* variants affects sugar release. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6300–6305.
20. Lapierre, C.; Pollet, B.; Petit-Conil, M.; Toval, G.; Romero, J.; Pilate, G.; Leplé, J.-C.; Boerjan, W.; Ferret, V.; Denadai, V.; *et al*. Structural alterations of lignins in transgenic poplars with depressed cinnamyl alcohol dehydrogenase or caffeic acid *O*-methyltransferase activity have an opposite impact on the efficiency of industrial kraft pulping. *Plant Physiol.* **1999**, *119*, 153–163.
21. Bose, S.; Francis, R.; Govender, M.; Bush, T.; Spark, A. Lignin content *versus* syringyl to guaiacyl ratio amongst poplars. *Bioresour. Technol.* **2009**, *100*, 1628–1633.
22. Luo, Z.; Polle, A. Wood composition and energy content in a poplar short rotation plantation on fertilized agricultural land in a future CO_2 atmosphere. *Glob. Change Biol.* **2009**, *15*, 38–47.
23. Telmo, C.; Lousada, J. The explained variation by lignin and extractive contents on higher heating value of wood. *Biomass Bioenergy* **2011**, *35*, 1663–1667.
24. Balatinecz, J.J.; Kretschmann, D.E. Properties and utilization of poplar wood. In *Poplar Culture in North America*; Dickmann, D.I., Isebrands, J.G., Eckenwalder, J.E., Richardson, J., Eds.; NRC Research Press: Ottawa, Canada, 2001; Part A, pp. 277–291.
25. Fengel, D.; Wegener, G. *Wood: Chemistry*, *Ultrastructure*, *Reactions*; Verlag Kessel: München, Germany, 2003; pp. 26–65.
26. Klasnja, B.; Orlovic, S.; Galic, Z. Chemical composition variability of juvenile wood of selected poplar clones. *Wood Res*. **2005**, *50*, 19–26.
27. Kürschner, K.; Hoffer, A. Ein neues Verfahren zur Bestimmung der Cellulose in Hölzern und Zellstoffen. *Tech. Chem. Pap. Zellst. Fabr*. **1929**, *26*, 125–129.
28. Seifert, V.K. Über ein neues verfahren zur schnellbestimmung der rein-cellulose. *Papier* **1956**, *10*, 301–306.
29. Seifert, V.K. Zur Frage der cellulose—schnellbestimmung nach der Acetylaceton-methode. *Papier* **1960**, *14*, 104–106.

30. Wright, P.; Wallis, A. Rapid determination of cellulose in plantation eucalypt woods to predict kraft pulp yields. *Tappi J*. **1998**, *81*, 126–130.
31. Kučerová, V.; Kačík, F.; Solár, R.; Sivák, J. Comparison of various methods of cellulose determination after thermal loading of spruce wood. *Acta Fac. Xylol. Zvolen* **2009**, *51*, 5−10.
32. Wyman, C.E.; Dale, B.E.; Elander, R.T.; Holtzapple, M.; Ladisch, M.R.; Lee, Y.Y.; Mitchinson, C.; Saddler, J.N. Comparative sugar recovery and fermentation data following pre-treatment of poplar wood by leading technologies. *Biotechnol. Prog*. **2009**, *25*, 333–339.
33. Chen, F.; Dixon, R.A. Lignin modification improves fermentable sugar yields for biofuel production. *Nat. Biotechnol.* **2007**, *25*, 759–761.
34. Davison, B.H.; Drescher, S.R.; Tuskan, G.A.; Davis, M.F.; Nghiem, N.P. Variation of S/G ratio and lignin content in a *Populus* family influences the release of xylose by dilute acid hydrolysis. *Appl. Biochem. Biotechnol*. **2006**, *130*, 427–435.
35. Li, L.; Zhou, Y.; Cheng, X.; Sun, J.; Marita, J.M.; Ralph, J.; Chiang, V.L. Combinatorial modification of multiple lignin traits in trees through multigene cotransformation. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4939–4944.
36. Stackpole, D.; Vaillancourt, R.; Alves, A.; Rodrigues, J.; Potts, B. Genetic variation in the chemical components of *Eucalyptus globulus* wood. *G3* **2011**, *1*, 151–159.
37. Yamada, T.; Yeh, T.F.; Chang, H.M.; Li, L.; Kadla, J.F.; Chiang, V.L. Rapid analysis of transgenic trees using transmittance near-infrared spectroscopy (NIR). *Holzforschung* **2006**, *60*, 24–28.
38. Demirbas, A. Relationships between lignin contents and heating values of biomass. *Energy Convers. Manag.* **2001**, *42*, 183–188.
39. Benetka, V.; Bartáková, I.; Mottl, J. Productivity of *Populus nigra* ssp. *nigra* under short-rotation culture in marginal areas. *Biomass Bioenergy* **2002**, *23*, 327–336.
40. Klasnja, B.; Kopitovic, S.; Orlovic, S. Wood and bark of some poplar and willow clones as fuelwood. *Biomass Bioenergy* **2002**, *23*, 427–432.
41. Wise, L.E.; Murphy, M.; D'Addieco, A.A. Chlorite holocellulose, its fraction and bearing on summative wood analysis and on studies on hemicelluloses. *Paper Trade J*. **1946**, *122*, 35–43.
42. Kačík, F.; Solár, R.; Baloghová, D. Analysis of lignin nitrobenzene oxidation products by high performance liquid chromatography (HPLC). *Wood Res*. **1995**, *40*, 13–20.