Journal of Analytical and Applied Pyrolysis 124 (2017) 230–238

Contents lists available at ScienceDirect

Journal of Analytical and Applied Pyrolysis

journal homepage: www.elsevier.com/locate/jaap

Application of orbitrap mass spectrometry for analysis of model bio-oil compounds and fast pyrolysis bio-oils from different biomass sources

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ARTICLE INFO

Article history:
Received 30 July 2016
Received in revised form 26 January 2017
Accepted 1 February 2017
Available online 10 February 2017

Keywords:
Ablative flash pyrolysis
Pyrolysis bio-oil
Orbitrap mass spectrometry
Electrospray
Atmospheric pressure chemical ionization

ABSTRACT

Pyrolysis bio-oils have great potential for the future use as biofuels and source of oxygenated chemicals. To optimize a pyrolysis process, detailed knowledge about the chemical composition of bio-oils is necessary. In recent years, high-resolution mass spectrometry (HRMS) has successfully been used to the characterization of pyrolysis bio-oils from lignocellulosic biomass. This method enabled to detect thousands of semivolatile and nonvolatile, high-molecular-weight bio-oil compounds and provided partial information about their structure. In this work, we used high-resolution orbitrap mass spectrometry to characterize semivolatile and nonvolatile, high-molecular-weight compounds of four bio-oils obtained from the ablative flash pyrolysis of different biomass sources. Before the analyses of these bio-oils, we analyzed model bio-oil compounds and commercially available bio-oil from fast pyrolysis of wood using positive-ion and negative-ion electrospray (ESI) and positive-ion and negative-ion atmospheric pressure chemical ionization (APCI) orbitrap mass spectrometry and compared the results. Based on this comparison, a combination of negative-ion ESI and APCI was found to be well suited for the characterization of pyrolysis bio-oils; these techniques were thus used for the study of bio-oils from different biomass sources and the obtained results were compared. In the studied bio-oils, mostly compounds with 1–8 oxygen atoms per molecule were detected and their degree of unsaturation (DBE) was about 1–10 (negative-ion ESI) and 1–17 (negative-ion APCI), respectively. Among the studied bio-oils, the differences were observed mostly in abundances of their major compounds (compound classes). The analyses of model bio-oil compounds brought valuable information about their behavior during the HRMS characterization of bio-oils. The presented results could help to improve the understanding of bio-oil composition and HRMS characterization of bio-oils and facilitate their further utilization.

1. Introduction

Pyrolysis bio-oils are liquids produced by pyrolysis of biomass. They are thus renewable and environmentally friendly feedstock and in the future, they could be widely used as biofuels or as sources of valuable oxygenated chemicals. Besides great potential for the future use, bio-oils also have some undesirable properties (e.g. chemical instability, high polarity and immiscibility with petroleum fuels) that need to be improved to achieve a more widespread use. To optimize a process of bio-oil production and obtain bio-oils with the desired properties, detailed knowledge of their chemical composition is necessary [1].

High-resolution mass spectrometry (HRMS) has successfully been used to characterize semivolatile and nonvolatile, high-molecular-weight bio-oil compounds [2–13]. This method enabled to detect thousands of bio-oil compounds and obtain partial information about their structure. HRMS characterization of complex mixtures requires powerful high-resolution mass spectrometers (FT-ICR, orbitrap) to uniquely and simultaneously resolve and

Abbreviations: APCI, atmospheric pressure chemical ionization; APPI, atmospheric pressure photoionization; Da, Dalton; DBE, double bond equivalents (number of rings and double bonds, degree of unsaturation); ESI, electrospray ionization; FT-ICR, Fourier transform ion cyclotron resonance; GC, gas chromatography; HRMS, high-resolution mass spectrometry; LC, liquid chromatography; LDI, laser desorption ionization; MS, mass spectrometry.

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http://dx.doi.org/10.1016/j.jaap.2017.02.002

Table 1
Physical and chemical properties of the studied bio-oils from ablative flash pyrolysis of different biomass sources (elemental composition corrected for the amount of water).

Bio-oil	Density 15 °C [g cm^{-3}]	Kin. viscosity 40 °C [mm^2 s^{-1}]	Water content [wt%]	C [wt%]	H [wt%]	N [wt%]	S [wt%]	O [wt%]
2SWO	1.184	18.69	45.0	54.8	7.1	0.029	0.004	38.1
7BWO	1.202	19.95	24.0	56.3	7.2	0.063	0.005	39.1
42PWO	1.167	6.69	32.8	60.4	7.3	0.150	0.013	36.6
95MO	1.158	4.99	37.0	54.3	7.7	0.444	0.041	37.5

identify each of the thousands of compounds present in the analyzed sample. In high-resolution mass spectrometry measurements, exact molecular masses of the present compounds are measured and from them, elemental compositions of these compounds are determined. The obtained elemental compositions are subsequently categorized into different compounds classes according to their heteroatom numbers ($N_nO_oS_s$), carbon numbers and DBE (double bond equivalents) to obtain a fingerprint of the sample [1]. In petroleomic analysis of pyrolysis bio-oils, negative-ion HRMS has typically been applied [3–13].

Negative-ion mass spectrometry is a well suited and widely applied method for the characterization of pyrolysis bio-oils due to its great ability to ionize and detect oxygen-containing bio-oil compounds [1]. Bio-oil analyses using negative-ion HRMS were usually focused on bio-oil characterization based on identified molecular formulas and following soft ionization techniques were mostly applied: ESI, APCI and laser desorption ionization (LDI) [3–13]. Negative-ion ESI is the most commonly applied ionization technique for the HRMS analysis of bio-oils, because this method enables to readily ionize oxygen-containing (medium polar and polar) bio-oil compounds and both carbohydrates and phenolic compounds can be detected [3–13]. Application of negative-ion LDI [2,6] and APCI [10] was also reported; APCI enables to ionize low polar to polar compounds and LDI ionizes nonvolatile species with low to medium polarity that absorb the light of the used laser (especially highly unsaturated compounds), but it usually fails to detect carbohydrates [1,2].

Positive-ion mass spectrometry was also reported for the pyrolysis bio-oil characterization, especially in recent time [11–14]. Cole et al. [11] used positive-ion ESI, positive-ion atmospheric pressure photoionization (APPI) and negative-ion ESI orbitrap mass spectrometry to analyze nitrogen-containing species in pyrolysis bio-oils from switchgrass. Among these techniques, positive-ion ESI was found to be the most appropriate for the analysis of nitrogen-containing species in bio-oils, while the other techniques enabled to detect especially oxygen-containing compounds. Using positive-ion ESI, about 300 different nitrogen-containing compounds were detected. Pyridine and imidazole were assigned to be the major structural motifs for N_1 and NO classes, respectively [11]. Santos et al. [12] used positive-ion and negative-ion electrospray FT-ICR mass spectrometry to analyze pyrolysis bio-oils from freshwater plants. In the positive-ion ESI spectra, $N_{1-3}O_{0-3}$ compounds were predominant and N_2 compounds were the most abundant of them. On the other hand, O_2–O_5 compounds (mainly phenols and carboxylic acids) and $N_{1-3}O_{2-3}$ compounds dominated in negative-ion ESI spectra [12]. Smith et al. [13] studied bio-oil aging using positive-ion APPI and negative-ion ESI. Both carbohydrates and phenolic compounds were observed in negative-ion ESI whereas aromatic compounds dominated in positive-ion APPI [13]. Bai et al. [14] used positive-ion APPI FT-ICR mass spectrometry to study the formation of phenolic compounds during the fast pyrolysis of lignin. Despite the fact that the application of positive-ion mass spectrometry in bio-oil analytics was already reported, direct comparison of positive-ion and negative-ion ESI and APCI for the HRMS characterization of pyrolysis bio-oils has not been performed yet.

Here, we used orbitrap mass spectrometry to analyze model bio-oil compounds, commercially-produced bio-oil obtained by fast pyrolysis of wood and four in lab-scale produced bio-oils obtained by ablative flash pyrolysis of different biomass sources (spruce wood, beech wood, poplar wood and miscanthus) using different ionization techniques. Based on the analyses of the model bio-oil compounds and commercial bio-oil, we chose a suitable combination of ionization techniques for the subsequent chemical characterization of the ablative flash pyrolysis bio-oils. The presented results can improve the understanding of bio-oil composition and HRMS characterization of bio-oils and facilitate their further utilization.

2. Experimental

2.1. Samples

A pyrolysis bio-oil sample used in this work was purchased from BTG Biomass Technology Group BV, Netherlands. The commercial BTG bio-oil was produced by the fast pyrolysis of wood at 550 °C in a rotating cone reactor. More details about the BTG rotating cone reactor technology can be found elsewhere [15]. Physical and chemical properties of the studied pyrolysis bio-oil were published elsewhere [10].

The other bio-oils used in this study were obtained from ablative flash pyrolysis of different biomass sources: spruce wood (2SWO), beech wood (7BWO), poplar wood (42PWO) and miscanthus (95MO). Physical and chemical properties of these bio-oils are presented in Table 1. The bio-oils were produced by ablative flash pyrolysis, in which the wood and grass samples (either wood logs or pelletized wood chips/grass, both with a diameter of 50 mm) were pressed onto a hot rotating disc at temperatures around 550 °C and a vapor residence time in the reactor of less than 1 s. The bio-oil was condensed at 2–5 °C and its yield amounted to 40–70 wt%. More details about the experimental setup are presented elsewhere [16].

2.2. Reagents and standards

All analytical standards (see Table S1) used except 5-methylfurfural were purchased from Sigma–Aldrich in purities ≥98%; 5–methylfurfural (98%) was purchased from Merck. An LC–MS grade methanol that was used for dissolution of analytical standards and pyrolysis bio–oil prior to the analyses was purchased from Sigma–Aldrich. Formic acid (eluent additive for LC–MS) was purchased from Fluka.

2.3. Bulk properties: density, viscosity, water content and elemental composition of the ablative flash pyrolysis bio-oils

Density and viscosity of the studied samples were determined according to EN ISO 12185 and ASTM D445, respectively. The details of water content and elemental composition (CH–O) measurements are described in detail elsewhere [10]. Sulfur a nitrogen contents were determined according to ASTM D5453 and ASTM D5762, respectively.

Table 2
Conditions of (+/−) ESI-MS and APCI-MS analyses of bio-oils and analytical standards.

Ionization	Parameter	Value
ESI (+/−)	Sheath gas flow	18 AU
	Auxiliary gas flow	7 AU
	Sweep gas flow	0 AU
	Ion source temperature	250 °C
	Spray voltage	3.5 kV (+), −2.5 kV (−)
	Scanning range	100–1000 Da
	Injection (via sample loop)	5 μl into the flow of CH_3OH (150 μl min^{-1})
	Capillary temperature	300 °C
APCI (+/−)	Sheath gas flow	40 AU
	Auxiliary gas flow	10 AU
	Sweep gas flow	0 AU
	Ion source temperature	300 °C
	Spray current	4 μA
	Scanning range	100–1000 Da
	Injection (via sample loop)	5 μl into the flow of CH_3OH (150 μl min^{-1})
	Capillary temperature	300 °C

2.4. Orbitrap mass spectrometry data acquisition and analysis

2.4.1. Sample treatment

Samples of the commercial BTG bio-oil and studied bio-oils were prepared as follows: stock solutions were prepared by diluting 10 μl of bio-oil in 1 ml of methanol (LC–MS grade, Sigma–Aldrich) and 250 μl of the stock solutions were further diluted in 1 ml of methanol to obtain final samples. Analytical standards were dissolved in methanol to obtain stock solutions with concentrations of 3 mg ml^{-1} and these were further diluted to obtain final concentrations of 15 μg ml^{-1}.

2.4.2. Data acquisition

A Thermo-Fisher Scientific LTQ Orbitrap Velos equipped with an Ion Max API source with HESI-II and APCI probes was used for analyses. Mass spectra were acquired in the positive- and negative-ion mode at mass resolution of 100 000 (*m*/*z* 400). Two ionization techniques were applied: ESI and APCI. Conditions of analyses of pyrolysis bio-oils and analytical standards are presented in Table 2. To ensure greater mass accuracy, following substances were applied as lock mass compounds in ESI-MS and APCI-MS spectra: diisooctylphtalate, *m*/*z* 391.2843 (positive-ion mode) and palmitic acid, *m*/*z* 255.2329 (negative-ion mode).

2.4.3. Data analysis

Instrument control and data acquisition were performed using Xcalibur 2.2 software (Thermo-Fisher Scientific). After acquisition of the raw data, two pre-processing steps were performed in Xcalibur: recalibration and generation of spectrum lists. For the recalibration, we used RecalOffline tool (a part of Xcalibur) that enables to perform a single-point recalibration. For the recalibration, following peaks were used as an anchor: $[C_6H_{10}O_5-H]^-$, *m*/*z* 161.0455 (negative-ion mode) and $[C_9H_{10}O_3+H]^+$, *m*/*z* 167.0701 (positive-ion mode). After the recalibration, spectrum lists were generated. The spectrum lists contained the *m*/*z* of the detected signal as well as its absolute and relative intensity, mass error in ppm, DBE and assigned elemental compositions. These spectrum lists were exported into MS Excel and further processed. Criteria for the assignments of elemental compositions are presented in Table 3. Only ions with relative abundances higher than 0.15% were analyzed. For the elemental compositions that were observed concurrently within the sample and blank spectra, final intensities were determined as difference of sample and blank spectra intensities. Mass peaks relevant to isotopic distributions were identified and deleted. A Kendrick mass defect analysis (tolerance ±0.001) was performed to confirm the correctness of the assignments of elemental compositions.

Table 3
Criteria for the assignments of elemental compositions to the detected peaks.

Parameter	Value
C	0–35
H	0–60
N	0–2[a]
O	0–20
S	0–1
Na	0–1[b]
DBE	0–30
Charge	±1
Tolerance error	3 ppm

[a] 0–5 for ESI (+).
[b] 0–1 for ESI (+), 0 for other techniques.

3. Results and discussion

3.1. Positive-ion and negative-ion ESI and APCI orbitrap mass spectrometry analysis of the commercial BTG bio-oil

Positive-ion and negative-ion ESI and APCI orbitrap mass spectra of the commercial BTG bio-oil are presented in Fig. 1. Negative-ion ESI and APCI orbitrap mass spectra of this bio-oil were already presented and discussed in our previous paper (Staš et al. [10]).

Briefly, in the *negative-ion ESI* spectrum, compounds with molecular weights in the range of ~100–450 Da were detected. These compounds contained 1–12 oxygen atoms per molecule and their DBE number was 1–18. Among the detected compounds, those with four oxygen atoms and DBE 2–3 were the most abundant in the spectrum (see Fig. 2). In the negative-ion ESI, bio-oil compounds were detected solely as $[M-H]^-$ions, i.e. in deprotonated form [10].

In the *negative-ion APCI* spectrum, compounds with molecular weights in the range of ~100–650 Da were observed. The detected compounds belonged into following heteroatom and DBE classes: O_1–O_{12} and DBE 1–22. Among the detected compounds, those with 3–5 oxygen atoms per molecule and DBE 5–7 were the most abundant in the spectrum. In the negative-ion APCI, most of bio-oil compounds formed only deprotonated ions, but some compounds formed both deprotonated and molecular ions (i.e. $M^{\bullet-}$); intensities of odd-electron molecular ions were significantly lower (~1:5) in comparison to even-electron deprotonated ions and only even-electron ions were thus considered [10].

Positive-ion APCI spectrum consisted of signals of compounds with the molecular weight range of about 100–650 Da. The number of oxygen atoms in molecules of the detected compounds was 1–12 and their DBE was 1–22. The most abundant compounds in the spectrum were those with 2–4 oxygen atoms in molecule and DBE 5–7. Bio-oil compounds were detected as protonated ions, i.e. $[M + H]^+$.

Positive-ion ESI spectrum differed significantly from the other spectra; O_1–O_{11} and $N_{1-3}O_{0-10}$ compounds with DBE 1–26 were detected. In the spectrum, compounds with DBE 2–6 were the most abundant. Among the O_x compounds, those with 2–4 oxygen atoms per molecule were predominant (see Fig. 3). Among the nitrogen-containing compounds, N_1O_{0-10} class was the most abundant (~20%). Within this class, N_1O_{2-4} compounds were the most abundant in the spectrum (3.3, 4.3 and 2.7%, respectively). Considering the low content of nitrogen in the studied bio-oil (~0.1 wt.%, see Staš et al. [10]), we can conclude that the ionization yields of nitrogen-containing compounds were high, because the relative intensity of nitrogen-containing compounds in the spectrum was

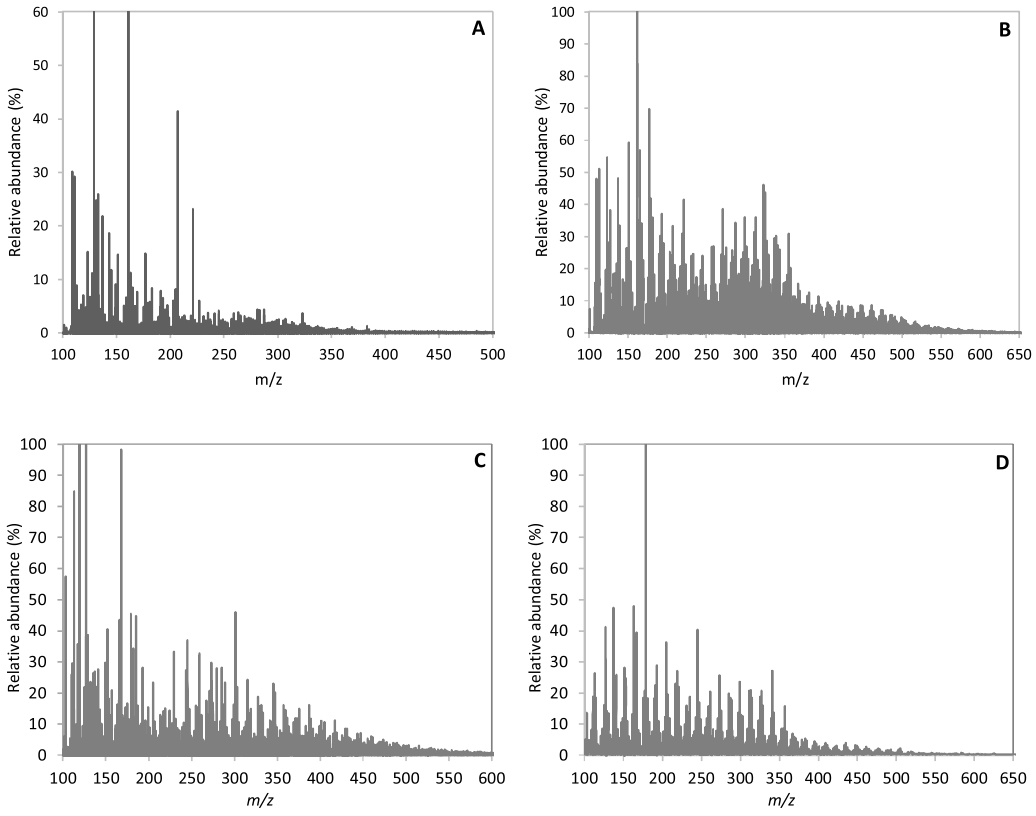

Fig. 1. Negative-ion ESI (A) and APCI (B) and positive-ion ESI (C) and APCI (D) orbitrap mass spectra of the commercial BTG bio-oil [10].

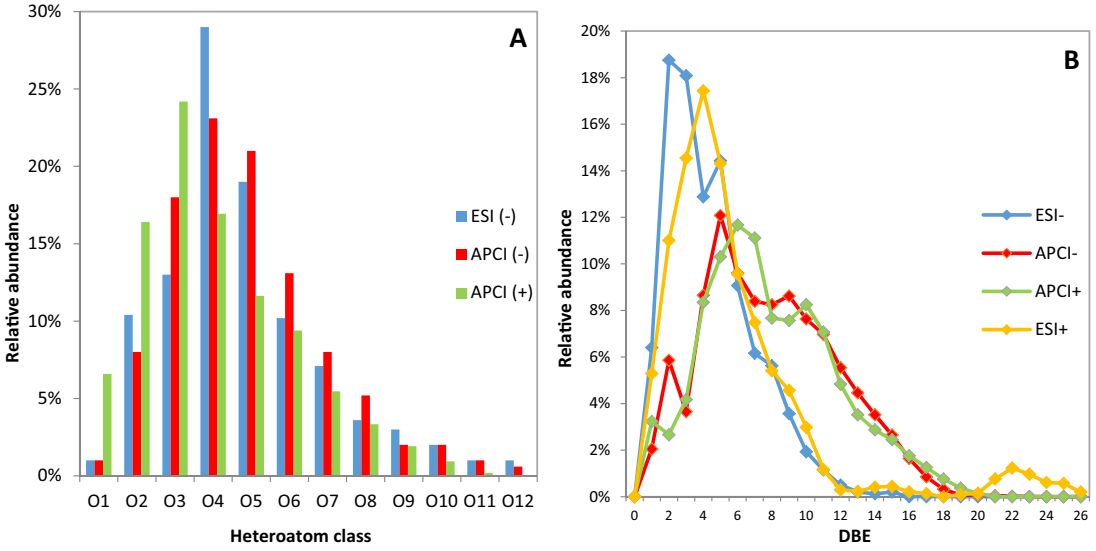

Fig. 2. Heteroatom class (A) and DBE (B) distributions obtained by orbitrap mass spectrometry using different ionization techniques (commercial BTG bio-oil).

about 25%. In the positive-ion ESI spectrum, mostly proton adducts $[M+H]^+$ or sodium adducts $[M+Na]^+$ were observed.

Overall, bio-oil compounds detected by positive-ion APCI and negative-ion ESI and APCI contained 1–12 oxygen atoms per molecule. Between these techniques, differences in relative abundances were observed within the heteroatom classes, but the overall trend in heteroatom class distributions was similar. DBE distribution obtained by positive-ion APCI showed similar trend as that of negative-ion APCI data, but different in comparison to negative-ion ESI data. Between positive-ion and negative-ion APCI, small differences were observed especially for DBE 1–2 and 5–9. In both positive-ion and negative-ion APCI spectra, the most abundant compounds were those with DBE 4–8, which should be mostly the decomposition products of lignin. On the other hand, negative-ion ESI preferred mostly decomposition products of holocellulose (DBE < 4). For this ionization technique, relative abundances of compounds with DBE 5 were higher and relative abundances of compounds with DBE > 6 were significantly lower in comparison to positive-ion and negative-ion APCI.

Our results indicate that positive-ion ESI did not provide representative information about the chemical composition of entire bio-oil, because it showed ability to predominantly ionize

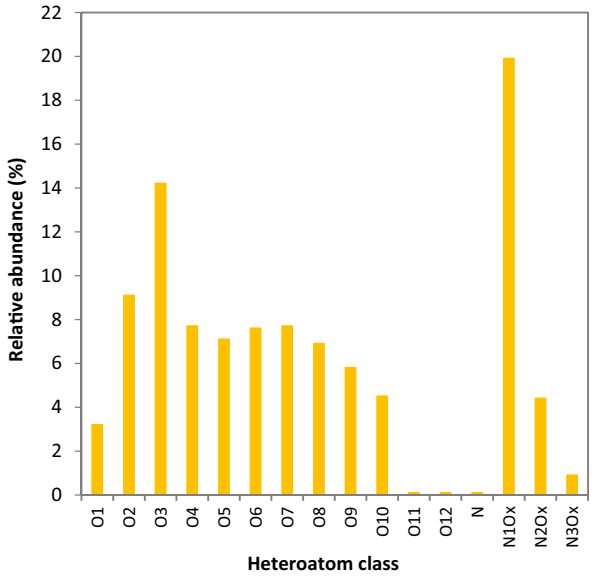

Fig. 3. Heteroatom class distribution obtained by positive-ion ESI orbitrap mass spectrometry (commercial BTG bio-oil).

the nitrogen-containing compounds of bio-oils that are typically present in low abundances. However, these results also indicate that positive-ion ESI could be used to characterize such nitrogen-containing species in bio-oils that are not detectable by other ionization techniques. Positive-ion APCI could be used to extend the knowledge of the chemical composition of pyrolysis bio-oils, because this method enabled to uniquely detect some bio-oil compounds that were not detectable by other ionization techniques. However, the sum of intensities of such compounds was very low (<0.5% of TIC).

3.2. Positive-ion and negative-ion ESI and APCI orbitrap mass spectrometry analysis of selected model bio-oil compounds

3.2.1. Selection of model bio-oil compounds

To obtain more information about the behavior of pyrolysis bio-oils (and their components) during an HRMS analysis, we performed positive-ion and negative-ion ESI and APCI analyses of model bio-oil compounds. For this study, we chose about 30 model compounds belonging to various compound classes that are typical for pyrolysis bio-oils from lignocellulosic biomass (e.g. methoxy- and dimethoxyphenols, methylphenols, carbohydrates, furans, ketones, pyrans, carboxylic acids, nitrogen-containing compounds etc.). Molecular weights of these compounds were mostly in the molecular weight range of ~100–200 Da. Structure of bio-oil compounds with molecular weights up to 220 Da is quite well known, because such compounds are detectable by GC-MS. On the other hand, information about the exact structure of bio-oil compounds with molecular weights more than 220 Da is only limited and thus, it is difficult to select model bio-oil compounds in this molecular weight range. We were aware that model compounds that were selected for this study may not representatively simulate behavior of entire pyrolysis bio-oils during their HRMS analysis. However, most of the selected model compounds are typically present in high abundances in pyrolysis bio-oils from lignocellulosic biomass. In addition, orbitrap mass spectrometers are known to have higher sensitivity for lower mass ions (<200 Da) in comparison to higher mass ions [3]. Therefore, we believed that analyses of our model compounds could bring at least more insight into orbitrap mass spectrometry characterization of entire pyrolysis bio-oils and also eventually evaluate possible applicability of different ionization techniques and/or ionization modes for HRMS characterization of bio-oils. Within the analyses of model compounds, we studied capabilities of positive-ion and negative-ion ESI and APCI to ionize and detect these model compounds and compared ionization yields (relative responses) of the model compounds within each ionization technique.

Table 4
Positive-ion and negative-ion ESI and APCI analyses of model compounds using orbitrap mass spectrometry.

Compound	Positive		Negative		Compound class
	ESI	APCI	ESI	APCI	
2-Acetylfuran	√	√	√	√	Furans
5-Methylfurfural	√	√	√	√	
5-(Hydroxymethyl)furfural	√	√	√	√	
Levoglucosan	√	–	√	√	Carbohydrates
D-Glucose	√	–	–	–	
D-Cellobiose	√	–	–	–	
3-Methyl-1,2,-cyclopentanedione	√	√	√	√	Ketones
Acetoxyacetone	√	√	–	√	
4-Hydroxy-4-methylpentanone	√	–	–	–	
o-Cresol	–	–	√	√	Methylphenols
m-Cresol	–	–	√	√	
p-Cresol	–	–	√	√	
3,5-Dimethylphenol	–	√	√	√	
Guaiacol	–	√	√	√	Methoxyphenols
4-Methylguaiacol	–	√	√	√	
Vanillin	√	√	√	√	
Eugenol	√	√	√	√	
Isoeugenol	–	√	√	√	
Acetoguaiacon	√	√	√	√	
Vanillic acid	–	√	√	√	
Isovanillic acid	–	√	√	√	
Coniferaldehyde	√	√	√	√	
Syringol	√	√	√	√	Dimethoxyphenols
4-Methylsyringol	√	√	√	√	
syringaldehyde	–	√	√	√	
Valeric acid	–	–	√	√	Others
2-Acetylpyrrol	√	√	–	–	
maltol	√	√	√	√	
2,6-Dimethylquinoline	√	√	–	–	

Fig. 4 and Table S2 present relative responses of the model compounds in the tested ionization techniques. For calculation of the relative responses, we chose the peak area of the positive-ion ESI signal of 4-methylsyringol to be the reference value. 4-Methylsyringol was detected by all tested ionization techniques and its signal in positive-ion ESI was very strong. The relative responses of the model compounds in the tested ionization techniques were obtained by dividing the peak areas of their signals by the reference value and subsequent multiplying the obtained value by 100. By this procedure, we obtained the relative responses of each model compound in each ionization technique.

3.2.2. Negative-ion ESI and APCI analysis of model bio-oil compounds

Both negative-ion ESI and APCI enabled successfully, as expected, ionization and detection of almost all of the selected model compounds (see Table 4). Except nitrogen-containing compounds, D-glucose, D-cellobiose and 4-hydroxy-4-methylpentanone were the only three model compounds that were detected neither by negative-ion ESI nor negative-ion APCI. In the *negative-ion ESI*, vanillin was the compound with the highest relative response (~71%). Other compounds with high relative responses were acetoguaiacon, vanillic acid, isovanillic acid, coniferaldehyde and syringaldehyde (i.e. methoxy- and dimethoxyphenol derivatives). Considering the obtained relative responses, model compounds belonging to furans, carbohydrates,

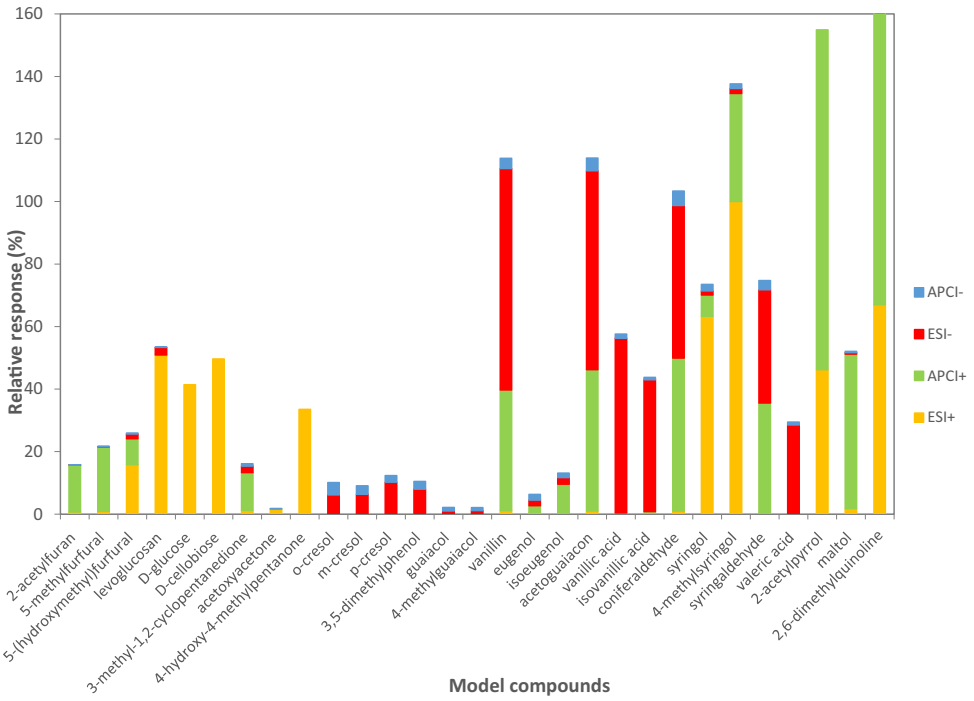

Fig. 4. Relative responses of the studied model compounds in positive-ion and negative-ion ESI and APCI (recalculated to the ESI (+) signal of 4-methylsyringol).

ketones, nitrogen-containing compounds and pyrans had significantly lower response (ionization yields) in comparison to vanillin. For furans and methylphenols, relative responses within each compound class were similar. However, great differences in relative responses were apparent within methoxy- and dimethoxyphenol compound classes.

In the *negative-ion APCI*, coniferaldehyde was the compound with the highest ionization yield. Overall, the relative responses observed in the negative-ion APCI were significantly lower in comparison to the negative-ion ESI. Furans, carbohydrates, ketones and pyrans were compounds with the lowest relative responses within the negative-ion APCI. Similarly to the negative-ion ESI, differences in relative responses were observed for methoxy- and dimethoxyphenol derivatives.

Overall, despite the lower relative responses of model compounds in negative-ion APCI, both negative-ion ESI and APCI showed good ability to ionize and detect the model compounds. Ionization yields (relative responses) differed significantly between the different compound classes. Relative responses of carbohydrates, furans, pyrans and ketones (products of hollocellulose degradation) and nitrogen-containing compounds were low. For methylphenols, methoxy- and dimethoxyphenols (degradation products of lignin), relative responses varied significantly within each compound class, i.e. no clear trends were observed within these classes. The obtained results indicate that both negative-ion ESI and APCI should have higher sensitivity to lignin decomposition products rather than to decomposition products of holocellulose.

3.2.3. *Positive-ion ESI and APCI analysis of the model bio-oil compounds*

Positive-ion ESI was not capable to ionize and detect methylphenols and some methoxyphenol derivatives. This method enabled the ionization and detection of the lowest number of model compounds among the applied methods (18 from 30, see Table 4). Overall, the relative responses of methoxyphenol derivatives were mostly very low. On the other hand, ionization yields of dimetoxyphenols were high and 4-methylsyringol was the compound with the highest ionization yield. Relative responses of carbohydrates were high (~41–52%). Nitrogen-containing compounds (as expected) showed good ionization yields as well.

Positive-ion APCI showed good capability to ionize and detect nitrogen-containing compounds; 2,6-dimethylquinoline was the compound with the highest ionization yield. Other compounds with high relative responses were maltol, coniferaldehyde, acetoguaiacon, syringaldehyde, 4-methylsyringol, 2-acetylpyrrol and vanillin. D-Glucose, D-cellobiose, levoglucosan, cresols, valeric acid and 4-hydroxy-4-methylpentanone were not detected. In general, relative responses of methylphenols, carbohydrates and ketones were low.

Overall, for both positive-ion techniques, relative responses of nitrogen-containing compounds were high. For both techniques, methylphenols were not detected and high relative responses for dimetoxyphenols were observed. Relative responses of hollocelulose degradation products differed between these two ionization techniques. The obtained results indicate good potential of positive-ion APCI for being used as alternative to negative-ion techniques for characterization of bio-oils. The capability of positive-ion ESI to ionize and detect model bio-oil compounds was lower in comparison to positive-ion APCI and negative-ion ESI and APCI. However, positive-ion ESI showed good sensibility to carbohydrates and nitrogen-containing compounds. To enhance the ionization yields of oxygen-containing compounds (and possibly measurement repeatability) in positive-ion ESI, we decided to apply a solution phase modifier (0.1 wt.% formic acid) for analyses of model compounds. However, we did not observe any significant influence upon ionization yields of the studied model compounds. The modifier increased the ionization yields of some compounds, but decreased those of the others (data not shown).

The analyses of the model compounds in positive-ion and negative-ion ESI and APCI revealed that HRMS cannot be used for quantitative analysis of bio-oils due to the significant differences in ionization yields (relative responses) of different oxygen-containing bio-oil compounds. However, we believe that such results can be used for a semi-quantitative comparison between

similar samples (e.g. from the same pyrolysis process), when the analysis is performed at the same experimental conditions. The presented results also indicate that a suitable combination of complementary ionization techniques is needed to perform a comprehensive HRMS characterization of bio-oils.

3.3. Orbitrap mass spectrometry analyses of the ablative flash pyrolysis bio-oils

Analyses of the commercial BTG bio-oil and model bio-oil compounds using different ionization techniques were performed to select an appropriate combination of ionization techniques for the subsequent characterization of the in lab-scale produced ablative flash pyrolysis bio-oils. When evaluating the results of analyses of model bio-oil compounds, we were taking into account not only relative responses of the model compounds in the tested ionization techniques, but also the number of the model compounds detected by each ionization technique. In addition, we considered also the results of analyses of the commercial BTG bio-oil. In terms of the relative responses of model compounds, positive-ion and negative ion ESI and positive-ion APCI might seem to be the most efficient. On the other hand, both negative-ion techniques seem to be more efficient in comparison with positive-ion techniques in terms of the number of model compounds detected. When considering the analyses of commercial BTG bio-oil, positive-ion ESI did not provide representative information about the chemical composition of entire bio-oil due to its ability to predominantly ionize nitrogen-containing compounds. Using positive-ion APCI, similar trends in heteroatom class and DBE distributions were observed as by negative-ion APCI. Positive-ion APCI also showed potential to detect some bio-oil compounds that were not detectable by other ionization techniques, but the sum of intensities of such compounds was very low. Based on these results, we decided to use negative-ion techniques for the analyses of ablative flash pyrolysis bio-oils.

Figs. 5 and 6 show the heteroatom class and DBE distributions of the studied bio-oils obtained by negative-ion ESI and APCI orbitrap mass spectrometry. In the *negative-ion ESI*, compounds with molecular weights in following ranges were observed: ~100–400 Da (2SWO and 7BWO) and ~100–350 Da (42PWO and 95MO). The majority of the detected compounds belonged into following heteroatom and DBE classes: O_1–O_8 and DBE 1–10, respectively. Among the detected compounds, those with four oxygen atoms per molecule and DBE 2–3 were the most abundant in the spectra. Solely deprotonated ions $[M–H]^-$ were observed. In the negative-ion ESI, there were observed differences mostly in relative abundances of the observed O_x and DBE classes (and major compounds) of the studied bio-oils. In comparison to the other bio-oils, 2SWO bio-oil had higher relative abundances of O_2 and O_3 compounds and lower relative abundances of O_4 compounds. The higher relative abundance of O_2 compounds was caused mostly due to the higher abundances of compounds with elemental compositions $C_7H_8O_2$ and $C_8H_{10}O_2$ (both O_2 DBE4) in the spectrum of the 2SWO bio-oil; these compounds were most likely guaiacol and methylguaiacol (these compounds were detected also by GC-MS, not shown in the manuscript). This could be substantiated by the structure of spruce (softwood) lignin, because unlike hardwood and grasses, the main constituent of softwood lignin is guaiacyl unit. On the other hand, the higher relative abundance of O_3 compounds in the negative-ion ESI spectrum of 2SWO bio-oil was caused mostly due to the higher abundance of $C_5H_6O_3$ and $C_6H_8O_3$ (O_3 DBE 3) compounds (probably furanones). The lower abundance of O_4 compounds was caused especially due to the lower abundance of O_4 DBE 3 compounds. Some nitrogen-containing compounds were also detected in the bio-oils and their relative abundances were ~0.1, 0.2 and 0.4% for 7BWO, 42PWO and 95 MO, respectively.

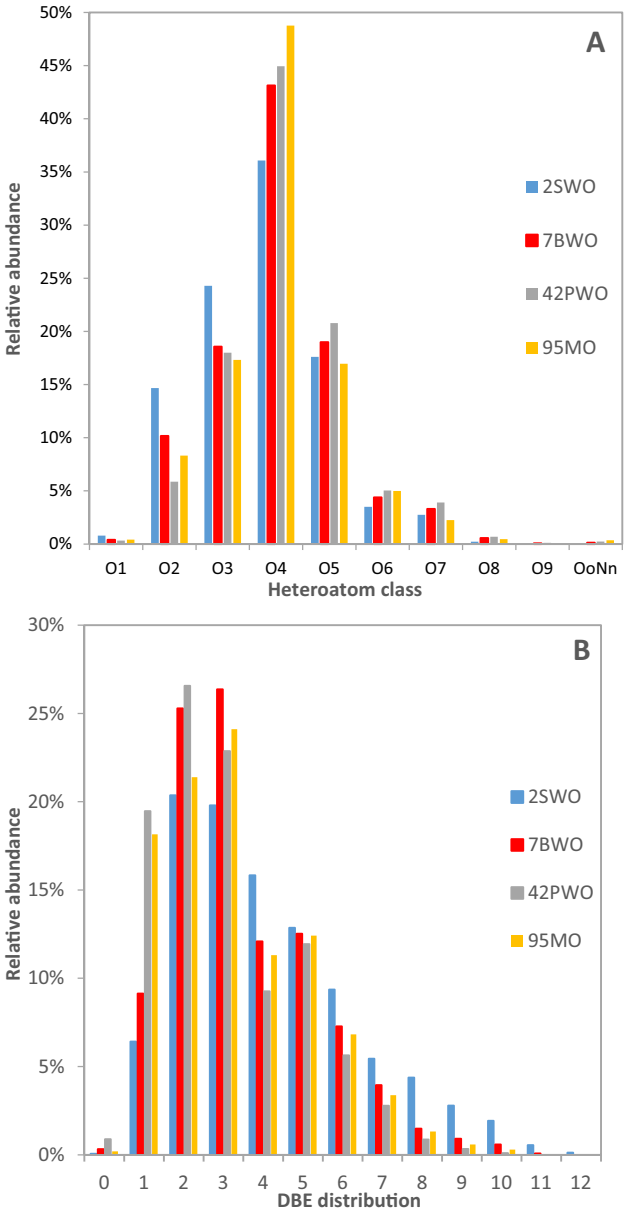

Fig. 5. Heteroatom class (A) and DBE distributions (B) obtained by orbitrap mass spectrometry analysis of the ablative flash pyrolysis bio-oils using negative-ion ESI.

In the *negative-ion APCI*, compounds with molecular weights in following ranges were observed: ~100–500 Da (2SWO, 7BWO and 42PWO) and ~100–450 Da (95MO). The detected compounds belonged mostly into following heteroatom and DBE classes: O_1–O_8 and DBE 1–17. Among them, the most abundant heteroatom and DBE classes were O_3–O_4 and DBE 5–8. In accordance with the analysis of the commercial BTG bio-oil, both even-electron molecular and odd-electron deprotonated ions were observed, but only deprotonated ions were considered due to their higher intensities. Similar results were observed in terms of heteroatom class distribution between the analyzed bio-oils. Differences between the bio-oils were observed mostly in the DBE distribution. The greatest difference was observed between 2SWO oil and other bio-oils. Relative abundances of DBE < 7 compounds (carbohydrates, lignin monomers and dimers) of 2SWO oil were significantly lower in comparison to the other bio-oils and on the other hand, relative abundances of DBE > 9 compounds (lignin dimers, trimers and tetramers) were significantly higher. Some nitrogen-containing

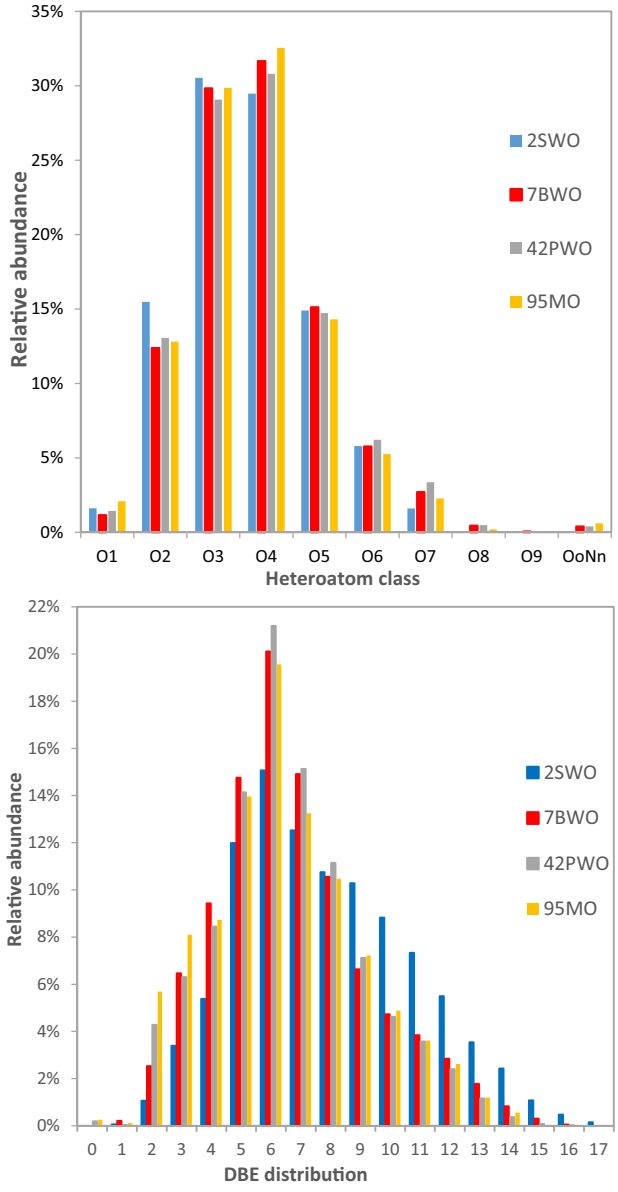

Fig. 6. Heteroatom class (A) and DBE distributions (B) obtained by orbitrap mass spectrometry analysis of the ablative flash pyrolysis bio-oils using negative-ion APCI.

compounds were also detected and their relative abundances were ~0.4, 0.4 and 0.6% for 7BWO, 42PWO and 95 MO, respectively.

Overall, similar results were obtained for all studied bio-oils from ablative flash pyrolysis of different biomass sources in terms of heteroatom and DBE classes detected. In terms of heteroatom and DBE classes detected, the results observed for the ablative flash pyrolysis bio-oils differed from those obtained for the commercial BTG bio-oil (produced by a different type of flash pyrolysis). The compounds detected in the ablative flash pyrolysis bio-oils had narrower molecular weight range, heteroatom class and DBE distributions. However, overall trend in relative abundances of the detected O_x and DBE classes was similar for the ablative flash pyrolysis bio-oils and commercial BTG bio-oil. For all ablative flash pyrolysis bio-oils, differences were observed mostly in relative abundances of the observed O_x and DBE classes (and major compounds) in both applied ionization techniques. When comparing the both ionization techniques used, similar results were obtained in terms of heteroatom classes detected. But differences were observed in DBE ranges and carbon numbers of the detected compounds; in the negative-ion APCI, the highest relative abundances were shifted towards more unsaturated compounds with higher carbon numbers (in comparison to negative-ion ESI). These trends are in agreement with those observed in the analyses of the commercial BTG bio-oil.

4. Conclusion

To our best knowledge, we have presented the first study of model bio-oil compounds and pyrolysis bio-oil from lignocellulosic biomass using positive-ion and negative-ion ESI and APCI orbitrap mass spectrometry. In addition, we have also presented the negative-ion ESI and APCI orbitrap mass spectrometry study of four bio-oils from ablative flash pyrolysis of different biomass sources (spruce wood, beech wood, poplar wood and miscanthus). Negative-ion orbitrap mass spectrometry has been found to be well suited to ionize (and detect) abundant bio-oil compounds. Thus, we have experimentally confirmed the widely accepted assumption about the suitability of negative-ion mass spectrometry for the characterization of pyrolysis bio-oils from lignocellulosic biomass. We have proved the non-versatility of common ionization techniques typically used in HRMS analysis of pyrolysis bio-oils. The presented results have also indicated that HRMS cannot be used for the quantitative analysis of bio-oils. Our study also enabled to elucidate structures of semivolatile, high-molecular-weight compounds of the studied bio-oils. These results could help to improve the understanding of HRMS characterization of bio-oils and bio-oil composition and facilitate their further utilization.

Acknowledgements

The results were obtained in the BioMates project. This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 727463. The publication reflects only the authors' view and the Commission is not responsible for any use that may be made of the information it contains.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jaap.2017.02.002.

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