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CORRECTION

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Correction: Reductive catalytic fractionation of agricultural residue and energy crop lignin and application of lignin oil in antimicrobials†

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Correction for 'Reductive catalytic fractionation of agricultural residue and energy crop lignin and application of lignin oil in antimicrobials' by Elvis Osamudiamhen Ebikade *et al.*, *Green Chem.*, 2020, **22**, 7435–7447, https://doi.org/10.1039/D0GC02781B.

Background

Herein the authors present further analysis of the relationship between feedstock properties and total monomer yields that were investigated previously. The authors conducted principal component analysis (PCA) in the original article to correlate the feedstock properties with the total monomer yields and the monomer selectivities obtained from reductive catalytic fractionation. They found a negative impact of ferulates reported as relative content to *p*-coumarates (%). In this work, we investigate mild and alkaline treatments to determine the absolute cinnamate (ferulates and *p*-coumarates) content (wt%) in the feedstocks and establish correlations. Our results indicate that existing methods provide unreliable determination of absolute cinnamate content (wt%). As a result, PCA cannot be utilized to inform correlations between total monomer yields and cinnamate content in the studied feedstocks. The PCA data and relevant discussion should be removed from the paper.

Introduction

In the original article, the authors performed reductive catalytic fractionation on five herbaceous feedstocks and utilized principal component analysis (PCA) to establish correlations between total monomer yields and feedstock properties (relative molar abundance of syringol (S), guaiacol (G), and hydroxyphenol (H) units in lignin, and relative molar abundance of *p*-coumarates and ferulates in biomass). The cinnamate content of the studied feedstocks was sourced from the Idaho National Lab (INL) biomass specification datasheet, which utilizes solution-state 2D NMR to determine the relative content (%) of ferulates and *p*-coumarates.[1] This method cannot provide absolute cinnamate content due to the varied relaxation times of bulk polymer and terminal end or pendant units. Here, the authors investigate the determination of absolute cinnamate content in the studied feedstocks to replace the relative content with absolute values and establish correlations. Further, the original authors regret that incorrect cinnamate relative content for sugarcane bagasse and miscanthus was reported in **Table 1** and **Table S3** of the original article.

Methods

Mild alkali treatment

The extractives from all five biomasses were removed by the sonication method using 80% ethanol in water and hexane solvents.[2] The mild treatment to quantify ester-linked cinnamates was performed following a literature method.[3] Each extractive free biomass of 0.1 g (dry biomass weight) was treated with 10 mL of 1 N NaOH for 24 h. Then, the resulting liquor was acidified to pH 2 with 12 M HCl and extracted with ethyl acetate three times. Around 20% of the ethylacetate was miscible in the aqueous

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phase. Both organic and aqueous phases were diluted 50 times and analyzed by LCMS. All the experiments were performed in triplicate.

Severe alkali treatment

Each extractive free biomass of 0.1 g was treated with 10 mL of 4 N NaOH and 0.5% anthraquinone at 170 °C for 2 h to release ester and ether-linked cinnamates.[4,5] 2-Hydroxycinnamic acid (~10 mg) was added as an internal standard. The resulting hydrolysate was acidified to pH 2 with 12 M HCl and extracted three times with ethyl acetate. The standards were treated under the same reaction procedure to correct for the partial degradation of hydroxycinnamic acids during the severe treatment. Both organic and aqueous phases were diluted 50 times and analyzed using LC-MS. The quantification was performed based on the calibrations developed using pure ferulic and *p*-coumaric acid. All the experiments were performed in triplicate.

Identification and quantification of cinnamates

The concentration of cinnamates was identified and quantified using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) on a Q-orbitrap mass spectrometer with a Waters Acquity UPLC BEH C18 column (1.7 μm 2.1 × 30 mm). The mobile phase included solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid) flowing at 0.5 mL min^{-1}. A gradient method was implemented to transition from 0% B to attain 95% B in 5 min.

The cinnamate (wt%) was determined using eqn (1).

$$\text{Cinnamate}_{\text{p}}\ (\text{wt\%}) = \frac{\text{cinnamate concentration}\,(\text{g mL}^{-1})}{\text{amount of biomass}\,(\text{g})} \times \text{volume}\,(\text{mL}) \times 100 \quad (1)$$

Eqn (2) accounts for cinnamate degradation (see ESI† for mass balance).

$$\text{Cinnamate}_{\text{f}}\,(\text{wt\%}) = \frac{\text{cinnamate}_{\text{p}}}{(1 - \text{IS}_{\text{degradation}}\,(\%))} \quad (2)$$

Here IS and $\text{cinnamate}_{\text{f}}$ denote internal standard and final absolute cinnamate content, respectively.

Results and discussion

Determination of absolute cinnamate content

The saponification method has been extensively utilized in the literature to quantify the absolute cinnamate content in herbaceous biomass.[3,4,6,7] Mild alkali treatment (≤2 N NaOH) releases ester-linked cinnamates, while severe treatment (~4 N NaOH) cleaves both ether and ester-linked cinnamates.[5] Consequently, severe alkali treatment was applied to quantify absolute cinnamate content. However, this method degrades cinnamates and their cyclo-dimers/trimers, making accurate quantification challenging. An internal standard (IS) was added before the reaction to quantify cinnamate degradation, in line with the literature.[3,4] ESI Fig. 1† illustrates varying IS degradation among feedstocks, potentially influenced by interactions with components released from the cell walls. The IS degradation also varied among triplicate experiments, affecting the reproducibility of absolute cinnamate content (a coefficient of variance of up to 50%; see ESI Table 1†). The cinnamate concentration is influenced by the breakdown of ester and ether-linked phenolic acids, followed by their dissolution or a simultaneous occurrence of both. The IS may degrade faster than the cinnamates under the same reaction conditions due to a higher initial concentration and each cinnamate may have different degradation kinetics compared to IS. Thus, quantifying cinnamate degradation using IS will likely provide inaccurate results (see ESI Fig. 2†). Other methods reported in the literature to account for cinnamate degradation during severe alkali treatment also have limitations. For example, Grabber *et al.*[4] compared the ratio of acids recovered from non-lignified walls after room temperature and high-temperature hydrolysis. The ratio was applied to correct losses of ferulic acid and di-ferulic acids during high-temperature hydrolysis of lignified walls. This could introduce errors, as non-lignified experiments disregard interaction effects with other phenolics released from lignin. Masarin *et al.*[5] conducted severe alkaline treatment at 170 °C on standard phenolics adsorbed to filter paper to quantify their degradation. The filter paper was likely used as a substitute for biomass. However, this does not accurately represent phenolics' release in biomass, driven by bond cleavage and dissolution. At 170 °C, standards may desorb and solubilize more rapidly than in biomass. Further, one method might not be suitable for all feedstocks, necessitating optimization of alkali concentration, time, and temperature.[6,7] Due to the unknown inherent ferulate/*p*-coumarate content (wt%) in various feedstocks, the method yielding the maximum amount would be optimal.[8] The cinnamates do not undergo degradation under the mild treatment, and we achieved excellent reproducibility for the triplicates in this case. Thus, determining absolute cinnamate content is challenging and influenced by the applied method

(see ESI Table 2† for mild alkali treatment results). On the contrary, solution-state 2D-NMR used by INL is a non-destructive reproducible technique that allows the study of the entire biomass and accurately determines relative ferulate and *p*-coumarates content, making it a versatile technique for comparative analyses.[1]

Correction of relative cinnamate content in sugarcane bagasse and miscanthus

The incorrect cinnamate content for sugarcane bagasse and miscanthus in Table 1 and **Table S3**, is corrected here as shown below.

Table 1 Composition of herbaceous biomass and lignin

Biomass	%Ash	%Lignin	%Holocellulose	%S	%G	%H	%Ferulate	%Coumarate
Corn stover	3.5	16.5	66.8	46.3	51.2	2.5	14.0	86.0
Sugarcane bagasse	7.0	25.6	63.5	53.2	44.8	2.1	18.0	82.0
Switchgrass	2.0	16.2	62.6	40.2	56.8	3.1	27.0	73.0
Miscanthus	0.5	20.4	70.8	42.8	54.8	2.4	34.0	66.0
Wheat straw	5.5	16.3	55.6	48.4	48.1	3.5	84.0	16.0

Ash, lignin, and holocellulose are reported on weight basis. S, G, H are reported on a relative molar abundance of the monolignols. Ferulate and coumarate percent is reported on a relative molar abundance of the hydroxycinnamates. Holocellulose = cellulose + hemicellulose. Full physico-chemical characterization data is in Table S1a and b.

Table S3 Monomer yield, average molecular weight, relative α-6′ content of the obtained lignin oils and ferulate content of feedstock

	Monomer yield (%)	Average oil molecular weight (g mol^{-1})	%Ferulate	α-6′, α
Corn stover	45.8	275	14.0	0.76
Sugarcane bagasse	33.7	360	18.0	0.82
Switchgrass	41.5	290	27.0	0.87
Miscanthus	43.8	275	34.0	0.85
Wheat straw	20.0	365	84.0	0.91

Relative α-6′ content is reported as the relative integrated contour area for α-6′, α normalized by the sum of relative integrated contour area for β–β′, γ; β-O-4′, γ; α-6′, α; and β–β′, β.

Since the absolute cinnamate content is unknown, PCA cannot be used to unravel correlations due to varying scaling factors of relative cinnamate content. Thus, we revise the following in the original article:

1. Omit from **Abstract**: contrary to woody biomass, where the monomer yields are positively correlated with the S-content of lignin, principal component analysis indicates that the monomer yields from herbaceous biomass depend on the content of lignin crosslinker – ferulate.
2. Omit machine learning analysis in **Materials and methods**: principal component analysis (PCA) of different datasets was conducted using the MINITAB software.
3. The following in the **Results and discussion**:
   a. Omit section '**Correlating monomer yields across feedstocks *via* simple machine learning**'
   b. Under the section '**Effect of ferulates on lowering the monomer yields**':
      i. We add to the line, "Table S3 … high relative ferulate contents have low monomer yields."
      ii. "This, along with the PCA results above, point to the ferulates as the component" is revised to "This suggests that ferulates are potentially"
   c. Omit Fig. 4, and S5 in the ESI, of the original article
4. **Conclusions**:
   a. Omit "Contrary to woody biomass, where the monomer yield is correlated with the syringol-content, PCA indicates that the ferulate, the lignin to carbohydrate crosslinker, negatively impacts the monomer yields."
   b. We add: "The …, potentially causing the low monomer yields … herbaceous biomass."
   c. We omit "with zero ferulate" and add to the line: "Overall, herbaceous biomass with low relative ferulate content … "lignin first" biorefinery."

Conclusions

We investigated the determination of absolute cinnamate content in various feedstocks to substitute relative cinnamate content (%) and establish correlations. The results indicate that existing methods are influenced by the applied method, feedstock type, and inaccurate quantification of degradation, leading to unreliable determination of absolute cinnamate content (wt%). In contrast, the solution-state 2D NMR method analyzes the entire biomass and provides reproducible relative cinnamate content, enabling accurate comparative analysis between diverse feedstocks. Currently, no ideal method exists for establishing correlations using absolute cinnamate content between various feedstocks, warranting further invest. Here, we corrected Table 1 and **S3** and omitted relevant PCA methods, results, and conclusions from the original article.

The Royal Society of Chemistry apologises for these errors and any consequent inconvenience to authors and readers.

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