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Research paper

# 4-Hydroxybenzoic acid from hydrothermal pretreatment of oil palm empty fruit bunches – Its origin and influence on biomass conversion

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ABSTRACT

An unknown major compound, characteristically occurring during processing of oil palm empty fruit bunches was identified with LC-DAD-ESI-MS/MS to be 4-hydroxybenzoic acid. Lignin from oil palm empty fruit bunches contains 4-hydroxybenzoic acid so a tempting conclusion was that the 4-hydroxybenzoic acid originated from lignin. However, another hypothesis to its origin was also tested. The route considered involves degradation of rhamnose to 5-methylfuran-2-carbaldehyde followed by reaction with formic acid. Experimental hydrothermal pretreatment of pure rhamnose in the presence of formic acid revealed that 5-methylfuran-2-carbaldehyde is in fact a degradation product from rhamnose, analogous to glucose degradation to 5-(hydroxymethyl)-2-furaldehyde. However, the subsequent step of carboxylation with formic acid to form 4-hydroxybenzoic acid was found not to take place in practice at realistic biomass hydrothermal pretreatment conditions. 5-methylfuran-2-carbaldehyde only differs from furfural by having an extra methyl group and the degradation route indicates that it may be a new important degradation compound to consider in other biomass feedstocks rich in deoxysugars such as rhamnose or fucose, e.g. pectin rich biomasses. Assessment of the influence of 4-hydroxybenzoic acid in the enzymatic hydrolysis of pretreated oil palm empty fruit bunches as well as its presence during fermentation showed that 4-hydroxybenzoic acid is not inhibiting or mediating neither on the enzymatic hydrolysis or fermentation in the quantified range from 0.1 g/L to 1 g/L, indicating an option for reaping the 4-hydroxybenzoic acid from the biomass liquor directly after hydrothermal pretreatment for bio-refinery value-addition.



## 1. Introduction

A wealth of biomasses as well as processing methods are available for biorefinery solutions – whether the goal is to supply environmentally friendly energy or valuable compounds available for further synthesis [1–5]. In order to avoid formation of undesirable products during processing and thus to attain optimal product yields it is a key prerequisite to know not only the structural composition of the biomass, but also to understand the reactions of the structural elements during processing. – Especially seen in the light of that undesired compounds that inhibit the enzymes and yeast can be formed during pretreatment in the production of 2nd generation bioethanol [6].

The empty fruit bunches left over from palm oil processing contain ~30–50% by weight of cellulose and have been prospected as an abundantly available lignocellulosic feedstock for cellulosic ethanol production, notably in the South East Asia and South America [7–10] with about $40 \times 10^6$ ton/year produced annually in Malaysia and Indonesia alone [11]. In order to implement oil palm empty fruit bunches (EFB) as a successful feedstock in 2nd generation bioethanol production much work is going on to obtain efficient conversion processes [12–14]. In the present work, the appearance of an unknown compound after hydrothermal EFB biomass pretreatment was consistently observed in HPLC chromatograms. The unknown compound was characteristic for EFB

*Abbreviations:* EFB, oil palm empty fruit bunches; 4-HBA, 4-hydroxybenzoic acid; LfHP, Liquid from Hydrothermal Pretreatment; HMF, 5-(hydroxymethyl)-2-furaldehyde.

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and was thus not present after hydrothermal pretreatment of wheat straw or corn stover at similar severity (Fig. 1). In order to rule out any undesirable effects and to understand the nature and origin of this compound, the objective of this work was to identify the compound and trace its possible origin.

## 2. Materials and methods

### 2.1. Chemicals

Buffer chemicals, solvents, acids and bases, avicel (avicel® PH-101) as well as 4-hydroxybenzoic acid were purchased from Sigma Aldrich (Steinheim, Germany). 5-Methylfuran-2-carbaldehyde was from Vitas-M Laboratory (Apeldoorn, The Netherlands).

### 2.2. Biomass

EFB was acquired by DONG Energy from Malaysia fresh from an oil mill, transported cooled (<5 °C) and stored at −18 °C. Prior to use it was thawn over 2 days to reach ambient temperature. The biomass composition before and after pretreatment was analysed according to [15].

### 2.3. Enzymes and yeast

Cellic CTec3 (1920 Biomass Hydrolysis Unit (2)/g) was obtained from Novozymes A/S (Bagsværd, Denmark). Apart from the cellulolytic enzyme base from *Trichoderma reesei* containing at least the two main cellobiohydrolases EC 3.2.1.91 (Cel6A and Cel7A), five different endo-1,4-β-glucanases EC 3.2.1.4 (Cel7B, Cel5A, Cel12A, Cel61A, and Cel45A), β-glucosidase EC 3.2.1.21, and a β-xylosidase [16], the Cellic CTec3 enzyme preparation also contains other proprietary hydrolysis-boosting proteins.

Dry yeast *Saccharomyces cerevisiae* was acquired from Lallemand, (Montreal, Canada).

### 2.4. Pilot plant pretreatment

Hydrothermal biomass pretreatment was performed at 200 °C for 18 min at 40% (w/w) dry matter with continuous feeding at a rate of approximately 50 kg (dry matter) EFB per hour. The pretreatment was performed in the Inbicon pilot pretreatment plant in Fredericia, Denmark. The pretreated material was separated into a fiber fraction and a liquid fraction. The liquid fraction was analysed as described below and both the fiber and liquid fractions were stored at −18 °C until enzymatic hydrolysis.

### 2.5. Isolation of 4-hydroxybenzoic acid from the pretreatment liquid

Preparative chromatographic purification was performed on an Äkta Purifier 100 system (GE Healthcare, Uppsala, Sweden) with a 6 mL Resource S column from GE Healthcare. Elution was carried out with 20 mM BIS-Tris (pH = 6.5) at 0.6 mL/min with isocratic conditions and fraction collection. The selected fraction was

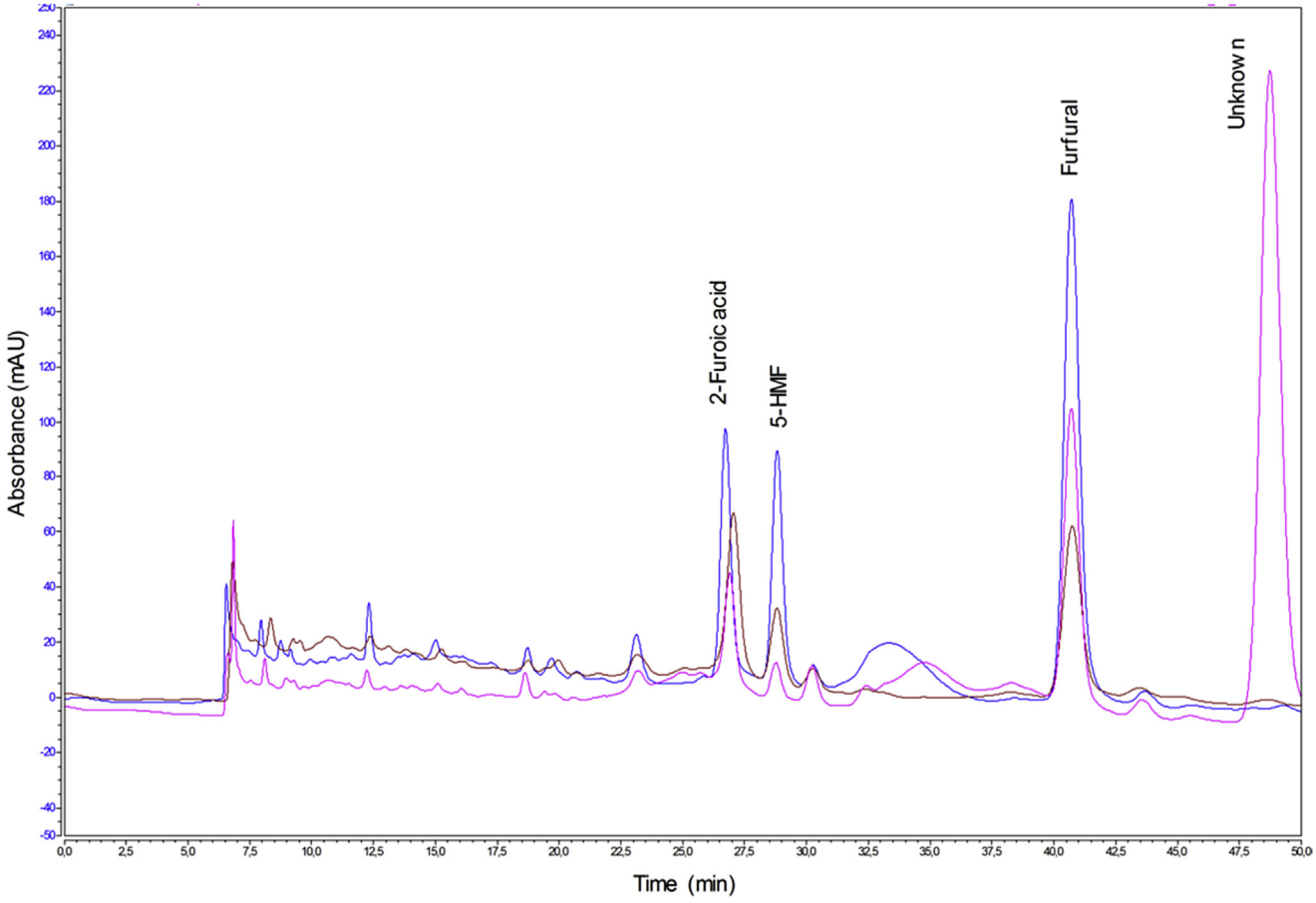


**Fig. 1.** HPLC analysis from pretreatment at a severity of approx. 4.0 of wheat straw (blue), corn stover (brown) and EFB (pink). Unknown compound rt 49.5 min. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)