Wood Technology/Products

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# Role of extractive compounds in emulsion stabilisation capacity of wood hemicelluloses

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**Abstract:** Hemicellulose-rich extracts obtained by pressurised hot water extraction of wood residues show promise as emulsion stabilisers. However, pressurised hot water extracts (PHWEs) from wood also contain lower molecular weight extractive components that may influence emulsion performance. This study investigated the effect of extractives on emulsion stability using PHWEs obtained from spruce and birch sawdust with or without solvent pre-extraction. The results indicated that extractives negatively affect the physical stability of emulsions in both spruce and birch and influence oxidative stability in spruce after prolonged storage. However, the roles of different extractives in emulsion performance require further investigation.

**Keywords:** pressurised hot water extraction; resin acid; fatty acid; lignan; particle size; oxidation

## 1 Introduction

To fully utilise side-streams such as sawdust from the forest industry, more value-added products are needed. Hemicelluloses can be extracted from hardwood and softwood by pressurised hot water flow-through extraction (Kilpeläinen et al. 2014) and utilised as emulsion stabilisers. Emulsions stabilised with wood-derived pressurised hot water extracts (PHWEs) have been extensively studied (Lahtinen et al. 2019; Lehtonen et al. 2016, 2018; Mikkonen et al. 2016; Valoppi et al. 2019). Their performance appears to be influenced by the presence of non-hemicellulose constituents such as lignin-derived phenolics (Lahtinen et al. 2019; Lehtonen et al. 2016, 2018), but the presence of wood extractives in the PHWEs and their effects on emulsion performance remain uncharacterised. This manuscript investigated the extractives found in spruce and birch PHWEs. The composition of extractives was determined in PHWEs obtained from sawdust with or without solvent pre-extraction, after which the physical and oxidative stability of emulsions stabilised using the PHWEs was investigated to better understand the role of extractives in emulsion performance.

## 2 Materials and methods

### 2.1 Sample preparation

Nordic birch (*Betula* spp.) and Norway spruce (*Picea abies*) sawdust were obtained from Finnish sawmills and freeze-dried. Portions of both were pre-extracted with hexane or acetone using an accelerated solvent extractor (ASE-350, Dionex, USA): sawdust (15 g) was placed into a 66 mL extraction vessel and extracted 3 × 15 min at 90 °C with 30 vol% addition of fresh solvent between extractions. Extracted sawdust was dried in a vacuum oven at 40 °C overnight.

Sawdust samples with and without pre-extraction were extracted with pressurised hot water with flow-through to obtain hemicellulose-rich extracts using a previously described laboratory-scale extraction system (Kilpeläinen et al. 2014). Sawdust (15 g) was extracted for 60 min at 180 °C (spruce) or at 170 °C (birch) with 4 mL/min flow-through. The PHWEs were collected, and their dry matter contents determined by oven drying an aliquot of the extract at 105 °C overnight. Extractions were performed in duplicate. The monosaccharide composition of each PHWE was determined in duplicate by acid methanolysis as before (Kilpeläinen et al. 2014).

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## 2.2 Analysis of extractives

A 1 ml aliquot of each PHWE was diluted with 3 mL of water and adjusted to pH 3 with 0.05 M $H_2SO_4$. A 2 mL aliquot of internal standard solution containing 0.02 mg/mL of C21 fatty acid, and 0.02 mg/mL of betulinol (for spruce) or cholesterol (for birch) in methyl-tert-butyl ether (MTBE) was added, after which the samples were shaken for one min and the phases separated. The water phase was further extracted twice with MTBE without internal standards. The MTBE phases were combined and the MTBE evaporated under $N_2$ flow at 60 °C. The extracts were dissolved in 100 µL of pyridine and silylated with 100 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 50 µL trimethylsilyl chloride (TMCS) for 20 min at 70 °C. Samples were prepared in duplicate. External calibration standards were prepared using sinapyl alcohol, coniferyl alcohol and syringic acid and silylated in the same way as the samples.

GC-MS analysis was performed on an Agilent 7890B/5977A system (Hewlett Packard, Palo Alto, CA, USA) using a Zebron ZB-SemiVolatiles column (Phenomenex, 30 m × 0.25 mm × film thickness 0.25 µm). The temperature gradient was: 150–230 °C, 7 °C/min; 230–290 °C, 4 °C/min; hold time 15 min. The injection temperature was 280 °C and the injection volume 1 µL. The injection was carried out in split mode with a ratio of 9.86:1. Helium was used as the carrier gas. The identification of extractives was done by comparing mass spectra to in-house and commercial databases (Wiley and NIST).

## 2.3 Emulsion preparation

Rapeseed oil (Keiju, Bunge Finland Ltd, Raisio) was purchased from a local supermarket and stripped of tocopherols as described earlier (Lampi et al. 1999). Oil-in-water emulsions were prepared using a previously optimised (Mikkonen et al. 2016) composition: 1 wt-% PHWE; 5 wt-% stripped oil, 25 mM Na-citrate (pH 4.5, total weight 80 g). The weight of the PHWE in the emulsion was based on its dry matter content. The preparation method starting with the preparation of coarse emulsion followed by passing through a high-pressure homogenizer has been described earlier (Lahtinen et al. 2019; Valoppi et al. 2019).

## 2.4 Physical and oxidative stability of emulsions

The physical and oxidative stability of the emulsions was monitored in an accelerated storage test at 40 °C by measuring the droplet size distribution and peroxide value as previously described (Lahtinen et al. 2019) over the course of 16 weeks. The droplet size distributions were measured in triplicate by the static light scattering technique using a Mastersizer Hydro 3000 (Malvern Instruments Ltd, Worcestershire, UK). Peroxide values were measured with a spectrophotometer as an indicator of primary oxidation by the ferric thiocyanate method as described before (Lehtonen et al. 2016). In addition, microscopy images were collected as before (Lahtinen et al. 2019) to visualise emulsion morphology.

# 3 Results and discussion

## 3.1 Composition of hot water extracts

The monosaccharide composition of the PHWEs obtained from spruce and birch sawdust without pre-extraction (PHWE-S and PHWE-B), with hexane pre-extraction (hPHWE-S and hPHWE-B) and with acetone pre-extraction (aPHWE-S and aPHWE-B) are given in Supplementary Table S1. The extractive composition of the PHWEs was analysed by GC-MS; the concentrations of key compound groups are summarised in Figure 1, while the concentrations of individual compounds can be found in

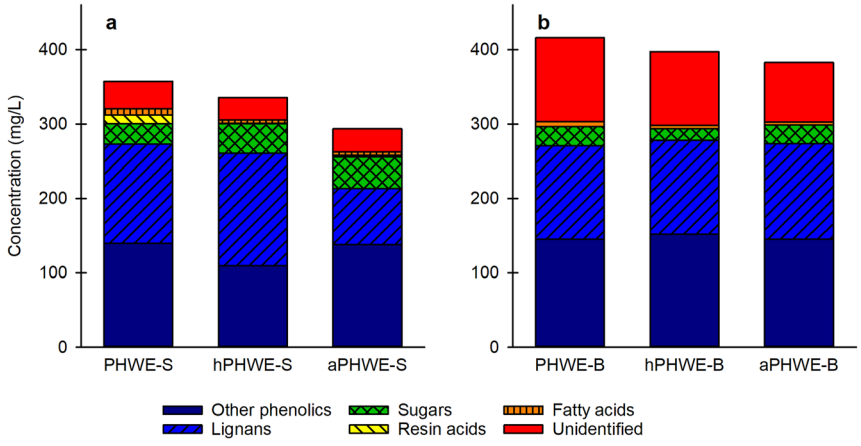


**Figure 1:** Concentrations (mg/L) of extractives grouped by compound type in PHWEs obtained from spruce sawdust without pre-extraction (PHWE-S), with hexane pre-extraction (hPHWE-S) and with acetone pre-extraction (aPHWE-S) (a), and in PHWEs obtained from birch sawdust without pre-extraction (PHWE-B), with hexane pre-extraction (hPHWE-B) and with acetone pre-extraction (aPHWE-B) (b).

Supplementary Table S2 for spruce and Supplementary Table S3 for birch.

PHWE-S contained 356 mg/L of extractives, which corresponds to 6 mg/g of extractives in the original sawdust. Lignans accounted for 134 mg/L and other phenolics 140 mg/L of the extractives, with hydroxymatairesinol and coniferyl alcohol as the dominant compounds. PHWE-S also contained resin acids, fatty acids and sugars in smaller amounts. Lignans, resin acids, and fatty acids are typical spruce stemwood extractives (Willför et al. 2003), and the low concentrations of the resin and fatty acids are likely caused by their poor water solubility. The other phenolics consisted mostly of lignin-derived compounds. The other phenolics as well as the sugars are likely extracted from the wood cell wall polymers during the PHWE. Pre-extraction of the spruce sawdust with hexane affected the hydrophobic extractives: resin acids were almost absent in hPHWE-S, and the concentration of fatty acids was reduced. The concentration of sugars and lignans increased slightly, possibly due to increased water extractability following the removal of the more hydrophobic components. Pre-extraction with acetone affected both the hydrophobic and hydrophilic extractives: resin acids were almost absent in aPHWE-S, and the concentrations of fatty acids and lignans were reduced to about half of the concentration found in PHWE-S. The concentration of the lignin-derived phenolics was reduced in hPHWE-S but not in aPHWE-S.

PHWE-B on the other hand contained 416 mg/L of extractives, corresponding to 7 mg/g of extractives in the original sawdust. Lignans (126 mg/L) and other phenolics (146 mg/L) derived from lignin were the major components, with syringaresinol as the dominant compound. PHWE-B also contained small amounts of fatty acids and sugars, and a large quantity of unidentified compounds. Syringaresinol, fatty acids and lignin-derived phenolics have been previously detected in birch stemwood, along with other lignans and flavonoids (Hiltunen et al. 2008) that were not detected in the PHWEs in this experiment. Pre-extraction of the birch sawdust had only a small effect on the extractive content of the PHWEs, with a decrease seen in the concentration of fatty acids, sugars and unidentified compounds. The poor extractability of birch extractives remains unexplained but may be influenced by factors such as particle size and the age of the industrial sawdust.

## 3.2 Physical stability of emulsions

Droplet size measurements were used to assess the physical stability of the PHWE emulsions. The droplet size distributions of the emulsions over the course of 16 weeks are shown in Figure 2, while the mean droplet sizes (Dx (10), Dx (50), Dx (90), D[3,2] and D[4,3]) are given in Supplementary Table S4.

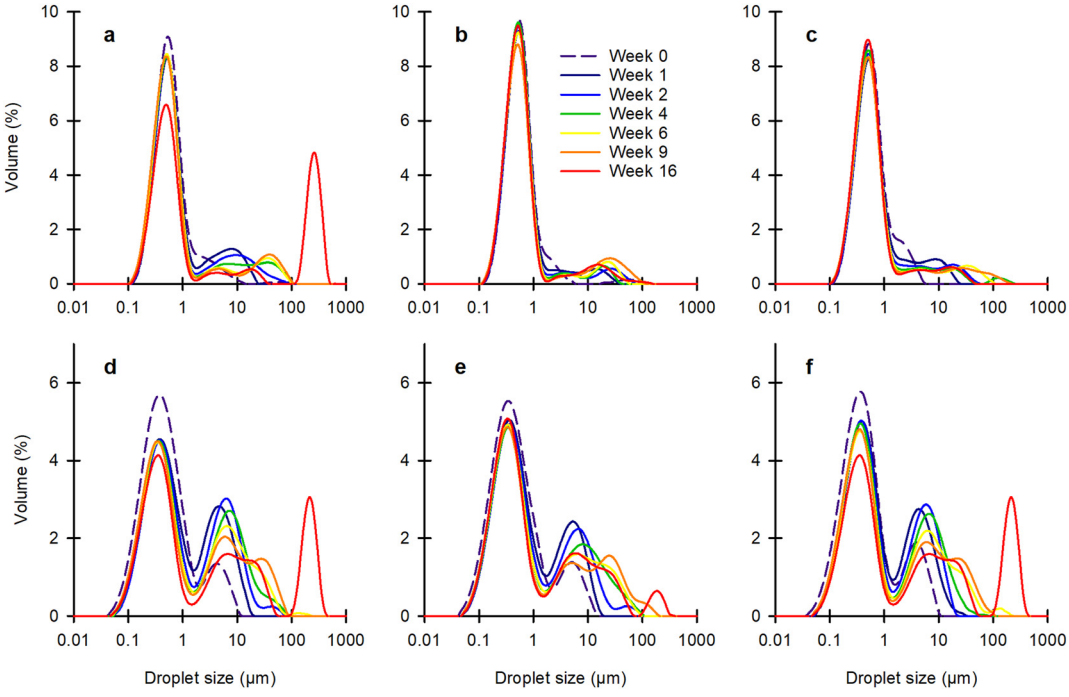


**Figure 2:** Particle size distributions of emulsions stabilised with PHWEs from spruce without pre-extraction (PHWE-S) (a), spruce with hexane pre-extraction (hPHWE-S) (b), spruce with acetone pre-extraction (aPHWE-S) (c), birch without pre-extraction (PHWE-B) (d), birch with hexane pre-extraction (hPHWE-B) (e), and birch with acetone pre-extraction (aPHWE-B) (f) over 16 weeks.

Emulsion morphology is visualised in Supplementary Figure S5. The emulsions stabilised with spruce PHWEs had similar initial distributions. Over the course of storage, larger droplets of increasing size appeared in the emulsions. The proportion and size of the larger droplets was more pronounced in PHWE-S than in hPHWE-S or aPHWE-S at every timepoint, and by week 16 the PHWE-S emulsion contained droplets over 100 µm in size. The improved stability of hPHWE-S and aPHWE-S suggests that spruce extractives have a destabilising effect on the emulsions. The destabilising effect may be linked to the resin and/or fatty acids as these two compound groups were removed by both pre-extraction solvents (see Figure 1 and Supplementary Table S2). The role of phenolic compounds in emulsion stability remains unclear; both hexane and acetone reduced the concentration of phenolics in the PHWE, but they affected different phenolic populations.

The emulsions stabilised with birch PHWEs also had similar initial distributions. The distributions were bimodal as has been previously documented for emulsions containing birch xylan (Lahtinen et al. 2019; Mikkonen et al. 2016). Over the course of storage, the distributions shifted towards larger droplet sizes, and by week 16, droplets over 100 µm in size had appeared in the emulsions. The emulsion stabilised with hPHWE-B showed better stability at every timepoint, which suggests that birch extractives also influence the stability of the emulsions. However, the extractive components responsible for the effect remain unidentified as no consistent extractive composition differences could be seen between the birch PHWEs (see Figure 1 and Supplementary Table S3).

### 3.3 Oxidative stability of emulsions

The oxidative stability of the prepared emulsions over 16 weeks in the accelerated oxidation test is shown in Figure 3. The spruce PHWE emulsions were stable for 12 weeks, with peroxide values remaining below 3 meq/kg oil. At 16 weeks the peroxide values increased dramatically, reaching approx. 40 meq/kg oil in the emulsions containing hPHWE-S and aPHWE-S. The peroxide value of the PHWE-S emulsion increased as well but only to an average of 22 meq/kg oil, which suggests that the extractives present in PHWE-S affect emulsion oxidation, either by inhibiting it or by increasing the conversion of primary oxidation products to secondary products. It remains unclear which extractive groups are involved. The similar behaviour of hPHWE-S and aPHWE-S suggests the involvement of the resin and fatty acids, which were removed by both solvents (see Figure 1). However, phenolics such as lignans are known for their

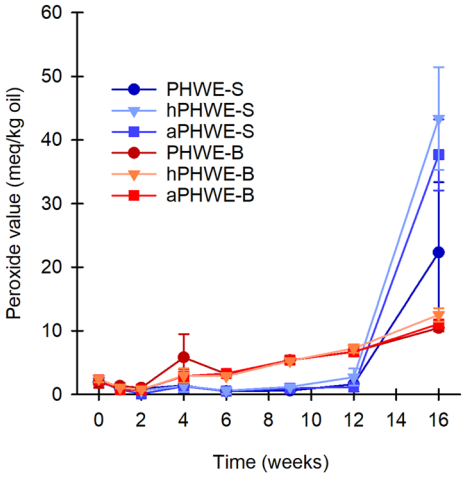


**Figure 3:** Peroxide values of emulsions stabilized with PHWEs obtained from spruce and birch sawdust without pre-extraction (PHWE-S and PHWE-B), with hexane pre-extraction (hPHWE-S and hPHWE-B) and with acetone pre-extraction (aPHWE-S and aPHWE-B) over 16 weeks.

ability to inhibit lipid peroxidation (Pietarinen et al. 2006), and both the hexane and acetone pre-extractions affected the phenolic fraction, just in different ways (see Figure 1). The peroxide values of the birch PHWE emulsions in turn increased gradually over the course of 16 weeks, reaching approx. 10 meq/kg oil by week 16. There was little difference between the different birch PHWE emulsions, as expected given the lack of differences in extractive composition (see Figure 1).

## 4 Conclusions

Extractives present in wood PHWEs influenced the physical and oxidative stability of emulsions stabilised using the PHWEs. The hydrophobic resin and fatty acids in particular appear to be involved; however, further information is required to fully understand the role of extractives in emulsion performance.

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**Data availability:** The raw data can be obtained on request from the corresponding author.

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**Supplementary Material:** This article contains supplementary material (https://doi.org/10.1515/hf-2024-0083).