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Short communication

# Extraction of flax shive using sodium ethoxide catalyst in anhydrous ethanol

Robert V. Parsons[a], Kari A.L. Parsons[b], John L. Sorensen[b,*]

[a] Department of Biosystems Engineering, University of Manitoba, Winnipeg, Canada
[b] Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

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ABSTRACT

This work investigated the yield and nature of solvent-soluble organic compounds extracted from flax shive using a room temperature reaction (20 °C) with sodium ethoxide catalyst at four different concentrations (0.2, 0.5, 0.7, and 1.0 M) in anhydrous ethanol. Results were compared with the use of aqueous sodium hydroxide (1.0 M) at two different reaction temperatures (20 °C and 100 °C). Quantitative yield from flax shive varied linearly with sodium ethoxide concentration and averaged 54.5 mg/g on a dry-mass basis (db) at 1.0 M. In contrast, the quantitative yield using 1.0 M sodium hydroxide was much lower, averaging 2.2 mg/g (db). Yield did not differ significantly due to changes of particle size in either case, or due to changes of temperature over the range considered in the case of sodium hydroxide.

Analyses using proton nuclear magnetic resonance ($^1$H NMR) confirmed all extracts to contain aromatic compounds, thus likely lignin derived, but found differences in chemical characteristics between the two extraction methods. One key difference was the presence of compounds with methyl ether groups in sodium hydroxide extracts that were absent in the case of sodium ethoxide extracts. Given that flax contains a mixed guaiacyl–syringyl lignin, methyl ether groups would be expected to be present. Control reactions on three model compounds were carried out to confirm that transesterification occurred with sodium ethoxide. These control reactions also demonstrated that methyl ether groups would be expected to remain intact under the extraction conditions reported here. In light of the higher yield of solvent soluble compounds recovered by extraction with basic ethanol, flax shive may represent a source of value-added phenolic constituents. This processing method may also represent a useful pre-treatment prior to the production of biofuels by cellulose degrading organisms.



## 1. Introduction

Flax shive is the woody by-product of decortication, the process used to remove valuable bast fibre from the stem periphery of flax (*Linum usitatissimum*). Composed primarily of xylem tissue from the stem core, it is the most lignified component of flax straw, with lignin content ranging from 23% to 31% (Buranov and Mazza, 2008). Flax shive is available in large quantities at low cost, with relatively consistent composition and size characteristics.

Given that flax shive is considered essentially a waste product, there has been strong interest to investigate alternative uses (Flax Council of Canada, 2002). One possibility is to use flax shive as a feedstock for the recovery of phenolic constituents. Such an approach could also support further processing of biomass for biofuel production, either by providing value-added by-products (Doherty et al., 2011), or through possible pretreatment prior to fermentation by microorganisms that can directly utilize residual cellulose as a substrate (Carere et al., 2008).

In this study, a preliminary assessment was undertaken for the extraction of flax shive using sodium ethoxide in anhydrous ethanol at room temperature. We report the yield and the preliminary chemical characterization of the compounds recovered using this novel method, compared to more conventional extraction using sodium hydroxide under similar conditions. Reactions of three model compounds were also examined to identify the possible outcome of treatment with sodium ethoxide catalyst on recovered phenolic compounds.

The nature of recovered phenolic compounds from plant materials depends both on the native lignin present and on the extraction process employed. In terms of lignin composition, flax primarily contains mixed guaiacyl–syringyl lignin (Gorshkova et al., 2000). Guaiacyl lignin compounds, which possess a single methyl ether bond, tend to dominate over syringyl lignin compounds, which have two methyl ether bonds. Hydroxyphenyl lignin compounds, without methyl ether groups, are less prevalent in flax shive, but may be associated with the fibre component at the stem periphery (Day et al., 2005). The number of different compounds that can be released from lignin is also typically very large, resulting in complex mixtures (Del Rio et al., 2007).

The recovery of phenolic compounds from flax shive has been examined recently (Akin et al., 1996; Lozovaya et al., 1999; Kim and

* Corresponding author. Tel.: +1 204 474 9504; fax: +1 204 474 7608.
*E-mail address:* John_Sorensen@umanitoba.ca (J.L. Sorensen).

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Mazza, 2006, 2007, 2009; Tapin et al., 2006; Buranov and Mazza, 2007, 2009; Buranov et al., 2010; Ross and Mazza, 2010). Extractions so far have tended to rely on some form of alkaline treatment, most typically using aqueous sodium hydroxide. This produces a mixture of phenolics, including guaiacyl and syringyl aldehyde, ketone and acid compounds.

A range of increasingly severe conditions with sodium hydroxide (i.e., higher concentrations and temperatures) can be considered to cleave ester and ether linkages of lignin in order to achieve higher levels of removal (Buranov and Mazza, 2008; Ross and Mazza, 2010). This includes "soda" pulping processes to yield essentially purified cellulose (Doherty et al., 2011). For this work, however, a concentration of 1.0 M sodium hydroxide at relatively low temperatures was selected as the benchmark. Firstly, this allowed comparing performance at mild process conditions, not requiring large energy inputs. Secondly, the use of simple alkaline solutions at relatively mild conditions has also been applied for the extraction of hemi-cellulose carbohydrates (Han, 1983). In order to develop an alternative process, we considered the use of alkoxide catalysts, such as sodium ethoxide in ethanol. Their use under ambient conditions to catalyze transesterification reactions is now common in the industrial production of biodiesel (Van Gerpen, 2005). By facilitating the reaction of ester linkages, alkoxide catalysts offer a novel approach for extracting phenolic compounds, while potentially leaving carbohydrate structures intact. The use of ethanol as the solvent for the reaction is also advantageous for environmental reasons. Alkoxide catalysts have already been applied to biomass processing, in analytical methods to quantify acetyl attachments (Browning, 1967) and in modifying natural fibre polymers (Persson, 2004). Transesterification also has been used as a key step in the recovery of secoisolariciresinol diglucoside (SDG), a valuable lignan found in defatted flax seed meal and seed hulls, but not in flax stem tissues (Westcott and Muir, 1998; Oomah and Hosseinian, 2008).

## 2. Materials and methods

Flax shive was obtained from the decortication plant of Schweitzer-Mauduit Canada Inc., located near Carman, Manitoba, and was screen-fractionated by E-Mission Free Inc. of Winnipeg, Manitoba. Two fractions were tested: (a) 1.0 mm flax shive (passing 10 mm and retained on 1.0 mm); and (b) 0.5 mm flax shive (passing 1.0 mm and retained on 0.5 mm). Moisture contents of both fractions on a wet basis (wb) were determined using ASAE standard S358.2 (ASAE, 2004), each performed in triplicate. Sodium ethoxide ($CH_3CH_2ONa$) catalyst was mixed with anhydrous ethanol to four different concentrations: 0.2 M; 0.5 M; 0.7 M; and 1.0 M. Tests were undertaken for both flax shive fractions at each concentration in triplicate. Catalyst solution (20 mL) was added to 1 g wb of flax shive in a closed beaker at room temperature (20 °C), and stirred constantly for a 1-h reaction period. The solution was filtered under vacuum and the retained flax shive then rinsed with an additional 100 mL anhydrous ethanol. The filtrate was adjusted to pH < 3 using concentrated HCl, and evaporated under reduced pressure. The residue was dissolved in dichloromethane (50 mL), washed twice with high purity water (30 mL, Millipore Q, >18 MΩ resistance), and once using saturated aqueous NaCl solution (30 mL). The organic phase was separated, dried using excess $Na_2SO_4$, filtered, and evaporated to dryness under reduced pressure to determine product mass.

NaOH was dissolved in high purity water to a concentration of 1.0 M. Tests were undertaken for both flax shive fractions in triplicate at each of two different reaction temperatures. The extraction similarly consisted of mixing a sample of 1 g wb of flax shive with 20 mL of 1.0 M NaOH solution, stirring constantly for a 1-h reaction period. Samples extracted at room temperature (20 °C) were contained in a closed beaker, while samples extracted at 100 °C were placed in a 50 mL round-bottom flask in a heated sand-bath with water condenser. Timing for the reaction period in the latter case was from the first indication of condensation in the condenser. The recovery procedure involved filtering the solution under vacuum and rinsing the retained flax shive with first a small amount of high purity water (20 mL) followed by 100 mL of 95% ethanol. All remaining steps were the same as those described for the extraction with alkoxide catalyst.

Three model compounds, all guaiacyl in nature, were tested for reaction with sodium ethoxide catalyst. These were methyl ferulate [(*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid], methyl vanillate (methyl 4-hydroxy-3-methoxybenzoate), and acetovanillone [1-(4-hydroxy-3-methoxyphenyl)ethanone]. Methyl vanillate and acetovanillone were commercially available. Methyl ferulate was synthesized by stirring 384 mg (2 mmol) of ferulic acid in 20 mL of acidic methanol (pH < 2) for 48 h. The solution was neutralized by the addition of $Na_2CO_3$. Dichloromethane (40 mL) was added, the solution washed with high purity water (2 × 30 mL), and saturated aqueous NaCl solution (30 mL). The organic phase was separated, dried using excess $Na_2SO_4$, filtered, and evaporated to dryness under reduced pressure to give 327 mg of crude product (i.e., 80% yield). Analysis by $^1$H NMR showed this product to be pure methyl ferulate. The characterization data are reported in Supplementary Data.

Each model compound (2 mmol) was separately placed in a round-bottom reaction flask with 20 mL of 1.0 M sodium ethoxide catalyst in anhydrous ethanol for 1 h at room temperature, with constant mixing. At the end of this period, the solution was adjusted to neutral pH using HCl, and evaporated to dryness under reduced pressure. The residue was then subjected to the same procedures as for the flax shive extracts.

For all flax shive extractions, one sample for each set of conditions was analyzed using $^1$H NMR ($CDCl_3$ solvent) on a Bruker Avance 300 MHz system. The $^1$H NMR spectra for flax shive extracts were compared to those of lignin-derived compounds in an existing database (Ralph et al., 2001). Model compound reactants and products were similarly analyzed using $^1$H NMR. The results for reactions of model compounds were compared to the same database. The $^1$H NMR spectra were also used quantitatively to estimate the extent of reactions for model compounds, comparing the integration for protons on carbons adjacent to the ester oxygen for both ethyl and methyl esters. The non-destructive nature of $^1$H NMR permitted further analysis of the same samples using gas chromatography-mass spectrometry (GC–MS).

The GC–MS used was a Varian model CP-3800 gas chromatograph coupled to a Varian model 320-MS TQ mass spectrometer. Samples were diluted in dichloromethane, filtered, and 1 μL volume manually injected directly into the GC without any derivatization. The injector temperature was set at 160 °C. The column was held at 75 °C for 1.5 min, then ramped linearly at a uniform rate of 30 °C per minute up to 265 °C, then held at 265 °C for 7 min.

## 3. Results and discussion

The moisture content of 1.0 mm flax shive averaged 76.1 mg/g (wb), with a standard deviation (±SD) of 2.2 mg/g, while that of 0.5 mm flax shive was 80.7 ± 1.7 mg/g (wb). Moisture contents of the two size fractions were significantly different, according to a two-sample pooled *t*-test (Moore and McCabe, 2006; $t_4 = 2.9$, $P < 0.05$), with these values used to adjust respective yields to a dry basis (db). The results obtained for the quantitative yield of solvent-soluble organics from flax shive (db) are summarized in Table 1 for all test conditions. Mean