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# Extraction of lipids from flax processing waste using hot ethanol

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ABSTRACT

The cuticle of flax stems contains lipids that provide a protective barrier to pathogens and control moisture loss. These lipids include wax esters and long chain fatty alcohols or policosanols. Cuticle fragments generated during several different fiber processing operations retain these lipid compounds that represent a potential co-product. Samples of flax shives (i.e., lignified core tissues), processing dust, and cuticular fractions recovered from enzyme retting waste water were extracted on a laboratory scale with hot ethanol to remove the lipid compounds. Ethanol extracts were analyzed by gas chromatography to determine the amount and type of lipids recovered. The results demonstrated that hot ethanol effectively extracted the lipid compounds from cuticle fragments in all these samples. When the extract was cooled, the longer chain wax esters (i.e., chain length of 44 carbon atoms or larger) precipitated and could be separated from the shorter chain lipid components (i.e., fatty alcohols and aldehydes less than 44 carbons). Similar results were obtained using absolute ethanol or 95% ethanol (aqueous). This technique provides a very economical method to recover lipid fractions as potential value-added co-product from flax processing waste.

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## 1. Introduction

Flax and other plants have a waxy or fatty cuticle layer of varying thicknesses on the outer walls of the epidermal layers (Akin et al., 2000; Stern et al., 2003). The cuticle protects the vulnerable internal plant tissues against microbial pathogens and moisture loss. Earlier research had shown that a variety of lipid compounds may be extracted from the flax cuticle including long chain acids, alcohols, and wax esters (Morrison and Akin, 2001; Gutierrez and Del Rio, 2003). The majority of these lipids are in the cuticularized epidermis, with small amounts on the bast fiber (Morrison and Akin, 2001). Histochemical staining for wax and near infrared-Raman spectroscopy also indicated that the preferential location of waxy compounds is in the cuticularized epidermis, with little of this substance on bast fibers (Himmelsbach et al., 1998; Akin et al., 2004).

Some of these cuticular lipid compounds display bioactive properties in mammalian systems. For example, the long chain alcohols or policosanols are reported to lower total blood cholesterol and improve the ratio of low-density to high-density lipoproteins in humans (Taylor et al., 2003; Varady et al., 2003). Fragments of cuticle that are generated when flax is processed for fiber become distributed in the dust, waste water, and shive fractions (Figs. 1 and 2). These low-value waste fractions contain varying amounts of cuticle with the associated lipids that could be recovered as a co-product for biomedical or nutraceutical formulations (Morrison et al., 2006).

The dust and other cuticle-containing waste fractions from flax are a major component in processing flax for fiber, both for linen and for composites or specialty papers. While flax in North America is primarily grown for linseed oil produc-

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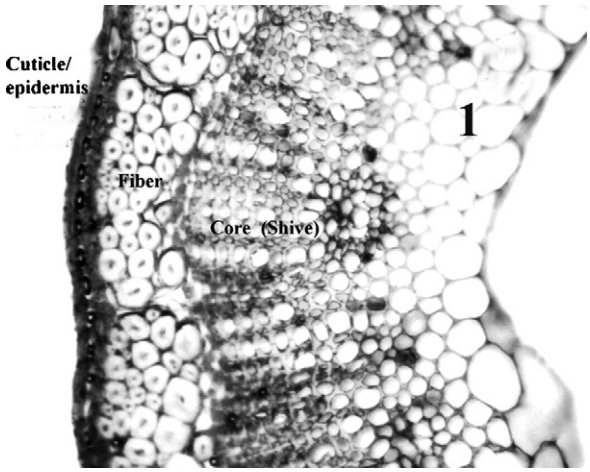


**Fig. 1 – Light micrograph of cross-section of flax stem showing the location of components. The cuticle is on the outside of the epidermis and helps protect the internal tissues. The bast fiber is the industrially important fibers for textiles, composites, and paper. The core (shive) contains the lignified cells that provide structural support and makes up the largest waste component in processing flax.**

tion, a small portion (15–20%) of the linseed straw is used for specialty paper. Strong potential exists to increase fiber production from linseed and to grow flax for fiber production in the southeast as a winter crop (Dodd et al., 2000; Akin et al., 2005). With linseed already a valuable commodity and flax fiber an increasingly sought-after product, an economical process to recover lipids from the waste cuticle fragments would support efforts to expand domestic flax production by introducing an additional value-added co-product (Gouni-Berthold and Berthold, 2002).

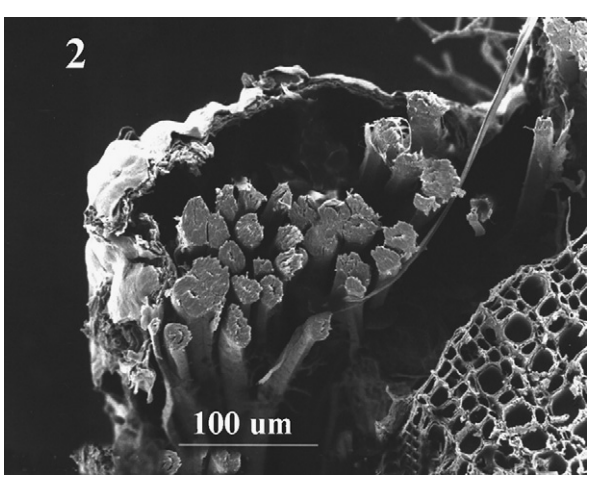


**Fig. 2 – Scanning electron micrograph of retted flax stem showing the separation of the bast fibers from the cuticularized epidermis and the shive fractions. During subsequent processing for clean bast fiber, the dust contains small particulates of cuticularized epidermis, shive, and fiber.**

## 2. Experimental

### 2.1. *Materials*

The flax dust and shive materials were obtained from commercial operations of Schweitzer-Mauduit International, Winkler, Canada. The dust was collected from the dust bag and the shive was from the last step before removal from the cleaning operation.

Waste water from pilot plant tests for the enzyme-retting of flax stems (Akin et al., 2007) was filtered in the laboratory through a double layer of cheese cloth to remove large particulates. This yielded 10 g solids/L waste water. These isolated solids were used in the study and are referred to as waste water solids. Ethanol was provided by AAper Alcohol (Shelbyville, KY, USA). Fatty acid, ester, and alcohol standards were purchased from Nu-Chek Prep, Inc. (Elysian, MN, USA).

### 2.2. *Microscopy*

Flax stems were free-hand sectioned, stained, and observed with a light microscope under bright-field illumination. Dew-retted sections of flax stems were excised and prepared for scanning electron microscopy as described (Akin et al., 2007).

### 2.3. *Solvent extraction*

Extractions were performed with the model 200 Accelerated Solvent Extractor, Dionex, Corp. (Sunnyvale, CA, USA). Samples, typically 5 g, were placed into stainless steel extraction chambers. The instrument was programmed to soak the sample for 5 min in the ethanol or aqueous ethanol solvent at the selected extraction temperature (50, 80, 90, 100 °C), flush the extraction chamber, and collect the extract. Samples were sequentially extracted three or more times with separate collections of each extract. The soak time was increased to 15 min after the initial extraction. Extracts were stored at −5 °C prior to analysis. Defatted solids were removed from the extraction chamber and subsequently hexane extracted to determine residual lipid content using an automated Soxhlet extractor, model 2050 Soxtech Avanti (Hoganas, Sweden).

### 2.4. *Solubility study*

The solubility of long chain waxes and alcohols in ethanol was determined as a function of temperature by placing an excess amount of a standard compound into a zero head space vial. The vial was equipped with a septum and valved port through which a syringe could draw a sample. The vial was heated to 80 °C and then cooled in a step-wise manner with samples taken by syringe. A temperature range of 80–40 °C was examined with samples analyzed at each 5 °C temperature level.

### 2.5. *Gas chromatographic analysis*

Gas chromatographic (GC) analysis of fatty compounds was performed on the Agilent 6890 gas chromatograph fitted with a DB5-HT column that measured 15 m × 0.250 mm × 0.1 μm.

Analyses were performed in duplicate. Nitrogen carrier gas was used with an oven temperature program starting at 120 °C for 1 min, increasing to 380 °C at 10 °C/min, and holding at 380 °C for 5 min.

## 3. Results and discussion

Analyses of ethanol extracts obtained from cuticular solids recovered from waste water (waste water solids) at different extraction temperatures are shown in Fig. 3. The first three extractions with ethanol removed significant but generally decreasing amounts of lipids at each temperature while the fourth and fifth extracts (not shown) contained no detectible amounts. An interesting relationship was observed between the extraction temperature and the amount of lipid removed. The first extraction at 90 °C removed the most lipids while the companion extractions performed at 80 and 100 °C removed lesser amounts. Extraction at 50 °C was not expected to be particularly effective although by the third extraction the amount of lipids removed was nearly comparable to the higher temperature extractions. The cumulative amounts of material recovered from the first three extractions are shown on the right side of the plot. The extraction at 90 °C appears to have removed the largest amount of lipids for the temperatures investigated with most recovered in the first fraction. It is unlikely that multiple extractions would be performed commercially due to process economics. Based on these preliminary results, a single extraction at 90 °C would be the most effective treatment for total lipid removal from waste water solids.

The compositions of the extracts of cuticular solids were determined by gas chromatography and exhibited variation with extraction temperature. Cuticle lipids include fatty alcohols, acids, aldehydes, and esters of different carbon chain lengths. The different components in the extracts have different potential applications and market values. The data presented in Table 1 show the composition of lipids in each fraction grouped by classes. Waxes are considered in one class and include ester compounds with total carbon chain lengths of 44 and greater. The other classes contain the shorter chain length compounds including the fatty acids, alcohols, and aldehydes of typically 30 carbons or less.

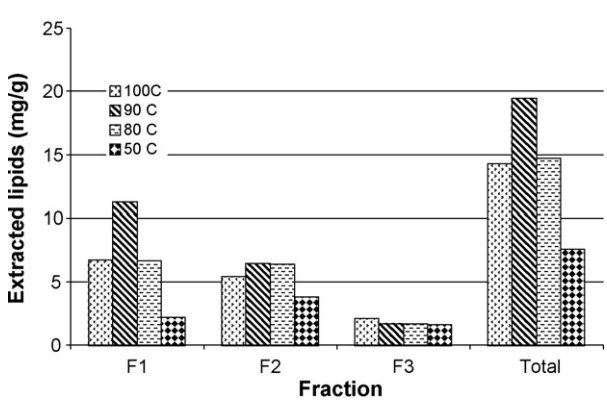


**Fig. 3 – Sequential extraction of flax waste water solids using ethanol at different temperatures.**

The first extract obtained at 100 °C was composed predominately of the shorter chain length compounds, i.e., between 16 and 30 carbon atoms. This extract contained approximately 35% fatty alcohols, 10% aldehydes, 26% fatty acids, and 26% waxes. Subsequent extractions at 100 °C removed increasing amounts of wax with the third extract comprised of 55% wax. This trend of increasing wax content was observed for extractions performed at 90 and 80 °C. The total amount of lipids extracted generally increased with extraction temperature. Extractions at 50, 80, and 90 °C yielded 7.6, 14.7, and 19.4 mg lipids/g solids, respectively. However, the highest extraction temperature, 100 °C, yielded an intermediate amount of extract, 14.3 mg/g.

Results for extractions performed on flax dust are presented in Table 2. The amounts of total lipid extracted from flax dust were greater than the amounts obtained from the cuticular waste water solids, i.e., the solids recovered by filtration of retting wash water. For example, 65.5 mg lipids/g flax dust was removed with 80 °C ethanol compared to 19.4 mg lipids/g waste water solids with 90 °C ethanol. However, these two fractions were obtained from different sources and may not be directly comparable. The proportion of wax in the extracts from the two fractions was also quite different. The flax dust extract obtained at 90 °C consisted of 6.1% wax compared to 34.3% for the waste water solids extract at this temperature. The highest extraction temperature, 100 °C, was also not the most effective for removing lipids from flax dust, yielding 43.4 mg/g compared to the maximum of 65.5 mg/g obtained at 80 °C. These results show the variation in the amount and composition of the extracts obtained from different sample types with extraction temperature.

Shive was also extracted with ethanol at 90 °C and yielded 10 mg lipids/g. The extract consisted only of 26 and 28 carbon length fatty acids, alcohols, and aldehydes. Subsequent extractions did not remove any additional lipids.

The role of moisture was briefly examined by performing an extraction of flax dust with 95% ethanol (aq) at 90 °C. These results were similar to those obtained with 100% ethanol although the wax content was reduced from 6.1 to 3.3%. This finding would be expected as the polarity of the 95% ethanol is increased and becomes a less effective lipid solvent.

The cuticle lipids, which are associated with the surfaces of plant tissues and are separated during retting and processing, allows a simpler recovery process than a conventional oilseed extraction. The latter involves movement of a solvent into a fractured solid matrix, solubilization of extractable components, and movement of the solvent with the extractable components out of the matrix. This multi-step mass transfer process is more complex than the removal of cuticle lipids on the plant tissue and which appears to be dominated by solubility effects.

The solubility of the wax compounds in ethanol and the related temperature dependence was examined further using individual wax standards. Solutions were prepared, cooled, and analyzed. Results obtained with wax compounds of 46–54 carbon chain length are shown in Fig. 4. Concentrations were determined by gas chromatography from a saturated solution of each standard. The solution was cooled from 80 to 40 °C in a stepwise manner and analyzed at each step. The longer chain length waxes of 50, 52, and 54 carbons showed a strong

**Table 1 – Amount and composition of ethanol extracts obtained at different temperatures from waste water solids**

| C atoms | Temperature (°C) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 100 | | | 90 | | | 80 | | |
| | F1[a] | F2[a] | F3[a] | F1 | F2 | F3 | F1 | F2 | F3 |
| Fatty acids | | | | | | | | | |
| 16 | 4.1 | 3.3 | | 3.2 | 3.0 | | 4.1 | 3.5 | |
| 18 | 1.9 | 1.7 | | 2.0 | 2.5 | | 2.3 | 2.0 | |
| 20 | 1.9 | | | 1.8 | | | 2.0 | | |
| 22 | 1.7 | | | 1.8 | | | | | |
| 24 | 2.0 | | | 1.5 | | | | | |
| 26 | 2.9 | | | 2.5 | | | | | |
| 28 | 5.5 | 3.2 | | 4.8 | 2.6 | | 6.6 | 2.7 | |
| 30 | 6.3 | 3.4 | | 4.4 | 2.5 | | 7.9 | 2.9 | |
| | 26.3 | 11.6 | 0.0 | 22.0 | 10.6 | 0.0 | 23.0 | 11.2 | 0.0 |
| Alcohols | | | | | | | | | |
| 26 | 10.8 | 11.2 | 15.7 | 9.9 | 15.4 | 21.8 | 11.8 | 12.7 | 23.3 |
| 28 | 12.1 | 10.0 | 11.7 | 10.2 | 13.8 | 18.4 | 11.9 | 11.7 | 20.1 |
| 30 | 11.7 | 6.6 | 10.2 | 7.8 | 4.7 | | 14.8 | 6.0 | |
| | 34.6 | 27.8 | 37.6 | 27.9 | 34.0 | 40.2 | 38.5 | 30.5 | 43.4 |
| Aldehydes | | | | | | | | | |
| 26 | 5.1 | 5.1 | 7.3 | 4.9 | 5.8 | | 6.2 | 5.8 | |
| 28 | 1.5 | | | 1.2 | | | 2.2 | | |
| 30 | 3.2 | 3.0 | | 3.4 | 4.3 | | 3.3 | 3.4 | |
| | 9.9 | 8.1 | 7.3 | 9.6 | 10.0 | 0.0 | 11.7 | 9.1 | 0.0 |
| Waxes | | | | | | | | | |
| 44 | | | | 1.8 | | | | | |
| 46 | 1.2 | 1.6 | | 1.7 | | | | | |
| 48 | 1.5 | 2.2 | | 1.5 | 2.3 | | | 1.8 | |
| 50 | 8.1 | 13.9 | 16.8 | 10.1 | 12.7 | 21.5 | 8.9 | 13.9 | 20.4 |
| 52 | 13.1 | 25.3 | 31.0 | 16.1 | 22.5 | 38.3 | 12.4 | 25.6 | 36.2 |
| 54 | 2.6 | 5.5 | 7.3 | 3.2 | 5.1 | | 2.2 | 5.6 | |
| 56 | | 1.5 | | | | | | | |
| | 26.5 | 49.9 | 55.1 | 34.3 | 42.7 | 59.8 | 23.5 | 46.9 | 56.6 |
| Other | | | | | | | | | |
| | 2.8 | 2.5 | 0.0 | 6.2 | 2.7 | 0.0 | 3.4 | 2.4 | 0.0 |

[a] Fraction.

decrease in solubility between 70 and 40 °C as the solution was cooled. The slight increase in solubility around 60 °C was unexpected but the general trend of decreasing solubility with temperature was followed. The shorter chain length waxes (46 and 48) did not exhibit a strong change in solubility over this temperature range.

These results indicate how the extraction temperature influences the composition of the extract through the solubility of the various wax components. The shorter chain length compounds are effected but to a lesser degree. This temperature dependence provides a variable that may be used to obtain a preferred composition of extract. However, the lower extraction temperatures, 50 °C, were less effective in removing total lipids. An alternative approach to obtain a lower wax fraction, for example, would exploit the temperature–solubility properties of the waxes to remove them from an extract obtained at a higher temperature, e.g., 90 °C. The practice of cooling vegetable oils to remove saturated triglycerides, winterization, takes advantage of differences in crystallization temperatures and achieves a separation of the components in a mixture. This scenario was tested with an ethanol extract of the flax lipids obtained at 80 °C. The extract initially contained 0.86 mg total lipids/mL with 25% of these lipids represented by the waxes. The extract was then cooled overnight at 10 °C and

**Table 2 – Amount of lipids extracted from flax dust with ethanol at different temperatures**

| Temperature (°C) | F1 (mg/g) | Wax (%) | F2 (mg/g) | Wax (%) | F3 (mg/g) | Wax (%) | Total (mg/g) |
|---|---|---|---|---|---|---|---|
| 100 | 23.43 | 4.8 | 12.41 | 4.68 | 7.57 | 0 | 43.42 |
| 90 | 17.61 | 6.10 | 13.71 | 7.77 | 5.90 | 0 | 37.25 |
| 80 | 34.74 | 3.39 | 23.18 | 2.51 | 5.61 | 0 | 65.53 |

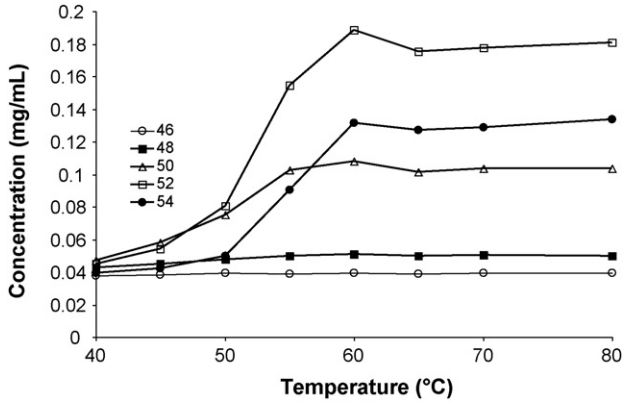


**Fig. 4 – Solubility of long chain waxes in ethanol as a function of temperature.**

the liquid phase was analyzed. After cooling the extract there were no waxes detected in the liquid phase. The precipitated waxes were recovered by filtration.

## 4. Conclusions

Hot ethanol was shown to dissolve the lipid components from flax cuticle fragments obtained from dust, shive, and cuticular solids recovered from process waste water. The solubility–temperature behavior of these components in ethanol was used to achieve a separation of the longer chain waxes from the shorter chain fatty compounds upon cooling the hot ethanol extract. This strategy could be implemented to recover lipid compounds of specific characteristics as value-added co-products from process waste or other low-value process streams generated during flax fiber production. The approach may be generalized to recover lipids from cellulosic materials such as perennial grasses or woodchips that are being considered as feedstocks for the production of ethanol.

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