Selective Break-Down of Flax Shive for the Recovery of High-Value Bio-Products

by

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THESIS ABSTRACT

A series of investigations was undertaken regarding the biorefining of biomass for the recovery of multiple high-value products. The overall objective was to develop a simple, selective breakdown approach for flax shive, addressing three specific research areas: material properties; extraction processing; and product purification. This thesis includes five published papers and additional research all related to the topic. Flax shive represents the woody residue left over after removing fibre from flax straw. It is composed of lignified xylem tissue. Flax shive is readily available in large quantities, at low cost, and with relatively consistent particle-size and composition characteristics.

Frictional behaviour investigations of bulk flax shive showed differing effects for addition of alcohols versus water. Adding any liquid significantly increased internal friction. The wall friction effects, however, depended on the liquid. Friction was increased significantly by water, but not as much by alcohols. Absorptive behaviour of flax shive, specifically liquid-holding capacity, was assessed using five liquids and compared to three other biomass materials. Flax shive was found to be a comparatively poor absorbent, a desirable behaviour for a feedstock used in aqueous- or alcohol-based processing.

The first step extraction employed sodium ethoxide catalyst in anhydrous ethanol. Yield of solvent-soluble organics varied linearly with catalyst concentration. At 1.0 M the yield was 54.5 ± 14.5 mg/g dry basis (db). Analyses using ^{1}H NMR consistently showed extracts to be phenolic in nature, and to contain no carbohydrate constituents. The second step extraction of hemicellulose polysaccharides was done using aqueous

1.0 M sodium hydroxide. The yield of carbohydrate precipitates was consistent, 99.4 ± 5.1 mg/g (db), and was unaffected by pretreatment. Analyses of polysaccharide backbone monomers showed consistently high molar ratios of xylose-to-glucose, i.e., 25.5 ± 3.4, with no mannose present. These results suggested a high concentration of glucuronoxylan polymer, likely greater than 90% by mass, with no glucomannan present.

Economic evaluation showed two-stage extraction of high-value products to be a potentially viable business. Such processing also tied directly to government polices aimed at increasing value-add from agricultural materials. Overall, flax shive was found to be a desirable feedstock for recovery of high-value bio-products.

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List of Abbreviations

^{1}H NMR	Proton nuclear magnetic resonance
ANCOVA	Analysis of co-variance
ANOVA	Analysis of variance
C of V	Coefficient of variation
db	Dry basis
df	Degrees of freedom
GC-MS	Gas chromatography - mass spectroscopy
HPLC	High pressure liquid chromatography
H-type	*p*-Hydroxyphenyl lignin type
G-type	Guaiacyl lignin type
LCC	Lignin-carbohydrate complex
min	minute
MS	Mean square
n/a	Not reported (not available)
N/D	Not detected
SD	Standard deviation
SS	Sum squares
S-type	Syringyl lignin type
TLC	Thin layer chromatography
wb	Wet basis

1 Introduction

Throughout history, the primary purpose of agricultural production has always been for food. At the same time, agricultural materials have been recognized as useful sources for products other than basic foodstuffs. Increasing attention, both in terms of research and commercial activity, is now being paid to agricultural materials as potential feedstocks for a variety of products. No where is this more evident than for biomass.

1.1 Biomass and Availability

There are many and somewhat varied definitions for the term "biomass". One authoritative version is provided in the standard ANSI/ASABE S593 (ASABE 2006), as "Organic materials that are plant or animal based, including but not limited to dedicated energy crops, agricultural crops and trees, food, feed and fiber crop residues, aquatic plants, forestry and wood residues, agricultural wastes, bio-based segments of industrial and municipal wastes, processing by-products and other non-fossil organic materials." There are associated distinctions differentiating primary (i.e., direct crops), secondary (i.e., processing byproducts), and tertiary (i.e., post-consumer) biomass streams.

Agricultural biomass represents the general subject of this work. Also known as agri-fibre residues, fibrous biomass, ligno-cellulosic biomass, biomass processing residuals, or related terms, these materials include straws or other plant residues left over after harvesting or processing. They are often referred to as biomass for simplicity, even though they represent only a subset of the definition under the above standard.

Chemically, biomass is a form of ligno-cellulose, essentially plant structural material. Biomass is composite in nature, being composed in varying proportions of

three readily identifiable polymeric constituents: cellulose; hemicellulose; and lignin. As described in more detail later, biomass is a heterogeneous aggregation of components involving carbohydrate and hydrocarbon sub-units. There are only relatively small amounts of other chemical materials involved.

Biomass, in general terms, represents a huge, but still largely untapped resource. The extent of biomass availability within Canada has already been extensively quantified. Wood and Layzell (2003) developed a broad-based inventory of various forms of biomass for Canada as a whole. This included both agricultural products and by-products, as well as forest residuals and municipal wastes. They used a macro-estimation approach and indicated that annual recoverable agricultural residuals within Canada alone represent in excess of 29 million tonnes on a sustainable basis. Stumborg and Townley-Smith (2004) considered agricultural residuals for Canada using a more micro-scale approach. They came to the conclusion after taking account of the implications of soil erosion and nutrient requirements, that ample biomass material is available in specific areas of Canada, particularly the Prairies, including the Province of Manitoba, to support industrial applications. They, however, did not consider an assessment of economic viability.

Jurisdiction-specific evaluations also have been underway. Studies by Prairie Practitioners Group (2008, 2009) within the Province of Manitoba evaluated the availability of crop straws, i.e., primary biomass, as well as a variety of other biomass processing byproducts, i.e., secondary biomass, including flax shive, oat hulls, sunflower hulls, and hemp hurds. These studies indicated the sustainable availability of roughly 1

to 2 million tonnes annually of primary crop straws of various types, as well as large quantities of secondary byproducts. Flax shive was identified as being the most significant secondary byproduct, with an available quantity of 60,000 to 70,000 tonnes annually, and it is the subject of investigations as a feedstock in this work.

1.2 Biomass Products

The impetus to find new useful and valuable feedstock applications for biomass is due to a series of converging societal trends, including: the decline in availability of inexpensive petroleum, and the associated need to address the rising costs of "synthetic" products of all types, not just energy; the increasing interest by consumers in products that are "naturally-derived"; the desire to obtain value from underutilized or wasted materials, particularly from an agricultural perspective; and the desire to enhance local economic development within agricultural communities.

Considering biomass as a feedstock has in part also led to popular use of the terms "bio-products" and "biorefining." Bio-products, or bio-based products, as outlined in the ANSI/ASABE Standard S593 (ASABE 2006), are defined as, "fuels, food, feed, chemicals, or industrial materials commercially produced in whole or in-part from biomass materials." Regarding biorefining, the U.S. Department of Energy had earlier adopted a definition based on Milne et al. (1990) to generically describe the conversion and extraction of a spectrum of multiple useful products from biomass via a single processing facility. This was mirrored in the ANSI/ASABE Standard S593 (ASABE 2006) as, "a facility that uses mechanical, thermal, chemical, and/or biochemical processes to convert biomass into value-added bio-based products or key intermediates

for the production of chemicals and other materials." A key implicit requirement in order to be considered under the umbrella of biorefining is that at least two distinct products be obtained from a single feedstock. The focus of this work was on separate sequential chemical extractions to obtain multiple products.

Given the plethora of possibilities for biomass, it is also important to consider the nature of and selection of bio-products with sufficient value to justify the expenses of both development and processing. Thimmanagari et al. (2010) presented a useful, brief categorization of potential bio-products into three major categories: firstly, bio-energy; secondly, bio-materials; and lastly, bio-based chemicals. The latter represents the broadest category, including basic and specialty industrial chemicals, resins, paints, lubricants, waxes, solvents, nutraceuticals, pharmaceuticals, bio-actives, cosmetics, platform intermediate chemicals, and others. In this work, the focus was on products in the category of chemicals, with applicabilities for resins and specialty chemicals.

1.3 Biomass Processing

Pursuing higher-value products is an intrinsically obvious approach to enhancing the value of biomass resources, however, product value is not the only important factor to determine economic outcome. Overall economic viability is determined by a combination of four factors, as outlined by Lange (2007): value of the end product(s), which determines the potential revenue; cost of input feedstock, which for biomass is typically low; capital cost of the conversion process; and product yield and operating cost. The last two aspects are both determined by the conversion process itself.

Product recovery from biomass, including high-value applications, continues to be fraught with technical and economic challenges. Examples of difficulties are ample, with two high profile cases, involving direct connections to Manitoba: Isobord; and Iogen Energy joint venture. The Isobord strawboard plant in Elie, Manitoba was initially heralded (Cooper et al., 1999). The plant, which was acquired and operated by Dow BioProducts, faced material-related problems through its history and ultimately ceased operations at the end of 2005 (Dow 2005). The Iogen ethanol-from-cellulose process had been under development for more than 25 years. Implementation of a commercial-scale ethanol plant was under recent consideration in Manitoba. Upon review by its ownership partners, Royal Dutch Shell and Iogen Corporation, the project was terminated (Shell and Iogen 2012). Processing costs or product yields were inadequate to proceed forward.

Problems of poor process performance and high-costs with biomass tend to be persistent. They stem from a variety of causes, including three identified main issues:

- Relatively poor yields of desired products due in part to heterogeneous feedstock composition, and in part from poor selectivity in recovery processes;
- Purification issues due in part to diverse components, and in part due to a frequent plethora of associated degradation by-products; and
- Materials handling related issues, due to inadequate feedstock characterization.

These three problem areas were specifically considered through the course of this work.

1.4 Research Objectives

The overall objective of this work was to investigate and to develop a simple, selective breakdown approach for the cost-efficient recovery of multiple high-value bio-

products from a biomass feedstock. Flax shive was the feedstock of choice in this work. As described in more detail later, flax shive is the woody residue left after removal of more-valuable bast-fibres from flax straw, and is available within Manitoba in large quantities, at low cost and with relatively consistent composition and size characteristics. The targeted products in this case were firstly phenolic-rich organic constituents, likely derived from lignin, and secondly novel oligosaccharides derived from hemicellulose. Three areas of research were tied together in this work:

- Investigating flax shive material properties, specifically frictional behaviour and liquid absorbency, that would effect processing;
- Identifying and evaluating selective breakdown processes for flax shive in order to achieve suitable yields of multiple products; and
- Considering product purification in process selection and development in order to ensure suitable purity, and thus value, of products.

1.5 Thesis Structure

This thesis was prepared using a publication-manuscript format. It incorporates five published papers, each presented in a separate chapter, as well as additional unpublished supporting experiments, presented in two additional chapters. All of this work relates to the central theme and objective of the thesis. For each chapter, the associated publication details are presented, as well as the contributions of Robert Parsons to each individual work.

Chapter 3 discusses unpublished preliminary experiments related to material characterization. This includes: screen-separation of flax-straw processing waste;

particle-shape characteristics of flax shive; density differences between flax shive and waste-fibre; and colour differences between various flax-straw processing waste components.

Chapter 4 and Chapter 5 deal with the frictional behaviour and the liquid-holding capacity of flax shive, respectively. Both chapters were published as separate papers in the peer-reviewed journal *Canadian Biosystems Engineering*, in 2008 and 2011 respectively.

Chapter 6 and Chapter 7 deal with the conversion process. Each covers one of the two separate extraction steps. These were, respectively, to recover phenolic-rich organics using sodium ethoxide catalyst, and to recover hemicellulose polysaccharides using aqueous sodium hydroxide. Both chapters were published as separate papers in the peer-reviewed journal *Industrial Crops and Products*, in 2011 and 2013 respectively.

Chapter 8 provides an overview of the entire process, as well as a preliminary evaluation of economic viability, market prospects for products, and policy aspects. Chapter 8 also directly links the pre-extraction of high-value constituents to expedite the development of next-generation bio-fuels, using left-over cellulose. Chapter 8 was published as a paper in 2011 as part of the CSBE/SCGAB 2011 Annual Conference.

Chapter 9 discusses additional, unpublished follow-up experiments dealing with larger-bench scale processing and compositional tests for sequential steps: pretreatment using sodium ethoxide; and subsequent alkaline extraction. The overall thesis discussion is presented in Chapter 10, and the overall thesis conclusions are presented in Chapter 11. These two final chapters tie all of these different research aspects back together.

2 Literature Review

The theoretical basis for the proposed selective breakdown of biomass is provided in the literature review. The literature review for this research is separated into three major components, providing overviews of: (i) relevant characteristics of ligno-cellulosic as typical for agricultural biomass; (ii) specific nature and characteristics of flax shive and related materials; and lastly (iii) breakdown processes applied to ligno-cellulose and their implications for intra- and inter-component bonding. Each of these aspects is described in separate sections. Additional literature review aspects are included in each of the chapters representing a published paper.

2.1 Ligno-Cellulose Characteristics

Ligno-cellulose represents the main structural material of all plants. The complexities of biosynthesis, the functions, and the micro- and macro-structures of native ligno-cellulose are not considered further in detail, given that the purpose of this work was to investigate biomass as a source for high-value products. Similarly no graphical or schematic representations of ligno-cellulose makeup or structure have been included, but are available in recent literature sources including Rubin (2008) (refer to Figure 2), and Foston and Ragauskas (2012) (refer to Figure 2). At the same time, there are still a number of key aspects of plant biology that are relevant to note.

Certain characteristics of plants and their associated ligno-cellulose structures are reasonably consistent. Plant tissues in angiosperms, i.e., flowering plants including grasses that represent the most widespread land plants, are generally divided into three types (Campbell and Reece 2002): dermal; vascular; and ground tissues. Dermal tissues

involve the specialized outer cellular layers primarily associated with protection and water retention. Vascular tissues are usually continuous throughout plants and primarily provide transport through two separate liquid transport networks. First is the xylem that acts as a water transport system, moving water upward from roots. It depends on transpiration and physical properties of water as the driving force. Second is the phloem that provide translocation of food materials throughout the plant from sugar sources to sugar sinks using pressure flow as the driving force. The last tissue type, called ground tissues, involve essentially any remaining tissues that are neither vascular nor dermal. In dicotyledonous plant stems, ground tissue is typically divided into pith, which is internal to the vascular tissue, and cortex, which is external to the vascular tissue.

As summarized by Campbell and Reece (2002), there are three major types of cells in plant tissues. Parenchyma cells are unspecialized, with thin, flexible primary cell walls, and are responsible for metabolic functions. Collenchyma cells have unevenly thickened primary cell walls, providing structural support to growing plant shoots. Sclerenchyma cells are much more specialized, rigid cells. They owe their rigidity to the progressive build up of thick secondary cell walls of a lignified cellulosic matrix inside the primary cell wall. Highly specialized sclerenchyma cells include vessels and tracheids in the xylem, which provide both support and water transport; and fibers and sclerieds, which are entirely for structural support. Fiber cells are long, slender and tapered, usually occurring in groups, while sclereids are shorter and more irregular in shape and occurrence.

Hydrostatic turgor pressure, resulting from the absorption of water, provides the motive force for cell expansion in growing plant tissue (Haigler 1985), i.e., parenchyma or collenchyma cells. The primary cell walls of such cells (Moller et al. 2006) are a highly hydrated, complex composite of polysaccharides, proteins and aromatic substances, albeit with little lignin. As plant tissue develops, sclerenchyma cells develop thick secondary cell walls incorporating cellulose and lignin, the latter of which acts as a hardening and binding agent, proving rigidity even in the absence of turgor. It is secondary cell walls that represent the main repository and source of ligno-celluloses.

Ligno-cellulose as a whole, is well understood to be heterogeneous in nature and, most importantly, to vary somewhat in composition, both between plant species and even between different tissues of the same plant. As such, targeting the recovery of high-value products depends both on the plant species selected and the specific portion of the plant selected, given potential differences in overall composition.

The main identified chemical constituents of ligno-cellulose (Haigler 1985) in approximate order of abundance are: cellulose; lignin; hemicellulose; pectic substances; extractives; ash; and other minor components, including lipidous material, proteins and others. Relevant aspects of each of these components to this work are described in the following separate sections.

2.1.1 Cellulose

Cellulose, or more properly α-cellulose, is recognized as the most prominent and most understood polymeric component of ligno-cellulose, and also the most abundant biopolymer in the world. The elucidation of cellulose as a common and consistent

chemical component of plant tissue dates to the work of Anselme Payen in 1838 (Nevell and Zeronian 1985). However, purified cellulose has an even longer history of economic use as a product, and remains the most important biopolymer exploited by humans, primarily for materials applications, notably paper and textile fibre.

Cellulose is well known, chemically, to be a linear condensation homo-polymer, consisting almost uniformly of individual D-anhydroglucopyranose units linked by β-1,4-glycosidic bonds (Haigler 1985 or Whistler and BeMiller 1997). These linkages result in a stiff, ribbon-like molecule readily oriented to inter- and intra-chain hydrogen bonding, leading to the ready formation of highly crystalline, structural fibrils. Cellulose polymers also exhibit a very high degree of polymerization (i.e. number of monomers), typically in the range of thousands to tens of thousands of D-glucose units per cellulose molecule.

The cellulose content in plants varies with the organism, the type of cell and the stage of individual cell development. Primary cell walls may contain upwards of around 10% cellulose, while secondary cell walls may be upwards of 50% cellulose. Specialized fiber cells, as described earlier, such as in cotton-seed hairs or in flax bast fibre, may contain an extremely high proportion of cellulose, upwards of 80%.

Through the biosynthesis of cellulose, plants effectively produce a strong and durable structural material, but, importantly, one that is made up of building blocks that are ultimately metabolic food units, i.e. D-glucose monomers. Cellulose is analogous to amylose and amylopectin polymers in starch, except that cellulose uniformly has β-1,4 linkages rather than α-1-4 linkages, and lacks any 1-6 side-chain linkages.

Although cellulose today is characterized according to its chemical structure, in the past it had been identified according to its chemical behaviour. Specifically, "alpha-cellulose" was defined as pulp or fibre constituents that did not dissolve in sodium hydroxide solutions at concentration levels as employed in mercerization processes, i.e., upwards of 5.2 M. Indeed, such a property-based definition for cellulose is still included in the TAPPI Standard T203 (TAPPI 2009).

Cellulose has a high degree of uniformity and consistency across different plant species and between different plant tissues. As such, cellulose is essentially a common commodity. From the perspective of high-value chemical or bioactive compounds, cellulose itself is relatively uninteresting. At the same time, cellulose does represent a potential low-cost source of glucose substrate for fermentation or other bioconversion (Carere et al. 2008). The potential to breakdown cellulose as a source of glucose has long been recognized (Duckworth and Thompson 1983, Wise 1983, or Litchtenthaler and Peters 2004). There are potential long-term cost advantages of using cellulose as a feedstock substrate for fuels or other fermentation-derived products, but in the meantime, D-glucose can be easily derived from plant-based starch and sugar sources.

2.1.2 Lignin

Lignin is one of the two feedstock components considered in this work for recovery of high value products. It is also the second largest constituent of ligno-cellulose. Lignin is, next to cellulose, the second most abundant polymeric plant material in the world (Baucher et al. 1998). It is, however, distinctly different from cellulose, being more hydrocarbon in nature, and being an irregular, three-dimensionally linked

heteropolymer, Although its presence has been well documented, the chemical nature of lignin is much less precisely defined than that of cellulose.

Boerjan et al. (2003) described lignin, chemically, as a complex mixture of phenyl-propyl subunits, based primarily on three hydroxycinnamyl alcohol monomers that differ in their degree of methoxylation: coniferyl alcohol; sinapyl alcohol and *p*-coumaryl alcohol. These three precursors lead to the formation respectively of guaiacyl (designated G), syringyl (designated S), and *p*-hydroxyphenyl (designated H) phenyl-propanoid units within lignin polymers. The quantity and composition of lignin, vary by the organism and type of cell, and are also influenced by developmental and environmental cues.

Some general trends in lignin composition have been observed. Gymnosperm (softwood) lignin tends to involve mostly G-units, with low levels of H-units. Angiosperm monocotyledonous lignin incorporates G- and S-units at comparable levels, with higher levels of H-units. Dicotyledonous lignin, including flax, involves a mixture of G- and S-units, with only traces of H-units.

The structures of the three lignin precursor alcohol molecules closely resemble those of the amino acids phenylalanine and tyrosine. Indeed, as described by Sjostrom (1993), the biosynthesis of these molecules all share certain common biochemical pathways, in particular the Shikimic acid pathway that leads to the formation of aromatic ring structures from glucose. Sjostrom (1993) noted that the bonds between lignin subunits are irregular but tend to be dominated by ether linkages.

Harmsen et al. (2010) indicated that ether bonds are generally considered to represent up to 70% of bonds between lignin subunits, with most of the remaining 30% being carbon-carbon bonds. Potentially more important for this work are the linkages between different polymeric components, particularly lignin with hemicellulose or cellulose. Harmsen et al. (2010) noted that hydrogen bonds and covalent ether bonds can exist between lignin and either cellulose or hemicellulose, but covalent ester bonds tend to exist between lignin and hemicellulose. These ester linkages tend to be part of so-called lignin-carbohydrate complexes (LCC), as described by Ebringerova et al. (2005).

Crawford (1981) described lignin as a peculiar biopolymer compared to polysaccharides, proteins or nucleic acids. Instead of having a linear backbone, with cleavable bonds at regular intervals, lignin is irregular, three-dimensional and amorphous in nature, with a random distribution of interconnecting bonds between subunits and linkages to carbohydrate components. Given this nature it has been not possible to isolate lignin in an unaltered state. This has been a well-recognized and ongoing issue (Pearl 1967, Forss and Fremer 2000). The nature of recovered lignin is changed irrespective of whether using methods to: (i) remove other components and leave lignin behind as an insoluble residual, including the acid-based Klason lignin method; or (ii) selectively recover or derivatize lignin constituents.

Lignin provides a variety of functions for plants (Whetten et al. 1998). These include compressive strength improvement and water impermeability to protect the polysaccharide structural matrix, as well as resistance to degradation by microbial attack and acting as an antioxidant. One key constituent is ferulic acid, which has been

specifically identified as a desirable antioxidant product and also found to be attached to certain plant derived oligosaccharides. Ferulic acid also shares a chemical structure that is very close to the three lignin precursors (Rosazza et al. 1995).

Despite substantial promise for exploitation to produce value-added products (Lora and Glasser 2002), actual lignin use for industrial applications remains very limited and largely disappointing so far. The largest practical application of lignin still today is simply in combustion for heat, as undertaken in Kraft pulping-process black liquor boilers, a common practice in the pulp and paper industry. The lack of exploitation is well recognized (Grosslink et al. 2004, Tuck et al. 2012).

Potential high-value product applications that might be possible with lignin were reviewed by Holladay et al. (2007). They identified three major categories of opportunities that they also associated with potential timeframes: (i) enhanced energy opportunities over the near-term, including heat and power generation, combustible fuels and synthesis gas production; (ii) macromolecule recovery opportunities over the medium-term, including carbon fibre precursors, polymer modifiers, and resin/adhesive/ binder ingredients; and (iii) aromatic chemical monomers over the long-term, including benzene/toluene/xylene, phenolic derivatives, such as ferulic acid, and specific lignin monomers such as propylphenol, eugenol, syringols, aryl ethers, and alkylated methyl aryl ethers. At the same time, a variety of continuing issues have been recognized regarding the recovery of specific products from lignin. These include: the high degree of complexity of lignin, especially in the nature of cross-linkages; the inconsistency and

variability of lignin composition; and the difficulties associated with extraction of usable lignin materials.

2.1.3 Hemicellulose

Hemicellulose is the second substrate considered in this work for recovery of high-value products, and is, in aggregate, the third major constituent of ligno-cellulose, behind both cellulose and lignin. Although in the past it was initially thought to be a potential precursor substance for cellulose biosynthesis in plants, hemicellulose is understood today to represent a class of heterogeneous polysaccharides that are formed through different biochemical pathways than cellulose (Sjostrom 1993).

Hemicellulose is almost always found in direct association with cellulose, and is a significant constituent in both primary and secondary cell walls, representing roughly 20% to 30% of mass (Whistler and BeMiller 1997). Carpita and McCann (2000) recommended these polymers would be better described as cross-linking glycans, reflecting their major function as cross-linking agents between individual cellulose fibrils. Thompson (1991) noted the term "polyose" had been suggested as an alternative name in Europe. However, despite its arcane origins, the term "hemicellulose" is widely recognized, and is used throughout this work to describe the compounds of interest.

Definitions of hemicellulose differ somewhat, as do the precise polymers that may or may not be included under this umbrella, as described later. A useful definition has been adopted, as outlined by Wilkie (1985), "hemicelluloses are non-cellulosic polysaccharides other than starches and fructosans found in the aerial, and normally lignified, parts of the organs of higher land plants from which they can be extracted by

dilute aqueous alkali after the removal of lignin. They can then be precipitated from neutral or slightly acidified solution by the addition of ethanol or acetone."

Hemicellulose polymers are not as regular in structure nor as uniform in composition as cellulose. This variability and diversity makes hemicellulose polymers more attractive, as a group, to investigate as sources of high-value products, specifically complex carbohydrates (Ebringerova et al. 2005). Although variable, hemicellulose polymers are not random, and exhibit consistent characteristics (Sjostrom 1993):

- Much smaller size than cellulose, typically no more than about 200 monomers per polymer molecule;
- Backbone frequently, but not always, consisting of a uniform sugar monomer in a pyranose form, most frequently D-glucose or D-xylose;
- Linear monomer-to-monomer backbone linkages typically consisting of β-1,4 glycosidic bonds, similar to cellulose, but in some instances with periodic mixtures of different linkages, such as β-1,3 glycosidic bonds as in β-glucans;
- Short side-chains on a high proportion of backbone monomers, typically a single monomer unit and no more than about three monomers as the longest side-chain;
- Consistent combinations of side groups associated with backbone structures within a given type of polymer;
- Side group linkage to O-2, O-3 or O-6 positions of individual backbone monomers, but consistent positions within a given polymer; and
- Occasional but consistent inclusion of acetyl groups via ester linkages.

The relatively smaller size of hemicellulose molecules has an important implication that blurs the distinctions between true polymers (i.e. polysaccharides) and oligosaccharides. As such, hemicellulose can be viewed as representing a combination of small polymers and/or large oligomers, although for the sake of consistency, hemicellulose components in this work are described as polymers.

The main monomer sugars found in individual hemicellulose polymers are in approximate order of abundance (Sjostrom 1993): D-glucose; D-xylose; D-galactose; D-mannose; and L-arabinose. In addition, smaller amounts of the deoxy sugars L-rhamnose and L-fucose, as well as D-glucuronic acid, 4-O-methyl-D-glucuronic acid, and D-galacturonic acid may be also included. All of these various sugars, deoxy sugars and sugar acids are biosynthesized by plants from glucose (Carpita and McCann 2000).

Hemicellulose polymers can be divided into four general classes based on chemical structure, reflecting the classifications as outlined by Ebringerova et al. (2005) and Brett and Waldron (1990). These four classes are:

- Xyloglycans, which involve a backbone of D-xylopyranose monomers typically linked linearly via β-1,4 glycosidic bonds, and with various side-attachments;
- Mannoglycans, which involve a backbone of either purely D-mannopyranose monomers or mix of D-mannopyranose and D-glucopyranose units linked linearly via β-1,4 glycosidic bonds, and with various side-attachments;
- Mixed-linkage glucans, which involve a backbone of D-glucopyranose monomers, but with mixed linear linkages other than solely β-1,4 glycosidic bonds; and

- Xyloglucans, which involve a backbone of D-glucopyranose monomers linearly linked via β-1,4 glycosidic bonds, like cellulose, but with regular side chain substitution of D-xylopyranose and/or other sugar monomers.

Schematic representations of the main hemicellulose polymers are provided in Ebringerova et al. (2005).

The nomenclature for hemicellulose polymers has evolved in a fairly arbitrary manner, with polymers typically named according to the main backbone constituent or constituents (Thompson 1991). One result of such development has been inconsistencies in classification. Ebringerova et al. (2005) noted that depending on historical circumstances, individual polymers may be considered as hemicelluloses, gums or pectic substances, or even some combination. There is also a significant degree of vagueness and overlap, particularly between identified sub-types of hemicellulose polymers. This is in part due to a relative lack of knowledge, particularly when compared to cellulose, and in part due to the extensive variability. Hemicellulose is known to vary in composition from plant species to species and even between different organs of the same plant.

2.1.4 Pectic Substances

Pectic substances represent a group of cell-wall matrix polymers rich in D-galacturonic acid, L-rhamnose, L-arabinose and D-galactose monomers (Brett and Waldron 1990). These polysaccharides are commonly found in dicotyledons, less so in monocotyledons. They are generally present at much lower overall concentrations than cellulose, lignin or hemicellulose constituents, i.e. in the range of only about 1% to 5% (Robyt 1998). Certain tissues in specific plants, however, may have relatively high

concentrations, and can be used for the extraction of commercial "pectin" products (Whistler and BeMiller 1997).

Just as in the case of hemicellulose polymers, pectic substances are not always described consistently. The term pectin is often used to generically describe water-soluble galacturonoglycan preparations, particularly commercial products used to produce stable gels (Whistler and BeMiller 1997). However, these represent only a portion of pectic substances, as described below. Their exploited gel-forming capabilities also reflect their natural function. Pectic substances tend to form a space-filling hydrophilic gel between cellulose microfibrils in plant primary cell walls (Cosgrove 1997).

Carpita and McCann (2000) identified five main types of polymers that are typically included as pectic substances. These are: (i) homogalacturonans; (ii) galacturonan derivatives; (iii) rhamnogalacturonan type I; (iv) arabinans; and (v) galactans. Carpita and McCann (2000) also noted that homogalacturonan and rhamnogalacturonan type I typically represent the main polymers in pectic substances.

Hemicellulose and pectic substances are both matrix polymers. The historical differentiation between them (Brett and Waldron 1990), has been based on differences in their means of extraction. i.e. alkaline solutions for hemicellulose versus hot solutions of chelating agent or dilute acid for pectic substance. Although arbitrary, this distinction is still useful from a practical perspective. An important structural distinction between the two polymers is that main backbone linkages in hemicelluloses typically involve β-1,4 glucosidic bonds, while in pectic substances backbone linkages typically involve α-1,4

glucosidic bonds. Pectic substances can be often covalently linked to phenolics, cellulose and proteins. Their cross-linking and gel-forming nature can interfere with the extraction of other desired compounds, such as hemicellulose components.

2.1.5 Extractives

The term "extractives" is used as a catchall to describe the constituents of plant biomass that are soluble in and can be extracted through the use of aqueous solutions or organic solvents. Again, as with the cases of pectic substances and hemicellulose, the definition is historical and based on recovery methods. A key implication is that such compounds tend to be largely non-polymeric in nature.

Extractives represent a small proportion of plant biomass, but historically have been economically important. Of particular importance have been secondary metabolite compounds, which traditionally have been high-value constituents derived from plants, including the broad classes of terpenoids, alkaloids, and phenolics (Harborne 1999). Materials of this type were not a priority for recovery in this work, although they are important to identify.

2.1.6 Ash

Ash represents in aggregate the residual mineral content present in biomass. Such constituents were not a priority for recovery in this work, however, they are important to identify. Nordin (1994) indicated that the mineral ash content of biomass materials can vary quite extensively, from as little as 0.5% to as much as 10% in some cases. Bakker and Elbersen (2005) identified five most frequently encountered minerals in biomass that are thought to have a potential impact on thermal or biochemical biomass conversion

processes. These are, in no specific order of priority: silicon; potassium; calcium; sulfur; and chlorine. Three of these (potassium, calcium and sulfur) are among the five essential macronutrient minerals required by plants (Kochian 2000). Chlorine is identified as an essential micronutrient for plants, but is a highly abundant element. Silicon is abundant, but is not an essential element for plants.

2.1.7 Others Including Proteins and Lipids

Finally there are a variety of other constituents that are also present in smaller concentrations, most notably proteins and lipids. Proteins and lipids represent important classes of products that are frequently obtained from plants. In the context of ligno-cellulose, which as defined earlier in Chapter 1 represents essentially plant structural materials, they are relatively unimportant for recovery applications. Dale (1983) observed that although proteins themselves have high-value as recovered products, research on biomass utilization has typically tended to not consider protein extraction, this simply given low content and associated difficulties for recovery and purification. Indeed, the priority in recent research on protein from ligno-cellulose has tended to focus on biomass as a substrate for microbial protein enhancement, rather than attempts at direct protein extraction (Ugwuanyi et al. 2009). Lipid-type materials tend to be primarily associated with cuticular outer layers of plants, consisting of waxes, cutins and suberins (Bidlack and Jansky 2011). Again, their content is low.

2.2 Flax and Flax Shive

2.2.1 Flax

Flax (*Linum usitatissimum*) is one the oldest crops to be exploited by humans, with its use known to extend back more than 10,000 years (Kozlowski et al. 2005). Flax is a dicotyledon of the Linaceae family in the order Linales (Salmon-Minotte and Franck 2005). It is easily recognized among farmed crops by its distinctive purple flower.

The flax stem has a range of 0.6 to 1.0 m in height and is approximately 2 mm in diameter (Kozlowski et al. 2005). It consists of eight major layers in sequential order moving inward from the outer surface: (i) epidermis, a single layer of dermal tissue cells covered by cuticular waxes; (ii) primary cortex or bark parenchyma, two to seven ground tissue layers of parenchyma cells; (iii) bast fibres, 20 to 40 bundles of fiber cells formed in a loose ring within the primary cortex; (iv) phloem, a layer of vascular tissue cells forming vascular bundles; (v) cambial zone or cambium, a growth origin layer of cells separating the phloem from the xylem; (vi) xylem, lignified vascular tissue of sclerenchyma cells that form the water transport network up the stem; (vii) core, the inner part of the xylem; and (viii) core channel, an empty space inside the middle of the stem along its whole length. In some instances, as noted by Salmon-Minotte and Franck (2005), the inner core of the stem is described to be tissue pith. Given that non-lignified parenchyma cells can only maintain rigidity by turgor pressure, once harvested, drying out of the stem results in a hollow core. Cross-sectional schematic representations of flax stems are provided in Salmon-Minotte and Frank (2005) and Kozlowski et al. (2005).

The primary portion of the flax stem of commercial interest is the long bast fibres located around the stem periphery. It is this material that is applied to textiles, paper and assorted other similar applications. Flax shive, on the other hand, which is the subject of this work, involves the more lignified xylem tissues from the central stem portions.

2.2.2 Flax Crop Applications and Flax Types

The initial and oldest application of flax was for fibre recovery to produce linen textiles, however, flax is also an oil seed crop. Flax or linseed oil, which is extracted from the seeds, has a variety of useful properties for both human-food and industrial applications. Given the duality of products, flax crop varieties and cultivars have essentially been tailored to one or other application, i.e. either fibre or seed oil. The former are typically described as "flax," with generally taller stems having less branches and less seed pods. The latter are often referred to as "linseed," with generally shorter stems having more branches and more seed pods (Salmon-Minotte and Franck 2005).

Kozlowski et al. (2005) noted that seed-oil dominates overwhelmingly world cultivation of flax. In 2003, for example, 2.4 million hectares were planted for seed-oil flax and only 0.5 million hectares for specialty fibre flax. They further noted that Canada is the world's leading flax cultivation country, representing more than one quarter of all the planted area. Canadian flax production is located almost entirely within Manitoba and Saskatchewan, and overwhelmingly oriented to seed-oil.

2.2.3 Flax Straw Processing

Despite domination of seed-oil flax in Western Canada, significant quantities of straw from seed oil flax are processed, particularly in Manitoba, to recover bast fibre

from the outer portion of the flax stem. There is one major flax straw processor and several additional players in the region.

The largest flax straw processor in Canada, and one of the largest in the world, is SWM International Inc., which was formerly known as Schweitzer-Mauduit Canada Inc. Their operations are based in Winkler, Manitoba, with round-baled straw collected and trucked to three sites. The largest of these is a permanent decortication facility just outside Carman, Manitoba in the Rural Municipality of Dufferin (Manitoba Conservation, 2001). SWM International processes in the range of 80,000 to 100,000 tonnes of flax straw annually, with recovered bast fibre shipped to their Spotswood, New Jersey mill where it is used to produce flax pulp. Flax shive from SWM International's Carman facility primarily tends to remain local. All of the flax straw processing wastes and flax shive samples used in this research work came from their Carman facility.

The scutching or decortication process for bast fibre removal is described overall by Salmon-Minotte and Franck (2005). The process involves a sequence of mechanical operations to separate desirable long fibres from remaining plant materials. Similar processes are employed for a variety of high-fibre plants, including leaf fibres from sisal or jute, and stem bast fibres from hemp. Decortication is also typically undertaken with moisture content less than about 19%, such that some straw drying may be necessary.

Decortication is generally preceded by retting of flax straw, although in some instances non-retted straw is employed. As described by Kozlowski et al. (2005), retting is effectively a "degumming process" although different from degumming as used in seed-oil processing. It involves primarily the decomposition of naturally-adhesive pectic

substances that bond the fibre bundles within the stem to surrounding cortex tissues. Morrison and Akin (2001) indicated that the retting process effectively delaminates the flax straw, permitting separation into differential components.

Pectic substances, as noted earlier, can be degraded under relatively mild conditions or using specifically selective enzymes (Kashyap et al., 2001). As described in Zhang et al. (2000), it has been common for pectolytic enzymes to be exploited both in natural flax retting methods, so-called field "dew-retting", and in synthetically induced methods, involving water retting or enzymatic retting. Koslowski et al. (2005) also outlined a variety of other chemical and physical methods that have been used in some instances for removal of pectic substances.

Decortication is the essential process in fibre recovery. Itself relies on differences in tensile strength between the long, bast fibres, which are strong, versus the other components of the stem, particularly the flax shive, which are relatively weak. As described by Munder et al. (2005), decortication can be undertaken using more specialized breaking-rollers that work the straw back and forth, or through adaptation of more conventional hammermills, either with or without internal cleaning incorporated, that shear the stems. Munder et al. (2005) noted that there is no single best method, with advantages and disadvantages in all cases. At the SWM International facility in Carman, hammermills were used at the time that samples of flax shive were obtained for testing. Although hammermills offer the significant advantage of much higher throughput, one major disadvantage is the generation of significant dust.

At the flax processing facility of SWM International, three major by-product components were indicated to be generated in roughly equal mass proportions: shorter waste-fibres, termed tow, which are undesirable for intended pulping applications; shards of lignified xylem tissue of the stem, which represent flax shive; and dust and fines, which is really a mixture of fines derived from the various other components of the stem.

Marshall et al. (2007) indicated in the processing of seed-oil flax straw that only roughly 25% to 30% of the mass of the stem can be recovered as higher-value fibre. The remaining 70% to 75% consists of the above by-product materials. The decortication process thus produces large quantities of a size-reduced and relatively consistent flax shive fraction, potentially suitable as a feedstock for further processing.

2.2.4 Current Uses of Flax Shive

The current applications of flax shive are very limited (Cooper et al. 1999), although interest has been long expressed to identify novel uses. Given limited market available, the price of flax shives has also tended to be very low, much lower than the long fibres, and lower even than tow fibres (Blouw 2007). Indeed, flax shive has tended to be treated as more of a waste for disposal. A summary of potential uses for flax shive, and respective status is presented in Table 2.1. Prospective opportunities remain limited.

2.2.5 Flax and Flax Shive Composition

The priority in terms of compositional analysis for flax, for obvious reasons, has been on the higher value bast fibre. Fibre composition is described in detail for example by Morvan et al. (2003). Given that flax shive has traditionally been less important, its precise composition has been less studied, with reported values not always consistent.

Table 2.1 Identified market applications for flax shive.

Identified Use	Reference(s)	Status
Animal bedding	Kasbo et al. (1994) Isman et al. (2003) Kim and Mazza (2006) Blouw (2007)	Commercially well-established
Solid combustion fuel	Sankari (2000) Morrison and Akin (2001) Kymalainen et al. (2004) Prairie BioEnergy (2008)	Commercially underway
Packing material/insulation	Sankari (2000) Kymalainen et al. (2004)	Commercially underway
Activated-carbon absorbent	Cox et al. (1999) Marshall et al. (2007)	Research
Composite cement additive	Khazma et al. (2008)	Research
Fibre-board input	Cooper et al. (1999) Kozlowski et al. (2005)	Research
Biochemical and extraction feedstock	Jacobs et al. (2003) Kim and Mazza (2006)	Research

Table 2.2 Overall mass composition of flax straw stems reported by various sources.

Component	Salmon-Minotte and Franck (2005)	Rowell et al. (2000)
Cellulose	49% to 71%	43% to 47%
Hemicellulose	17% to 29%	24% to 26%
Lignin	18% to 27%	21% to 23%

Table 2.3 Reported mass composition of flax shive from various sources, by mass (db).

Component	Ramaswamy and Tschirner (2005)	Sain and Fortier (2002)	Mazza and Kim (2006)	Kymalainen et al. (2001)	Cox et al. (1999)	Ross and Mazza (2010)	Agbor (2011)
Cellulose	53.1%	53%	43%	< 50%	46.0%	33.3%	50.2%
Hemicellulose	13.2%	13%	15%	~ 25%	23.1%	21.9%	18.2%
Lignin	24.0%	24%	34%	25% to 30%	26.2%	27.3%	22.7%
Pectic substances	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Extractives	1.5%	1.5%	n/a	n/a	n/a	n/a	n/a
Crude protein	n/a	n/a	n/a	n/a	n/a	n/a	2.0%
Wax	n/a	n/a	n/a	n/a	n/a	3.5%	n/a
Ash	n/a	< 2%	2%	n/a	3.1%	1.5%	1.9%
Unaccounted (difference)	9.2%	< 6.5%	6%	—	1.6%	12.5%	5.0%

Note: n/a = data not available.

Flax straw, as a whole, has α-cellulose content of 40% to 50%; hemicellulose content of 20% to 25%; and lignin content of 20% to 25%. Ranges for these three constituents reported by Salmon-Minotte and Franck (2005) and Rowell et al. (2000) are summarized in Table 2.2. The data from Rowell et al. (2000) were also similarly reported in Han (1998) and Rowell and Cook (1998).

The extraction of bast fibre from flax straw gives rise to different component compositions in recovered long fibre versus residual flax shive. Bast fibre is higher in cellulose content, and lower in both lignin and hemicellulose, while flax shive is the reverse (Ramaswamy and Tschirner 2005; Sain and Fortier 2002). Given the origin of flax shive, which is xylem tissue made up of lignified sclerenchyma cells, this is logical.

A summary of flax shive component analyses is presented in Table 2.3. This table includes data from Ramaswamy and Tschirner (2005), Sain and Fortier (2002), Mazza and Kim (2006), Kymalainen et al. (2001), Cox et al. (1999), and Ross and Mazza (2010). Also included are more recent data for flax shive sourced from SWM International and similar to the feedstock used in this work (Agbor 2011, personal communication). This latter analysis was undertaken by the Feeds Innovation Institute at the University of Saskatchewan, with methods employed being consistent with those outlined in Walter et al. (2010). The data in Table 2.3 show a consistent order of abundance in all cases for the three major constituents, namely cellulose, then lignin, then hemicellulose. There are, however, significant differences in absolute values for the three, and minor constituents are more uncertain. These results clearly illustrate significant variabilities in reported flax shive component composition.

Table 2.4 Carbohydrate monomer composition of flax shive by mass (db).

Carbohydrate Monomer	Akin et al. (1996)		Han (1998)	Jacobs et al. (2003)
	Natasja	*Ariane*		
Glucose	27.0%	27.7%	34.9%	58.3%
Xylose	16.7%	13.7%	18.5%	33.3%
Mannose	1.3%	1.5%	2.0%	2.6%
Galactose	0.9%	1.0%	0.7%	1.3%
Arabinose	0.9%	0.7%	0.3%	0.6%
Rhamnose	1.7%	1.0%	0.3%	n/a
Uronic acids	0.3%	0.3%	n/a	n/a
4-O-Methyl-glucuronic acid	n/a	n/a	n/a	3.8%

Note: n/a = data not available.

Table 2.5 Calculated molar ratios of sugar monomers relative to glucose in flax shive.

Molar Ratio of Monomer to Glucose	Akin et al. (1996)		Han (1998)	Jacobs et al. (2003)
	Natasja	*Ariane*		
Xylose	0.740	0.591	0.636	0.685
Mannose	0.048	0.053	0.057	0.046
Galactose	0.032	0.035	0.021	0.022
Arabinose	0.042	0.031	0.010	0.012

2.2.6 Flax Shive Hemicellulose

Analyses of the carbohydrate monomer composition of flax shive are presented in Table 2.4, showing data from Akin et al. (1996), Han (1998) and Jacobs et al. (2003). These analyses included cellulose as well as hemicellulose. As such the fact that glucose was the most abundant monomer in all cases made sense. Not all constituents were measured in all cases, but for the four sugar monomers that were consistently determined, the calculated molar ratios of the sugars relative to glucose are presented in Table 2.5. These ratio values were all relatively consistent, showing the monomers in descending order of mean molar ratios relative to glucose to be: xylose (0.66); mannose (0.05); galactose (0.03); and arabinose (0.02). This is slightly different than the typical order for hemicellulose (Sjostrom 1993). Rhamnose and uronic acid constituents, when measured, appeared to be less consistent.

Flax is a member of the order Linales, for which the major cross-linking glycan within primary cell walls is xyloglucan, in particular fucogalactoxyloglucan (Carpita and McCann 2000). As such, this polymer would be expected to be present in flax shive, and the monomer fucose also could be expected to be present at least in minor amounts. As noted by Fry (1989), however, although xyloglucan is abundant and well identified within primary cell walls of dicotyledonous plants, it is not as abundant in lignified secondary cell walls, which is the nature of the xylem tissue in flax shive.

The important work of Jacobs et al. (2003) specifically identified three major types of hemicellulose polymers shown to be present in flax shive. In order of abundance, the three hemicellulose polymers they identified were: O-acetyl-4-O-methyl-

Table 2.6 Characteristics of major hemicellulose polymers in flax shive.

Hemicellulose Polymer	Characteristics	Reference(s)
O-Acetyl-4-O-methyl-glucuronoxylan	Main backbone of D-xylose monomer	Ebringerova et al. (2005)
	4-O-methyl-glucuronic acid side attachment on roughly 1 in 13 backbone monomers	Jacobs et al. (2003)
Glucomannan	Main backbone roughly equal proportions of D-glucose and D-mannose	Jacobs et al. (2003)
	D-galactose as major periodic side attachment monomer	Ebringerova et al. (2005)
Xyloglucan	Main backbone of D-xylose monomer	Ebringerova et al. (2005)
	Single D-xylose side-attachment on each three of four backbone monomers in repeat sequence	Ebringerova et al. (2005)

glucuronoxylan; xyloglucan; and glucomannan. Characteristics of these three polymers are summarized in Table 2.6. The carbohydrate monomer makeup of flax shive, as described in Table 2.4, is consistent with the presence of these three main hemicellulose polymers.

2.2.7 Flax Shive Lignin

Lignin, as noted earlier, is a complex material. Although its overall content in biomass can be evaluated using standard gravimetric techniques, such as Klason lignin, its *in-situ* chemical structure is more difficult to precisely evaluate. This is given the fact that extraction and analysis methods all affect the nature of recovered lignin (refer to Section 2.1.2).

Evaluations of lignin composition have been undertaken for flax straw and flax shive, but results are not always consistent, due to the inherent complexity of lignin as described above. Buranov and Mazza (2008) included discussion of flax straw and flax shive lignin in their general review of lignin from herbaceous crops, and suggested some general trends in the nature of the lignin do appear consistent. The lignin of flax is understood to primarily involve a mixture of guaiacyl-syringyl compounds (Gorshkova et al. 2000), with G-type lignin being most abundant. Although *p*-hydroxyphenyl lignin compounds are less prevalent in flax shive, lignin associated with the periphery components of the stem has been identified to be different, involving a more significant proportion of H-type compounds (Day et al., 2005).

Work by Tapin et al. (2006) on the pulping of whole flax straw suggested that less than 10% of overall flax straw lignin was H-type. For remaining lignin, the molar ratio

was approximately 3 for G-to-S type lignin compounds. Ross and Mazza (2010) specifically evaluated flax shive lignin. They suggested a somewhat higher molar ratio of approximately 3.6 for G-to-S type lignin, and with very low content of H-type lignin.

del Rios et al. (2011) undertook a more comprehensive evaluation of the lignin associated with both flax fibre and flax shive fractions, in this case based on a pyrolysis technique. They confirmed much lower lignin content in fibre, i.e., roughly 4% by mass versus 29% by mass for flax shive in their case. They also confirmed that lignin in the flax fibre and the flax shive were distinct. Based on their analysis techniques, the estimated molar ratios of H:G:S type lignin were 13:72:15 for the fibre fraction versus 5:87:8 for flax shive. Compared to other work, they showed a very strong dominance of G-type lignin compounds. From their work the calculated ratio was close to 11 for G-to-S type lignin compounds. del Rios et al. (2011) also undertook $^{13}C/^{1}H$ NMR analyses of derivatives. One important observation from this work is that they identified only two ester-based derivatives, methyl vanillate and methyl syringate, and these were present in very low proportions. Ester bonds are understood to be present, especially in lignin-carbohydrate complexes, as described earlier. The low presence of ester products may have been an impact of their pyrolysis-based analysis technique.

Methods for recovering lignin affect the resulting nature of the lignin obtained, as noted earlier in Section 2.1.2. Pyrolysis, in general, results in the progressive breakdown of biomass into smaller organic fragments and chars. These thermal breakdown processes depend significantly on temperature, and there can be catalysis via commonly present metal ions, e.g., sodium and calcium (Brebu and Vasile 2010, Varhegyi et al.

1997). As such, the nature of final products from pyrolysis-based analysis may be not entirely consistent with initial *in-situ* structure.

Much of the work on lignin constituents in flax shive has involved extraction of free phenolic compounds by various techniques (Akin et al., 1996; Lozovaya et al., 1999; Kim and Mazza, 2006, 2007, 2009; Tapin et al., 2006; Buranov and Mazza, 2007, 2009; Buranov et al., 2010; Ross and Mazza, 2010). This appears to be due in part to the complexities of lignin, noted earlier, and in part to the potential value of these compounds as products. Phenolic constituents that have been deliberately included in analyses are:

- H-type compounds: *p*-coumaric acid, and *p*-hydroxybenzaldehyde:
- G-type compounds; ferulic acid, vanillin, vanillic acid and acetovanillone; and
- S-type compounds: syringic acid, syringaldehyde and acetosyringone.

Of these constituents, vanillin has been typically found to be the highest concentration product in the above analyses for flax and flax shive. In the work by del Rios et al. (2011), by contrast, the most abundant constituents from both flax fibre and flax shive fractions were guaiacol-based compounds (i.e., top three constituents in order: 4-methyl-guaiacol; 4-vinyl-guaiacol; and guaiacol), although, like vanillin, these are also G-type lignin derivatives. This difference appeared to be the result of their use of a pyrolysis-based approach.

2.3 Extraction Process Assessment and Selection

The overall objective of this work was to develop a process to extract multiple high-value products from flax shive. Two extraction processes were selected for experimental testing from ten categories of processes that were initially considered. The two selected were, firstly, extraction using sodium ethoxide catalyst in anhydrous ethanol, and, secondly, extraction using aqueous sodium hydroxide. The assessment and selection method for the extraction processes is described in this section.

Ten major categories of prospective extraction processes were considered, as summarized in Table 2.7, with each described separately in the following sections. Given the large number of prospective approaches that could be used, and the range of potential factors to consider in their comparison, a multi-criteria based selection method was employed. Such selection methods are by nature highly subjective, but provide a means to systematically assess a large number of alternatives. Similar, but not identical, selection methods were used by both Werpy and Petersen (2004) and Holladay et al. (2007) to identify prospective biomass product opportunities.

The selection method itself was relatively simple, using a ranked-scoring methodology. Seven different selection criteria were employed, as summarized in Table 2.8, with each criterion stated as a question. Each of the selection criteria was scored for each of the ten processes, as follows: scoring +2 if the process strongly fulfilled the criterion; scoring +1 if the process was satisfactory for the criterion; scoring 0 if the process was neutral or uncertain relative to the criterion; scoring -1 if the process was somewhat deficient relative to the criterion; and scoring -2 if the criterion was

Table 2.7 Prospective extraction processes considered and evaluated for flax shive.

Process Category	Brief Description	Refer to Section
Acid hydrolysis	Acids used as catalysts for hydrolysis (of carbohydrate components)	2.3.1
Autohydrolysis	Elevated temperatures used without external acid addition	2.3.2
Steam processing	Saturated or superheated steam used without acid hydrolysis	2.3.3
Alkaline processing	Alkaline reagents used to remove hemicellulose and/or lignin	2.3.4
Solvent/organosolv	Solvents used for selective dissolution of components	2.3.5
Enzymes	Enzymes used to selectively cleave connecting linkages	2.3.6
Selective chemical reactions	Specific chemical reactions used to selectively cleave connecting linkages	2.3.7
Supercritical fluids	Fluids, like carbon dioxide, used as solvents at above critical conditions	2.3.8
Subcritical water	Fluids, like water, used at close to critical conditions for breakdown	2.3.9
Other liquids and reagents	Other liquids or reagents not covered by other process categories	2.3.10

Table 2.8 Selection criteria used for evaluation of prospective extraction processes.

Criterion	Explanation
(i) Selectivity	Can the extraction process yield targeted product(s) from hemicellulose or lignin, without creating a plethora of multiple products that would require extensive separation (purification)?
(ii) Reduced by-products	Can the extraction process avoid the generation of excessive by-products or degradation products requiring extensive additional post-purification?
(iii) Minimized breakdown	Can the extraction process avoid excessive corollary damage to other, non-targeted feedstock components that could impact recovery of further product(s)?
(iv) Low energy inputs	Can the extraction process be accomplished with low energy inputs?
(v) Low cost inputs	Can the extraction process recover usable product(s) from hemicellulose or lignin component using reasonably low cost material inputs?
(vi) Low environmental effects	Does the extraction process avoid the use of chemical inputs that may be toxic or problematic in terms of environmental effects; and avoid generating any products or by-products that might be similarly problematic in terms of environmental effects?
(vii) Simple and practical	Is the extraction process relatively simple, without extensive controls or requirements, and could it be practically implemented?

strongly deficient. Respective scores for all criteria were then summed to provide an overall score. A summary of scoring for all processes is provided later in Table 2.8.

Each of the selection criteria addressed a distinct aspect, but were clustered in three areas: (i) product purification, addressed under criteria #1, #2, and #3; (ii) process inputs and externalities, addressed under criteria #4, #5, and #6; and (iii) overall process simplicity and practicality, addressed under criterion #7. The three criteria relating to product purification were particularly important, given product purification was identified in Section 1.4 as one of the three research focusses for this work. Although no separate research was undertaken on purification, product purity was addressed in the selection, i.e., to select extraction processes recovering relatively pure products that would not require extensive further purification.

The ten different process categories considered for flax shive extraction are described in more detail in the following separate sections. Outlined for each case are: the basic principles and/or reaction mechanisms involved; relevant background in terms of the application of the process to biomass; and the scoring and associated rationale for each of the seven selection criteria.

2.3.1 Acid hydrolysis

Acid hydrolysis is a process that has been long applied to biomass. Different types of acids at different concentrations, both dilute and concentrated, have been considered. Although concentrated acids can act effectively as solvents to dissolve ligno-cellulose and components, it is the catalytic hydrolytic action of acids on carbohydrates that is most important. The reaction mechanisms are well understood (Saeman 1945,

Nevell 1985), and proceed in three sequential stages. In the presence of suitable acid, and conditions of temperature and pressure, inter-monomer glycosidic bonds in cellulose and hemicelluloses are firstly cleaved to produce a mixture of monomers, but in an indiscriminate manner. The initial breakdown of polymers to monomer sugars is followed by dehydration of sugars to aldehydes, and then subsequent degradation of aldehydes to acids and other humic substances. Acid hydrolysis is also relevant, indirectly, to lignin. Strong acid can dissolve and digest virtually all cellulose and hemicellulose, leaving lignin behind. This is the basis for Klason lignin and related gravimetric lignin determinations.

Acid hydrolysis has been used in the past for the production of sugar monomers for fermentation (Saeman 1983), and, most notably, for degradation of high hemicellulose content biomass feedstocks for the production of furfural (Dunlop 1948). As noted by Aien and Sjostrom (1985), hemicelluloses are hydrolyzed much more easily compared to cellulose. If hemicellulose polymer structures are to be retained, then exposure to aggressive acid conditions needs to be avoided.

Overall, in terms of scoring, acid hydrolysis was deemed as: (i) not selective (-1), affecting all carbohydrates in an indiscriminate manner, but hemicellulose most strongly; (ii) problematic in generating a bevy of degradation products requiring further purification (-2); (iii) problematic in degrading both hemicellulose and cellulose, thus preventing further feedstock applications (-1); (iv) requiring high energy inputs, given that reactions occur at elevated temperatures (-2); (v) involving relatively low costs

otherwise (+1); (vi) having no major environmental concerns (+1); and (vii) relatively simple and well understood (+1), being already used extensively in the field.

2.3.2 Autohydrolysis

Autohydrolysis can be described as a form of acid hydrolysis, in which biomass is treated with steam at elevated temperatures but without externally-added acid as catalyst (Ramos 2003), typically through direct contact with steam. Under these conditions acetic acid, which can be released from acetylated hemicelluloses, has been considered the main acid catalyst. Other acids, such as formic and levulinic acids, also may be produced and may play a role. The reactions and kinetics involved are understood (Garrote et al. 1999).

Autohydrolysis has been identified as a means for the recovery of xylo-oligosaccharides. The limitations of autohydrolysis are also well understood, and the technique is not necessarily desirable. Extensive purification is usually required, as described in more detail by both Moure et al. (2006) and Vasquez et al. (2000).

Overall, in terms of scoring, autohydrolysis was deemed as: (i) not selective (-1); (ii) causing unintended byproducts requiring further purification (-1); (iii) not problematic in terms of cross-component degradations (+1); (iv) requiring relatively high energy inputs, given that reactions occur at elevated temperatures (-2); (v) having relatively low costs otherwise (+1); (vi) having no major environmental concerns (+1); and (vii) relatively simple and well understood (+1).

2.3.3 Steam Processing

Steam processing is differentiated from autohydrolysis as representing solely thermal effects of flushing a product with water vapour. This could be undertaken using

saturated or superheated steam conditions, but is not necessarily intended to result in acid-induced degradation processes, as in the case of autohydrolysis. Steam processing is employed for recovery of extractives from biomass, such as alkanoids. (Harborne 1999).

A detailed review of steam processing of ligno-cellulose was undertaken by Ramos (2003), albeit with an emphasis on autohydrolysis as described above. Saturated steam, at temperature ranging from 140ºC to 240ºC, has been employed with ligno-cellulose. In addition to acid hydrolysis of polysaccharides, four relatively distinct degradation mechanisms have been identified. First is acid catalyzed dehydration, which is well recognized in acid hydrolysis, converting xylose and glucose monomers to respective aldehydes, furfural and hydroxymethylfurfural (HMF). Next are oxidation and pyrolysis, which are both thermal degradation processes occurring at elevated temperatures, the former in the presence of oxygen and the latter in the absence of oxygen. Lastly at high temperatures, in the range of 220ºC to 240ºC, condensation reactions can occur, involving lignin, hemicellulose-derived by-products and acid-soluble lignin, leading to the build-up of insoluble polymeric substances that are distinctly different from lignin.

The application of steam involves a number of compromises, not just in the severity of conditions, i.e. higher temperature and/or acid content leading to more enhanced breakdown, but also in the presence/absence of oxygen. A key problem in the use of steam for biomass, however, is how to prevent the liberation of acids. Without this ability, steam processing is little different from autohydrolysis.

Overall, in terms of scoring, steam processing was deemed as: (i) not particularly selective (-1); (ii) likely to cause some unintended byproducts requiring further purification (-1); (iii) not problematic in terms of cross-component degradations (+1); (iv) requiring high energy inputs, given saturated or superheated steam conditions (-2); (v) having relatively low costs otherwise (+1); (vi) having no major environmental concerns (+1); and (vii) relatively simple and well understood (+1). Steam processing could offer more significant potential if it were possible to prevent with certainty the release of acid groups from the biomass. One speculative possibility could be through the use of transesterification reactions, described later in Section 2.3.7, to remove acetyl side-groups and other possible acid sources prior to steam processing. As noted earlier in Section 2.1.3, hemicellulose polymers can involve significant acetyl side-groups. This is particularly the case for flax shive hemicellulose polymers, as indicated in Table 2.6. Steam processing thus could assist in the selective mobilization of lignin, subject to key conditions: substantial reduction or elimination of acid groups prior to steam; absence of oxygen to prevent oxidative reactions; and temperature sufficient to act on lignin, but not so high to cause thermal degradation.

2.3.4 Alkaline Processing

The use of alkaline reagents represents a common approach used to process ligno-cellulosic materials. Specificity and effectiveness, however, depend directly on the conditions used, in terms of reagent concentration, temperature and extraction-period. At issue is that alkaline reagents can affect both hemicellulose and lignin, but with relative

effects dependent on the precise conditions. Appropriate use of alkaline processing thus depends on careful selection of reagents and processing conditions.

Alkaline extraction has been a long-known method for recovery of hemicellulose polymers at relatively mild conditions (Yanovsky 1939). Ready-extractability using alkaline solutions was indeed how hemicellulose was initially defined, i.e., as "beta cellulose", and this definition continues even today in the TAPPI Standard T203 (TAPPI 2009). The defined conditions for alkaline extraction, however, have tended to be very ad hoc in nature (Watson and Williams 1959). This is not surprising given the diverse nature of biomass and hemicellulose polymer types. If lignin can be removed first, alkaline solutions can be extremely effective for hemicellulose extraction, including significantly increased yields and more rapid rates at elevated temperatures (Han 1983).

Applying more aggressive conditions directly to biomass results in the breakdown of both lignin and hemicellulose, which is not desirable relative to the research objectives. Such behaviour, at the same time, forms the basis of conventional alkaline chemical-pulping processes, including soda, Kraft, polysulfide, alkaline sulfite, neutral sulfite and anthraquinone processes (Gratzl and Chen 2000). The key objective in commercial pulping is the removal of lignin, through both dissolution and reaction, in order to permit the recovery of relatively pure cellulose. Hemicellulose, which is also present, happens to be simultaneously degraded as a collateral impact, although there has been some consideration recently of pre-extracting hemicellulose as part of pulping processes (Al-Dajani and Tschirner 2008).

At temperatures in the range of 140ºC to 170ºC, heated aqueous solutions, typically of sodium hydroxide, are known to disrupt ether linkages within lignin. Additives are used to assist in degradation and solubilization of lignin, and in stabilization of carbohydrates. Common additives include: reduced-sulfur compounds to promote lignin degradation; polysulfides to enhance carbohydrate stabilization; bisulfite and sulfite ions to promote lignin degradation; and anthraquinone and anthrahydroquinone to promote both lignin degradation and carbohydrate stabilization.

In addition to direct attack on ether linkages, alkaline solutions can also lead to saponification reactions of ester bonds. These mirror the reactions used in soap and detergent manufacturing. However, saponification is an endothermic reaction, unlike transesterifiation described later in Section 2.3.7, and requires heat input to occur.

An additional important benefit of alkali is the disruption of hydrogen bonding between hemicellulose and cellulose, allowing the two components to be more easily separated. As described by Biliaderis and Izydorczyk (2007) and Ebringerova et al. (2005), one of the reasons alkali is employed in extraction processes for various hemicellulose derived polymers is for this purpose.

Overall, in terms of scoring, alkaline processing was deemed as: (i) likely to be selective, but dependent on the ability to select suitable processing conditions (0); (ii) unlikely to create unintended byproducts (+1); (iii) likely to be able to avoid collateral breakdown of other components, but again dependent on selection of suitable processing conditions (0); (iv) involving low energy inputs, if mild conditions can be employed (0);

(v) having relatively low costs otherwise (+1); (vi) having no major environmental concerns (+1); and (vii) relatively simple and well understood (+1).

2.3.5 Solvent (Organosolv) Processing

The use of organic solvents has been investigated extensively for application to ligno-cellulosic materials. Indeed, the use of organic solvents at elevated temperatures is the basis of so-called "organosolv" pulping technologies. In such cases, the solvents serve primarily for delignification, through solubilizing and removing the more hydrophobic lignin components, with the objective of leaving behind cellulose pulp.

A detailed review of organic solvents for organosolv pulping was prepared by Muurinen (2000). A diverse range of organic chemicals have been considered, including: methanol; ethanol; other alcohols; formic (methanoic) acid; acetic (ethanoic) acid; other organic acids; phenols; cresols; ethyl acetate; amines and amine oxides; ketones, particularly acetone; dioxane; and others.

Lignin is separated and recovered in a relatively undamaged form, opening up possible product opportunities. A relatively pure cellulose pulp is also recovered. However, there are two recognized issues that cause concerns with this type of process. Firstly, these processes all share the characteristic of essentially shattering the hemicellulose, producing a stream of monomers, rather than intact polymers. This reduces both the value and applicability for the hemicellulose. Secondly, the environmental suitability of the process depends on the specific organic solvent selected. Some, such as ethanol or methanol, are positive (Capello et al. 2007). Other solvents could be problematic.

Overall, in terms of scoring, solvent processing was deemed as: (i) relatively selective (+1); (ii) likely not to create significant unintended byproducts, but depending on the solvent (0); (iii) problematic in being known to shatter hemicellulose structure, rendering it less valuable (-1); (iv) requiring relatively high energy inputs, given operation at elevated temperatures (-2); (v) uncertain in terms of costs, depending on the selected solvent and associated losses (0); (vi) uncertain in terms of environmental concerns, depending on the selected solvent (0); and (vii) relatively straightforward (+1).

2.3.6 Enzyme Processing

The use of enzymes for the potential recovery of high-value products from biomass presents a contradiction. Enzymes and associated biotechnologies have advanced significantly in the recent past, in particular commercial enzymes falling under the classes of cellulase, xylanase and pectinase. However, despite their efficiency and specificity, enzymes do not directly appear to be as useful as would be expected as part of a deliberate extraction process. The root of the problem is indeed their specificity.

Enzymes catalyze specific reactions, including specific bond breakdowns for polymers such as cellulose, hemicellulose and lignin. The β-1,4-glycosidic bonds of cellulose are acted upon by a variety of cellulase enzymes. This exact same linkages, however, are also present in the backbone of xyloglucan hemicellulose, and also can be acted upon by the same types of enzymes, such as endo-β-D-glucanases (Carpita and McCann 2000). The use of cellulase-type enzymes is thus less selective than desired.

The bonds within hemicellulose polymers can be acted upon by various enzymes, of which there are at least ten different types (Jeffries 1994). Given a desire to retain

hemicellulose polymers as intact as possible, it is not necessarily desirable to employ such enzymes. The nature of enzymes, whereby all suitable bonding sites are attacked equally, makes it inherently difficult to ensure only partial breakdown occurs.

The situation regarding potential selective breakdown of lignin is even more problematic. The state of understanding of bacterial and fungal enzyme for the breakdown of lignin was recently reviewed by Bugg et al. (2011). These enzyme systems are still largely at an exploratory stage of development. Without availability of low-cost commercial enzymes, this sort of approach is too expensive to be practical.

One final area regarding enzymes is pectic substances. While present in flax straw, the extent of their presence in flax shive is uncertain. As noted earlier in Section 2.2.3, retted flax straw has undergone enzymatic degradation of pectic substances to some degree. As noted in Table 2.5, recent analyses of flax shive composition did not include pectic substances. The bonds involved in pectic substances are distinctly different from both cellulose and hemicellulose, and they can be selectively acted upon by a variety of commercially-available pectinase enzymes (Kashyap et al. 2001), with minimal collateral damage to cellulose or hemicellulose. Ideally, flax shive should free of pectic substances, however, if not, the use of pectinase enzymes could be appropriate.

Overall, in terms of scoring, enzyme processing was deemed as: (i) uncertain in selectivity (0); (ii) unlikely to create unintended byproducts (+1); (iii) could result in unanticipated collateral breakdown of other components (0); (iv) involving low energy inputs, given reactions at ambient conditions (+1); (v) having high costs given the state of

current enzyme development (-2); (vi) having no major environmental concerns (+1); and (vii) complex and still relatively unproven for practical application in this case (-2).

2.3.7 Selective Chemical Reaction

The use of selective chemical reaction mirrors the application of enzymes, just not biological in nature. As in the case of enzymes, the specific target reaction or reaction site must be identified, and reagents to achieve a desired reaction outcome must be available at reasonable cost. There are obviously many possibilities, but the review of the nature of constituents of ligno-cellulose, and their inter- and intra-component bonding, as discussed in earlier sections, quickly identified ester bonds as a priority.

Ester bonds are not involved in the main backbones of hemicellulose polymers, these instead involving β-1,4-glycosidic bonds, and are not significantly involved in links between lignin subunits, these being primarily ether bonds. Ester bonds tend to be present in locations such that if they could be selectively and systematically cleaved, it would be beneficial to the recovery of products, particularly hemicellulose- and lignin-based, or at least not likely detrimental in terms of collateral component damage.

Specifically, ester bonds are know or suspected to be involved in the following main sites within ligno-cellulose:

- Present significantly in linkages between lignin and hemicellulose, as discussed earlier, that would be desirable to separate;
- Present significantly within hemicellulose structures in the attachment of acetyl side-groups, as discussed earlier, that would be desirable to remove in order to prevent autohydrolysis, described earlier in Sections 2.3.2 and 2.3.3;

- Potentially present in polymer to polymer linkages between pectic substances with both lignin and hemicellulose that would be beneficial is disrupted; and
- Present to some extent within lignin that also would be not detrimental if disrupted.

Ester bonds can be readily catalyzed to undergo transesterification at room temperature and ambient pressure conditions. Transesterification reactions are, today, most commonly involved in the commercial production of biodiesel (Van Gerpen, 2005), whereby base-catalyzed conversion of plant oil or animal fat derived triacyl-glycerides is typically undertaken using methanol as the added alcohol. As described by Fry (1986), such conversions have been applied to plant cell wall polymers using sodium methoxide as catalyst. Indeed transesterification has already been employed for analytical purposes for the removal and measurement of acetyl side groups on ligno-cellulose, as outlined by Zhu et al. (2008), and also employed for modification of fibre polymers such as cellulose, as described by Persson (2004).

Although sodium methoxide in methanol is the most common catalyst considered for transesterification, it is also possible to use sodium ethoxide in anhydrous ethanol. Both ethanol and methanol have been identified as comparatively desirable solvents from environmental and occupational health and safety perspectives (Capello et al. 2007), but ethanol has been judged as somewhat better, likely given much lower acute-toxicity for humans. Importantly, the ability to use a potable alcohol and basic catalyst that upon addition of neutralizing hydrochloric acid yields only salt is highly advantageous for processing of materials that could ultimately find some application into food products.

Overall, in terms of scoring, the use of selective chemical reactions, specifically transesterification with sodium ethoxide, was deemed as: (i) likely to be selective, but not entirely certain in practice (0); (ii) unlikely to create unintended byproducts (+1); (iii) unlikely to cause collateral damage to other components, but not entirely certain in practice (0); (iv) involving low energy inputs, given operation at room temperature (+1); (v) having relatively low costs otherwise (+1); (vi) having no major environmental concerns (+1); and (vii) not complex but still unproven for practical application (0).

2.3.8 Supercritical Fluid Processing

Supercritical fluid processing refers to the use of specific fluids above critical conditions for extraction. The basic features and advantages of this technology, as applied to biomass, were described in detail by Demirbas (2001). The most commonly considered fluid, for a variety of reasons, is carbon dioxide. At supercritical conditions,i.e. at a combination of pressures above 7.4 MPa and temperatures above 31ºC, it acts as an inert, non-polar solvent with a fluid density more liquid-like in nature than a gas. Subsequent reduction of pressure to a suitable lower condition reduces solvent capacity, and products can be easily collected without solvent residuals. Carbon dioxide has no acute toxicity and is thus highly suitable even for food grade products.

Supercritical carbon dioxide extraction (SCFE) has potential application for the recovery of extractive constituents as could be recovered using more conventional solvents, discussed earlier in Section 2.3.5, or any other hydrophobic materials. Significant attention has also been paid recently to using supercritical carbon dioxide explosion as a benign pretreatment for enzymatic hydrolysis of ligno-cellulosics (Brodeur

et al. 2011). The greatest limitation of SCFE, however, is its high cost, which means that it can only realistically be employed as part of a process where at least one of the resulting products has an extremely high value. The use of SCFE often can provide utility, nevertheless, for research in terms of acting as an analytical standard by which to compare performance of other organic solvents.

Overall, in terms of scoring, supercritical fluid processing was deemed as: (i) uncertain in selectivity, depending on the product material (0); (ii) uncertain in terms of co-extracting unintended byproducts requiring further purification (0); (iii) not problematic in terms of cross-component degradations (+1); (iv) involves moderate energy inputs, due to high pressures but lower temperature involved in processing (-1); (v) having very high costs, given the sophisticated nature of the high pressure equipment (-2); (vi) having no major environmental concerns (+1); and (vii) highly complex and unproven in terms of practical application (-2).

2.3.9 Subcritical Water and Solvent Processing

A novel, advanced technology has been under development by Mazza and colleagues at Agriculture and Agri-Food Canada for the selective breakdown of biomass materials, including specifically flax shive. This process essentially involves the use of water at sub-critical conditions, i.e., pressures of about 5 MPa and temperatures generally in the range of 180ºC to 230ºC, noting that critical conditions for water involve approximately 22 MPa and 374ºC. Variations have involved operation in two stages, and inclusion of different reagent additives. These have involved aqueous sodium hydroxide (up to 0.6 M), aqueous ammonia (15% solution), or aqueous ethanol (30% solution).

Investigations of this process involving flax shive have been reported in a series of publications covering different aspects of this technology.

The process was initially applied to the recovery of free-phenolic compounds from flax shive, notably *p*-hydroxybenzaldehyde, vanillic acid, syringic acid, vanillin, acetovanillone and ferulic acid (Kim and Mazza 2006, Kim and Mazza 2007), achieving a level of recovery of identified compounds of about 5.7 mg/g (db). The process was subsequently altered to consider simultaneous recovery of both lignin and carbohydrates from flax shive (Buranov and Mazza 2007). This was undertaken in stages, but done to achieve increased recovery, rather than separate recovery of lignin and carbohydrates. The process was specifically applied for the recovery of ferulic acid (Buranov and Mazza 2009), although this was not successful for flax shive, showing a lower yield than simple alkaline extraction. The recovery of hemicellulose was addressed in Buranov and Mazza (2010), showing that pressurized water, without additional reagents, could achieve up to 90% recovery of hemicellulose from flax shive, albeit with significant lignin co-extracted. The recovery of lignin was addressed in Buranov et al. (2010) and Ross and Mazza (2010), showing that pressurized aqueous ethanol (30%) could remove up to approximately 96% of lignin present in flax shive. In this latter case, however, only about 55% of product was recovered as separate lignin product, the remainder incorporated in co-extracted carbohydrates or in solution. This use of aqueous ethanol was noted by Buranov et al. (2010) to be similar to organosolv pulping, as was discussed earlier in Section 2.3.5, but employing a lower ethanol concentration.

Although representing an advanced extraction achieving very high yields, the subcritical water technology was not considered further for this research. It was nevertheless used as a benchmark for recovery, in particular for hemicelluloses. Overall, in terms of scoring, the subcritical water process was deemed as: (i) relatively selective to lignin or hemicellulose (+1); (ii) potentially generating cross-contaminating extractions that might require some further purification (0); (iii) unlikely to result in damage of other constituents (+1); (iv) requiring high energy inputs, due to high pressures and high temperature involved (-2); (v) having very high costs, given the sophisticated nature of the high pressure equipment (-2); (v) having no major environmental concerns (+1); and (vi) complex and unproven for practical application (-2).

2.3.10 Other Reagents and Reactants

The complex nature of ligno-cellulose and its polymeric constituents means that a wide variety of complex chemical interactions are possible. Thus a variety of compounds and mixtures may act as solvents, or as agents for derivatization or breakdown. A more detailed discussion was provided by Heinze and Koscholle (2005), as well as by Ramos (2003). Additional compounds that have been investigated for ligno-cellulose, and not otherwise described, include the following in no particular order: sulfur dioxide; dimethyl sulfoxide; alkaline hydrogen peroxide; inorganic salts with acidic properties; molten inorganic salt hydrates; Lewis acids; cuprammonium hydroxide and cupriethylenediamine hydroxide; transition metal complexs of ammonia or amines; thioisocyanide derivatives; chlorine or acidic sodium chlorite; nitrobenzene; thioacidolysis; and complex multi-component mixtures. Although it was important to at

least mention these reagents, noting that some are already used for analytical purposes, none were considered further, given complexities, environmental compatibility concerns and costs. Overall, in terms of scoring, these methods were deemed as: (i) uncertain in terms of selectivity, depending on the reagent involved (0); (iiv) uncertain in terms of byproducts (0); (iii) uncertain in terms of impacts on other components (0); (iv) uncertain in terms of energy inputs (0); (v) high cost otherwise, given likely cost of reagents (-1); (vi) likely to be environmentally problematic to some degree, given reagents (-1); and (vii) likely to be complex and unproven in terms of practical application (-4).

2.3.11 Overall Results of Comparison

The overall scoring results of prospective processes are provided in Table 2.9. Based on the evaluation as undertaken, the processes cluster into three groups, in terms of desirability for further consideration in high-value product recovery:

- Top ranked processes, alkaline processing and selective chemical reactions, which had the highest overall scores, +4 in both cases;
- Intermediate ranked processes, autohydrolysis and steam processing followed closely by solvent and enzyme processing, which had overall scores of 0 to -1; and
- Lowest ranked processes, supercritical fluid extraction, subcritical processing, acid hydrolysis, and other liquid reagents, which had overall scores of -3 to -4.

The top two ranked processes were selected for further investigation, however, uncertainties were still identified in both cases. Selective chemical reaction, specifically based on transesterification using sodium ethoxide catalyst in anhydrous ethanol, is

considered further in Chapter 6. In this case there were three main uncertainties that needed to be confirmed: (i) whether the process was sufficiently selective; (ii) whether or to what extent there might be collateral impacts on other components; and (iii) whether the process could be undertaken in a simple manner. Alkaline processing, specifically using mild conditions for reagents, is considered further in Chapter 7. In this case there were also three main uncertainties that needed to be confirmed: (i) whether the process was sufficiently selective; (ii) whether or to what extent there might be collateral impacts on other components; and (iii) whether the process could be sufficiently effective at mild conditions in order to minimize energy inputs.

In terms of the four intermediate ranked processes, all were adversely affected in scoring primarily by their high energy inputs or high costs. Autohydrolysis and steam processing both appear to offer promise, but this depends significantly on whether the release of acid constituents could be adequately prevented. This question was not considered further in this work. Of the four lowest ranked processes, acid hydrolysis, which has been commonly used in the past, was most adversely affected in scoring by high energy inputs and the inherent lack of selectivity. The other three processes in this group were adversely affected in scoring mostly by a combination of high costs, environmental issues, and excessive complexity. Supercritical extraction and subcritical processing, in particular, may have significant merit, but simply do not appear to be sufficiently cost effective or practical at this time.

Table 2.9 Summary of selection criteria scores for prospective extraction processes, with scoring values as explained in Section 2.3.

Potential Extraction Process / Criterion	Selectivity	Byproducts	Collateral Breakdown	Energy Inputs	Cost Inputs	Environmental Effects	Simplicity and Practicality	**Total Score**
Acid hydrolysis	-1	-2	-1	-2	+1	+1	+1	-3
Autohydrolysis	-1	-1	+1	-2	+1	+1	+1	0
Steam processing	-1	-1	+1	-2	+1	+1	+1	0
Alkaline processing	0	+1	0	0	+1	+1	+1	+4
Solvent/organosolv	+1	0	-1	-2	0	0	+1	-1
Enzymes	0	+1	0	+1	-2	+1	-1	-1
Selective chemical reactions	0	+1	0	+1	+1	+1	0	+4
Supercritical fluids	0	0	+1	-1	-2	+1	-2	-3
Subcritical water	+1	0	+1	-2	-2	+1	-2	-3
Other liquids and reagents	0	0	0	0	-1	-1	-2	-4

3 Preliminary Flax Shive Characterizations

A series of preliminary experiments were undertaken, but not published, to better understand the composition and separation of flax-straw processing waste; the particle-shape characteristics of flax shive; the density differences between flax shive and waste-fibre; and colour differences between various flax-straw processing waste components. These investigations are presented in the following sections.

3.1 Flax Straw Processing Waste Separation

Quantities of flax straw processing wastes were obtained from the production line of SWM Intl. Inc., located in Carman, Manitoba, as illustrated in the photograph in Figure 3.1. The materials were unsegregated, and were indicated by staff from SWM Intl. to consist of roughly equal proportions of spongy mid-core shive, the subject of this work; waste-fibre, termed "tow" that was deemed too short to be marketable and not worthwhile to recover; and dust and fines. The particle-size distinctions between these various components, however, were not precisely defined. The SWM Intl. facility in Carman uses a hammer-mill approach for decortication, which as noted by Jagannadh and Kolla (1997) is common in specialty paper-product applications. This approach is well known for it high through-put capacity advantage, but also well known for damaging (i.e., shortening) fibre, and for creating significant fine dust residuals.

For the purpose of this work, three fractions were initially defined, based on particle-size differences, as follows:

- "Waste-fibre" fraction, designated as all materials that would not pass through a #10 mesh screen (nominal opening of 1.68 mm);

Figure 3.1 Photograph of SWM Intl. Inc. flax straw decortication facility located in Carman, Manitoba.

- "Mid-core" fraction, designated as all materials that would pass through a #10 mesh screen (nominal opening of 1.68 mm), but not pass through a #20 mesh screen (nominal opening of 850 μm); and
- "Fines" fraction, designated as all material that would pass through a #20 mesh screen (nominal opening of 850 μm).

The composition of flax-straw processing waste was evaluated based on these defined fractions.

3.1.1 Objectives

The objectives of this initial testing were to evaluate the separation of flax-straw processing wastes using sieve screening, and to evaluate the mass proportions of the recovered fractions, as defined, in particular compared to expected proportions, as indicated by staff at the SWM Intl. facility.

3.1.2 Materials and Methods

Four 20 Litre buckets of flax-straw processing waste were taken from one of the receiving trailers at the SWM Intl. facility. This is the point in the process where waste materials are discharged from the pneumatic conveying system, and are still largely mixed, without significant time for settling and segregation. The moisture content of this waste material was not determined, but was indicated to be around 10% on a wet basis by staff at the SWM Intl. facility. All samples were kept in the sealed buckets in a cool dry location until used (i.e., basement storage room consistently at about 20ºC).

Flax-straw processing wastes were split into individual batch samples of approximately 70 to 150 g, with each sample weighed. Each of 13 different samples was

then separated using sieves in order: #10 mesh, #20 mesh, and then the pan (Canadian Standard Sieve Series, Tyler, St. Catharines, ON). Samples were shaken for a total of 600 s each (Model CL 305-A-1, Soil Test Engineering, Evanston, IL). The mass of each of the three resulting fractions for each sample was manually recorded to the nearest 0.1 g. Sample fractions were finally aggregated.

3.1.3 Results and Discussion

A total of 13 batch samples, representing total mass of close to 1.8 kg of flax-straw processing waste, was separated using the selected sieves. The breakdowns for fractions for individual batch samples are presented in Table 3.1, as well as the aggregate. The aggregate mass of the resulting fractions accounted for roughly 96% of the original material mass. Although individual portions varied somewhat, the breakdown of the final accumulated fractions was roughly 40% waste fibre, 23% mid-core and 33% fines. This did not quite conform to the expectation of equal proportions, but could be reasonably explained.

The waste-fibre fraction for individual samples tended to remain on the top screen as essentially a fibre mat that could be removed largely in one piece. Indeed this material would not flow freely, and had to be manually removed from the sieve. This fraction was found to be largely free of dust, but contained significant embedded smaller mid-core fragments. The obvious trapping of mid-core materials in the mesh of fibre explained the difference in mass proportion between test results from what was anticipated. This led directly to changes being made in the separation method, as discussed later.

Table 3.1 Sieve screening separation results for flax-straw processing wastes.

Initial Mass of Processing Wastes (g)	Mass of Recovered Fractions (g)				Accounted Recovery (%)
	Waste-Fibre Fraction	Mid-Core Fraction	Fines Fraction	Total Mass	
141.6	46.6	30.9	60.1	137.6	97.2%
116.8	51.9	26.6	32.5	111.0	95.0%
153.8	76.5	33.7	39.2	149.4	97.1%
164.0	64.0	45.3	48.8	158.1	96.4%
138.8	46.8	40.5	46.2	133.5	96.2%
159.0	46.6	33.4	45.9	125.9	79.2%
102.4	28.6	28.7	43.8	101.1	98.7%
70.2	13.9	21.4	33.5	68.8	98.0%
120.6	42.0	29.3	47.7	119.0	98.7%
140.5	53.2	37.0	49.5	139.7	99.4%
147.7	72.7	29.6	44.9	147.2	99.7%
151.2	87.1	24.3	39.1	150.5	99.4%
145.9	78.1	27.7	39.3	145.1	99.4%
1,752.2	708.0	408.4	570.5	1,686.9	96.3%
Proportions	40.4%	23.3%	32.6%	96.3%	

The mid-core fraction had a noticeably lighter colour than the fibrous material. It too was essentially free of dust, but contained a few noticeable "knots" of fibre. These knots of waste-fibre were readily visible as dark-coloured, tight balls that were always found on the top of the sample. Mid-core material would flow much more readily, being able to be poured out of the sieve, however, it could still easily bind and bridge, impeding flow.

The fines fraction was an obvious mixture of dust and smaller-sized mid-core material. These two components of the fines fraction were visibly distinct by their respective colours. Dust was much darker in colour, while mid-core material was much lighter. Again a few visible knots of fibre were often present. The fines, like the mid-core material, would tend to flow more readily than the waste-fibre, and could be poured from the sieve. However, the fines too could still easily bind.

Separation of flax-straw processing wastes by screen fractionation was simple. Screening was found to be effective for the removal of dust and fines (see further evaluation in Section 3.4), but more problematic for separation of waste fibre. The finding both of an apparently reduced proportion of mid-core material, and of the entrapment of mid-core materials in fibre led to three changes in the method of screening, as employed in work outlined in later chapters, particularly Chapter 4, Chapter 5, Chapter 7 and Chapter 9.

First, the sieve selection was altered somewhat, with slightly larger particle-sized mid-core material used as the flax shive feedstock in most further testing. In later work, the selected material for testing was altered to primarily use that passing through a #8

mesh screen (nominal opening of 2.36 mm), and retained on a #16 mesh screen (nominal opening of 1.18 mm). In Chapter 6, two slightly different flax shive fractions were used to assess particle size impacts on extraction processing: (i) retained on a 1.0 mm screen; and (ii) passing 1.0 mm screen and retained on 0.5 mm screen (refer to Section 3.4).

Second, smaller individual batches of flax-straw processing waste were sieved at one time. This reduced the likelihood of fibre matting that inadvertently could retain flax shive. Lastly, multiple larger-sized mesh-screens were included in the sieving stack, both above and below the desired screen sizes. As noted later in Chapter 4 (Section 4.4.1), the order of sieves involved: #6 (3.36 mm); #8 (2.36 mm); #16 (1.18 mm); #14 (1.20 mm); #14 (1.20 mm); and then the pan. The use of additional sieves was intended to reduce hold-up and in particular to help prevent fibre matting. This same screen order was also used for feedstock materials for Chapter 5, Chapter 7 and Chapter 9.

3.2 Flax Shive Shape Characteristics

Feda (1982) outlined a three-part approach to characterizing the shape characteristics of particulate materials, that was used to evaluate flax shive. Under Feda's method, a generalized particle, as illustrated in Figure 3.2, is first defined by three measurements, described as follows: thickness (T), which is the distance between the plane of greatest stability of the particle and a plane touching the top surface of the particle that is parallel to the plane of greatest stability; width (B), which is the minimum distance between two planes touching the particle and perpendicular to the plane of greatest stability; and length (L) which is the distance between two planes touching the particle and perpendicular to both the planes defining T and B.

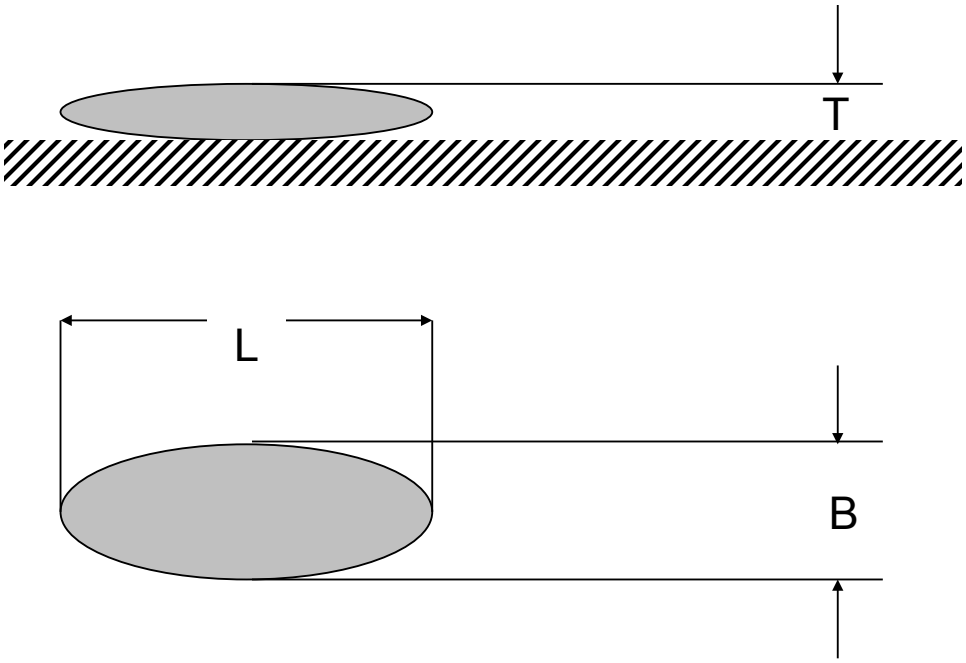

Figure 3.2 Particle characteristic dimensions as outlined by Feda (1982).

Figure 3.3 Example scan of flax shive sample for shape dimensions (grid = 1/4 inch).

Feda secondly outlined two dimensionless ratios used to characterize particles, as defined by the following two equations:

$$\text{Elongation} = L / B \tag{3.1}$$

$$\text{Flatness} = B / T \tag{3.2}$$

Random samples of flax shive (i.e., mid-core fraction) were scanned, as illustrated in Figure 3.3, and scans used for preliminary evaluation of the dimensionless elongation and flatness parameters. Although inherently two-dimensional in nature, the third dimension was also visible in scans due to shadowing. Scans showed, most obviously, that the elongation ratio for flax shive was very high, ranging from a minimum of about 3 to as high as around 20. At the same time, the B and T dimensions were much more similar, with the flatness ratio ranging from 1 to about 2.

Feda lastly outlined the plotting of the inverse of elongation ratio versus the inverse of flatness ratio, as illustrated in Figure 3.4. This plot defines four quadrants representing four types of particles: disk-type; platy/flaky; isometric (bulky); and rod/ needle type particles. The positioning of flax shive is presented on the same plot, based on the evaluated ratios. Using Feda's method, flax shive is characterized as involving rod/needle type particles.

3.3 Flax Shive and Waste-Fibre Densities

3.3.1 Objective

The objectives of this testing were to determine the volume, and thus density, of samples of two of the earlier defined and separated fractions of flax-processing waste, namely the waste-fibre fraction, and the mid-core fraction (i.e., considered as flax shive).

3.3.2 Materials and Methods

Samples of the designated waste-fibre and mid-core fractions were taken from the fractionated materials as described earlier. These samples were weighed and respective volumes determined using an multivolume gas (helium) pycnometer model 1305 (Micromeritics Instrument Corporation, Norcross, GA). Prior to sample testing a "calibration" test was undertaken using a spherical steel ball of known volume, this being 16.758 cm^3.

The principle of the gas pycnometer is based on ideal gas law behaviour. A known sample mass is placed in a cell chamber of known total volume, and pressurized to a measured level higher than atmospheric pressure. Opening a valve to an attached expansion chamber, also of known total volume, causes overall pressure to equilibrate. The discrepancy in pressure from what would be expected if the two cells were empty is due to the volume of the sample material inserted into the cell. The calibration and sample volumes are calculated using the following equation:

$$V_{sample} = V_{cell} - V_{expansion\ chamber} / (P_{initial}/P_{final} - 1) \quad (3.3)$$

where V is volume in units of cm^3, and P is gauge pressure in units of psig.

The waste-fibre sample volume was determined using the largest sample cup for the system, and was calculated using reference volumes, as follows:

$V_{cell} = 142.50\ cm^3$

$V_{expansion\ chamber} = 68.21\ cm^3$

The mid-core fraction volume, on the other hand, was determined using the medium-sized sample cup, and was calculated using reference volumes, as follows:

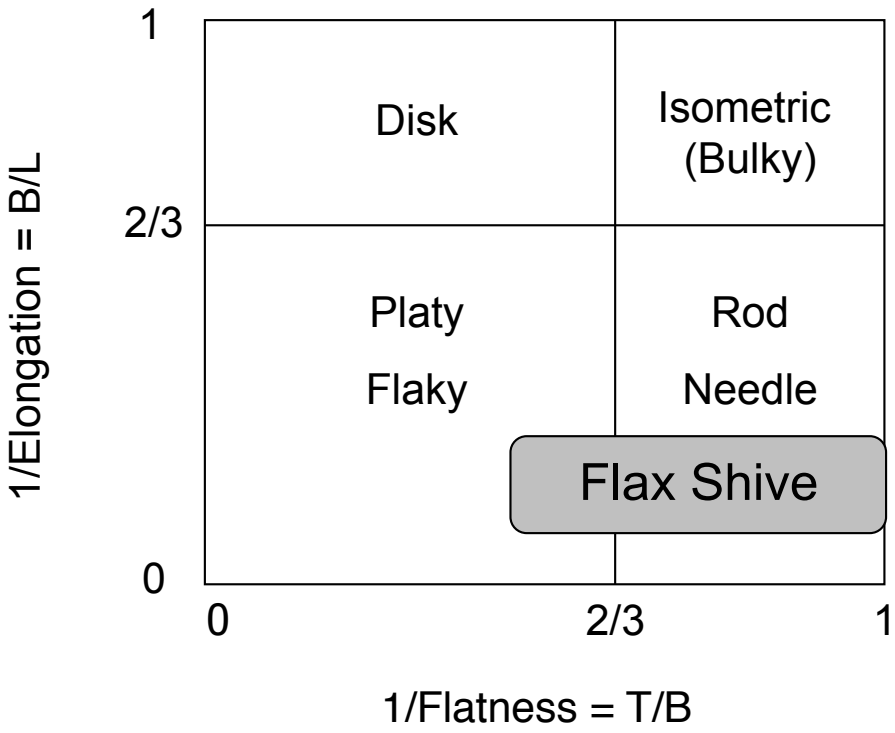

Figure 3.4 Particle characterization matrix as outlined by Feda (1982).

Table 3.2 Gas pycnometer test results for flax-straw processing waste fractions.

Item	Calibration Sphere	Waste-Fibre Fraction	Mid-Core Fraction
Sample Mass (g)	n/a	7.428	3.396
Initial Pressure (psig)	19.667	19.340	19.413
Final Pressure (psig)	9.698	12.925	12.139
Calculated Volume (cm^3)	16.725	5.07	2.61
Calculated Density (g/cm^3)	n/a	1.465	1.301

V_{cell} = 36.45 cm^3

$V_{expansion\ chamber}$ = 20.28 cm^3

3.3.3 Results and Discussion

Results of tests on the calibration sphere and the two fraction samples are presented in Table 3.2. The test of the calibration sphere suggested a volume of 16.725 cm^3, a difference of less than 0.2% compared to the known volume of 16.758 cm^3. As such, the calibration of the device was confirmed. A particle density of approximately 1,300 kg/m^3 for the mid-core fraction, representing flax shive, made sense given that this value was roughly comparable to known densities of other plant materials, such as wheat kernels. The higher particle density of 1,465 kg/m^3 for the waste-fibre fraction also made sense given this material consisted of the stronger fibre of the flax stem, but was somewhat lower (i.e., 5%) than the reported particle density for flax bast fibre from Western Canada of about 1,540 kg/m^3 (Jagannadh and Kolla 1997).

The finding of differences in density between the fractions was also important in explaining differences in the two types of fine particles encountered, as discussed later, particularly in smaller-sized fractions. Light-coloured material appeared to be composed of lighter-density xylem tissue. Dark-coloured material on the other hand appeared to be composed of higher-density fibre. It was observed subsequently with clear plastic bags of loose flax-straw processing waste that darker-coloured fines tended to migrate to the bottom of the bag.

3.4 Flax Straw Processing Waste Colour Differences

The nature of colour differences between flax-straw processing waste fractions was not measured directly using analytical equipment, but was explored further simply using photographs. E-Mission Free Inc. of Winnipeg, Manitoba provided samples for testing of flax materials from SWM Intl. that were more completely fractionated using a series of screens. Photographs of four of the screen fractions were taken under controlled conditions, involving a consistent measured distance (2 feet), the same background material, and the same lighting conditions. Photographs of these four fractions are presented in Figure 3.5, as follows:

- Figure 3.5(a), fraction passing 1.0 mm screen and retained on 0.5 mm screen;
- Figure 3.5(b), fraction passing 0.5 mm screen and retained on 0.3 mm screen;
- Figure 3.5(c), fraction passing 0.3 mm screen and retained on 0.1 mm screen; and
- Figure 3.5(d), fraction passing 0.1 mm screen and retained in the pan.

Two observations were obvious comparing these photographs. First, the progressively finer fractions showed an overall colour transition from lighter to progressively darker. Second, the progressively finer fractions were not uniform in colour but rather contained distinct lighter and darker particles. These appeared to correspond to xylem tissue and fibre fragments respectively, as discussed earlier. The fraction retained on the 0.5 mm screen was composed overwhelming of light-coloured materials, and thus appeared to be almost entirely xylem tissue. The finer fractions appeared to contain increasing proportions of fibre-derived fragments, with the finest appearing to be made up of almost uniform darker-coloured fibre material.

(a) Material < 1.0 mm; ≥ 0.5 mm

(b) Material < 0.5 mm; ≥ 0.3 mm

(c) Material < 0.3 mm; ≥ 0.1 mm

(d) Material < 0.1 mm

Figure 3.5 Photographs of progressively finer screen fractions of flax processing waste.

Two screen fractions provided by E-Mission Free were used as feedstock for the first-step extraction experiments, outlined in Chapter 6. These were the 0.5 mm fraction (i.e., passing 1.0 mm and retained on 0.5 mm), described above as the finest fraction that appeared to be composed uniformly of xylem tissue, and a larger 1.0 mm fraction (i.e., passing 10 mm and retained on 1.0 mm). These two fractions were used to assess the effects of particle-size on extraction yield during pretreatment using sodium ethoxide in anhydrous ethanol solution. The evaluations of colour differences, combined with earlier screening results permitted the preparation of feedstocks for experiments that were composed largely of flax shive, without significant waste-fibre or fines present.

4 Flax Shive Frictional Behaviour

This chapter was previously published as the following paper: Parsons, R.V., S. Cenkowski and Q. Zhang. 2008. Effects of alcohols versus water addition on the bulk solids frictional behaviour of flax shive. *Canadian Biosystems Engineering* 50: 3.67–3.75. Robert Parsons acted as primary author for the published paper, and conducted all of the experiments undertaken.

4.1 Abstract

Understanding the frictional behaviour of biomass fibre is critical for its effective utilization. There is growing research on the handling of fibrous biomass, including the effects of moisture content; however, the impacts of non-water solvents on frictional behaviour have not been addressed in the literature. In particular, the implications of adding alcohol solvents to flax shive, as could occur as part of a high-value product recovery process, remain undefined. The bulk solids frictional behaviour of flax shive was investigated using a standard shear test system. The angles of internal friction and wall friction were evaluated to determine the effects of adding varying proportions of one of three different liquids, methanol, water or isopropanol. The shear strength of flax shive, as measured by the angle of internal friction, was increased by the addition of any of the three liquids, but did not depend on which liquid or what quantity of liquid was added over the range tested. Given differences in surface tension and viscosity of the three liquids, neither property appeared to affect the angle of internal friction. The frictional behaviour of flax shive against a standard galvanized-metal wall material was measured by the angle of wall friction. Changes in the angle of wall friction were different, with a much larger increase found in the case of water addition than for either of the alcohols. Change in the angle of wall friction appeared to be related to the surface

tension of the added liquid. Keywords: frictional behaviour, bulk solids, flax shive, biomass, fibre, internal friction, wall friction, alcohol, methanol, isopropanol.

4.2 Introduction

Fibrous ligno-cellulosic biomass, which consists of residual straw or other plant materials left over after harvesting, has been recognized as a potential renewable source for the production of fuels, materials, and high-value compounds (BioProducts Canada 2004). For these varied uses, it will be necessary to significantly process the biomass feedstock using unit operations such as fractionation and chemical modification that will include the use of water and in some instances non-water solvents, particularly alcohols. Ileleji (2005) and Zhou and Ileleji (2005) noted that the processing of biomass can be difficult and costly if handling characteristics are not properly understood. There is a growing body of research on the handling of fibrous biomass, including the effects of moisture content, however, the impacts of non-water solvents on the bulk solids frictional behaviour of biomass have not been addressed in the literature, and remain ill defined.

The objective of this work was to determine the effects of adding selected alcohols versus water on the angle of internal friction and the angle of wall friction for flax shive, in particular reflecting the conditions of both the feed material and liquid addition that would be encountered in a pretreatment process for the recovery of high-value constituents, such as oligosaccharides. At the same time, alcohol solvents with differing viscosity properties were included, permitting the opportunity to also assess the impacts of variations in the surface tension and the viscosity of the added liquid on frictional behaviour.

Research has been underway at the University of Manitoba on the potential recovery of nutraceutical constituents from flax shive, including recovery through the use of alcohol solvents. The selection of flax shive as a candidate feedstock was straightforward. Kozlowski et al. (2005) identified that Western Canada is a leading world production area for flax (*Linum ussitatissimum*), albeit dominated primarily by oilseed flax. Significant quantities of flax straw are processed, nevertheless, to recover bast fibre from the outer portion of the flax stem. This generates large volumes of flax shive, a waste residual composed primarily of the woody core of the flax stem. The scutching or decortication process, described in general by Salmon-Minotte and Franck (2005), produces a size-reduced and relatively consistent shive fraction, potentially suitable as a feedstock for further processing.

The use of alcohols was also straightforward. As described by Wyman (1999), alcohol solvents have been well identified for use in organosolv fractionation to separate ligno-cellulosic biomass into major components (refer to Section 2.3.5 for further explanation). More specifically, Vazquez et al. (2000) noted that pretreatment of raw biomass materials with alcohols can be a potential initial step in the manufacture of selected oligosaccharides. Being able to exploit alcohols for this purpose, however, requires that their effects on the frictional behaviour of flax shive be quantified.

4.3 Literature Review

The basic theory of bulk solids frictional behaviour has been well established, described for example in Mohsenin (1986) or Shamlou (1988). Bulk solids frictional

behaviour is characterized using plasticity theory and evaluated in terms of the classical, linear Mohr-Coulomb relationship:

$$\tau = \mu \sigma + C \tag{4.1}$$

where: τ is the shear stress (kPa), σ is the normal stress (kPa), μ is the slope or coefficient of friction (dimension-less), and C is the intercept (cohesion or adhesion) (kPa).

The slope term represents the coefficient of internal friction (μ_i) or the coefficient of wall friction (μ_w), depending on whether friction is assessed against the solid material itself or against a wall surface. Similarly, the meaning of the intercept term depends on which type of frictional interaction is being assessed. In the case of internal friction, it represents cohesion, the sticking together of particles, while in the case of wall friction, it represents adhesion, the sticking of particles to another surface material. The coefficients of friction are often expressed as angles of friction (ϕ), whether angle of internal friction (ϕ_i) or angle of wall friction (ϕ_w), by calculating the arctangent of the respective coefficient of friction:

$$\phi = \text{arctangent}\,(\mu) \tag{4.2}$$

In terms of liquid addition to bulk solids, the formation of liquid bridges between particles has been long recognized as an important and sometimes problematic factor determining bulk solids behaviour (Peleg 1977). Shamlou (1988) indicated that as the content of liquid in a dry bulk solid is increased, attractive forces arising from physically absorbed liquid films can readily dominate the interactive forces between individual particles or between particles and an adjacent surface. The magnitude of these forces,

termed capillary forces, is related to the surface tension of the liquid. If the content of liquid in a bulk solid continues to be increased, as described by Fekete et al. (2007), at some level the porosity of the solid material will be exceeded and it will not be able to retain internally any additional liquid. Up to this saturation point, wall friction at an adjacent surface will be a combination of solid particle sliding friction and liquid capillary forces. Beyond a saturation level, the adjacent surface will be completely wetted by liquid alone, and friction will be dominated entirely by the liquid and its viscosity. Thus, both the surface tension and viscosity properties of the liquid can be important under different conditions.

As summarized by Schulze (2006), it is not possible to theoretically deduce the frictional behaviour of a specific bulk solid material or the extent to which it may be able to freely flow. This is firstly because behaviour depends on too many variables, both of the handling system and the particulate material itself, including size, size distribution, shape, and composition, and, secondly, because of potential changes in the dominant forces. It makes sense to empirically derive the necessary quantitative information. In considering the frictional behaviour of flax shive, three general groups of materials that may have potential relevance have been extensively evaluated in the literature. These are other agricultural bulk materials, including seed crops and foods; powders, particularly food products; and, most relevant, other ground fibrous biomass.

Seed kernels, fruits and other related products share compositional similarities with fibrous biomass, but have quite different particle characteristics, being generally larger in size, more regular in shape, and less fibrous in character. Of greatest relevance

is the impact of liquid addition on such agricultural materials, with only water being considered in the literature. The general indication for agricultural materials is that adding moisture has a monotonic increasing impact, i.e., both internal friction and wall friction increase as moisture content is increased (Mohsenin 1986), although the precise nature of variation with moisture over a range of values remains less certain. Mohsenin (1986) summarized various earlier studies in this area for agricultural materials in general, both relevant to wall friction and internal friction, but suggested no consistent relationships with moisture content.

Fine powders have been extensively addressed in the literature, and, as described by Prescott and Barnum (2000), moisture content is well recognized as an important issue in their handling, even to the extent of impeding the ability of powders to flow. However, the nature of powders is not necessarily similar to fibrous biomass. Powder products are generally much finer in particle size and also can be vastly different in composition. An important insight from fine powder handling is in the investigation of viscosity and surface tension modifiers. The use of bulk solid lubricant additives, particularly magnesium stearate, is common in the pharmaceutical industry, as described by Faqih et al. (2007). The established effect is to provide a surface coating to reduce inter-particle friction and particle-wall friction, however, the precise causative action of the lubricant is less clear, i.e., whether the effect results from viscosity or from surface tension. Scoville and Peleg (1981) specifically considered the impacts of liquid films on the bulk properties of model powders of glass beads. They observed:

> "Perhaps the most interesting result of this work is that neither the film viscosity in the range of up to 2000 mPa·s nor its surface tension at 35-75 mN/m had a decisive

> effect on the powder bulk characteristics. This was found in all the tested powders regardless of their particle size and also in sand where the shape of the particle was more irregular. Apparently, the presence of the liquid film by itself was sufficient to cause drastic modifications in the bulk properties.''

For flax shive, the frictional behaviour of ground ligno-cellulosic biomass residuals is most relevant, having both similar composition and fibre-like particle nature. However, only recently has the behaviour of such biomass begun to be studied significantly. As observed by Ileleji (2005), ''There is limited basic research that has been conducted to characterize their physical and flow proper- ties with respect to handling because, until recently, they were not considered a major feedstock for conversion.''

Mani et al. (2003, 2004) evaluated wall friction and adhesion coefficients for corn stover, based on variations in both grind size and moisture content. The coefficient of wall friction was found to increase with increased moisture content, with the relationship in this case suggested to be quadratic in nature. However, the moisture content was relatively low, ranging from 7.2 to only 15.2% wb. Shaw and Tabil (2006) compared properties for a broader range of materials including peat moss, oat hulls, wheat straw, and flax shive, but did not study the effect of moisture content or grind size. For sample moisture content in the range of 9-10% wb, they found ground flax shive to have a similar coefficient of wall friction as ground wheat straw or oat hulls, but with a relatively higher adhesion value.

Zhou and Ileleji (2005, 2006) assessed the frictional behaviour of various grinds of corn stover and switch- grass, specifically samples ground through hammermill screen sizes of 6.4, 3.2 and 1.6 mm. They identified moisture content and particle size as key variables. Zhou and Ileleji (2005) evaluated the angle of repose (ϕ^o), which, as noted by

Reimbert and Reimbert (1987), is closely related to the angle of internal friction. Only corn stover was assessed at different moisture conditions by Zhou and Ileleji (2005). They consistently found an increase in the angle of repose for higher moisture content. However, for these samples only two moisture conditions were assessed, a dry condition, in the range of 5.4 to 6.0% wb, and a wet condition, in the range of 25.6 to 48.5% wb. No comprehensive relationship could be deduced, given the lack of intermediate data points. Zhou and Ileleji (2005) also found the angle of repose to be generally higher for a larger grind size for both corn stover and switch grass, i.e., higher for 6.4 mm grind than smaller grinds. At the same time, they found that the angles of repose for both ground corn stover and switch grass were generally much higher than for grains, with the expectation that these material would be problematic for transport and storage.

Fasina (2006) and Fasina et al. (2006) investigated flow properties for various grinds of peanut hulls, switchgrass, and poultry litter, but in this case using relatively smaller hammer-mill screen sizes of 3.2, 1.6 and 0.8 mm. They did not include the effect of moisture content. They found, in contrast, that screen size did not significantly affect the unconfined yield strength or the cohesion of samples in this case. For all samples, they found the angle of internal friction and cohesion were greater than critical values of 30º and 2 kPa, respectively, such that none of the materials would be expected to flow under gravity discharge from a bin or silo.

Available literature suggests that flax shive, like other fibrous biomass, likely would be a problematic bulk solid. Both the angle of internal friction and wall friction

would be expected to be relatively high. Flow behaviour would be expected to significantly worsen through the addition of moisture.

An important consideration in recent literature where moisture content was a variable is that higher moisture content levels were obtained by using harvested wet material stored at reduced temperatures until used, adding distilled water to relatively dry samples followed by equilibration periods of up to several days, or exposing samples to a very high relative humidity environment, again for periods of up to several days. Such procedures to set or adjust moisture content prior to bulk solids frictional evaluation, however, do not reflect the anticipated conditions in a pretreatment process, whereby relatively dry biomass feedstock would be dosed with liquid and then processed within a relatively short period of time, with processing likely to begin within about 3 h and concluded in under 24 h.

Additional issues in the handling and evaluation of fibrous biomass have been identified in the literature. In conventional shear testing for frictional behaviour, particularly for powders, it has been typical for samples to be preconsolidated (Shamlou 1988). However, as described by O'Dogherty (1989), compressed fibrous straw exhibits elastic recovery, rendering preconsolidation impractical. Sieving of biomass samples is also recognized to be problematic, whether for separation or analysis. Sieves are only two-dimensional in nature while individual fibrous particles are three-dimensional, potentially having varying lengths, and as noted by Bartl et al. (2004), sieves cannot distinguish between width and length of biomass fibres. Sieving also represents effectively a form of staged gravity-flow through openings of decreasing size. As

outlined by Paulrud et al. (2002) the highly irregular and often elongated particle shape associated with size-reduced biomass has a significant impact on the passage of materials through sieves, in particular increasing the potential for bridging. Naita et al. (1998) found in the measurement of particle size distribution for non-biomass materials by various analytical techniques that shape had a much more important impact for rod-like particles than for block-shaped particles. Yang et al. (2006) specifically compared image analysis versus standard sieves for ground biomass, and confirmed that caution must be exercised in the use of conventional sieve methods given the highly irregular nature of the fibrous biomass. Concerns regarding sieving were considered in this research in the separation of the flax shive samples.

4.4 Methods

4.4.1 Shive sample preparation

Sufficient shive for testing was obtained during the early summer of 2006 from the production line of the flax decortication facility of SWM Intl. Inc. (formerly Schweitzer-Mauduit Canada Inc.), located near Carman, Manitoba. The material from the decortication line was double-sealed in plastic bags and stored in a cool, dry location until used in order to minimize changes in moisture content or other properties.

SWM Intl. Inc. staff described their decortication waste as composed of three roughly equal mass components: mid-core shive itself; residual short waste fibre, termed tow; and dust. Shive was segregated prior to testing using standard sieves (Canadian Standard Sieve Series, Tyler, St. Catharines, ON). Only selected sieves were used, and in the following order: #6, #8, #16, #14, #14 and then the pan (refer to Section 3.1.3). The

material was shaken for approximately 120 s in a shaker (Model CL 305-A-1, Soil Test Engineering, Evanston, IL). The material retrieved for testing was that passing through the #8 mesh screen (nominal opening size of 2.38 mm), but retained on the #16 mesh screen (nominal opening size of 1.19 mm). This pretreatment was not done for analysis purposes, but rather ensured that the samples for testing consisted predominantly of the spongy stalk mid-core, with the residual longer fibres largely excluded and the fine dust removed.

The two-dimensional deficiency of sieving was recognized. To mitigate this issue and ensure consistency of test samples, batches of mid-core shive were prepared by aggregating and mixing multiple sieve retentate samples into a single batch. Three separate batches were prepared in this way, with batch sizes of approximately 1500 g for Batch #1 and Batch #2, and 3000 g for Batch #3. Comparative shear test runs were conducted using samples from the same mixed batch.

4.4.2 Moisture content

SWM Intl. Inc. staff indicated that the moisture content of feed flax material to the decortication plant tended to be in the range of 10 to 14% wb, prior to processing. Three random samples of shive were retained in sealed plastic bags and assessed afterward for moisture content using ASAE standard S358.2 (ASAE, 2004).

4.4.3 Liquid additions

Samples of approximately 450 g of shive were withdrawn from respective mixed batches and placed into 20 L sealed plastic buckets. The samples were dosed with one of three liquids: methanol; water; or isopropanol (2-propanol). The liquids involved are all

hydrophilic in nature, but the two alcohols have much lower surface tension and the three liquids have varying viscosity properties.

Relevant liquid property data were obtained from the CRC Handbook (Lide 2006) for the three liquids, and linearly interpolated to 20ºC, the approximate temperature of the laboratory. The resulting surface tension values were 22.5, 72.7, and 21.3 mN/m for methanol, water and isopropanol, respectively, and viscosity values were 0.59, 1.07, and 2.55 mPa·s in the same order, respectively. Industrial grade methanol and isopropanol (i.e., 99.9%) were obtained from Canadian Tire and Safeway Pharmacy, respectively. Commercially available distilled water was used for water-dosed samples, this also obtained from Safeway. Four levels of liquid addition were used:

- No liquid, representing the control;
- Approximately 203 g liquid, representing 45% of the shive sample mass;
- Approximately 405 g liquid, representing 90% of the shive sample mass; and
- Approximately 608 g liquid, representing 135% of the shive sample mass.

These liquid doses were in addition to the residual moisture content already present in the shive samples, but were set sufficiently high to be significantly dominant in all cases.

All liquid-dosed samples were left for 3 h in the sealed buckets prior to testing in order to approximate the conditions that could be experienced as part of an actual shive processing operation. Similarly, all shear testing of liquid-dosed samples was completed within 24 h of liquid addition.

4.4.4 Shear testing

The shear test unit used for the experiments had been earlier constructed internally at the Department of Biosystems Engineering at the University of Manitoba, and was described briefly in Zhang et al. (1994). This unit is consistent with the standard shear apparatus originally developed by Jenike for determination of flow properties, and described in more detail in Mohsenin (1986). Important features of the device and associated methods are noted as follows:

- Unit incorporated a square rather than round test cell, with two compartments constructed of clear plastic of a thickness of approximately 13 mm. As a result, no rotation of the test cell was employed during testing. The elevation-view of the unit when used to assess internal friction is represented schematically in Figure 4.1. The plan-view internal dimensions of the square cell were approximately 127 × 127 mm, with the upper and lower compartments each 75 mm high.
- Upper cell compartment was pulled at a constant rate of 0.12 mm/s, as opposed to being pushed, with the shear force on the drawn load cell being data-logged at 1 s intervals using a data acquisition unit (Model 3852A, Hewlett-Packard, Santa Clara, CA).
- Normal loads on the cell were achieved using a mass slung underneath the unit by means of a cradle sled suspended at the centre of the cell top plate using a stainless steel ball to ensure uniform weight distribution (Figure 4.1).

- Lower cell portion was removable and could be replaced by a block with a standard flat galvanized sheet-metal top surface to permit testing for the angle of wall friction. The elevation-view of the unit when used to assess wall friction is represented schematically in Figure 4.2.
- No pre-consolidation loading of the samples was undertaken.

Individual test runs were conducted over a period of approximately 120 to 180 s each. For each individual batch of shive, random control samples prior to adding any liquids were withdrawn for shear testing, then returned and remixed into the batch.

It was not possible to completely prevent evaporation of alcohols during testing, given volatility, but exposure was limited. Samples incorporating liquids were removed quickly from a respective sealed bucket into the test cell. When the top plate of the cell was fitted, the total exposed area of the cell was only the gap between the upper and lower portions. This clearance was limited to no more than 2 mm, such that the total open area was less than 10^{-3} m^2, reducing potential evaporation of the alcohols. Once tests were completed, samples were then quickly returned back to their bucket, remixed into the remaining sample, and resealed.

At least four test runs were conducted using different compressive normal loads at each liquid-addition level for each liquid, and for each batch control sample to derive a linear regression based on Eq. 4.1. The mass of the entire sled apparatus was about 14 kg, to which additional increments of 20 kg were added. Four different normal stresses were thus employed: 8.5, 20.7, 32.8, and 45.0 kPa. The respective angle of internal friction or wall friction was calculated from each derived slope using Eq. 4.2.

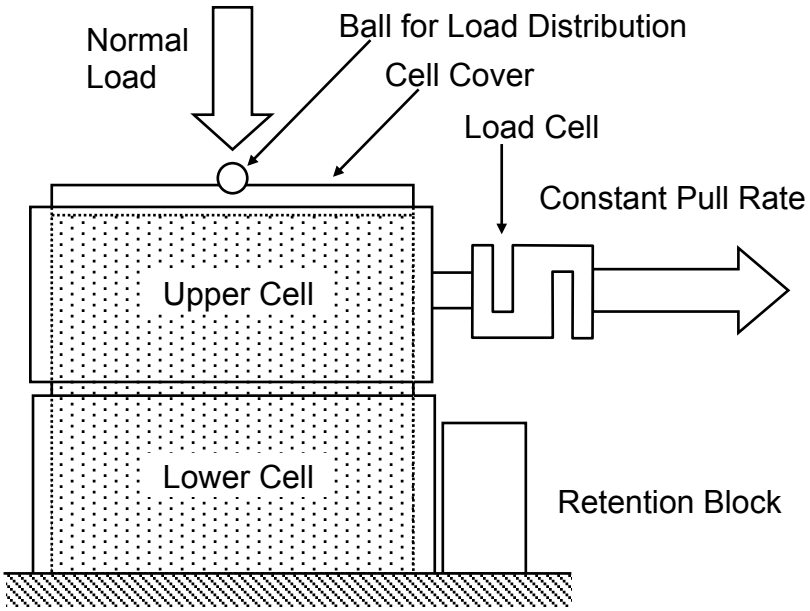

Figure 4.1 Schematic elevation view of test unit as used for internal friction assessment.

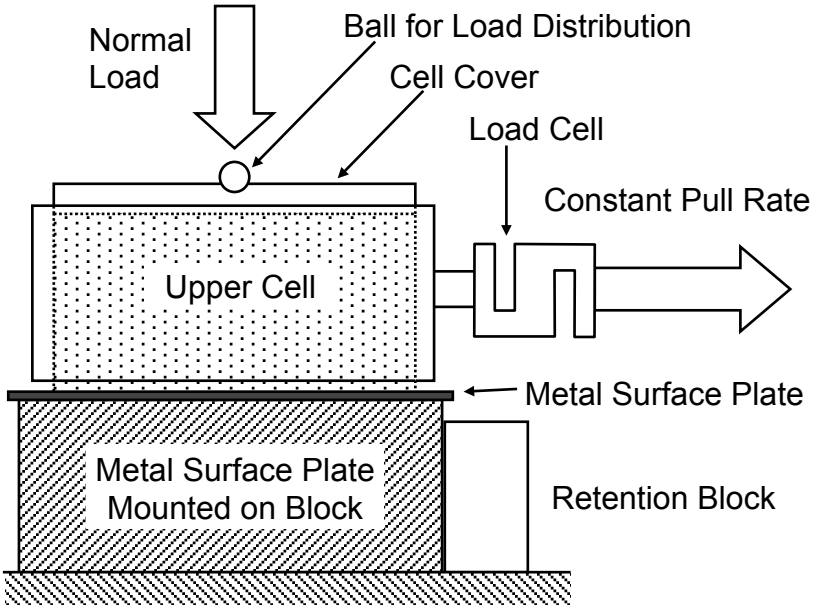

Figure 4.2 Schematic elevation view of test unit as used for wall friction assessment.

One data point was missing from the results for both the angle of internal friction and wall friction. This was for the shive sample dosed with 45% isopropanol. A problem developed with the shear test unit preventing this final set of runs from being completed.

4.4.5 Data analysis

Data analysis was performed using Microsoft Excel X for Mac, Service Release 1. Two key statistical analyses of data were conducted manually, given the small number of data points involved, to compare water-dosed versus alcohol-dosed samples for both angles of friction. These involved F-tests, calculating F statistics to assess the similarly of sample variances, and two-sample pooled t procedures, calculating two-sample pooled t statistics to assess the similarity of sample means. Procedures used are as outlined in Moore and McCabe (2006), with the use of the two-sample pooled t-test being based on the assumption of equivalent population standard deviations, verifiable from the corresponding F-test.

4.5 Results and Discussion

The moisture content of three random shive samples was found to be 8.3% wb ± 0.1%. This was lower than the indicated moisture content of the flax straw prior to processing, but consistent with the findings of Zhou and Ileleji (2005), who found that the moisture content of biomass was reduced through hammermilling. These results also confirmed that the added liquids were the dominant liquids present, representing at minimum more than five times the residual moisture content.

The selection of shear testing within a period of 3 to 24 h after liquid addition was dictated by process considerations. It is important to note that when shear testing was

started after the 3 h period, all shive samples were found to have completely absorbed all added liquid, with no evidence of free liquid in any bucket. More importantly, there was no exuding of liquids evident from any of the samples even under the highest compressive loads. In all cases, the liquid added to the shive was completely absorbed, and none of the samples achieved a liquid saturation condition.

The slope data for the internal friction assessments are presented in Table 4.1 in the order conducted. Shear stress versus normal stress data provided excellent fit in all cases, with the lowest value of the bivariate coefficient of determination (r^2) being 0.97 (i.e., for Batch #3 test using 135% isopropanol). A total of four control runs were conducted without liquid addition. The mean value of the angle of internal friction for these control tests translated to approximately 28º, with a standard deviation of 3º based on a sample size n = 4.

The calculated angles of internal friction for the various liquid-dosed samples are summarized in Table 4.2. An increase in the angle of internal friction clearly occurred due to the addition of water or either of the alcohols. However, the results for all the liquid-dosed samples were relatively similar, despite differences in the type of liquid added or the level of dosing. Verification is provided later in Table 4.5. The mean value of the angle of internal friction for all the liquid-dosed samples was approximately 37º, with a standard deviation of 2º based on a sample size n = 11. The increase in the angle of internal friction appeared to result from liquid addition, and not depend on which liquid or what quantity of liquid was added over the range tested. Given differences in surface tension and viscosity properties for the three liquids, the further implication is

Table 4.1 Data for internal friction assessments of liquid-dosed flax shive samples (n = 4 data points in all cases, except * where n = 5 data points).

Batch number	Liquid		Slope or coefficient of internal friction (μ_i)	Intercept or cohesion (kPa)	r^2
	Type	Addition (%)			
#1	Control	0	0.43	2.10	0.99
	Methanol	90	0.70	1.41	0.99
	Water	90	0.73	1.17	0.99
	Isopropanol	90	0.78	-0.61	0.99
#2	Control *	0	0.53	0.95	0.98
	Methanol	90	0.79	-1.05	0.99
	Water	90	0.75	1.13	0.99
	Isopropanol	90	0.72	2.14	0.99
#3	Control	0	0.56	1.38	0.98
	Control *	0	0.59	0.10	0.99
	Methanol	135	0.72	1.13	0.99
	Water	135	0.82	-0.99	0.99
	Isopropanol	135	0.66	4.32	0.97
	Methanol	45	0.74	1.37	0.99
	Water	45	0.75	1.68	0.99

that neither of these liquid properties had an important impact on the angle of internal friction. This result is consistent with the earlier findings of Scoville and Peleg (1981).

The slope data for the wall friction assessments are presented in Table 4.3 in the order conducted. Again, the shear stress versus normal stress data provided excellent fit in all cases, with the lowest value of r^2 being 0.89 (i.e., for Batch #2 control test). A total of three control runs were conducted without liquid addition. The mean value of the angle of wall friction for these control tests translated to approximately 12º, with a standard deviation of 2º based on a sample size n = 3.

The calculated angles of wall friction for the various liquid-dosed samples are summarized in Table 4.4. An increase in the angle of wall friction occurred due to liquid addition, but was more complex. In this case, the values for the addition of water were clearly higher and different from those for the two alcohols. The mean value of the angle of wall friction for the water-dosed samples translated to approximately 28º, while the mean value of the angle of wall friction for all alcohol-dosed samples translated to approximately 23º (Table 4.5). Given differences in surface tension and viscosity properties for the three liquids, the further implication is that the surface tension, but not the viscosity, of the added liquid affected the angle of wall friction. At the same time, the angle of wall friction appeared to be unaffected by the quantity of liquid added over the range tested, just as in the case for the angle of internal friction.

The experiments showed a clear difference between the nature of changes in the angle of wall friction versus the angle of internal friction when an alcohol versus water was added. As illustrated by the two-sample pooled t statistics presented in Table 4.5, for

Table 4.2 Summary of angles of internal friction for liquid-dosed tests (For explanation of missing data point * see Section 4.4.4).

Liquid addition (%)	Angle of internal friction (ϕ_i,º)		
	Methanol	Water	Isopropanol
45	37	37	*
90	35	36	38
90	38	37	36
135	36	39	33

(Note: mean control value, with no liquid addition, $\phi_i = 28º \pm 3º$, n = 4)

Table 4.3 Data for wall friction assessments of liquid-dosed flax shive samples (n = 4 data points in all cases, except * where n = 5 data points).

Batch number	Liquid		Slope or coefficient of wall friction (μ_w)	Intercept or cohesion (kPa)	r^2
	Type	Addition (%)			
#2	Control	0	0.22	1.16	0.89
	Methanol	90	0.48	1.06	0.98
	Water	90	0.56	0.42	0.99
	Isopropanol	90	0.48	0.77	0.99
#3	Control	0	0.24	-0.20	0.99
	Control *	0	0.18	0.67	0.98
	Methanol	135	0.38	1.05	0.99
	Water	135	0.53	0.29	0.99
	Isopropanol	135	0.43	0.94	0.99
	Methanol	45	0.36	0.86	0.99
	Water	45	0.54	0.16	0.99

a 95% confidence interval the difference between the mean angles of wall friction for water versus alcohol addition was sufficient to show distinct behaviour, while the difference between the mean angles of internal friction for water versus alcohol addition was due to random variation. The associated F statistics in both cases indicated that the assumption of equivalent population standard deviations was reasonable (Table 4.5).

It would appear from the results that the angle of wall friction depends on the surface tension of the added liquid, but the angle of internal friction does not. This situation was verified by qualitative observation. Both the alcohol- and the water-dosed samples formed solid plugs in the test cell as a result of compaction. However, while the water- dosed samples tended to cling to the wall, forming a stable bridge that was difficult to remove, the alcohol-dosed samples all tended to slip out easily as a whole plug. This may represent a useful behaviour that could be exploited. Given test results, further investigation of the impacts of adding alcohols on the frictional behaviour of flax shive and other biomass is warranted.

4.6 Conclusions

The shear strength of bulk solid flax shive, as measured by the angle of internal friction, was increased by the addition of any of the three liquids tested, whether methanol, water, or isopropanol. The angle of internal friction increased consistently by about 9º as a result of liquid addition, but with no relationship to which liquid or what quantity of liquid was added over the range tested. Neither the liquid properties of surface tension nor viscosity appeared to have any effect on the angle of internal friction.

Table 4.4 Summary of angles of wall friction for liquid-dosed tests (For explanation of missing data point * see Section 4.4.4).

Liquid addition (%)	Angle of wall friction (ϕ_w,º)		
	Methanol	Water	Isopropanol
45	20	28	*
90	26	29	26
135	21	28	23

(Note: mean control value, with no liquid addition, $\phi_w = 12º \pm 2º$, n = 3)

Table 4.5 Evaluation of differences between water-dosed versus alcohol-dosed flax shive samples based on F statistic and two-sample pooled t statistic.

	Parameter	Internal friction	Wall friction
Water-dosed tests	Mean angle of friction	37º	28º
	Standard deviation	1º	1º
	Sample size (n)	4	3
Alcohol-dosed tests	Mean angle of friction	36º	23º
	Standard deviation	2º	3º
	Sample size (n)	7	5
F-test	F statistic	4.0	9.0
	Confidence interval	95%	95%
	Degrees of freedom	6,3	4,2
	Critical F statistic	8.9	19.3
	Analysis result	Null hypothesis accepted	Null hypothesis accepted
	Implication	Population variances the **same**	Population variances the **same**
t-test	Difference in mean	1º	5º
	Pooled t statistic	0.9	2.7
	Confidence interval	95%	95%
	Degrees of freedom	9	6
	Critical t statistic	2.3	2.4
	Analysis result	Null hypothesis accepted	Null hypothesis rejected
	Implication	Population means the **same**	Population means **different**

The frictional behaviour of flax shive against a standard flat galvanized-metal wall material was measured by the angle of wall friction. Changes in the angle of wall friction were found to be higher by about 5º for the addition of water than for the addition of either of the two alcohols. In this case the surface tension, but not the viscosity, of the added liquid appeared to affect the angle of wall friction.

A clear difference was found to exist between the nature of changes in the angle of wall friction versus the angle of internal friction as different liquids were added. This appeared to occur because the angle of wall friction depended on the surface tension of the liquid, whereas the angle of internal friction did not. It may be possible to usefully exploit this difference in behaviour in the handling of flax shive or other fibrous biomass bulk solids, in particular as part of a process to recover high-value constituents. (Refer to Section 10.1 for discussion of the significance of this research work to the overall thesis objectives).

5 Flax Shive Absorptive Behaviour

This chapter was previously published as the following paper: Parsons, R.V and S. Cenkowski. 2011. Liquid-holding capacity of flax shive versus other biomass materials using distilled water, alcohols, and hexane. *Canadian Biosystems Engineering* 53: 3.19-3.27. Robert Parsons acted as primary author for the published paper, and conducted all of the experiments undertaken.

5.1 Abstract

The liquid-holding capacity of flax shive was characterized, compared to three other types of biomass with five different liquids. Flax shive is essentially a waste product of fibre recovery that is available in large quantities at low cost, with consistent composition and size characteristics. The other biomass materials tested were hemp hurd, commercial pine shavings, and the Simply Straw™ product, a flaked commercial absorbent produced primarily from wheat straw. The five liquids tested were distilled water, methanol, anhydrous ethanol, isopropanol, and hexane. All tests used samples with the same particle size at room temperature and atmospheric pressure, with liquid-exposure for one hour followed by draining for one hour. Precautions were included to address the evaporation of volatile liquids and to reduce disturbance of bulk solids, which could potentially alter liquid retention. Flax shive exhibited comparatively low liquid-holding capacity across the range of liquids tested. Hemp hurd and commercial pine shavings exhibited good liquid-holding capacity for all liquids, particularly for non-aqueous liquids. The Simply Straw™ product showed variable behaviour. It had the highest liquid-holding capacity for water, but low for all non-aqueous liquids. The results were found to be statistically significant using two-way analysis of variance (ANOVA), with liquid-holding capacity affected in order of importance by the type of liquid selected

and then by the biomass material selected. The interaction between the main effects (biomass and liquid) was also significant, most evident with the anomalous behaviour of the Simply Straw™ product. Liquid-holding capacity results correlated most strongly to the liquid properties of surface tension and inverse molar volume. Flax shive was shown to be less desirable as an absorbent, but its behaviour could be beneficial when used as a feedstock for extraction of potential higher-value constituents, reducing losses of solvent agents and products. Key words: absorbency, liquid-holding capacity, biomass, flax shive, hemp hurd, pine shavings, wheat straw, Simply Straw™, water, methanol, ethanol, isopropanol, hexane.

5.2 Introduction

Absorbency relates to the ability of solid materials, usually porous, to take up and to hold liquids. Absorbents have been identified as one potential market for agricultural crop residues, including industrial by-products such as flax shive (Flax Council of Canada 2002) and hemp hurd (Agricola Group 2008). Use of biomass as animal bedding for water uptake is obvious, but applications are diverse, including use as absorbents for non-aqueous liquids in spills of oils or solvents (Tavisto et al. 2003).

Depending on the process or application involved, increased absorbency can be either beneficial or detrimental. An important but less-obvious example of the latter is the retention of solvents, whether water, alcohols or other liquids that may be used in recovery processes for higher-value constituents. Work has been underway at the University of Manitoba on the recovery of possible high-value products from flax shive using both aqueous and alcohol-based recovery processes. In this case, understanding the

absorbency of flax shive is important in order to minimize losses of solvents and extracted products.

A key constraint for all biomass-based materials, including flax shive, is the lack of objective data in the literature to understand the absorbency performance of biomass. Most development in this field has tended to be of a commercial nature (Chatterjee and Gupta 2002a), typically without peer review. The objective of this work was thus to characterize the absorbency of flax shive, in particular the liquid-holding capacity, relative to three other biomass materials: hemp hurd, commercial pine shavings, and the commercial absorbent product Simply Straw™. Five liquids were tested, namely distilled water, the alcohols methanol, ethanol, and isopropanol, and hexane, which is a representative non-polar alkane and a so-called "neutral solvent."

Flax shive and the other biomass materials examined in this study are all plant-derived and ligno-cellulosic in nature, albeit with differing compositions (Han 1998) and structures. Flax shive, the primary focus of this work, represents the woody by-product of decortication, the process involved in removing bast-fibres from the outer stem of the flax plant (*Linum usitatissimum*). It is composed primarily of broken pieces of xylem tissue from the stem core, although contaminating fibre fragments from the stem periphery are usually present. Given the nature of flax shive, essentially being a waste product (i.e., 70-85% of stem mass), there has been strong interest in investigating and developing alternative uses for this material (Flax Council of Canada 2002).

Hemp hurd is a parallel by-product to flax shive, but instead is derived from the decortication of the stalk of the industrial hemp plant (*Cannabis sativa*). It too consists

primarily of woody xylem tissue. Kymalainen et al. (2001) characterized both flax shive and hemp hurd as highly capillarated in nature, and thus potentially absorbent. Pine shavings represent a well-established commercial product for various absorbent applications, produced either as an incidental by-product of sawing and milling, or deliberately via planing. Shavings are derived from the woody trunk core of pine trees (*Pinaceae*). As such, they are composed of dense, non-porous heartwood rather than active, porous xylem tissue. The Simply Straw™ product is a flaked commercial absorbent that is produced from major cereal straws. The primary source of the product is wheat (*Triticum* spp.) straw. One additional common characteristic of all four biomass materials was that all were subjected, either deliberately or incidentally in the processes from which they were produced, to physical treatments involving shear stress, whether hammer-milling, sawing, planing, extrusion or others.

5.3 Literature Review

Liquid absorbency is only one manifestation of the broader field of sorption. Sorption describes the penetration and dispersal of a gas, vapour, or liquid onto and throughout a solid to form a mixture (Machin and Rogers 1970). Sorptive processes are generally subdivided into adsorption, referring to attachment at surface interfaces, and absorption, referring to penetration within the bulk structure of a solid material. In the case of liquid absorbency, absorption is primarily involved, but in practice the distinction between the two can be blurred. These transport phenomena also can be highly complex, and are not completely understood. Chatterjee and Gupta (2002a) noted that although attempts have been made to define and predict absorbency based on both classical and

contemporary theories, none have been completely satisfactory in explaining the intricacies of liquid uptake in actual materials. Hence, the focus of development has been on empiricism, with a trial-and-error approach being common.

Liquid absorbency as a property has relevance in a variety of fields. It can be characterized by liquid-holding capacity, which is defined as the volume or mass of liquid taken up by the absorbent material per unit dry mass. For water, the distinction between volume- versus mass-based measures is immaterial, given water density around unity, but the difference could be important for other liquids. More importantly, the method of measuring liquid-holding capacity is not uniform across applications, depending on corresponding specific requirements (Gupta and Chatterjee 2002).

Data on the liquid-holding capacity of flax shive for water have been published recently in two separate patents (Kasbo et al. 1994; Isman et al. 2003), but with somewhat contradictory results. In both cases, flax shive was not altered chemically, but physical separation was undertaken. Even though different methods of physical separation were employed, primarily screen-sizing for the former versus air classification for the latter, the nature of particle sizes was relatively similar.

The patent by Kasbo et al. (1994) showed the liquid-holding capacity of flax shive for distilled water to be about 2.7 mL/g (db). This was based on a liquid-exposure time of one hour, with the particle size range from #10 mesh (2.00 mm nominal opening) to #22 mesh (0.76 mm nominal opening). Their data further showed important effects were exerted by the particle size, discussed later. The authors also showed that saturation-equilibrium was not reached quickly; rather exposure for at least 3 to 4 h was required.

The patent by Isman et al. (2003) claimed a liquid-holding capacity for flax shive with water to be in the range of 4 to 5 mL/g (db), almost double that of Kasbo et al. (1994). This was based on a liquid-exposure time and draining period of one hour each, but with the quality of the water being unspecified. In their case, the dimensions of the flax shive particles were specified, with length from about 5 to 23 mm, and both width and thickness from about 0.3 to 0.8 mm.

The liquid-holding capacity of an absorbent material at equilibrium is determined by two distinct major liquid uptake mechanisms (Chatterjee and Gupta 2002b). First is the attraction of the liquid to solid surfaces. Surface interaction leads to the spreading of liquids on solid surfaces, and to the bulk movement of liquids into and through internal capillaries. Second is the partial solubilization in the liquid of the actual matrix polymers that make up the solid, leading to swelling. Liquid diffusion is involved in this latter case. Although some concerns have been raised regarding the rigour of liquid-holding capacity when it is used as a measure of absorbency (Gupta and Chatterjee 2002), a key advantage is that it fully encompasses the aggregate effects of both these absorbency mechanisms as may be at play for a particular material.

Liquid attraction to the solid surface is the more conventionally understood phenomenon, with the absorbent material typically treated as a porous medium with defined capillaries. Liquid attraction, in combination with the pore size distribution, determines associated capillary pressure and the resulting uptake of liquid into internal capillary spaces. When different liquids are involved, various properties can be implied from theoretical equations to be of potential influence on liquid-holding capacity

(Chatterjee and Gupta 2002b). Possible properties of the liquid alone include viscosity, surface tension, density or specific gravity, molecular weight, molar volume, and vapour pressure. The contact angle of the liquid against the solid, and the gross permeability of the liquid in the solid are also noted, but these are interactive properties that are also dependent on the specific solid material.

An important complication for biomass residues, such as flax shive, is that they are bulk solids, composed of discrete loose particles. Thus, in addition to internal capillary structure, there are significant interstitial spaces between individual particles. Two obvious effects of this situation are that overall liquid-holding capacity will depend partly on particle size and its associated distribution, in addition to internal capillary characteristics, and that any disturbance of a bulk-solid bed could change the interstitial spaces and thus alter the liquid-holding capacity (e.g., bed-shearing leading to plastic deformation). Kasbo et al. (1994) found that liquid-holding capacities were higher for smaller-sized flax shive (i.e., particles smaller than #22 mesh compared to particles in the range of #10 to #22 mesh, or larger).

Absorption rates due to capillary action for both flax shive and hemp hurd were included in investigations by Kymalainen et al. (2001) and Tavisto et al. (2003). Kymalainen et al. (2001) considered uptake of distilled water into prepared columns of flax shive and hemp hurd materials over periods up to 7 hours. They found similar uptake for flax shive and hemp hurd, but noted that rates for fine shive/hurd fractions were higher than for coarse shive/hurd fractions, supporting the importance of particle size distribution. Tavisto et al. (2003) evaluated flax shive and hemp hurd in experiments

on capillary rise and droplet absorption rates for three liquids: distilled water, ethanol, and lubricating oil. For water, they found absorption rates for hemp hurd to be higher than flax shive. For ethanol, they found hemp hurd to be slightly higher than flax shive, but for lubricating oil, they found no practical difference. Droplet absorption rates for water were faster with hemp hurd than flax shive. Droplet absorption rates in all cases were much longer for oil than water, and very rapid in all cases for ethanol. Although capillary absorption rates are related to liquid-holding capacity, and thus provide insights, they are not necessarily fully indicative of the liquid-holding capacity, given separate impacts of the second absorbency mechanism.

The second absorbency mechanism involves partial solubilization and dissolution of the polymers that make up the solid matrix itself into the liquid, resulting in swelling (Chatterjee and Gupta 2002b). This effect is typically associated with so-called "superabsorbent" products for water absorbency. The mechanism is also observed for many organic polymers in combination with selected organic solvents (Machin and Rogers 1970). Properties of the liquid alone that may be relevant to this mechanism include the molecular weight, molar volume, partial pressure, and functionality of the liquid (i.e., specific functional groups involved, including polarity). Relevant interactive properties, which are also dependent on the solid matrix polymers involved, include the diffusivity of the liquid in the solid, and the solubility of the matrix polymers in the liquid (Chatterjee and Gupta 2002b; Machin and Rogers 1970).

Purified, cross-linked cellulose polymers and cellulose derivatives definitely exhibit partial dissolution and swelling with water (Young 2002), and have been

employed for superabsorbent applications. Indeed, even cellulose derived from flax shive has been used for the preparation of superabsorbents (Feng et al. 2010). In the context of native ligno-cellulosic materials, which are composite in nature, superabsorbent behaviour may be observed with water, but not as pronounced. Kymalainen and Pasila (2000) noted that absorptive behaviour of flax shive and hemp hurd, in terms of reaching equilibrium moisture content, was anticipated to be very similar to that of wood, given relatively similar lignin contents. As water is absorbed, cell walls swell, but only to a saturation level, termed the water saturation point.

The mechanism of partial dissolution and swelling also would be anticipated to be less pronounced for liquids other than water given the limited solubility of cellulose. Cellulose at ambient conditions tends to be only soluble in highly specialized solvents (Heinze and Koschella 2005), not simple alcohols or alkanes as employed in this work. It is important to note that methanol and ethanol have been used as agents in organsolv processes for ligno-cellulosic feedstocks, whether for direct pulping or for pretreatment prior to enzymatic hydrolysis (Zhao et al. 2009). The role of the alcohol solvents in such processes is specifically for the solubilization and dissociation of the lignin component, however, without the use of catalysts, these chemical processes require elevated temperatures (greater than about 180°C). No appreciable dissolution or swelling of the lignin component would be expected at ambient conditions. Some of the non-aqueous solvents used in this work have also been considered previously for the solubilization of gums and cuticular waxes from flax, such as alkane hydrocarbons (Thellier 1926) and

ethanol (Holser and Akin 2008). Again, whether done for removal or recovery, only elevated processing temperatures have been employed.

One last consideration for liquid-holding capacity determination is that all the liquids in this current study have been used for the recovery of "extractive" constituents from ligno-cellulosic materials (Sluiter et al. 2005). The mass loss of extractive constituents into the liquid, if significant, could be considered to affect the calculation of liquid-holding capacity, given that the dry mass of absorbent solid is considered constant in the calculation. Deliberate extractions are typically, however, undertaken at elevated temperatures (i.e., with solvents under reflux). At ambient conditions the mass of recovered extractives would be substantially lower in all cases, and unlikely to be of significance in impacting liquid-holding capacity results.

Naik et al. (2010) recently included extraction results for wheat straw, flax straw and pinewood, using methods as outlined in Sluiter et al. (2005), with distilled water, ethanol, and hexane employed as solvents, although at elevated temperatures in all cases. Given the biomass materials and liquids considered, their results were at least indicative of expectations for this current work. They found distilled water generally to yield the largest quantity of extractives, ethanol to be intermediate, and hexane to be the lowest. They also found flax straw to be the most extractable of the three materials.

5.4 Methods and Materials

5.4.1 Biomass Materials

Samples of unseparated flax shive were provided from the decortication plant of SWM Intl. Inc. (formerly Schweitzer-Mauduit Canada Inc.), located near Carman,

Manitoba. These materials were taken directly off the process-line and also included waste fibre components and dust. Samples of hemp hurd were obtained from Emerson Hemp Distribution Company, which is located near Emerson, Manitoba. Commercial pine wood shavings were obtained from a local pet supplies store. Samples of the Simply Straw™ product were provided by E-Mission Free Inc. of Winnipeg, Manitoba.

5.4.2 Screen Sizing

In all cases, samples were screened to ensure consistent particle size, and, in particular, to eliminate dust residuals. Screening of unseparated flax shive was undertaken using the method outlined earlier by Parsons et al. (2008), involving a specific sequence of additional screens, with retained material passing through a #8 mesh screen (2.36 mm nominal opening), and retained on a #16 mesh screen (1.18 mm nominal opening). All the other biomass materials were screened as well, but only using the #8 mesh screen, followed by the #16 mesh screen, with samples being shaken for a period of approximately one minute.

5.4.3 Moisture Content

Three random samples of each biomass material were used for moisture content determination using ASAE standard S358.2 (ASAE 2004). This involved retention in a controlled temperature oven at 103°C ± 2°C for a period of twenty-four hours. Resulting moisture content values were expressed on a wet basis (wb) as percentages, and were used to calculate the dry mass of biomass samples in the liquid-holding capacity experiments, as described later.

5.4.4 Test Liquids and Properties

Commercial distilled water was obtained from a local supermarket. Industrial-grade methanol was obtained from a local hardware store. Anhydrous ethanol was obtained from Commercial Alcohols (Brampton, ON). Industrial-grade isopropanol (2-propanol) was obtained from a local pharmacy. Reagent-grade hexane (mixed hexanes) was obtained from EMD Chemicals Inc. (Gibbstown, NJ). Property data for all five liquids are presented in Table 5.1. Values for molecular weight, density, viscosity, surface tension and vapour pressure were obtained from the Lide (2006). Temperature dependent property values were either obtained for 20ºC, the approximate room temperature for all experiments, or interpolated as required to that temperature. Molar volume was calculated by dividing molecular weight by density.

5.4.5 Test Method

The method used for testing liquid-holding capacity involved five main features to take account of the bulk-solid nature of the biomass materials and the volatile nature of some of the liquids, and to reflect realistic performance under conditions likely to be encountered as part of aqueous or alcohol-based extraction processes. These are summarized as follows:

(i) All biomass material samples were enclosed in spherical, stainless steel mesh ball containers (mesh balls) in order to maintain the contained bed of bulk-solid particles as static as possible through the course of testing. This was done to minimize any movement that could alter inter-particle spacing and thus affect the quantity of liquid held. The mesh balls were approximately 50 mm in diameter, with mesh nominal

Table 5.1 Summary of relevant liquid properties at room temperature (20ºC).

Liquid	Molecular Weight (g/mole)	Density (g/mL)	Viscosity (mPa·s)	Surface Tension (mN/m)	Vapour Pressure (kPa)	Molar Volume (mL/mole)
Water	18.02	1.00	1.19	72.70	2.34	18.05
Methanol	32.04	0.79	0.63	22.50	16.90	40.49
Ethanol	46.07	0.79	1.31	22.40	7.87	58.37
Isopropanol	60.10	0.77	2.90	21.30	6.02	76.51
Hexane	86.18	0.66	0.34	18.40	20.20	131.61

(Note: data from Lide 2006)

opening size smaller than 0.5 mm, significantly smaller than #16 mesh (i.e., 1.18 mm nominal opening), the smallest mesh used for prescreening of all biomass materials.

(ii) Liquid immersion and draining were undertaken inside a sealed container in order to avoid evaporation. Samples thus could drain, but not undergo additional drying, particularly for the more volatile solvents.

(iii) Draining of liquids was undertaken only by gravity, without centrifugation.

(iv) Liquid immersion and draining periods of one hour each were employed, with the mass of the mesh balls also ensuring that samples were fully submerged for the entire immersion period. These timings were consistent with methods used by Kasbo et al. 1994) and Isman et al. (2003), but more importantly, corresponded to approximate conditions likely to be experienced as part of a biomass extraction process.

(v) Liquid-holding capacity results were corrected for the additional liquid that would be retained on the surface and within the screen of the mesh balls themselves, and that could lead to an overestimate of the liquid retained by the biomass inside. Two separate mesh ball containers were used, with each one tested separately for the retention of each liquid, as described later.

Each biomass material sample was loaded into a mesh ball and weighed. The difference between this value and the mesh ball mass provided the sample mass (i.e., mass of the absorbent material). This was converted to a dry mass value using the wet-basis moisture content measurement for the biomass material.

The mesh ball with sample was placed inside a sealed 2 L polypropylene container and immersed in approximately 500 mL of liquid for a period of one hour. At

the end of this time, the mesh ball was lifted out of the liquid to permit draining, but still maintained within the sealed container for a period of one hour. After this drainage period, the mesh ball was removed and reweighed. The difference between this value and the mass of the mesh ball with biomass sample provided the gross liquid retention mass. For each combination of biomass material and liquid, four replicate experiments were undertaken.

In order to correct for liquid retention by the mesh balls themselves, separate immersion tests were undertaken. For each test, the completely dried mesh ball was weighed, immersed in approximately 500 mL of liquid inside the sealed container for a period of five minutes, lifted out of the liquid to permit draining for a period of 15 minutes, then removed from the sealed container and reweighed. For each combination of liquid and mesh ball four replicate experiments were undertaken.

The liquid-holding capacity value for each sample test was calculated by taking the gross liquid retention mass, subtracting the liquid retention associated with the specific mesh ball for the liquid involved, and then dividing this value by the dry mass of biomass sample within the mesh ball, which was assumed to remain constant. Liquid-holding capacities were expressed per mass of absorbent on a dry basis (db), in units of g/g (db).

5.4.6 Statistical Analyses

To evaluate liquid-holding capacity tests, two-way analysis of variance (ANOVA) was employed, with calculation methods as outlined in Weiss (2006). The liquid (five levels) and the biomass material (four levels) were treated as the fixed-effect factors.

There were four replicate experiments per treatment combination, for a total of (N =) 80 observations. A key requirement for the application of ANOVA is the homogeneity of variances. This was evaluated using the Levene Test (Levene 1960). Monotone data transformation, as also outlined by Weiss (2006), was considered to address any non-homogeneity of variances. A 95% confidence level was presumed for significance for all resulting F-statistics (i.e., $P < 0.05$). Probability levels for calculated F-statistics were obtained from Moore and McCabe (2006).

5.5 Results and Discussion

5.5.1 Moisture Content

Results for moisture content, including mean and standard deviation (SD), for each biomass material on a wet basis, are presented in Table 5.2. All materials exhibited moisture content values less than 10% (wb), with flax shive being the least dry and commercial pine shavings being the most dry. The mean dry mass content for each biomass material is also presented in Table 5.2.

5.5.2 Biomass Sample Mass Consistency

The consistency of the quantity of biomass used for the tests was evaluated, with a summary of biomass sample mass statistics presented in Table 5.3. The mean sample mass for all 80 tests undertaken was approximately 6.7 g (wb) with SD just under 1.3 g (wb). The resulting coefficient of variation (CV) for sample mass was thus about 19%. As such, the quantity of biomass used for each test was confirmed to be relatively consistent, and any variability between sample mass values was not excessive.

Table 5.2 Moisture content (wb) and resulting dry mass content of biomass materials, with each mean and standard deviation value based on three replicate experiments.

Biomass Material	Moisture Content (wb)	Mean Dry Matter
Flax Shive	9.97% ± 0.32%	90.03%
Hemp Hurd	8.93% ± 0.54%	91.07%
Pine Shavings	6.97% ± 0.10%	93.03%
Simply Straw™ Product	7.49% ± 0.11%	92.51%

Table 5.3 Biomass sample mass statistics, based on eighty total samples tested.

Parameter	Mass Value (g, wb)
Mean Biomass Sample Mass	6.70
Standard Deviation	1.27
Maximum Biomass Sample Mass	10.21
Minimum Biomass Sample Mass	4.87
Median Biomass Sample Mass	6.30

5.5.3 Liquid-Holding Capacity for Biomass Materials

Liquid-holding capacity results for all twenty combinations of liquid and biomass material are presented in Table 5.4, including mean and SD values for each. Reviewing the raw data using the Levene Test, however, showed variances to be non-homogeneous in nature, in particular with skewing of residuals. Results of the Levene Test are presented in Table 5.5, with the value of the derived test-ratio being about 2.7. Given the assumption of a 95% confidence level for significance, the critical value for the test-ratio, i.e., $F_{19,60}$ value, was about 1.8. As such, the higher test-ratio value confirmed that variances for the raw data were not homogeneous. Further review showed that for each individual liquid, variances were uniform across biomass materials in all cases. This was evidenced in Table 5.4 by the relatively consistent SD values exhibited for each liquid. At the same time, for each individual biomass material, variances were generally not homogeneous across the range of liquids, which was not entirely unexpected because the liquids tested were very different.

The overall non-homogeneity of variances was addressed by transforming the raw data using base-ten logarithms. Corresponding results for the Levene Test as applied to transformed data are also presented in Table 5.5. In this case, the derived test-ratio was only about 1.5, confirming variances to be homogeneous, as required for the application of ANOVA.

A summary of ANOVA results using transformed data is presented in Table 5.6. The results showed that the biomass material, the liquid, and the interaction of biomass by liquid all had statistically significant effects on liquid-holding capacity, with $P < 0.001$

Table 5.4 Liquid-holding capacity for twenty combinations of biomass material and liquid, with each mean and standard deviation value based on four replicate experiments.

Biomass Material	Liquid	Liquid-Holding Capacity (g/g, db)
Flax Shive	Water	2.68 ± 0.25
	Methanol	1.53 ± 0.07
	Ethanol	1.30 ± 0.03
	Isopropanol	1.18 ± 0.07
	Hexane	1.09 ± 0.04
Hemp Hurd	Water	4.26 ± 0.26
	Methanol	2.51 ± 0.06
	Ethanol	1.97 ± 0.02
	Isopropanol	1.71 ± 0.05
	Hexane	1.63 ± 0.05
Pine Shavings	Water	4.19 ± 0.22
	Methanol	2.40 ± 0.04
	Ethanol	2.16 ± 0.14
	Isopropanol	1.90 ± 0.13
	Hexane	1.65 ± 0.05
Simply Straw™ Product	Water	4.59 ± 0.25
	Methanol	1.57 ± 0.09
	Ethanol	1.26 ± 0.07
	Isopropanol	0.80 ± 0.10
	Hexane	0.84 ± 0.10

in all cases as noted in Table 5.6. The main effect of liquid explained most of the variation in the data (69%), followed by the main effect of biomass (22%) and then the interaction between the two factors (8%). Interactive effects were most evident with the anomalous behaviour exhibited by the Simply Straw™ product, as described later. It is important to note that a preliminary ANOVA on raw data (not shown) produced similar results, albeit with slightly larger F-values.

Flax shive exhibited a generally lower liquid-holding capacity when compared to the other biomass materials. It showed the lowest mean value both for distilled water and for methanol, and close to the lowest value for ethanol, isopropanol, and hexane. Flax shive thus does not appear to be promising as a deliberate absorbent product. At the same time, these results are desirable when considering the use of flax shive as a feedstock for aqueous or alcohol-based processing. When compared to hemp hurd or pine shavings, liquid retention was 30% to 40% lower for flax shive, which is important in minimizing solvent and extracted-product losses.

The liquid-holding capacity of flax shive for distilled water, with a mean value of 2.68 g/g (db), agreed well with the earlier results of Kasbo et al. (1994). This was despite slight differences in particle screen-sizing, i.e., #10 to #22 mesh for Kasbo et al. (1994) compared to #8 to #16 mesh used in the current work. The value for distilled water was also substantially lower than the range of 4 to 5 mL/g (db) suggested by Isman et al. (2003). There was no clear explanation as to why the performance of flax shive suggested by Isman et al. (2003) was so much better, in particular, given relatively similar particle sizes.

Table 5.5 Levene Test of raw data and logarithm-transformed data assessing homogeneity of variance.

Data Set	df	Levene Test-Ratio	P-value
Raw Data	19, 60	2.74	0.003
Logarithm-Transformed Data	19, 60	1.52	0.107

df = degrees of freedom

Table 5.6 Two-way ANOVA of logarithm-transformed liquid-holding capacity.

Source	df	SS	MS	F-value	P-value
Biomass	3	0.79	0.264	357	< 0.001
Liquid	4	2.48	0.619	838	< 0.001
Interaction	12	0.279	0.023	31.5	< 0.001
Error	60	0.044	0.001		

df = degrees of freedom; SS = sum of squares; MS = mean square = SS ÷ df

As part of an earlier frictional behaviour study conducted by Parsons et al. (2008) [refer to Chapter 4], flax shive samples of similar particle size were dosed with distilled water, methanol and isopropanol at levels up to 1.35 g/g (wb), but without any observed separation of liquid. For both distilled water and methanol, the liquid-holding capacity of flax shive was found in the current work to be higher than this level, i.e., 2.68 and 1.53 g/g (db) respectively, consistent with the earlier results. In the case of isopropanol, however, the liquid-holding capacity was found in the current work to be lower, i.e., 1.18 g/g (db). This represented an apparent contradiction, in that some liquid separation potentially should have been seen in the earlier work. Liquid addition methods in the two cases, however, were different. Exorption from liquid saturation (i.e., complete immersion followed by draining) was used in the current work versus the addition of set amounts of liquid to the solid in the earlier work. More importantly, liquid exposure times were different, only one hour in the current work versus a minimum of three hours in the earlier work. The difference in behaviour could be thus reasonably explained.

Results for hemp hurd and pine shavings were quite similar to one another, with both materials exhibiting generally good liquid-holding capacity across the full range of liquids tested. Although somewhat lower for water than the Simply Straw™ product, they showed the highest mean values for all non-aqueous liquids tested, ranging from 40% to 80% higher compared to the other biomass materials. Based on these results, hemp hurd and pine shavings appear to be promising as deliberate absorbent products, especially for non-aqueous liquids. The substantial differences in behaviour between flax shive and hemp hurd were somewhat surprising, given both the similarities of the

decortication processes involved, and the relative similarities in taxonomic origin, both being derived from the inner stems of angiosperm dicotyledonous plants.

The Simply Straw™ product exhibited the most unusual and variable behaviour among the materials tested. It showed the highest liquid-holding capacity for distilled water, with a mean value of 4.59 g/g (db), yet the lowest for ethanol, isopropanol and hexane, and near the lowest for methanol. Based on these results, the Simply Straw™ product appears to be promising as a deliberate and selective absorbent product for water, for example as may be important in animal bedding. It is not promising as a deliberate absorbent product for non-aqueous liquids.

Good linear correlations (i.e. $R^2 > 0.8$) were obtained for liquid-holding capacity as a function of two of the liquid properties identified earlier as potentially relevant to liquid retention. These were the surface tension and the inverse of molar volume of the liquid. Results are presented in Figure 5.1 and Figure 5.2, respectively. Liquid viscosity and vapour pressure at room temperature did not show good correlations to mean liquid-holding capacity.

The strong linear correlation for surface tension, as shown in Figure 5.1, involved a classic "dumb-bell" regression problem, with data only at extremes. Nevertheless, the effect was present. All of the biomass materials showed a much higher mean liquid-holding capacity for water, which has a high surface tension around 73 mN/m, compared to the other liquids, which all have much lower surface tensions in the range of 18 to 23 mN/m at ambient conditions. Surface tension of a liquid is relevant only to the absorbency mechanism associated with liquid attraction to solid surfaces.

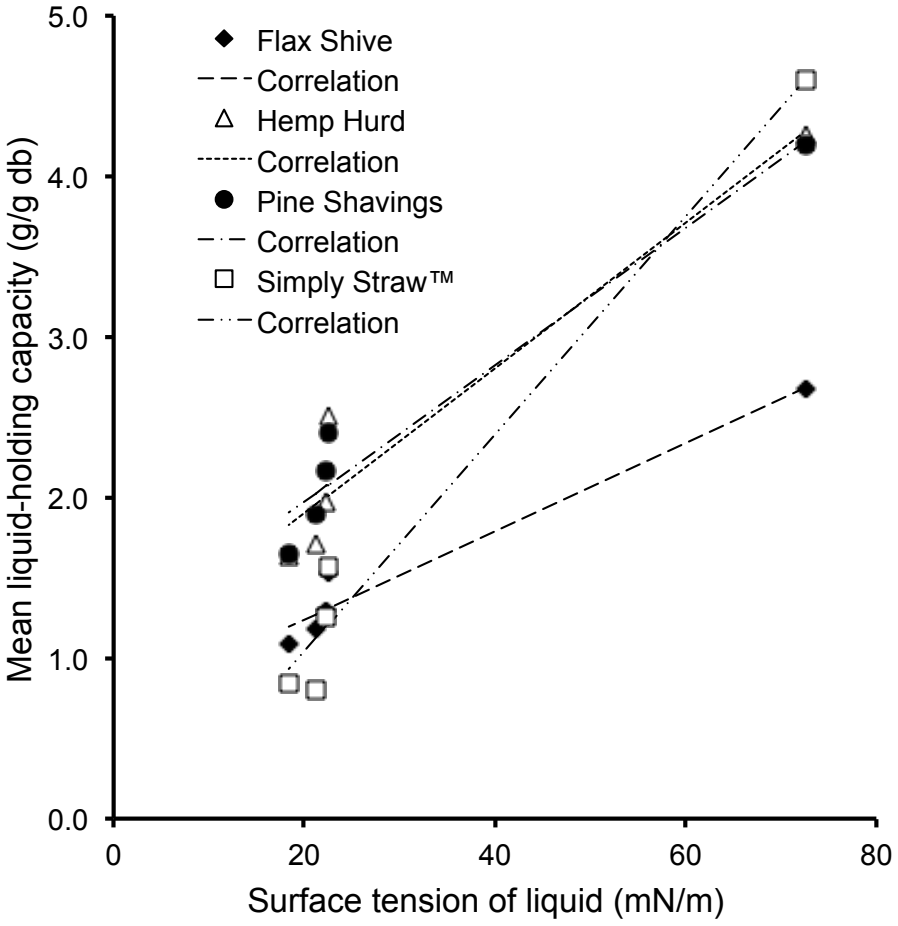

Figure 5.1 Liquid-holding capacity of biomass materials as a function of liquid surface tension.

Inverse molar volume showed the strongest correlation to mean liquid-holding capacity of all liquid properties, and is illustrated in Figure 5.2. Reasonable correlations were separately found for liquid-holding capacity as a function of both density and inverse molecular weight, from which inverse molar volume is calculated, but neither as strong as for inverse molar volume itself. The molar volume of a liquid, in contrast to surface tension, can be relevant to both of the absorbency mechanisms, including partial dissolution and swelling.

The nature of the curves in Figure 5.1 and Figure 5.2 showed the Simply Straw™ product to have absorbency behaviour that was very different from the other biomass materials. In both figures, the slopes for flax shive, hemp hurd, and pine shavings were relatively parallel. Hemp hurd and pine shavings, in particular, were almost identical. The Simply Straw™ product was distinct, showing much steeper slopes in both cases.

This discrepancy in behaviour was explored further by looking at the ratios of mean liquid-holding capacities relative to distilled water. Ratios were calculated for each biomass material by dividing the mean liquid-holding capacity for each liquid by the respective mean value for distilled water. The results are shown in Figure 5.3, with the liquids presented in order of increasing molecular weight and molar volume (i.e., water, methanol, ethanol, isopropanol, hexane), and with the value for water in each case being unity. Trend lines are also shown for the purpose of comparison.

As illustrated in Figure 5.3, for flax shive, hemp hurd and pine shavings the ratio values tended to cluster together, with the trend in relative order being identical in all cases. For all three biomass materials, the liquid-holding capacity for methanol was

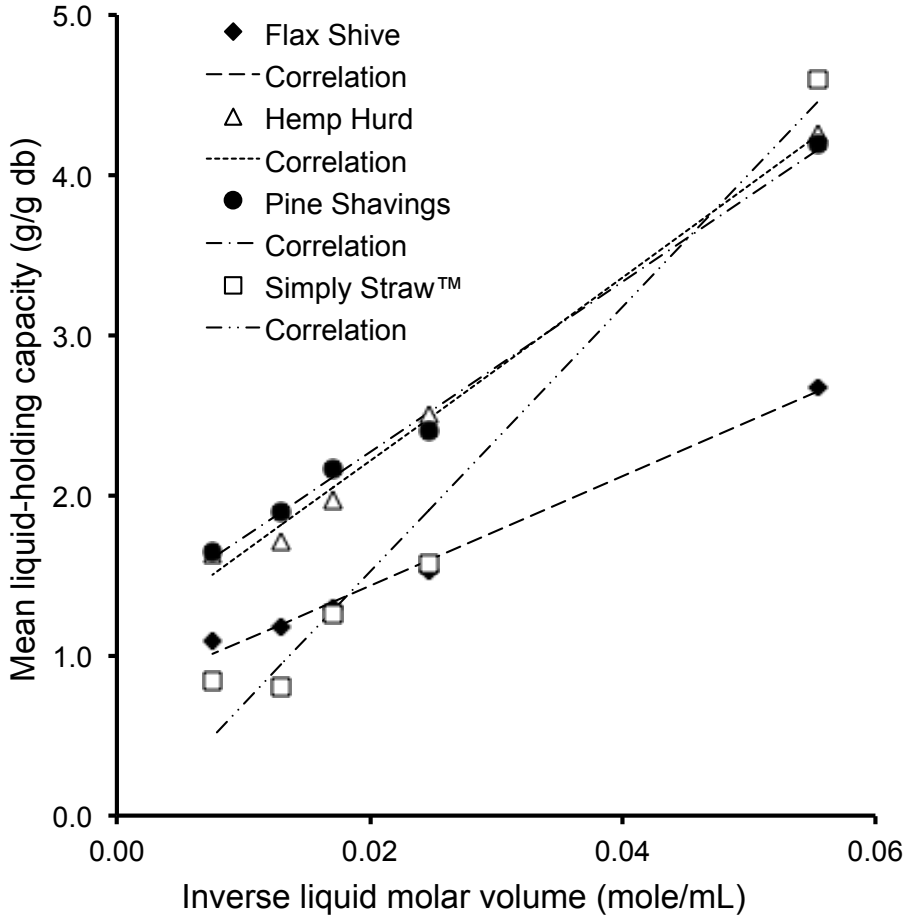

Figure 5.2 Liquid-holding capacity of biomass materials as a function of liquid inverse molar volume.

about 58% that of water, followed by ethanol at about 49%, isopropanol at about 43%, and finally hexane at about 39%. The Simply Straw™ product, on the other hand, showed very different values, with generally lower ratios compared to water. It also showed differences in the trend of relative order for the liquids.

5.6 Conclusions

Liquid-holding capacity was evaluated for flax shive and three other biomass materials, all having similar particle sizes, using five different liquids at conditions of room temperature and atmospheric pressure. The differences in results were shown to be statistically significant using two-way ANOVA. Liquid-holding capacity was most affected by the liquid selected, and then by the biomass material selected. Interaction between these two main factors was also found to be statistically significant. The liquid-holding capacity results correlated most strongly to the liquid properties of surface tension and inverse molar volume.

Flax shive exhibited a generally low liquid-holding capacity across the range of liquids tested. The results obtained specifically for flax shive using water as the liquid, were in good agreement with one earlier patent, but contradicted the results of a second patent. Hemp hurd and commercial pine shavings exhibited generally good liquid-holding capacity across all liquids, being particularly high for non-aqueous liquids. The Simply Straw™ product showed variable behaviour. It had the best liquid holding-capacity for water among all materials tested, but low for all non-aqueous liquids. (Refer to Section 10.1 for discussion of the significance of this research work to the overall thesis objectives).

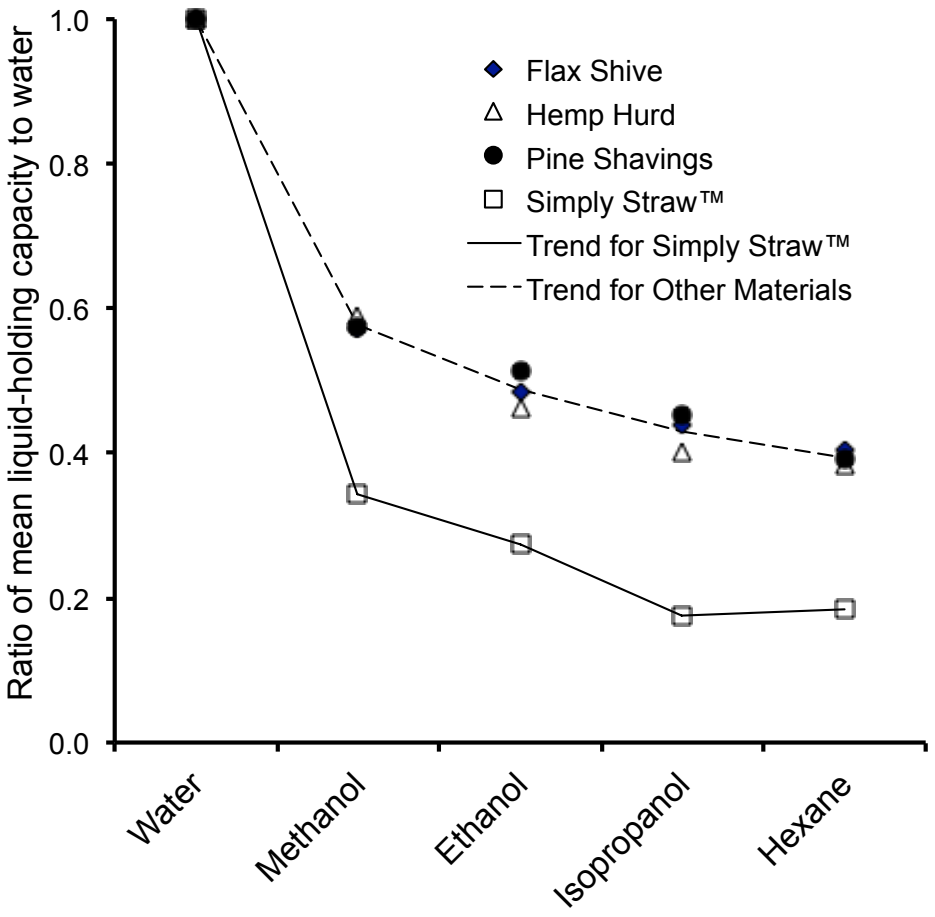

Figure 5.3 Ratios of mean liquid-holding capacity to water for biomass materials, with liquids listed in order of increasing molecular weight and molar volume, and trend-lines shown for the purpose of comparison.

6 First Step Extraction of Phenolics from Flax Shive

This chapter was previously published as the following paper: Parsons, R.V., K.A.L. Parsons and J.L. Sorensen. 2011. Extraction of flax shive using sodium ethoxide catalyst in anhydrous ethanol. *Industrial Crops and Products* 34: 1245–1249. Robert Parsons acted as primary author for the published paper, and conducted all of the experiments involving flax shive. The model compound reaction component of the paper was undertaken by a student under Professor Sorensen.

6.1 Abstract

This work investigated the yield and nature of solvent-soluble organic compounds extracted from flax shive using a room temperature reaction (20ºC) 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 (20ºC and 100ºC). 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. Key words: flax, *Linum usitatissimum*, shive, biomass, lignin, phenolics extraction, sodium ethoxide, alkoxide, sodium hydroxide, cellulose.

6.2 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 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 hemicellulose 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).

6.3 Methods and Materials

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 (20ºC), 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 (20ºC) were contained in a closed beaker, while samples extracted at 100ºC 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 ^1H NMR showed this product to be pure methyl ferulate. The characterization data are reported in Supplementary Data (Appendix A).

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 160ºC. The column was held at 75ºC for 1.5 min, then ramped linearly at rate of 30ºC per minute up to 265ºC, then held at 265ºC for 7 min. (Note: The column used for the GC–MS system was not identified in

the published paper, but was a FactorFour™ Capillary Column, Model VF-5ms: 30 m, 0.25 mm, 0.25 μm).

6.4 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 6.1 for all test conditions. Mean and SD values are presented for each set of test conditions.

For extractions using aqueous sodium hydroxide the yields were all relatively low. Analysis of variance (ANOVA; Weiss, 2006), showed there was no significant effect due to particle size ($F_{1,8} < 1$), reaction temperature ($F_{1,8} = 3.74$, $P = 0.09$), or interaction of the two factors ($F_{1,8} < 1$). As such, all these data points were combined, with the mean yield for all tests conducted at 1.0 M being 2.2 ± 1.5 mg/g (db) for n = 12. Details of statistical analyses are included in Supplementary Data (Appendix A).

For extractions using sodium ethoxide catalyst in ethanol the yields appeared to increase linearly as catalyst concentration increased, but seemed unaffected by changes in particle size. The use of analysis of covariance (ANCOVA; Kutner et al. 2005) confirmed that the yields did not differ significantly between particle size fractions ($F_{1,20} = 2.24$, $P = 0.15$). This finding would have an impact on the use of this method for

Table 6.1 Quantitative yield of solvent-soluble organics recovered from flax shive under varying conditions, with each mean and standard deviation based on three replicate measurements.

Reagent	Concentration (M)	Temperature (ºC)	Yield (mg/g db)	
			1.0 mm fraction	0.5 mm fraction
Sodium ethoxide in ethanol	0.2	20	15.7 ± 0.7	15.3 ± 1.7
	0.5	20	32.3 ± 6.4	32.9 ± 3.1
	0.7	20	37.7 ± 12.9	43.1 ± 18.9
	1.0	20	49.6 ± 13.7	59.4 ± 16.2
Sodium hydroxide in water	1.0	20	1.7 ± 0.5	1.1 ± 0.5
	1.0	100	3.4 ± 2.4	2.5 ± 1.1

processing given that being able to use larger-sized particles requires less equipment and lower energy inputs. All these data points were combined into a single linear regression, with a resulting $R^2 = 0.68$. The mean yield for all tests conducted at a catalyst concentration of 1.0 M was 54.5 ± 14.5 mg/g (db) for n = 6. The predicted value at 1.0 M according to the linear regression was 55.0 mg/g (db), which was consistent with the observed mean value.

The ^{1}H NMR spectra collected on all of the extraction residues tests showed a mixture of compounds. In the spectra for all extracts using both methods, there were multiple sets of overlapping peaks apparent. It was not possible to identify individual compounds in the ^{1}H NMR of these mixtures. However, we were still able to make some conclusions about the functional groups that were present in the extracted molecules. All ^{1}H NMR spectra and details of analysis of functional group interpretations are provided in Supplementary Data (Appendix A).

In all of the extract residues, the analysis of the ^{1}H NMR suggested the presence of aromatic protons in the recovered compounds, as indicated by signals in the range of 6.8–7.6 ppm. This suggested the compounds were likely of lignin origin. The ^{1}H NMR also indicated the absence of any carbohydrates in the extracts. Carbohydrates generate signals in ^{1}H NMR spectra over a range from 3 ppm to 5 ppm (Cui 2005), with diagnostic peaks between 4.3 ppm and 4.8 ppm resulting from the proton attached to the anomeric carbon. Signals in these chemical shift ranges were absent in all samples analyzed by ^{1}H NMR. This finding also suggested that none of the extracts included any phenolic constituents with carbohydrate linkages, such as certain types of tannins.

The ^{1}H NMR spectra for all the sodium hydroxide extracts showed essentially the same overall set of signals. This confirmed there were no significant differences in chemical makeup due to changes in particle size or reaction temperature. The ^{1}H NMR spectra suggested the presence of aromatic protons (6.8–7.6 ppm), aldehyde protons (9.8 ppm), non-aromatic double bonds (5.4 ppm), methyl ether groups (singlets at 4.0 ppm), methyl ketone groups (singlets at 2.5 ppm), and aromatic methyl substituents (singlets at 2.3 ppm). These groupings of signals were consistent with the presence of guaiacyl and syringyl aldehyde and ketone compounds. Comparison of the ^{1}H NMR spectra for the extracts at all concentrations of sodium ethoxide catalyst in ethanol showed similar patterns of signals to one another. This result suggested that there were no significant differences in the composition of extracted compounds as a result of changes in particle size or catalyst concentration. The ^{1}H NMR spectra indicated the presence of aromatic protons (6.8–7.6 ppm), ethyl esters (2-hydrogen quartet at 4.2 ppm and 3-hydrogen triplet at 1.3 ppm), methyl ketone (singlets at 2.5 ppm), and methyl-substituted aromatic rings (singlets at 2.3 ppm).

The sodium ethoxide and sodium hydroxide extracts were analyzed using GC–MS, however the results were inconclusive. In all of the extracts analyzed, there were no compounds that could be detected above baseline noise levels. The most likely explanation is that all the extract constituents have boiling points above the maximum temperature of the GC column which was 265ºC. This would occur if extracts consisted of oligomeric lignin fragments. This result is consistent with the purification procedure that involved multiple extractions against aqueous solutions and evaporation. However,

such oligomeric constituents nevertheless would be still amenable to analysis by ^{1}H NMR.

The data obtained by ^{1}H NMR on the flax shive extracts indicated that there was a difference in the chemical composition of the extracts that was dependent on the extraction method used, but independent of the particle size and catalyst concentration. The signals consistent with the presence of methyl ethers were present for sodium hydroxide extracts, but absent in all of the ^{1}H NMR for the sodium ethoxide extracts. This last observation was important given that the presence of methyl ether containing guaiacyl or syringyl compounds would have been expected in extracts from flax shive.

The apparent lack of any methyl ether functional groups in the sodium ethoxide extracts was examined through the use of the three model compounds: methyl ferulate; methyl vanillate; and acetovanillone. All three model compounds contain guaiacyl type methyl ether groups, permitting a determination of the effect of the extraction conditions on these types of chemical bonds. Methyl esters of the two acid compounds were selected as starting materials in order to demonstrate that transesterification was occurring under selected conditions.

All three model compounds were, separately, reacted with 1.0 M sodium ethoxide and the reaction worked up under conditions identical to those used to extract the flax shive. The residue left after evaporation of the solvent was then analyzed by ^{1}H NMR in an identical manner used for the flax shive extracts. The conversion of methyl ferulate to ethyl ferulate occurred with a 70% yield based upon the integration of ^{1}H NMR signals for the diagnostic ester protons. Methyl vanillate, on the other hand, was converted to

ethyl vanillate with only a 14% yield based on ^{1}H NMR integration. These results demonstrated that transesterification would occur under the conditions used for the extractions of the flax shive. In the control reactions using methyl ferulate and methyl vanillate the methyl ether groups appeared to be intact, based upon the integration for the signal at 4 ppm. The acetovanillone appeared identical in the ^{1}H NMR both before and after exposure to the catalyst suggesting no reaction with the methyl ether groups. This set of control reactions demonstrated that there would be no hydrolysis expected of the methyl ether groups under conditions identical to those used for extraction of the flax shive. These results were in contrast to the apparent lack of methyl ether containing compounds observed in the ^{1}H NMR from the sodium ethoxide extracts.

The reasons for the significant difference in behaviour are still uncertain. One possible explanation is that the sodium ethoxide selectively hydrolyzed only hydroxyphenyl lignin constituents. Alternatively the hydrolysis of the methyl ether groups may have been facilitated by the complex matrix of the flax shive in combination with the basic ethanol.

The flax shive extracts using sodium ethoxide appeared to be phenolic in nature, likely lignin-derived, and also likely oligomeric, with high boiling points. This conclusion is based on the signals identified in the ^{1}H NMR and the lack of peaks in the GC–MS. Based on their preliminary characteristics, these materials may be applicable as resin constituents (Holladay et al. 2007). The origin of the extracted compounds from lignin will be the subject of future studies. In addition the suitability of the shive residue for further biotechnological processing will be examined in detail. Subsequent

experiments will involve determining changes in acid-soluble (Klason) lignin to confirm the source of isolated phenolics as well as assessing the effect of sodium ethoxide on subsequent yield and composition of extracted hemi-cellulose carbohydrates. Additionally the comparison of the effects of sodium ethoxide pretreatment on the rates and yield of degradation of cellulose residuals through fermentation to biofuels using cellulose-degrading microorganisms (Carere et al. 2008) will be determined.

6.5 Conclusions

We report here for the first time the use of sodium ethoxide in ethanol as a processing method for extracting phenolic constituents from flax shive. The extraction is effective at room temperature, reducing the need for large process-energy inputs. The amounts of extracted compounds were significantly higher than the amounts observed when the extraction was done in aqueous sodium hydroxide. This suggested that basic ethanol may be a more effective extraction method for the processing of flax shive. Under both extraction conditions tested the amount and profile of extracted compounds was independent of particle size. This demonstrates that the extraction method described here is effective on larger particle sizes, cutting down on the need for extensive processing of the flax shive. The ethanol extracted compounds lack the expected methyl ether functional groups in contrast to the profile of compounds generated by aqueous sodium hydroxide. Control reactions with model compounds confirm that basic ethanol would be expected to leave these bonds intact. None of the extraction conditions tested appeared to extract carbohydrates from the flax shive. Follow-up research will focus on confirming the lignin-origin of the extracts, and assessing impacts of pretreatment on subsequent bio-

processing steps. Other efforts will include extending this method to other sources of plant-derived cellulose. (Refer to Section 10.2 and Section10.3 for discussion of the significance of this research work to the overall thesis objectives).

7 Second Step Extraction of Polysaccharides from Flax Shive

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7.1 Abstract

Flax shive is a by-product of decortication of flax straw for fibre recovery, and consists of ligno-cellulosic xylem tissue. It is available in large quantities at low cost, and shows promise as a biorefining feedstock for recovery of multiple products. The objectives of this work were to investigate hemicellulose polysaccharide extractions from flax shive using alkaline reagents at mild conditions, and to determine impacts of pretreatment using sodium ethoxide in anhydrous ethanol. Sodium ethoxide is similar to the catalyst for industrial biodiesel, and had been shown previously to recover phenolic constituents from flax shive. In this work, carbohydrates extracted from flax shive were evaluated quantitatively, in terms of recovered precipitate mass, and qualitatively, in terms of backbone monomer composition. Extracts obtained using 1.0 M sodium hydroxide, alone or following pretreatment, showed no difference in mass yield, with mean recovery of 99.4 ± 5.1 mg/g on a dry basis. In terms of composition, extracts obtained using 1.0 M sodium hydroxide, alone or following pretreatment, showed no detectable mannose and no difference in molar concentration ratio of xylose-to-glucose, with mean ratio of 25.5 ± 3.4. Results inferred that no glucomannan polymer was extracted, and that glucuronoxylan polymer was present at high concentration, greater than 90% of the product stream. Tests using saturated barium hydroxide showed poor

mass yield, with possible inhibitory effects in mixed solutions. The use of saturated barium hydroxide or elevated sodium hydroxide resulted in lower glucuronoxylan concentration. Flax shive showed good potential for carbohydrate extraction, and the technical feasibility of recovering multiple products was demonstrated. Key words: flax shive; carbohydrate; sodium ethoxide; ethanol; sodium hydroxide; barium hydroxide

7.2 Introduction

Biorefining has been described as the sustainable processing of biomass into a spectrum of marketable products and energy (IEA Bioenergy 2008). Energy applications continue to be a major focus of biomass research; however, there is recognition that the recovery of high-value products is important for the economic viability of biomass processing, especially involving ligno-cellulosic feedstocks (Sims et al. 2008, Zhang 2011). Previous work identified flax shive as a potential biorefining feedstock for the recovery of multiple high-value products (Parsons et al. 2011a), including hemicellulose-derived polysaccharides.

Flax shive is the woody by-product of decortication; the process used to remove fibre, which are high in cellulose content, from the stem periphery of flax (*Linum usitatissimum*). Flax shive is available in large quantities at low cost, with relatively consistent composition and size characteristics. Given that flax shive is essentially a waste, there has been strong interest in identifying alternative uses (Flax Council of Canada, 2002).

Parsons et al. (2011b) recently reported a method for selectively recovering phenolic constituents from flax shive at room temperature and pressure, involving sodium

ethoxide in anhydrous ethanol. This is a catalyst similar to that used for industrial biodiesel production, reacting with ester linkages. The method did not appear to remove carbohydrates, suggesting that their subsequent extraction could be considered as part of a multi-product bio-refining process.

Potential high-value products considered from carbohydrate components (cellulose and hemicellulose) have primarily involved sugar-monomer derivatives and other small molecules (Werpy and Petersen 2004, de Jong et al. 2012). One frequently overlooked opportunity is the recovery of complex, non-cellulose polysaccharides from hemicellulose. Applications of these polysaccharides include adhesives, thickeners, stabilizers, film formers and emulsifiers (Doner and Hicks 1997). The nature and possible uses of such products, however, depend both on the feedstock (Ebringerova et al. 2005), and on the extraction process (Watson and Williams 1959).

Flax shive is composed primarily of xylem tissue, and contains significant hemicellulose, in the range of about 16% to 24%. It contains negligible starch residual (Myers and Olson 1958), little protein (Dinusson et al. 1973), and little lipid or waxy material (Holser and Akin 2008). Flax shive, however, does contain significant amounts of lignin (Ross and Mazza 2010) that can contribute to contamination, such as excessive colour extracts (Watson and Williams 1959), or can constrain extraction given cross-linkages to hemicellulose (Ebringerova and Heinze 2000).

Analyses of the combined carbohydrate components of flax shive typically have shown the following monomers to be present in approximate order of abundance: glucose, xylose, mannose, galactose, and arabinose (Akin et al. 1996, Han 1998, Jacobs

et al. 2003, Buranov and Mazza 2010, Tamaki and Mazza 2010). Rhamnose and uronic acids have been also identified, but their relative abundance is less consistent. Aside from cellulose itself, which is made up solely of glucose, the monomer composition of flax shive is indicative of the three main groups of hemicellulose polymers that are predominant. These are, in order of abundance: 4-O-methyl-glucuronoxylan; xyloglucan, and glucomannan (Jacobs et al. 2003). Only three carbohydrate monomers are present in the backbone chains of these polymers, namely xylose, glucose, and mannose (Ebringerova 2005), simplifying analysis.

The extraction of hemicellulose polymers from plant materials using alkaline reagents is well established. Indeed, hemicellulose was originally defined based on it being readily solubilized by alkali even before chemical structure hypotheses were developed (Yanovsky 1939). Sodium hydroxide (NaOH) has been commonly used for extraction of hemicellulose, and was selected as the main reagent for this work. Other alkaline reagents have included potassium hydroxide, ammonium hydroxide, sodium carbonate, calcium hydroxide and barium hydroxide (Rutenburg and Herbst 1957, Watson and Williams 1959, Timell 1964 and 1965). Saturated barium hydroxide ($Ba(OH)_2$) was selected as a second reagent, based on recent food science applications (Bergmans et al. 1996), and given that it has been identified to behave differently than NaOH (Izydorczyk 2005, Izydorczyk and Dexter 2008).

Documented conditions for alkaline extraction of hemicelluloses, however, have varied significantly in terms of reagent concentration, temperature and extraction-period. Cited concentrations have ranged from 0.03 M to 20 M, and temperatures from room

conditions to upwards of 160ºC (Rutenburg and Herbst 1957, Watson and Williams 1959). At the same time, alkaline reagents also have been employed extensively to react with and break down lignin. This is particularly the case for NaOH, the use of which formed the basis of soda pulping (Doherty et al. 2011). This was the first commercialized chemical pulping process, involving reactions under pressure at temperatures of 140 to 170ºC, with NaOH concentrations of 13 to 16% (3.25 to 4 M). Later pulping advancements, particularly the Kraft process (NaOH and sodium sulfide), are based on soda pulping, but with the consistent objective to break down and remove both lignin and hemicellulose.

There is significant overlap in conditions identified for alkaline reagents to recover hemicelluloses versus to break down lignin. As a further complication, alkaline processing, described in reviews by Kumar et al. (2009) and Brodeur et al. (2011), has recently become an important topic of research for biomass pretreatment prior to enzymatic conversion of cellulose to glucose for fermentation. In this case, however, the success criteria are characterized by cellulose accessibility and glucose yield, not by whether hemicelluloses are recovered in a pure state. Some instances have been noted whereby relatively mild conditions, for example involving 1.5% NaOH (0.375 M), room temperature and extraction-period of 114 h, have removed significant hemicellulose and lignin from biomass. Ross and Mazza (2010) specifically investigated lignin removal from flax shive. They used relatively mild conditions involving 1.25 M NaOH, temperature of 80ºC, and extraction-period of 5 h, to remove approximately 92 mg/g (db) of lignin, fully one-third of the total lignin content of tested flax shive.

Watson and Williams (1959) described alkaline extraction methods as ad hoc, being variable and not systematically defined. This situation continues to the present, and is due in part to the highly diverse nature of hemicellulose polymers and biomass feedstocks. There is no simple set of optimum conditions.

Mild conditions were deliberately selected for alkaline extraction in this work, specifically to help reduce purification requirements. Previous work by Parsons et al. (2011b) had also shown that extraction conditions involving 1.0 M NaOH, room temperature, and 1 h extraction-period would recover only minimal quantities of solvent-soluble organics from flax shive, i.e., 1.4 ± 0.6 mg/g (db).

The objectives of this research were two-fold. The first objective was to compare at small-scale the recovery of hemicellulose polysaccharides from flax shive using common alkaline reagent solutions, 1.0 M NaOH and saturated $Ba(OH)_2$, at room temperature, using 1 h extraction-periods. The second objective was to determine at small-scale the effect of pretreatment of flax shive at room temperature using sodium ethoxide in anhydrous ethanol on the subsequent extraction of hemicellulose carbohydrates. Both extraction techniques were evaluated quantitatively, in terms of recovered mass, and qualitatively, in terms of carbohydrate backbone monomer composition.

7.3 Materials and Methods

7.3.1 Flax Shive Sampling, Pre-screening and Moisture Content

Flax shive was provided from the decortication plant of SWM Intl. Inc. (formerly Schweitzer-Mauduit Canada Inc.), located near Carman, Manitoba. Material was pre-

screened to ensure consistent particle size and to eliminate dust residuals, as outlined by Parsons et al. (2008). Selected material passed through a #8 mesh screen (2.36 mm nominal opening), and was retained on a #16 mesh screen (1.18 mm nominal opening). Pre-screened flax shive was double-sealed in plastic containers and stored in a cool, dry location until used. Prior to experiments, materials were checked manually to remove any residual seeds still present. The moisture content of the flax shive was assessed using ASAE standard S358.2 (ASAE 2004) in triplicate. All flax shive samples were measured on a wet basis (wb), whereas yields were calculated on a dry basis (db), based on derived moisture content.

7.3.2 Experimental Design for Small-Scale Tests

Experiments were all done at small-scale, using 1 g flax shive samples, and involved two main variables. These were the alkaline extraction reagent and the pretreatment method. A total of seven different extraction conditions were tested, as follows: (i) 1.0 M aqueous NaOH alone; (ii) 1.0 M aqueous NaOH after pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol; (iii) 1.0 M aqueous NaOH after pretreatment using azeotropic ethanol (95.6%); (iv) elevated concentration 2.0 M aqueous NaOH; (v) saturated aqueous $Ba(OH)_2$ alone; (vi) saturated aqueous $Ba(OH)_2$ after pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol; and (vii) mixed reagent solution of 1.0 M NaOH added to saturated aqueous $Ba(OH)_2$. Four replicate experiments were undertaken for each set of conditions. All alkaline extractions and pretreatments were conducted at room temperature and pressure. Extraction periods of 1 h were used for all pretreatments, where applied, and for all alkaline extractions.

7.3.3 Pretreatment Methods

For sodium ethoxide pretreatments, the method outlined in Parsons et al. (2011b) was used, with minor variations. A 1 g (wb) sample of flax shive was added to 20 mL of 1.0 M sodium ethoxide (Sigma Aldrich) in anhydrous ethanol (Commercial Alcohols). The solution was sealed using sealing film in a 50 mL beaker fitted with a stir bar, and placed on a magnetic stir-plate to ensure thorough mixing. After 1 h, the flax shive was filtered under vacuum using a stainless steel mesh screen with approximate opening of 200 μm. A total of 100 mL of fresh anhydrous ethanol was used to wash flax shive completely out of the beaker and fully rinse flax shive retained on the mesh screen. The flax shive was left under vacuum for a further 30 min. For ethanol pretreatment, the same procedure was used, except using azeotropic ethanol (Commercial Alcohols) and no catalyst. For both cases the mesh screen was removed and retained flax shive transferred to a beaker for alkaline extraction, using methods described later. No further analysis was undertaken for collected sodium ethoxide solution filtrates.

7.3.4 Alkaline Extractions

Alkaline extraction reagent solutions were prepared using reagent grade NaOH or $Ba(OH)_2$ (Fisher Scientific) respectively using high purity water (Millipore Q). For experiments involving pretreatment prior to the alkaline reagent, the same initial 1 g (wb) sample was carried through from pretreatment. For experiments involving alkaline reagents without pretreatment a new 1 g (wb) sample of flax shive was used.

For each experiment, the flax shive sample was added to 20 mL of reagent solution in a 50 mL beaker. The beaker was fitted with a stir bar, sealed using sealing

film, and placed on a magnetic stir-plate to ensure thorough mixing. After extraction for 1 h, the solution was filtered under vacuum through a stainless steel mesh screen with approximate opening of 200 μm, and rinsed with 80 mL of high purity water while under vacuum. The pH of the recovered filtrate was adjusted to 3 using concentrated HCl, and then 400 mL of azeotropic ethanol was added to precipitate carbohydrates (Rutenburg and Herbst 1957, Han 1983). This solution was thoroughly mixed and retained for precipitate mass measurement, with a 5 mL sample of the solution withdrawn for composition analysis. This sample and remaining solution were stored in a commercial freezer unit at a measured temperature of -12ºC.

7.3.5 Carbohydrate Precipitate Mass

For each replicate experiment under all extraction conditions, a filter paper was pre-dried in an oven at 103ºC for a period of 4 h, and weighed. The precipitate solution in each case was filtered under vacuum. The filter paper with retained matter was dried at 103ºC for a period of 15 min and reweighed. The difference in weight provided the mass of recovered precipitate.

7.3.6 Carbohydrate Composition Analyses

Samples from all replicate experiments under all extraction conditions were evaluated for carbohydrate composition. Sugar monomers were analyzed using high pressure liquid chromatography (HPLC). Hydrolysis of extracted polysaccharides to monomers was adapted from the method of Sluiter et al. (2010), using sulphuric acid (H_2SO_4), but adjusted to smaller analyte quantities for digestion.

For each extract sample, a total of 3 mL of precipitate solution was sequentially centrifuged and decanted using an Eppendorf centrifuge tube. The tubes with retained precipitates were dried under vacuum. To each tube, 20 µL of 98% H_2SO_4 and 14 µL of high purity water were added to form a 72% acid solution. Tubes were sealed and placed in a 30ºC water bath for 60 min. The acid concentration in each tube was then reduced to 4% by adding 840 µL of high purity water. The tubes were resealed and placed in a small autoclave at 120ºC for 60 min. After digestion, the pH was adjusted to 6 by slow addition of potassium carbonate (K_2CO_3), and 300 µL of high purity water added. Each sample was lastly filtered using a 0.2 µm syringe filter. Blank samples (water only) were also included, and carried through all digestion steps.

The HPLC analysis of sugar monomers was adapted from the method of Sluiter et al. (2008). All samples were analyzed using a Dionex HPLC system equipped with a Dionex Carbopac PA1 column and electrochemical (EC) detector (model ED40). No guard column was employed. Dilute NaOH solution (3.5 mM) was employed as the eluent, with a constant flow rate of 1.0 mL per min. Column temperature was maintained at 36°C. For each digested sample, 100 µL was mixed with 900 µL of high purity water (i.e., 1:10 dilution) into an injection vial. The injection volume was 25 µL.

HPLC was used to analyze for the three backbone monomers, xylose, glucose and mannose. Reagent grade D(+)-xylose, D(+)-glucose, and D(+)-mannose (Sigma-Aldrich) were used to prepare 10 mM stock solutions in high purity water. These were then mixed with high purity water as required to provide 2, 20, 100, and 200 µM standards into injection vials. Data for these standards were used to construct separate calibration

curves for each monomer (see Supplementary Data, Appendix B). Retention times were 8.4 min for glucose, 9.6 min for xylose and 10.4 min for mannose. Selected samples were also spiked with known concentrations of xylose, glucose or mannose to confirm peaks in HPLC analyses.

In order to reduce the number of variables and address possible concentration differences between samples, composition data were presented as molar concentration ratios using glucose as the denominator. Ratios were determined by dividing the concentration of xylose or mannose, as calculated from peak area using the respective calibration curve, by the concentration of glucose, calculated in the same way.

7.3.7 Statistical Analyses

Experimental data for precipitate mass recovery and carbohydrate composition were separately assessed using one-way analysis of variance (ANOVA). Data sets were evaluated to confirm homogeneity of variances using the Levene Test (Levene 1960). ANOVA was undertaken manually using procedures as outlined in Weiss (2006) and based on a 95% confidence level. Critical F-statistic and associated probability (P) values were obtained from Moore and McCabe (2006). Post-hoc tests of data were undertaken using the method of Student-Newman-Keuls for pair-wise comparisons. Critical q-statistic values for these evaluations were obtained from Fisher and Yates (1963), based on a 95% confidence level.

7.4 Results

7.4.1 Moisture Content

On a wet basis, the moisture content of the flax shive averaged 88.9 ± 0.2 mg/g. This translated to an average dry mass for the flax shive of 911.1 mg/g. This latter value was used to convert carbohydrate precipitate yields to a dry basis.

7.4.2 Carbohydrate Precipitate Mass Recovery

The quantitative results for carbohydrate precipitate recovery are presented in Table 7.1 on a dry basis. The mean and standard deviation values are presented for each case, with the extraction conditions ordered from the highest to the lowest mean yield value. Comparing these results as a one-way ANOVA with seven different treatment levels confirmed that significant differences existed (see Supplementary Data, Appendix B). Results of the Levene Test (not presented) showed variances to be homogeneous.

Post-hoc pair-wise tests between the different treatment levels were based on 4 replicates for each condition. These tests showed extraction using 1.0 M NaOH after pretreatment with 1.0 M sodium ethoxide to have the highest precipitate yield. The next highest values were for the treatment involving 1.0 M NaOH after pretreatment with azeotropic ethanol, followed by 1.0 M NaOH alone, and then followed by elevated concentration 2.0 M NaOH. There was no significant difference between the yields for these four conditions ($P > 0.05$), but they were found to be different compared to the other three treatments.

Table 7.1 Quantitative mass yield of carbohydrate precipitates from flax shive for treatment conditions at small scale ordered from highest to lowest, with mean and standard deviation presented based on four replicate experiments.

Treatment Condition	Precipitate Yield (mg/g db)
1.0 M NaOH after pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	102.4 ± 5.8 *a*
1.0 M NaOH after pretreatment using azeotropic ethanol	100.2 ± 2.6 *a*
1.0 M NaOH alone	99.3 ± 5.5 *a*
Elevated concentration 2.0 M NaOH	95.7 ± 5.0 *a*
Mixed reagent 1.0 M NaOH added to saturated aqueous $Ba(OH)_2$	45.2 ± 4.2 *b*
Saturated $Ba(OH)_2$ after pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	16.5 ± 3.6 *c*
Saturated $Ba(OH)_2$ alone	12.7 ± 7.3 *c*

Note: Values with the same letter are not significantly different according to the method of Student-Newman-Keuls for pair-wise comparisons, $P < 0.05$.

Of the remaining treatments, the next highest yield value was for the mixed reagent solution of 1.0 M NaOH added to saturated $Ba(OH)_2$. This yield value was found to be different from all other conditions. The lowest yields were obtained using saturated $Ba(OH)_2$ after pretreatment with 1.0 M sodium ethoxide, and saturated $Ba(OH)_2$ alone, which had the lowest overall yield. There was no significant difference between the results for the last two conditions ($P > 0.05$).

For the NaOH extractions, none of the pretreatments or the use of elevated concentration resulted in any quantitative enhancement in product recovery. At the same time, mass yield was not adversely affected, in particular by pretreatment using sodium ethoxide in anhydrous ethanol. The mean yield of carbohydrate precipitates for NaOH extractions was 99.4 ± 5.1 mg/g (db) (n = 16). For saturated $Ba(OH)_2$, whether alone or following pretreatment, the mean yield of carbohydrate precipitates was 14.6 ± 5.7 mg/g (db) (n = 8). This was less than 15% of the yield obtained using NaOH extraction. For the mixed reagent solution of NaOH and $Ba(OH)_2$, the mean yield of carbohydrate precipitates was 45.2 ± 4.2 mg/g (db) (n = 4). Although higher than for $Ba(OH)_2$, this value was much lower than for NaOH.

7.4.3 Carbohydrate Composition

Peak retention times for the monomers in samples were 8.4 ± 0.4 min for glucose, 9.6 ± 0.3 min for xylose, and 10.4 ± 0.2 min for mannose. Peaks were confirmed by spiked samples. Two of the monomers, xylose and glucose, were detected in all samples, and the xylose peak was the largest of the three in all cases. Mannose was detected only

in samples for certain extraction conditions. (For details refer to Supplementary Data, Appendix B).

Mannose was not detected in any of the samples from extractions using 1.0 M NaOH, whether alone or following pretreatment. Samples for the other four treatment conditions all showed detectable mannose. The concentrations of mannose in all these latter cases were much lower than the lowest standard level of 2 μM. The fact that mannose could not be detected for some treatments and was present only in small amounts in remaining treatments, meant that only the molar ratio of xylose-to-glucose could be calculated.

Ratios of xylose-to-glucose molar concentration and the presence/absence of mannose are summarized in Table 7.2 for all extraction conditions, ordered from highest to lowest mean ratio value. Comparing ratio data as a one-way ANOVA with seven different treatment levels confirmed that significant differences existed (see Supplementary Data, Appendix B). Results of the Levene Test (not presented) showed variances to be homogeneous.

Post-hoc pair-wise tests between the different treatment levels were based on 4 replicates for each condition. These tests showed extraction using 1.0 M NaOH after pretreatment with 1.0 M sodium ethoxide to have the highest mean ratio of xylose-to glucose. The next highest values were for 1.0 M NaOH after pretreatment with azeotropic ethanol, followed by 1.0 M NaOH alone. There was no significant difference between the results for these three conditions ($P > 0.05$), but they were found to be different compared to the other four treatments. The overall mean ratio of xylose-to-

Table 7.2 Ratio of xylose-to-glucose molar concentration and presence/absence of mannose in carbohydrate precipitate samples from different treatment conditions at small scale, with mean and standard deviation presented based on four replicate experiments.

Treatment Condition	Xylose-to-Glucose Ratio	Mannose
1.0 M NaOH after pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	27.5 ± 2.2 *a*	Absent
1.0 M NaOH after pretreatment using azeotropic ethanol	24.7 ± 5.3 *a*	Absent
1.0 M NaOH alone	24.4 ± 1.5 *a*	Absent
Elevated concentration 2.0 M NaOH	15.3 ± 2.7 *b*	Present
Mixed reagent 1.0 M NaOH added to saturated aqueous $Ba(OH)_2$	13.2 ± 2.1 *b*	Present
Saturated $Ba(OH)_2$ after pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	10.9 ± 9.1 *b*	Present
Saturated $Ba(OH)_2$ alone	7.0 ± 2.2 *b*	Present

Note: Values with the same letter are not significantly different according to the method of Student-Newman-Keuls for pair-wise comparisons, $P < 0.05$.

glucose for these three treatments was 25.5 ± 3.4 (n = 12). These were also the same treatments identified to have no mannose present.

The remaining four treatment conditions, in the order of the mean xylose-to-glucose ratio value, were: elevated concentration 2.0 M NaOH; mixed reagent 1.0 M NaOH added to saturated $Ba(OH)_2$; saturated $Ba(OH)_2$ after pretreatment with 1.0 M sodium ethoxide; and saturated $Ba(OH)_2$ alone, which had the lowest overall ratio. There was no significant difference between the results for these remaining four treatments ($P > 0.05$), with the overall mean ratio of xylose to glucose for these cases being11.6 ± 5.5 (n = 16). As noted earlier, mannose was identified to be present in all extract samples for these treatment conditions.

7.5 Discussion

The most important overall finding of this work was that pretreatment of flax shive using 1.0 M sodium ethoxide in anhydrous ethanol did not affect the subsequent recovery of hemicellulose polymers using 1.0 M aqueous NaOH, either in terms of mass recovery or carbohydrate composition. When coupled with earlier work by Parsons et al. (2011b), this showed two sequential extraction steps could be employed with flax shive to successfully recover two separate product streams.

The extraction of flax shive using 1.0 M NaOH produced a stream of carbohydrate polymers that had both reasonable mass yield, and relatively high concentration. In terms of mass yield, earlier work by Buranov and Mazza (2010) evaluated a series of advanced high-pressure carbohydrate extraction processes for flax shive, and they achieved an upper-end yield of about 150 mg/g (db). The results from

this current work showed about 66% of their upper-end yield, but without the need for elevated temperature or pressure.

Consistently high molar ratios of xylose-to-glucose were found in all 1.0 M NaOH extracts. The mean ratio was more than twice that achieved earlier by Jacobs et al. (2003) using microwave processing of flax shive. The comparable ratio of xylose-to-glucose for their work was calculated to be approximately 11 for unpurified extracts.

The fact that no mannose was detected for any of the samples involving 1.0 M NaOH also meant that glucomannan polymer was likely not extracted under the conditions employed. This lack of mannose permitted calculating the mass proportions of remaining major polymers, 4-O-methyl-glucuronoxylan and xyloglucan, based on composition assumptions (Ebringerova 2005, Jacobs et al. 2003). Using the mean and standard deviation values for the ratio of xylose-to-glucose, 4-O-methyl-glucuronoxylan polymer was estimated to represent 92 to 94% of the recovered carbohydrates in 1.0 M NaOH extracts (see Supplementary Data, Appendix B). Given the low level of phenolic organics extracted under the same conditions (Parsons et al. 2011b), 4-O-methyl-glucuronoxylan polymer likely represented more than 90% by mass of the recovered stream. Finding a high product concentration is important for biorefining to be viable.

Variance in the molar ratios of xylose-to-glucose using 1.0 M NaOH was much lower than either variance in measured xylose or glucose concentrations (see Supplementary Data, Appendix B). This confirmed the suitability of using ratio data, in that monomer concentrations might vary, but proportionate composition remained consistent. At the same time, given the low concentrations of glucose involved, a

recognized concern was that ratios were more sensitive to unit molar changes in the concentration of glucose, whether due to variations in flax shive composition or measurement errors.

The specific nature of recovered carbohydrate polymers needs to be clarified beyond the preliminary monomer analyses undertaken. Additional properties to investigate include: molecular weight/degree of polymerization, side attachments, extent of acetyl groups, and any unexpected chemical changes that may have occurred as a result of pretreatment with sodium ethoxide in anhydrous ethanol. The absence of mannose in 1.0 M NaOH extracts, combined with the known presence of glucomannan polymer in flax shive (Jacobs et al. 2003), raises the possibility for further investigations into sequential processing to specifically target glucomannan as an additional separate biorefining product.

A last area for further work is to evaluate the effects of the sodium ethoxide and alkaline extraction processes, as investigated, on the subsequent use of residual cellulose as a substrate for fermentation. Next-generation fermentation processes can yield a variety of products, including ethanol, hydrogen and other high-value materials (Carere et al. 2008). Using the cellulose as a substrate makes sense both as a means to address final residuals from extractions and to replenish ethanol used as an input for pretreatment.

Saturated $Ba(OH)_2$ was found to be a poor extraction reagent for flax shive under the conditions employed. The mass yield was significantly lower than for NaOH, and in mixed solutions $Ba(OH)_2$ appeared to exhibit inhibitory effects. For all extractions using $Ba(OH)_2$ the molar ratio of xylose-to-glucose was much lower than for 1.0 M NaOH, and

mannose was detected. Izydorczyk (2005) noted that saturated $Ba(OH)_2$ tends to remove carbohydrate polymers that are more difficult to extract. This observation was borne out. The use of elevated concentration NaOH, at 2.0 M, provided no increase in mass yield, but also resulted in a different composition of polymers.

7.6 Conclusions

Extraction of flax shive using aqueous NaOH at mild conditions, i.e., 1.0 M concentration, room temperature, and 1 h extraction-period, provided a reasonable mass yield of hemicellulose polymers with a high concentration of glucuronoxylan. The mean recovery of precipitated polysaccharides was 99.4 ± 5.1 mg/g (db) in small-scale tests. The yield was not affected by pretreatment using sodium ethoxide in anhydrous ethanol. Hydrolysis and subsequent analysis of monomers in extract samples showed the mean ratio of xylose-to-glucose to be 25.5 ± 3.4, and with no mannose present. These results suggested glucuronoxylan polymer was recovered at greater than 90% by mass. Monomer composition was not affected by pretreatment.

Overall, technical feasibility was confirmed that flax shive could be processed using two sequential extraction steps to yield two separate product streams. Solvent-soluble phenolic organics could be recovered first using sodium ethoxide in anhydrous ethanol, as investigated previously. Then, carbohydrate polymers from hemicellulose could be recovered using aqueous NaOH at mild conditions, as investigated in this work. Further development of this biorefining opportunity is warranted.

Additional comparative tests involving saturated aqueous $Ba(OH)_2$ as extraction reagent showed poor results. The mass yield was lower, and the composition was

different, including the presence of mannose. When used in mixed solution with NaOH, there even appeared to be inhibitory effects on mass yield. Using an elevated concentration of 2.0 M NaOH provided no enhancement of mass yield, but also resulted in a different composition. (Refer to Section 10.2 and Section 10.3 for discussion of the significance of this research work to the overall thesis objectives).

8 Biorefining Process Opportunity Evaluation

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8.1 Abstract

Next-generation biofuels from ligno-cellulosic feedstocks face cost hurdles. Market values for derived energy products, such as ethanol, remain relatively low, making new, costly conversion technologies difficult to economically justify solely based on energy. One means to address this problem, particularly during early development and demonstration phases for bioconversions, is to pre-extract higher-value products. This paper outlines the evaluation of available feedstocks in Manitoba for product-based biorefining, leading to the selection of flax shive as a model feedstock. This paper then describes a series of process research investigations undertaken on the selective breakdown of flax shive to recover high-value bio-products, both lignin-derived phenolics and hemicellulose-derived novel carbohydrates, leaving behind residual cellulose as a potential substrate for subsequent bioconversion. Lastly, the economic benefits of such a biorefining approach for biofuel production are presented.

8.2 Introduction

Fibrous ligno-cellulosic biomass, which consists of plant straw or other residues left over after harvesting or processing, has been long recognized as a potential renewable feedstock for liquid and gaseous fuels, for materials, and for high-value compounds (BioProducts Canada, 2004). The constituent carbohydrate polymer cellulose, which is

composed chemically of the diamer cellobiose and in turn the monomer glucose, is of particular importance as a carbon-source in the fermentative production of next-generation biofuels (Carere et al., 2008).

Ligno-cellulosic biomass is not just cellulose, but rather a composite of varying proportions of three main components: cellulose, hemicellulose and lignin (Han, 1998). Cellulose is a relatively consistent material, with uniform structure and composition. Both hemicellulose (Ebringerova et al., 2005) and lignin (Buranov and Mazza, 2008) are heterogeneous and highly variable.

For fermentation-based bioconversions, the non-cellulose components are typically disadvantageous. At best, their presence reduces overall yield. Worse, they can act as a technical barrier via inhibition of desired microbial processes, particularly in the case of lignin (Ragauskas et al., 2006). At the same time, there is an inherent economic barrier, whereby it is difficult for biofuel-products alone, given their relatively low-value commodity nature, to economically support expensive new process developments (IEA, 2011).

A way to simultaneously address both technical and economic barriers is through what is now popularly termed "biorefining." The U.S. Department of Energy adopted a definition of this term based on Milne et al. (1990) to generically describe the conversion and extraction of a spectrum of multiple useful products from biomass in a single processing facility. Biorefining can imply the production of multiple products via a single consolidated bioconversion or even plant species being adapted to produce multiple valuable products. In this paper, biorefining refers specifically to the pre-

extraction of multiple higher-value products from a biomass feedstock prior to fermentation.

The economic rationale for a product pre-extraction approach is described in more detail later, but in simple terms it means that recovery processes do not depend fundamentally on the fermentation itself in order to be economically viable. Such an approach also links inherently to government policies intended to increase the diversification of products derived from agricultural sources, as for example outlined in the Manitoba Bioproducts Strategy (Manitoba, 2011). Given the diverse, but ordered-structure of hemicellulose and lignin, these components of ligno-cellulose represent a priority source for recovering higher-value compounds of various types.

This paper considered the use of novel technology, as well as policy, economic and business development aspects. It includes an evaluation of available feedstocks in Manitoba for product-based biorefining, leading to the selection of flax shive as a model feedstock. This paper then describes a series of process research investigations undertaken on the selective breakdown of flax shive to recover high-value bioproducts, both lignin-derived phenolics and hemicellulose-derived novel carbohydrates, leaving behind residual cellulose as a potential substrate for subsequent fermentation. Lastly, the economic benefits of such a biorefining approach for biofuels production are presented.

8.3 Feedstock Opportunity Selection

In his seminal work on competitive strategy, Porter (1980) identified two major generic advantages that can form the basis of a competitive industrial strategy: (i) cost advantage, whether in terms of production, inputs, processing, logistics, etc.; or

(ii) differentiation, based on some unique feature or characteristic of inputs, processes, products or marketing. With regard to higher-value chemical products from agricultural biomass, any resulting competitive advantage for a specific jurisdiction, whether cost- or uniqueness-based, ultimately would be due to the feedstock materials that are available.

Competitive advantages can also emerge from a specific extraction or bioconversion process. However, the application of any process will invariably migrate to the location offering the most appropriate or lowest-cost feedstock. This reinforces the available feedstock material as the key determining-feature for choosing appropriate opportunities in any given jurisdiction.

For an individual jurisdiction, like Manitoba, the first critical step is to gain an understanding of what biomass feedstock materials may be realistically available. For Manitoba, fortunately, this evaluation has been conducted recently (Prairie Practitioners Group 2008, and Prairie Practitioners Group 2009). Five major biomass feedstock materials were identified, summarized in Table 8.1, along with approximate annual quantities and associated prices.

Given these available materials, the conundrum was how to rank them in terms of priority opportunities for bio-products. To do this, a standard business-based selection-criteria evaluation was used, employing eight separate criteria for each of the biomass materials in terms of suitability for pre-extraction of higher-value constituents. These are summarized as follows:

1. **Availability**, in particular whether material could be realistically available in any given year in quantities greater than 10,000 tonnes per year, sufficient to support the requirements of a likely-sized processing plant.
2. **Cost** of material, including unit costs of available material, and logistics costs to a processing site.
3. **Physical nature** of available material, including the extent of pre-processing already conducted and the available particle size.
4. **Uniqueness** of material composition, in the context of providing a strategic advantage.
5. Additional high/medium-value **co-products** that already are or may be extracted in a non-intrusive manner to add extra value.
6. **Technical complexity** of conversion using the material, including any necessary pre-processing requirements.
7. Current and prospective **alternative market uses** for the material that could compete with its use as a feedstock.
8. **Intellectual property**, reflecting essentially the extent of constraints on pre-extraction processes (i.e., significant versus little intellectual property currently).

Under the evaluation method, each criterion for each biomass material was scored, as follows: +2, if strongly fulfilled criterion; +1, if met requirements; 0, if neutral or uncertain; -1, if somewhat deficient; and -2, if strongly deficient. The results of the evaluation are presented in Table 8.2. Presented for each biomass material are firstly, the

Table 8.1 Major biomass feedstock materials available in Manitoba.

Biomass	Annual Quantity Available	Rough Price
Wheat and other monocotyledonous cereal crop straws	Dependent on specific area: Range of 1 to 2 million tonnes available in aggregate	$5-$25 per tonne
Flax shives	60,000 to 70,000 tonnes/y	$25-$30 per tonne
Oat hulls	48,500 tonnes/y	$30-$40 per tonne
Sunflower hulls	11,000 to 13,500 tonnes/y	$30-$45 per tonne
Hemp hurds	Small quantity available	$440 per tonne

(Note: data from Prairie Practitioners 2008, 2009).

Table 8.2 Results of evaluation of biomass materials for value-added product pre-extraction.

Evaluation criterion	Flax shive	Oat hulls	Sunflower hulls	Hemp hurd	Cereal straws
1. Availability	+2	+2	+1	-2	+2
2. Cost	+1	+1	+1	-2	+2
3. Nature	+1	+2	+1	+1	-1
4. Uniqueness	+2	+1	0	+2	-1
5. Co-products	-1	+1	0	0	0
6. Complexity	+1	+1	0	+1	-1
7. Alt Markets	+2	-1	0	-1	+1
8. IP	+1	-1	+1	+1	-1
Total Score	9 of 16	6 of 16	5 of 16	1 of 16	1 of 16
Overall Rank	1	2	3	4	5

score for each of the criteria based on this scoring allocation, secondly, the total score for each biomass material, and lastly, the corresponding priority ranking overall.

Flax shive was identified as the top priority feedstock to consider in Manitoba for pre-extraction of higher-value products. Oat hulls and sunflower hulls ranked next. Hemp hurd and cereal straws were lowest. The selection-criteria and assigned scores were obviously subjective in nature, but were deemed reasonable in this case as reflecting the objective of the selection, i.e., for pre-extraction of higher-value products from the biomass.

8.4 Rationale for Selection of Flax Shive

Flax shive, the top ranked biomass feedstock for pre-extraction, is the woody by-product of the decortication process used to remove valuable bast fibre from the stem periphery of flax (*Linum usitatissimum*). Flax shive consists primarily of broken xylem tissue from the stem core, although, given the nature of the decortication process, it is impossible to prevent the intrusion of at least some matter from the stem periphery. Flax shive is available at low cost, and with consistent particle size and composition, although dust and waste-fibre residues would need to be separated via screening.

Western Canada, specifically the provinces of Saskatchewan and Manitoba, represents the largest production area for flax in the world, albeit dominated by seed oil flax rather than fibre flax (Kozlowski et al., 2005). Hence flax shive has an important degree of uniqueness for Western Canada. There is also significant decortication being undertaken, notably by SWM Intl. (formerly Schweitzer-Mauduit Canada Inc.) at its

facility near Carman, Manitoba. The nature of their process also inherently ensures low intrusion of contaminating non-flax matter.

The Flax Council of Canada (2002) identified two other key points that are advantageous for flax shive in this case, firstly the lack of any significant alternative markets, and secondly the lack of significant existing intellectual property. Further, with regard to both alternative-uses and to processing characteristics, Parsons and Cenkowski (2011) recently showed that flax shive is a relatively poor absorbent, across a range of liquids. Although flax shive has been applied as a bedding material in a number of instances, compared to other biomass materials it has a relatively poor liquid-holding capacity. At the same time, a relatively low liquid-holding capacity is advantageous for pre-extraction processes, as it reduces solvent and product losses.

For the development of a suitable pre-extraction process, it was next important to understand the specific chemical makeup of the feedstock. Fortunately, again, flax shive has been sufficiently investigated. Its nature and composition are quite well understood, in particular regarding lignin and hemicellulose.

Salmon-Minotte and Franck (2005) outlined the rough composition of flax straw as a whole to include cellulose content of 49% to 71%, hemicellulose content of 17% to 29%, and lignin content of 18% to 27%. The extraction of bast fibres gives rise to different component compositions. Bast fibre has high cellulose content, while flax shive features the reverse, with lower cellulose but higher lignin and hemicellulose (Ramaswamy and Tschirner, 2005 and Sain and Fortier, 2002). In terms of the major three constituents, although variable, flax shive can be generally considered on mass

basis to consist of less than about one-half cellulose, and roughly one-quarter each of hemicellulose and lignin (Ross and Mazza, 2010). As such, flax shive contains high proportions of non-cellulose components, from which higher-value compounds could be extracted. It also still contains a significant proportion of cellulose, useable as a carbon-source for fermentation.

In terms of lignin composition, the flax plant primarily contains mixed guaiacyl-syringyl lignin (Gorshkova et al., 2000). Guaiacyl lignin compounds, which possess a single methyl-ether bond, tend to predominate over syringyl lignin compounds, which have two methyl-ether bonds. Hydroxyphenyl lignin compounds, lacking methyl-ether groups, are less prevalent in flax shive, but may be a relatively significant constituent of lignin associated with the fibre component at the stem periphery (Day et al., 2005). The recovery of lignin-based phenolic compounds from flax shive has been examined recently by a number of investigators (Akin et al., 1996; Lozovaya et al., 1999; Kim and Mazza, 2006; Tapin et al., 2006; Kim and Mazza, 2007; Buranov and Mazza, 2007; Buranov and Mazza, 2009; Kim and Mazza, 2009; Buranov et al., 2010).

The major carbohydrate monomers in flax shive, including those found in both cellulose and hemicellulose, in approximate order of prevalence are: glucose, xylose, mannose, galactose, glucuronate, and arabinose (Akin et al., 1996; Han, 1998; Jacobs et al., 2003; Buranov and Mazza, 2010; Tamaki and Mazza, 2010). This composition reflects the presence of three main groups of hemicellulose polymers that are predominant in flax shive. These are glucuronoxylan, glucomannan, and xyloglucan (Jacobs et al., 2003).

Glucuronoxylan is the most prominent hemicellulose polymer in flax shive. As outlined by Ebringerova (2005), this represents the major group of hemicellulose polymers generally found in the woody tissues of dicotyledonous plants, consisting of a main backbone of β-(1→4) linked D-xylopyranose units, with periodic side attachments of α-D-glucuronic acid, or its 4-O-methyl derivative, linked to the 2-position of the xylose backbone unit. Periodic acetylation is also typically present. Jacobs et al. (2003) characterized the specific nature of this polymer in flax shive, finding it to be relatively easy to extract, in their case using hydrothermal microwave treatment, and also readily separable to a high degree of purity using subsequent ion-exchange chromatography, i.e., roughly 90% of that recovered stream.

Glucomannan was the second most prevalent type of polymer recovered from flax shive by Jacobs et al. (2003). As outlined by Ebringerova (2005), this group of polymers is also commonly present in smaller amounts in the woody tissues of dicotyledonous plants, consisting of β-(1→4) linked D-mannopyranose and D-glucopyranose units in the main backbone, and frequently with D-galactopyranose side attachments at the 2-position of the mannose backbone units. Jacobs et al. (2003) characterized the recovered glucomannan from flax shive as consisting of roughly equal proportions of glucose and mannose. Although not as prevalent as glucuronoxylan, they found this recovered neutral polymer was readily extracted, but also found it required more extensive purification steps after ion-exchange chromatography, suggested to be due primarily to the co-presence of xyloglucan.

The third major polymer type, xyloglucan, is also well known to be present in flax straw and flax shive. As outlined by Ebringerova (2005), this type of polymer consists of a backbone of β-(1→4) linked D-glucopyranose units, analogous to α-cellulose, but with D-xylopyranose side units also linked to the 6-position of the backbone glucose units. Xyloglucan is a common type of polymer in dicotyledonous plants, particularly in the primary cell wall. For flax, the xyloglucan is "Type I" in nature, involving a repeating sequence of three xylose-substituted glucose units and a fourth non-substituted glucose unit. Although it is a commonly present type of polymer, xyloglucan tends to be difficult to extract (Ebringerova, 2005), due to strong hydrogen bonding with cellulose. Xyloglucan was not identified by Jacobs et al. (2003), but they inferred this polymer was likely present in neutral carbohydrate polymers recovered from flax shive along with glucomannan.

8.5 Pre-Extraction Process Research on Flax Shive

A series of research investigations have been undertaken on the pre-extraction of higher-value compounds from flax shive. The overall process is represented schematically in Figure 8.1, and is quite simple, involving two separate extraction steps at room temperature:

- First step extraction using sodium ethoxide catalyst in anhydrous ethanol to recover lignin- derived phenolic constituents; and
- Second step extraction using aqueous sodium hydroxide to recover hemicellulose carbohydrate constituents.

Preliminary results from experiments undertaken on the first extraction step have been presented by Parsons et al. (2011b). A yield of approximately 55 mg per g dry basis flax shive was obtained (i.e., around 5% mass yield). Analyses using ^{1}H nuclear magnetic resonance (NMR) confirmed that the recovered material was phenolic in character and thus likely lignin-derived. NMR also confirmed the recovered material to be a mixture, not any single pure compound. Further analysis using gas chromatography-mass spectrometry (GC-MS) suggested the material to be likely oligomeric, rather than consisting of lignin-derived monomers. This process step was found to not extract any carbohydrates, essentially leaving them intact. The recovered material was identified as potentially usable as an input for natural-based resins as a potential market application (Holladay et al., 2007). A unique feature of the extracts was the absence of methyl-ether groups on constituent phenolic-rings. This is advantageous for resin-use, given that demethylation of lignin-derived phenolics has been identified as important for enhancing resin reactivity (Hu et al., 2011).

Investigations of the second extraction step are underway, but not yet published (note: Chapter 8 was published chronologically before Chapter 7). In this case, the yield of carbohydrates has been quantitatively confirmed, but not yet the composition. Importantly, the carbohydrates have been found to be polymeric, rather than monomers; quite different from typical pretreatment processes. The mass yield in this case was found to be around 10%.

Earlier work by Parsons and Cenkowski (2008) determined that some hemicellulose carbohydrates found in flax shive, specifically glucomannans, closely

match materials currently used as selective absorbents for mycotoxins in animal feed, thus a potential market application. They also may be useable as selective absorbents in other applications. The recovered carbohydrate material has been qualitatively observed to have very high-liquid holding capacity in solution, and may have application as a thickener or gelation agent. As such, just as in the case of lignin-derivatives, higher-value markets appear to be available for the recovered novel carbohydrates.

The impacts of the sequential pre-extraction steps on subsequent bioconversion of the residual cellulose to biofuels have not yet been evaluated, but testing is planned. Importantly, although there could be synergistic improvements in technical yield, these are not required. The benefit of pre-extraction is primarily economic, as described next. Bioconversion of cellulose to ethanol is most advantageous in this case. Ethanol is an important solvent agent in both pre-extraction steps. Self-production of ethanol eliminates the need to purchase ethanol externally.

8.6 Economic Benefit of Pre-Extraction

A preliminary economic and financial analysis was undertaken for the flax shive pre-extraction process in order to determine both its own viability and its impacts on subsequent biofuels production. The analysis is presented in Table 8.3, using an annual cost/revenue basis. Assumptions are listed as follows:

- Production plant to be located in Manitoba, in close proximity to Winnipeg.
- Production plant based on 10,000 tonnes annual flax shive input, with typical moisture content of around 8%.

- Order of magnitude estimate of capital cost for production plant and associated facilities of approximately $20 million, including ethanol bioconversion as target biofuel. This value was based on the relative complexity of the facility as compared to other recent projects.
- Project life of 20 year, and cost of money of 14% overall, including both equity and financing, translating to a present value interest factor for annuity (PVIFA) of 6.62 (i.e., present value to annual cost conversion factor).
- Combined higher-value product output of 15% by mass, translating to 1.5 million kg annually.
- Ethanol makeup requirement for extraction processes annually, due to losses, assumed as 10% of flax shive mass, or approximately 1.4 million Litres.
- Cost of flax shive assumed as $100 per tonne, including prescreening to remove dust and waste fibre, and delivery to plant.
- Ethanol bioconversion yield of 150 Litres per tonne flax shive (conservative), yielding output of 1.5 million Litres annually, with assumed ethanol value of $0.70 per Litre.
- Annual labour and other operating costs for facility of $3 million annually, based on facilities of similar complexity, and excluding make-up ethanol.

The results of the preliminary evaluation, as presented in Table 8.3, showed the project to be viable if the average higher-value product price was at least $4.70 per kg. This price is realistic for the products considered. Although more detailed follow-up is required, the results showed that producing higher-value products from flax shive via

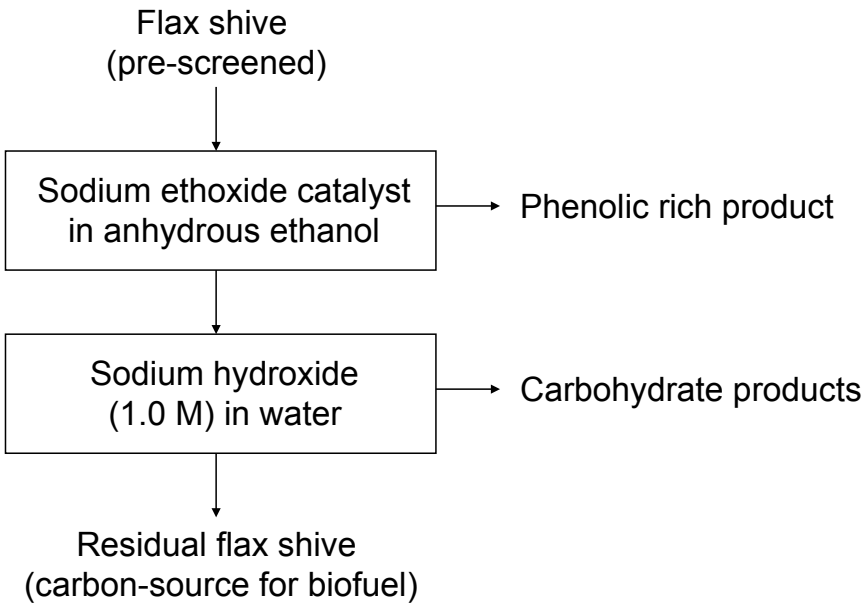

Figure 8.1 Schematic of flax shive pre-extraction process for higher-value compounds.

Table 8.3 Project economic analysis on annual cost/revenue basis.

Item	Basis	Annual Value
Expenses		
Feedstock cost	10,000 tonnes at $100 per tonne	$1,000,000
Ethanol makeup cost	$0.70 per Litre for 1.4 million Litre	$1,000,000
Labour and operating	Single assumed value	$3,000,000
Annual payment for project capita	$20 million capital cost with PVIFA = 6.62	$3,000,000
Total expenses		$8,000,000
Revenues		
Ethanol revenue	$0.70 per Litre for 1.4 million Litre	$1,000,000
Required revenue from higher-value products	Average product price of $4.70 per kg (for 1.5 million kg of products)	$7,000,000
Total revenue required		$8,000,000

(Note: analysis was based on covering all costs including appropriate profit).

sequential pre-extractions is highly attractive as a stand-alone business, and project viability could be achieved in the near-term.

At the same time, it was clear that the proposed facility, as discussed above, would involve primarily a higher-value product pre-extraction business, rather than biofuels. There was also a significant, obvious disconnect in terms of operational-scale; the above facility would produce only about 1.5 million Litres of ethanol annually, effectively pilot-scale. A world-scale production level for ethanol would be two orders of magnitude larger (i.e., 150+ million Litres ethanol annually). Ethanol production is still useful, contributing around 12% of the total annual revenue for the project, in the form of ethanol makeup back to the process.

Sensitivity analysis also provided some important insights. The viability of the project was most sensitive to the price or yield of the higher-value products, both of equal importance. As such, enhancing yield and securing sufficient prices are priorities for any future business involving flax shive pre-extraction. The project was sensitive next most to changes in the annual labour and operating cost. On a relative basis, changes in ethanol yield or cost, capital cost, or flax shive cost had much less impact.

If ethanol production from residual cellulose were totally excluded, the required average higher-value product price would rise to only $5.30 per kg. As such, the project could tolerate developmental problems or even complete disruptions in ethanol production, such as could be encountered in the scale-up of a next-generation ethanol process to pilot-scale operation. The key benefit of such a project to biofuels production

thus would be through providing an already economically viable platform for pilot-scale development and optimization of bioconversions.

Next-generation biofuels are only now tentatively moving toward commercial status. The costs and risks associated with pilot-scale operations are very high. Having a partnering facility to fully develop bio-conversions in a commercial environment is desirable. There is also a less-obvious need to be able to operate pilot-scale facilities for long periods, increasing associated costs and risks. For example, in 1997 Iogen Corporation initiated a pilot-plant in Ottawa to demonstrate their wheat straw-to-ethanol bioconversion process. Their facility is of roughly the same order of magnitude in terms of ethanol output as the proposed flax shive pre-extraction project. Iogen's pilot-plant has operated for roughly a decade, with no intent indicated yet to cease operations.

8.7 Conclusions

One important means to address the twin problems of the low-value for biofuel-products and the potential detrimental impacts of ligno-cellulosic feedstocks is to pre-extract higher-value products. Systematically considering potential biomass materials within Manitoba led to the selection of flax shive as a model feedstock for pre-extraction followed by bioconversion of residual cellulose to ethanol. Research investigations on an ethanol-based pre-extraction process under development have shown reasonable yields of lignin-derived phenolics and hemicellulose derived novel carbohydrates under moderate operating conditions. The economics of pre-extraction appear to be highly attractive. The key benefit for next-generation biofuels is through providing a means to implement pilot-scale ethanol production in an already commercially justified facility.

9 Additional Larger-Bench Scale Tests

Additional experimental investigations were undertaken, but not published, involving larger-bench scale processing and compositional tests for pretreatment and alkaline extraction processing steps. All of the processing for the sequential extraction steps in Chapter 6 and Chapter 7 were undertaken at small-scale, using only 1 g (wb) samples of flax shive. The next logical phase for evaluation of the process was to undertake processing of larger quantities of flax shive, and to assess changes in the composition of the flax shive as carried through sequential steps.

9.1 Objective

The objective of this additional work was to evaluate overall compositional changes in flax shive, specifically relative changes in the content of cellulose, hemicellulose and lignin components, through the course of extractions.

9.2 Methods and Materials

In order to determine changes in the overall composition through pretreatments and extractions, larger samples of at least 20 g each of flax shive were prepared and analyzed externally by the Feeds Innovation Institute at the University of Saskatchewan. Six different treatment conditions were assessed for composition covering the course of processing steps, as follows: (i) untreated flax shive, as base-case material; (ii) flax shive pretreated using 1.0 M sodium ethoxide in anhydrous ethanol; (iii) flax shive pretreated using azeotropic ethanol; (iv) flax shive extracted with 1.0 M aqueous NaOH alone; (v) flax shive extracted with 1.0 M aqueous NaOH, after pretreatment using 1.0 M

sodium ethoxide in anhydrous ethanol; and (vi) flax shive extracted with 1.0 M aqueous NaOH, after pretreatment using azeotropic ethanol.

Each of the samples was assessed for content of cellulose, hemicellulose, lignin and ash, with the methods employed being consistent with those outlined in Walter et al. (2010). The accuracy of these analyses was indicated to be ±3%. Given that none of the processes undertaken should significantly affect the cellulose component of the flax shive, for further comparison the results were also reported for each sample in terms of the mass ratio of hemicellulose-to-cellulose (db) and the mass ratio of lignin-to-cellulose (db), essentially using the cellulose content of the samples as an internal standard.

Sample preparation necessitated the processing of much larger quantities of materials. As was done in the case of small-scale extractions, sufficient quantities of flax shive were processed through pretreatments to permit materials to be carried through the subsequent alkaline extraction, with allowance for losses. This meant processing 70 g using 1.0 M sodium ethoxide solution in anhydrous ethanol, and using azeotropic ethanol. For pretreatments and alkaline extractions, similar procedures were used as for small-scale samples, as outlined in Chapter 7, with selected changes.

Pretreatments were undertaken using a 1,000 mL beaker with an aspect ratio of 2-to-1. A 10% mass per volume solution was used rather than 5% as for smaller-scale tests. A higher solids proportion was used in order to ensure a total volume requirement of less than 1,000 mL. After 70 g of flax shive was added to the respective 700 mL of solution, the beaker was fitted with a stir bar, sealed using sealing film, and placed on a magnetic

stir-plate. Adequate mixing in these cases was found to be more difficult than for small-scale samples.

After pretreatment for 1 h, the flax shive was filtered under vacuum and retained, using a mesh screen with larger-sized nominal openings of approximately 500 μm. The flax shive was then rinsed using only an equal volume of either anhydrous ethanol or azeotropic ethanol respectively (i.e., 700 mL total in each case). The retained flax shive remained under vacuum for 30 min.

For 1.0 M aqueous NaOH extractions involving pretreatment, flax shive samples were carried over, while for NaOH-only extraction, fresh flax shive was used. The NaOH extraction was undertaken using the same 1,000 mL beaker, again using 10% mass per volume of flax shive. After flax shive was added to the alkaline solution, the beaker was fitted with a stir bar, sealed using sealing film, and placed on a magnetic stir-plate. Again mixing was found to be more difficult than for small-scale samples.

After extraction for 1 h, the flax shive was filtered under vacuum using the mesh screen with nominal opening of approximately 500 μm, and then rinsed using an equal volume of high purity water. Filtrate solutions were discarded. The retained flax shive samples were removed and dried in an oven at 103ºC for 24 h.

9.3 Results and Discussion

Results of compositional analyses for the larger-bench scale samples are presented in Table 9.1 in terms of cellulose, hemicellulose, lignin and ash content (db), and in Table 9.2 in terms of the ratios of hemicellulose-to-cellulose, and lignin-to-cellulose by mass (db). The most obvious result was the consistency of ratios of

hemicellulose-to-cellulose in Table 9.2 across the three treatments involving 1.0 M NaOH extraction, whether alone or following pretreatment. The mass proportion of hemicellulose was significantly reduced, in the range of 25% to 30% across the three conditions.

Testing at larger-bench scale thus showed that 1.0 M NaOH was effective in reducing the relative hemicellulose content of flax shive. This confirmed the extraction of hemicellulose-derived polysaccharides. Pretreatment either by sodium ethoxide in anhydrous ethanol or by azeotropic ethanol had no effect on the subsequent reduction of hemicellulose content, confirming pretreatments did not affect polysaccharide extraction.

Given that the hemicellulose content of untreated flax shive for the larger-bench scale tests was determined to be 169 mg/g (db) as outlined in Table 9.1, the extraction yield from hemicellulose using 1.0 M NaOH could be calculated based on an average compositional change of -27.3%. This translated to 46.1 mg/g (db) of removed hemicellulose material. This value, however, was much lower than the precipitate yield of 99.4 ± 5.1 mg/g (db) found for smaller-scale experiments in Chapter 7.

The nature of the method used to determine precipitate mass yield in smaller-scale experiments was understood to not differentiate between constituents retained on filter paper, and, as such, could be erroneously elevated by the presence of any contaminants. At the same time, for the larger-bench scale treatments, a much higher solids proportion was used, specifically in order to limit the total volume to a manageable level. Significant mixing issues were noted. As such, larger-bench scale treatments were likely more subject to mass-transfer constraints that also could reasonably explain the lower

apparent yield of hemicelluloses. Further work is required for larger-scale processing to firstly ensure adequate mixing and extraction involving high-solids content solutions, and then to confirm the nature and yield of carbohydrates through the course of process steps.

Also regarding carbohydrates, pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol appeared to significantly increase the ratio of hemicellulose-to-cellulose by +12%, as indicated in Table 9.2. Reviewing the composition of components in Table 9.1 showed that this pretreatment method had the lowest content of cellulose, the highest content of hemicellulose, and the highest content of ash of any of the tested conditions. Elevated ash content was also seen with NaOH extractions, and these increased values could be readily explained in terms of residual sodium ethoxide or NaOH, respectively. However, ash content in absolute terms was relatively small, and the change in the ratio of hemicellulose-to-cellulose was primarily due to the combination of decreased cellulose content and increased hemicellulose content. These were not expected. The use of sodium ethoxide in anhydrous ethanol was not expected to affect either cellulose or hemicellulose. Parsons et al (2011b) showed using ^{1}H NMR analyses that recovered organic extracts contained no signature signals that would indicate the presence of carbohydrates or attached carbohydrate fragments. Also unexpected in this case was that in absolute terms the content of lignin was virtually unchanged from the untreated flax shive sample. This aspect is discussed next.

For all treatment conditions evaluated, the mass ratios of lignin-to-cellulose were found to be higher than for untreated flax shive, as shown in Table 9.2. These results were unexpected. The largest increases were for the NaOH extractions. Consistent with

the effects observed for hemicellulose, the ratio values for NaOH extractions were found to be similar to one another, whether or not pretreatment was involved.

The use of 1.0 M NaOH might be expected, if anything, to decrease the content of lignin, although the conditions for NaOH extraction were specifically selected to try to minimize lignin dissolution. More important was the lack of any apparent effect on lignin by sodium ethoxide in anhydrous ethanol. It was expected that this pretreatment would likely reduce the content of lignin. Parsons et al. (2011b) had shown that processing of flax shive using this catalyst at a 1.0 M level produced a mean yield of 54.5 ± 14.5 mg/g (db) of organic extracts. These extracts were confirmed using ^{1}H NMR to be phenolic in nature, and thus likely lignin-derived. The results of the larger-bench scale composition tests, however, showed the relative proportion of lignin to apparently be increased. As noted earlier, for sodium ethoxide pretreatment without NaOH extraction, the absolute content of lignin was no different than for untreated flax shive. There were no explanations for the unexpected increase in lignin content across all conditions. For treatment of flax shive using sodium ethoxide in anhydrous ethanol, further investigation is required to confirm the nature and precise origins of the phenolic extracts.

Table 9.1 Composition results for larger-bench scale tests.

Treatment Condition	Content of Component (mg/g db)			
	Cellulose	Hemicellulose	Lignin	Ash
Untreated flax shive	495	169	224	19
Pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	479	183	223	34
Pretreatment using azeotropic ethanol	491	174	231	18
Extraction with 1.0 M NaOH alone	533	130	261	26
Extraction with 1.0 M NaOH after pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	532	135	266	25
Extraction with 1.0 M NaOH after pretreatment using azeotropic ethanol	532	128	268	29

Table 9.2 Mass ratios (db) of hemicellulose-to-cellulose and lignin-to-cellulose for larger-bench scale samples evaluated for overall composition through the course of processing, with changes from untreated flax shive composition also indicated.

Treatment Condition	Hemicellulose-to-Cellulose		Lignin-to-Cellulose	
	Mass Ratio (db)	Change (%)	Mass Ratio (db)	Change (%)
Untreated flax shive	0.341	—	0.452	—
Pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	0.382	+ 12.0%	0.466	+ 3.1%
Pretreatment using azeotropic ethanol	0.354	+ 3.8%	0.471	+ 3.9%
Extraction with 1.0 M NaOH alone	0.244	- 28.4%	0.489	+ 8.4%
Extraction with 1.0 M NaOH after pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	0.255	- 25.5%	0.501	+ 10.6%
Extraction with 1.0 M NaOH after pretreatment using azeotropic ethanol	0.241	- 29.3%	0.504	+ 11.4%

10 Overall Thesis Discussion

The overall results of all research work undertaken for this thesis are discussed in the context of the central theme and objective, i.e., addressing different aspects of the development of a multi-step process using flax shive as feedstock to recover multiple, separate high-value bio-products. Discussions are divided into the three main research areas that were covered, namely material properties, process development, and purification.

10.1 Material Property Aspects

The material property aspects of the research were covered in Chapter 3, which addressed particle characteristics; Chapter 4, which addressed frictional behaviour when liquid additions were involved, and Chapter 5, which addressed liquid absorbency. These material properties affect the separation, materials handling and extractive processing of flax shive. Although not all relevant properties were evaluated, the research provided important insights, including a number of unexpected discoveries.

The waste materials from flax straw processing were found to consist of a mixture of three main byproduct components: xylem tissue (i.e., flax shive); waste fibre; and dust and fines. Of these, only the mid-core flax shive was considered for use as a feedstock material in extraction work. This necessitated pre-separation to recover flax shive, which would be also required at larger scale for industrial operation.

Preliminary testing (Chapter 3) showed that simple sieves/screens could be adapted to satisfactorily separate flax shive from the other components of flax straw processing waste, albeit with some concerns. In general terms, flax shive involved an

intermediate particle size, requiring effectively two separations: firstly from waste fibre; and secondly from dust and fines. It was found that the two separations could be undertaken simultaneously. At the same time, the size-based transitions between the components were not entirely clear cut.

Screening was found to be very effective for the removal of dust and fines. A minimum screen size of 1.18 mm was used for experiments in Chapter 4, Chapter 5, Chapter 7 and Chapter 9. The two flax shive fractions tested in Chapter 6 involved minimum screen sizes of 1.0 mm and 0.5 mm respectively. In all cases the flax shive samples used in experiments were found to be essentially free of dust and fines.

Preliminary testing (Chapter 3) showed flax shive (i.e., mid-core xylem) to have both a noticeably lighter colour than waste fibre (i.e., light beige versus dark brown), and a significantly lower density than waste fibre (i.e., 12% lower based on pycnometer tests). These characteristics were most important in comparing and distinguishing flax shive relative to dust and fines.

The dust and fines component was identified strictly on the basis of having a smaller particle-size, but also was observed to be made up of discrete fragments of both xylem and fibre tissues. Comparisons of sieved samples (Figure 3.5) showed that below a screen size of 0.5 mm, progressively smaller-sized fractions contained a progressively higher proportion of dark particles. This implied an increasing proportion of waste fibre-derived tissue that would have a different composition than xylem-based flax shive. It was observed that screen fractions larger than 0.5 mm had a relatively uniform light beige colour, implying this retained material was composed predominantly of flax shive.

Tamaki and Mazza (2010) investigated differences in chemical composition when comparing three screen fractions of flax shive: fine (i.e., < 150 μm); medium (i.e. 150 to 850 μm); and course (i.e., 850 to 2,000 μm). The calculated molar ratios of xylose-to-glucose from their data were 0.67 for the course fraction and 0.65 for the medium fraction, both consistent with the ratio values in Table 2.5 from analyses by other researchers. For their fine fraction, however, a lower value of 0.42 was calculated, implying higher glucose, and thus, higher cellulose content. Tamaki and Mazza (2010) also found fine flax shive to have much higher ash content than the other fractions, i.e., greater than 30% versus 2% to 3% by mass (db). The flax shive used in all experiments in this work was most similar in size to the medium fraction of Tamaki and Mazza.

As part of the first step extraction experiments in Chapter 6, two flax shive size-fractions were tested: (i) passing 10 mm and retained on 1.0 mm; and (ii) passing 1.0 mm and retained on 0.5 mm. The mass yields of phenolic extracts from the two fractions were found to be not statistically different, suggesting the composition of the two fractions were indeed the same. A minimum screen size of 0.5 mm was thus found to be a useful limit to ensure separation of flax shive from dust and fines. Some smaller xylem materials would be obviously still lost, but consistent composition maintained.

The separation of flax shive and waste fibre was found to be more problematic. In Chapter 4, the two-dimensional limitations of sieves were noted. Potential problems can occur in the separation of rod-like or fibrous particles where one dimension is significantly longer than the other two, i.e., the ability for a particle to pass through a sieve in this case depends significantly on the orientation of the particle. Although less of

a concern for the dust and fines, for which particle dimensions were generally much smaller, this was important for separation of flax shive and waste fibre. Both these components fit generically into the rod/needle-like quadrant of the particle classification matrix of Feda (1982), as outlined in Figure 3.2.

Aside from density and colour differences, as noted earlier, the main size difference between flax shive and waste fibre components was in their respective elongation ratios (Equation 3.1). As noted in Section 3.2, the elongation ratio of flax shive was high, estimated in the range of 3 to 20. The elongation ratio of waste fibre was even higher. Although not measured directly, reasonable assumptions from the data of Han (1998) suggested ratios for waste fibre greater than 300, i.e. cross-sectional dimension of 20 to 30 μm and length of 10 to 30 mm, This is more than an order of magnitude higher than flax shive.

For the first step extraction experiments (Chapter 6), the screen size used to separate flax shive from waste fibre involved a nominal opening of 10 mm. For the remaining experiments (Chapter 4, Chapter 5, Chapter 7 and Chapter 9), the screen size involved a nominal opening of 2.36 mm. Given the known limitations of screening, these dimensions did not reflect per se actual particle sizes, but, rather, a sequence of screens that ensured fibrous material was mostly excluded.

It was also necessary to still manually review all feedstock samples of flax shive prior to experiments. This was to remove any errant balls of fibre that may have worked their way through the sieves. Manual checking was also important to identify and remove any residual broken or whole flax seeds, which were occasionally present. Flax

seed material was a particular concern given potential reaction of lipids in the presence of alcohol and alkoxide catalyst to form fatty acid esters. The separation methods as employed were satisfactory for laboratory scale work. As the process is moved toward larger-scale industrial operation, however, it will be necessary to identify or develop improved methods for separating flax shive from waste fibre.

Operation at industrial scale will also necessitate identifying suitable storage and materials handling methods for biomass bulk solids through the course of the complete process. Williams et al. (2008) summarized the range of available options for storage and materials handling in biomass processing facilities, current status, and key material properties for design. The latter include: bulk density; density pressure relationships; angle or coefficient of internal friction; angle or coefficient of wall friction; cohesion and flow function; and liquid content. Some of the relevant properties of flax shive were evaluated as part of this work, but not all, and not through all processing stages.

Materials handling will be complicated by the diverse range of materials necessary to be handled, including: unsegregated flax straw processing wastes; separated waste fibre; separated dust and fines; separated dry flax shive; flax shive inundated with alcohol-based solutions (i.e., alkoxide catalyst solutions); and flax shive inundated with water-based solutions (i.e., NaOH solutions). Materials handling techniques applicable for biomass in general were identified by Williams et al. (2008), and include: screw (or auger) conveyors; bucket elevators; belt conveyors; drag (or chain) conveyors; and pneumatic based conveyor systems. Not all techniques will be applicable for all specific

applications through the course of the process. Additional investigation and testing under relevant conditions will be necessary to confirm all materials handling methods.

Particularly important and unique to the process outlined in this research work is materials handling of wetted flax shive. The frictional behaviour investigations, as described in Chapter 4, evaluated the differential impacts of alcohol versus water additions on bulk-solid flax shive. This is directly relevant given that two different liquid solutions were employed in sequential extractions. First was extraction using 1.0 M sodium ethoxide in anhydrous ethanol (Chapter 6), followed by extraction using 1.0 M NaOH in aqueous solution (Chapter 7).

Levinspiel (1972) described that in transitioning from batch-based, laboratory-scale reaction tests, as employed in this research, to a continuous, industrial-scale operation, two types of idealized reactor configurations can be considered as part of the reactor design procedure. These are: (i) continuous stirred tank reactor, or CSTR, which involves constant feeding of ingredients into a continuously mixed tank with consistent outflow, with the key characteristic of complete mixing, sometimes termed, “complete back-mixing”; or (ii) plug flow reactor, or PFR, which involves continuous feeding of ingredients through an extended tubular length, with the key characteristic of no back-mixing of ingredients.

The practical handling of a slurry of flax shive in either aqueous or alcohol solution tends to preclude the use of a CSTR. Work on larger-bench scale processing (Chapter 9) for compositional tests showed the mixing of flax shive in alcohol and aqueous solutions in a 1,000 mL beaker to be very difficult. This was even with a solids

content of only 10% mass per volume, which was still relatively dilute. On the other hand, the PFR configuration is still feasible to consider. As such, further investigation of a PFR type system for extracting flax shive is warranted, although it would need to be adapted in order to adjust to the frictional behaviours involved.

It was found in Chapter 4 that the addition of any liquid would significantly increase the internal friction of wetted flax shive. The angle of internal friction for control samples of flax shive (i.e., no liquid addition) was 28º ± 3º. For flax shive dosed with liquid, the angle of internal friction was 37º ± 1º when distilled water was added, and 36º ± 2º when alcohol (i.e., methanol or isopropanol) was added. These values were not statistically different, with an overall average for any liquid addition of 37º ± 2º.

The wall friction of wetted flax shive, however, depended differentially on the type of liquid added. The angle of wall friction for control samples of flax shive (i.e., no liquid addition) was 12º ± 2º. For flax shive dosed with liquid, the angle of wall friction was 28º ± 1º when distilled water was added, and 23º ± 3º when alcohol (i.e., methanol or isopropanol) was added. These values were significantly different, and could impact how these different slurries might be moved.

Some method of contacting and mixing flax shives with the respective liquid solutions, without clogging or binding, would be still ultimately required, and is not yet determined, but the most obvious choice for moving the saturated slurries forward in an enclosed tubular manner, consistent with PFR principles, is the use of a screw conveyor or screw extruder. These technologies are commonly used in agricultural and food

applications, however, their ability to operate in practice in this case is uncertain, in particular when alcohols are involved.

Woodcock and Mason (1987), in describing the operational principles of screw conveyors for bulk solids, noted that they effectively create two components of motion: axial movement, which is desired to move solids forward; and rotational movement. Each helical flight essentially acts as a wedge, lifting solids and pushing them forward, however, being able to achieve this intended motion depends on relative friction values. The internal friction between particles, and the friction of particles against the sidewall casing surface of the conveyor must be significantly higher than the friction of particles against the surface of the helical flight of the screw. Under the extreme condition whereby there is no wall friction against the casing surface, the solids move only in rotation, creating a solid cylindrical plug. This same situation is recognized in the operation of screw extruders (Moscicki and van Zuilichem 2011).

The finding of a significantly lower wall friction for addition of alcohols compared to water (Chapter 4) raises the concern that the operation of these screw-devices could be significantly impeded or even compromised. The specific friction values involved, however, depend on the particular surface materials, i.e., wall friction against helical flight and against wall casing, and these are not yet known. Actual testing will be required to confirm whether or not screw conveyors or screw extruders could be used.

Alternative approaches to materials handling of wetted flax shive also need to be considered. The use of a drag conveyor system is another option (Williams et al. 2008),

that could potentially move saturated slurries of flax shive in an enclosed tubular manner, consistent with PFR principles. In this case the solids would be essentially "pulled" forward. Some sort of tubular piston or plunger system also could be considered, with solids instead being "pushed" forward. More detailed investigation of these alternatives for handling wetted flax shive also will be required.

The liquid-holding capacity of flax shive emerged as a important material property affecting the development of an extraction process involving the use of aqueous- and alcohol-based solutions. The investigations into biomass liquid absorbency (Chapter 5) showed flax shive to be a relatively poor absorbent compared to other biomass materials.

Of the four biomass materials tested, flax shive consistently showed either the lowest or near the lowest liquid-holding capacity for all of the liquids tested. Specifically for distilled water, the liquid holding capacity was 2.68 ± 0.25 g/g (db). This was significantly the lowest value, by 36% to 42% compared to all the other materials. For ethanol, the liquid holding capacity was 1.30 ± 0.03 g/g (db), which was the second lowest value, but only 3% higher that the lowest value. At the same time, the liquid-holding capacity for ethanol was 34% to 40% lower than the best performing biomass materials.

Although flax shive does not appear promising as an absorbent product, its behaviour is highly desirable when considering its use as a feedstock for aqueous- and alcohol-based processing. Low liquid-holding capacity is important for minimizing losses of solvents/reagents and extracted products that would be retained with the solid

biomass material. As noted, these losses would be 30% to 40% lower for flax shive compared to other biomass materials, a significant benefit. An additional impact not identified in Chapter 5 is that the bulk density of flax shive saturated with water or alcohol would be much lower compared to other biomass materials, given much lower liquid holding capacity across the entire range of liquids tested. This has implications for materials handling, as discussed earlier.

10.2 Selective Breakdown Process Development Aspects

The technical aspects of process development in this research were investigated in two steps, reflecting the two sequential extractions undertaken. The first step extraction (Chapter 6) involved recovery of phenolic organics from flax shive using solutions of sodium ethoxide in anhydrous ethanol. The second step extraction (Chapter 7) involved the subsequent recovery of hemicellulose-derived polysaccharides from flax shive using aqueous NaOH solutions. An overview of the entire process (Chapter 8) included preliminary evaluation of economic viability, market prospects for derived products, and related policy aspects. Additional larger-bench scale composition tests (Chapter 9) were undertaken in order to provide confirmation for the overall process.

Of the two extraction steps, the most novel was the application of the alkoxide catalyst to flax shive as a ligno-cellulosic feedstock (Chapter 6). This extraction approach had never been undertaken previously. It provided an organic product stream with both reasonable yield and consistent chemical make-up. In terms of the three uncertainties identified in Section 2.3.11, this extraction: (i) was confirmed to be

selective; (ii) was confirmed to not affect yield or composition of carbohydrates in subsequent extraction; and (iii) was found to be relatively simple in practice.

Quantitatively, the mass yield of recovered organics varied linearly with the concentration of sodium ethoxide catalyst, at least up to a concentration of 1.0 M. Yield was unaffected by the particle size of the flax shive within the size-ranges tested. In small-scale experiments using a concentration of 1.0 M sodium ethoxide in anhydrous ethanol, the yield was found to be 54.5 ± 14.5 mg/g (db).

Qualitatively, the extracted organics were shown consistently, using ^{1}H NMR, to involve aromatic ring structures, suggesting phenolic organics of some type. This result further suggested that the organics were likely derived from the lignin of the flax shive, given lignin is the most obvious source of constituents with aromatic ring structures. The lack of ability to register any individual compounds using GC-MS suggested that the recovered products were likely oligomeric fragments, with boiling points too high to be evaporated in a GC-MS system.

The results of larger-bench scale experiments (Chapter 9) identified an inconsistency that will need to be resolved for the further development of the process. Smaller-scale experiments showed a reasonable yield of phenolic organics that were likely lignin-derived. Larger-bench scale compositional tests, on the other hand, showed the relative mass proportion of lignin in samples to increase rather than decrease after pretreatment using sodium ethoxide in anhydrous ethanol. If the proportion of lignin was increased, the extracts that were removed using sodium ethoxide logically could not originate from lignin.

Further investigation will be necessary to confirm both the nature and the precise origins of the phenolic extracts. Continuing with the assumption that these materials are indeed composed of lignin fragments, the next step logically would involve applying analytical methods as would be typically used to characterize lignin. As a starting point, Brunow (2004) recently summarized the relevant methods for lignin molecular mass and chemical structure evaluation, including both degradative/derivative and non-destructive techniques.

The second step extraction involved the use of 1.0 M aqueous NaOH to recover polysaccharides from flax shive (Chapter 7). Extraction using NaOH was generally known in the literature for the recovery of hemicelluloses, but had been not specifically applied to flax shive as a feedstock. In terms of the three uncertainties identified in Section 2.3.11, the extraction: (i) was confirmed to be selective for glucuronoxylan polymer at mild conditions involving 1.0 M NaOH reagent concentration, room temperature and 1 h extraction-period; (ii) was confirmed to not extract significant solvent-soluble organics under the same conditions; and (iii) was confirmed to have low energy requirements, given effectiveness at mild conditions. A carbohydrate product stream was obtained with both reasonable yield and consistent composition.

Quantitatively the mass yield of recovered carbohydrate precipitates in small-scale experiments was not affected by pretreatment using sodium ethoxide in anhydrous ethanol. The mean recovery of carbohydrate precipitates was 99.4 ± 5.1 mg/g (db). This yield compared well with other flax shive extractions in the literature (Buranov and Mazza 2010), particularly given mild conditions.

Larger-bench scale compositional tests (Chapter 9) showed a consistent reduction in the proportion of hemicellulose content in flax shive after extraction using 1.0 M NaOH, with or without pretreatment (i.e, 25% to 29% reduction). These results confirmed that hemicellulose polysaccharides were being extracted. The yield calculated for these tests was lower than for small-scale experiments. However, the use of a higher proportion of flax shive solids in solution for larger-bench scale tests, combined with identified mixing issues, suggested that the lower observed recovery could be reasonably explained by mass transfer limitations (Chapter 9).

The yield of carbohydrate polymers needs to be confirmed, particularly at larger scale. The nature of the recovered carbohydrate polymers needs to be further clarified. Additional properties that need to be investigated include: molecular weight/degree of polymerization, side attachments, extent of acetyl groups, and any chemical changes that may have occurred as a result of pretreatment.

Economic evaluation of the multiple-product extraction process based on flax shive (Chapter 8), showed it had good potential to be an economically attractive business. In order to be viable, the average price for all recovered products needed to be $4.70 per kg. A series of recent studies, including Werpy and Petersen (2004), Holladay et al. (2007), and de Jong et al. (2012), suggest that higher-value products from biomass typically involve prices in the range of $1 to $10 per kg. The calculated average price level for proposed flax shive extraction was within this range, meaning that the project is potentially viable, at least on an order of magnitude basis, and does not require unrealistically exorbitant product prices. The viability of the project was found to be

most sensitive to the price or yield of the products, both of equal importance. As such, enhancing yield and securing sufficient prices are priorities for any future business involving flax shive extraction. Preliminary evaluation showed that the process merits further consideration, however, more detailed economic assessment still will be necessary on an ongoing basis to confirm viability. As the nature of the process operation at larger scale become more clear, uncertain cost items also will need to be clarified or added, such as waste disposal costs, energy costs, catalyst costs, labour costs, and others.

Two additional areas for research were identified for the overall process that were not included in this work. Firstly, the sequential extraction process was found to work well with flax shive as feedstock, and might be applicable to other biomass materials. Priority feedstocks for consideration were identified as oat hulls, sunflower hulls, hemp hurds, and cereal straws (Chapter 8). Secondly, the process steps as undertaken, appeared to leave behind residual cellulose that itself could become a further usable substrate, particularly for cellulosic fermentations. The extraction of high-value constituents, as undertaken in this research, was identified as a potential means to expedite the development of next-generation biofuels, using left-over cellulose (Chapter 8).

10.3 Purification Considerations

No separate experiments were undertaken on product purification, but product purity was an intrinsic consideration in process selection and development. Each of the two extraction steps, (Chapter 6 and Chapter 7), resulted in a stream of products with consistent composition. The two extractions also appeared to be independent, and had no adverse impacts on each other, particularly in terms of the purity of derived products.

The product stream from the first step extraction step involved a complex mixture of recovered organics that could not be entirely characterized, but in all cases was shown to be consistently phenolic in nature using ^{1}H NMR. A further important insight from ^{1}H NMR (Chapter 6), was that no characteristic signals associated with sugar molecules were observed. This precluded the presence of carbohydrate constituents in the recovered products, whether as polysaccharides, attached sugar fragments, or tannins.

A single uniform extraction period of 1 h was used for all experiments with sodium ethoxide catalyst. It was not practical to measure product concentrations as a function of extraction time, given the extensive post-extraction work-up required. The linear dependence of yield on catalyst concentration (i.e., with catalyst rather than reactants being the limiting factor), as well as no apparent inhibition, suggested that the reaction(s) involved could be categorized as first-order in nature (Levenspiel 1972). In general terms, PFR reaction systems, discussed earlier, are well suited to first-order reactions.

The recovered product stream of polysaccharides from the second step extraction (Chapter 7) showed consistent and high ratios of xylose-to-glucose molar concentrations, and no mannose was detected. These results suggested that the product stream contained a high concentration of glucuronoxylan polymer, estimated to be more than 90% by mass, and no glucomannan polymer present. Watson and Williams (1959) identified colour as a key product-purity characteristic for hemicelluloses, with products desired to be as white as possible. Further colour characterization of the recovered polysaccharides is an additional important step for investigation of this extraction.

The finding of no mannose in the analyses of extracts using 1.0 M aqueous NaOH also led to the identification of a follow-up opportunity (Chapter 7). Glucomannan polymer did not appear to be extracted, yet glucomannan is known to be present in flax shive hemicellulose (Jacobs et al. 2003) and represents a potentially valuable product (Chapter 8). The potential arises to consider one further extraction step after recovery of glucuronoxylan polymer. This would require different extraction conditions to specifically target the recovery of glucomannan as a separate product stream.

A final consideration in terms of purity was the discovery that the two separate extraction steps did not interfere with each other, and did not result in cross-contamination. Sodium ethoxide extracts did not contain carbohydrate constituents and did not appear to adversely affect hemicellulose. The subsequent aqueous NaOH extracts were unaffected by sodium ethoxide pretreatment, and appeared to contain little solvent-soluble organics. When these results were taken together (Chapters 6 and Chapter 7) it meant that two completely separate product streams could be recovered sequentially from flax shive. This is highly desirable from the perspective of biorefining, where the deliberate intent is to recover multiple different products from the same biomass feedstock.

11 Overall Thesis Conclusions

The overall objective of this research work was to investigate and to develop a simple, selective breakdown approach for the cost-efficient recovery of multiple high-value bio-products from flax shive as a biomass feedstock. This included consideration of feedstock material properties, process development and purification.

Two sequential extraction procedures were applied to flax shive. They each yielded a separate product stream, each of potential high-value. The first step extraction, involving sodium ethoxide in anhydrous ethanol, was novel in that it had not been undertaken previously. The second step extraction, involving aqueous NaOH, was more conventional in nature, but had not been applied to flax shive previously nor had been combined with other product extractions.

Importantly, these two extraction steps were found to not interfere with each other. The separate product streams involved virtually no cross contamination, with high concentrations of products in both cases, greatly simplifying purification. All of the extractions were undertaken deliberately at mild conditions (i.e., room temperature and atmospheric pressure) to reduce energy inputs. Reasonable product yields were still achieved. When taken together, the sequential extractions represented the basis of a biorefining process applicable to flax shive, and, potentially, to other biomass materials.

All of the experimental work undertaken, whether published or unpublished, was directed toward the overall research objective. Similarly, the various findings all contributed toward achieving the research objective.

Preliminary work (Chapter 3) showed that flax straw processing waste consists of three distinguishable components: flax shive; waste fibre; and dust and fines. Flax shive, which is made up of xylem tissue and involves an intermediate particle size, required effectively two separations. Screening was found to be simple and effective for removing smaller-sized dust and fines. Although the size transition was not entirely clear cut, the use of a screen size no smaller than 0.5 mm ensured that retained material was uniformly composed of flax shive.

The separation of flax shive from waste fibre was more problematic. Screen sizes were less directly meaningful, given the rod-like/fibrous nature of both components. It was found that flax shive and waste fibre tissues were readily distinguished by differences in particle elongation ratio, particle density and colour characteristics. Arrangements of screens were employed that could mostly separate these two components, but manual review of samples was still required to remove errant fibre and flax seed materials. As such, identifying or developing better methods to separate waste fibre from flax shive will be necessary.

Bulk solids friction investigations of flax shive dosed with liquids (Chapter 4) found that internal friction was increased by the addition of any liquid, whether water or alcohol, but that wall friction was affected differentially by the liquid added. Wall friction was increased significantly by water addition, but much less by alcohols. This finding had not been previously reported in the literature, and has implications for the materials handling of flax shive in a process involving sequential alcohol- and water-based reagent solutions.

Liquid absorbency investigations (Chapter 5) compared flax shive to three other biomass materials in terms of liquid-holding capacity for five different liquids, including water and alcohols. Flax shive exhibited comparatively low liquid-holding capacity across the range of liquids. While not advantageous as an absorbent material, the behaviour of flax shive showed it to be well suited as an extraction feedstock, with lower losses of extraction solvent/reagents and products due to lower retention of liquids with flax shive solid particles.

The first step extraction of flax shive using sodium ethoxide catalyst in anhydrous ethanol solution was investigated separately at small-scale (Chapter 6). The mass yield of solvent-soluble organics varied linearly with the concentration of sodium ethoxide, and was significantly higher than for extraction by aqueous NaOH. At a concentration of 1.0 M sodium ethoxide, the yield was found to be 54.5 ± 14.5 mg/g (db). At the same time, yield was not affected by flax shive particle-size over the size-range tested. Analyses using ^{1}H NMR consistently showed the extracts to be phenolic in nature, and to contain no carbohydrate constituents.

The second step extraction of hemicellulose polysaccharides using aqueous NaOH was also investigated at small-scale, including the effects of pretreatment (Chapter 7). The mass yield of carbohydrate precipitates using 1.0 M NaOH was found to be consistent, 99.4 ± 5.1 mg/g (db), and unaffected by pretreatment. Analyses of polysaccharide backbone monomers using HPLC showed extracts using 1.0 M NaOH, with or without pretreatment, to have consistently high ratios of xylose-to-glucose molar concentrations, 25.5 ± 3.4, with no mannose present. These results suggested a high

concentration of glucuronoxylan polymer, estimated to be more than 90% by mass, and no glucomannan polymer present.

The overall process was evaluated in terms of economic, market and policy aspects (Chapter 8). Evaluation of a range of biomass feedstocks available within Manitoba, showed flax shive to be the most desirable to consider for high-value product recovery. This also tied directly to government polices to increase value-add from agricultural materials. The economics of two-stage extraction of high-value products appeared to be potentially attractive, at least on a preliminary basis. A conceptual full-scale facility would require less than 15% of flax shive currently generated in the Province, not stressing feedstock availability. In order to be economically viable, a calculated average price of less than $5 per kg was necessary for the recovered products.

Additional investigations were undertaken at a larger-bench scale in order to evaluate compositional changes through the course of the process, and to corroborate the results of small-scale experiments (Chapter 9). In these experiments the relative content of hemicellulose was found to be consistently and significantly reduced using 1.0 M aqueous NaOH extraction (i.e., 25% to 29% reduction), whether with or without pretreatment. The calculated yield of recovered hemicellulose was not as high as for small-scale experiments, but this may have been due to identified mass transfer limitations. Also in these tests, the relative content of lignin was found to be increased by all of the extraction procedures undertaken. This was problematic, given that for all of the tested conditions, it was anticipated that lignin content would be reduced, particularly

regarding treatment using sodium ethoxide in anhydrous ethanol. Further testing was recommended to resolve this apparent inconsistency.

Flax shive was found to be a suitable feedstock for the recovery of high-value bio-products. Good yields of separate product streams, each with consistent composition, were achieved for individual extraction steps. The two extraction steps were also found to not interfere with each other, either in terms of product mass yield or product composition. Based on these initial results, further development of the two-step flax shive extraction process is warranted.

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Appendix A: Supplementary Data for Chapter 6

Supplementary Data included with published paper: Parsons, R.V., K.A.L. Parsons, and J.L. Sorensen. 2011. Extraction of Flax Shive using Sodium Ethoxide Catalyst in Anhydrous Ethanol. *Industrial Crops and Products* 34: 1245-1249.

A.1 Details of Methods

A.1.1 Flax Shive and Moisture Content

Flax shive was from the decortication plant of Schweitzer-Mauduit Canada Inc., located near Carman, Manitoba. No further grinding or size reduction was undertaken, but flax shive was pre-separated to remove waste short-fibre and screen-fractionated by E-Mission Free Inc. of Winnipeg, Manitoba. Two fractions were provided for testing: (i) 1.0 mm flax shive, passing through approximately 10 mm nominal opening and retained on 1.0 mm nominal opening; and (ii) 0.5 mm flax shive, passing through 1.0 mm nominal opening and retained on 0.5 mm nominal opening. Moisture contents of both fractions (wet basis) were determined using ASAE standard S358.2 (ASAE, 2004), with three replicate tests each. The flax shive fractions were stored in sealed plastic bags in a cool, dry location until used.

A.1.2 Flax Shive Extraction Procedures

Sodium ethoxide (CH_3CH_2ONa) catalyst (Aldrich) was mixed with anhydrous ethanol (Commercial Alcohols) to four different concentrations: 0.2 M; 0.5 M; 0.7 M; and 1.0 M. Extraction tests using approximately 1 g samples were undertaken for both 1.0 mm flax shive and 0.5 mm flax shive fractions at each concentration, in each case using 20 mL of catalyst solution in a closed beaker, at room temperature (approximately 20ºC) and stirred constantly for a 1 hour reaction period. At the end of this time, the

solution was vacuum-filtered via a Buchner filter (Whatman No. 1 paper) to retain flax shive solids, with the flax shive rinsed with 100 mL of additional fresh anhydrous ethanol. Filtrate was adjusted to about pH 3 using concentrated HCl, and then evaporated. Remaining residuals were rinsed into a separatory funnel using 50 mL dichloromethane (reagent grade) and 30 mL high purity water (Millipore Q). The solvent phase was retained, rinsed a second time using 30 mL high purity water, and a third time using 30 mL saturated NaCl solution in high purity water. Aqueous phases were discarded. Excess Na_2SO_4 was added to the final solvent phase to absorb water, the solution filtered and evaporated, residuals transferred using additional dichloromethane to a pre-weighed vial, and finally evaporated and weighed to determine recovered mass. Three replicate tests were undertaken for each set of conditions.

NaOH (Fisher Scientific) was dissolved in high purity water to a concentration of 1.0 M. Extraction tests, again using approximately 1 g samples, were undertaken for both 1.0 mm flax shive and 0.5 mm flax shive fractions, using 20 mL of NaOH solution, which was mixed for a 1-hour reaction period. Samples extracted at room temperature were contained in a closed beaker, while samples extracted at 100ºC were placed in a 50 mL round-bottom flask in a heated sand bath with reflux condenser. Timing for the reaction period in the latter case was from the first indication of condensation. At the end of the reaction period, the solution was filtered under vacuum via a Buchner filter (Whatman No. 1 paper), with the flax shive rinsed sequentially with a small amount of high purity water (up to about 20 mL) followed by 100 mL of 95% ethanol (Commercial

Alcohols). All remaining steps were the same as above. Three replicate tests were also undertaken for each set of conditions.

A.1.3 Model Compound Reactions

Three model compounds, all guaiacyl in nature, were tested for reaction with sodium ethoxide catalyst in anhydrous ethanol at room temperature. 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 esters of the two acids were selected as starting materials to show potential ester change.

Ferulic acid was obtained from Aldrich and converted to methyl ferulate via acidic methanolysis. Approximately 0.002 moles of ferulic acid were reacted for a two-day period in a solution of 19 mL methanol and 1 mL HCl. The product solution was rinsed into a separatory funnel using 40 mL of dichloromethane and washed twice with 30 mL of high purity water, followed by 30 mL of saturated NaCl in high purity water. The separated solvent phase was evaporated to recover converted methyl ferulate. Both methyl vanillate and acetovanillone were obtained directly from Aldrich.

Separately for each of the three model compounds, approximately 0.002 moles of sample were placed in a round-bottom reaction flask with 50 mL of 1.0 M sodium ethoxide catalyst in anhydrous ethanol for 1 hour at room temperature, mixing constantly. At the end of this period, the solution was acidified using HCl to react with any remaining catalyst, and evaporated to dryness. Remaining procedures for the residuals were the same as for the flax shive extracts.

A.1.4 Chemical Analyses

For all of the model compounds, the extent of reactions and associated formation of product compounds were monitored through the course of conversions by thin film chromatography (TLC), using a combination of dichloromethane and methanol as solvents. The initial compounds, intermediates, and final products were all analyzed to assess chemical characteristics, using ^{1}H nuclear magnetic resonance (NMR) on a Bruker Avance 300 MHz system. Deuterated chloroform was used as solvent for all determinations except ferulic acid, for which deuterated water was employed instead. Given the use of relatively pure compounds (i.e., single reactant and single target product), the ^{1}H NMR analyses were used quantitatively to estimate the extent of conversions achieved for model compounds by comparing integration areas for hydrogens associated with ethyl esters to hydrogens associated with the aromatic ring structures.

For the flax shive extractions, samples were analyzed using ^{1}H NMR for chemical characteristics using the same system. One extract sample was selected for analysis for each of the eight different processing conditions using sodium ethoxide catalyst in ethanol, and for each of the four different processing conditions using sodium hydroxide. All flax shive extract samples were dissolved in deuterated chloroform as solvent. The ^{1}H NMR scans for derived compounds were interpreted using Ralph et al. (2001).

Table A.1 Wet-basis moisture content data for all flax shive tests.

Sample Material	Replicate	Moisture Content (mg/g wb)
1.0 mm Flax Shive Fraction	1	78.3
	2	76.0
	3	74.0
Mean		76.1
Standard Deviation		2.2
Coefficient of Variation		2.8%
0.5 mm Flax Shive Fraction	1	82.3
	2	80.7
	3	78.9
Mean		80.7
Standard Deviation		1.7
Coefficient of Variation		2.1%

A.2 Details of Results

A.2.1 Moisture Content

Moisture content on a wet basis (wb) for all tested flax shive samples are provided in Table A.1 (supplementary materials), with each of three replicates presented. On a wet basis (wb), the moisture content of 1.0 mm flax shive averaged 76.1 mg/g, 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; $t_4 = 2.9$, $P < 0.05$). These moisture content estimates were used to adjust organics recovery to a dry-mass basis (db).

A.2.2 Quantitative Organics Recovery

Results for the quantitative yield of solvent-soluble organics from flax shive (db) are presented in Table A.2 for each replicate test in the order undertaken. Summary quantitative yield results are presented in Table A.3, with mean and standard deviation values presented for each set of test conditions.

For sodium ethoxide catalyst in ethanol, the yield appeared to increase in a linear manner as catalyst concentration was increased, but to be largely unaffected by changes in particle size. Use of analysis of covariance (ANCOVA; Kutner et al. 2005) confirmed that yields did not differ significantly between particle size fractions (i.e., $F_{1,20} = 2.24$, $P = 0.15$), with results for the ANCOVA presented in Table A.4.

Given the lack of significant difference due to particle size changes, all data points for sodium ethoxide in ethanol were combined into a single linear regression,

Table A.2 Solvent-soluble organics recovery data for all flax shive tests, presented in the order undertaken.

Reagent	Concentration (M)	Temperature (ºC)	Fraction	Yield (mg/g db)
Sodium ethoxide catalyst in anhydrous ethanol	1.0	20	1.0 mm	47.3
	1.0	20	0.5 mm	60.8
	0.5	20	1.0 mm	26.2
	0.5	20	0.5 mm	29.8
	0.5	20	1.0 mm	38.9
	0.5	20	0.5 mm	36.0
	0.7	20	1.0 mm	39.7
	0.7	20	0.5 mm	51.1
	0.7	20	1.0 mm	49.4
	0.7	20	0.5 mm	56.8
	0.2	20	1.0 mm	15.4
	0.2	20	0.5 mm	16.5
	0.2	20	1.0 mm	16.5
	0.2	20	0.5 mm	15.9
	1.0	20	1.0 mm	64.3
	1.0	20	0.5 mm	74.9
	0.5	20	0.5 mm	32.9
	0.5	20	1.0 mm	31.7
	0.2	20	1.0 mm	15.3
	0.2	20	0.5 mm	13.3

Table A.2 Solvent-soluble organics recovery data for all flax shive tests, presented in the order undertaken (continued).

Reagent	Concentration (M)	Temperature (ºC)	Fraction	Yield (mg/g db)
Sodium ethoxide catalyst in anhydrous ethanol	1.0	20	1.0 mm	37.2
	1.0	20	0.5 mm	42.5
	0.7	20	0.5 mm	21.5
	0.7	20	1.0 mm	23.9
Sodium hydroxide in water	1.0	20	1.0 mm	1.9
	1.0	20	1.0 mm	1.2
	1.0	20	0.5 mm	1.3
	1.0	100	1.0 mm	2.4
	1.0	20	0.5 mm	0.4
	1.0	100	0.5 mm	1.3
	1.0	100	1.0 mm	6.2
	1.0	100	0.5 mm	2.6
	1.0	100	1.0 mm	1.6
	1.0	100	0.5 mm	3.6
	1.0	20	1.0 mm	2.1
	1.0	20	0.5 mm	1.4

Table A.3 Summary of quantitative yield of solvent-soluble organics recovered from flax shive under varying conditions, with each mean and standard deviation based on three replicate measurements.

Reagent	Conc. (M)	Temp. (ºC)	Yield (mg/g db)	
			1.0 mm Fraction	0.5 mm Fraction
Sodium ethoxide in ethanol	0.2	20	15.7 ± 0.7	15.3 ± 1.7
	0.5	20	32.3 ± 6.4	32.9 ± 3.1
	0.7	20	37.7 ± 12.9	43.1 ± 18.9
	1.0	20	49.6 ± 13.7	59.4 ± 16.2
Sodium hydroxide in water	1.0	20	1.7 ± 0.5	1.1 ± 0.5
	1.0	100	3.4 ± 2.4	2.5 ± 1.1

Table A.4 ANCOVA for effect of flax shive particle size on the extraction yield of solvent-soluble organics with varying concentration of sodium ethoxide catalyst in ethanol.

Source	df	SS	MS	F-Value	P-Value
Particle Size	1	234.2	234.2	2.24	0.15
Concentration (covariate)	1	4739.6	4739.6	45.3	< 0.001
Interaction	1	93.49	93.49	0.89	0.36
Error	20	2091.1	104.56		

Notes: df = degrees of freedom; SS = sums of squares; MS = mean square (= SS/df); F = calculated F statistic; P = associated probability value, with P ≦ 0.05 as assumed requirement for significance.

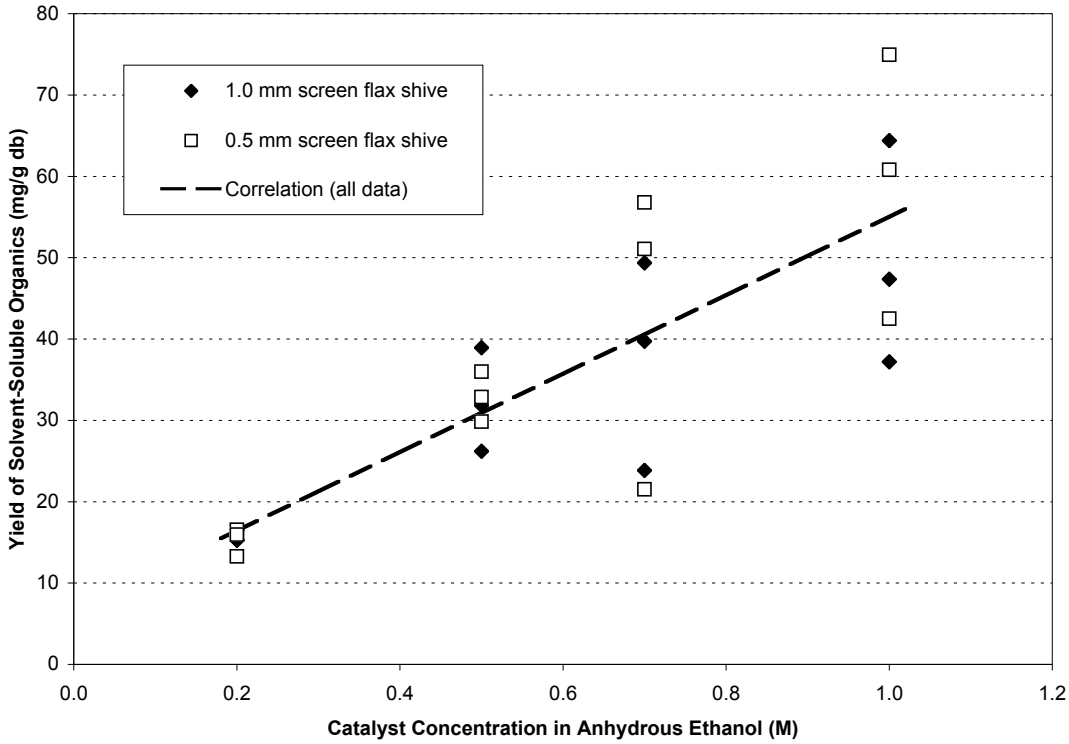

Figure A.1 Linear regression of all data for yield of solvent-soluble organics as a function of the concentration of sodium ethoxide catalyst in ethanol.

Table A.5 ANOVA of flax shive particle size and reaction temperature effects on the extraction yield of solvent-soluble organics using aqueous sodium hydroxide.

Source	df	SS	MS	F-Value	P-Value
Particle Size	1	1.84	1.84	0.95	0.36
Temperature	1	7.27	7.27	45.3	0.09
Interaction	1	0.03	0.03	0.89	0.67
Error	8	15.55	1.94		

Notes: df = degrees of freedom; SS = sums of squares; MS = mean square (= SS/df); F = calculated F statistic; P = associated probability value, with P ≦ 0.05 as assumed requirement for significance.

illustrated in Figure A.1 and with resulting $r^2 = 0.68$. The mean yield for all tests conducted at a catalyst concentration of 1.0 M was 54.5 ± 14.5 mg/g (db) for n = 6. The predicted value at 1.0 M concentration according to the aforementioned linear regression was 55.0 mg/g (db), essentially consistent with the observed mean yield.

For sodium hydroxide in water, yields were all relatively low but were slightly higher for the reaction at 100°C compared to 20°C. Analysis of variance (ANOVA; Weiss, 2006), with summary results presented in Table A.5, however, showed for sodium hydroxide that there was no significant effect due to particle size ($F_{1,8} < 1$), reaction temperature ($F_{1,8} = 3.74$, $P > 0.09$), or interaction of the two factors ($F_{1,8} < 1$). In all cases the critical $F_{1,8}$ value was 5.32, which was not achieved in any case. As such, all data points for extraction using sodium hydroxide in water were combined. Thus, the mean yield for all tests conducted at 1.0 M was 2.2 ± 1.5 mg/g (db) for n = 12.

Overall, the extraction yield results obtained using sodium ethoxide in ethanol were significantly higher at all catalyst concentrations than for aqueous sodium hydroxide at 1.0 M. Comparing results at 1.0 M concentration in particular showed the yield using sodium ethoxide in ethanol to be approximately twenty-five times higher.

A.2.3 Chemical Nature of Flax Shive Products

The ^{1}H NMR spectra obtained for flax shive extraction samples under all conditions are presented in Figures AA.1 through AA.12, summarized in Table A.6. The all cases, the spectra suggested the presence of aromatic ring structures in the compounds, with peaks in the range of 6.8 ppm to 7.6 ppm in all cases. This confirmed the likely phenolic character and lignin origin of the extracts. The spectra also all

Table A.6 Summary of ^{1}H NMR spectra for all flax shive extraction samples.

Reference	Description of Extract Sample
Figure AA.1	Sodium ethoxide in ethanol, 0.2 M; 20ºC; 1.0 mm flax shive
Figure AA.2	Sodium ethoxide in ethanol, 0.2 M; 20ºC; 0.5 mm flax shive
Figure AA.3	Sodium ethoxide in ethanol, 0.5 M; 20ºC; 1.0 mm flax shive
Figure AA.4	Sodium ethoxide in ethanol, 0.5 M; 20ºC; 0.5 mm flax shive
Figure AA.5	Sodium ethoxide in ethanol, 0.7 M; 20ºC; 1.0 mm flax shive
Figure AA.6	Sodium ethoxide in ethanol, 0.7 M; 20ºC; 0.5 mm flax shive
Figure AA.7	Sodium ethoxide in ethanol, 1.0 M; 20ºC; 1.0 mm flax shive
Figure AA.8	Sodium ethoxide in ethanol, 1.0 M; 20ºC; 0.5 mm flax shive
Figure AA.9	Sodium hydroxide in water, 1.0 M; 20ºC; 1.0 mm flax shive
Figure AA.10	Sodium hydroxide in water, 1.0 M; 20ºC; 0.5 mm flax shive
Figure AA.11	Sodium hydroxide in water, 1.0 M; 100ºC; 1.0 mm flax shive
Figure AA.12	Sodium hydroxide in water, 1.0 M; 100ºC; 0.5 mm flax shive

suggested the absence of any carbohydrate compounds. Carbohydrates produce a number of signature peaks in the range of 3 ppm to 5 ppm (Cui, 2005), but with key peaks in the range of 4.3 to 4.8 ppm, which were absent in all cases.

The ^{1}H NMR spectra for the sodium hydroxide extracts showed essentially the same overall set of signals under different conditions. This confirmed there were no significant differences in chemical makeup due to changes is particle size or reaction temperature. Interpretations of these spectra are presented in Table A.7. There were seven major groups of peaks present. The spectra suggested the presence of aromatic ring structures as noted earlier, aldehyde groups (peaks around 9.8 ppm), non-aromatic double bonds (peaks around 5.4 ppm), methyl-ether groups (peaks around 4.0 ppm), ketone groups (peaks around 2.5 ppm), and methyl-substituted aromatic rings (peaks around 2.3 ppm). These spectral characteristics were consistent with compounds like vanillin and acetovanillone that would be expected to be present in sodium hydroxide extracts.

The ^{1}H NMR spectra for the extracts using sodium ethoxide in ethanol showed essentially the same overall set of signals under different conditions. This confirmed there were no significant differences in chemical makeup in this case due to changes in particle size or catalyst concentration. Interpretations of these spectra are presented in Table A.8. There were eight major groups of peaks present. The spectra suggested the presence of aromatic ring structures as noted earlier, ethyl esters (combination of peaks around 1.3 ppm and 4.2 ppm), ketone groups (peaks around 2.5 ppm), and methyl-substituted aromatic rings (peaks around 2.3 ppm).

Table A.7 Interpretation of ^{1}H NMR peak cluster results for flax shive extracts using aqueous sodium hydroxide.

Group	^{1}H NMR Spectra Feature	Interpretation
1	Peak cluster from about 0.7 to 1.8 ppm with significant peaks around 0.9 ppm, 1.3 ppm and 1.7 ppm	Likely associated with saturated primary or secondary carbons, with 0.9 ppm being likely primary, 1.3 ppm being likely secondary. Note in this case there was no peak cluster around 4.2 ppm (associated with ethyl ester group together with peaks around 1.3 ppm) so not likely indicative of an ester.
2	Cluster of smaller peaks from 1.8 to 2.7 ppm with apparent peaks at 2.0 ppm, 2.1 ppm (small), 2.3 ppm, and 2.5 ppm (small)	Peak at 2.3 ppm may be associated with methyl group attachment on an aromatic ring. Peak at 2.5 ppm likely indicates a ketone group, as present in the case of acetovanillone.
3	Cluster of three peaks at about 3.6 ppm (smallest), 3.8 ppm and 4.0 ppm	Peak around 3.6 may be associated with hydroxyl group, and the two around 4.0 ppm appear related to methoxyl group, as present in the case of vanillin.
4	Small peak around 5.4 ppm	May be associated with hydrogen on beta carbon (double bond) on cinnamyl alcohol derivatives.
5	Small peak around 6.3 ppm	May be indicative of non-aromatic double bond (hydrogen on beta carbon) as present in ferulic acid, although it is a doublet peak. It more likely associated with hydroxyl group associated with 4-position on vanillin, present at about 6.4 ppm.
6	Peak cluster from 6.8 to 7.6 ppm with distinct major peak at 7.3 ppm	All likely associated with aromatic ring, but central peak at 7.26 associated with deuterated chloroform solvent (Gottlieb et al. 1997).
7	Significant peak around 9.8 ppm	Likely associated with aldehyde group.

Table A.8 Interpretation of ^{1}H NMR peak cluster results for flax shive extracts using sodium ethoxide catalyst in anhydrous ethanol.

Group	^{1}H NMR Spectra Feature	Interpretation
1	Peak cluster from 0.6 to 1.4 ppm with distinct major peak at 1.3 ppm (note overlapping)	Likely saturated primary or secondary carbons, with major peak at 1.3 ppm likely one of two associated with ethyl ester group.
2	Peak cluster from 1.5 to 2.5 ppm with distinct major peak at 2.3 ppm	Peak at 2.3 ppm likely methyl group attachment on an aromatic ring.
3	Distinct major peak at 2.6-2.7 ppm	Uncertain, although possibly associated with ketone. Ketone on acetovanillone is around 2.5 ppm.
4	Small peak around 3.5 ppm	Likely hydroxyl group.
5	Small peak cluster around 4.2 ppm (overlapping)	Likely one of two peak clusters associated with ethyl ester group.
6	Peak cluster from 6.7 to 6.9 ppm	Likely associated with aromatic ring.
7	Peak cluster from 7.0 to 7.6 ppm with distinct major peak at 7.3 ppm	Likely associated with aromatic ring, but central peak at 7.26 associated with deuterated chloroform solvent (Gottlieb et al. 1997).
8	Small peak around 8.1 ppm	Uncertain. Likely associated with aromatic ring, but not present for model compounds.

At the same time, these spectra were distinctly different from those obtained for sodium hydroxide extracts, suggesting that the chemical natures of products from the two methods were likely different. Key differences with the sodium ethoxide extracts, in addition to the presence of ethyl esters in the compounds, were the apparent absence of aldehyde groups, non-aromatic double bonds, and methyl-ether groups. This last observation was important in that it precluded the presence of guaiacyl or syringyl compounds.

A.2.4 Model Compound Reactions

The ^{1}H NMR spectra obtained for the model compound reactions are presented in Figures AB.1 through AB.7 , summarized in Table A.9.

The extents of reactions of methyl ferulate and methyl vanillate respectively were evaluated based on a comparison of NMR peak integration to assess the ratio of ethyl ester associated-hydrogen to aromatic ring associated-hydrogen, as follows:

- For the ethyl esters of both ferulic acid and vanillic acid, five hydrogen are associated with the ethyl ester involving two sets of ^{1}H NMR peaks, represented by a triplet around 1.3 ppm and a quadruplet around 4.0 ppm, while three hydrogen are associated with the aromatic ring structure, represented by peaks in the range of about 6.5 ppm to 7.6 ppm, although also noting the peak for deuterated chloroform solvent occurs around 7.3 ppm, needing to be excluded from consideration.
- For pure ethyl esters, representing full conversion, the ratio of respective integrated areas would be expected to be approximately 5 to 3 or 1.66 to 1.

- For the reacted methyl ferulate product, the ratio from the ^{1}H NMR spectra of product was approximately 1.16 to 1. Dividing by the expected ratio for pure ethyl ester, above, suggested a conversion of around 70%, i.e., 1.16 divided by 1.66.
- For the reacted methyl vanillate product, the ratio from the ^{1}H NMR spectra of product was approximately 0.23 to 1. Dividing by the expected ratio for pure ethyl ester, above, suggested a conversion of around 14%, i.e., 0.23 divided by 1.66.

As such, the reaction of methyl ferulate to ethyl ferulate using sodium ethoxide catalyst in ethanol had a high conversion, approximately 70% on a molar basis. Methyl vanillate, on the other hand, was poorly converted to ethyl vanillate, with a conversion of only about 14% on a molar basis under the same conditions. These results confirmed that transesterification reactions occurred, but there were substantial differences in conversion rates for the two acid esters.

The ^{1}H NMR spectra for products of methyl ferulate and methyl vanillate reactions were both found to contain peaks around 4.0 ppm. These confirmed the presence of methyl-ether groups on compounds in both cases.

There were effectively no differences in spectra for acetovanillone before and after exposure to the catalyst. Impacts on acetovanillone were also evaluated based on a comparison of NMR peak integration to assess any change in the ratio of methyl ether side-group associated-hydrogen to aromatic ring associated-hydrogen, as follows:

- For acetovanillone, three hydrogen are associated the methyl ether side-group, and three hydrogen are associated with the aromatic ring. As such the expected ratio is 3 to 3 or 1.00 to 1.

- The evaluated ratio for unreacted acetovanillone from the ^{1}H NMR spectra of the reactant was approximately 1.04 to 1. The evaluated ratio for reacted acetovanillone from the ^{1}H NMR spectra of the product was approximately 1.02 to 1. As such, there was essentially no difference.

As expected, acetovanillone was unaffected by the extraction process. For all model compounds the catalyst had no apparent effect on methyl-ether side-groups.

Table A.9 Summary of ^{1}H NMR spectra for model compound reactions.

Reference	Description of Model Compound Sample
Figure AB.1	Ferulic acid prior to methanolysis reaction
Figure AB.2	Methyl ferulate prior to reaction with sodium ethoxide
Figure AB.3	Methyl ferulate to ethyl ferulate reaction product
Figure AB.4	Methyl vanillate prior to reaction with sodium ethoxide
Figure AB.5	Methyl vanillate to ethyl vanillate reaction product
Figure AB.6	Acetovanillone prior to reaction with sodium ethoxide
Figure AB.7	Acetovanillone after reaction with sodium ethoxide

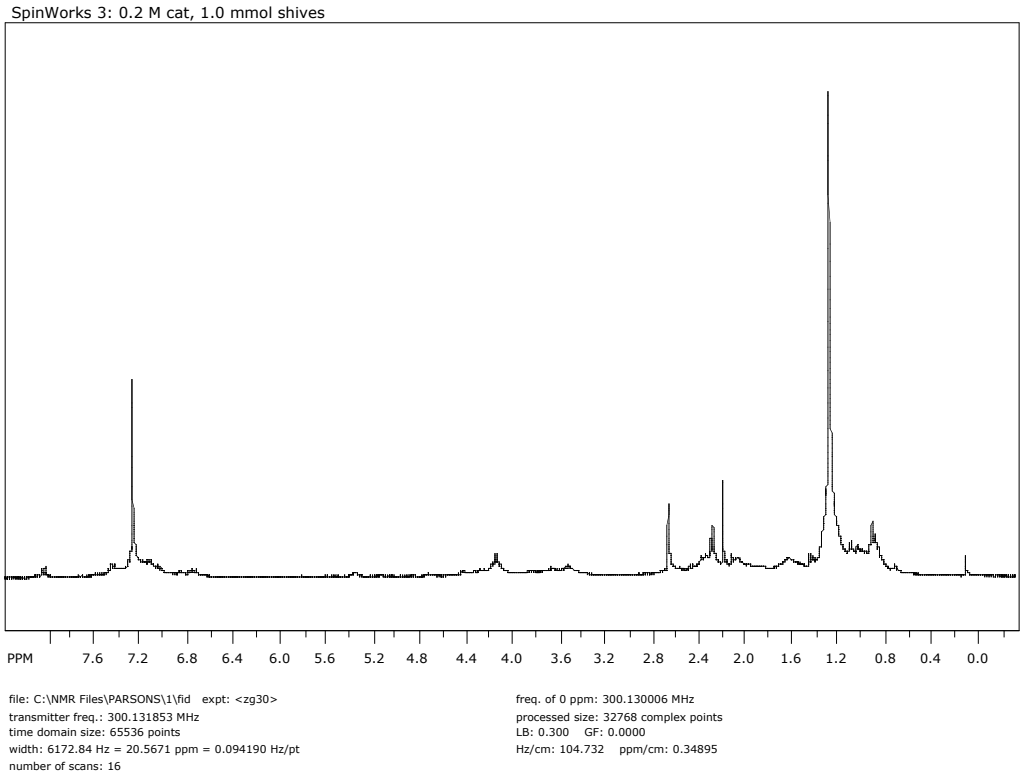

Figure AA.1 ^{1}H NMR results for extract sample: Sodium ethoxide in ethanol, 0.2 M; 20ºC; 1.0 mm flax shive.

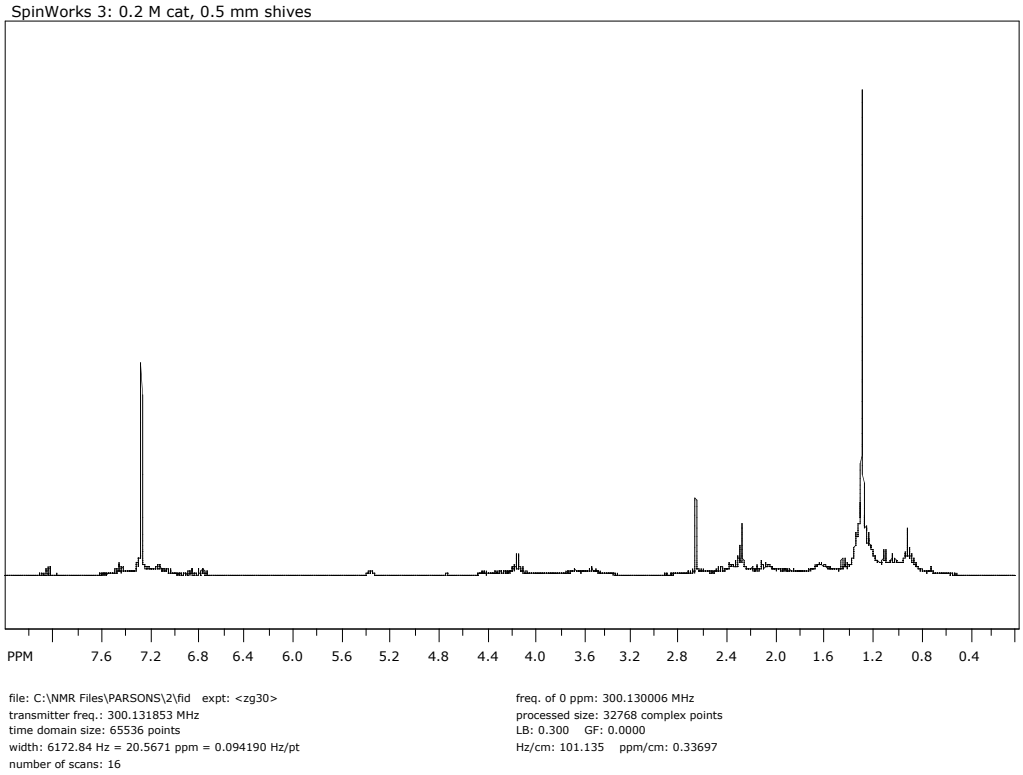

Figure AA.2 ^{1}H NMR results for extract sample: Sodium ethoxide in ethanol, 0.2 M; 20ºC; 0.5 mm flax shive.

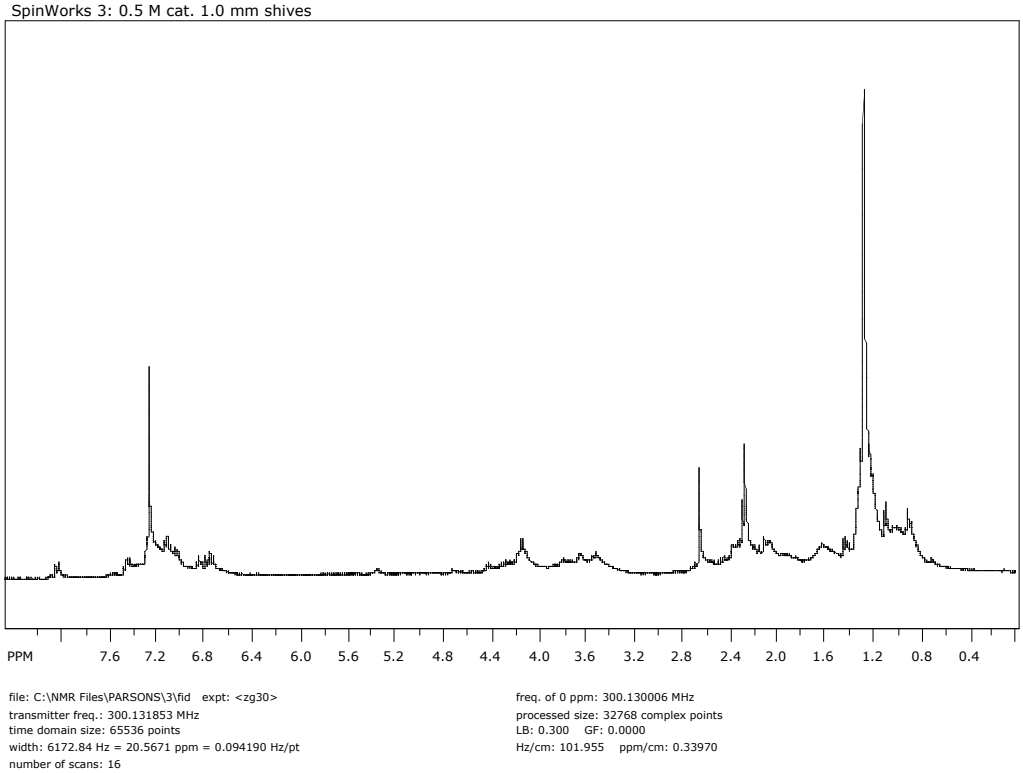

Figure AA.3 ^{1}H NMR results for extract sample: Sodium ethoxide in ethanol, 0.5 M; 20ºC; 1.0 mm flax shive.

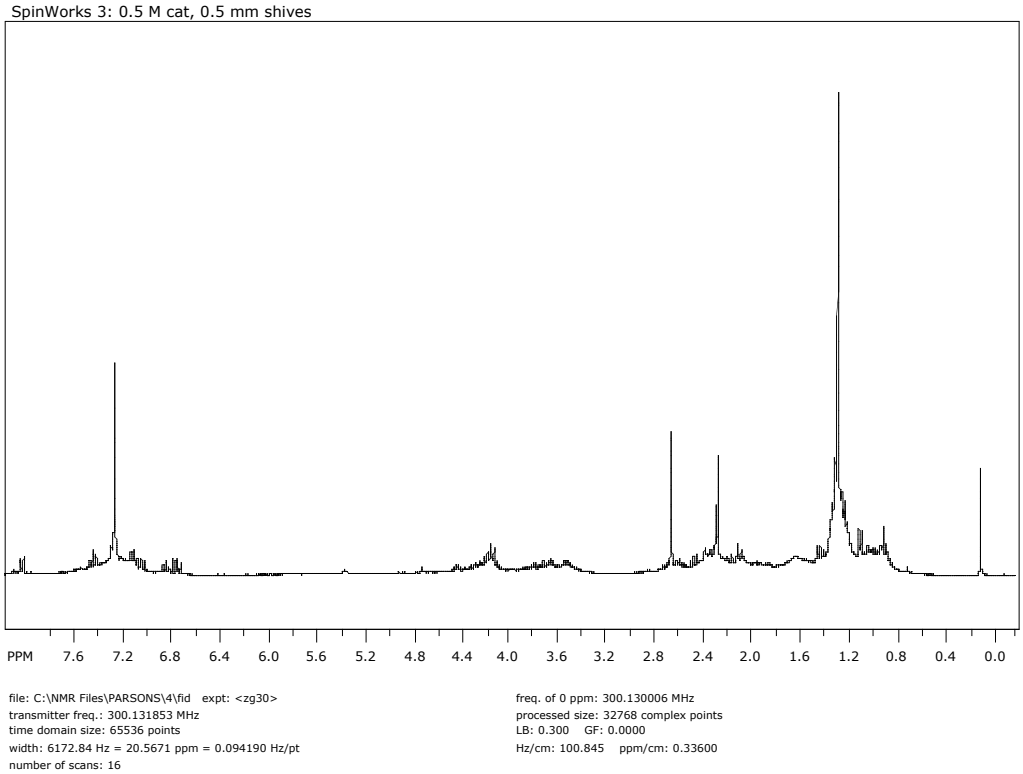

Figure AA.4 ^{1}H NMR results for extract sample: Sodium ethoxide in ethanol, 0.5 M; 20ºC; 0.5 mm flax shive.

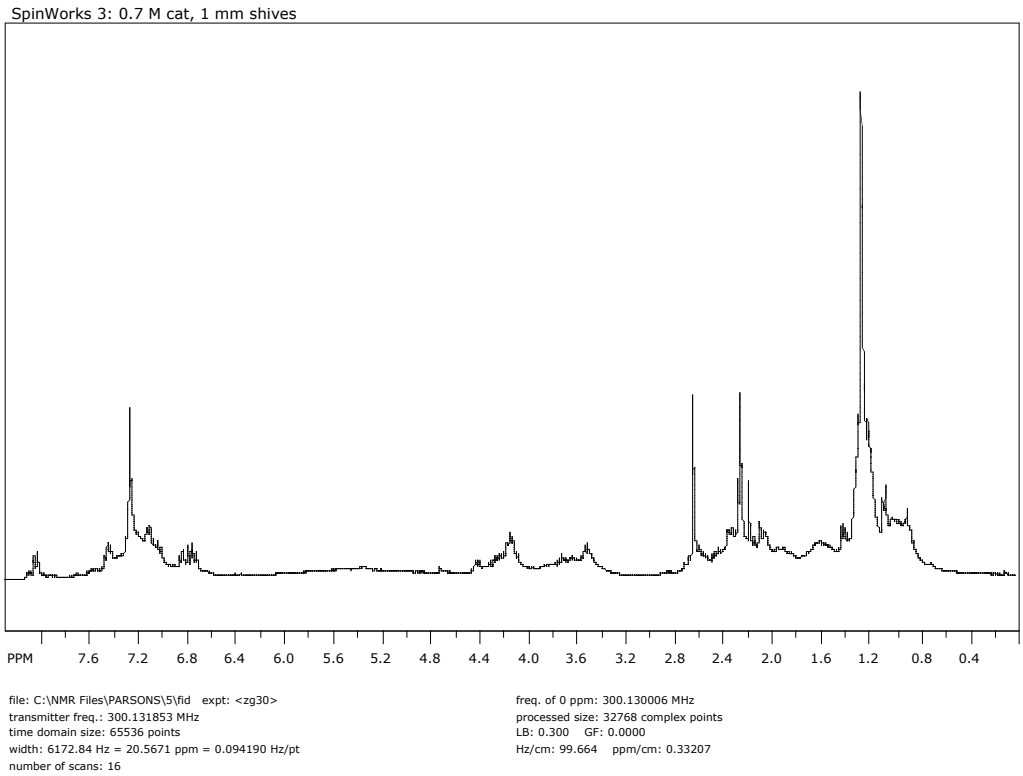

Figure AA.5 ^{1}H NMR results for extract sample: Sodium ethoxide in ethanol, 0.7 M; 20ºC; 1.0 mm flax shive.

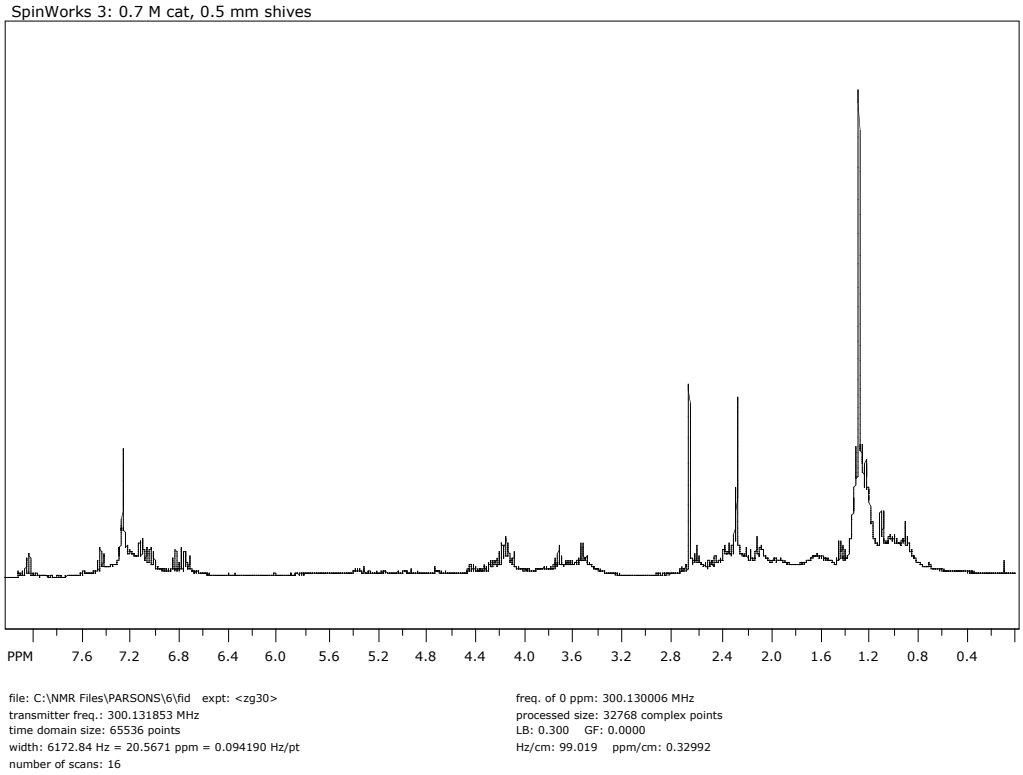

Figure AA.6 ^{1}H NMR results for extract sample: Sodium ethoxide in ethanol, 0.7 M; 20ºC; 0.5 mm flax shive.

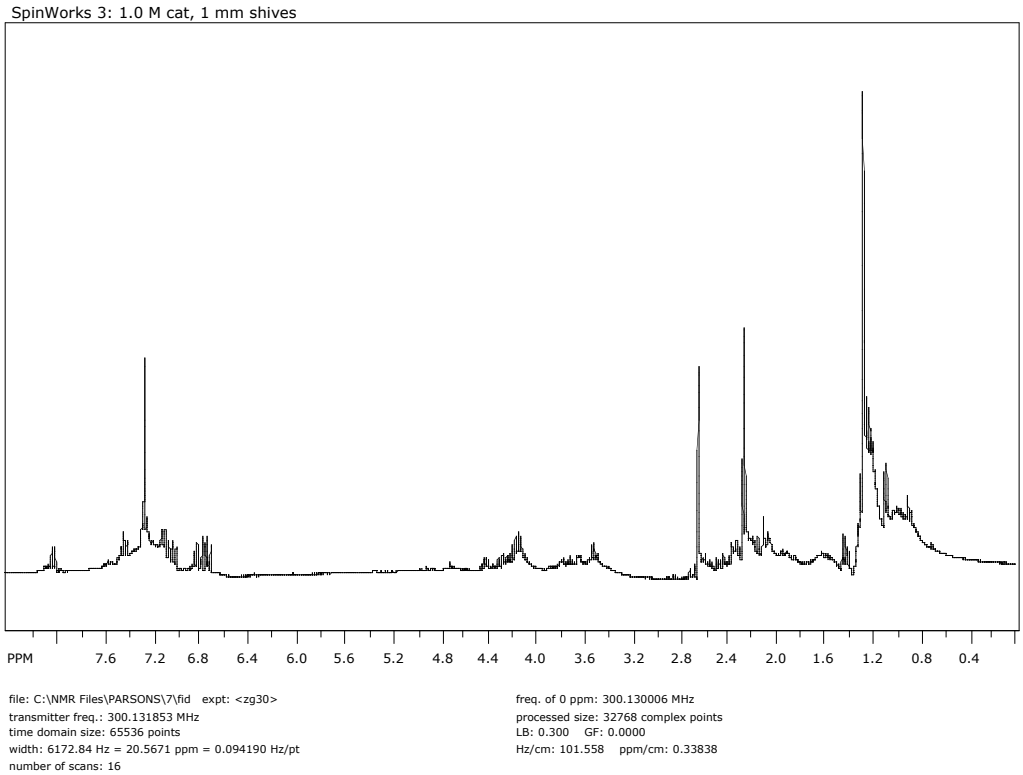

Figure AA.7 ^{1}H NMR results for extract sample: Sodium ethoxide in ethanol, 1.0 M; 20ºC; 1.0 mm flax shive.

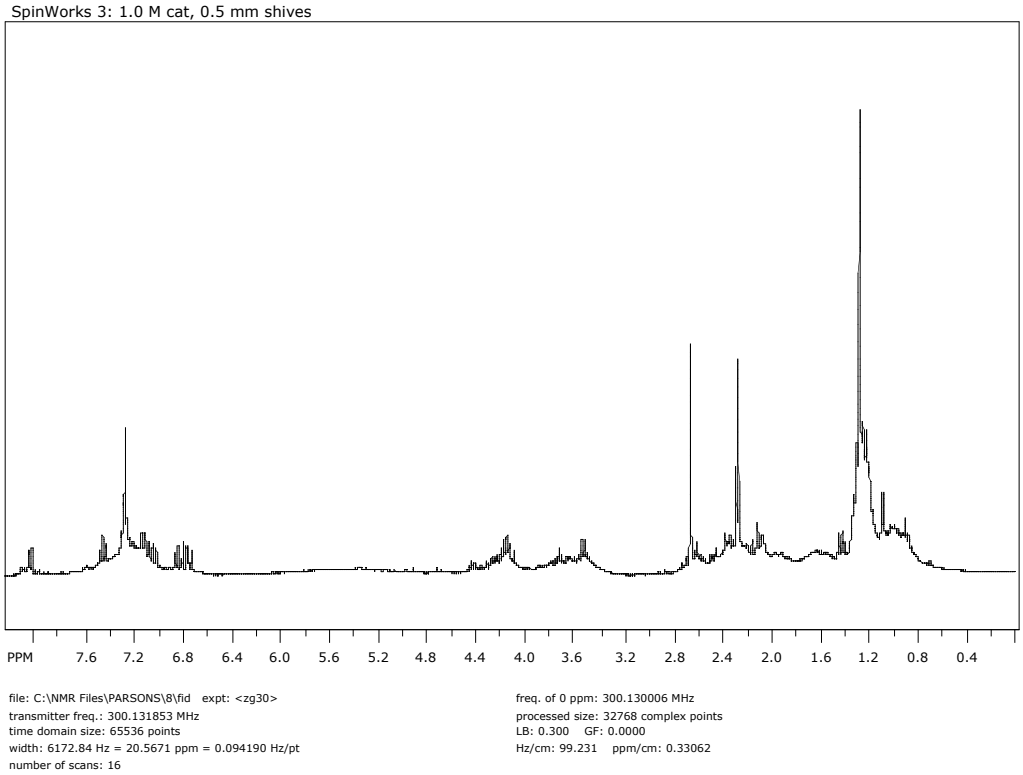

Figure AA.8 ^{1}H NMR results for extract sample: Sodium ethoxide in ethanol, 1.0 M; 20ºC; 0.5 mm flax shive.

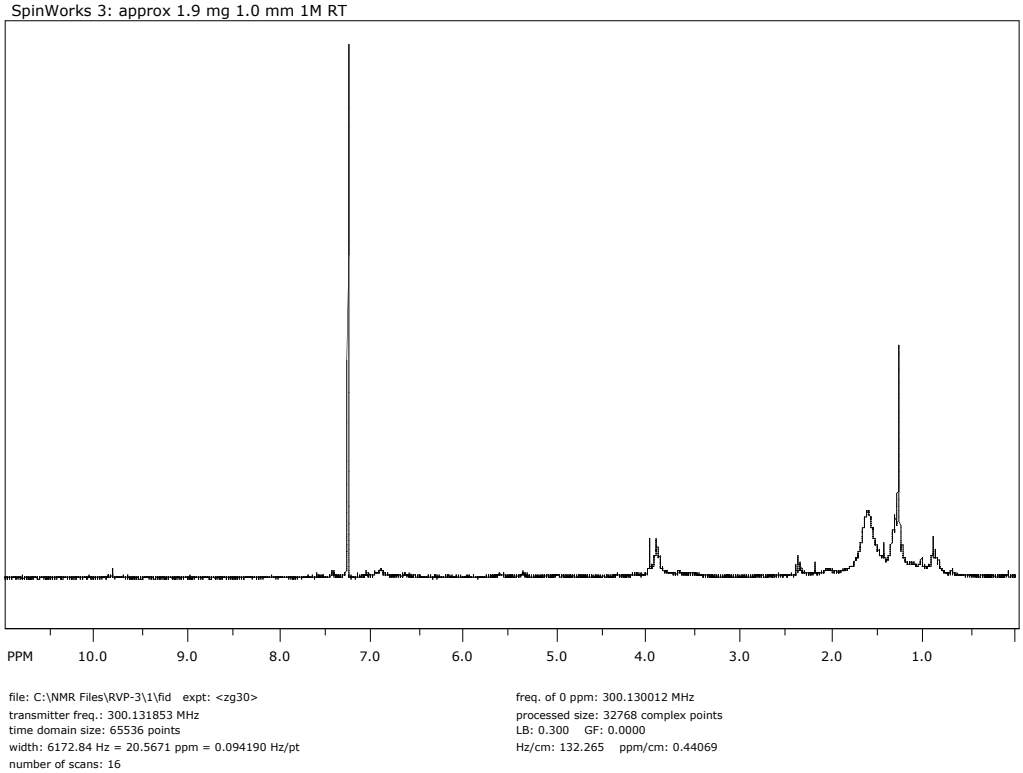

Figure AA.9 ^{1}H NMR results for extract sample: Sodium hydroxide in water, 1.0 M; 20ºC; 1.0 mm flax shive.

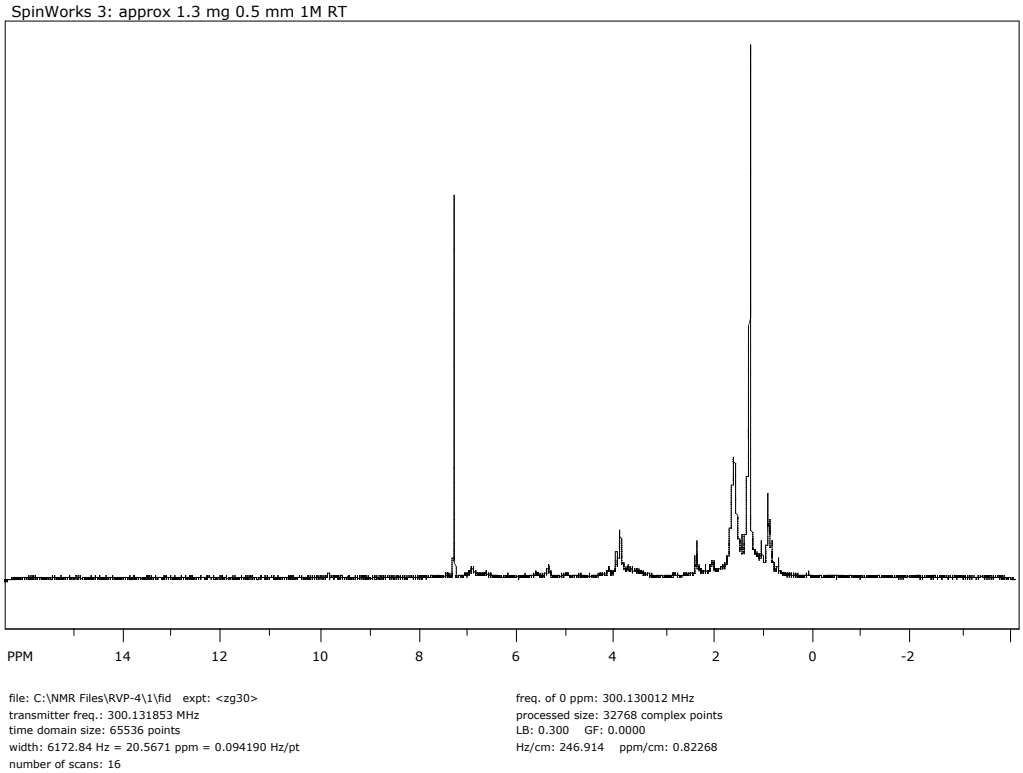

Figure AA.10 ^{1}H NMR results for extract sample: Sodium hydroxide in water, 1.0 M; 20ºC; 0.5 mm flax shive.

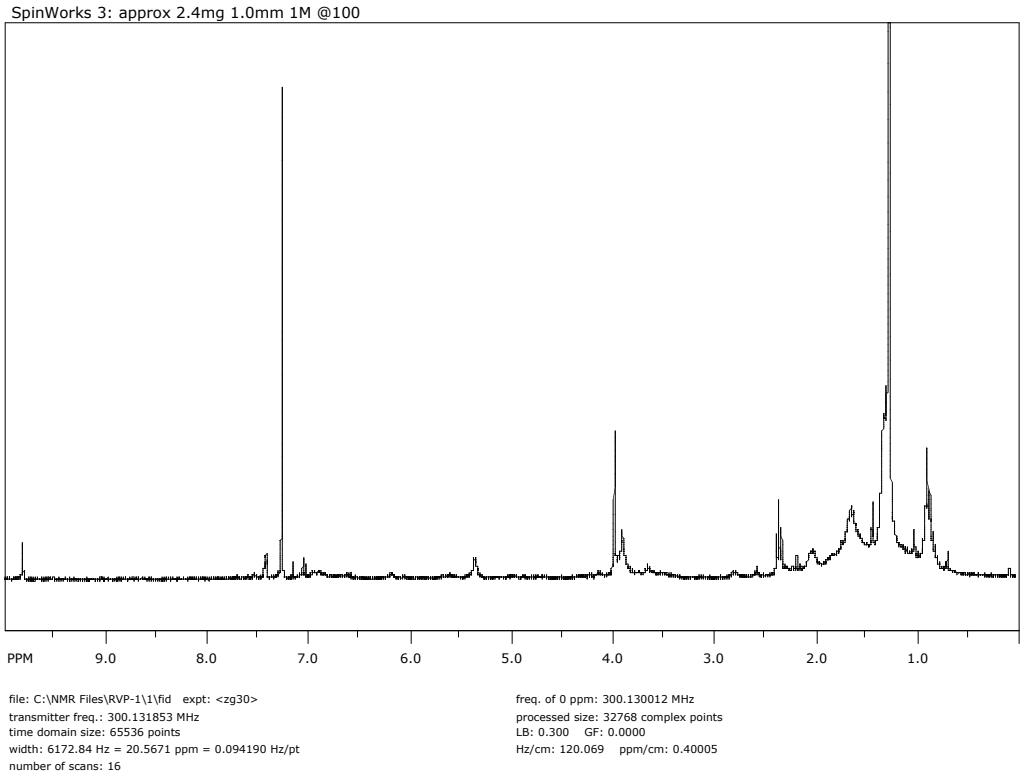

Figure AA.11 ^{1}H NMR results for extract sample: Sodium hydroxide in water, 1.0 M; 100ºC; 1.0 mm flax shive.

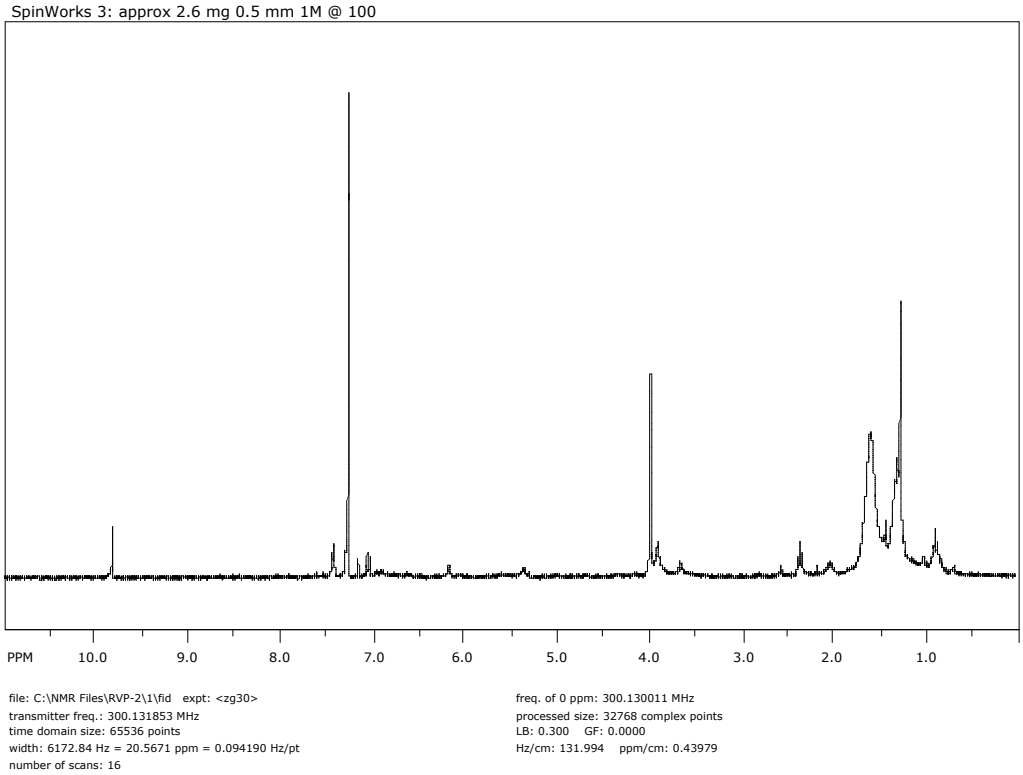

Figure AA.12 ^{1}H NMR results for extract sample: Sodium hydroxide in water, 1.0 M; 100ºC; 0.5 mm flax shive.

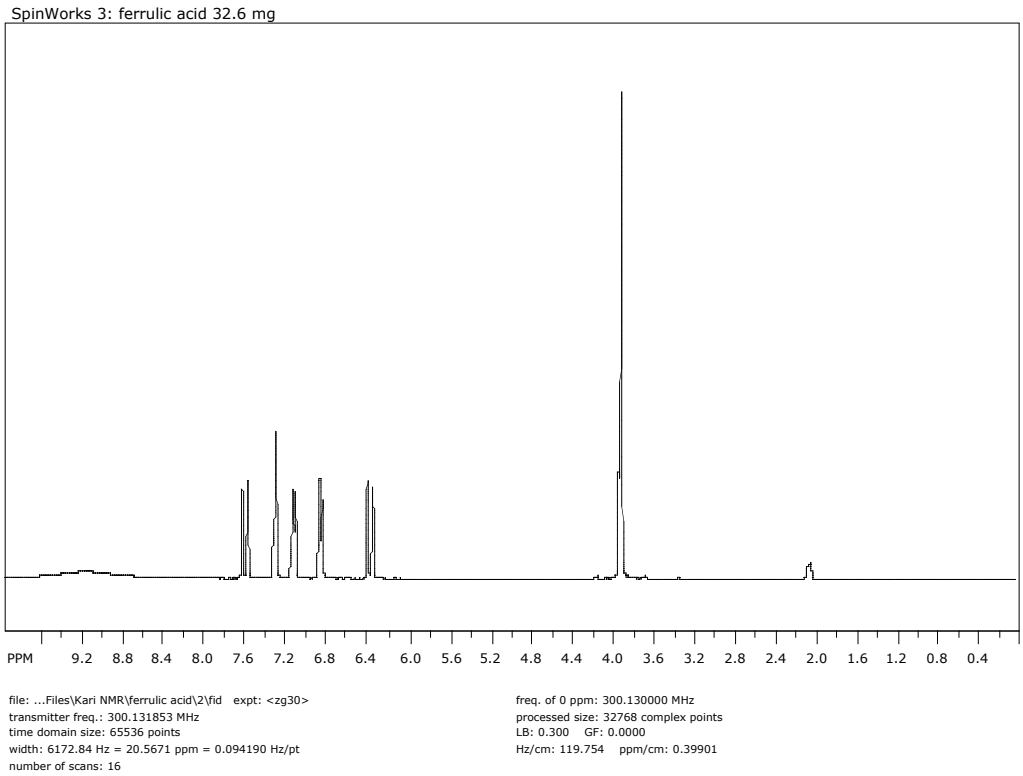

Figure AB.1 ^{1}H NMR results for model compound: Ferulic acid prior to methanolysis reaction.

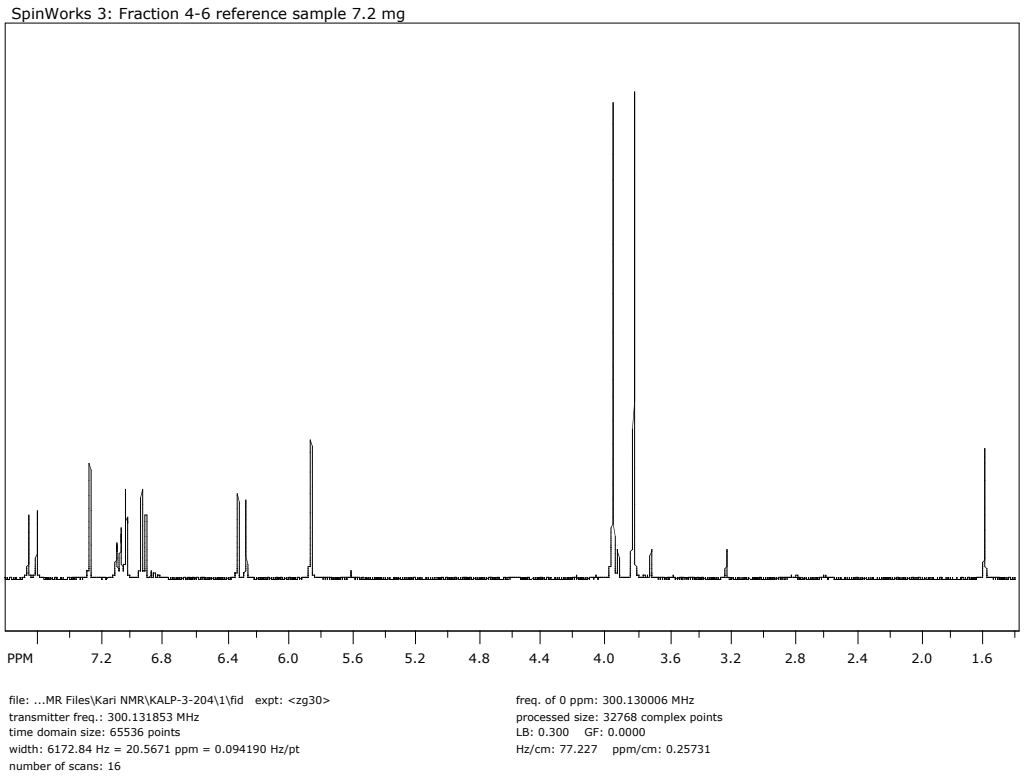

Figure AB.2 ^{1}H NMR results for model compound: Methyl ferulate prior to reaction with sodium ethoxide.

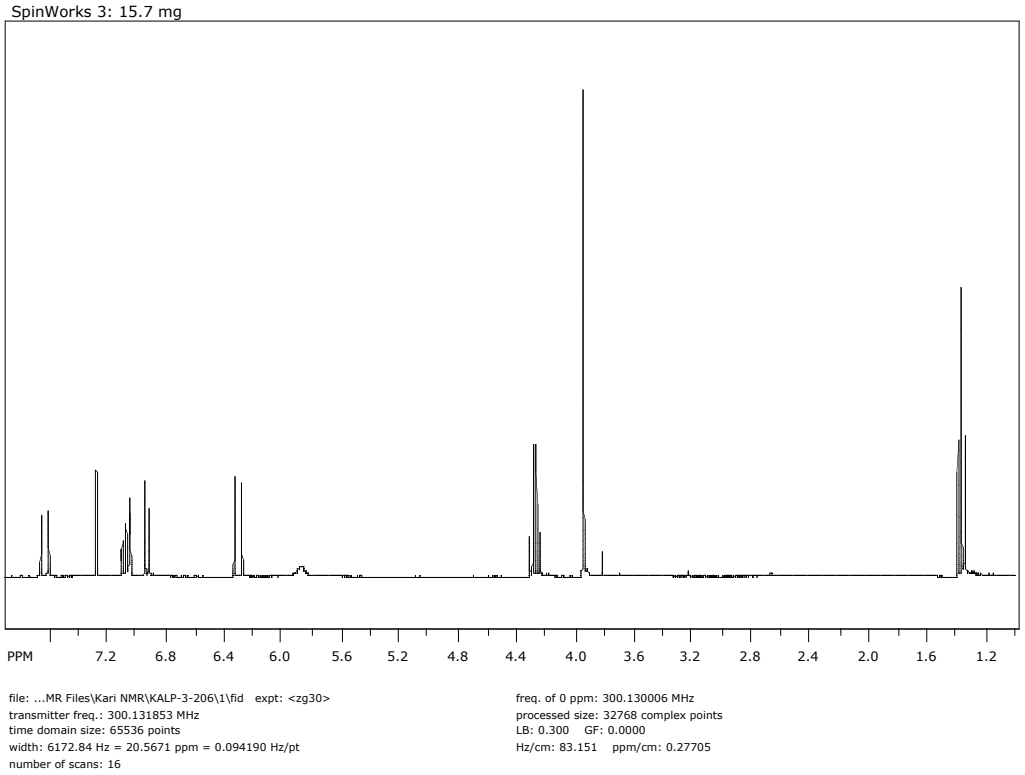

Figure AB.3 ^{1}H NMR results for model compound: Methyl ferulate to ethyl ferulate reaction product.

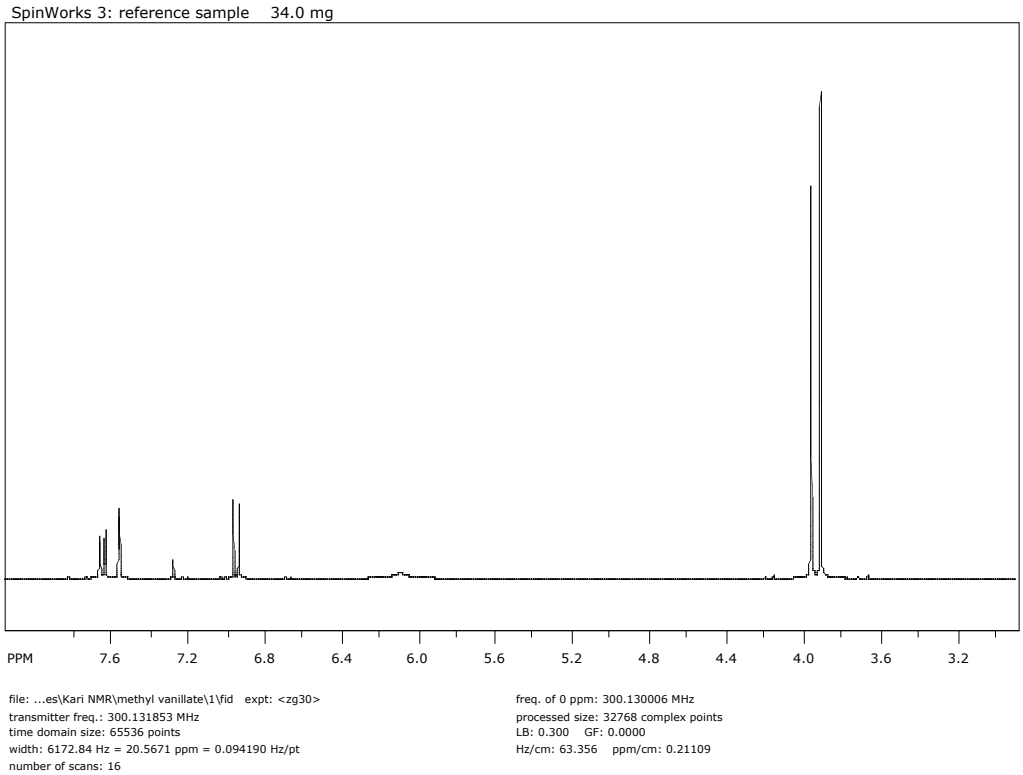

Figure AB.4 ^{1}H NMR results for model compound: Methyl vanillate prior to reaction with sodium ethoxide.

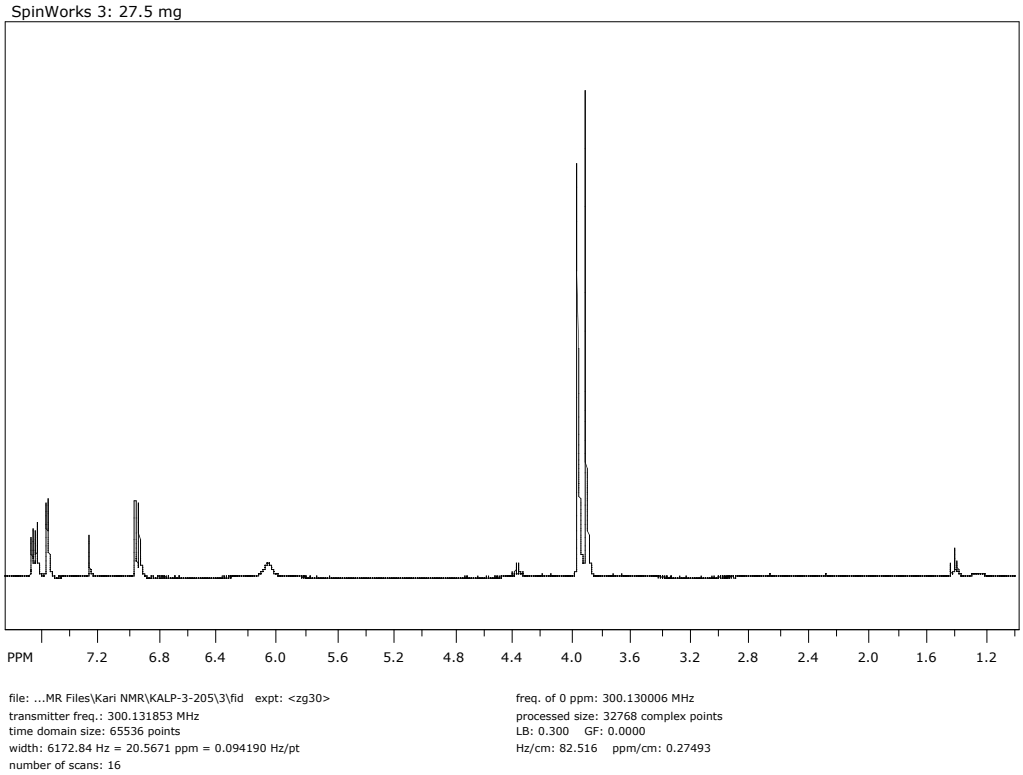

Figure AB.5 ^{1}H NMR results for model compound: Methyl vanillate to ethyl vanillate reaction product.

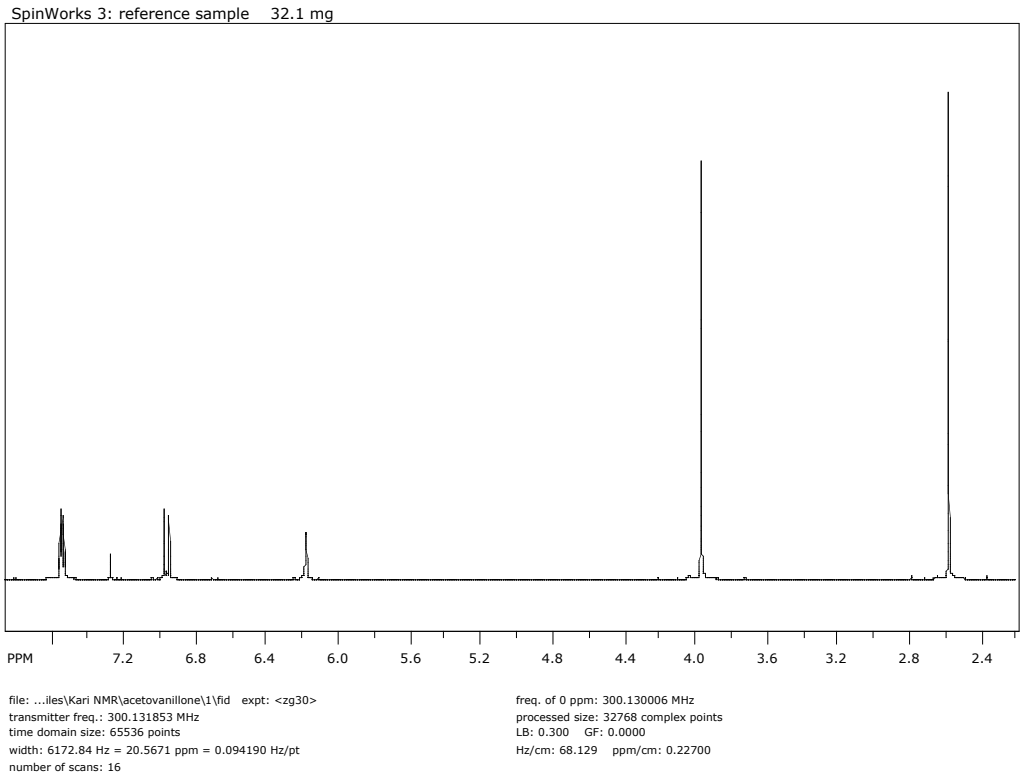

Figure AB.6 ^{1}H NMR results for model compound: Acetovanillone prior to reaction with sodium ethoxide.

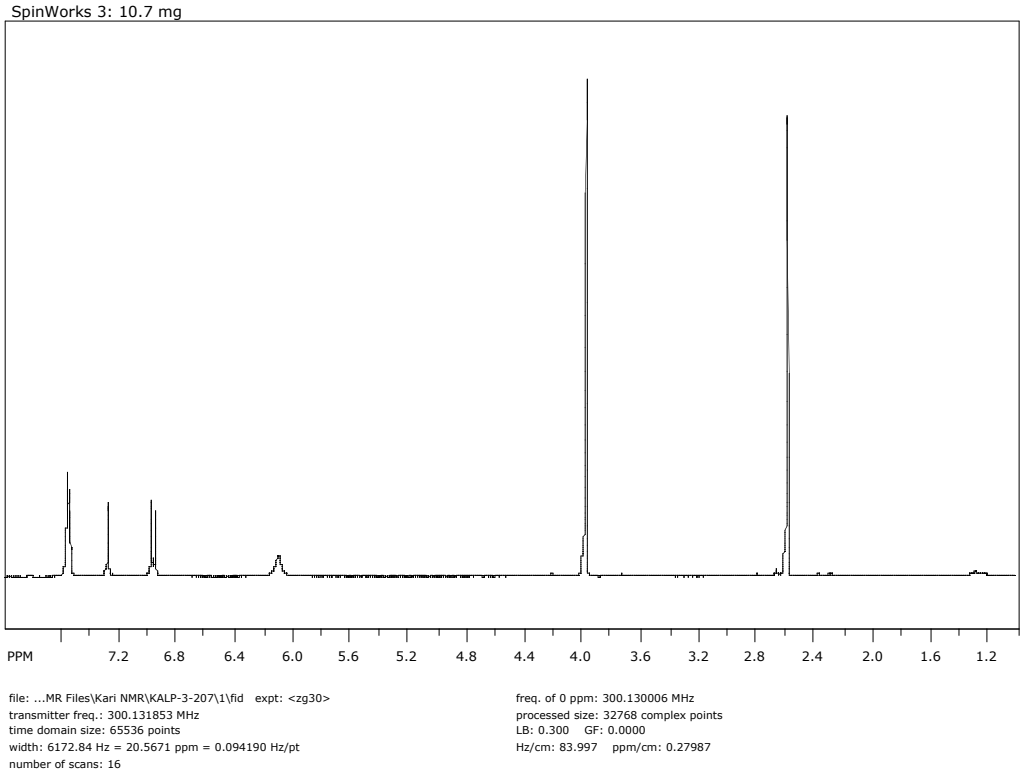

Figure AB.7 ^{1}H NMR results for model compound: Acetovanillone after reaction with sodium ethoxide.

Appendix B: Supplementary Data for Chapter 7

Supplementary Data included with submitted manuscript: Parsons, R.V., S. Cenkowski, J.L. Sorensen, T. Beta and S.D. Arntfield. Hemicellulose Polysaccharide Recovery from Flax Shive using Alkaline Solutions with Sodium Ethoxide Pretreatment.

B.1 Detailed Moisture Content Results

Moisture content results for all triplicate tests are presented in Table B.1.

B.2 Detailed Carbohydrate Precipitate Mass Yield Results

The quantitative yield results of carbohydrate precipitates from all individual flax shive extraction experiments are presented in Table B.2 on a dry basis.

B.3 Detailed Statistical Analyses of Precipitate Mass Yield Results

All the data for carbohydrate precipitate recovery were analyzed as a one-way ANOVA based on seven different treatment levels. The results of the ANOVA are presented in Table B.3. This confirmed there were significant differences between treatment conditions (i.e., F-ratio = 263 compared to critical $F_{6,21} = 2.6$).

Comparisons were undertaken using post-hoc pair-wise tests between mean yield values for the different treatment conditions, based on the method of Student-Newman-Keuls. Treatments were first reordered from highest to lowest mean yield value, with these results presented in Table B.4. The comparative analysis of means was based on 4 replicates for each condition, and a mean square error value of 25.8 from the ANOVA. The results for the pair-wise comparisons are presented in Table B.5.

Table B.1 Results for individual moisture analysis tests.

Trial Sample	Moisture Content (mg/g wb)	Dry Matter Content (mg/g wb)
1	89.1	910.9
2	88.9	911.1
3	88.7	911.3
Mean	88.9	911.1
Standard Deviation	0.2	0.2
Coefficient of Variation	0.2%	

Table B.2 Carbohydrate precipitate recovery yield-values for all experiments.

Treatment Condition	Replicate	Precipitate Yield (mg/g db)
1.0 M aqueous NaOH alone	1	96.9
	2	104.9
	3	102.7
	4	92.8
1.0 M aqueous NaOH following pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	1	101.9
	2	110.1
	3	101.5
	4	95.9
Elevated concentration 2.0 M aqueous NaOH	1	100.8
	2	99.2
	3	91.5
	4	91.2
1.0 M aqueous NaOH following pretreatment using azeotropic ethanol	1	101.0
	2	98.0
	3	98.3
	4	103.5
Saturated aqueous $Ba(OH)_2$ alone	1	4.4
	2	8.9
	3	19..9
	4	17.6

Table B.2 Carbohydrate precipitate recovery yield-values for all experiments (continued).

Treatment Condition	Replicate	Precipitate Yield (mg/g db)
Saturated aqueous $Ba(OH)_2$ following pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	1	17.5
	2	12.2
	3	15.4
	4	20.9
Mixed reaction 1.0 M NaOH added to saturated aqueous $Ba(OH)_2$	1	40.3
	2	45.7
	3	44.4
	4	50.6

Table B.3 ANOVA for carbohydrate precipitate recovery as affected by treatment condition (one-way ANOVA with 7 treatment levels).

Source	df	Sum Squares	Mean Square	F-statistic	P-value
Treatment	6	40,800	6,800	263	< 0.001
Error	21	542	25.8		

Note: df = Degrees of freedom

Table B.4 Treatment conditions reordered from highest to lowest mean yield.

Label	Treatment Condition	Mean Yield (mg/g db)
T1	1.0 M NaOH after pretreated using 1.0 M sodium ethoxide in anhydrous ethanol	102.4 ± 5.8
T2	1.0 M NaOH after pretreated using azeotropic ethanol	100.2 ± 2.6
T3	1.0 M NaOH alone	99.3 ± 5.5
T4	Elevated concentration 2.0 M NaOH	95.7 ± 5.0
T5	Mixed reagent 1.0 M NaOH added to saturated aqueous $Ba(OH)_2$	45.2 ± 4.2
T6	Saturated $Ba(OH)_2$ after pretreated using 1.0 M sodium ethoxide in anhydrous ethanol	16.5 ± 3.6
T7	Saturated $Ba(OH)_2$ alone	12.7 ± 7.3

Table B.5 Post-hoc pair-wise comparisons of mean values for carbohydrate precipitate yield under different treatment conditions based on method of Student-Newman-Keuls.

Lower Value	Difference from Higher to Lower Pair Value							Critical Difference
	T7	T6	T5	T5	T3	T2	T1	
T7	—	3.8	32.5*	83.0*	86.6*	87.5*	89.7*	15.6
T6		—	28.7*	79.2*	82.8*	83.7*	85.9*	14.9
T5			—	50.4*	54.1*	55.0*	57.1*	14.2
T4				—	3.6	4.5	6.7	13.3
T3					—	0.9	3.0	12.0
T2						—	2.2	9.9
T1							—	

Note: Significant differences between different treatment conditions (i.e., difference greater than critical difference) denoted by an asterisk.

A summary of post-hoc analysis results is as follows:

- Mean mass recoveries from the top four treatments were not statistically different from each other, but were significantly different from the other three treatments. These four all involved use of NaOH, and included 1.0 M NaOH alone, elevated concentration 2.0 M NaOH, and 1.0 M NaOH following pretreatment, whether by sodium ethoxide in anhydrous ethanol or azeotropic ethanol.
- Mean mass recovery for the mixed NaOH/$Ba(OH)_2$ solution was significantly different from all other treatments.
- Two lowest mean mass recovery values, both involving $Ba(OH)_2$, whether alone or following pretreatment, were not different from each other.

B.4 HPLC Calibration Curves and Spiked Samples

Calibration curves for reagent xylose, glucose and mannose standards mixed using high purity water are presented in Figure B.1. Standards covered the range from 2 μM to 200 μM. All spiked samples used to confirm identification of peaks in HPLC analyses are summarized in Table B.6.

B.5 HPLC Monomer Peak Retention Times

Monomer peak retention times are provided in Table B.7 for all samples. Spike sample monomers are indicated in the table using an asterisk (*). Scans for all HPLC runs for samples are presented later in Figures BA.1 through BA.28. Mean retentions

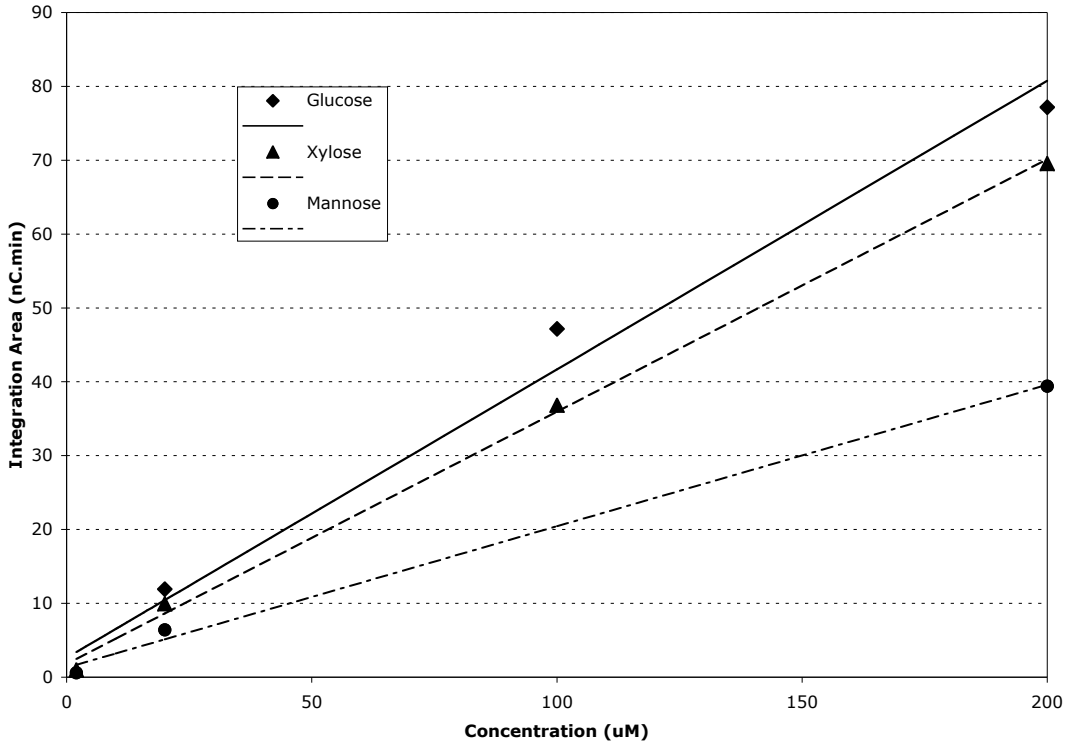

Figure B.1 Calibration curves for xylose, glucose and mannose standards.

Table B.6 Summary of spiked HPLC samples.

Sample	Nature of Spiking
1.0 M NaOH alone, replicate sample 2	5 µL of 10 mM mannose
1.0 M NaOH alone, replicate sample 3	5 µL of 10 mM xylose
1.0 M NaOH alone, replicate sample 4	5 µL of 10 mM glucose
1.0 M NaOH after pretreated using azeotropic ethanol, replicate sample 2	2 µL of 10 mM mannose
1.0 M NaOH after pretreated using azeotropic ethanol, replicate sample 3	2 µL of 10 mM xylose
1.0 M NaOH after pretreated using azeotropic ethanol, replicate sample 4	2 µL of 10 mM glucose

Table B.7 Monomer peak retention times for all samples.

Treatment Condition	Replicate	Retention Time (min)		
		Glucose	Xylose	Mannose
1.0 M aqueous NaOH alone	1	8.70	9.90	N/D
	2	8.48	9.68	10.30*
	3	8.31	9.45*	N/D
	4	8.13*	9.27	N/D
1.0 M aqueous NaOH following pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	1	8.73	9.97	N/D
	2	8.63	9.83	N/D
	3	8.43	9.62	N/D
	4	8.35	9.53	N/D
Elevated concentration 2.0 M aqueous NaOH	1	8.78	10.00	10.65
	2	8.78	10.00	10.81
	3	8.73	9.97	10.61
	4	8.73	9.95	10.73
1.0 M aqueous NaOH following pretreatment using azeotropic ethanol	1	8.17	9.28	N/D
	2	8.21	9.33	9.98*
	3	8.25	9.37*	N/D
	4	8.28*	9.42	N/D
Saturated aqueous $Ba(OH)_2$ alone	1	8.63	9.85	10.65
	2	8.57	9.78	10.58
	3	8.50	9.70	10.52
	4	8.45	9.63	10.53

Table B.7 Monomer peak retention times for all samples (continued).

Treatment Condition	Replicate	Retention Time (min)		
		Glucose	Xylose	Mannose
Saturated aqueous $Ba(OH)_2$ following pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	1	8.53	9.73	10.57
	2	8.50	9.70	10.55
	3	8.23	9.40	10.35
	4	8.18	9.32	10.23
Mixed reaction 1.0 M NaOH added to saturated aqueous $Ba(OH)_2$	1	8.22	9.38	10.27
	2	8.13	9.28	10.27
	3	8.07	9.20	10.15
	4	8.05	9.20	10.13

times for monomers in samples were 8.4 ± 0.2 min for glucose (n = 28), 9.6 ± 0.3 min for xylose (n = 28), and 10.4 ± 0.2 min for mannose (n = 18).

B.6 Detailed Carbohydrate Composition Results

Carbohydrate composition results for all samples are presented in Table B.8. These results were used to calculate xylose-to-glucose molar ratios. Given that mannose was only at trace levels where present, ratios were not calculated for mannose-to-glucose. Known concentrations of sugar monomers added to selected samples were backed out and not included in data as presented.

B.7 Detailed Statistical Analyses of Xylose-to-Glucose Molar Ratio Results

All the data for xylose-to-glucose molar ratios were analyzed as a one-way ANOVA based on seven different treatment levels. The results of the ANOVA are presented in Table B.9. This confirmed there were significant differences between treatment conditions (i.e., F-ratio = 13.1 compared to critical $F_{6,21} = 2.6$).

Comparisons were undertaken using post-hoc pair-wise tests between mean yield values for the different treatment conditions, based on the method of Student-Newman-Keuls. Treatments were first reordered from highest to lowest mean yield value, with these results presented in Table B.10. The comparative analysis of means was based on 4 replicates for each condition, and a mean square error value of 19.2 from the ANOVA. The results for the pair-wise comparisons are presented in Table B.11.

Table B.8 Carbohydrate composition results for all samples.

Treatment Condition	Replicate	Concentration (μM)		
		Glucose	Xylose	Mannose
1.0 M aqueous NaOH alone	1	1.7	42.6	Absent
	2	2.6	62.4	Spike only
	3	3.5	90.2	Absent
	4	2.2	48.8	Absent
1.0 M aqueous NaOH following pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	1	2.1	60.5	Absent
	2	6.5	193.0	Absent
	3	5.3	130.0	Absent
	4	3.1	86.0	Absent
Elevated concentration 2.0 M aqueous NaOH	1	6.6	91.5	Present
	2	5.2	93.2	Present
	3	2.7	32.5	Present
	4	6.5	111.0	Present
1.0 M aqueous NaOH following pretreatment using azeotropic ethanol	1	5.2	153.0	Absent
	2	7.0	141.0	Spike only
	3	6.4	190.0	Absent
	4	9.5	192.0	Absent
Saturated aqueous $Ba(OH)_2$ alone	1	1.1	8.8	Present
	2	0.6	5.4	Present
	3	1.2	6.9	Present
	4	0.9	4.1	Present

Table B.8 Carbohydrate composition results for all samples (continued).

Treatment Condition	Replicate	Concentration (μM)		
		Glucose	Xylose	Mannose
Saturated aqueous $Ba(OH)_2$ following pretreatment using 1.0 M sodium ethoxide in anhydrous ethanol	1	2.8	20.7	Present
	2	0.9	21.1	Present
	3	2.2	16.5	Present
	4	1.9	8.6	Present
Mixed reaction 1.0 M NaOH added to saturated aqueous $Ba(OH)_2$	1	1.7	18.1	Present
	2	3.3	50.5	Present
	3	5.2	70.3	Present
	4	6.2	83.2	Present

Table B.9 ANOVA for xylose-to-glucose molar ratio as affected by treatment condition (one-way ANOVA with 7 treatment levels).

Source	df	Sum Squares	Mean Square	F-statistic	P-value
Treatment	6	1,510	252	13.1	< 0.001
Error	21	402	19.2		

Note: df = Degrees of freedom

Table B.10 Treatment conditions reordered from mean xylose-to-glucose molar concentration ratio.

Label	Treatment Condition	Ratio
T1	1.0 M NaOH after pretreated using 1.0 M sodium ethoxide in anhydrous ethanol	27.5 ± 2.2
T2	1.0 M NaOH after pretreated using azeotropic ethanol	24.7 ± 5.3
T3	1.0 M NaOH alone	24.4 ± 1.5
T4	Elevated concentration 2.0 M NaOH	15.3 ± 2.7
T5	Mixed reagent 1.0 M NaOH added to saturated aqueous $Ba(OH)_2$	13.2 ± 2.1
T6	Saturated $Ba(OH)_2$ after pretreated using 1.0 M sodium ethoxide in anhydrous ethanol	10.9 ± 9.1
T7	Saturated $Ba(OH)_2$ alone	7.0 ± 2.2

Table B.11 Post-hoc pair-wise comparisons of mean values for xylose-to-glucose molar concentration ratio under different treatment conditions based on method of Student-Newman-Keuls.

Lower Value	Difference from Higher to Lower Pair Value							Critical Difference
	T7	T6	T5	T5	T3	T2	T1	
T7	—	4	6.2	8.3	17.4*	17.8*	20.6*	10.1
T6		—	2.2	4.3	13.4*	13.8*	16.6*	9.7
T5			—	2.1	11.2*	11.6*	14.3*	9.2
T4				—	9.1*	9.5*	12.2*	8.6
T3					—	0.4	3.1	7.8
T2						—	2.8	6.4
T1							—	

Note: Significant differences between different treatment conditions (i.e., difference greater than critical difference) denoted by an asterisk.

A summary of post-hoc analysis results is as follows:

- Mean ratios of xylose-to-glucose molar concentration for the top three extraction treatments were not statistically different from each other, but were significantly different from the other four treatments. These three treatments were also the same ones identified to have no mannose detected, namely 1.0 M NaOH alone, and 1.0 M NaOH following pretreatment using either sodium ethoxide in anhydrous ethanol or azeotropic ethanol.
- Mean ratios of xylose-to-glucose molar concentration for the other four treatments were not statistically different from each other. These four treatments all showed detectable mannose in all samples.

A comparison of grouped composition statistics for the two distinct clusters of treatments is provided in Table B.12.

B.8 Calculated Mass Proportion of Recovered Carbohydrate Polymers

The lack of any mannose in samples for the three top treatments, all involving 1.0 M aqueous NaOH, meant that the mass proportions of 4-O-methyl-glucuronoxylan and xyloglucan could be calculated directly, based on assumptions. The assumptions used for calculations are as follows:

- Recovered glucose monomer was all assumed to be associated with xyloglucan polymer;
- Xyloglucan polymer was assumed to have three xylose units to each four glucose units in the backbone; and

- 4-O-methyl-glucuronoxylan polymer was assumed to have one 4-O-methyl-glucuronate unit as a side-attachment for each thirteen xylose units in the backbone.

From these assumptions, a calculation curve was determined for the mass proportion of 4-O-methyl-glucuronoxylan as a function of the molar ratio of xylose-to-glucose, as presented in Figure B.2, with remaining mass being xyloglucan.

Specific calculation results, based on these assumptions, are as follows:

- Mean molar ratio of xylose-to-glucose for all extractions involving 1.0 M aqueous NaOH was 25.5 ± 3.4 (n = 12), whether with or without pretreatment.
- Molar ratios thus ranged, based on ± 1 SD, from a lower value of 22.1, to an upper value of 28.9.
- In turn, these translated to proportions of 4-O-methyl-glucuronoxylan ranging from 92.4% to 94.1% by mass, respectively, of total carbohydrate.

BA EC Detector Scans from HPLC for All Extraction Tests

Consistent vertical axis scales are presented for treatment conditions in Figures as presented. The scale for extractions involving $Ba(OH)_2$, with or without treatment, which had smaller peaks, ranged from 0 to 100 nC (Figures BA.17 through BA.24). For all other extractions, as presented, the ranged ranged from 0 to 200 nC.

Table B.12 Grouped composition statistics for distinct clusters of treatments.

Group / Value	Mean	SD	C of V
Top three treatment levels: 1.0 M NaOH, with or without pretreatment (n = 12)			
Xylose concentration	121.6 µM	53.6 µM	44.1%
Glucose concentration	4.8 µM	2.4 µM	50.0%
Xylose-to-glucose molar ratio	25.5	3.4	13.4%
Other four treatment levels: $Ba(OH)_2$, elevated NaOH, mixed reagents (n = 16)			
Xylose concentration	36.3 µM	32.2 µM	88.8%
Glucose concentration	3.1 µM	2.2 µM	70.8%
Xylose-to-glucose molar ratio	11.6	5.5	47.2%

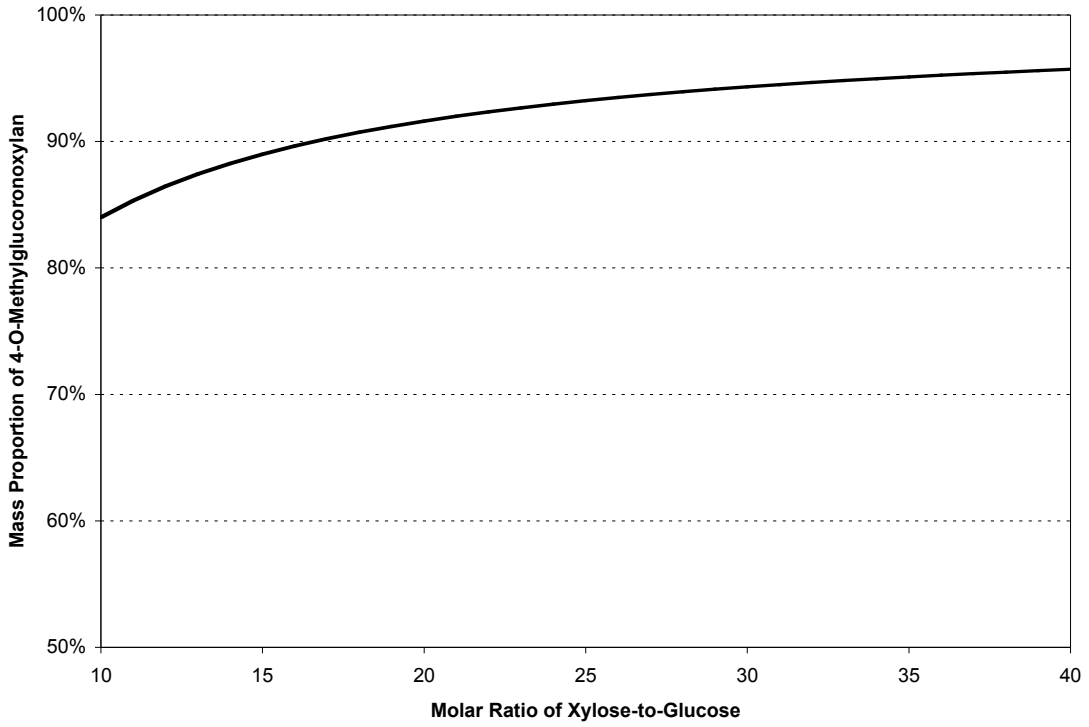

Figure B.2 Mass proportion of 4-O-methyl-glucuronoxylan polymer calculated from molar ratio of xylose-to-glucose in samples.

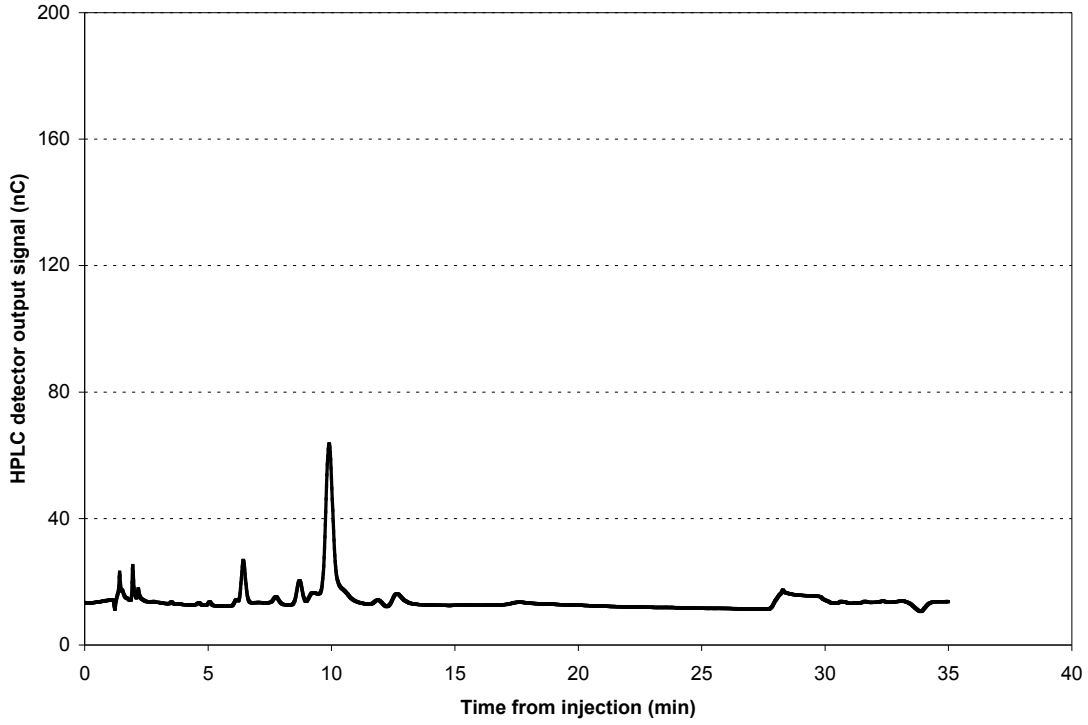

Figure BA.1 EC detector scan from HPLC for extraction sample: 1.0 M NaOH only, replicate 1.

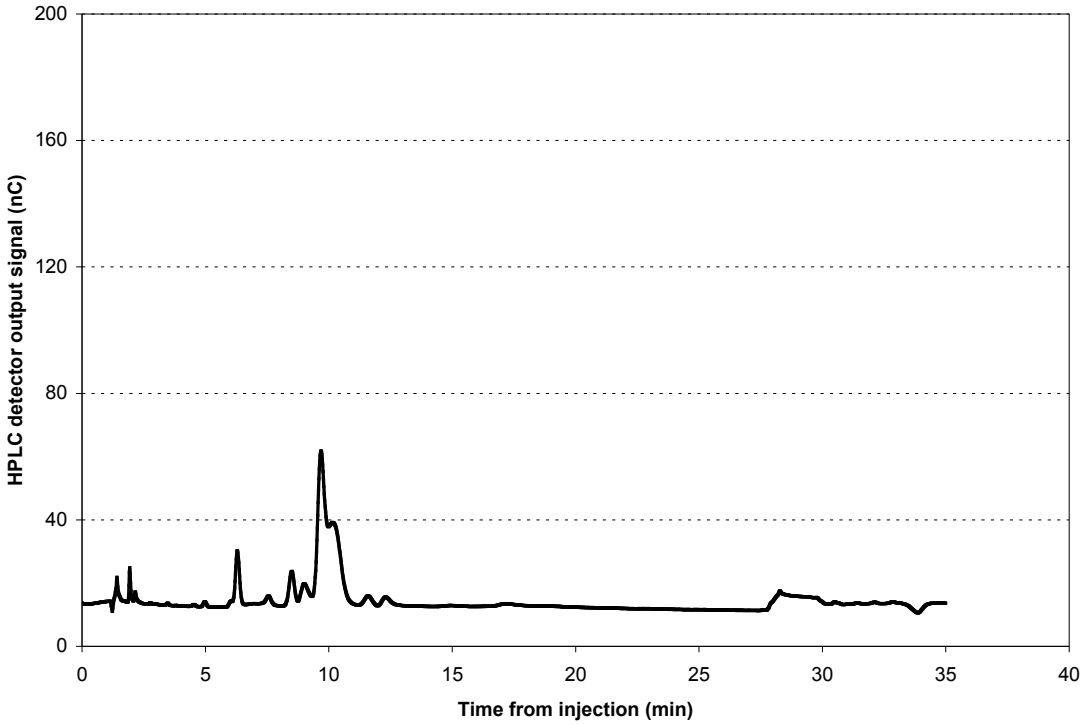

Figure BA.2 EC detector scan from HPLC for extraction sample: 1.0 M NaOH only, replicate 2 (mannose spike).

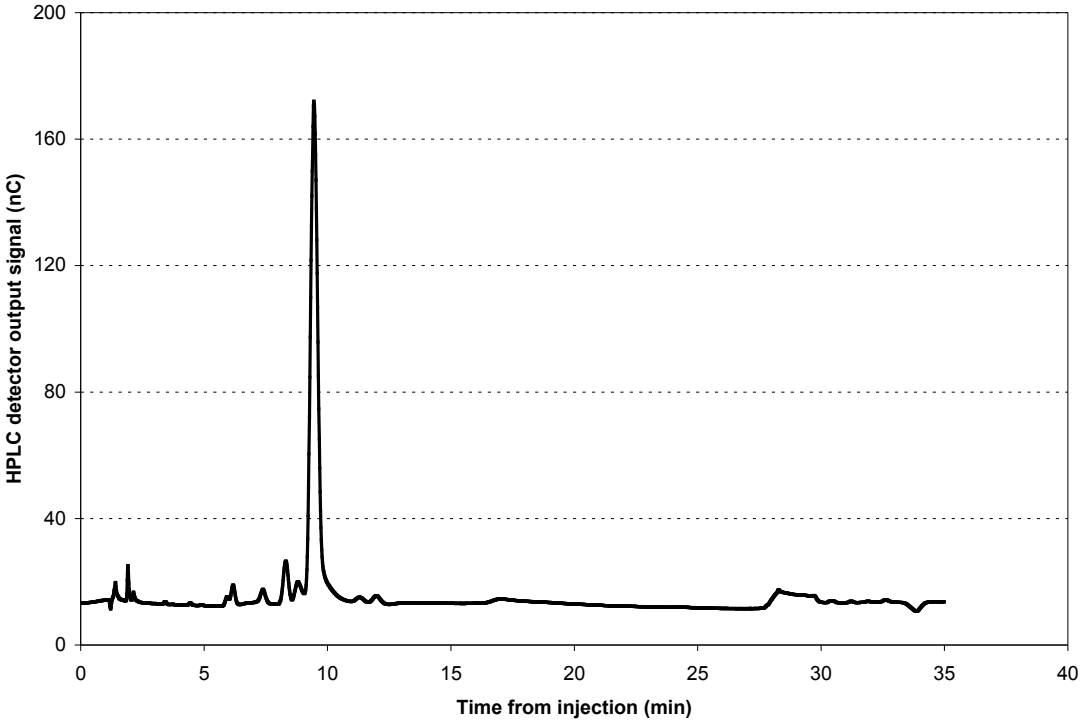

Figure BA.3 EC detector scan from HPLC for extraction sample: 1.0 M NaOH only, replicate 3 (xylose spike).

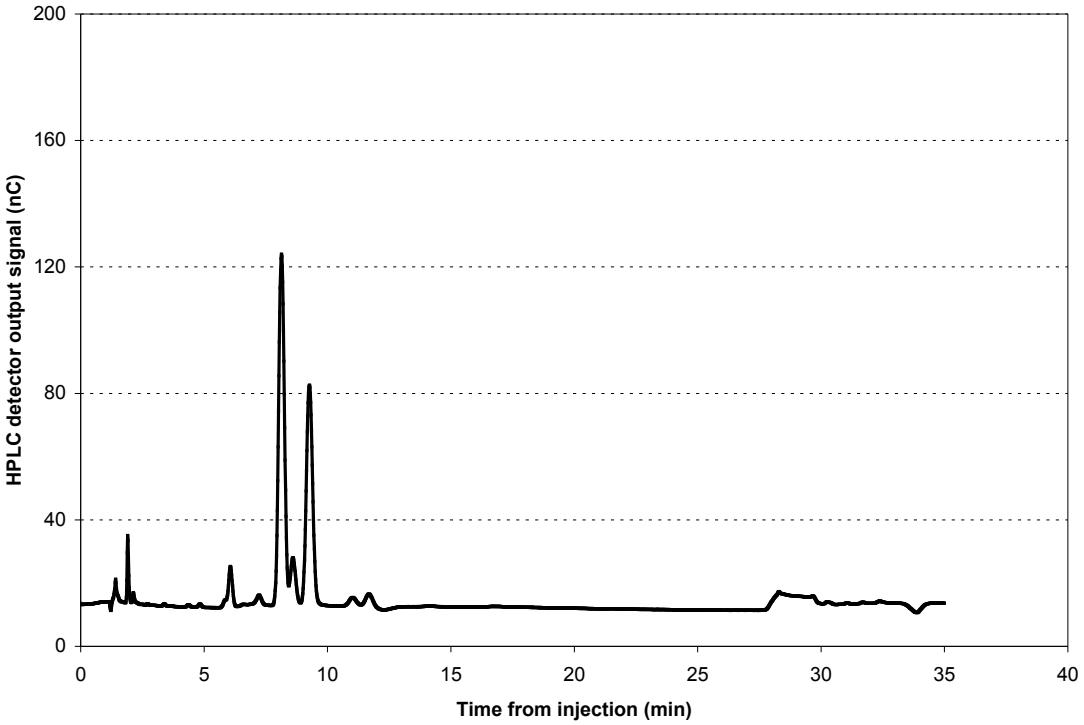

Figure BA.4 EC detector scan from HPLC for extraction sample: 1.0 M NaOH only, replicate 4 (glucose spike).

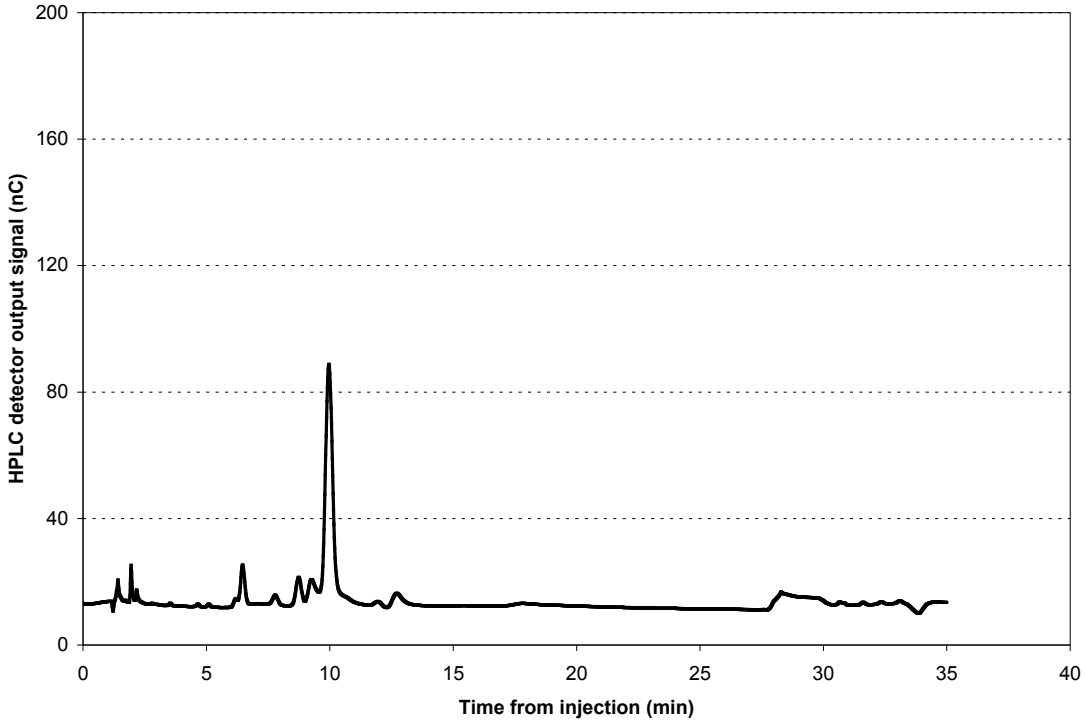

Figure BA.5 EC detector scan from HPLC for extraction sample: 1.0 M NaOH following sodium ethoxide, replicate 1.

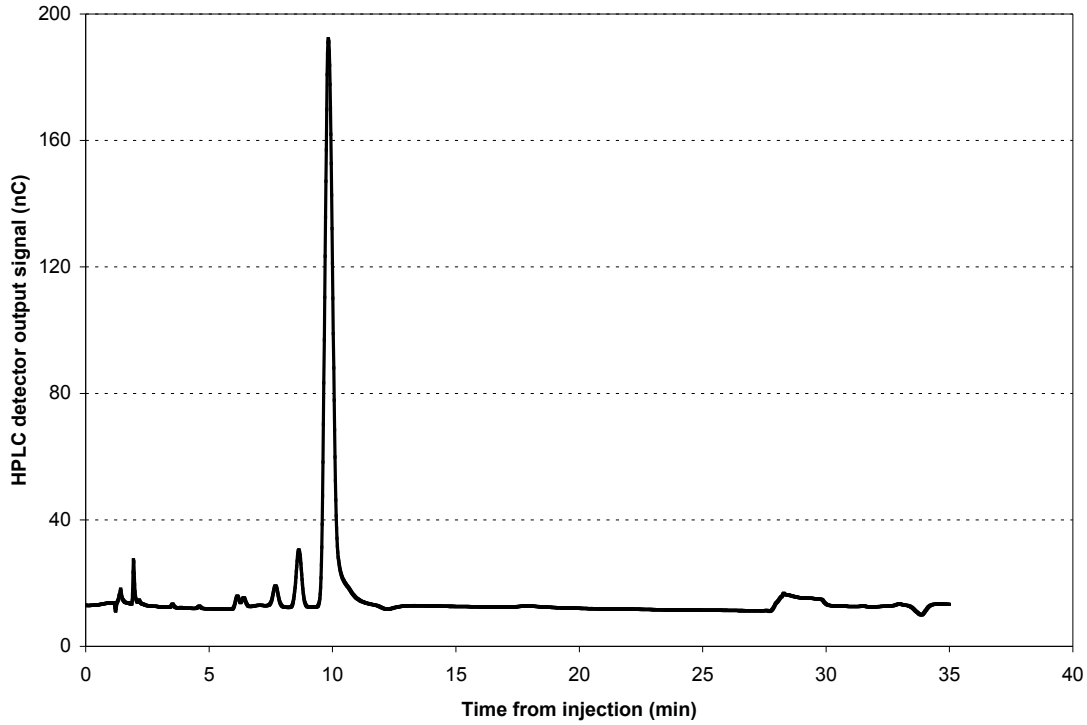

Figure BA.6 EC detector scan from HPLC for extraction sample: 1.0 M NaOH following sodium ethoxide, replicate 2.

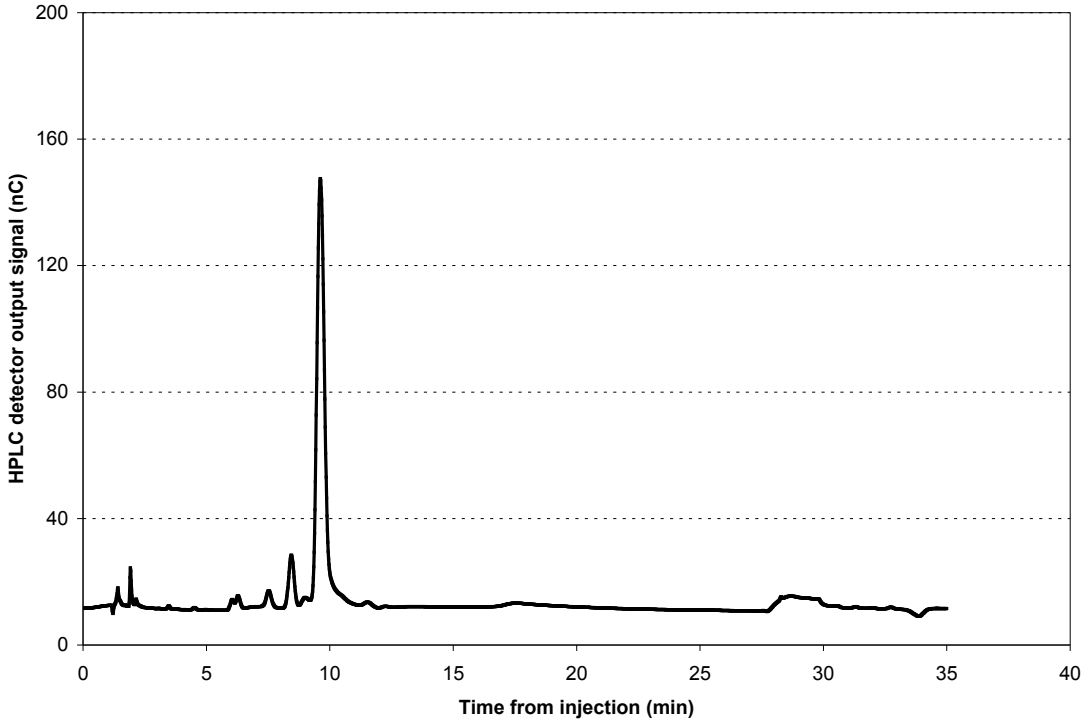

Figure BA.7 EC detector scan from HPLC for extraction sample: 1.0 M NaOH following sodium ethoxide, replicate 3.

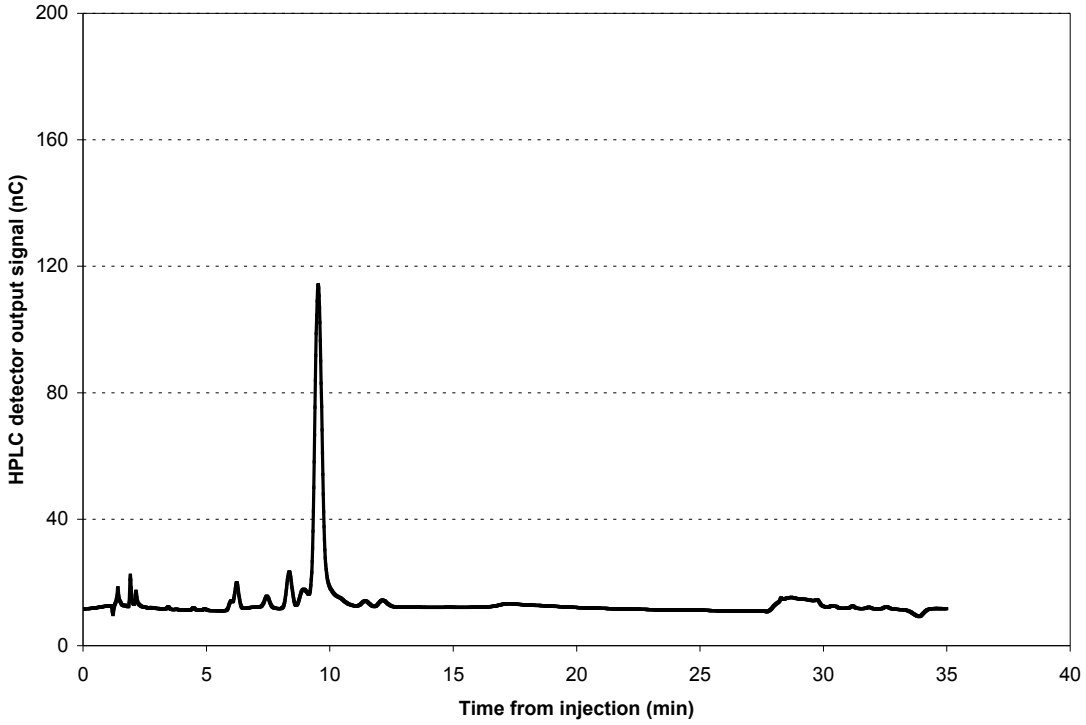

Figure BA.8 EC detector scan from HPLC for extraction sample: 1.0 M NaOH following sodium ethoxide, replicate 4.

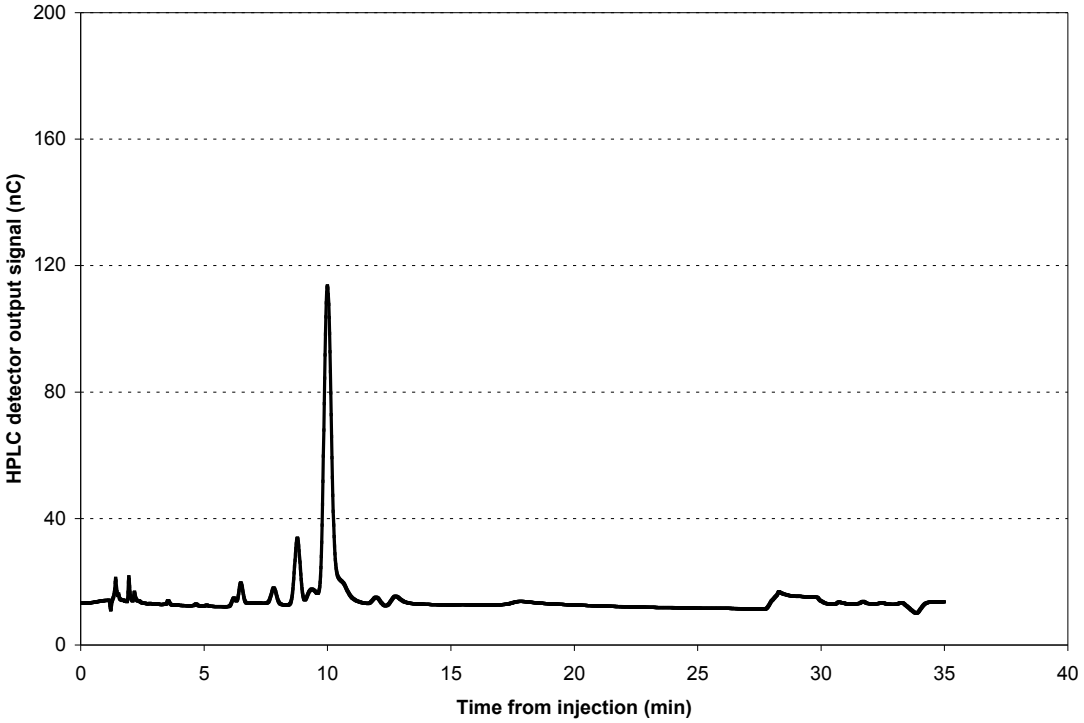

Figure BA.9 EC detector scan from HPLC for extraction sample: Elevated 2.0 M NaOH, replicate 1.

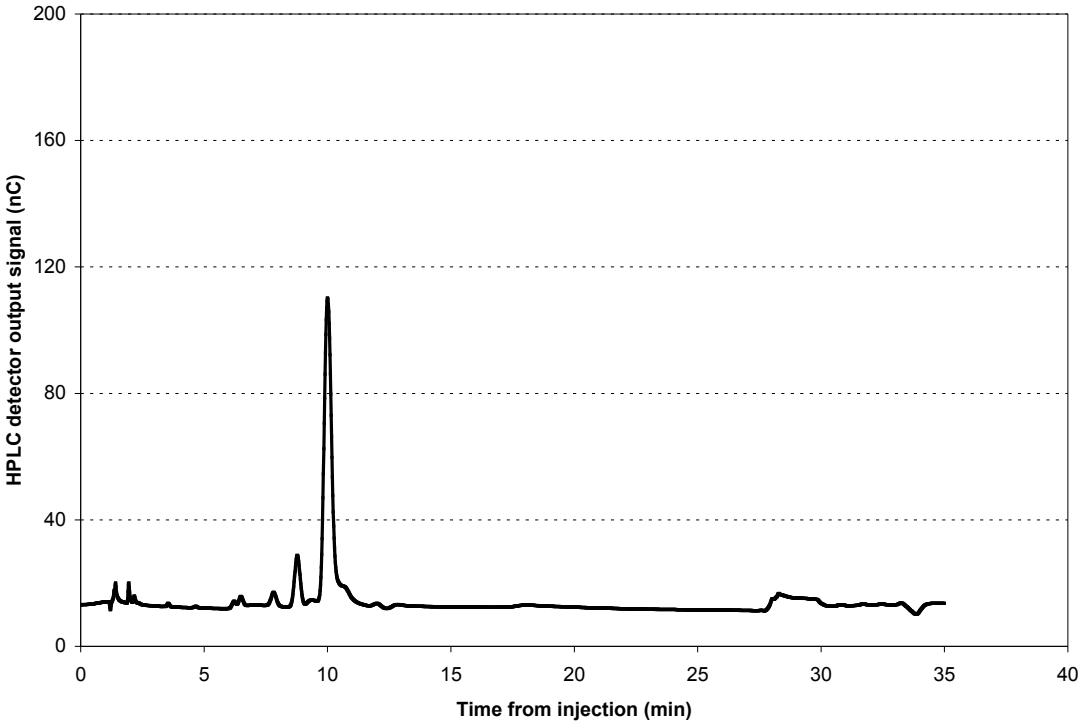

Figure BA.10 EC detector scan from HPLC for extraction sample: Elevated 2.0 M NaOH, replicate 2.

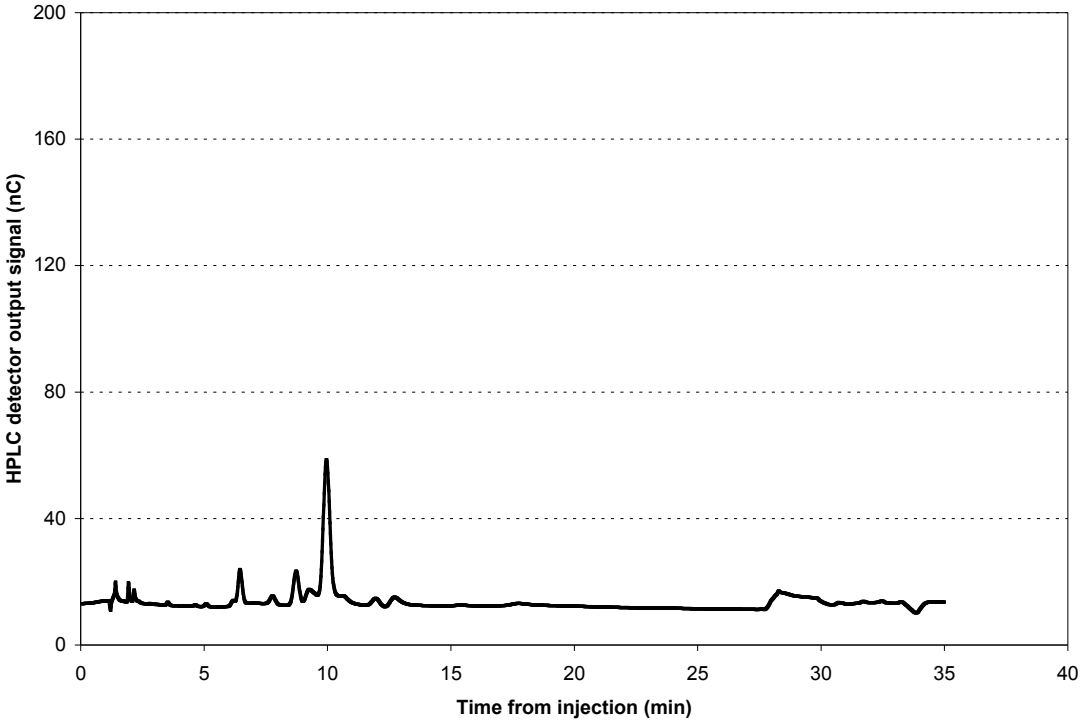

Figure BA.11 EC detector scan from HPLC for extraction sample: Elevated 2.0 M NaOH, replicate 3.

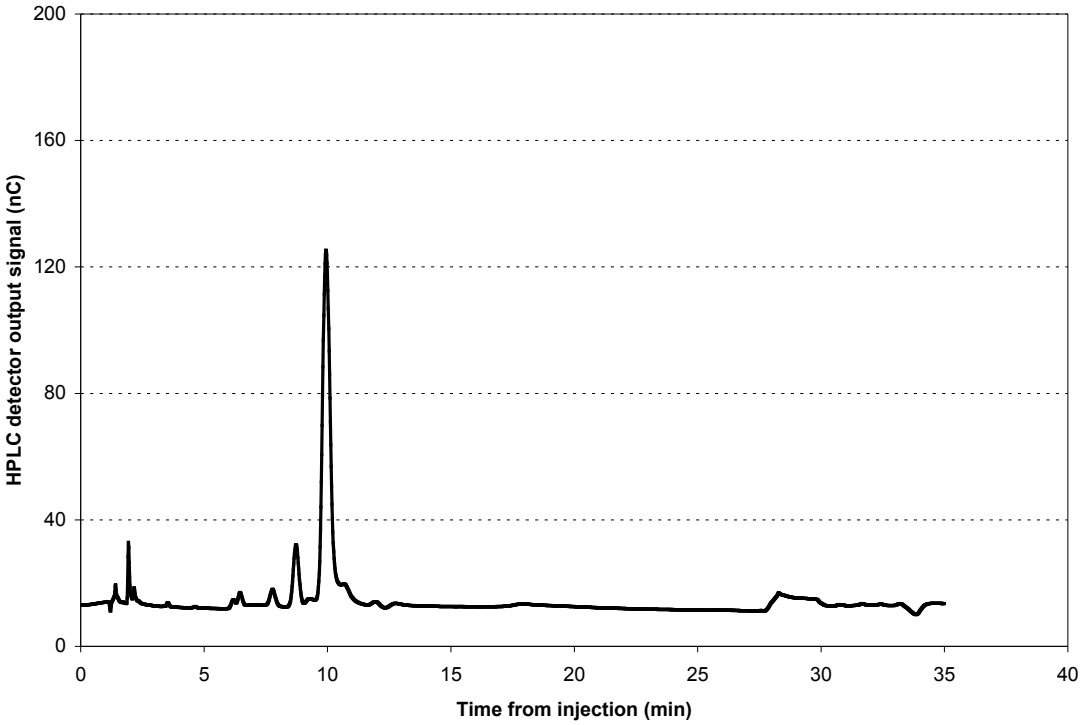

Figure BA.12 EC detector scan from HPLC for extraction sample: Elevated 2.0 M NaOH, replicate 4.

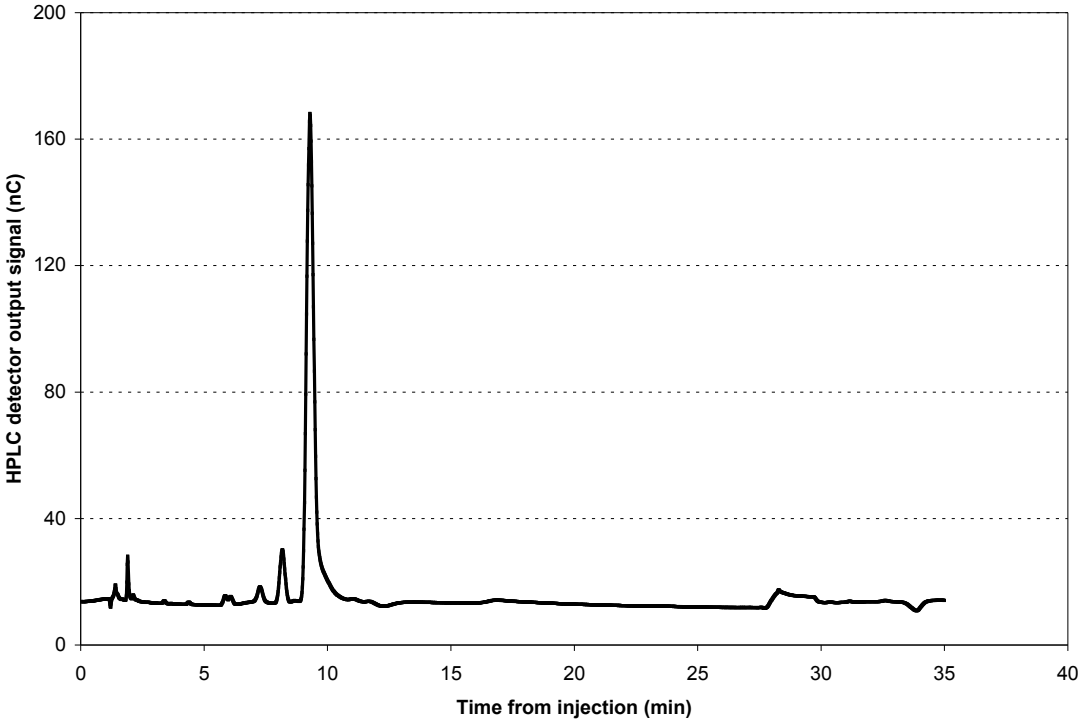

Figure BA.13 EC detector scan from HPLC for extraction sample: 1.0 M NaOH following ethanol, replicate 1.

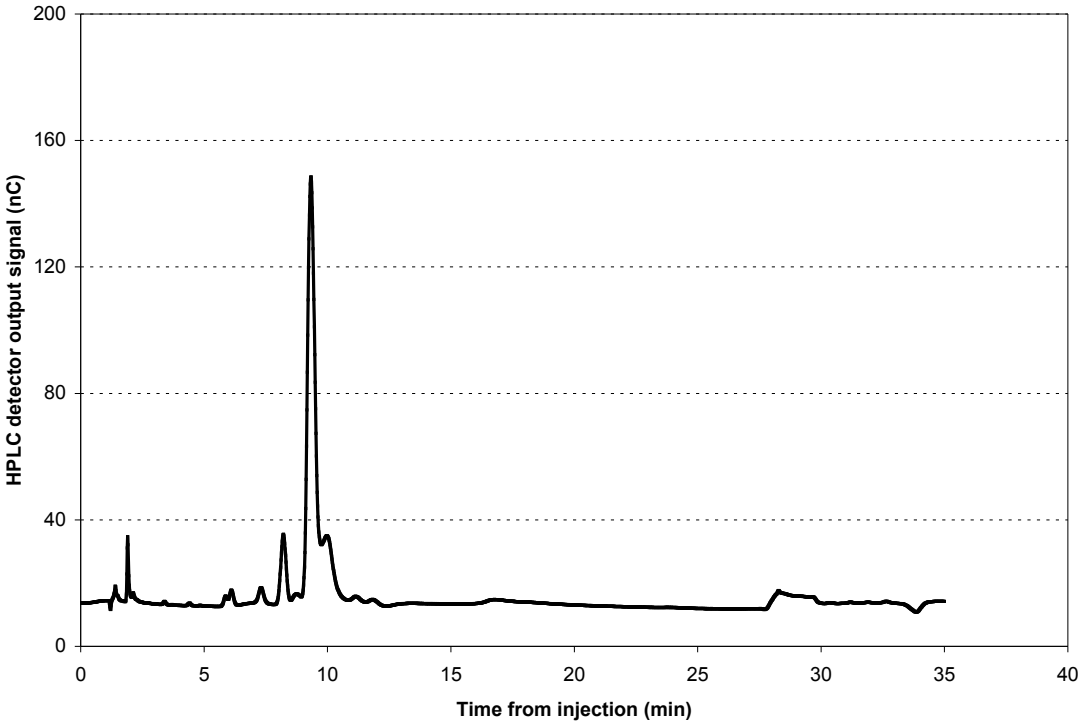

Figure BA.14 EC detector scan from HPLC for extraction sample: 1.0 M NaOH following ethanol, replicate 2 (mannose spike).

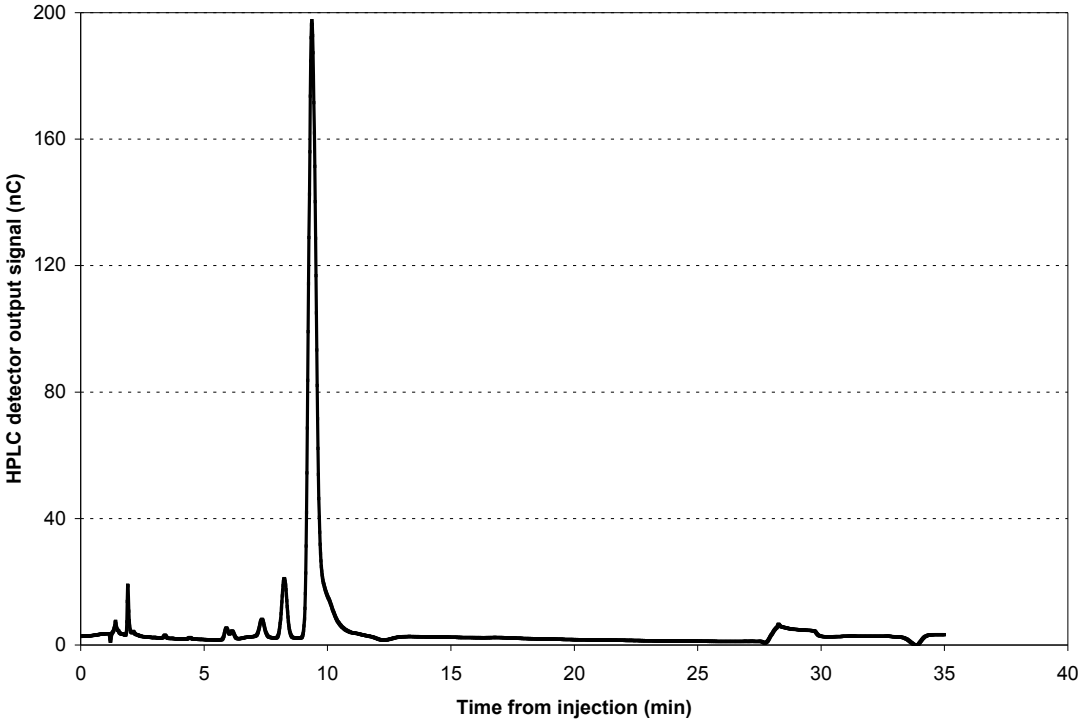

Figure BA.15 EC detector scan from HPLC for extraction sample: 1.0 M NaOH following ethanol, replicate 3 (xylose spike).

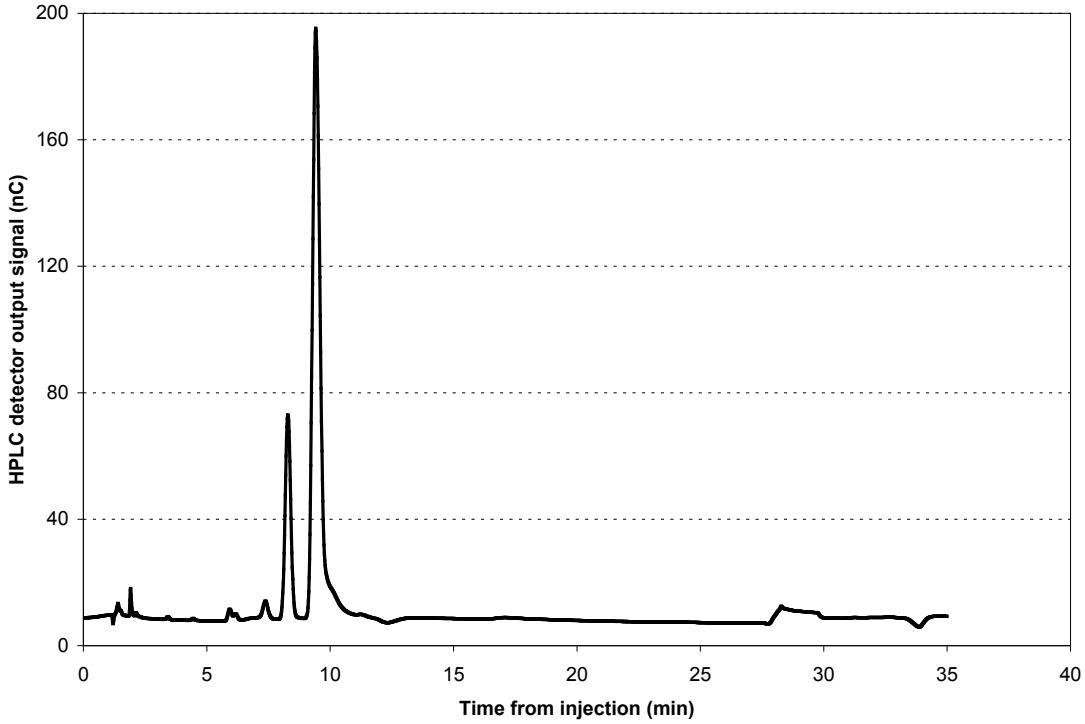

Figure BA.16 EC detector scan from HPLC for extraction sample: 1.0 M NaOH following ethanol, replicate 4 (glucose spike).

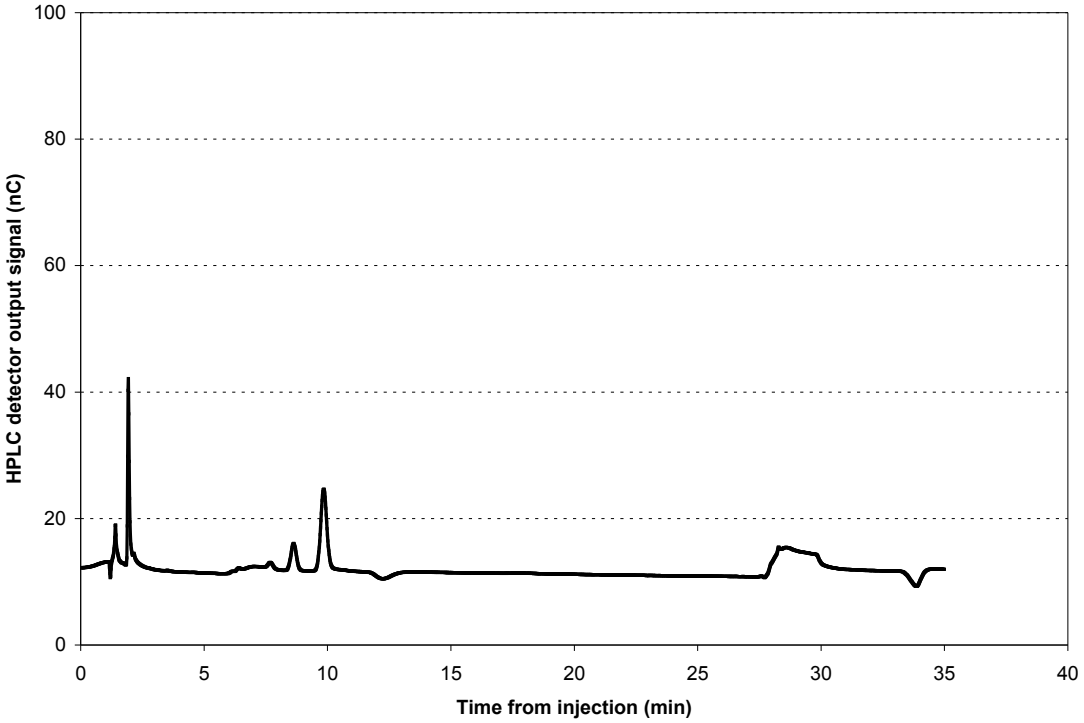

Figure BA.17 EC detector scan from HPLC for extraction sample: Saturated $Ba(OH)_2$ only, replicate 1.

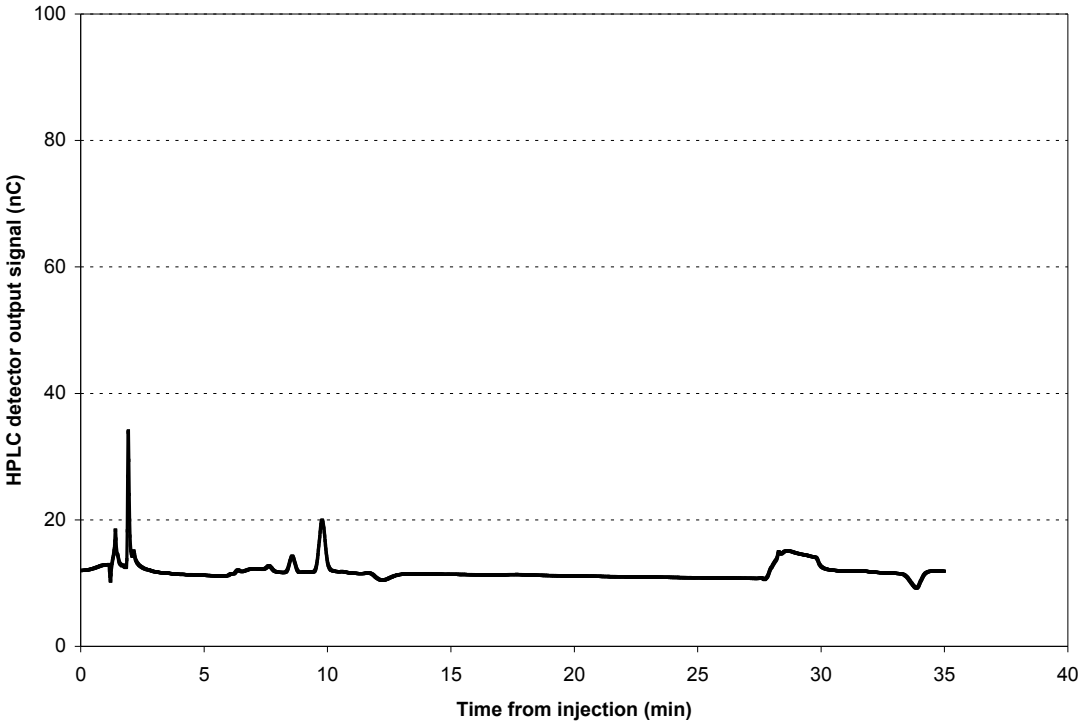

Figure BA.18 EC detector scan from HPLC for extraction sample: Saturated $Ba(OH)_2$ only, replicate 2.

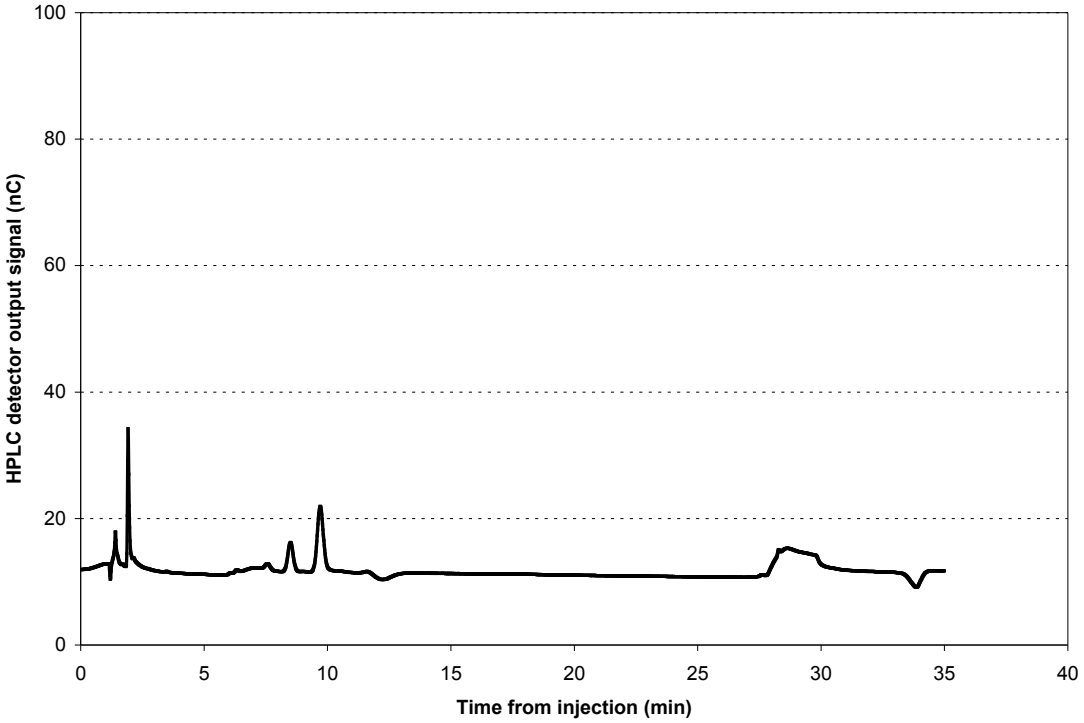

Figure BA.19 EC detector scan from HPLC for extraction sample: Saturated $Ba(OH)_2$ only, replicate 3.

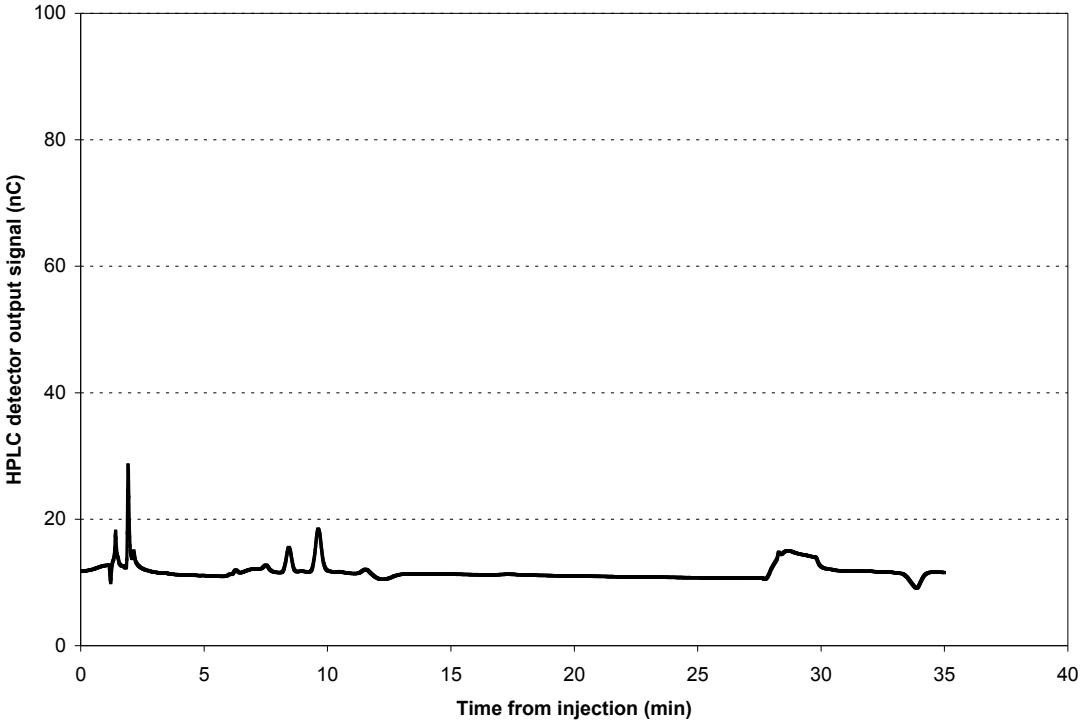

Figure BA.20 EC detector scan from HPLC for extraction sample: Saturated $Ba(OH)_2$ only, replicate 4.

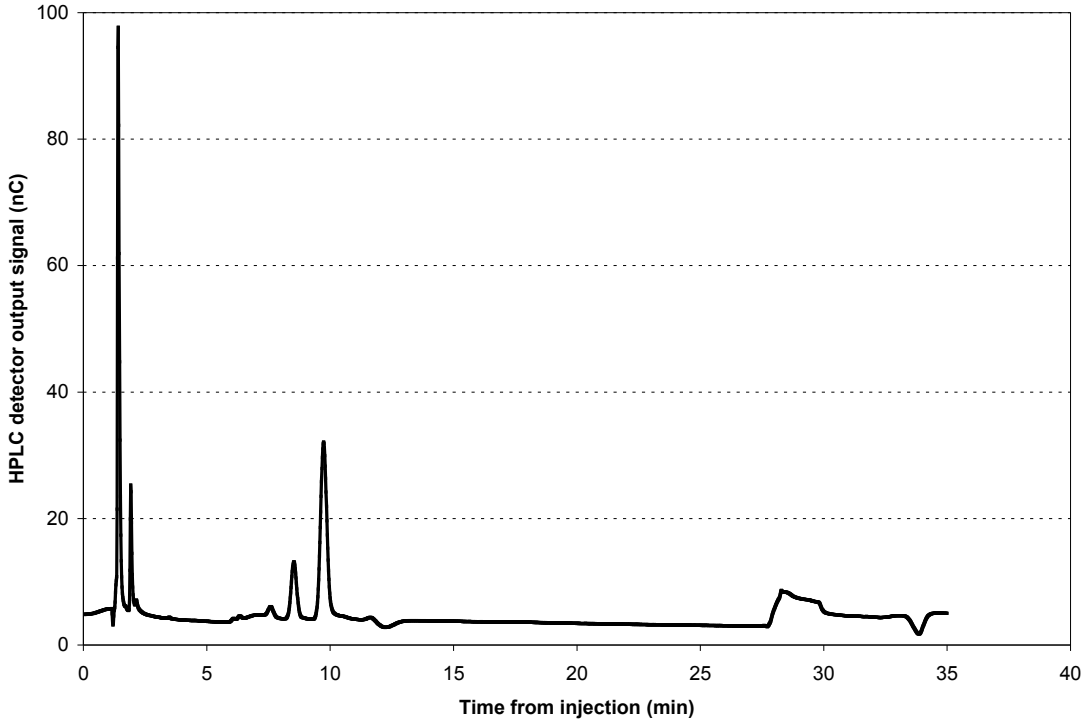

Figure BA.21 EC detector scan from HPLC for extraction sample: Saturated $Ba(OH)_2$ following sodium ethoxide, replicate 1.

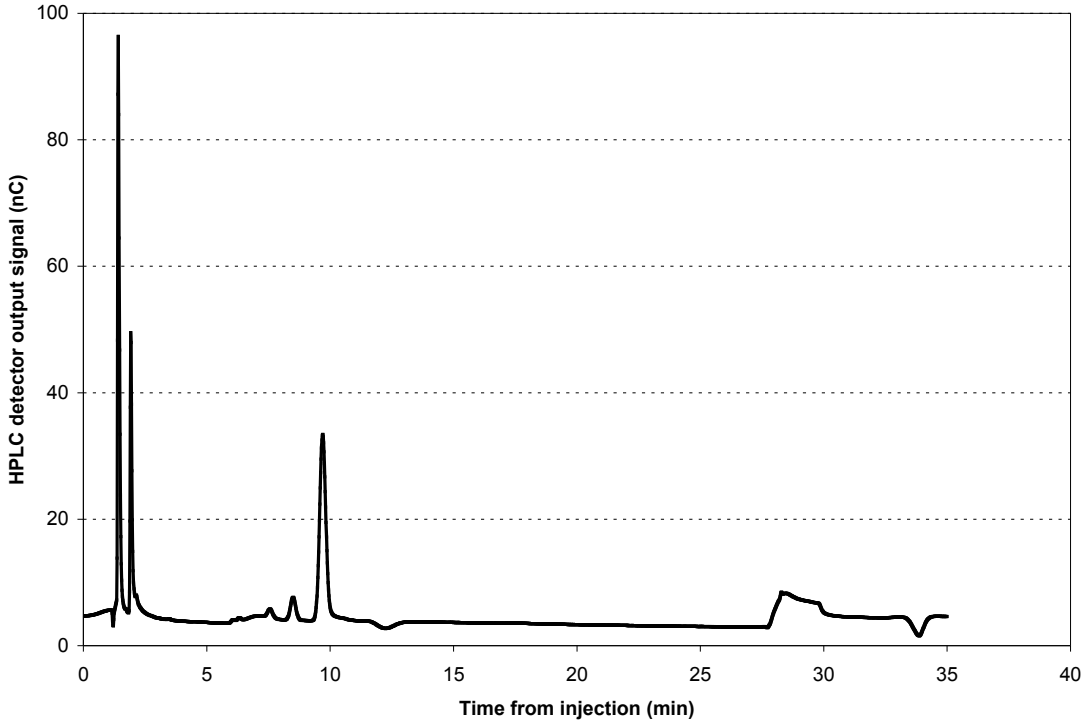

Figure BA.22 EC detector scan from HPLC for extraction sample: Saturated $Ba(OH)_2$ following sodium ethoxide, replicate 2.

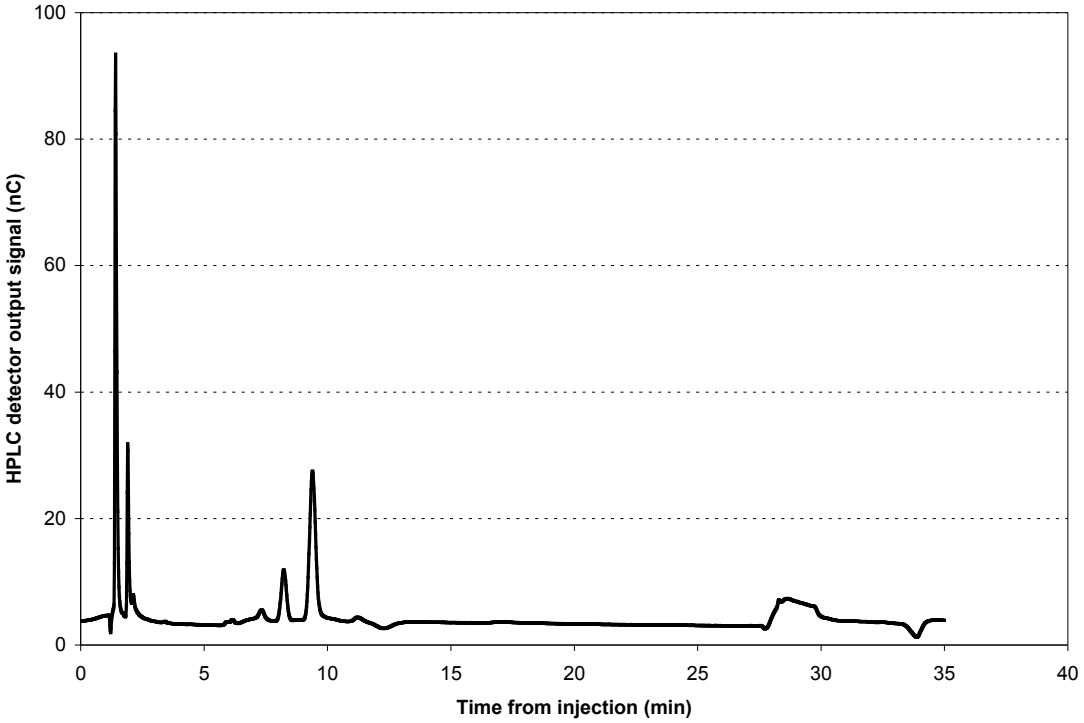

Figure BA.23 EC detector scan from HPLC for extraction sample: Saturated $Ba(OH)_2$ following sodium ethoxide, replicate 3.

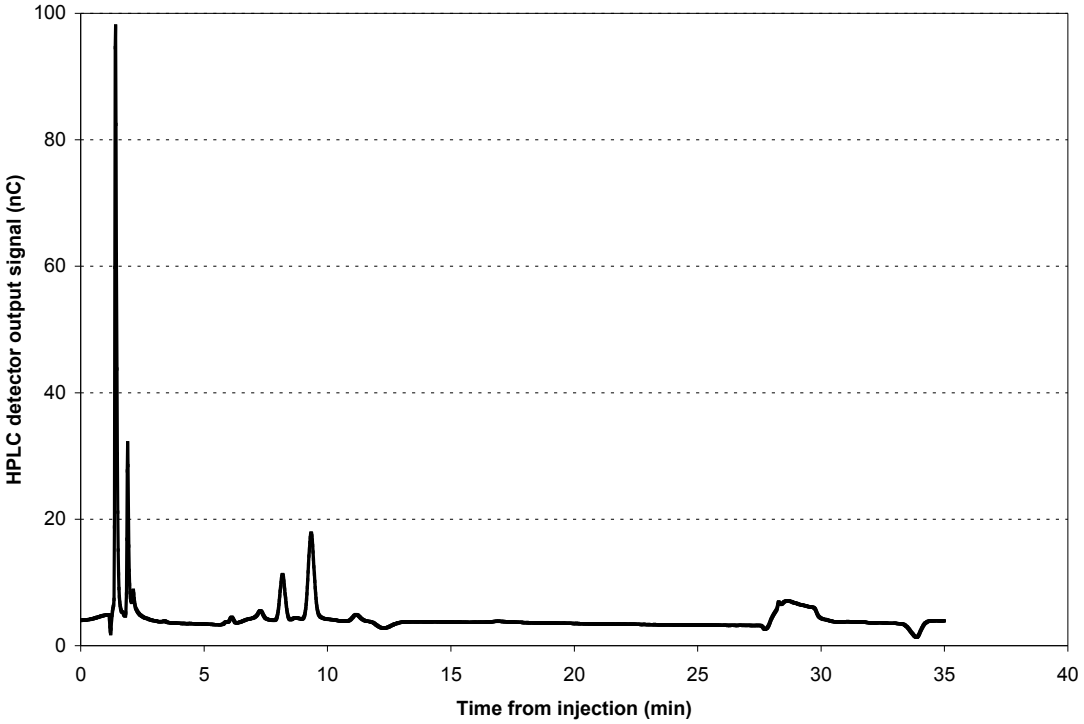

Figure BA.24 EC detector scan from HPLC for extraction sample: Saturated $Ba(OH)_2$ following sodium ethoxide, replicate 4.

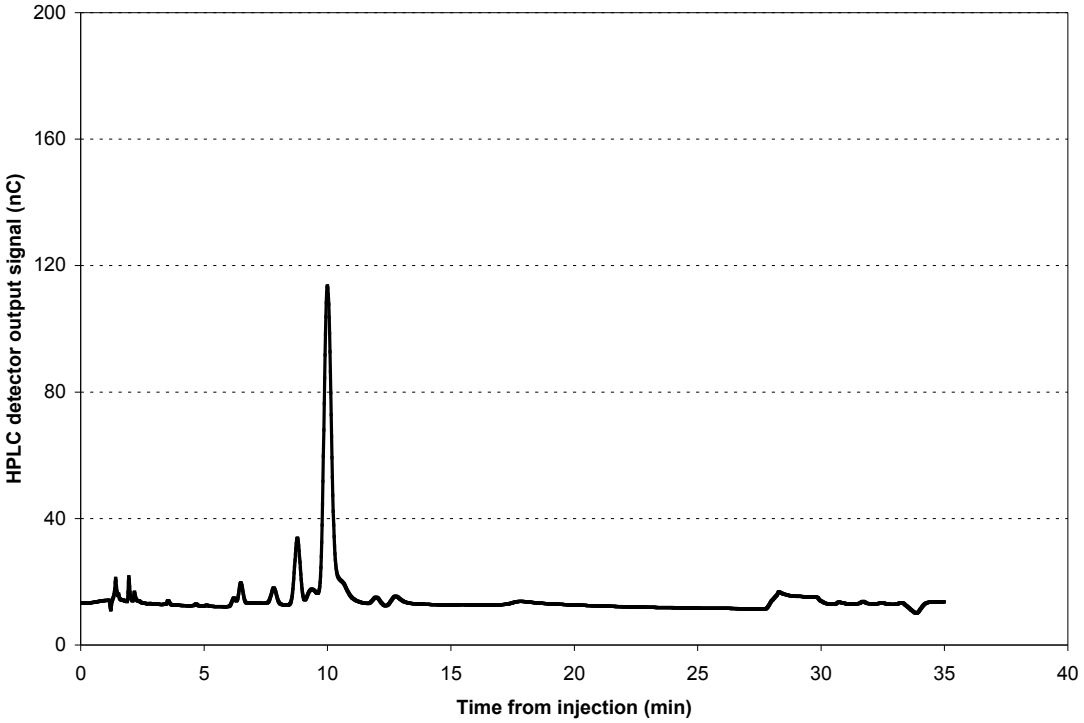

Figure BA.25 EC detector scan from HPLC for extraction sample: Mixed 1.0 M NaOH in saturated $Ba(OH)_2$, replicate 1.

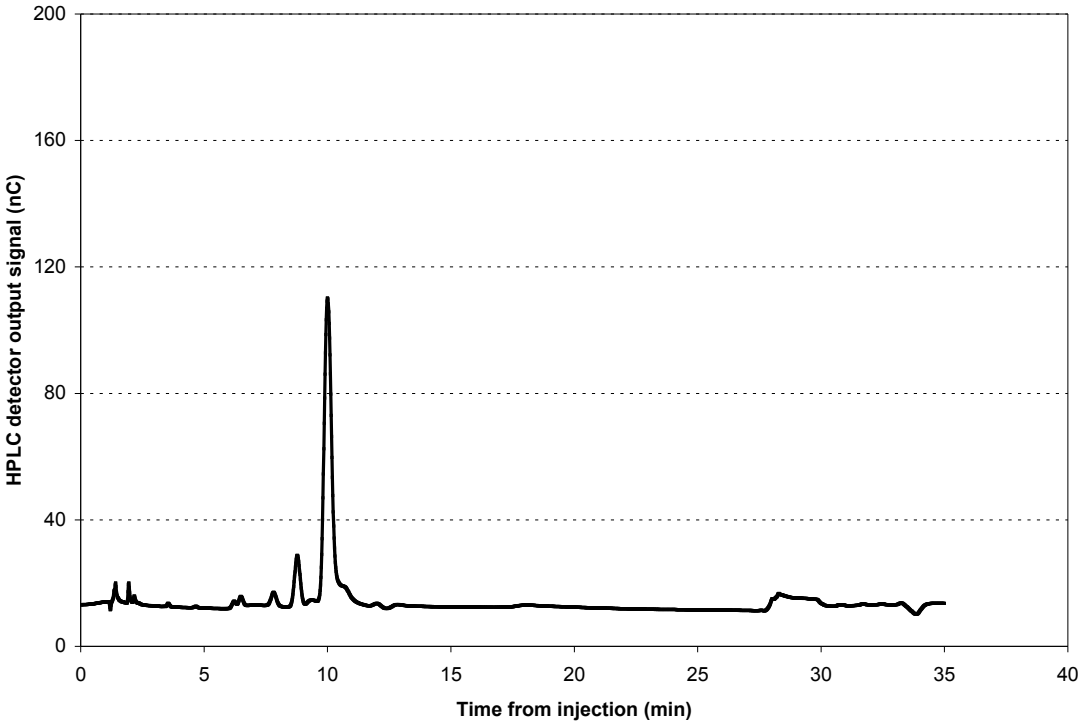

Figure BA.26 EC detector scan from HPLC for extraction sample: Mixed 1.0 M NaOH in saturated $Ba(OH)_2$, replicate 2.

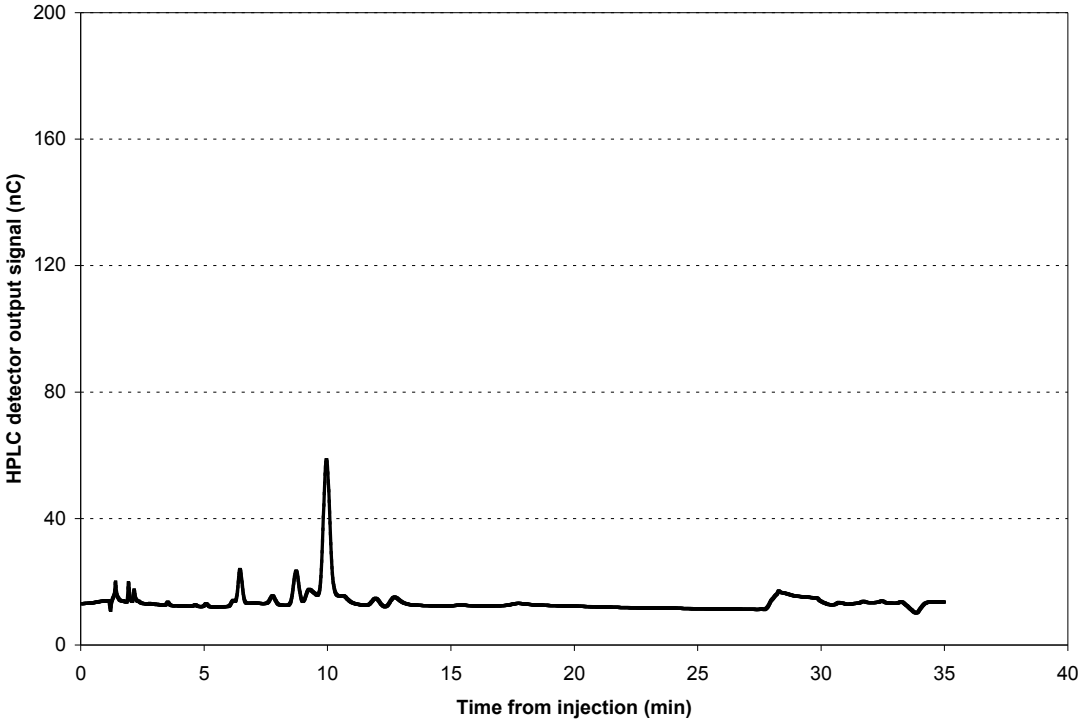

Figure BA.27 EC detector scan from HPLC for extraction sample: Mixed 1.0 M NaOH in saturated $Ba(OH)_2$, replicate 3.

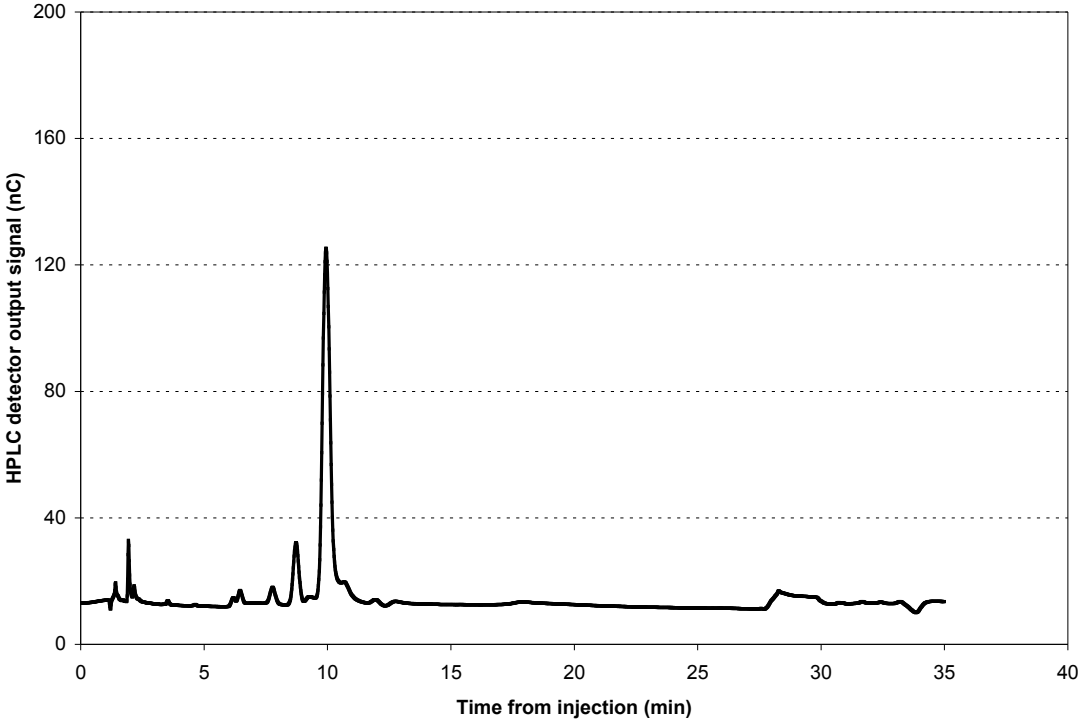

Figure BA.28 EC detector scan from HPLC for extraction sample: Mixed 1.0 M NaOH in saturated $Ba(OH)_2$, replicate 4.