AN ABSTRACT OF THE DISSERTATION OF

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Title: Application of Infrared Spectroscopy for Determination of Wood Natural Durability

Abstract approved:

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Jeffrey J. Morrell

Natural durability remains one of the most attractive characteristics of wood, and helps wood obtain a premium price. A worldwide shift towards the use of younger trees from intensively managed forests has created greater concerns about wood quality, especially the wood's resistance to fungi and insects. Wood durability is assessed using a variety of standards. Some standards are based on destructive methods that measure weight loss after exposure to wood degrading organisms. These tests are useful but there are concerns about variabilities in durability classifications according to different testing methods. Furthermore, durability can be heavily influenced by variations within and between trees, sites, regions, genetic origin, and

age. Thus, there is a need for a faster, non-destructive and economically viable technique for screening wood durability. Fourier transform infrared spectroscopy with attenuated total reflectance (ATR-FTIR) and near infrared spectroscopy (NIR), with chemometrics analysis was explored for classifying wood durability. The extractive contents of Alaska yellow cedar (*Callitropsis nootkatensis*) and western juniper (*Juniperus occidentalis*) were investigated to understand the variability that existed between and within trees, and the relationships between brown-rot decay (*Gloeophyllum trabeum* and *Rhodonia placenta*), termite (*Reticulitermes flavipes*) resistance, and the spectroscopic results were examined. FT-IR showed sensitivity in detecting to one of the extractive concentrations (carvacrol) as differences were observed on 3% concentration. The majority of the Alaska yellow cedar and western juniper samples were classified as resistant to highly resistant against decay fungi and termites. A moderate to poor correlation between extractives and mass loss to wood biodegradations agents (fungi and termites) was observed, indicating the possibility for other factors may contribute to wood superior durability. Chemometrics analysis using principal component analysis (PCA) and hierarchical cluster analysis (HCA) on the spectral data was unable to accurately classify wood based on their durability. Nevertheless, results suggest FT-IR and NIR can be used for analyzing wood extractives, as well as the possibility for producing more accurate predictions on species with greater variability in durability.

Application of Infrared Spectroscopy for Determination of Wood Natural Durability

by

Shahlinney Lipeh

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Shahlinney Lipeh, Author

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CONTRIBUTION OF AUTHORS

Dr. Jeffrey Morrell assisted in the experimental design, reviewing and editing of each chapter. Dr. Laurence R. Schimleck helped in reviewing and editing of each chapter.

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Chapter 1. Introduction

1.1. Background and justifications

Natural durability is one of the most important and highly valued characteristics of wood. A worldwide shift towards the use of young trees from intensively managed forests has increased concerns regarding quality, especially related to resistance to fungi and insects. Younger trees contain more sapwood, a light color region with little or no extractives compounds (Hillis, 2011). Extractives have been linked to the durability of many wood species, acting as either toxicants or inhibitors to fungal growth, and deterring termite feeding (Ajuong et al., 2014; Rudman, 1958; Taylor et al., 2006; Woodward and Pearce, 1988).

Wood biodegradation has a massive economic impact with losses of USD 1 billion and USD 300 million attributed to termites and decay fungi (Clausen and Yang, 2007; Ghaly and Edwards, 2011; Nicholas, 1982). Furthermore, wood biodegradation creates hazardous conditions owing to weakened wood in the structure (mass losses of 5-10% may reduce 60-80% of wood strength). Variations in durability between and within trees of the same species further complicates proper wood utilization (Gierlinger and Wimmer, 2004; Guilley et al., 2004; Lukmandaru and Takahashi, 2009; Nault, 1988). Durability gradients within trees may be caused by biological detoxification, natural

oxidation of heartwood extractives, or continued polymerization of extractives to produce less toxic compounds (Anderson et al., 1963; Hillis, 2011).

Wood durability is assessed using a variety of procedures. Some national standards use destructive methods that measure weight loss after exposure to wood degrading organisms. These tests are useful, but there are concerns about variability in classifications developed using different test methods. Furthermore, durability can be heavily influenced by various factors including variation within and between trees, sites, regions, genetic origin, and age. A number of alternative approaches to standardized methods have been developed. These include measuring phenolic content, and evaluation of wood density and lignin content (Harju and Venalainen, 2006; Humar et al., 2008; Niamké et al., 2011). However, these methods are destructive, time-consuming and limited to a small number of samples that may not represent the overall durability of a species. Thus, there is a need for faster, non-destructive and economically viable techniques for screening wood durability.

One possible solution for assessing durability is infrared (IR) spectroscopy, a surface-based analytical method, that examines the vibration of molecules when a material is exposed to infrared light to generate chemical 'fingerprints' unique to the analyzed material (Siesler et al., 2008). IR combined with chemometrics, (the multivariate analysis of chemical data) is a powerful tool for identifying spectral features that

discriminate between two or more groups (Stuart, 2004). Vibrational spectroscopy-based methods, such as Fourier transform infrared (FT-IR) spectroscopy, near infrared (NIR) reflectance spectroscopy, and Raman spectroscopy are more rapid, non-destructive and reproducible as opposed to the typical wet chemical analysis methods such as high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS).

Spectroscopic approaches have been widely utilized to assess wood constituents, including extractives (Gierlinger and Wimmer, 2004; Taylor et al., 2011; Wang et al., 2015). Additionally, combining spectral datasets with chemometric analysis of wood chemical properties has been successfully linked to physical and mechanical properties of wood (Kelley et al., 2004; Meder et al., 1999; Schimleck et al., 1998). However, there are relatively few examples of using the techniques to assess wood durability. For larch (*Larix* spp.), strong correlations between predicted and measured values of larch durability to decay fungi (Gierlinger et al., 2002) were reported. Moderate to poor correlations were obtained for predicting durability to decay fungi or termites as seen on highly durable species, such as western red cedar (*Thuja plicata*) and teak (*Tectona grandis*) (Niamké et al., 2014; Stirling et al., 2014).

These studies have mainly used NIR spectroscopy techniques. Data are still lacking on the application of FT-IR for predicting wood durability. Advances in the FT-IR

technology allows faster and more accurate analysis, and development of powerful software that is user-friendly for performing complex analysis on spectral data have markedly increased use of this technique. The goal of this study was to assess the potential for using FT-IR and NIR to determinate wood durability against fungi and termites.

1.2. Objectives

The primary objective of this work was to establish a rapid, non-destructive technique for determining natural durability using infrared spectroscopy (ATR-FTIR and NIR) with chemometric analysis. The extractive contents of Alaska yellow cedar (*Callitropsis nootkatensis*) and western juniper (*Juniperus occidentalis*) were investigated to understand the variability that existed between and within trees, and the relationships between these levels and resistance to brown-rot decay (*Gloeophyllum trabeum* and *Rhodonia placenta*), and termite (*Reticulitermes flavipes*). To achieve this objective, the work was divided to three components:

1. Investigate the ability of the ATR-FTIR and NIR to detect extractives content in wood using various concentrations of carvacrol, a known biocide in Alaska yellow cedar heartwood. **[CHAPTER 3]**

2. Explore the existence of durability gradients from pith to bark and relationships with extractives content in Alaska yellow cedar lumber and western juniper disks. **[CHAPTER 4 and CHAPTER 5]**

Utilize spectral information with chemometric methods (hierarchical component analysis and principal component analysis), and to relate wood resistance to decay fungi or termite attack and extractives content. **[CHAPTER 4 and CHAPTER 5]**

1.3. Dissertation structure

All of the chapters in this dissertation, with the exception of chapter 1 (this chapter) and chapter 6 (conclusion), are prepared in manuscript format for dissemination in scientific journals.

1. Chapter 1 describes the motivation for this study, basic background to the subjects and ways that this study contribute to the current knowledge.
2. Chapter 2 provides a comprehensive review on wood extractives and their contribution to wood durability with background on infrared spectroscopy usage in wood and other lignocellulosic materials. Draft prepared for submission in *Wood Science and Technology*.
3. Chapter 3 explores the utilization of two infrared spectroscopy techniques, attenuated transmission reflectance fourier transform-infrared (ATR-FTIR) and near

infrared (NIR) spectroscopy for characterization of extractives in Alaska yellow cedar. Draft prepared for submission in *Bioresources*.

4. Chapter 4 utilizes the feasibility of ATR-FTIR and NIR for sorting wood durability using boards of Alaska yellow cedar. Draft prepared for submission in *Canadian Journal of Forest Research*.
5. Chapter 5 explores the feasibility of ATR-FTIR combined with chemometrics analysis for sorting wood durability of western juniper disks along the radius. Draft prepared for submission in *Holzforschung*.
6. Chapter 6 concludes the findings from the present study, future works and recommendations.

Additionally, results from the current study were presented in the following international conferences:

1. Rapid detection of the Alaska yellow cedar, *Callitropsis nootkatensis* (Cupressaceae) extractives using Fourier transform infrared (FT-IR) spectroscopy. Paper prepared for the IRG48 Scientific Conference on Wood Protection. Ghent, Belgium. 4-8 June 2017. IRG/WP 17-20612.
2. Characterization of the Alaska yellow cedar extractives using Fourier transform infrared (FT-IR) spectroscopy. IUFRO 2017 Division 5 Conference. 12-16 June 2017, Vancouver, Canada.

3. Application of ATR-FTIR for determination of Alaska yellow cedar and western juniper durability. Poster prepared for Society of Wood Science and Technology (SWST) and the Japan Wood Research Society (JWRS) Joint Convention. November 5-9 2018, Nagoya, Japan.

CHAPTER 2

BIOCIDAL PROPERTIES OF WOOD EXTRACTIVES AND POTENTIAL APPLICATION OF INFRARED SPECTROSCOPY TO DETERMINATE NATURAL DURABILITY

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Chapter 2. Biocidal Properties Of Wood Extractives And Potential Application Of Infrared Spectroscopy To Determine Natural Durability

Abstract

Naturally durable wood offers broader application especially in exterior, ground contact and marine applications where wood is exposed to decay fungi, insects and marine borers. Wood extractives are a dominant factor in determining resistance to biodeterioration. However, the complexity and variability in extractives content / type within and between individual trees provide can affect durability classifications. Development of infrared spectroscopy (Fourier transform-infrared spectroscopy and near infrared spectroscopy) techniques combined with advanced statistical analyses could provide a powerful tool for rapid, non-destructive determination of wood chemistry, including extractives. Determining a durability classification is laborious, slow, and destructive, while the small number of samples analyzed might not be representative of the overall population. Continuous improvements in technology and chemometric techniques create potential for rapid lumber sorting and to quantify durability.

Keywords: wood extractives, natural durability, infrared spectroscopy, fourier transform-infrared spectroscopy, near infrared spectroscopy

2.1. Introduction

As a biological material, wood is susceptible to biodegradation including discoloration, decay and insect attack. These defects cause major losses in timber production and wood utilization. Wood degradation agents (fungi, insects, bacteria, marine borers) use structural polymers found in cell walls as well as compounds in storage tissues as their nutritional sources (Zabel and Morrell 1992). Brown and white rot fungi utilize cellulose and hemicellulose in cell walls, although only white rot fungi are able to degrade lignin. Subterranean termites consume the carbohydrates in the wood either directly or via symbiotic protozoa present in their gut. However, some tree species produce chemicals in the heartwood region that are highly toxic and confer resistance to wood-degrading organisms.

Durable wood receives a premium price in the market and lumber with this appealing quality has been used throughout history for building houses, boats, marine construction and other applications requiring exposure under extreme conditions. For example, Native Americans along the Pacific Coast used the highly durable western red cedar (*Thuja plicata*) and coast redwood (*Sequioa sempervirens*) for building structures, totem poles and canoes. Both remain important species for interior and exterior applications. Teak (*Tectona grandis*), originally from Southeast Asia, was one of the earliest commercial timbers with trade dating back to 4000 BC. This species possesses excellent resistance against wood decay fungi and insects, high strength,

good dimensional stability, attractive color and aesthetic properties. The highly prized timber is used as a building material and in exterior applications such as boat decking and outdoor furniture. Durable wood can remain structurally sound for hundreds of years as demonstrated by some of the oldest temples and royal palaces in China built in the early 15th century, built mainly using *Persea nanmu* (Lauraceae) (Hillis 1989).

In the late 1800s, increasing demand for wood led to the introduction of synthetic wood preservatives as alternatives to naturally durable wood. These materials performed well, but were broad-spectrum pesticides, affecting non-targeted organisms that might be beneficial to the environment. In the 1980s, growing public concerns about possible adverse effects of wood preservatives renewed interest in naturally durable wood and the possibility of using these biocidal properties as natural, "green" preservatives.

As a biological material, wood durability varies across species, among individual trees of the same species and within a given tree. Most of this variability is controlled genetically, while some is due to tree age and environment. The current method for determining wood durability is a slow, destructive process taking months to years, and only allows testing of a limited number of samples. There is growing interest in development of new methodologies for assessing durability using non-destructive methods. Infrared (IR) spectroscopy has been used successfully for chemical analysis of

wood and is a possible solution. This chapter will discuss the roles of extractives in wood durability, review the concept of infrared spectroscopy, and summarize wood-related research using infrared spectroscopy for assessment of wood durability.

2.2. Heartwood extractives and natural resistance of wood

Natural durability or the inherent ability of wood to resist biological degradation, is an important wood quality parameter for some applications. Durable wood allows broader application, including outdoor use or applications where utilization of preservative treated wood is a concern. While a number of wood properties have been explored for their roles in natural durability (e.g. density, lignin content, moisture content), heartwood extractives - the secondary metabolites produced during heartwood formation - are the major factor affecting wood durability (Scheffer and Cowling 1966).

Table 2.1 Examples of extractives found in wood (from Alén 2011).

Extractives	**Terpenoids**	**Fats**	**Phenolic substances**	**Carbohydrates**	**Inorganic**
Subclasses	Monoterpenoids Resin acids Other terpenoids	Triglyceride Fatty acids	Lignans Flavonoid Stibenes Tannins	Sugars Starch Proteins Gum	Various salts
Function in tree	Protection	Physiological	Protection	Biosynthesis Nutrient reserve Protection	Photosynthesis Biosynthesis
Occurence	Oleoresin canals Heartwood Knots Bark	Parenchyma Cell	Heartwood Knots Bark Foliage	Sapwood Cambium Heartwood	Sapwood Sap in inner bark
Tree species	Softwoods	All	All	All	All

Extractives are low molecular weight compounds that broadly include terpenoids, tropolones, flavonoids, stilbenes, and other aromatic materials that are removable using organic solvents and water (Scheffer and Cowling 1966) (Table 2.1). Extractives are restricted to the heartwood which is defined as the region where “the inner layers of the wood in the growing tree have ceased to contain living cells, and in which the reserve materials (e.g. starch) have been removed or converted into heartwood substance” (IAWA 1964). Extractives constituents and content vary from 1% to 30% of wood, depending on the wood species, growth conditions, and time of the year when the tree is felled (Hill 2006; Miller 2010). In addition to durability, extractives also influence other wood properties such as color, odor, taste, density, hygroscopicity, and flammability (Miller 2010; Rowell 2012).

Historically, durable wood has been used as a construction material and can resist degradation in outdoor applications. However, the importance of wood extractives in determining durability was only recognized in the 1920s, beginning with comparative studies of wood durability for sapwood, heartwood and water extracted heartwood (Hawley et al., 1924). This study also showed that extracted compounds originating from durable wood applied to non-durable wood increased protection against wood decay fungi and insects. Commencing in the 1950s, multiple publications linked wood durability with extractives content (Rudman 1959, 1963, 1965). In the 1980s and 1990s, public concerns regarding possible adverse impacts of wood preservatives on

the environment and human health spurred research on alternative 'green' preservatives using the biocidal properties of heartwood extractives. In addition, the feasibility of using extractives as additives to engineered wood composite products to limit biodegradation has been explored (Kamke and Winandy 2008). Despite several promising studies, field applications have proven elusive because of compound stability, precursors (e.g. anthocyanins and anthocyanidins) and other confounding factors influencing effectiveness (Rowe 1979).

More recent studies have demonstrated the importance and mechanism of extractives (including wood non-biocidal compounds) in protecting wood against decay fungi and termites (Haupt et al. 2003; Aloui et al. 2004; Gierlinger et al. 2004b; Ragon et al. 2008; Pometti et al. 2010; Feraydoni and Hosseinihashemi 2012; Kirker et al. 2013; Ateş et al. 2015). Phenolic components are recognized as critical owing to their ability to scavenge radicals and active oxygen including singlet oxygen, free radicals, and hydroxyl radicals (Hall and Cuppett 1997). Flavonoids are also important and consist of fused aromatic and benzopyran rings with phenyl substituents that have active hydroxyl groups and antioxidactive properties. Flavonoids also provide UV protection and color to plants and fruits (Laks et al. 1988).

Extractives constituents of commercially durable species have been well studied. For example, nootkatone found in western redcedar and Alaskan yellow cedar (*Callitropsis nootkatensis*) is highly toxic to termites and decay fungi (Barton 1976). The family Cupressaceae includes a number of highly durable species whose heartwood contains tropolones such as β-thujaplicins (Haluk et al. 2000; Hillis 2011). Tropolones are unique 7 carbon rings with broad ranges of toxicity. Studies of extractives found on other durable species are listed in Table 2.2. In addition, extensive lists of durable species have been compiled for North America (Scheffer and Morrell 1998; Clausen 2010) and Europe (EN 350-2), but it is important to remember that wood durability varies widely.

Table 2.2 Examples of durable species with known extractives content related to their durability.

Species	Major extractives	Group	Reference
Alaska yellow cedar (*Callitropsis nootkanensis)*	Nootkatone	Sesquiterpene	(De Groot et al., 2000; Gao et al., 2007; Grace and Yamamoto, 1994)
	Chamic acid, chaminic acid	Terpenoid	
Western juniper (*Juniperus occidentalis)*	Cedrol	Sesquiterpene	(Liu, 2009; Mun and Prewitt, 2011; Tumen et al., 2012)
Western redcedar (*Thuja plicata*)	Plicatic acid	Lignans	(Chedgy et al., 2009; Morris and Stirling, 2012; Nault, 1988)
	α-, β- and γ-thujaplicin, β-thujaplicinol, β-dolabrin, Thujic acid	Tropolone	
Teak (*Tectona grandis*)	Quinones, tectoquinone	Terpenoid	(Lukmandaru and Takahashi, 2009; Moya et al., 2014)

2.2.1. Variations in extractives composition

Wood is a heterogeneous material, with properties varying among and within trees (Hillis 2011). Inter-specific variation (e.g. between species) is mainly controlled genetically, while variation on an intra-specific level (e.g. between individual trees) can be due to cambial age, soil, and other environmental factors. In general, extractives content increases from the pith towards the outer heartwood, and reaches a maximum at the boundary between heartwood and sapwood. While longitudinally, concentration decreases with tree height. Durability gradients are believed to be caused by biological detoxification, natural oxidation of heartwood extractives, and continued polymerization of extractives to produce less toxic compounds (Anderson et al. 1963; Hillis 2011).

Light colored sapwood, comprised of living xylem, is considered as non-durable in most species (Hillis 2011). However, some species such as white oak have durable sapwood, particularly in the transition zone between newly formed heartwood and innermost sapwood (Eslyn and Highley 1976; Highley 1995). Sapwood surrounding a previously injured zone may also be more resistant to decay compared to undamaged sapwood (Shigo 1965). The inner sapwood zone is more resistant than newly formed sapwood in species with indistinguishable sapwood and heartwood (Hillis 2011).

Genetics, age and silvicultural practices such as fertilization and growth site conditions are the three primary factors affecting wood durability (Taylor et al. 2006b; Hillis 2011). Trees with superior quality can be selected through tree breeding and genetic modification programs to produce trees with desirable traits, but this is a very slow process. Fertilization is primarily performed to promote the tree growth, but it can also produce more susceptible sapwood. Higher nitrogen levels from fertilization may also promote fungal attack (Merrill and Cowling 1965). Conversely, fertilizer may also increase the amount of carbohydrates that can be utilized for the synthesis of toxic compounds (Taylor et al. 2006a).

An important durability issue concerns durability differences between wood sourced from plantations or regrowth in native forests and wood from old growth forests of the same species. These wood have more sapwood region than heartwood, therefore less extractives accumulation in heartwood compared to old-growth trees. Comparisons between naturally grown teak and plantation teak showed decreased durability due to reduced levels of tectoquinone in plantation trees (Haupt et al. 2003; Lukmandaru and Takahashi 2009). Redwood is classified as highly durable, but has been downgraded to moderately durable in second-growth trees (Clark and Scheffer 1983; Jones et al. 2014). A similar effect has been reported with Port Orford cedar (*Chamaecyparis lawsoniana*) (Ajuong et al. 2014). Conversely, western red cedar decay resistance was comparable between the younger and old-growth trees (Freitag and

Morrell 2007), despite the supposedly lack of heartwood (i.e. extractives) in the former. Thus, it is still unclear why reduced durability occurs in second growth, but it is a major concern as future wood supply will increasingly rely on plantation forests.

Another factor affecting durability is lignification. Lignin provides stiffness to stem tissues, facilitating aerial development and protection against destruction by organisms (Zabel and Morrell 1992). Lignin type and amount are crucial in resistance to soft-rot fungi, in hampering growth of wood decay fungi and deterring termite feeding (Zainal 1976; Gierlinger et al. 2004b; Shanbhag and Sundararaj 2013). While lignin is an important factor in determining durability, it is clear that decay fungi have evolved mechanisms to degrade it (Kirk et al. 1976; Blanchette 1984, 1984). Crystals, when present, reduce moisture-holding capacity, making wood harder to wet, and thus limiting attack by organisms (e.g. wood decay fungi) that require moisture for enzyme diffusion and cellulose hydrolysis to simple sugars. Harder woods containing high amounts of silica or calcium carbonate can affect the ability of termites and marine borers to attack wood (Taniguchi et al. 1986). It is a common assumption that denser wood is associated with higher durability, but there are numerous exceptions. Sugar maple (*Acer saccharum*) has high specific gravity (0.56 basic, 0.71 at 12% moisture content) but is susceptible to decay and insect attack (Clausen 2010).

2.2.2. Wood durability assessment and the need for a rapid determination

Wood susceptibility to biodegradation is usually determined using mass loss or visual evaluation after exposing pieces of the wood to biodeterioration agents over certain periods of time (Willeitner and Peek 1997). These assessments are based on standardized criteria and can be conducted under field or laboratory conditions. Field tests were introduced in the early 1920s and were originally devised to find alternative species for the durable chestnut and cedar species (Humphrey 1916; White 1922).

Accelerated laboratory tests were developed in the 1940s for in-depth durability studies and to identify specific toxic heartwood compounds (Southam and Ehrlich 1943; Zabel 1948; Scheffer et al. 1949; Kennedy 1955; Scheffer 1957; Anderson et al. 1963). Solvent-extracted compounds from durable woods were tested against common wood decay fungi or wood-destroying insects. A number of standardized durability assessment methods have been developed, with different regions of the world opting for their own standard versions. Standards also vary to accommodate differences in specific wood degrading species, their optimal requirements, test duration and sample sizes (Willeitner and Peek 1997; Stirling 2009).

Laboratory tests are often combined with field tests to develop more accurate durability classifications. Results may vary due to variations in climate and types of degradation organisms present. Species now grown in plantations, for example teak

(*Tectonia grandis*), larch (*Larix* spp.), Eucalyptus (*Eucalyptus* spp.) or sessile oak (*Quercus petraea*) are difficult to accurately classify because tests require a large number of samples and a long times to produce meaningful results (Gierlinger et al. 2004b; Guilley et al. 2004; Lukmandaru and Takahashi 2009; França et al. 2017).

Extractives have a significant role in wood durability (Scheffer and Cowling 1966; Taylor et al. 2006b) and chemical analysis may provide a more rapid predictor of resistance than long term field trials. Chemical analysis of extractives from Schorger's method was developed almost a century ago (Schorger 1917), and many of the destructive methods remain unchanged till now. Wet chemical analysis using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) provide comprehensive and specific identification of the chemicals in wood that may contribute to durability (Daniels and Russell 2007). Despite their precision, laboratory analysis can be tedious, expensive, and often requires destructive sampling. Additionally, data obtained from such analyses are difficult to interpret without detailed technical knowledge of wood chemistry, especially for wood extractives and their relationship with durability.

Changes in wood durability associated with plantation forests as well as growing interest in alternative durable species suggest the need for a rapid method for assessing extractives content that accounts for the variability in wood durability

(Amusant et al. 2017). Several methods have been suggested, including using the Folin-Ciocalteu (FC) assay for determination of phenolic content (Harju and Venäläinen 2006), evaluation of wood density and measuring lignin content. However, predictions based on one or several chemical components can be difficult as the presence of major extractive compounds alone may not exclusively explain durability. Interactions between several extractives and not just those with biocidal properties may contribute to durability and their interactions remain poorly understood (Ragon et al. 2008). Heartwood color, which has been directly linked to extractives concentration has also been analyzed to determine wood durability (Gierlinger et al. 2004a; Kijidani et al. 2012), but this technique lacks accuracy.

There remains a need for a non-destructive method for rapid assessment of durability. Spectroscopic methods offer one alternative for rapidly assess wood chemistry, including extractives content and other chemical constituents (e.g. lignin) related to wood durability. These methods, while primarily surface oriented, can still provide relatively robust chemical data. Spectroscopic techniques, such as infrared spectroscopy, utilize the infrared (IR) spectra to generate chemical 'fingerprints' that are unique to biological samples. Data from this region, combined with chemometrics (multivariate analysis of chemical data) are used to identify spectral features that discriminate between two or more groups. Successful development of a non-

destructive spectral technique could allow for rapid determination of durability and facilitate lumber sorting.

2.3. Infrared spectroscopy and application in wood-related studies

2.3.1. Background

Infrared spectroscopy is an analytical technique that can be used for characterizing major wood compounds. It has become increasingly popular due to its speed, high selectivity and ease of analysis using samples in many forms, e.g. liquids, solutions, pastes, powders, films, fibers, gases, and solids. Additionally, the availability of portable infrared systems create the possibility of on-site measurement for rapid chemical analysis. Infrared spectroscopy utilizes differences in molecular vibrations under infrared light to identify chemical compounds (Stuart 2004a). Primary use includes material identification and authentication, quality control of products, and detection of contaminants (Greene et al. 1992; Rodriguez-Saona and Allendorf 2011). The robustness of this technique is illustrated through applications across fields from chemistry to biology, and life sciences to environmental analysis (Yeh et al. 2005; Roggo et al. 2007; dos Santos et al. 2017).

Advancements in sampling accessories and development of software (user friendly interface, library searching capabilities, complex algorithm spectral analysis) have increased ease of use. The availability of hand-held equipment also allows field application under variable environmental conditions, and has been successfully used for identification of unknown substances and produce adequate predictive ability (Sorak et al. 2012; Alcalà et al. 2013). However, more work is needed to test the ability of these portable tools, as they might lack the sensitivity and accuracy of the lab-benched spectrometers especially in analyzing complex materials such as wood.

Infrared spectroscopy as discussed in this chapter focuses on the two main techniques, Fourier-transform infrared (FT-IR) and near-infrared (NIR), which are based on absorption spectroscopy. Another infrared technique, Raman spectroscopy, is not discussed as it has not yet progressed to field practicality; however, it is included in Table 2.3 which provides a comparison of the three IR spectroscopy techniques.

2.3.2. Fourier transform-infrared spectroscopy (FT-IR)

Fourier transform-infrared spectroscopy (FT-IR) is the most common vibrational technique used for material identification and authentication (Faix 1992). It measures light absorption in the mid infrared region between wavelengths 400 to 4000 cm^{-1} (25000 – 2500 nm) corresponding to fundamental molecular vibrations. Output is an IR

spectrum - a plot of % absorbance, transmittance or reflectance against wavenumber (Günzler and Gremlich 2002). Transmittance spectra are usually obtained for qualitative analysis and for interpretation of chemical groups, while absorbance is used for quantitative analysis because it is independent of sample concentration. Absorption corresponding to the vibrational frequencies is then used for identification of a particular functional group. One region that is especially useful for identification is the 'fingerprint' region (1500-600 cm^{-1}), as this region contains the greatest number of unique vibrations. Other regions of interest include the C-H, N-H, O-H stretch region (4000-2500 cm^{-1}), the triple-bond region (2500-2000 cm^{-1}), and the double-bond region (2000-1500 cm^{-1}) (Stuart 2004a).

An ATR-FTIR spectrometer is usually fitted with a diamond sensor for analysis of solids, liquids, pastes and gels that are highly absorbing or non-reflective (Stuart, 2004). A germanium crystal is used for highly absorbing materials such as carbon filled elastomers and rubbers. These sensors analyze the outer 2-3 microns and 0.5-2 micron of the sample surface respectively. Contact pressure between samples and the ATR sensors (diamond or germanium crystal) and sample thickness are the two factors that may affect spectral quality.

Alternatively, the DRIFT method is a non-contact technique with a penetration depth between 200 to 500 microns that is used to analyze solid samples with low reflectance.

This technique is mainly used for analysis of artwork, soils, rocks and minerals, composites, fabrics and corrosion on metal surfaces. Several wood-related studies using DRIFT have been explored (Nuopponen et al. 2006; Toivanen and Alén 2006; Monteiro Pastore et al. 2008). Toivanen and Alén (2006) used DRIFT for analyzing extractives variations in wood and showed the spectral results as comparable to the wet analysis. However, this technique still require preparation by mixing samples with KBr as opposed to the surface scan using ATR-FTIR (Stuart 2004a).

Table 2.3 Comparisons between Raman, FT-IR and NIR spectroscopy (modified from Siesler, 2002).

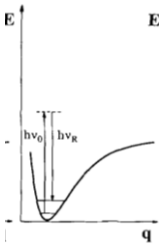
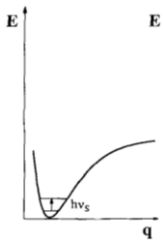
	Raman	FT-IR	NIR
			
Bands	Fundamentals 4000-500 cm^{-1}	Fundamentals 4000-400 cm^{-1}	Overtones combinations 12500-4000 cm^{-1}
Vibrational technique	Scattering technique	Absorption techniques	
Source	Monochromatic radiation (laser VIS-NIR)	Polychromatic (dispersed) radiation	
		Globar	Tungsten
Information	Information contained in scattered radiation	Information contained in absorbed radiation	
Selectivity	High structural selectivity		Low structural selectivity
Functionalities	Homonuclear (e.g. C=C, C-C, S-S)	Polar (e.g. C-F, Si-O, C=O, C-O)	CH / OH / NH

Table 2.3 (Continue) Comparisons between Raman, FT-IR and NIR spectroscopy (modified from Siesler, 2002).

	Raman	FT-IR	NIR
Sample preparation	None	Required (except for ATR)	None
Sample size	Small sample volume (μl) or sample thickness (μm)		Large sample thickness (up to cm)
Current monochromator / detection principle	NIR-Raman (FT) VIS- Raman (CCD)	FT-IR	Grating FT-NIR AOTF DIODE-ARRAY
Advantages	Fast (minutes)	Fast (minutes)	Fast (minutes) No / Minimal sample preparations Non-destructive
Disadvantages	Weak sensitivity to minor constituents	Sensitivity to moisture	Weak sensitivity to minor constituents

Despite the advantages of FT-IR (fast, simple, sensitivity, versatility), it is highly sensitive to moisture or samples with high water content. The presence of water molecules produces strong peaks that may interfere with spectral absorption and mask the absorbent making it difficult to identify compounds. This limitation is much more prominent in natural products with heterogeneous properties such as wood.

2.3.3. Near infrared spectroscopy (NIR)

Near infrared spectroscopy (NIR) is similar to FT-IR and is an absorption-based technique that utilizes similar vibrational overtone (harmonic) and combination modes of fundamental vibrations (Bokobza 1998). Minimal or no sample preparation is required, resulting in faster spectral acquisition.

The NIR region (4000 to 12500 cm-1 or 2500-800 nm), is dominated by overtone and combination bands of CH, OH and NH functionalities, but none of the corresponding overtones of FT-IR fundamental absorptions of polar groups (C-F, Si-O, C=O and C-O) (Stuart 2004a) (Figure 2.2). NIR has a lower sensitivity than FT-IR, producing peaks that are poorly defined and are often overlapped, making it unsuitable for analysis of compounds at low concentrations or for qualitative analysis (Stuart 2004a). Nevertheless, in the NIR region positions of different functional groups can be detected and used for quantitative analysis.

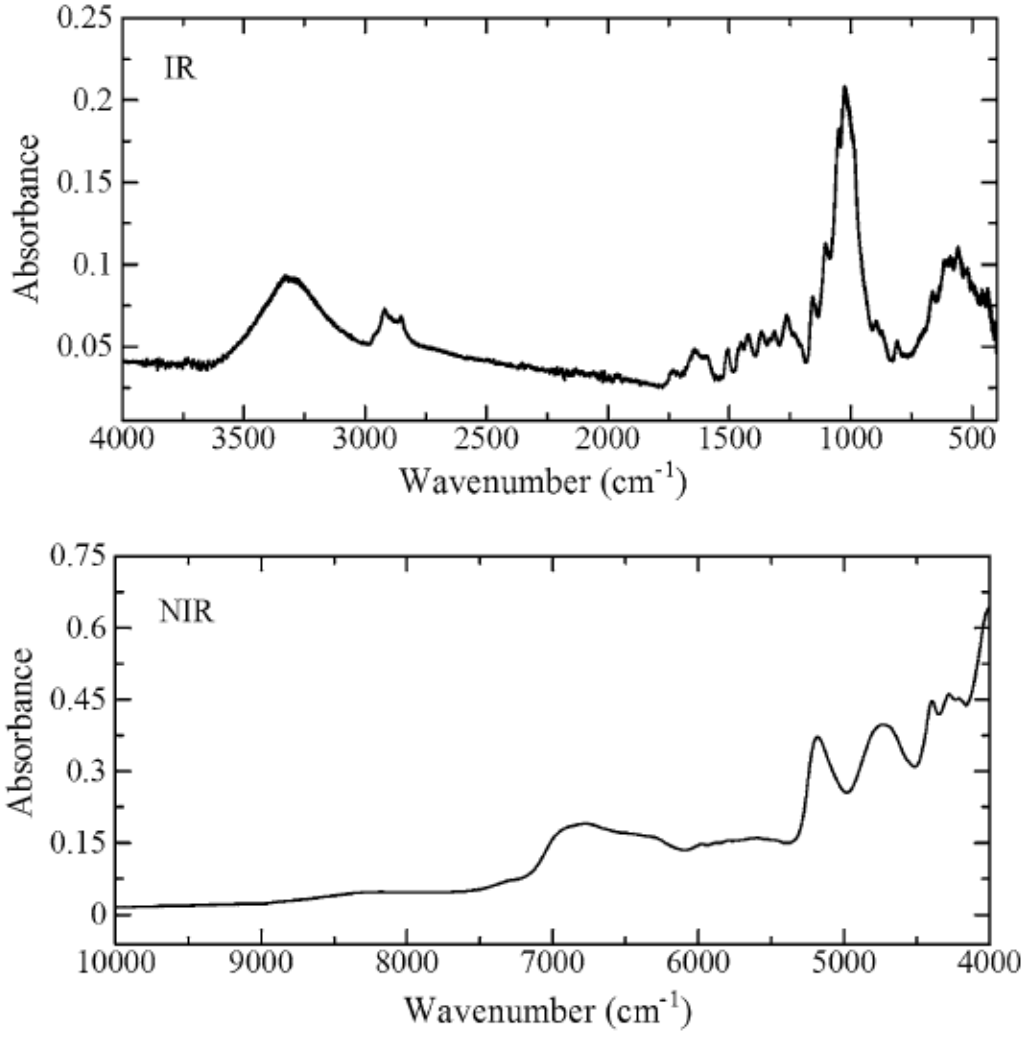


Figure 2.2 Examples of FT-IR (top) and NIR (bottom) spectra of Douglas-fir wood (from Tsuchikawa and Kobori 2015).

NIR intensities are also 10-1000 times lower than FT-IR range, with smaller peaks, making them unsuitable for analysis of compounds present at low concentrations. However, availability of highly sensitive NIR spectrometers (e.g. with efficient detectors and brighter light sources) allow quick, easy scanning and analysis of dense materials and liquids (Cozzolino, 2009; McClure, 2003).

Common NIR applications include food and agricultural product analyses that require large volume sampling (Williams and Norris 1987; Prieto et al. 2009; Cozzolino 2014). NIR spectra have wider separation and minimization of features of symmetric vibrations, leading to an isolation of anti-symmetric bands. The weaker overtones and combination bands compared with fundamental absorption bands allows NIR spectroscopy to be used for analysis of samples up to several millimeters thick (Stuart 2004a).

2.3.4. Chemometric analysis for spectral data

Spectral data obtained from IR spectroscopy are often combined with appropriate analyses to reveal underlying relationships either qualitatively or quantitatively. This includes identifying spectral patterns that may be associated with variations between groups and developing prediction models. General steps for spectral analysis include:

(1) pre-processing of raw spectral data, (2) chemometric methods (multivariate analysis of spectral data), and (3) model validation.

2.3.4.1. Data pre-processing

Pre-processing of the raw spectral data employees functions to minimize background noise and improve model fit. There are several methods for pre-processing depending on sample type and technique used for collecting spectra. The most common techniques (Table 2.4) are baseline correction, normalization and smoothing (Stuart 2004a).

2.3.4.2. Chemometrics methods

Chemometrics is defined as "a chemical discipline that uses statistical and mathematical methods, to design or select optimum procedures and experiments, and to provide maximum chemical information by analyzing chemical data" (Varmuza and Filzmoser 2009). Spectral data sets rely on a suitable chemometric analysis to relate the physical or chemical properties of samples to absorption in the spectral wavelength range (Bokobza 1998). This includes determining the groupings of similar objects (clusters), correlations between objects, and detecting outliers. Analysis is performed either by selecting the peak at the specific wavenumber directly using multiple regression, or utilizing the whole spectrum data using, for example, principal components regression (PCR) or partial least squares (PLS) regression. The most

important aspects of chemometrics are the multivariate statistical data analysis (the analysis of data with many observed variables) and inspection of patterns with many significant types of variations (Martens and Martens 2001). Chemometrics is used for exploratory analysis, calibration and classification / prediction (Table 2.5).

Table 2.4 Common pre-processing methods for IR spectral analysis using FT-IR and NIR (Stuart, 2004).

Pre-processing method	**Function**
Baseline correction	Correct spectra with sloped or varying baselines
Smoothing	Reduce random noise, resulting in removal of narrow spikes in a spectrum
Derivatives	Enhance resolution by examining changes in first derivative, and provide negative peak for each band and shoulder in absorption spectrum for second derivative
Deconvolution	Resolve overlapping bands by producing narrower bands that is distinguishable within closely spaced features
Curve-fitting	Estimate parameters of the component curves for overlapping bands

The main purpose of exploratory data analysis is to learn about data distribution using clusters or groups of similar objects. The most common exploratory technique for analysis of spectral data is principal component analysis (PCA). PCA uses several scoring systems (i.e. principal components) that summarize the systematic patterns of variation between samples and allows graphical observation of clustering objects or variables (Martens and Martens 2001). Variability can be from a single measurement (e.g. wood density), but often it represents derived characteristics of several measurements e.g. relationships between wood density and mass loss due to termite feeding. PCA has limitations as often groupings are not clear cut and interpretation may be difficult. Unlike other statistical analysis techniques, there is no hypothesis testing, no P-value, and no exact rule regarding a positive or negative result. Users must be able to interpret and justify the scoring systems and any groupings produced from the data. PCA analysis has been used successfully to differentiate between wood of different species, provenances and origin (Schimleck et al. 1996).

Multivariate calibration allows development of models for optimal predictions between variables, for example partial least square (PLS) regression. Multivariate classification aims to assign objects correctly to given classes. It can be used to classify unknown materials or for validating the classification of known materials. This includes using latent variables with a good discrimination power such as linear discriminant analysis (LDA) and *k*-nearest neighbor classification (*k*-NN).

Development of powerful software has enabled preprocessing for large datasets such as those found in spectral data. Common spectral analysis software include Unscrambler and R. R is a free, powerful analysis tool with several packages (e.g. *ChemoSpec, hyperSpec*) for processing FTIR and NIR data. Other software that can be used for spectral analysis (such as FT-IR and NIR) is discussed elsewhere (Wehrens 2011; Costa et al. 2016).

2.3.4.3. Model validation

The last step in spectral analysis involves validating the model to ensure accuracy and model fit. The best technique for this process is to split the data in three sets – a 'training set' for creating models (50% of objects), a 'validation set' (25% of objects) for model optimization, and a 'test set' or 'prediction set' to evaluate the accuracy and prediction performance (25% of objects) (Varmuza and Filzmoser, 2009).

Table 2.5 Summary of common multivariate analyses methods for assessing spectral data (Varmuza and Filzmoser, 2009).

Multivariate analysis	Description
Exploratory data analysis	
Principal component analysis (PCA)	Produce dimension reduction for explaining maximum variability using minimal number of principal component
Hierarchical cluster analysis (HCA)	Complimentary to PCA that produce dendogram, providing more insight on relations between objects, with objects hierarchy build based on similarity
Multivariate calibration	
Partial least square (PLS) regression	A method to relate a matrix X (IV) to a vector y (response) or to a matrix Y
Principal component regression (PCR)	Combination of PCA and the multiple linear regression
Multivariate classification	
Linear discriminant analysis (LDA)	Find the differences between two or more classes of objects
k-nearest neighbor classification (*k*-NN)	Pattern recognition based on distance between objects related to the similarity of the objects
Soft Independence Modelling of Class Analogy (SIMCA)	Developing two or more separate bilinear modelling-based class models, followed by checking how each sample fits into each of these class models

2.3.5. FT-IR and NIR application in wood extractives and durability studies

FT-IR and NIR spectra combined with chemometrics have been successfully used since the late 1980s for qualitative and quantitative analysis of wood chemical composition, microstructure, fiber architecture, characterization of interfaces, and properties of both natural fibers and related composites (Stuart 2004b; Siesler et al. 2008). Typical band assignments for FT-IR and NIR in relation to wood are given in Table 2.6.

The primary applications of FT-IR and NIR in relation to wood and wood-based studies can be generalized as follow:

1. Characterizing major wood constituents (cellulose, hemicellulose, lignin and extractives) and moisture content (Wright et al. 1990; Ajuong and Breese 1998; Schwanninger and Hinterstoisser 2001; Raymond and Schimleck 2002; Chen et al. 2010; Jones et al. 2014).
2. Estimating physical and mechanical properties, including surface roughness, density, grain angle, tracheid length, microfibril angle, stiffness, modulus of elasticity, modulus of rupture and tensile strength (Meder et al. 1999; Schimleck et al. 1999; Kelley et al. 2004).

Table 2.6 NIR (top) and FT-IR (bottom) major absorbance bands for wood (from Xu et al. 2013).

Wavelength (nm)	Bond vibration	Related structure
1520	O—H stretch first overtone	$CONH_2$
1616	C—H stretch first overtone	$=CH_2$
1688	C—H stretch first overtone	Aromatic
1724	C—H stretch first overtone	$-CH_2$
1740	S—H stretch first overtone	–SH
1782	C—H stretch first overtone	Cellulose
1896	O—H stretch C—O stretch	C=O, CO_2H
1910	O—H stretch first overtone	Ar—OH
2028	C=O stretch second overtone	$CONH_2$
2074	N—H2 deformation second overtone	Amide II
2266	O—H C—O combination bands	Cellulose
2332	C—H stretch, C—H deformation	Cellulose, starch
2386	C—O stretch O—H deformation second overtone	Primary alcohols

Wavenumber (cm^{-1})	Assignment/functional group	Polymer
875	Glycosidic linkage	Hemicellulose[a]
930	Glycosidic linkage	Cellulose, hemicellulose[a]
990	C—O valence vibration	Cellulose[b]
1035	C—O, C=C, and C—C—O stretching	Cellulose, hemicellulose, lignin[a]
1160	C—O—C asymmetrical stretching	Cellulose, hemicellulose[a]
1200	O—H bending	Cellulose, hemicellulose[a]
1215	C—C + C—O stretch	Lignin (wood)[c]
1270	Aromatic ring vibration	Guaicyl lignin[a]
1280	C—H bending	Crystalline cellulose[a]
1310	CH2 wagging	Cellulose, hemicellulose[a]
1327	C—O of syringyl ring	Lignin (wood)[c]
1335	C—H vibration, O—H in-plane bending	Cellulose, hemicellulose, lignin[a]
1380	C—H bending	Cellulose, hemicellulose, lignin [a]
1425	C—H in-plane deformation	Lignin (wood)[c]
1440	O—H in-plane bending	Cellulose, hemicellulose, lignin[a]
1465	C—H deformation	Lignin[a]
1500	Aromatic ring vibration	Lignin[a]
1595	Aromatic ring vibration + C=O stretch	Lignin[a]
1682	C=O stretching (unconjugated)	Lignin (wood)[c]
1730	Ketone/aldehyde C=O stretch	Hemicellulose[a]
1750	Free ester	Hemicellulose[a]
2840, 2937	C—H stretching	Lignin (wood)[c]
3421	O—H stretching	Lignin (wood)[c]

3. Classifying woods e.g. by species and geographic site (Pastore and Pastore 2011; Carballo-Meilán et al. 2016; Mauruschat et al. 2016; Haartveit and Flæte 2018).
4. Monitoring changes after treatment or exposure in terms of chemical, biological, thermal / radiative degradation, and assessment of natural durability (Kotilainen et al. 2000; Gierlinger et al. 2003; Pandey and Pitman 2003; Flæte and Haartveit 2004; Stirling et al. 2007; Taylor et al. 2008; Poletto et al. 2012).

NIR has been used more frequently than FT-IR as sample preparation is easier and the process is not as sensitive to wood moisture content. NIR allows wood to be analyzed in its solid form as opposed to powdered form for FT-IR analysis. The limited effect of water makes NIR suitable for analyses of samples containing water such as wood (Osborne et al. 1993). Early applications of NIR concentrated on pulp related properties including pulp yield as well as cellulose and lignin content (Schimleck et al. 1998; Schwanninger and Hinterstoisser 2001; Raymond and Schimleck 2002; Kelley et al. 2004). Spectral peak assignments (Schwanninger et al. 2011; Xu et al. 2013) and applications specific to wood have been reviewed elsewhere (Schimleck et al. 2000; So et al. 2004; Tsuchikawa 2007; Sandak et al. 2009; Tsuchikawa and Schwanninger 2013).

Although spectroscopic approaches have been widely utilized to assess chemical properties of wood, including extractives (Holmgren et al. 1999; Da Silva et al. 2013; He and Hu 2013), few studies have explored relationships among spectral data,

extractives content and durability (Gierlinger et al. 2003; Sykacek et al. 2006; Stirling et al. 2007; Rana et al. 2010). Gierlinger et al. (2002) used FT-NIR to predict larch heartwood durability to two wood decay fungi (*Coniophora puteana* and *Rhodonia placenta*) and found strong correlations ($r > 0.9$) between predicted and measured values. Sykacek et al. (2006) extended the study by examining another wood decay fungus species, *Gloeophyllum trabeum*, and showed that spectra from the radial surface provided better correlations. More importantly, the studies indicated that the predictive model was applicable to any larch wood species, regardless of origin. This suggests the possibility for broader application of wood assessment without the need to differentiate based on species within the same genus. Furthermore, NIR predictions between extractives content to durability were also improved in moderately resistant woods, such as Scots pine (*Pinus sylvestris*) (Flæte and Haartveit 2004).

Conversely, Stirling et al. (2014) used NIR for predicting western red cedar resistance to a decay fungus (*C. puteana*) and a species of termite (*Coptotermes formosanus*). However, their predictive model was poor for decay resistance and moderate for termite resistance. They also reported a low correlation between extractives content and decay or termite resistance. This indicated that NIR might not be effective for extractives-based prediction on samples with lower correlations to their extractives.

FT-IR has only recently been explored for predicting wood durability, although the technique has been used to predict extractives levels (Ajuong and Breese 1998; Ajuong and Redington 2004; Poletto et al. 2012; Mattos et al. 2014; Wang et al. 2015). Several studies have utilized both FT-IR and NIR to examine the relationship between wood compounds and physical or mechanical properties (Zhou et al. 2015).

The goal of this research was to utilize both FT-IR and NIR spectroscopy and suitable chemometrics analysis to predict wood durability of two highly durable wood species, Alaska yellow cedar and western juniper, against economically-significant wood decay fungi and termites. Preliminary studies showed the potential use of FT-IR for predicting the durability of Alaska yellow cedar based on the extractives concentration (Shahlinney and Morrell 2017).

2.4. Limitations of infrared spectroscopy

Despite the rapid advancement in infrared spectroscopy applications, especially FT-IR and NIR, several limitations exist. The high moisture sensitivity of FT-IR necessitates that moisture content is minimized prior to collection of spectra e.g. by proper drying and storage. Other factors that may increase variability include ambient temperature and humidity, surface texture, and variation in wood density.

Spectral pre-processing is also required prior to performing multivariate analysis. Standardized pre-processing is necessary so that direct comparisons can be made

across similar studies and ensure reproducibility and validity of the data. Optimization of protocols for sample analysis either using fresh samples or processed samples is also required.

2.5. Conclusions and future trends

The inherent variability of resistance against biological degradation poses problems in current durability classifications. Recent developments in infrared spectroscopy and the availability of powerful statistical methods for spectral data analysis have the potential to create a rapid, non-destructive tool to assess wood durability. This method could be further developed to predict wood durability and utilizing the latest generation of field portable instruments (Sorak et al. 2012) might be used for lumber sorting on a real-time and for in-situ durability classification (Conrad et al. 2014) instead of depending on species-based ratings that may not reflect the current resource. This would allow producers to remove non-durable materials from a stock of lumber. It could also be used to sort materials when a species mixture contains species with varying degree of durability.

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CHAPTER 3

Characterization of the Alaska yellow cedar (*Chamaecyparis nootkatensis*) extractives using FT-IR and NIR spectroscopy

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Chapter 3. Application of ATR-FTIR and NIR Spectroscopy for Characterization of Carvacrol in Alaska Yellow Cedar

Abstract

Extractives, such as carvacrol, play major roles in the excellent durability of Alaska yellow cedar. The feasibility of ATR-FTIR and NIR, coupled with hierarchical cluster analysis (HCA) and principal component analysis (PCA) was investigated for detecting carvacrol in wood. Alaska yellow cedar was extracted using sequential Soxhlet extraction with toluene-ethanol, followed by ethanol and hot water. Extracted samples were then milled, and the powders treated with different concentrations of carvacrol. Spectral analysis of the wood with carvacrol was conducted using ATR-FTIR and NIR spectroscopy, and peaks indicative of carvacrol identified. Chemometric analysis on spectral data using PCA and HCA was able to classify between wood with high (>34%) to lower carvacrol concentrations (<3.5%). The results suggest that infrared spectroscopy could be a tool for non-destructive qualitative and quantitative evaluation of extractives, and possibly for rapid assessment of Alaska yellow cedar durability.

Keywords: Alaska yellow cedar, durability, carvacrol, infrared spectroscopy, ATR-FTIR, NIR

3.1. Introduction

Alaska yellow cedar, *Callitropsis nootkatensis* (D. Don.) Oerst. Ex D.P. Little (Cupressaceae) is native to western North America and found along the coasts of southeast Alaska and British Columbia and at higher elevations as far south as northern California (Harris 1990; Sturrock 2010). The high strength and excellent durability of the wood of this species makes it suitable for a variety of exterior applications (Grace and Yamamoto 1994; De Groot et al. 2000; Hennon et al. 2000). Alaska yellow cedar uses include decks, play structures, poles, furniture and totem poles for indigenous peoples of coastal North America. This species is also a popular choice in Japan for building temples and teahouses due to its termite resistance and similarity to hinoki cypress (*Chamaecyparis obtusa* (Siebold & Zucc.) Endl.).

The durability of Alaska yellow cedar heartwood is related to its extractives content (Barton 1976; Taylor et al. 2006). Extractives refer to the low molecular compounds in wood that are removed using organic solvents and water (Scheffer and Cowling 1966). Wood extractives are present in low concentrations (1-5%) in temperate species, and up to 30% for tropical species (Haluk et al. 2000; Hillis 2011). Alaska yellow cedar contains high amounts of extractives from the tropolone group including nootkatin, as well as carvacrol, a terpenoid (Kelsey et al. 2005; Manter et al. 2007; Karchesy et al. 2018). Carvacrol has been found to be an effective control for arthropod pests including ticks, mosquitoes and fleas (Panella et al. 2005; Anderson and Coats 2012).

Durability can vary widely between and within individual trees of the same species due

One possible non-destructive technique for measuring extractives, is infrared spectroscopy. Infrared radiation activates/excites molecular vibrations that are characteristic of a given chemical compound, or a class of compounds (Stuart 2004). Two main vibrational spectroscopy techniques used in wood analysis are near infrared (NIR) and Fourier-transform infrared (FT-IR) spectroscopy (Pandey 1999; Schimleck et al. 2000; Gierlinger et al. 2003; Poletto et al. 2012; Xu et al. 2013).

Near infrared spectroscopy (NIR) has been used for numerous wood analysis studies due to its simplicity, requiring minimal sample preparation and low sensitivity to wood moisture content (Siesler et al. 2008). NIR spectra range from 4000 to 12500 cm^{-1} (2500-800 nm), with absorption bands resulting from overtones and combinations of vibrations of C-O, O-H, C-H and N-H bonds (Siesler et al. 2008). Extractives analysis using NIR has been explored for mahogany, white oak, eucalyptus, larch and poplar (Gierlinger and Wimmer 2004; Poke and Raymond 2006; Da Silva et al. 2013; He and Hu 2013). He and Hu (2013) showed the ability of NIR to predict hot-water-soluble extractive contents of different hardwood and softwood species and identify their extractives chemical components. Other wood quality studies include the determination of basic density, mechanical strength, stiffness and wood chemical components (Wright et al. 1990; Michell and Schimleck 1996; Schwanninger and

Hinterstoisser 2001; Schimleck and Evans 2002; Schwanninger et al. 2004; Schauwecker et al. 2013).

FT-IR has been used more extensively in wood-related analysis. This mainly due to equipment enhancements that increase the sensitivity of the spectrometers. FT-IR spectroscopy detects functional groups of compounds at the mid infrared region of 4000 cm^{-1} to 400 cm^{-1} (Lin-Vien et al. 1991). Several applications, combined with multivariate data analysis, have been explored for qualitative and quantitative determination of wood properties including lignin content, as well as chemical changes during and after treatment (Ajuong and Breese 1998; Rodrigues et al. 2001; Pandey and Pitman 2003; Rana et al. 2010; Fabiyi et al. 2011; Shangguan et al. 2014). However, extractives studies using ATR-FTIR are still lacking, especially those relating extractives to durability using spectral data. Extractives studies on eucalyptus, rosewood and Scots pine indicated the potential of FT-IR for detection of extractives important to durability (Holmgren et al. 1999; Nuopponen et al. 2003; Popescu et al. 2007; Wang et al. 2015b).

The current study investigated the feasibility of ATR-FTIR and NIR spectroscopy to detect, characterize and quantify carvacrol in Alaska yellow cedar at varying concentration levels using the resultant spectral information with chemometric analysis.

3.2. Materials and methods

3.2.1. Sample collection and preparation

Fifteen Alaska yellow cedar (AYC) boards (130 x 80 x 200 mm) were provided by a sawmill in British Columbia, Canada. No obvious color differences were observed between the heartwood and sapwood. One board was selected randomly for this study, while the rest were used for a separate durability study (Shahlinney et al. 2018). The lumber was cut into smaller strips along the radial face (15 x 90 x 140 mm, r x t x l). These strips were further divided into five subsamples measuring 15 x 15 x 140 mm. The subsamples were conditioned for one month at 20 ± 2°C and 65 ± 5% relative humidity. Samples were then oven-dried (50 ± 2°C) for 48 hours and density determined using ASTM Standard D4442-16 (ASTM 2016). Low temperatures were used to minimize the possibility of extractives degradation (Scheffer 1973). These subsamples were further cut into thirty 15 mm cubes (Please refer to Appendix A).

3.2.2. Extractives free wood blocks

Thirty cubes (15 x 15 x 15 mm) were used to prepare extractive-free wood using the soxhlet extraction method of Kirker et al. (2013), based on a modified ASTM Standard D 1105-96 (ASTM, 2013). Blocks were conditioned at 9% moisture content at room temperature (20-23°C and 30% relative humidity) for a week and weighed prior to the test.

Six blocks from each radial location were retained as the unextracted control samples. The remaining 18 blocks were extracted in 320 mL of 95% ethanol–toluene (2:1 v/v) in a soxhlet for six hours at 60°C to remove low molecular compounds typically found in the cell lumen and on cell surfaces. These compounds are predominantly non-polar, aliphatic compounds (such as fatty acids and their monohydric esters, gummy substances and minor amounts of aromatic compounds) (Ajuong & Breese 1998, ASTM, 2013). The samples were then rinsed with 95% ethanol and ovendried (50 ± 2°C) overnight. Once dried, blocks were weighed and extracted again with 320 mL of 95% ethanol for six hours at 60°C. This process removed compounds mainly from within cell walls, consisting of cyclic aromatic compounds of increasing polarity (such as tannins). The samples were then drained, washed with 95% ethanol, ovendried (50 ± 2°C) overnight and weighed. Finally, the blocks were boiled in a hot water bath for eight hours at 100°C for the removal of tannins, gums, sugars, starches and coloring matter (ASTM 2013). Blocks were then washed with distilled water and air-dried overnight.

Extracted blocks were then ovendried at 50 ± 2°C for 72 hours and weighed to determine total loss due to extractive removal. Six blocks representing each radial face were ground using a Wiley mill (Arthur H. Thomas Co., US) to pass through a 60 mesh screen and then sealed inside air-tight bags that were stored in the dark at 5°C until

used. The remaining wood blocks were used for a separate durability study (Shahlinney et al. 2018) (Please refer to Appendix 2).

3.2.3. Carvacrol preparation

Carvacrol (Sigma-Aldrich, 99%) was diluted in ethanol (Sigma-Aldrich, ≥99.5%) to produce concentrations of 0%, 1%, 3%, 5%, 10%, 25%, 50%, and 100% (wt/wt basis). All samples were placed into 4 ml glass vials that were stored in the dark at 5°C until used.

3.2.4. Collection of spectra

The powdered samples of extracted and non-extracted Alaska yellow cedar (0.01 ± 0.001 g) were measured three times, using three different extracted samples for each concentration by ATR-FTIR and NIR spectroscopy. Two drops of a given concentration of carvacrol was applied to the extracted wood powders. These powders were allowed to dry for one hour at room temperature prior each analysis. Actual carvacrol content in each wood after treatment was calculated and recorded (Table 3.1) using the following formula:

$$\%\ carvarol\ in\ wood = \frac{(b-a)(c)}{b}$$

With, a= Weight of extracted wood (g); b= Weight of carvacrol with wood (g); c= Carvarol concentration (%)

Table 3.1 Labelling scheme used for extracted yellow cedar samples treated with carvacrol at varying concentrations. Each was evaluated on three different samples that were each scanned three times (n=3).

Label	% Carvacrol	% Carvacrol in wood (wt/wt)
A	0	0.0
B	1	0.7
C	3	2.0
D	5	3.5
E	10	6.8
F	15	10.8
G	50	34.7
H	100	78.2

*3.2.4.1. **ATR-FTIR spectroscopy***

All attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were acquired on a Nicolet iS50 FT-IR spectrometer equipped with Smart iTR ATR (Thermo Scientific, USA). Samples were pressed against a single-reflection diamond crystal with a torque knob. The internal reflectance element was a small, diamond prism that allowed a sampling diameter of approximately 2.0 mm. OMNIC 9.2 software (Thermo Scientific, USA) was used for instrument management, spectra acquisition, spectra preprocessing, and file transformation from OMNIC spectra to spreadsheet (CSV).

Approximately 0.1 g of extracted Alaska yellow cedar powder treated with carvacrol was placed on top of the optical medium, and spectra were immediately acquired. Prior to analysis, wood powders were ovendried at 50°C for 2h to reduce the present of moisture inside the wood that could interfere with the spectral readings. The spectra were collected against air as a background (repeated every 15 minutes) over the wavenumber range 4000 - 650 cm^{-1} , with a resolution of 4 cm^{-1} to exclude the signals not relevant within the sample. For each spectrum, 64 scans were co-added and averaged to obtain a good signal-to-noise ratio.

A total of 6942 data points were recorded from 650 to 4000 cm^{-1}. ATR-FTIR spectra were available for 24 yellow cedar samples treated with carvacrol concentrations from 0% (solvent only) to 100% (w/w). ATR-FTIR spectra were collected in triplicate by

taking a newly treated sample for each carvacrol concentration, and the average of the three spectra was used for further analysis. Raw spectra were pretreated for baseline correction and smoothing to help reduce nonlinearity and multicollinearity among variables. (Please refer to Appendix 3)

3.2.4.2. NIR analysis

Near Diffuse reflectance near infrared (NIR) spectra of ground Alaska yellow cedar samples were acquired on a FOSS NIRSystems 6500 spectrometer (Foss NIRSystems Inc, Silver Spring, MD, USA) fitted with a feed and forage analyzer spinning module (Foss NIRSystems Inc, Silver Spring, MD, USA). Samples were placed inside an insert (diameter of 13 mm) within a ring cup (36 mm diameter and 9 mm thick). The Vision 4.1 software (Metrohm, Switzerland) was used for instrument management and spectra acquisition. Approximately 0.1 g of extracted Alaska yellow cedar powder treated with carvacrol was placed inside the ring cup, and spectra were immediately acquired. Each spectrum was obtained for 32 scans and averaged. A total of 700 data points were recorded from 1100 to 2500 nm (9090 to 4000 cm^{-1}). NIR spectra were available for 24 Alaska yellow cedar samples treated with carvacrol concentrations from 0% to 100% (w/w). NIR spectra were collected in triplicate by taking a new

treated sample for each carvacrol concentration, and the average of the three spectra was used for further analysis (Table 1).

3.2.5. **Statistical analysis**

All analyses were done on R Studio version 1.0.136 (RStudio Team, 2016) using the R package "Chemospec"(Hanson 2017) on the spectral dataset. Hierarchical cluster analysis (HCA) was done using Euclidean distance to identify groupings based on the similarity of IR spectra (Siesler et al. 2008). These spectra were grouped into a new object called a ¨cluster¨ or ¨hierarchical group¨. Cluster analysis is used for grouping of concentrated groups, with no information on group membership (i.e. finding groups containing similar objects). Principal component analysis (PCA) was performed for differentiating between samples with varying carvacrol concentrations. PCA was carried out using classical or robust methods (Varmuza and Filzmoser 2009; Wehrens 2011). The classical methods use all the data provided to compute the scores and loadings, while the robust methods use the core of the data, meaning some samples might be down weighted. The robust technique is useful when there are outliers, as it is less sensitive compared to classic PCA (Gharibnezhad et al. 2011). Both methods search for the components that explain as much variance in the data as possible.

3.3. Results and discussion

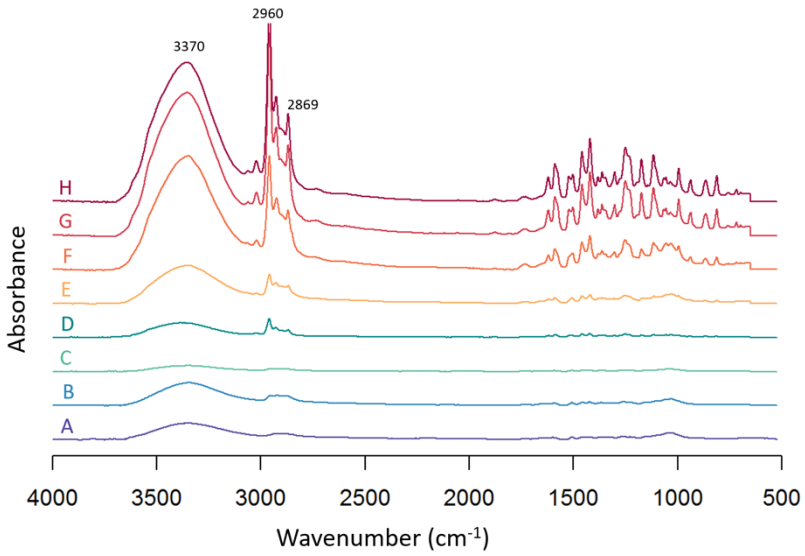
3.3.1. Spectral assignment for carvacrol

3.3.1.1. ATR-FTIR

Changes in ATR-FTIR spectra were detected at different carvacrol concentrations (Figure 3.1). A broad band with increasing absorbance at higher carvacrol contents was observed from the stretching vibrations of O-H at 3417 cm^{-1}. In addition, an aliphatic C-H around 2950 cm^{-1} was detected at 5% carvacrol (D) and the signal increased with increasing carvacrol concentrations (Figure 3.1A).

The region from 1800 to 600 cm^{-1} showed the greatest evidence of unique characteristics (fingerprint) (Figure 3.1B). This region typically shows evidence of complex deformations of molecules and may arise from specific characteristics of molecular symmetry or combination bands arising from multiple bands deforming simultaneously (Siesler et al. 2008). Carvacrol consists of functional group phenolic -OH and aromatic rings. The presence of double bonds and / or aromatic rings was signified by the weak absorption of C=C near 1650 cm^{-1}, while the presence of an aromatic ring was implied by the medium to strong absorptions at 1600-1450 cm^{-1}. In the current study, the aromatic ring region showed increasing absorbance with increasing carvacrol concentrations, while the weak C=C absorption (1650 cm^{-1}) was almost undetectable in the spectra (Figure 3.1B).

(A)



(B)

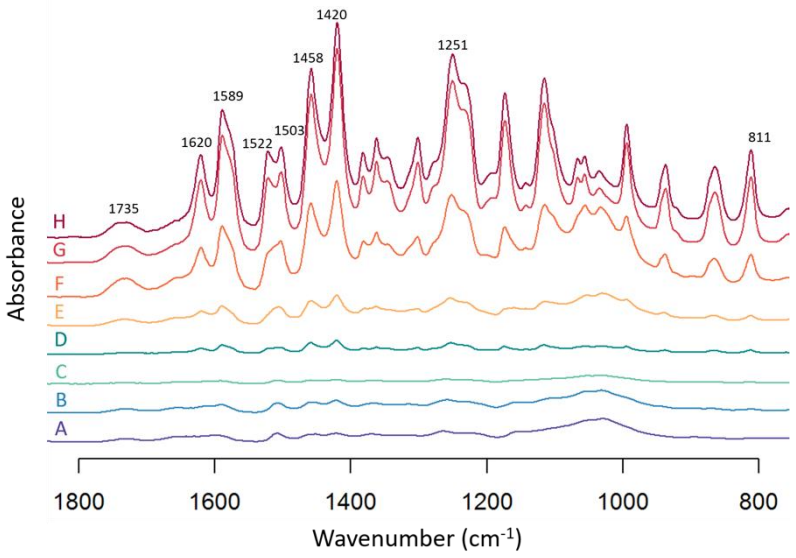


Figure 3.1 Representative FT-IR spectra of extracted yellow cedar treated with the eight carvacrol concentrations at 4000 – 650 cm-1 (A), and 1800 – 650 cm-1 (B)

[A=0.0%, B=0.7%, C=2.0%, D=3.5%, E=6.8%, F=10.8%, G=34.7% and H=78.2% carvacrol].

The peak at 1503 cm^{-1} was indicative of the deformation vibration within benzene rings in carvacrol (Lin-Vien et al. 1991; Stuart 2004), and became cleaved at higher carvacrol concentrations (34.7% and 78.2%). Additionally, observations made on the extracted and non-extracted Alaska yellow cedar from the same samples used in the current study (Shahlinney and Morrell 2017) indicated a reduction in peak height on extracted samples suggesting the loss of some phenolic compounds after extraction.

The band at 811 cm^{-1} (corresponds to out-of-plane CH wagging vibrations) was an indicator of carvacrol (Schultz et al. 2005). The peak for this band increased with carvacrol concentration. This band has also been used for differentiating different types of aromatic ring substitution (Lin-Vien et al. 1991). The 811 cm^{-1} peak was detected on samples with a minimum of 3.5% carvacrol (this concentration indicates the sensitivity of the ATR-FTIR spectrometer), although it is difficult to observe in the resulting spectra due to the scale used for absorbance (Figure 1).

Extractive-related peaks have been observed in wood occurring at 1730 cm^{-1}, 1633 cm^{-1}, 1600 cm^{-1}, 1510 cm^{-1} and 1271 cm^{-1} (Pandey and Pitman 2003; Nuopponen et al.

2003; Colom and Carrillo 2005; Schauwecker et al. 2013; Mattos et al. 2014; Zhou et al. 2015). All these peaks, with slight shifts in peak position, were observed in the current study (Table 2). Similar findings were also observed by Shahlinney and Morrell (2017) using a transmission FT-IR. Broad peaks at 1735 cm^{-1} corresponded to C=O bonds typical of non-conjugated ketones and conjugated carboxylic acids in hemicellulose and lignin. This peak did not appear to change with increasing carvacrol and may be indicative of other extractive groups in Alaska yellow cedar that were not removed in the extraction process (Moore and Owen 2001). Based on the resulting spectra (Figure 1), only the region between 4000-2500 cm^{-1} and 1800-650 cm^{-1} was retained for further analysis.

Table 3.2 FT-IR bands related to wood extractives found in the Alaska yellow cedar heartwood.

Peak (cm^{-1})	Reference peak (cm^{-1})	Description	Associated wood compounds	References
1735	1730	C=O stretching vibrations produced by ester carbonyl	Fat, wax or esterified resin acids	Zhou et al. 2015
1620-1589	1600	C=C stretching or aromatic ring deformation	Aromatic compounds, phenolic group	Zhou et al. 2015, Pandey & Pitman 2003
1620	1633	Olefinic double bond	-	Zhou et al. 2015
1503 - 1522	1510	Deformation vibration within benzene rings	Aromatic compounds	Zhou et al. 2015
1251	1271	Carbon single bonded oxygen	-	Zhou et al. 2015
811	811	Out-of-plane CH wagging vibrations	Carvacrol	Schulz et al. 20015

3.3.1.2. NIR spectra

NIR spectra of extracted and non-extracted Alaska yellow cedar were compared along with extracted Alaska yellow cedar treated with 0% carvacrol (A) or 78.2% carvacrol (H) (Figure 2). No differences were detected between the extracted and control samples (non-extracted samples with no added carvacrol). Samples with 78.2% carvacrol (H) showed changes in peaks at 1200 nm, 1700 nm, and 2400 nm, compared to samples with 0% carvacrol. The band at 1700 nm denotes the C-H stretch first overtone ($-CH_2$), and is one of the major bands for wood (Xu et al. 2013). Absorption at 1200 nm is the second overtone of the C-H stretching vibration (Siesler et al. 2008).

NIR spectra for Alaska yellow cedar treated with different concentrations of carvacrol were observed at 1100 to 2500 nm (9090 to 4000 cm^{-1}) (Figure 3.2). Peaks indicative of carvacrol (1700 nm, 1200 nm and 2400 nm) increased with rising carvacrol concentration. The peak around 2400 nm appeared at a carvacrol concentration of 3.5% (D), with a stronger peak observed with increasing carvacrol concentrations. However, sample B appeared to have slightly higher absorbance than expected, being between the spectrum with samples treated with 6.8% (E) and 10.8% (F) carvacrol (Figure 3). There is a possibility that some of the samples did not received equal mixing during treatment process with carvacrol, resulting in a higher or lower concentration

that influenced the resulting spectrum. Additionally, conversion of spectral data to derivatives might highlight the differences between spectra and be more suitable for analysis. As the ATR-FTIR spectra showed more information compared to the NIR spectraonly the ATR-FTIR spectral information was considered for the chemometrics analysis to assess carvacrol concentration.

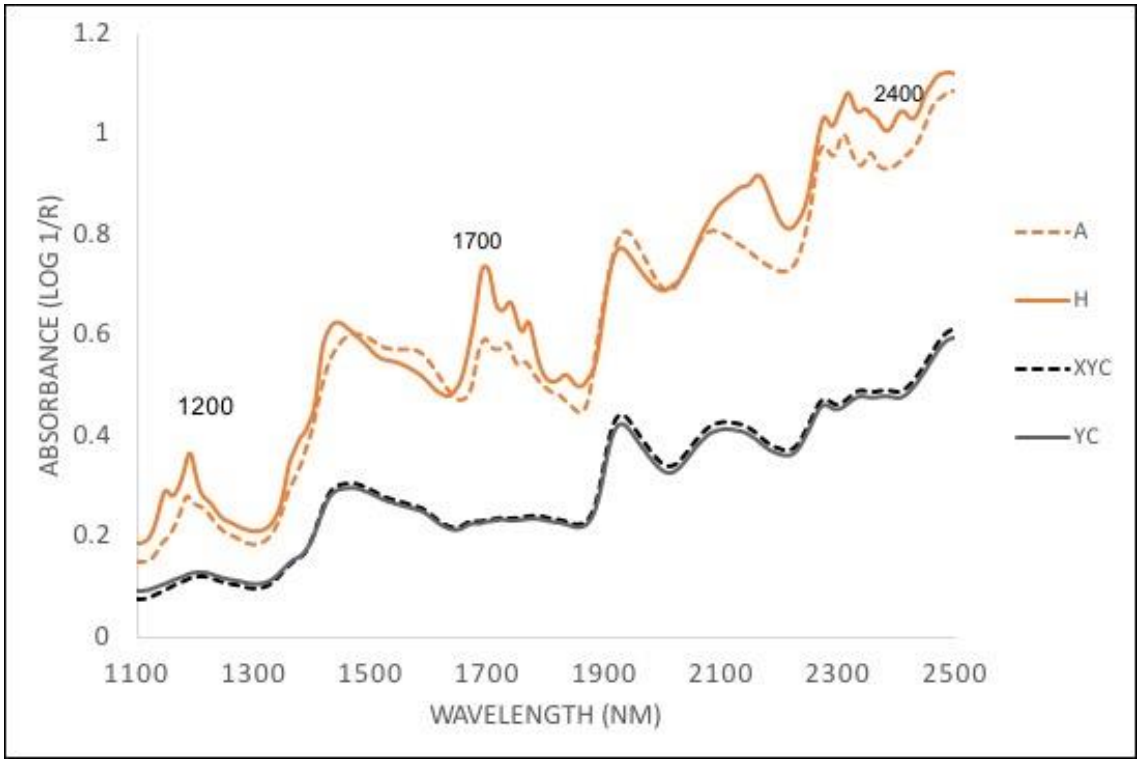


Figure 3.2 Representative NIR spectra (1100 to 2500 nm) for Alaska yellow cedar treated with carvacrol at eight concentrations [A=0.0%, B=0.7%, C=2.0%, D=3.5%, E=6.8%, F=10.8%, G=34.7% and H=78.2% carvacrol].

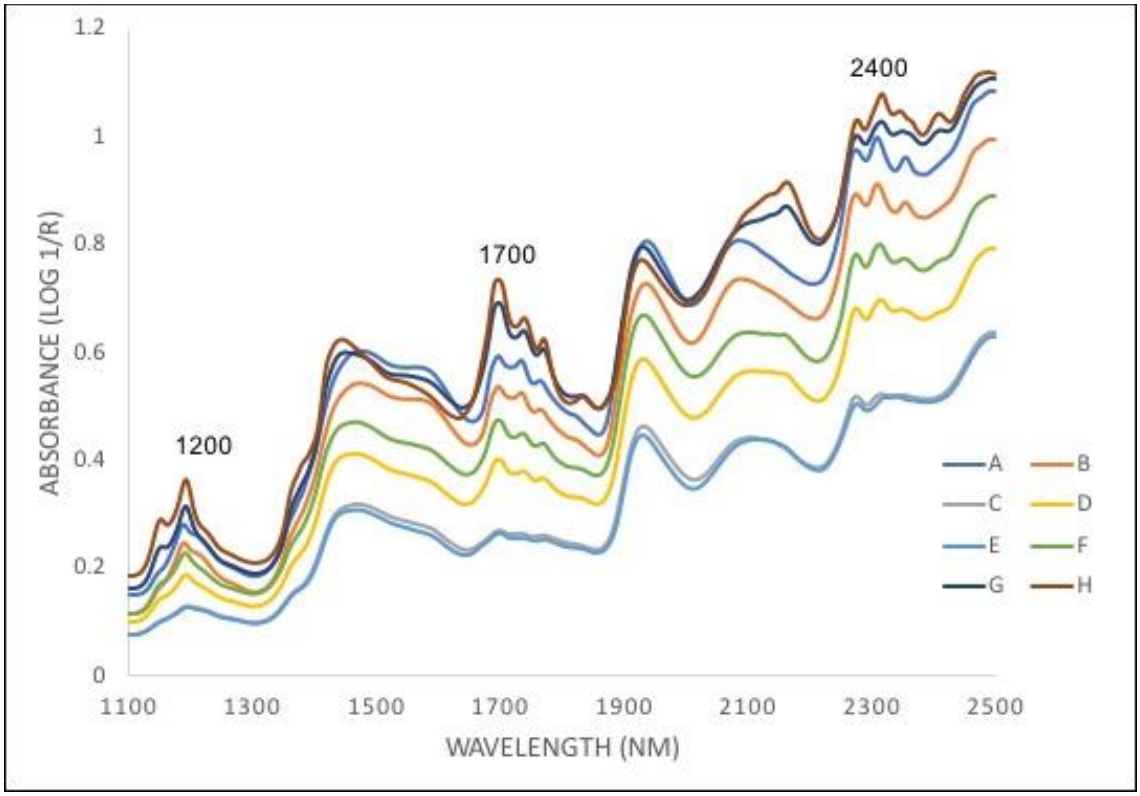


Figure 3.3 Representative NIR spectra (1100 to 2500 nm) for Alaska yellow cedar treated with carvacrol at eight concentrations [A=0%, B=1%, C=3%, D=5%, E=10%, F=15%, G=50% and H=100% carvacrol].

3.3.2. Chemometrics analysis

3.3.2.1. Hierarchical cluster analysis

The ATR-FTIR data for all the samples treated with different concentrations of carvacrol were examined to determine if specific differences could be identified for each treatment. The resulting HCA dendogram produced two major clusters (Figure 3.4). The left cluster consisted of samples with carvacrol concentrations lower than 10.8%, while the right cluster contained samples with higher carvacrol concentrations (over 10.8%). However, the sample containing 6.8% carvacrol (E) did not follow the trend. The left cluster was further separated into one group mainly with carvacrol concentrations from 0 to 2.0% (A, B and C), and another with concentrations between 3.5 and 6.8% (D and E).

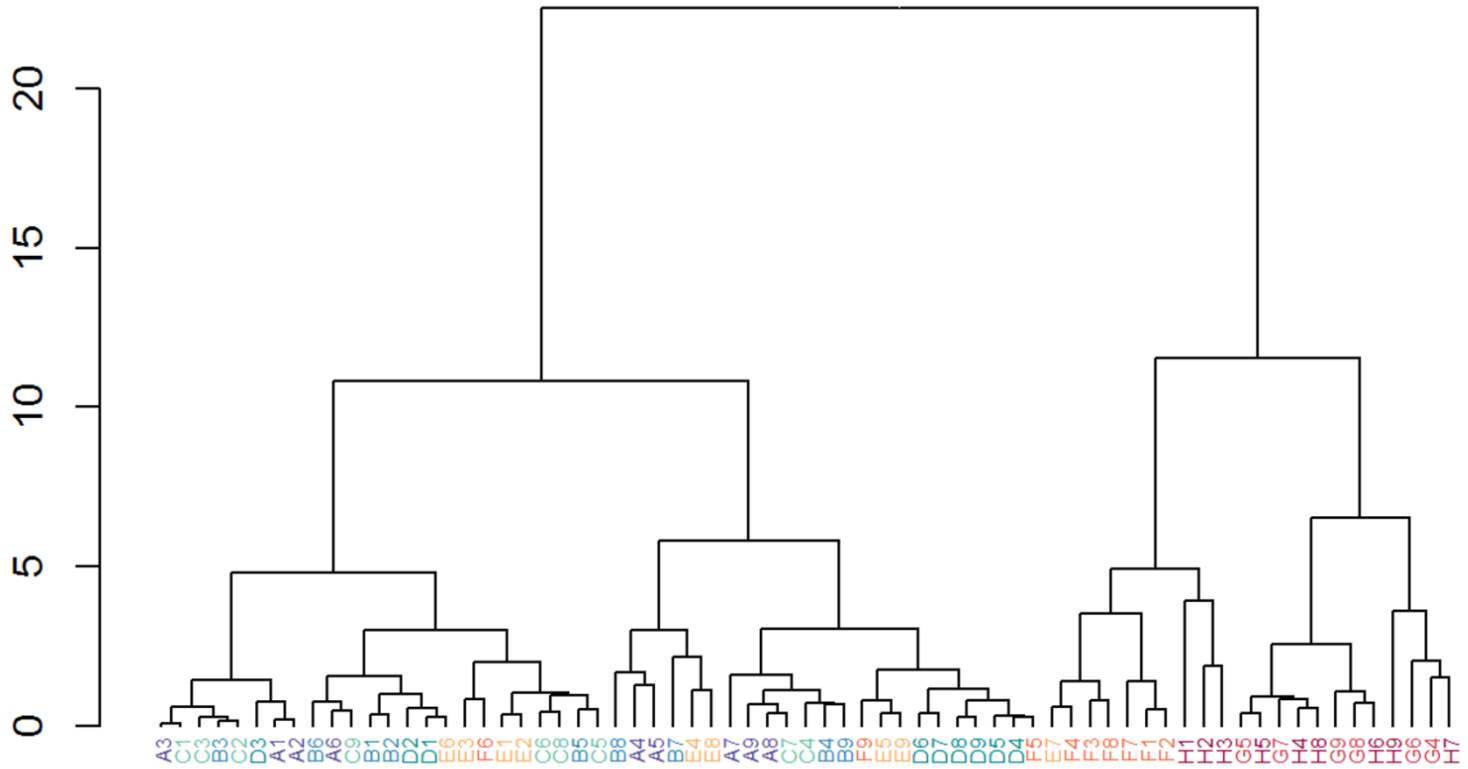


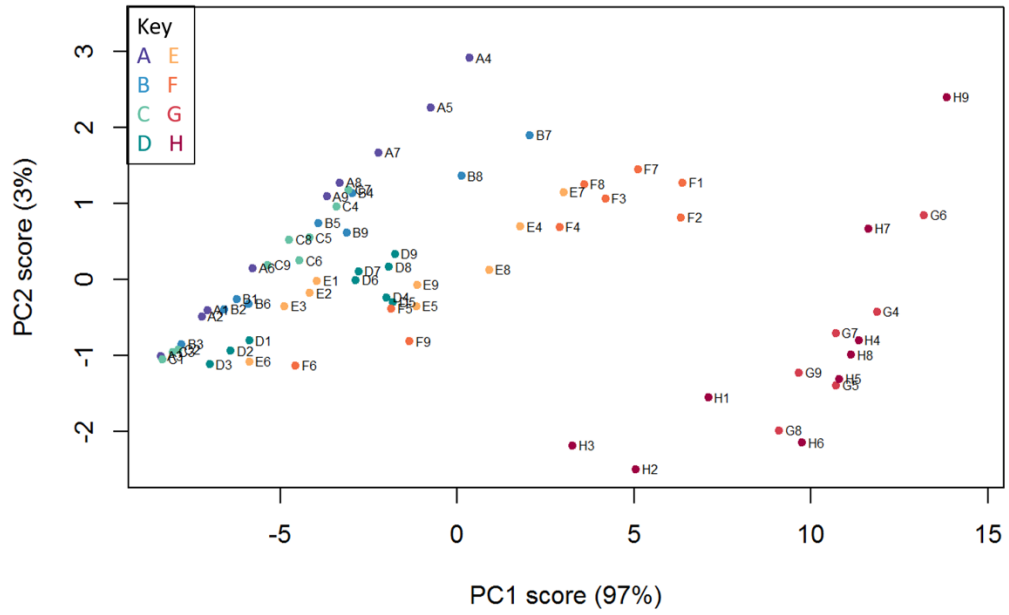
Figure 3.4 Dendogram of hierarchical clustering (with Euclidean distance) of extracted yellow cedar treated with the eight carvacrol concentrations [A=0.0%, B=0.7%, C=2.0%, D=3.5%, E=6.8%, F=10.8%, G=34.7% and H=78.2% carvacrol].

3.3.2.2. Principal component analysis

In this study, PCA was performed on the 4000-2500 cm^{-1} and 1800-650 cm^{-1} region on 24 samples with three replicates at each carvacrol concentration. Classic and robust PCA showed similar values of the first two principal components (PCs), but with different directions (Figure 3.5). Both analyses showed that the first two PCs explained more than 99% of the variations in our data. No outliers were detected in the study, as observed from the orthogonal distance analysis on the spectral data sets. Thus, classic PCA was used for further analysis.

Samples with 34.7% (G) or 78.2% (H) carvacrol were grouped together on the far right of PC1 scores and at the negative values along PC2 score (Figure 5A). Samples with 6.8% (E) and 10.8% (F) carvacrol were grouped closed together along PC1 and PC2 scores. Samples treated with 3.5% carvacrol or lower concentrations appear inseparable in Figure 5A. However robust PC showed that 3.5% carvacrol (D) samples were located close to the '0' value along PC2 scores (Figure 5B). Robust PC also showed separation between the 34.7% and 78.2% carvacrol treatments and samples receiving lower carvacrol concentrations.

(A)



(B)

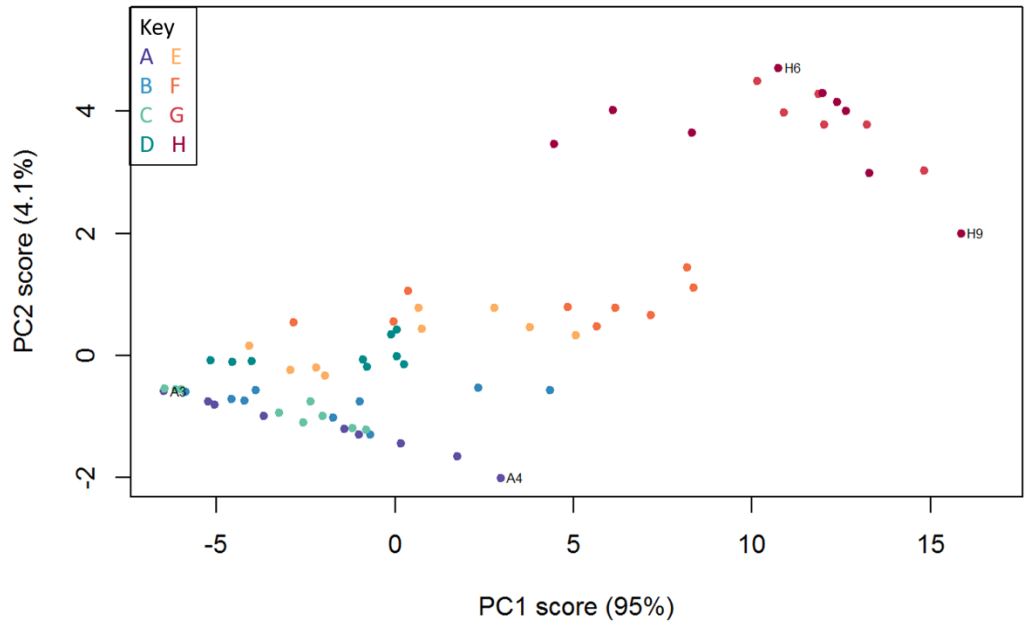


Figure 3.5 Principal component analysis showing the first two principal component (PC) scores for classical PCA (A), and robust PCA (B) on Alaska yellow cedar treated

with different concentrations of carvacrol [A=0.0%, B=0.7%, C=2.0%, D=3.5%, E=6.8%, F=10.8%, G=34.7% and H=78.2% carvacrol].

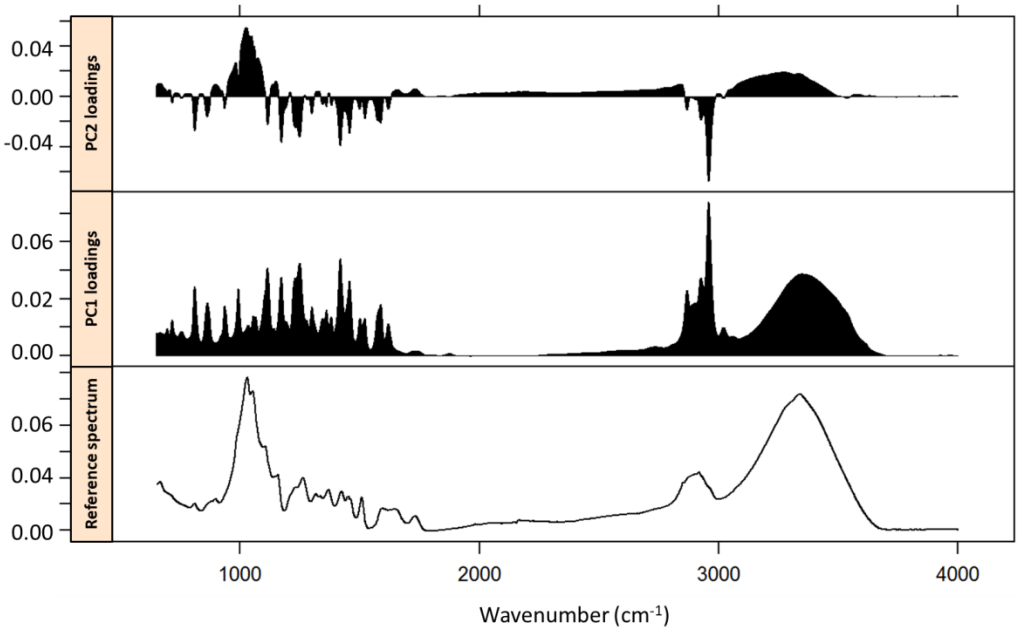


Figure 3.6 Loading plots for the PC1 and PC2 for ATR-FTIR spectra of Alaska yellow cedar treated with varying concentrations of carvacrol.

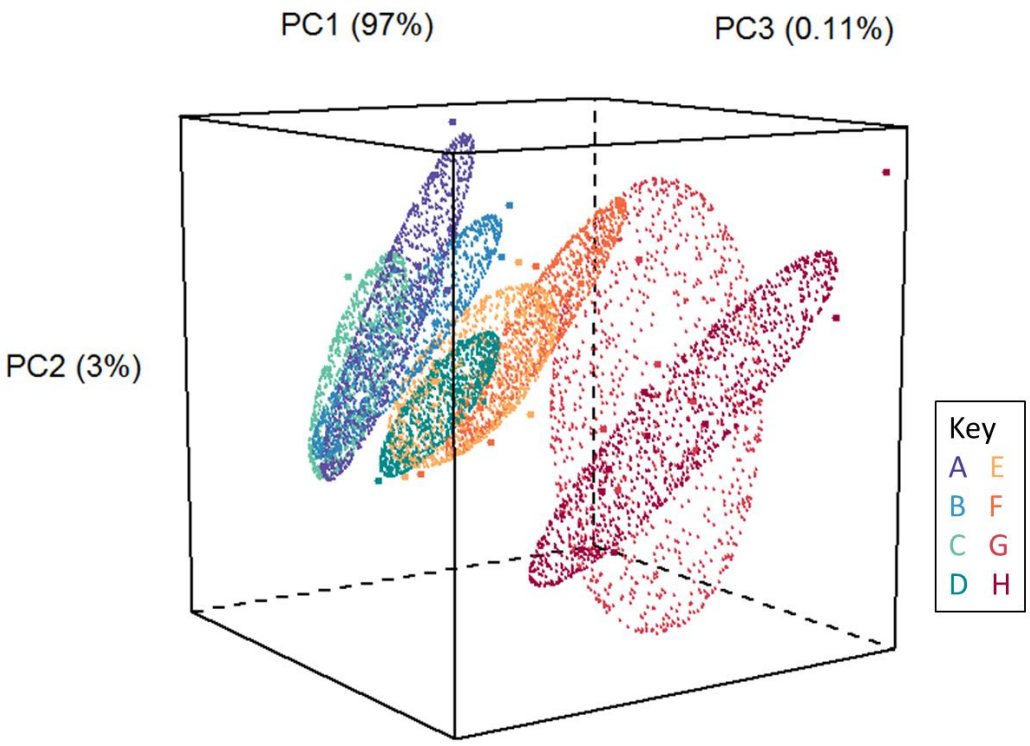


Figure 3.7 Principal component analysis using the classic method showing 3D representations of the first three principal components (PCs) of ATR-FTIR spectra of Alaska yellow cedar treated with different concentrations of carvacrol [A=0%, B=1%, C=3%, D=5%, E=10%, F=15%, G=50% and H=100%].

The loading plots for PC1 and PC2 indicated that the peak at 2950 cm^{-1} appeared to have a strong influence on PC1 scores, allowing separation between high (50-100%) and low carvacrol concentrations (Figure 3.6). Differences in PC2 scores were attributed to the strong negative values at 2950 cm^{-1} and positive values at 1115 cm^{-1}.

Samples showed strong peaks with increasing carvacrol concentrations, suggesting a relationship between peak height and carvacrol concentration as an indirect measure of durability. ATR-FTIR showed sensitivity for detecting carvacrol at concentrations above 3.5%. These concentration differences might be difficult to observe based on visual inspection of spectra alone. However, chemometrics analysis using PCA or HCA enabled discrimination between carvacrol concentrations.

In Alaska yellow cedar, extractives content was reported to be between 3 – 5% (wt/wt) (Kirker et al. 2013). The ability of infrared spectroscopy to detect a single extractive at 3.5%, as seen in current study, provides the potential for quantitative and qualitative assessment of extractives and, indirectly, wood durability. Better prediction might be possible using wood species with higher extractives content (Sjöström 1993; Amusant et al. 2007), although more extensive testing will be required to establish relationship between extractives and durability.

However, the heterogenous nature of wood made spectral analysis complex and challenging. Continued advancement in chemometrics analysis, and more sensitive

spectrometers might improve the process, but the technique will still provide a relative guide to durability. Furthermore, spectral pretreatments and examination of more wavelength ranges prior to chemometrics analysis could help improve predictions. Various data pre-processing (e.g. no preprocessing, offset correction, multiple scatter correlation, first and second derivatives) have been explored (Candolfi et al. 1999; Byrne et al. 2016), but additional studies specifically for extractives could help optimize the quantification and prediction of wood extractives. Data pre-processing, specifically the use of first and second derivatives should be explored in the future and may improve the classification. Several studies have utilized derivatives in spectral analysis that showed better prediction models relating to other wood quality parameters (Schwanninger et al. 2004; Wang et al. 2015a).

3.4. **Conclusions**

Changes in ATR-FTIR and NIR spectra of extracted Alaska yellow cedar treated with various concentrations carvacrol were evaluated in this study. Spectral information on ATR-FTIR detected changes in carvacrol levels at low concentrations, suggesting the potential for qualitative and quantitative analysis of extractives in wood. Further studies on the relationship between extractives content, resistance to fungal or insect attack, and spectral information will be required to determine the feasibility of using infrared spectroscopy for rapid, non-destructive determination of wood durability.

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CHAPTER 4

ATR-FTIR STUDY OF ALASKA YELLOW CEDAR EXTRACTIVES AND RELATIONSHIP WITH THEIR NATURAL DURABILITY

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Chapter 4. ATR-FTIR Study of Alaska Yellow Cedar Extractives and Relationship with Their Natural Durability

Abstract

New approaches for determination of wood durability are needed as timber utilization shifts towards plantation species or native forest regrowth that generally has reduced durability. This study focuses on the application of Fourier transform-infrared with attenuated total reflectance (ATR-FTIR) spectroscopy combined with principal component analysis (PCA) for distinguishing between groups of wood which vary in susceptibility to two decay fungi (*Gloeophyllum trabeum* and *Rhodonia placenta*) and eastern subterranean termites (*Reticulitermes flavipes*). Mass losses and extractives yield using sequential extractions (toluene:ethanol > ethanol > hot water) showed moderate to weak relationships. PCA analysis revealed weak ability to distinguish amongst levels of wood durability to all tested organisms.

Keywords: wood durability, infrared spectroscopy, Fourier transform infrared spectroscopy, Near infrared spectroscopy, chemometrics

4.1. Introduction

Wood quality, including its ability to resist degradation against fungi and insects, is highly variable – not just between different species but also amongst individual trees of the same species (Hillis 2011). Short rotation plantation trees and native forest regrowth are increasingly harvested to accommodate the demand of wood for construction. These woods are generally more susceptible to wood degradation compared to old growth trees due to the decreased availability of toxic chemicals known as extractives (Haupt et al. 2003; França et al. 2017).

Extractives are non-structural, low molecular weight chemical compounds in wood (Scheffer and Cowling 1966). Quantitative determination of extractives content can be done using organic or inorganic solvents and water. These solvents provide different yields based on polarity (Hillis 2011). In particular, the wood phenolic extracts exhibit high toxicity against wood decay fungi and insects (Rudman 1959; Niamké et al. 2014), while terpenoid compounds possess toxic, antifeedant and repellant properties against termites (Lajide et al. 1995, Watanabe et al. 2005).

Differences in heartwood extractives content and concentration have been related to the existance of durability gradients in wood (Nault 1988; Gierlinger and Wimmer 2004; Lukmandaru and Takahashi 2009; Hashida et al. 2014). In general,

extractives content increases from the pith towards the outer heartwood, and reaches a maximum at the boundary between heartwood and sapwood (Hillis 2011). Longitudinally, concentration decreases with tree height. Durability gradients are also believed to be caused by biological detoxification, natural oxidation of heartwood extractives, and continued polymerization of extractives to produce less toxic compounds (Anderson et al. 1963; Dizhbite 2004; Gao et al. 2007). This makes durability classification a complex process.

Durability classification can be determined using the weight loss method or by visual rating after exposing woods to biodegradation agents over certain periods of time (Stirling 2009). Representative wood samples are exposed in the environment where the wood is to be used, or as exposed as smaller samples using specific decay fungi or insect species in the laboratory. Mass loss before and after test is used to classify durability based on the standardized methods. These tests are laborious, destructive and can require long term periods (months to years) before durability can be classified, especially for highly durable species.

Infrared spectroscopy techniques, such as Fourier transform-infrared spectroscopy with attenuated total reflectance (ATR-FTIR) or near infrared (NIR) spectroscopy are a rapid, surface-based screening technique that is used for characterization of

many wood properties (Pandey 1999; So et al. 2004; Tsuchikawa and Schwanninger 2013). These techniques are used mainly for determination and prediction of major wood compounds (cellulose, hemicellulose, lignin and extractives content) or wood moisture content (Ajuong and Breese 1998; Schwanninger and Hinterstoisser 2001; Jones et al. 2002; Hodge and Woodbridge 2004; Poke and Raymond 2006). Many wood compounds can be related to physical and mechanical properties or can be used to predict wood density, surface roughness, tensile strength, modulus of elasticity and modulus of rupture (Schimleck and Evans 2002; Meder et al. 2002; Kelley et al. 2004; Schimleck et al. 2006).

Despite the wide utilization of spectroscopic approaches to access chemical properties, few studies have explored the relationships between spectral information, extractives composition and durability (Gierlinger et al. 2003; Sykacek et al. 2006; Stirling et al. 2007, 2015; Li and Altaner 2018). Previous studies produced varying results. Gierlinger et al. (2002) showed that FT-NIR accurately predicted larch heartwood durability to decay fungi. Striling et al. (2014) showed that NIR produced poor prediction for western red cedar decay resistance. FT-IR applications for durability prediction has been employed to a limited extent since it requires sample preparations such as mixing wood powder with KBr before it can be analysed. However, advancement in FT-IR with Attenuated Total Reflactance (ATR-FTIR), allow samples to be analysed with minimal or no preparation.

Developments in FT-IR technology also offers high signal-to-noise ratio and better wavelength precision. To the best of our knowledge, durability study using ATR-FTIR has not been examined.

Spectral information can be combined with appropriate chemometrics analyses to reveal the underlying relationships. Hiearchical cluster analysis (HCA) and principal component analysis (PCA) are exploratory methods that can be used for spectral data analysis (Schimleck et al. 1996; Martin and Martin 2007; Toscano et al. 2017). These techniques can be used to identify spectral patterns related to variations between groups and to develop prediction models. Infrared spectroscopy combined with reliable chemometrics analysis is a powerful way for predicting wood properties (Meder et al. 1999; So et al. 2004; Nascimbem et al. 2013).

Alaska yellow cedar, *Callitropsis nootkatensis* (D. Don.) Oerst. Ex D.P. Little (Cupressaceae) is native to western North America and is found along the coasts of southeast Alaska and British Columbia, and at higher elevations as far south as northern California (Harris 1990; Sturrock 2010). The wood is highly prized due to the excellent durability against biodegradation (Kelsey et al. 2005; Karchesy et al. 2018), and is popular in Japan as replacement for native hinoki (*Chamaecyparis obtusa* (Siebold & Zucc.) Endl.) as a structural materials, for ceremonial boxes and

as a raw material for restroration of temples and shrines (Jozsa 1991). However, Alaska yellow cedar supplies have been declining in the last decade and reforestation is on-going to recover the population (Hennon et al. 1990; Beier et al. 2008). Alaska yellow cedar old-growth trees (~1000 years old) grow slowly, with 12 annual rings mm^{-1} (Hennon 1992). There is a concern that the wood quality, including durability to biodegradation of the planted Alaska yellow cedar might be inferior to that of old growth trees.

This study examined the feasibility of using ATR-FTIR spectroscopy with chemometrics analysis for estimating natural resistance of Alaska yellow cedar to attack by brown-rot fungi and termites.

4.2. Material and methods

4.2.1. Samples and sample preparation

Ten Alaska yellow cedar boards measuring 130 x 80 x 200 mm (3-3/4" x 5-3/4" x 8') were provided by a sawmill in British Columbia, Canada. No obvious color differences were observed between heartwood and sapwood. The cross section of each board was determined and numbered based on the distance from pith using the direction of growth rings as reference (Please refer to Appendix 4).

The lumber was cut into smaller strips along the radial face (15 x 90 x 140 mm, r x t x l). These strips were further divided into five subsamples measuring 15 x 15 x 140 mm. The subsamples were conditioned for one month at 20 ± 2°C and 65 ± 5% relative humidity. Samples were then oven-dried (50 ± 2°C) for 48 hours and density determined according to ASTM Standard D4442-16 (ASTM 2016). Low temperatures were used to minimize the possibility of extractives degradation (Scheffer 1973). These subsamples were further cut into eleven 15 mm cubes (Please refer to Appendix 4).

4.2.2. Extractives analysis

A total of 204 blocks (15 x 15 mm) taken from the middle section of each board were ground to pass a 60 mesh screen using a Wiley mill (Arthur H. Thomas Co., US) and sealed individually inside air-tight plastic bags that were stored in the dark at 5°C until used. The ground wood powder was weighed (1.0 ± 0.05 g), and placed inside an individual fabric filter bag (Nuiby unbleached tea filter bags, 6.1 x 8.1 cm, mesh size 2.4” x 3.2”), labeled and weighed again to obtain the initial wet weight with bag. Three individual tea bags (replicates) were prepared for each wood powder from each radial location from the ten boards. The bags were oven-dried overnight at 50°C and reweighed. The low temperature was used to reduce degradation of extractives, especially the heat-sensitive volatile compounds.

The bags were extracted using a modification of ASTM standard D1105 (ASTM D1105-96 2013) as described by Taylor et al. (2006) which allow simultaneous analysis of large quantities of samples. This extraction method was choosen instead of the conventional Soxhlet extraction owing to the large number of extractions required for this study. However, we recognize that complete extraction may not occur using this method. Thus, results can only be compared between tested wood samples, and not used to assess absolute extractives content.

The samples were then sequentially extracted using toluene:ethanol 1:2 (Fisher Scientific, 99.9%), followed by 95% ethanol (Fisher Scientific, 99.9%) and finally using hot water. A ten liter Erlenmeyer flask was filled with nine liters of toluene-ethanol mixture along with the filter bags and stir bar. Three extraction cycles were made for tea bags filled with Alaska yellow cedar powder from one or two disks for all radius locations per cycle. The flask was then placed on a hot plate with continuous stirring and heated at 60°C for 24 hours. The bags were removed, rinsed with ethanol and oven-dried at 50 ± 2°C for 24 hours. Mass loss after extraction was recorded as the extractives content of each sample. These steps were then repeated using 95% ethanol. For hot water extraction, the bags were placed in a six litre Erlenmeyer flask with distilled water and boiled in a water bath for 6 hours. The bags were removed from flasks and rinsed with distilled water, then air-dried overnight before they were oven-dried at 50 ± 2°C for 48 hours and

weighed. Weight loss after all three extractions was recorded as the total extractives content.

4.2.3. Extractives compounds identification using GC-MS

Extracts (1 μL of toluene-ethanol or ethanol) were analysed on Shimadzu GC-2010 gas chromatograph equipped with an flame ionization detector and an autosampler / injector. Analyses were performed using a Rtx-5 capillary column (30 mm long, 0.25 mm inner diameter) using helium (grade 5) as the carrier gas at a linear flow velocity of 1 ml / min of column flow. The temperature was set at 40°C for two min, then increased to 80°C by 5°C / min until 260°C was reached. The injector and detector temperature were maintained at 280°C. Compounds were identified by spectral comparison and relative retention times with those in the NIST standard reference #107 (Lindstrom and Mallard 2001) and a spectral extractives library (Adams 2007).

4.2.4. Durability study

4.2.4.1. Wood decay testing

The decay resistance of the blocks was assessed following procedures described in AWPA Standard E10 (AWPA, 2017). A total of 384 Alaska yellow cedar blocks were conditioned at 20°C and 65% relative humidity to constant weight. Blocks were

then oven-dried at 50 ± 2°C and initial oven-dried weights were recorded. Blocks were then placed in plastic bags and sterilized by exposure to 2.5 mrad of ionizing radiation from a ^{60}Co source at the Oregon State University Radiation Center. The blocks were kept in a sealed sterile container for no longer than 2 weeks until testing.

Decay chambers were prepared by half filling a 454 ml french square test jar with soil dampened with 12 ml of water. A western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) wood feeder strip (25 x 20 x 3 mm) was placed on the soil surface. The bottles were loosely capped and sterilized by autoclaving at 121°C for 45 minutes. The flasks were placed in a fume hood to cool overnight. A small plug (10 mm diameter) was taken from the edge of an active culture of *Rhodonia placenta* (Fr.) Niemelä, K.H. Larss. & Schigel (isolate Madison #698, USDA Forest Products Laboratory, Madison, Wisconsin) or *Gloeophyllum trabeum* (Pers.) Murrill (isolate Madison #617) grown on 1.5% PDA (potato-dextrose agar) and placed on one edge of the wood feeder. These fungi cause brown-rot degradation and are among the principle degraders of wooden structures, especially in temperate regions (Green and Highley 1997).

The jars were incubated at 26 ± 2°C for one week for *G. trabeum*, or two weeks for *R. placenta*, which allowed the fungal mycelium to cover the feeder strips. A sterilized Alaska yellow cedar block (with cross-section down) from each disk and radius location was then placed on the feeder strip and the jar was incubated at 26 ± 2°C for 12 weeks. Three replicate blocks from each radius location of each board were tested (with a total of 192 block) for each fungus. Additionally, ten blocks of non-durable ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson) sapwood were used as a control for each fungus. Two pine blocks were removed, oven-dried and weighed each week starting from week 8 to monitor whether weight loss had reached 50%. The test was terminated once weight loss of the controls was greater than 50%.

The blocks were removed, scraped clean of soil and fungal mycelium, and weighed to determine the wet weight. The blocks were then oven-dried overnight at 50°C and weighed again. Differences between the oven-dried weight before and after testing were used to determine the wood mass loss using the following formula:

$$Mass\ loss\ (\%) = \frac{Ovendried\ weight\ before\ test - Ovendried\ weight\ after\ test}{Overndried\ weight\ before\ test} \times 100$$

Decay resistance was classified using the scale described in the ASTM Standard D2017 (ASTM, 2005) where: 0 -10 % weight loss is highly resistant, 11 - 24 % is resistant, 25 - 44 % is moderately resistant, and > 45 % is non-resistant to decay. (Please refer to Appendix 5)

4.2.4.2. Termite testing

Alaska yellow cedar (15 x 15 x 6 mm) from all ten boards and radius locations were tested against the eastern subterranean termite, *Reticulitermes flavipes* (Oshima) following a modified no-choice termite test (termite forced to feed to only one type of wood block) described in AWPA Standard E1 (AWPA 2016). Testing was conducted at the U.S. Forest Products Laboratory, Starkville, Mississippi. Test jars were filled with 50 g white sand and 9 ml of distilled water and allowed to stand for two hours prior to use. Wood blocks were oven-dried for 24 hours at 50°C, weighed to the nearest 0.001 g and conditioned to room temperature before the test was initiated. Each test block was placed on top of a piece of aluminum foil on the surface of the damp sand and 3g of termites (approximately 150 termites including 1-3 soldiers) were added to each test jar. Three replicates were used for each test, with a total of 192 blocks used. All test jars were incubated for 28 days at 26 $\pm$ 2 °C and 50 $\pm$ 5% relative humidity. A tub of water was placed inside the incubator to maintain relative humidity. At the end of the test, the blocks were cleaned, and the

mass loss due to termite feeding was calculated based on differences between oven-dried weight before and after exposure.

4.2.5. Collection of ATR-FTIR spectra

Mid-infrared spectra in the range 4000 and 650 cm^{-1} were recorded using an attenuated total reflectance (ATR) system with a ZnSe crystal head Smart iTR (Thermo Scientific, USA) mounted on a Nicolet iS50 spectrometer (Thermo Scientific, USA). Spectral analysis of the powdered Alaska yellow cedar, including extracted samples from the extractives analysis, was done using OMNIC software version 9.2 (Thermo Scientific, USA). A background spectrum was obtained every 15 minutes to exclude signals that were not relevant to the sample. An individual spectrum represented an average of 32 scans at a resolution of 4 cm^{-1}. Samples were analyzed three times and averaged to obtain representative spectra.

The resulting spectra were pre-processed by performing a baseline correction (for correcting vertically sloped, curved or shifted spectra) and smoothing. The preprocessed spectra were used for further analysis.

4.2.6. Statistical analysis

All analyses were conducted using R Studio version 1.0.136 (RStudio Team, 2016). Average fungal and termite feeding weight losses along the radial transect were averaged and standard deviation determined. Variables tested for weight loss on each fungal and termite species included disk, radial location, and extractives content.

Chemometrics analysis using principal component analysis (PCA) was performed using the R package "Chemospec" (Hanson 2017) on the spectral dataset. Data for these analyses were categorized as N = non-resistant (consist of non-resistant and moderately resistant samples), R = resistant, or RR = highly resistant based on the results of durability tests against *G. trabeum*, *R. placen4ta* or *R. flavipes*. The moderately resistant and non-resistant samples were grouped together due to the low numbers of non-resistant materials in this study.

PCA is an unsupervised pattern recognition technique, with no assumptions made on group membership, and is commonly used to detect groups in a dataset. PCA can reduce the number of original spectral variables (wavenumber dimensions) to smaller sets of variables (principal components). Graphical representations of correlations between samples, principal components, and wavenumbers allow

visual detection of groups (i.e. durability classifications). PCA was applied to the spectral region 1800 to 650 cm^{-1} (fingerprint region) and the factor loadings calculated. The highest peaks in the first, second and third factor loadings were identified and assigned. The resulting PC plots (as a function of the wavenumber) were analyzed visually to detect spectral regions with high positive or negative factor loadings.

4.3. Results and Discussions

4.3.1. Extractives analysis

Toluene-ethanol extracts were dark yellow color, while ethanol extracts were light yellow. Total extractives content range from 0.84% to 3.39% of the dry weight (Figure 4.1). These results were lower than those from previous studies (Kirker et al. 2013) Kirker et al. (2013) obtained 4% extractives yield from Alaska yellow cedar using the same solvents as in present study, but with a different extraction technique (Soxhlet extraction).

Toluene-ethanol solvents remove waxes, fats, some resins, and wood gums, while hot water removes tannins, gums, sugars, starches and coloring matter. Ethanol extracts consist of phenolic substances, terpenoids, fats and carbohydrate (Sjöström 1993). Compounds identified by GC-MS from the ethanol extracts consist of valencene-11,12-diol and kudtdiol, which has also been found in Alaska yellow

cedar methanol extracts (Khasawneh and Karchesy 2011). However carvacrol and nootkatone were not identified. These compounds are usually recovered from the essential oil of Alaska yellow cedar heartwood (Khasawneh and Karchesy 2011; Kelsey et al. 2015). Nootkatone is highly volatile and easily degraded (Bharadwaj et al. 2012), and Soxhlet extraction might not be suitable for detecting extractive compounds from Alaska yellow cedar.

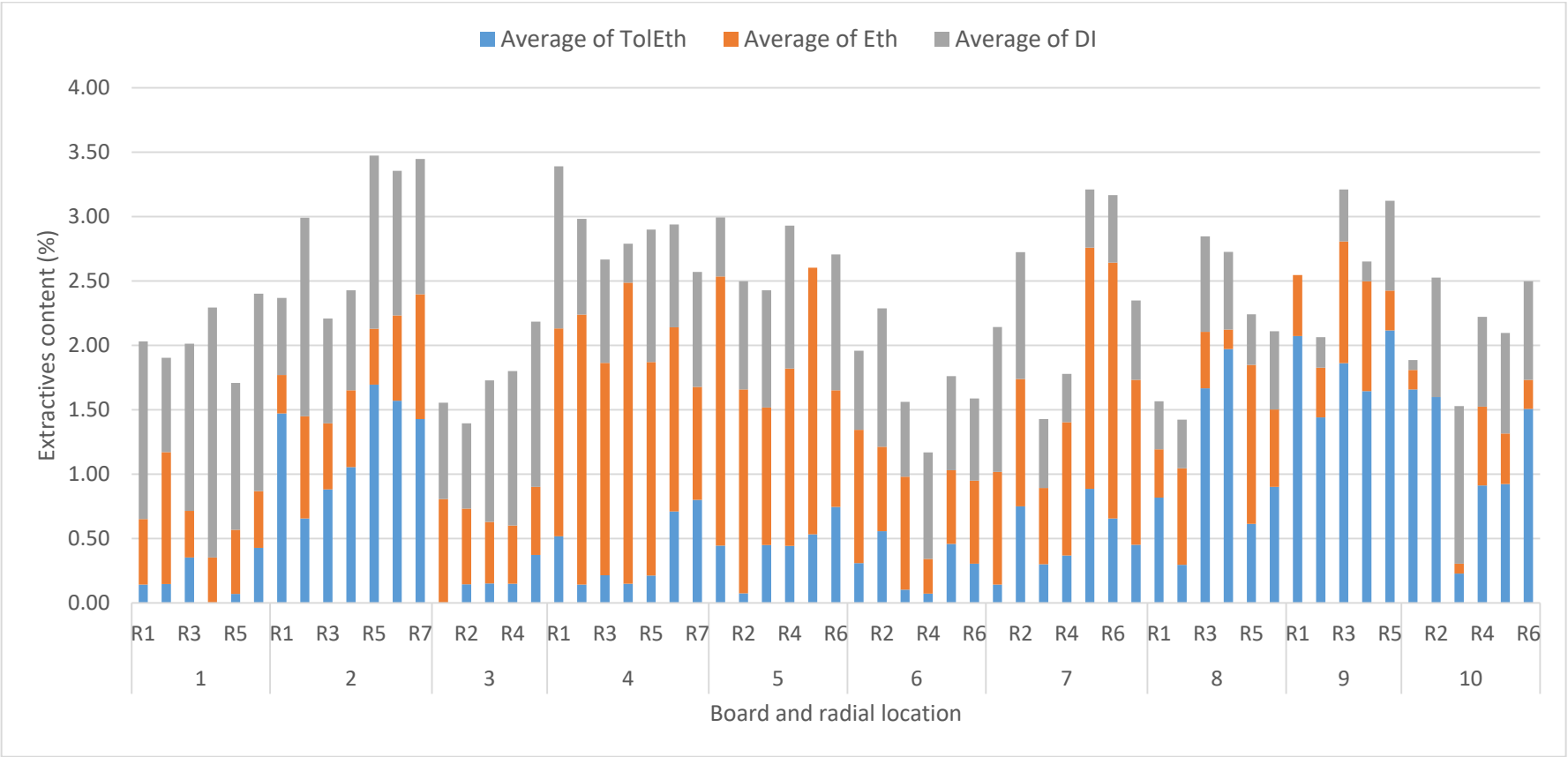


Figure 4.1 Extractives content (%) from ten boards (1-10) of Alaska yellow cedar radially from near the pith (R1) to bark (R6 or R7) after sequential extraction using toluene:ethanol 1:2 (TolEth), ethanol (Eth) and hot water (DI). Each value represents the average oven dry weight for three extractions.

4.3.2. Mass loss by decay fungi and termites

Mass losses due to fungal exposure for 12 weeks showed considerable variability in decay resistance (Figure 4.2). *G. trabeum* produced higher weight losses than *R. placenta*, especially on boards 1, 2, 9 and 10. Board 6 was highly resistant against both fungi, with all samples having mass loss less than 5%.

Mass losses due to *G. trabeum* showed variability with most blocks classified as highly resistant to resistant, while a small number were moderately resistant to non-resistant. Most of the non-durable samples came from the same boards (boards 1, 2, and 7). In previous study, *R. postia* produced higher mass losses than *G. trabeum* (Kirker et al. 2013). Exposure to the eastern subterranean termites, *R. flavipes* showed that wood ranged from highly resistant to resistant (Table 4.1), except for some samples on board 1 that were moderately resistant. Board 6 showed highly resistant to *R. flavipes* attack, similar to results of wood decay fungi.

Variability in mass losses by *G. trabeum*, as indicated by the higher standard deviation values were observed in some of the samples, particularly those from board 2. Some of this variability was also observed on other samples exposed to *G. trabeum*, especially those further from the pith. Interestingly, some blocks from board 1 exposed to *G. trabeum*, *R. placenta* and *R. flavipes* displayed similar high variability (Table 4.1).

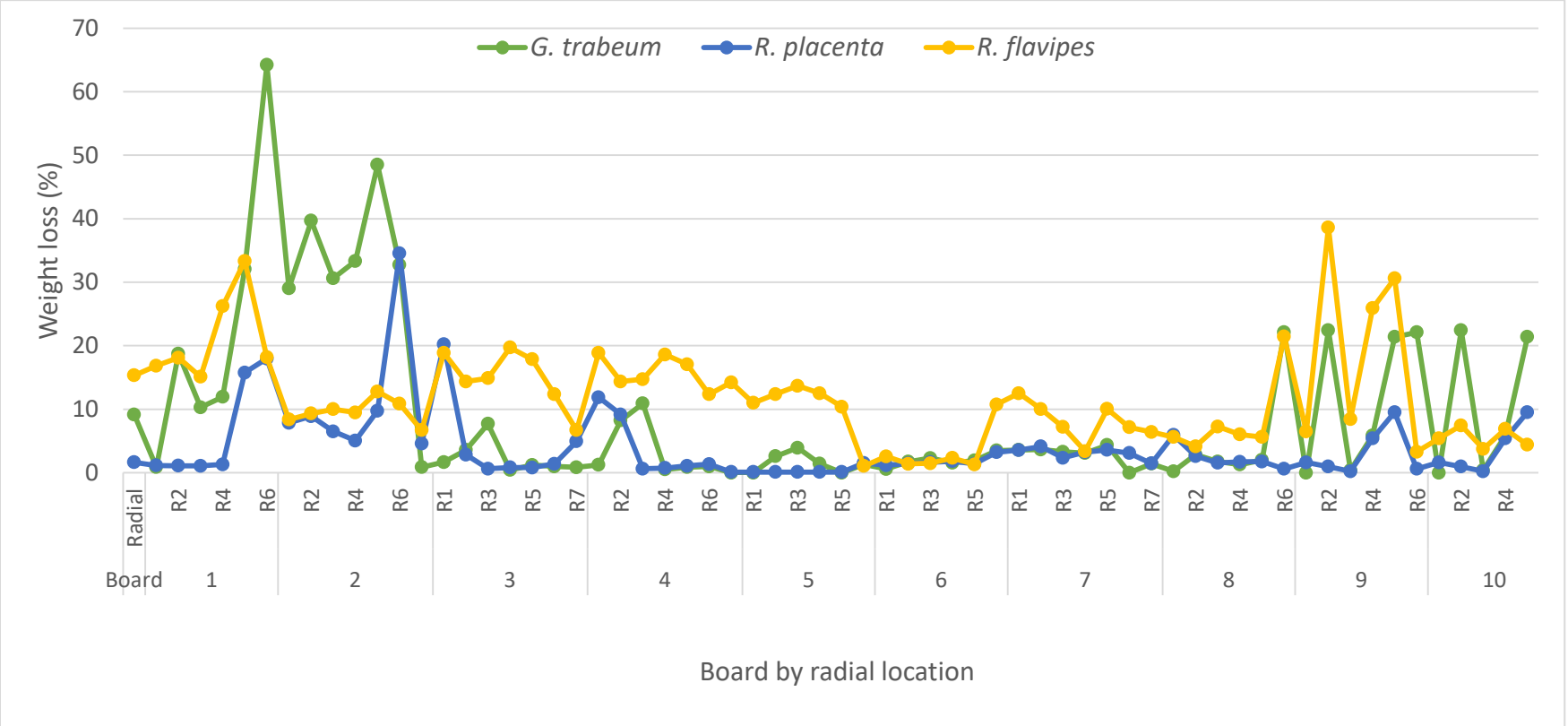


Figure 4.2 Mass losses (%) for Alaska yellow cedar after exposure G. trabeum, R. placenta, or R. flavipes by disk and radial position. Each value is the average of nine specimens per organism and wood location (n=9).

4.3.3. Relationship between extractives content and mass losses

Relationships between extractives content and weight loss were established to assess the variability in the mass losses for each organism (Figure 4.3 – 4.5). Increased extractives in ethanol extracts indicated a moderate relationship with reduced mass loss by *G. trabeum* (r = -0.60), *R. flavipes* (r = -0.40) and *R. placenta* (r = -0.30). Ethanol extracts consisted of phenolic substances and terpenoids (Sjöström 1993) that are known to contribute to the high durability of Alaska yellow cedar (Grace and Yamamoto 1994; Arango et al. 2006; Kirker et al. 2013).

Extracts of Alaska yellow cedar contain nootkatone (sesquiterpene), nootkatin (tropolone) and carvacrol (terpenoid) that are insecticidal and / or fungicidal and have been used for control of many arthropod pests (Rao et al. 2010; Panella et al. 2018). Furthermore, Kirker et al. (2013) showed significantly higher mass losses in extracted Alaska yellow cedar compared to non-extracted samples exposed to *G. trabeum*, *R. placenta* or *R. flavipes*. Their study also employed similar sequential extractions using the same solvents as in present study, but with Soxhlet extraction.

Table 4.1 Average total extractives content and weight losses of Alaska yellow cedar samples exposed to the *G. trabeum*, *R. placenta* or *R. flavipes*.

Board	Radial	Extractives content (%) (n=3)	Weight loss (%)		
			G. trabeum (n=9)	*R. placenta* (n=9)	*R. flavipes* (n=3)
1	R1	1.96 (0.44)	12.29 (9.70)	1.18 (0.86)	15.32 (4.00)
	R2	1.83 (0.36)	6.53 (6.48)	2.01 (0.76)	16.85 (1.98)
	R3	1.94 (0.67)	18.41 (3.69)	1.01 (0.09)	18.10 (0.41)
	R4	1.94 (0.23)	11.97 (2.77)	1.03 (0.11)	15.11 (6.99)
	R5	1.13 (1.30)	17.10 (14.14)	3.02 (2.87)	26.21 (15.34)
	R6	2.10 (0.15)	37.58 (28.73)	19.01 (19.38)	33.33 (17.54)
2	R1	2.37 (0.76)	28.73 (30.74)	17.26 (0.62)	18.17 (2.28)
	R2	2.99 (0.42)	17.42 (18.79)	8.96 (1.58)	8.38 (3.41)
	R3	2.21 (0.37)	16.19 (20.50)	4.80 (3.63)	9.32 (2.66)
	R4	2.43 (0.44)	15.37 (13.35)	5.47 (4.06)	9.99 (0.66)
	R5	2.81 (3.39)	12.91 (17.70)	5.49 (2.21)	9.48 (4.22)
	R6	2.84 (3.59)	18.78 (25.77)	8.36 (1.97)	12.78 (1.70)
	R7	2.45 (3.73)	12.74 (17.45)	17.17 (15.43)	10.85 (2.16)

*Value represents the mean of n samples for each test with standard deviation.

Table 4.1 (continue) Average total of extractive content and weight losses of Alaska yellow cedar samples exposed to the *G. trabeum*, *R. placenta* or *R. flavipes*.

Board	Radial	Extractives content (%) (n=3)	Weight loss (%)		
			G. trabeum (n=9)	*R. placenta* (n=9)	*R. flavipes* (n=3)
3	R1	1.48 (0.88)	0.85 (0.04)	4.94 (0.50)	6.70 (2.84)
	R2	1.17 (0.62)	1.24 (0.38)	11.87 (9.12)	18.87 (1.76)
	R3	1.69 (0.49)	8.23 (10.51)	9.17 (12.25)	14.35 (0.69)
	R4	1.80 (1.18)	10.89 (12.11)	0.62 (0.35)	14.88 (0.34)
	R5	1.17 (0.49)	0.50 (0.08)	0.73 (0.31)	19.71 (3.01)
	R6	0.84 (0.60)	0.88 (0.33)	1.07 (0.24)	17.85 (0.55)
	R7	2.18 (0.13)	0.99 (0.44)	1.34 (0.30)	12.35 (0.60)
4	R1	3.39 (1.23)	3.44 (0.70)	3.82 (0.29)	5.07 (1.00)
	R2	2.76 (0.47)	3.42 (0.33)	3.75 (0.91)	11.81 (2.21)
	R3	2.59 (0.36)	2.26 (0.00)	9.06 (0.00)	6.65 (6.59)
	R4	2.64 (0.56)	3.96 (1.05)	6.10 (4.82)	5.26 (1.39)
	R5	2.90 (0.50)	2.31 (0.66)	4.43 (1.81)	7.85 (2.09)
	R6	2.94 (0.55)	2.20 (0.90)	2.23 (0.63)	8.10 (1.29)
	R7	2.57 (0.51)	2.76 (0.06)	4.15 (1.54)	6.29 (2.43)

*Value represents the mean of n samples for each test with standard deviation.

Table 4.1 (continue) Average total of extractive content and weight losses of Alaska yellow cedar samples exposed to the *G. trabeum*, *R. placenta* or *R. flavipes*.

Board	Radial	Extractives content (%) (n=3)	Weight loss (%)		
			G. trabeum (n=9)	*R. placenta* (n=9)	*R. flavipes* (n=3)
5	R1	2.99 (0.31)	0.15 (4.39)	0.34 (1.87)	14.21 (3.18)
	R2	2.50 (0.57)	-1.10 (3.51)	-0.53 (0.91)	10.99 (2.83)
	R3	2.43 (0.41)	0.86 (3.83)	-0.52 (1.06)	12.37 (0.93)
	R4	2.93 (0.35)	3.21 (1.02)	-0.25 (2.67)	13.68 (2.07)
	R5	2.60 (0.11)	0.39 (1.93)	-0.97 (1.59)	12.50 (2.71)
	R6	2.71 (0.19)	5.22 (12.11)	-0.21 (1.89)	10.38 (0.80)
6	R1	1.89 (0.98)	1.01 (0.89)	0.99 (0.86)	1.06 (0.92)
	R2	2.22 (0.91)	0.61 (0.53)	0.87 (0.77)	2.57 (1.03)
	R3	1.17 (0.49)	1.12 (0.97)	0.95 (0.82)	1.38 (1.35)
	R4	0.84 (0.60)	1.95 (0.36)	1.49 (0.16)	1.49 (0.13)
	R5	1.82 (0.50)	1.50 (0.09)	1.79 (0.13)	2.31 (1.66)
	R6	1.59 (0.80)	2.02 (0.10)	1.38 (0.13)	1.31 (1.27)

*Value represents the mean of 3 samples for each test with standard deviation.

Table 4.1 (continue) Average total of extractive content and weight losses of Alaska yellow cedar samples exposed to the *G. trabeum*, *R. placenta* or *R. flavipes*.

Board	Radial	Extractives content (%) (n=3)	Weight loss (%)		
			G. trabeum (n=9)	*R. placenta* (n=9)	*R. flavipes* (n=3)
7	R1	1.92 (0.40)	33.62 (26.34)	11.40 (14.02)	10.74 (2.59)
	R2	2.72 (0.04)	26.95 (20.26)	7.93 (7.27)	12.51 (1.89)
	R3	1.20 (0.38)	31.59 (39.52)	8.36 (8.37)	10.01 (0.43)
	R4	1.78 (0.80)	27.07 (33.63)	6.22 (5.20)	7.19 (3.38)
	R5	3.21 (0.39)	14.13 (15.63)	5.66 (3.87)	3.38 (1.98)
	R6	3.17 (0.66)	12.75 (11.86)	7.60 (6.79)	10.04 (2.78)
	R7	2.35 (0.38)	N/a	6.17 (4.37)	7.15 (1.40)
8	R1	1.27 (1.10)	1.22 (0.23)	1.52 (0.43)	6.40 (1.89)
	R2	1.20 (0.90)	0.30 (0.26)	4.55 (1.19)	5.62 (1.00)
	R3	2.69 (0.27)	-0.76 (3.13)	3.15 (1.89)	4.15 (0.92)
	R4	2.14 (0.73)	1.54 (0.24)	1.74 (0.21)	7.26 (3.76)
	R5	2.24 (0.52)	1.50 (0.22)	1.62 (0.14)	6.01 (4.90)
	R6	2.11 (0.33)	1.82 (0.27)	1.43 (0.38)	5.61 (2.34)

*Value represents the mean of n samples for each test with standard deviation.

Table 4.1 (continue) Average total of extractive content and weight losses of Alaska yellow cedar samples exposed to the *G. trabeum*, *R. placenta* or *R. flavipes*.

Board	Radial	Extractives content (%) (n=3)	Weight loss (%)		
			G. trabeum (n=9)	*R. placenta* (n=9)	*R. flavipes* (n=3)
9	R1	2.15 (0.45)	13.72 (12.81)	0.46 (0.61)	21.44 (2.19)
	R2	3.10 (0.22)	-1.20 (1.43)	1.00 (0.72)	6.47 (0.99)
	R3	2.90 (1.01)	21.22 (5.77)	1.22 90.60)	38.62 (4.15)
	R4	2.65 (0.48)	6.26 (8.82)	1.00 (0.79)	8.43 (1.57)
	R5	3.12 (0.83)	10.94 (6.48)	12.22 (8.21)	25.90 (2.85)
	R6	N/a	19.82 (3.41)	16.85 (11.63)	30.59 (3.72)
10	R1	1.80 (0.30)	13.72 (12.81)	0.46 (0.61)	3.29 (0.86)
	R2	2.14 (0.64)	-1.20 (1.43)	1.00 (0.72)	5.38 (1.36)
	R3	1.37 (0.22)	21.22 (5.77)	1.22 (0.60)	7.45 (1.64)
	R4	2.22(0.48)	6.26 (8.82)	1.00 (0.79)	3.75 (0.88)
	R5	2.09(0.22)	10.94 (6.48)	12.22 (8.21)	6.89 (0.22)
	R7	2.34 (0.62)	19.82 (3.41)	16.85 (11.63)	4.39 (1.60)

*Value represents the mean of n samples for each test with standard deviation.

N/a: Missing data due to contamination and insuffient number of blocks available.

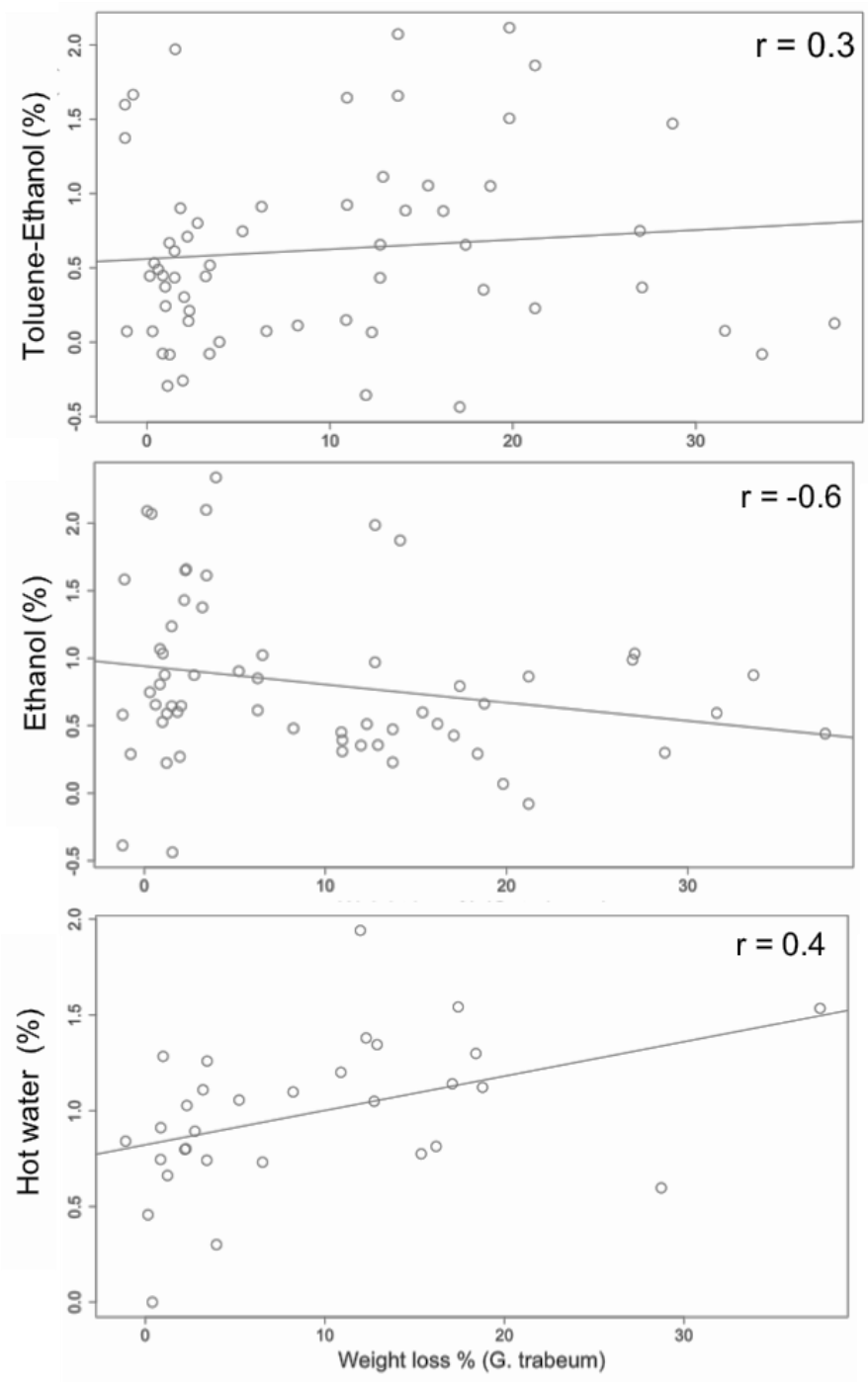


Figure 4.3 Relationship between weight losses (%) caused by exposure to *G. trabeum* and extractives yield using different solvents.

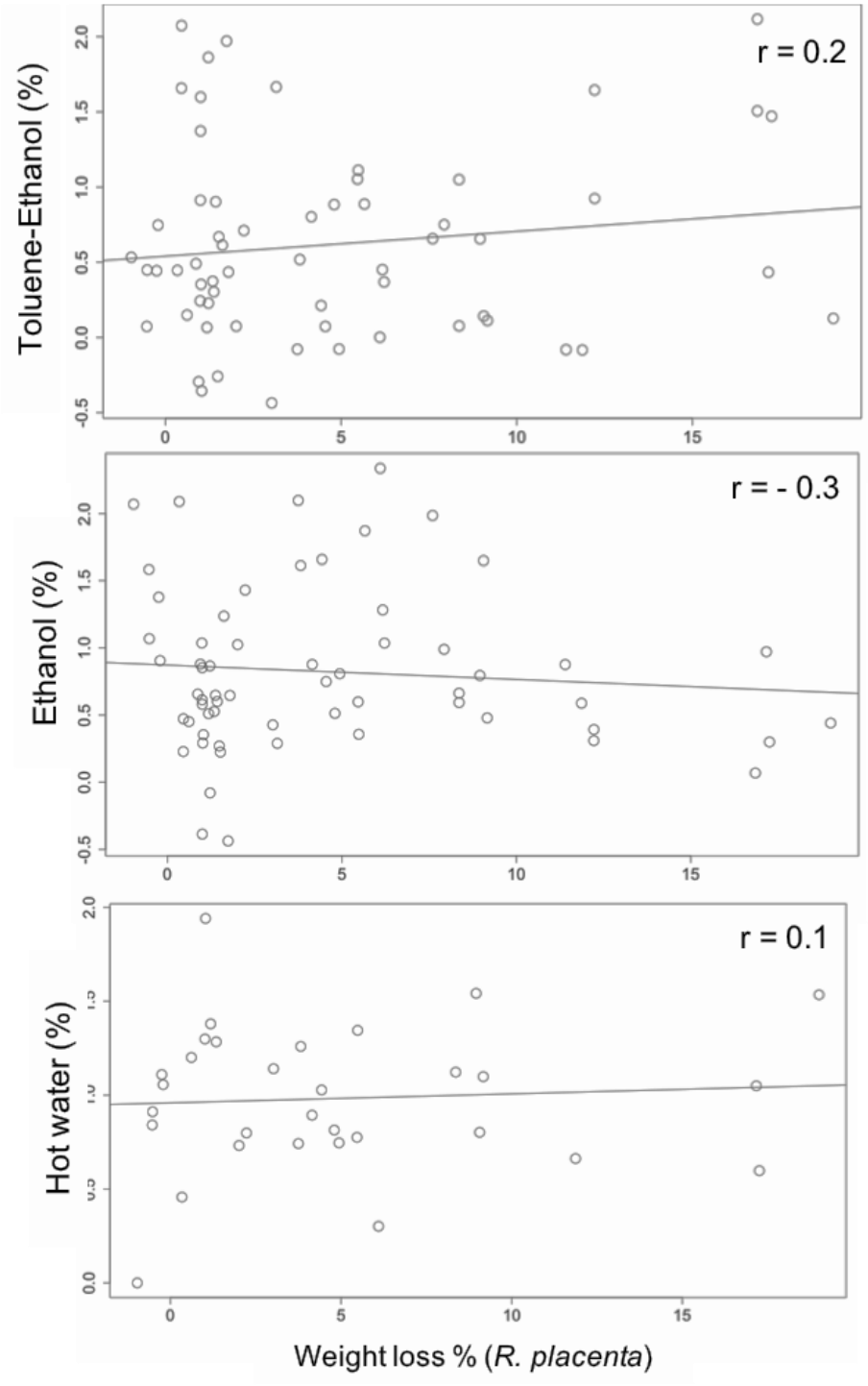

Toluene-Ethanol (%)
r = 0.2
Ethanol (%)
r = - 0.3
Hot water (%)
r = 0.1
Weight loss % (R. placenta)

Figure 4.4 Relationship between weight losses (%) caused by exposure to *R. placenta* and extractives yield using different solvents.

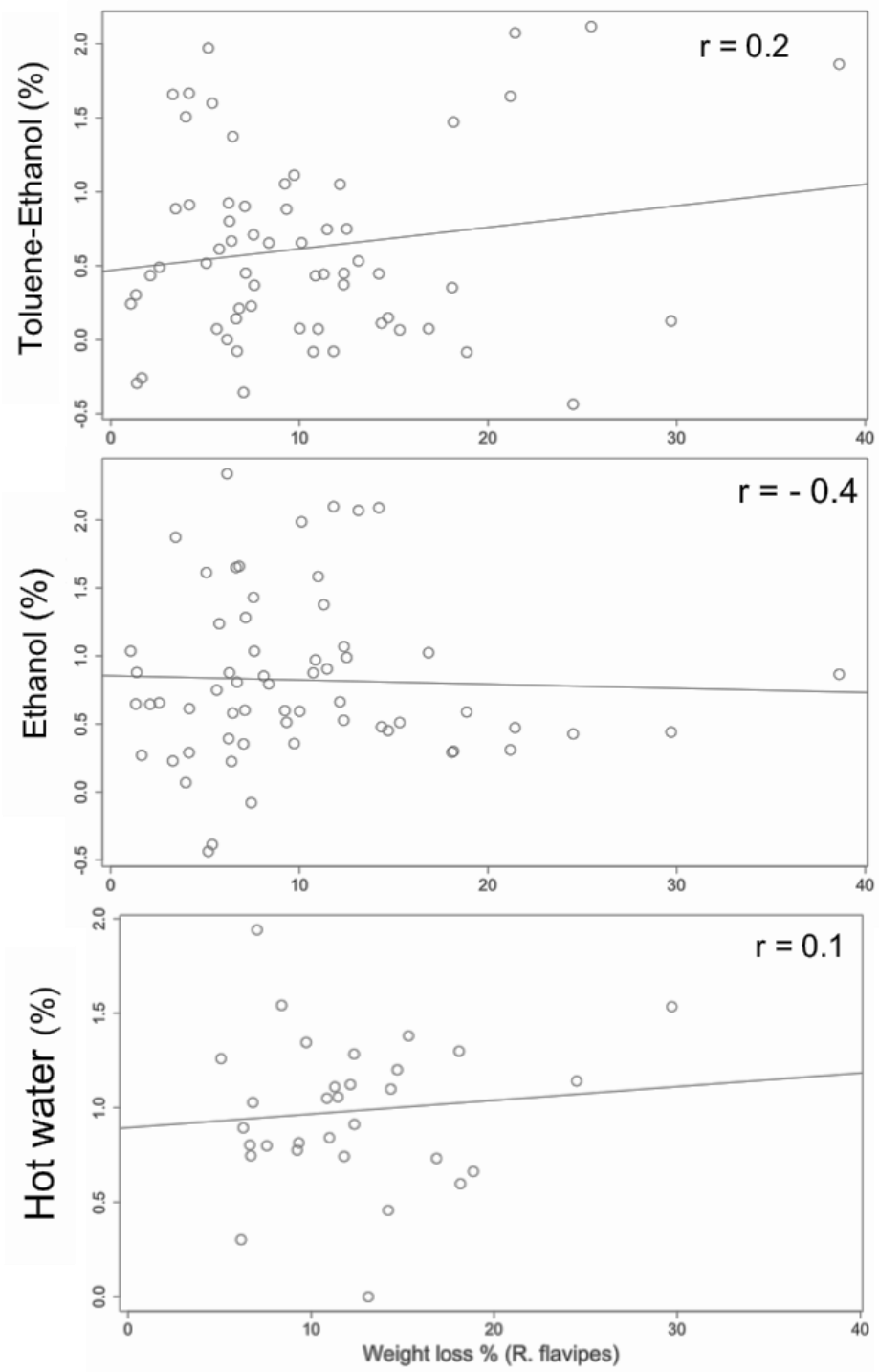


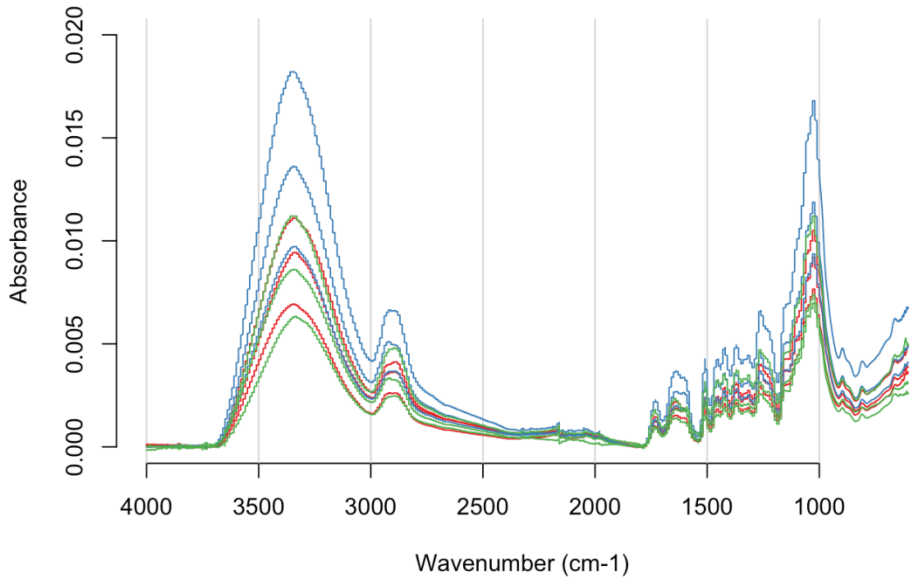
Figure 4.5 Relationship between weight losses (%) caused by exposure to *R. flavipes* and extractives yield using different solvents.

Poor relationships were observed between toluene:ethanol and hot water extracts and mass losses by *R. placenta* or *R. flavipes*. The moderate relationship between increases in toluene:ethanol and hot water extracts and increasing mass losses by *G. trabeum* (Figure 4.3), indicated that some of the compounds from these extracts might contribute to the wood being more susceptible to *G. trabeum*. Hot water extracts contain sugar and starches that are easily utilized by decay fungi (Zabel and Morrell 1992), and might contribute to increasing mass loss by *G. trabeum*.

4.3.4. ATR-FTIR spectra

Averaged ATR-FTIR spectra (3 spectra/sample) were investigated for their potential to distinguish between Alaska yellow cedar of different durability classes after exposure to *G. trabeum* (Figure 4.6), *R. placenta* (Figure 4.7) and *R. flavipes* (Figure 4.8). The lack of representatives for 'moderately durable' and 'non-durable' groups (Table 4.1) led us to classify both as 'non-durable'. Comparisons between averaged spectra between the three different durability classes for *G. trabeum* showed no clear differences between the highly resistant, resistant and non-resistant Alaska yellow cedar (Figure 4.6). Similar findings were observed in spectra representing different durability classifications for *R. placenta* (Figure 4.7) or *R. flavipes* (Figure 4.8).

(A)



(B)

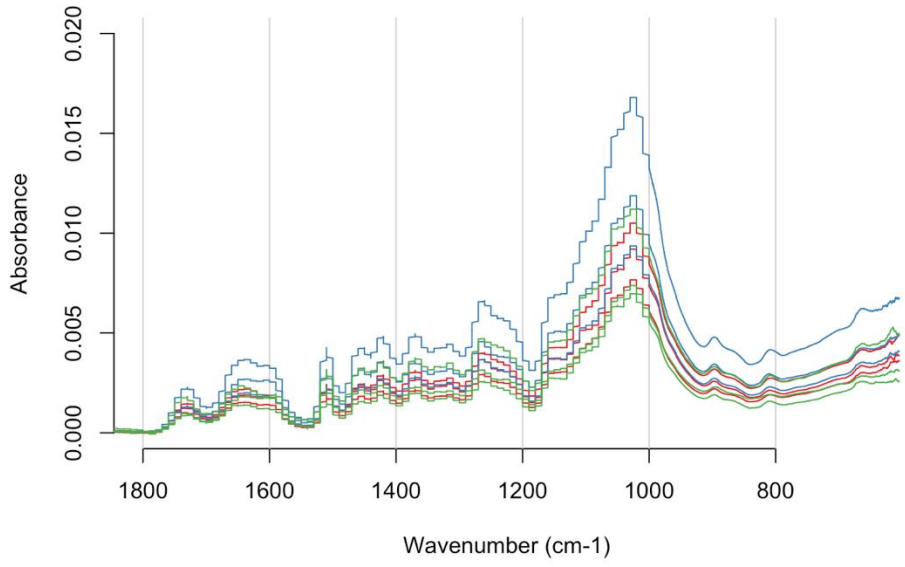
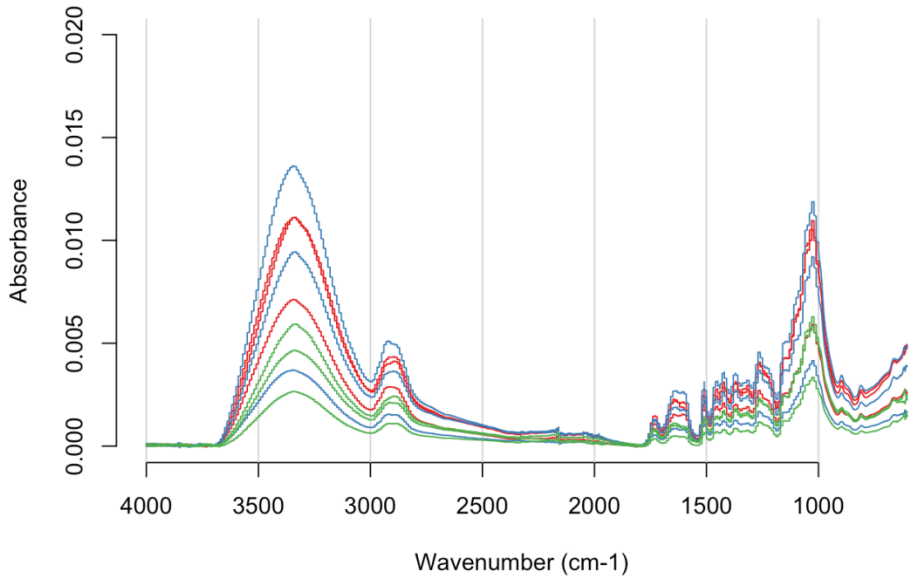


Figure 4.6 Representative FT-IR spectra of Alaska yellow cedar samples according to durability classifications established by exposure to *G. trabeum* at wavenumber 4000 –

650 cm^{-1} (A), and 1800 – 650 cm^{-1} (B). Green = highly resistant, blue = resistant, red = non-resistant.

(A)



(B)

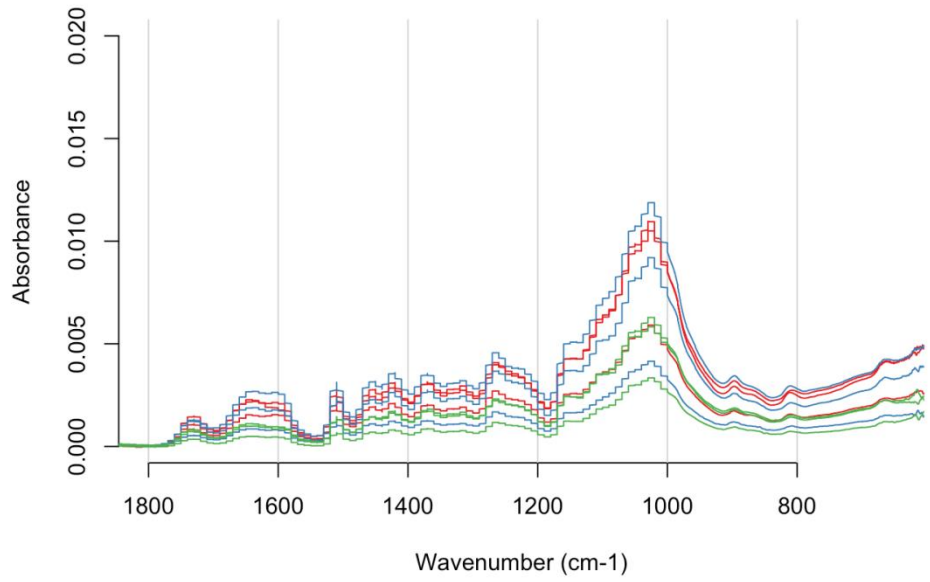
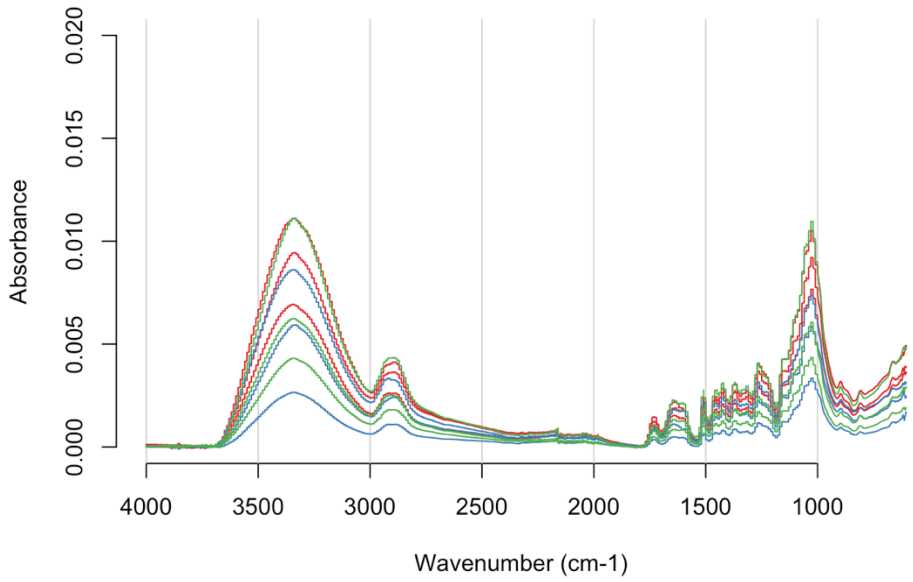


Figure 4.7 Representative FT-IR spectra of Alaska yellow cedar samples according to durability classifications established by exposure to *R. placenta* at wavenumber 4000 – 650 cm^{-1} (A), and 1800 – 650 cm^{-1} (B). Green = highly resistant, blue = resistant, red = non-resistant.

(A)



(B)

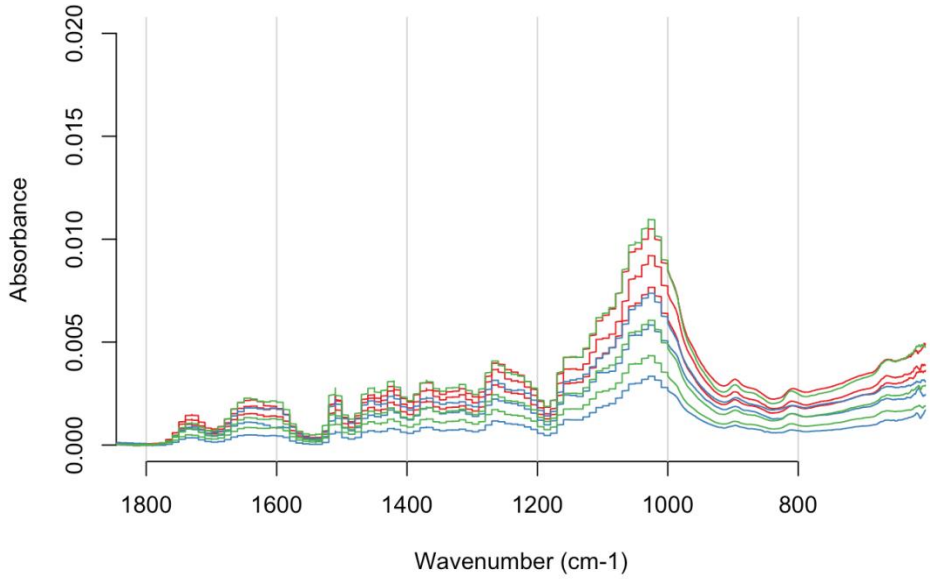


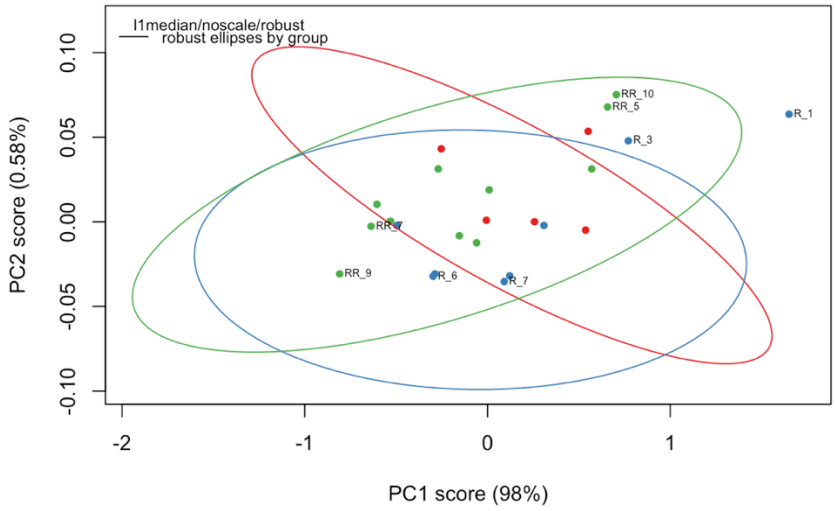
Figure 4.8 Representative FT-IR spectra of Alaska yellow cedar samples according to durability classifications established by exposure to *R. flavipes* at wavenumber 4000 –

650 cm^{-1} (A), and 1800 – 650 cm^{-1} (B). Green = highly resistant, blue = resistant, red = non-resistant.

Principal component analysis (PCA) was used for qualitative recognition of different durability groups. The spectral data (1800-650 cm^{-1}) were subjected to PCA analysis, and the results were examined. Robust PCA was used in this analysis as outliers were detected in the spectral data. Classical PCA is sensitive to outliers and the presence of anomalous data can lead to a first principal component (PC1) that explains the high variance towards the anomalous data (Gharibnezhad et al. 2011). Outlier removal can resolve these issues, but a simpler option is using robust PCA that is less influenced by outliers. In this analysis, robust PCA using median absolute deviation was used to measure the spread of the the data instead of using standard deviation (Hanson 2017).

The first two principal components (PC) explained 98.58% of the variation in the dataset for spectral data on durability to *G. trabeum*, with PC1 representing 98% and PC2 0.58% of the variations (Figure 4.9). The distribution of samples along PC1 (Figure 4.9A) showed that the majority of the non-resistant samples had positive PC scores, while the highly durable samples were distributed within the negative PC1 and on the positive PC2 (Figure 4.9A). A majority of the resistant group also had positive PC1. However, they appeared randomly distributed along PC2. Groupings with the 95% confidence interval did not clearly show any separations between the groups with all three durability classes overlapping.

(A)



(B)

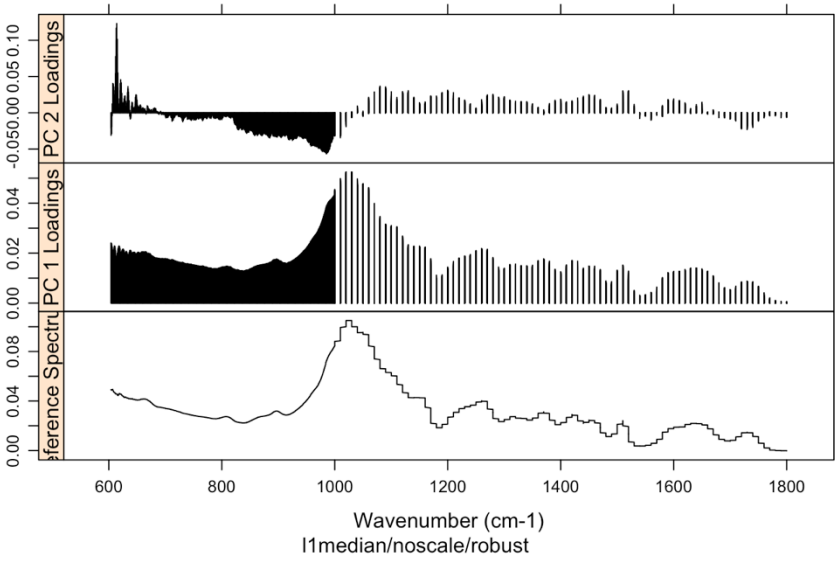
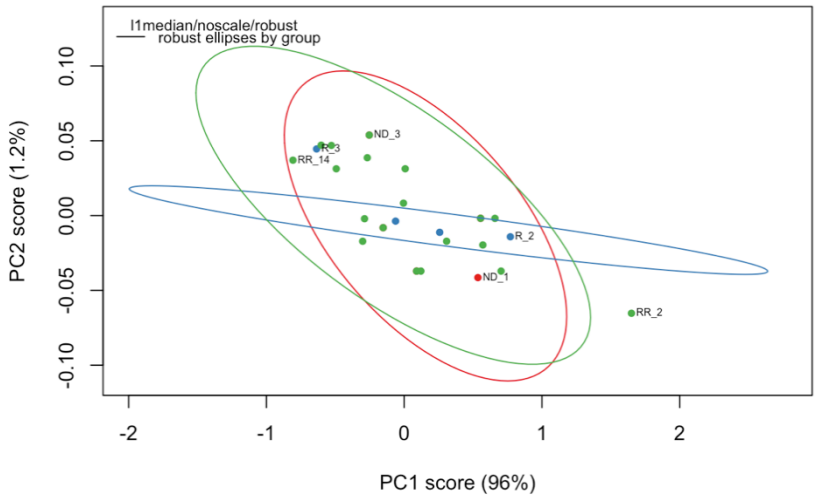


Figure 4.9 PCA analyses with (A) PC1 vs PC2 scores plotted using robust PCA techniques for the different durability classes to *G. trabeum*, and (B) the loadings profile of the first two PCs. Solid lines on the PC1 vs PC2 plot indicate the 95% confidence interval for each group. N = non-resistant (red) , R = resistant (blue), RR = highly resistant (green).

The loadings profile for *G. trabeum* (Figure 4.9B) on the first two PCs suggested that PC1 variation was related to the regions at 1050 cm^{-1}. This is the region of C-O stretching in cellulose and hemicellulose (Pandey 1999; Kuo et al. 2007). PC2 loadings showed variations mainly at 1600 cm^{-1} (C=C stretching or aromatic ring formation) which is the region associated with extractives. However, this variation only made a small contribution (0.58%) to the variability in the spectral data. It is possible that the sensitivity of ATR-FTIR to wood particle size and pressure between the ATR-FTIR crystal's tip may have affected the differences seen in the 1050 cm^{-1} regions. The use of wood powders produced a homogeneous conditions. However, care must be taken to ensure that powder sizes are similar to minimize effects on spectral absorbance.

Similar findings were also observed for the effects of spectral output on durability classifications for *R. placenta* and *R. flavipes* (Figure 4.9 and 4.10). The first two PCs explained 97.2% of variation in the dataset in PCA analysis on spectra attributed to durability to *R. placenta*, (Figure 4.10). The highly resistant and non-resistant groups had positive PC1 scores, and negative scores for PC2. The resistant group had positive PC1 and negative PC2 scores. The loading plot on PC1 showed that variation was mainly from 1020 cm^{-1}, similar to the finding for *G. trabeum*. PC2 variations was from the 1650 cm^{-1} region associated to keto-carbonyl conjugated with benzene rings (Figure 4.9B). The negative PC2 scores can be explained by the variation at 895 cm^{-1} which is the C1 group frequency in cellulose and hemicellulose.

(A)



(B)

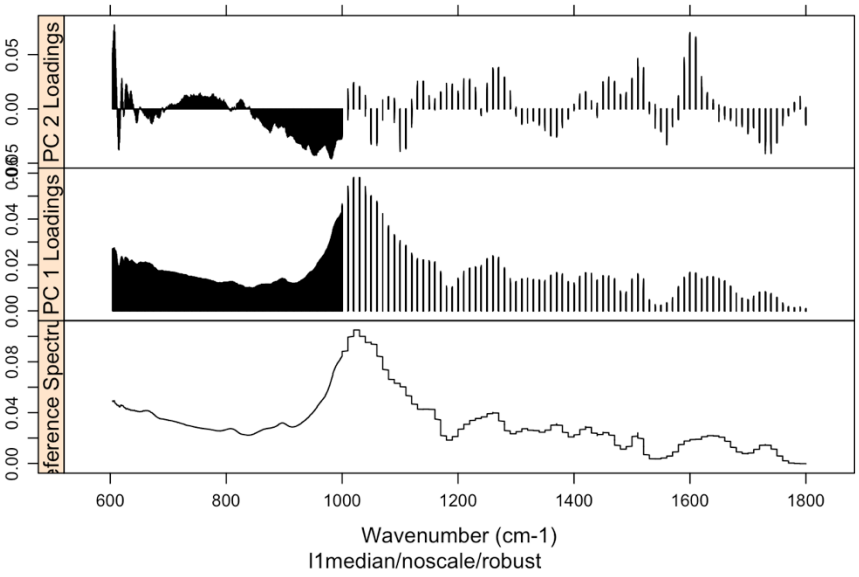
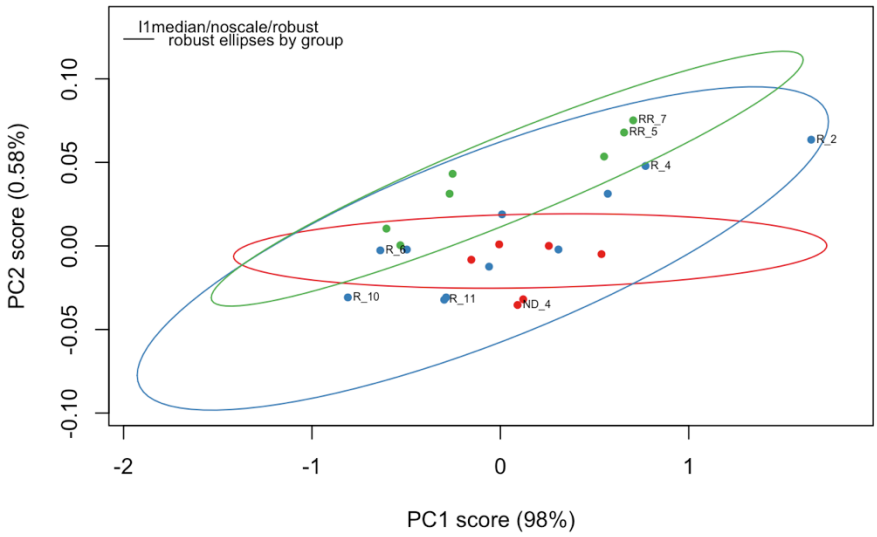


Figure 4.9 PCA analyses with (A) PC1 vs PC2 scores plotted using robust PCA techniques for the different durability classes to *R. placenta*, and (B) the loadings profile of the first two PCs. Solid lines on the PC1 vs PC2 plot indicate the 95% confidence interval for each group. N = non-resistant (red) , R = resistant (blue), RR = highly resistant (green).

(A)



(B)

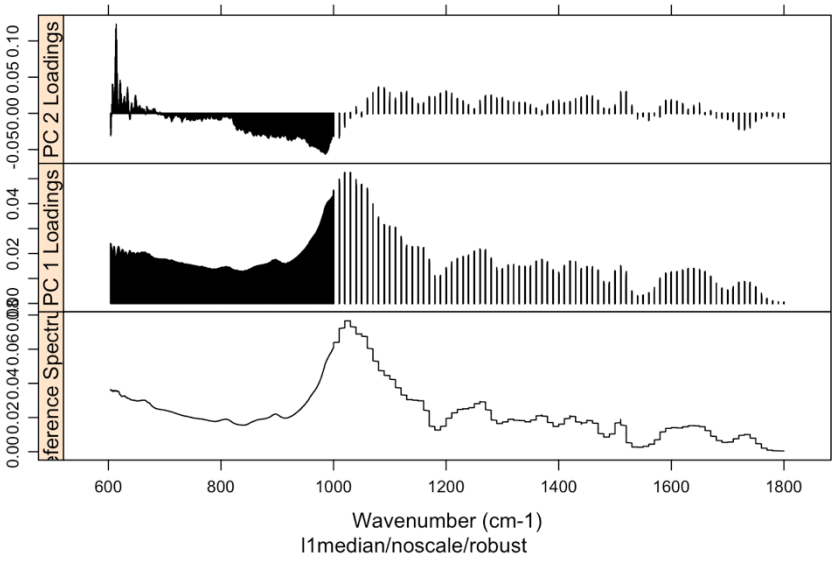


Figure 4.10 PCA analyses with (A) PC1 vs PC2 scores plotted using robust PCA techniques for the different durability classes to *R. flavipes*, and (B) the loadings profile of the first two PCs. Solid lines on the PC1 vs PC2 plot indicate the 95% confidence

interval for each group. N = non-resistant (red) , R = resistant (blue), RR = highly resistant (green).

PCA analyses of spectra related to resistance to *R.flavipes* indicated that the first two PCs explained 98.58% of the variation (Figure 4.10A). Positive PC1 and negative PC2 scores were found for the non-resistant group (Figure 4.10A). The highly resistant had some samples with positive PC1 and negative PC1 scores Similar to *G. trabeum* and *R. placenta*, PC1 variations arised from the region 1050 cm^{-1} (Figure 4.10B). However PC2 loadings are caused by variation at 990 cm^{-1} which is indicative of C-O valence vibration on cellulose (Xu et al. 2013).

Although the PCA analysis failed to recognize different groupings based on Alaska yellow cedar durability to the test organisms, however, to the influence of wood extractives on the resulting spectra was seen in a previous study by comparing extracted and non-extracted Alaska yellow cedar (Shahlinney and Morrell 2017). Furthermore, ATR-FTIR spectra also showed sensitivity to carvacrol, one of the main biocides of Alaska yellow cedar. Differences between carvacrol levels in extracted Alaska yellow cedar were detected using ATR-FTIR, but only at greater than 5% concentration (Shahlinney et al. 2018). The extractives level in Alaska yellow cedar used in the presence study might be insufficient to detect changes between spectra and consequently different durability classes.

4.4. Conclusions

Variations in extractives content and resistance to brown-rot fungi and termites was observed for the ten Alaska yellow cedar boards across the radius examined in this study. In general, Alaska yellow cedar displayed high resistance to the tested organisms, although some samples were more susceptible to fungal and termites attack. Increased ethanol extractives contributed moderately to decreased mass losses by fungi and termites. The use of ATR-FTIR with PCA for determination of the Alaska yellow cedar durability was unable to accurately predict durability classifications. Insufficient representatives from the non-resistant and moderately resistant groups may have contributed to the poor predictions.

Despite the lack of predictive ability, infrared spectroscopy could still be applied to detect chemical compounds important to wood durability. This study showed that interpreting infrared spectra was complicated by the complex nature of individual wood components (cellulose, hemicellulose, lignin, and extractives). In addition, discerning extractives from other major wood components, including those contributing to wood durability, is difficult as two or more components may contribute to the same absorption band. Future studies should compare spectral and durability

information of younger plantation trees with naturally grown samples. Younger trees might offer more variability in their durability and extractives content and these results could be used to improve the current predictive model.

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CHAPTER 5

RELATIONSHIP BETWEEN ATR-FTIR SPECTROSCOPY OF WESTERN JUNIPER EXTRACTIVES AND NATURAL RESISTANCE TO FUNGAL AND TERMITE ATTACK

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Chapter 5. Relationship Between ATR-FTIR Spectroscopy Of Western Juniper Extractives And Natural Resistance To Fungal And Termite Attack

Abstract

Wood extractives are considered as the major factor contributing to wood natural durability. Fourier transform infrared with attenuated total reflectance (ATR-FTIR) spectroscopic study for rapid determination of western juniper (*Juniperus occidentalis* Hook. var. *occidentalis*) durability based on extractives in the heartwood, sapwood-heartwood and sapwood regions was presented. Wood was exposed to brown-decay fungi (*Gloeophyllum trabeum* (Pers.) Murrill and *Rhodonia placenta* (Fr.) Niemelä, K.H. Larss. & Schigel) or eastern subterranean termite (*Reticulitermes flavipes* (Oshima)) for 12-week and 4-week, respectively. Durability classifications were compared to their extractive contents, along with the spectral data of the extracted and non-extracted blocks to establish relationship using hierarchical cluster analysis (HCA) and principal component analysis (PCA). Results showed variability in durability of western juniper to tested organisms, with majority showing high resistance to fungi and termite attack. A moderate to weak connection was observed between durability and extractive content. HCA and PCA analysis also failed to classify the durability with accuracy.

Keywords: *Juniperus occidentalis, ATR-FTIR, extractives, natural durability, decay fungi, termite*

5.1. Introduction

Durability is a highly desirable quality that contributes to the premium paid for some wood species. Durable woods have broad applications in exterior exposures such as poles, fences, flooring or even marine pilings. The phase-out of certain wood preservatives, such as chromated copper arsenate (Schultz and Nicholas, 2000) and increasing public concerns about chemical effects on health and the environment have encouraged the use of naturally durable woods. The compounds in these wood may also serve as models for synthesizing naturally protective compounds (Binbuga et al., 2008).

The economic losses due to biodegradation are massive. Decay is estimated to cost 300 million (US) dollars in losses annually, while termites cause over 1 billion (US) dollars of damage (Clausen and Yang, 2007; Ghaly and Edwards, 2011; Nicholas, 1982). This damage also creates hazards due to weakening of structures. Mass losses of 5-10% may reduce wood strength by as much as 60-80% in bending properties (Wilcox 1978, Ibach and Lebow 2014). Increases in the use of woods from plantation forests that are perceived as less durable than old growth trees have also created growing concerns about the durability properties of future forest.

Resistance to wood decay fungi and insects in many durable wood species has been linked to their extractives content (Rudman, 1958). Extractives are minor wood components, mostly ranging from 1-5% of total mass, but increasing up to 30% for some tropical species (Hillis, 2011). Extractives consist of a broad group of non-structural compounds that are soluble with organic or inorganic solvents, or water (Scheffer and Cowling, 1966). Non-polar solvents (e.g. hexane, dichloromethane and diethyl ether) remove lipophilic extractives, while hydrophilic extracts are obtained using water or other polar solvents (e.g. acetone, methanol). Extractives may act as toxicants, repellents, or antioxidants (Ajuong and Breese, 1997; Dizhbite, 2004; Rudman, 1958; Woodward and Pearce, 1988), although the exact protective mechanisms of many extractives remain poorly understood (Onuorah, 2002; Schultz and Nicholas, 2000; Taylor et al., 2006).

Durability gradients in wood within a single tree and between trees further complicate extractive durability studies (Gierlinger and Wimmer, 2004; Guilley et al., 2004). Extractives production is genetically controlled; while cambial age, soil and environmental factors can cause durability differences among individual trees (Taylor et al., 2002). Furthermore, extractives production is controlled by many genes, making breeding for durability a major challenge (Bush et al., 2011; Harju et al., 2001; Paques and Charpentier, 2015). Extensive lists of durability classifications are available for wood of many species (Clausen, 2010; Scheffer and Morrell, 1998), but the durability

of wood species may vary from durable to non-durable. For example teak (*Tectona grandis* Linn. F.), has a reputation for high durability to wood degrading organisms, but shows reduced durability in plantation grown material (Haupt et al., 2003; Lukmandaru and Takahashi, 2009).

The genus *Juniperus* spp. (Cupressaceae) contains a number of highly durable species such as eastern red cedar (*Juniperus virginiana* L.) and western juniper (*Juniperus occidentalis* Hook.). Western juniper is found in the western United States (Farjon, 2013). The heartwood of this species contains cedrol, widdrol, and sesquiterpene alcohol, compounds that show strong termiticidal and antifungal properties (Craig et al., 2004; Liu, 2009; Mun and Prewitt, 2011; Orejuela, 1995). Western juniper is increasingly abundant on Oregon's eastern rangeland, but its utilization is hampered by a lack of viable markets (Leavengood, 2008). These species might be used in exterior applications where durability has value but data are lacking on variation among individual western juniper trees.

Durability testing generally exposes wood in the field or uses smaller scale laboratory studies that expose wood blocks to a specific wood decay fungus or insect species (American Wood Protection Association, 2014; EN 350-2, 1994). The process is laborious, time-consuming, and limited to a small number of samples that may not represent the overall durability of a species. This may prove problematic, when a

species that is considered durable fails in a building. There is a need for more rapid techniques that can properly classify actual durability of large numbers of samples rather than using a presumed durability based on limited testing.

One possible solution for assessing durability is infrared spectroscopy, a surface-based analytical method that examines the vibration of molecules when a material is exposed to infrared light to generate chemical 'fingerprints' unique to the analyzed material (Siesler et al., 2008). Attenuated total reflectance fourier transform infrared (ATR-FTIR) spectroscopy is a well-established technique, especially in the food industry (Azizian et al., 2004; Cozzolino, 2014; Washburn and Birdwell, 2013). ATR-FTIR application to forest products has been gaining popularity due to advances in FT-IR spectrometers that allow fast, more accurate analysis. The simplicity of the technique (no sample preparation using other chemicals e.g. KBr pellet) as well as development of powerful software to perform chemometrics analysis on complex infrared spectra have markedly increased the use of this technique. Examples of ATR-FTIR application have focused on chemical analysis and predictions of wood constituents such as cellulose, hemicellulose and lignin (Pandey, 1999; Stark and Matuana, 2007; Zhou et al., 2015a).

The sensitivity of the FT-IR spectrometer also allows the study of extractive components that are often present at low concentrations in wood (1-5%) (Hillis, 2011).

Furthermore, the correlation of wood chemical properties with physical properties, has resulted in several studies linking these relationships to FT-IR spectral information. Extractives studies conducted on Scots pine (Holmgren et al., 1999; Nuopponen et al., 2003), eucalyptus (Popescu et al., 2007), and rosewood (Wang et al., 2015), have produced promising results for detection of extractives important to durability.

The present study was designed to explore the relationships between western juniper extractives content and variations in resistance to fungal and termite attack using ATR-FTIR. Spectral data were assessed using principal component analysis and hierarchical cluster analysis to determine if these techniques could be used to establish durability classes for this wood species.

5.2. Materials and Methods

5.2.1. Samples and sample preparation

Wood disks (150 mm of thick) were cut at breast height from five freshly felled Western juniper (*Juniperus occidentalis* Hook. var. *occidentalis*) (Cupressaceae) trees in northern California. There was a clear color differentiation between sapwood and heartwood. Disk diameter, sapwood and heartwood depth, and number of growth rings were recorded for each disk (Table 1). The disks were air dried under cover with regular air flow for approximately three months. Radial strips (50 mm wide) were then cut from each disk, with strip lengths varying from 100 to 150 mm. A five millimeter

radius area around the pith was discarded. The remainder of each strip was cut into 15 mm squares moving from the heartwood (HW) to the sapwood-heartwood (SH) boundary and finally to the sapwood (SW) (Appendix 1). Blocks with defects were discarded then the remainder were labelled and separated for extractives analysis, infrared spectroscopy, wood decay tests, and termite tests (Appendix 1). Prior to testing, each block was oven-dried at 50 ± 2°C for 24 hours to determine density according to ASTM Standard D4442-16 (ASTM 2016), except that 50°C was used instead of 103°C to minimize extractives degradation, as certain extractives are known to be highly volatile and sensitive to elevated temperatures (Scheffer, 1973).

Table 5.1 Characteristics of the five western juniper disks used to evaluate extractives content and durability.

Disk	Diameter (mm)	Heartwood diameter (mm)	Sapwood diameter (mm)	Heartwood: sapwood ratio	Growth rings
1	344	175	169	1.04	92
2	425	260	165	1.58	103

3	400	195	205	0.95	102
4	458	310	148	2.09	107
5	520	356	164	2.17	77

5.2.2. Extractives analysis

A total of 102 blocks of 15 cm^2 from the heartwood, sapwood-heartwood boundary and sapwood of each disk were ground to pass a 60 mesh screen using a Wiley mill (Arthur H. Thomas Co., US) and sealed individually inside air-tight plastic bags that were stored in the dark at 5°C until used. Extractives analysis was conducted using a soaking technique that allowed the extraction of many samples simultaneously (Taylor et al. 2006). This extraction method was chosen instead of the conventional Soxhlet extraction owing to the large number of extractions required for this study. However, complete extraction may not occur using this method. Thus, results can only be compared between tested wood samples, and not as used as absolute extractives content.

The ground wood powder was weighed (0.5 ± 0.05 g) and placed inside an individual fabric filter bag (Nuiby unbleached tea filter bags, 6.1 x 8.1 cm), labelled and weighed again to obtain the initial wet weight with bag. Three individual tea bags (replicates)

were prepared for each wood powder from each radius location from the five disks. The bags were then oven-dried at 50 ± 2°C for 24 hours and an initial oven-dried weight was recorded. The samples were then sequentially extracted using hexane (Fisher Scientific, 99.9%), methanol (Fisher Scientific, 99.9%) and hot water. The solvent selection was based on previous literature that showed effective removal of extractives from western juniper using these solvents (Mun and Prewitt, 2011; Tumen et al., 2012). Hexane is a non-polar solvent that removes mainly aliphatic compounds, while methanol (polar solvent) mainly removes phenolic substances, terpenoids, fats, and some carbohyrates. Hot water removes carbohydrates such as sugars and starch along with proteins, gum, pectins and coloring material (ASTM D1105-96, 2013; Sjöström, 1993; Yang and Jaakkola, 2011).

A ten liter Erlenmeyer flask was filled with nine liters of hexane along with the filter bags and a stir bar. Three cycles of extraction were made by placing tea bags filled with western juniper powders from one or two disks for all radius locations per cycle. The flask was then placed on a hot plate with continuous stirring and heated at 60°C for 24 hours. The bags were removed, rinsed with hexane and oven-dried at 50 ± 2°C for 24 hours. Mass loss after extraction was recorded as the extractives content of each sample. These steps were then repeated using 95% methanol. For hot water extraction, the bags were placed in a six litre Erlenmeyer flask distilled water and boiled in a water bath for 6 hours. The bags were removed from flasks and rinsed with

distilled water, then air-dried overnight before they were oven-dried at 50 ± 2°C for 48 hours and weighed. Weight loss after all three extractions were recorded as the total extractives content.

5.2.3. Extractives compounds identification using GC-MS

Identification of the extracted components was done on samples obtained from the same population of trees from a separate study (Miyamoto et al., 2018). Extracts were analysed on Shimadzu GC-2010 gas chromatograph equipped with an flame ionizote detector and an autosampler / injector. Analyses were performed on a Rtx-5 capillary column (15 mm long, 1.25 mm inner diameter, 0.25 µm thick; 5% diphenyl / 95% dimethyl polysiloxane coating) (Restek, Bellfonte, PA, USA), using helium as the carrier gas at a linear flow velocity of 1.98 ml / min of column flow. The temperature was set at 100°C for one min, then 5°C / min until 250°C was reached. The injector and detector temperature were maintained at 275°C.

5.2.4. Durability study

5.2.4.1. Wood decay testing

The decay resistance of the blocks was assessed following procedures described in AWPA Standard E10 (AWPA, 2017). A total of 204 western juniper blocks were conditioned at 20°C and 65% relative humidity to constant weight. Blocks were then

oven-dried at 50 ± 2°C and initial oven-dried weights were recorded. Blocks were then placed in plastic bags and sterilized by exposure to 2.5 mrad of ionizing radiation from a ^{60}Co source at the Oregon State University Radiation Center. The blocks were kept in a sealed sterile container for no longer than 2 weeks until testing.

Decay chambers were prepared by half filling a 454 ml french square test jar with soil dampened with 12 ml of water. A western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) wood feeder strip (25 x 20 x 3 mm) was placed on the soil surface. The bottles were loosely capped and sterilized by autoclaving at 121°C for 45 minutes to reach sterility. The flasks were placed in a fume hood to cool overnight. A small plug (10 mm diameter) was taken from the edge of an active culture of *Rhodonia placenta* (Fr.) Niemelä, K.H. Larss. & Schigel (isolate Madison #698, USDA Forest Products Laboratory, Madison, Wisconsin) or *Gloeophyllum trabeum* (Pers.) Murrill (isolate Madison #617) grown in 1.5% PDA (potato-dextrose agar) and placed on one edge of the wood feeder. These fungi cause brown-rot degradation and are among the principle degraders of wooden structures, especially in temperate regions (Green and Highley, 1997).

The jars were incubated at 28°C for one week for *G. trabeum*, or two weeks for *R. placenta*, which allowed the fungal mycelium to cover the feeder strips. A sterilized western juniper block (with cross-section down) from each disk and radius location

was then placed on the feeder strip and the jar was incubated at 28°C for 12 weeks. Three replicate blocks from each radius location of each disk were prepared for each fungus. Additionally, ten blocks of non-durable ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson) sapwood were used as a control for each fungus. Two pine blocks were removed, oven-dried and weighted each week starting from week 8 to monitor whether weight loss had reached 50%. The test was terminated once weight loss was greater than 50%.

The blocks were removed, scrapped clean of soil and fungal mycelium, and weighed to determine the wet weight. The blocks were then oven-dried overnight at 50°C and weighed again. The difference between the oven-dried weight before and after test was used to determine the wood mass loss using the following formula:

$$Mass\ loss\ (\%) = \frac{Ovendried\ weight\ before\ test - Ovendried\ weight\ after\ test}{Overndried\ weight\ before\ test} \times 100$$

Decay resistance was classified using the scale described in the ASTM Standard D2017 (ASTM, 2005) where: 0 -10 % weight loss is highly resistant, 11 - 24 % is resistant, 25 - 44 % is moderately resistant, and > 45 % is non-resistant to decay.t

5.2.4.2. Termite testing

Western juniper blocks (15 x 15 x 6 mm) from all five disks and radius locations were tested against the eastern subterranean termite, *Reticulitermes flavipes* (Oshima)

following a modified no-choice termite test (termite forced feed to only one type of wood block) described in AWPA Standard E1 (AWPA 2016). Testing was conducted at the Forests Products Laboratory, Starkville, Mississippi. Test jars were filled with 50 g white sand and 9 ml of distilled water and allowed to stand for two hours prior to use. Wood blocks were oven-dried for 24 hours at 50°C, weighed to the nearest 0.001 g and conditioned to room temperature before the test was initiated. Each test block was placed on top of a piece of aluminum foil on the surface of the damp sand and 3g of termites (approximately 150 termites including 1-3 soldiers) were added to each test jar. Three replicates were used for each test. All test jars were incubated for 28 days at 26 $\pm$ 2 °C and 50 $\pm$ 5% relative humidity. A tub of water was placed inside the incubator to maintain relative humidity. At the end of the test, the blocks were cleaned, and the mass loss due to termite feeding was calculated based on differences between oven-dried weight before and after exposure.

5.2.5. Collection of spectra

5.2.5.1. ATR-FTIR spectroscopy

Mid-infrared spectra in the range 4000 and 650 cm^{-1} were recorded using an attenuated total reflectance (ATR) system with a ZnSe crystal head Smart iTR (Thermo Scientific, USA) mounted on a Nicolet iS50 spectrometer (Thermo Scientific, USA). Spectral analysis of the powdered western juniper, including extracted samples from the extractives analysis, was done using OMNIC software version 9.2 (Thermo

Scientific, USA). A background spectrum was obtained every 15 minutes to exclude signals that were not relevant to the sample. An individual spectrum represented 32 scans at a resolution of 4 cm^{-1}. Samples were analyzed three times and averaged to obtain representative spectra.

The resulting spectra were pre-processed by performing a baseline correction (for correcting vertically sloped, curved or shifted spectra), smoothing and converted to second derivatives (smoothed and derived using Savitzky and Golay with 7 smoothing points filter and a three-order polynomial). The use of second derivatives eliminated the effects of baseline shift and resolved overlapping bands. The raw spectra and the second derivatives were used for further analysis.

5.2.6. Statistical analysis

All analyses were carried out using R Studio version 1.0.136 (RStudio Team, 2016). Average fungal and termite feeding weight losses along the radial transect were compared by two-way analysis of variance (ANOVA) tests using the Tukey's Honest Significant Difference (Tukey HSD) test procedure for multiple comparisons (α=0.05). Variables tested for weight loss on each fungal and termite species included disk, radial location, and extractives content.

Chemometrics analysis using hierarchical cluster analysis (HCA) and principal component analysis (PCA) were carried out using the R package "Chemospec"

(Hanson, 2017) on the second derivatives spectral dataset. Data for this analysis were categorized to N = non-resistant (consist of non-resistant and moderately resistant samples), R = resistant, and RR = highly resistant based on the results of durability tests against *G. trabeum*, *R. placenta* or *R. flavipes*. The moderately resistant and non-resistant samples were grouped together due to the low numbers of non-resistant materials in this study.

Both HCA and PCA are unsupervised pattern recognition techniques, with no assumptions made on group membership, and are commonly used to detect groups in a dataset. HCA uses distances or similarity measures when objects grouped together based on similarity of their spectral features. This produces HCA plots (dendrograms) calculated based on the Euclidean distance. Principal component analysis of spectral dataset can reduce the number of original spectral variables (wavenumber dimensions) to smaller sets of variables (principal components). Graphical representations of correlations between samples, principal components, and wavenumbers allow visual detection of groups (i.e. durability classifications). PCA was applied to the spectral region at 1800 to 650 cm^{-1} (fingerprint region) and the factor loadings calculated. The highest peaks in the first, second and third factor loadings were identified and assigned. The resulting PC plots (as a function of the wavenumber) were analyzed visually to detect spectral regions with high positive or negative factor loadings.

PCA is the most common method used for data compression by using an orthogonal matrix compression, while HCA uses distances or similarity measures when objects are grouped together based on their spectral features (Siesler et al., 2008). For PCA, loadings and score plots were used to visualize data and identify the spectra regions that best explained the observed variations, respectively.

5.3. Results and discussion

5.3.1. Extractives analysis

Hexane extracts were almost colorless, while methanol extracts were light yellow. Total extract yields were between 1.5 to 4.5% of the dry weight (Figure 5.1). Methanol extracts gave the highest yields, followed by hot water and hexane. These results are consistent with the soxhlet extraction results of Tumen et al. (2013) who found 4.3% extraction for hexane, and 7.3% for methanol. However, this contradicted with the hexane (0-1.6%) and methanol (0.2-3.0%) extraction yields were slightly lower than in previous reports (Tumen et al., 2012). Similarly, distillation of western juniper heartwood produced low essential oil yields, ranging from 1.1% (top of trees) to 2.3% (bottom of trunk) (Kurth & Ross 1954). Adams (1987) recovered similar yields of essential oils from the heartwood (2.33%). In general, the extractive contents were highest towards the sapwood-heartwood boundary and lowest in the mid-heartwood and sapwood regions. This follows the general pattern of extractives distribution as observed in many wood species, for example western red cedar, *Thuja plicata* Donn ex

D. Don (DeBell et al., 1999) and European larch, *Larix decidua* Mill. (Gierlinger and Wimmer, 2004).

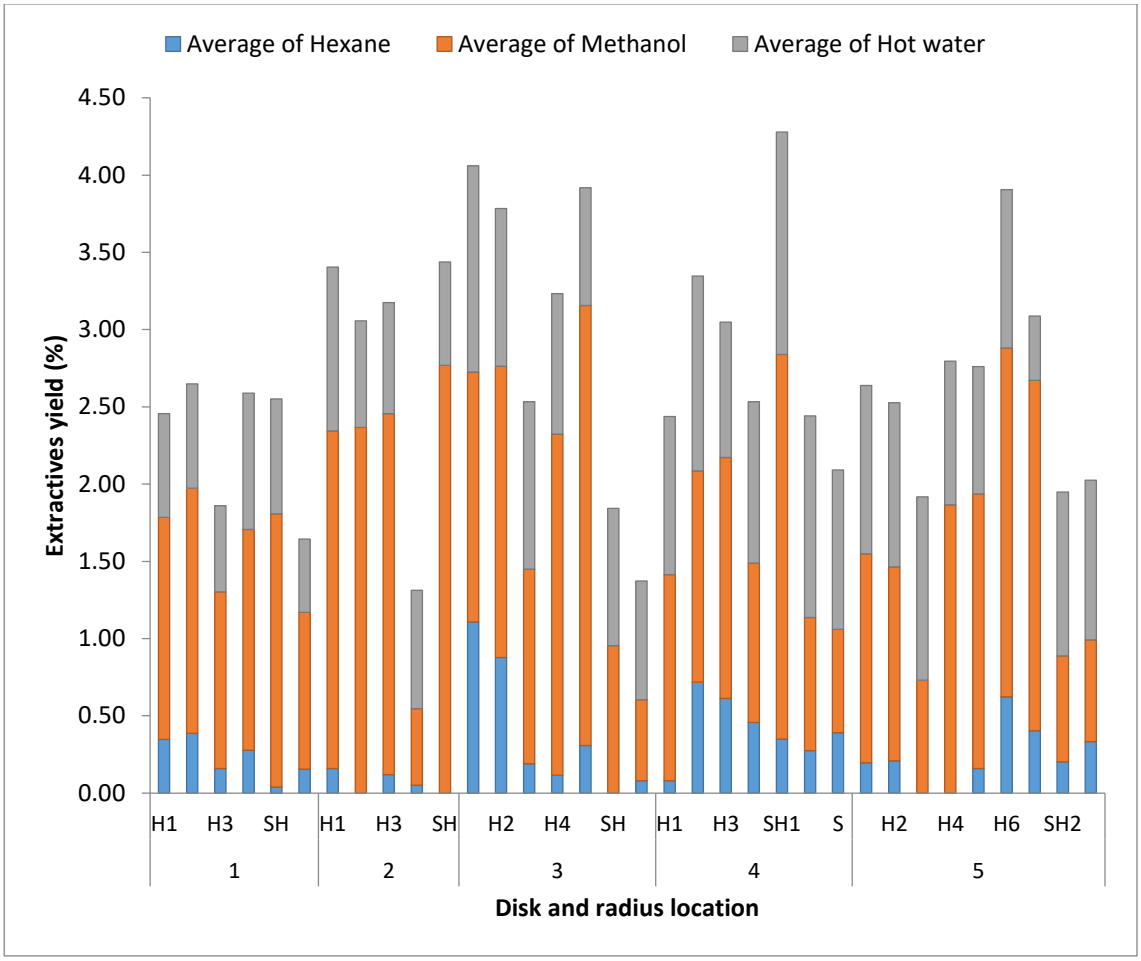


Figure 5.1 Extractives yield (%) from five disks (1-5) of western juniper radially from the pith (H1) to sapwood (S) after sequential extraction using hexane, methanol and hot water. Each value consists of the average oven dry weight basis (n=3) for each extraction radially. H = Heartwood, SH = sapwood-heartwood, S = sapwood.

A previous study of western juniper from northern California (Miyamoto et al. 2018) identified the main extractive compounds as cedrol (51%), α-cedrene (5%), and widdrol (3%). Cedrol was also reported as the main compound found in essential oils of western juniper heartwood (Adams 1987). These compounds, particularly cedrol, contribute to the durability of several *Juniperus* spp. against decay fungi, including *G. trabeum* (Mun and Prewitt, 2011). The might have contributed partially to western juniper durability to brown-rot fungi and termite used in this study.

5.3.2. Mass loss by decay fungi

Weight losses after the 12 week exposure to *G. trabeum* and *R. placenta* indicated that there was considerable variability in decay resistance (Table 5.2 and Table 5.3). The majority of the samples were classified as either highly resistant (mass loss <10%) or resistant (11 - 24%), especially against *G. trabeum*. As expected, the inner heartwood (closer to the pith) and outer heartwood (closer to the heartwood-sapwood boundary) showed higher decay resistance, compared to the sapwood regions where the majority of samples were classified as moderately resistant with mass losses ranging from 25 to 44%.

Table 5.2 Weight losses (%) for western juniper after exposure to *G. trabeum*, *R. placenta*, or *R. flavipes* by disk and radial position. Each value is the average of three specimens per organism and wood location. The same letters in a column indicate that there were no statistical differences between the specimens according to Tukey HSD at α=0.05. IH=inner heartwood, OH=outer heartwood, SH=sapwood-heartwood, S=sapwood.

Disk	Label	Radial position	Weight loss (%)					
			G. trabeum		*R. placenta*		*R. flavipes*	
			Average	Std Dev	Average	Std Dev	Average	Std Dev
1	H1	IH	3.53 b	3.06	11.75 b	0.69	0.45 c	0.78
	H2	IH	1.98 b	3.42	15.03 b	2.55	3.72 bc	0.90
	H3	OH	6.64 b	7.79	12.04 b	2.11	3.47 bc	0.88
	H4	OH	7.01 b	1.16	12.91 b	0.93	7.75 b	2.04
	SH	SH	3.17 b	1.05	12.83 b	2.31	17.55 a	0.57
	S	S	16.70 a	0.32	33.81 a	1.64	16.31 a	4.21
2	H1	IH	2.82 a	1.43	4.19 b	0.38	0.90 c	0.78
	H2	IH	3.05 a	1.90	13.53 b	2.01	1.04 c	0.90
	H3	OH	1.97 a	1.51	28.19 a	2.70	2.19 bc	0.90
	H4	OH	14.60 a	20.10	29.62 a	7.96	5.33 b	1.79
	SH	SH	10.09 a	8.14	11.01 b	1.13	10.63 a	2.03
3	H1	IH	3.72 cd	0.36	3.42 b	1.89	1.19 bc	0.01
	H2	IH	4.19 cd	0.66	5.96 b	2.04	2.50 b	1.25
	H3	OH	9.82 c	0.56	9.81 b	7.20	1.24 bc	0.03
	H4	OH	4.04 cd	0.43	10.83 b	4.02	0.00 c	0.00
	H5	OH	2.10 d	1.37	4.38 b	1.48	0.00 c	0.00
	SH	SH	36.73 b	0.12	10.36 b	5.34	3.60 b	0.02
	S	S	44.80 a	6.27	29.58 a	1.86	10.74 a	2.05
4	H1	IH	14.98 c	3.59	15.99 bc	2.80	0.69 c	0.59
	H2	IH	5.45 d	1.49	6.02 c	3.31	0.00 c	0.00
	H3	OH	7.32 d	0.79	14.92 bc	1.29	3.26 bc	0.73
	H4	OH	15.13 c	4.67	15.19 bc	3.67	1.58 c	0.67
	SH1	SH	3.18 d	3.11	9.79 bc	1.76	5.64 b	2.49
	SH2	SH	54.29 a	11.36	18.73	8.92	13.23 a	2.25

Disk	Label	Radial position	Weight loss (%)					
			G. trabeum		*R. placenta*		*R. flavipes*	
			Average	Std Dev	Average	Std Dev	Average	Std Dev
	S	S	29.37 b	3.42	27.72 a	0.90	13.99 a	0.73
5	H1	IH	6.82 d	1.27	4.13 de	3.08	0.99 a	0.85
	H2	IH	13.44 d	4.80	11.87 cd	5.47	1.51 cd	0.03
	H3	IH	25.35 bc	5.81	10.61 de	3.55	6.63 b	2.29
	H4	OH	17.82 cd	5.02	1.8 e	0.36	3.27 c	1.60
	H5	OH	7.43 d	2.79	4.17 de	2.67	1.32 d	0.01
	H6	OH	4.69 d	2.28	0.92 e	0.83	2.31 cd	0.80
	SH1	SH	9.23 d	2.56	16.80 bc	4.30	0.00 d	0.00
	SH2	SH	48.23 a	3.69	23.47 ab	3.55	11.16 a	1.63
	S	S	38.72 ab	5.18	27.04 a	5.12	11.44 a	0.86

.

Table 5. 3 Durability classifications of western juniper samples exposed to *G. trabeum*, *R. placenta* or *R. flavipes* according to radial position in accordance with ASTM Standard D2017 (ASTM, 2005).

Organism	Radial position	*n*	Durability classification (% of samples)			
			Highly resistant	Resistant	Moderately resistant	Non-resistant
G. trabeum	Inner heartwood	33	75.8	18.2	6.1	0.0
	Outer heartwood	33	72.7	3.0	24.2	0.0
	Sapwood-heartwood	24	54.2	8.3	16.7	20.8
	Sapwood	12	0.0	25.0	66.7	8.3
R. placenta	Inner heartwood	33	51.5	42.4	6.1	0.0
	Outer heartwood	33	33.3	51.5	15.2	0.0
	Sapwood-heartwood	24	25.0	66.7	8.3	0.0
	Sapwood	12	0.0	8.3	91.7	0.0
R. flavipes	Inner heartwood	33	100.0	0.0	0.0	0.0
	Outer heartwood	33	100.0	0.0	0.0	0.0
	Sapwood-heartwood	24	54.2	45.8	0.0	0.0
	Sapwood	12	8.3	91.7	0.0	0.0

Western juniper is classified as very resistant (Scheffer and Morrell, 1998) to resistant to decay fungi (Clausen, 2010). These classifications are in agreement with the results obtained from the present study. Similarly, western juniper heartwood displayed high resistance to decay in above-ground field test under the tropical conditions of Hilo,

Hawaii (Morrell, 2011). However, the sapwood and sapwood-heartwood samples in that study displayed lower resistant to decay as opposed to the current study. This might be due to the samples exposed to extreme field conditions during the test making it more susceptible to decay, as well as the presence of more aggressive wood decay species. Nevertheless, western juniper fence posts have been observed to provide service lives ranging from 23 to 66 years (Morrell et al., 1999).

Higher standard deviation values (high variability) were observed in some of the weight loss samples exposed to *G. trabeum* (Figure 5.3). A possible explanation is that, despite the controlled settings in the incubation chambers, some variables might appear inside the test jars due to placement either further or closer from the air vent during the incubation period as micro-climates can be produced inside the jars. As fungi are sensitive to moisture content and temperature, they will decay wood more rapidly when the optimal parameters (above 30% MC and temperature 23 - 28°C) are met (Clausen, 2010; Zabel and Morrell, 1992). Another possibility is the presence of included sapwood, which is a common occurance in heartwood of *Juniperus* spp., including western juniper. The existence of included sapwood is unclear (Shigo and Hillis 1973), but it might be more susceptible to decay, causing higher weight loss compared to matched samples.

5.3.3. Mass loss by termite attack

Wood was highly resistant or resistant to *R. flavipes* attack at all radial positions (Table 5.3). Excellent termiticidal activity was noted with all samples (including sapwood). Complete mortality was observed at the end of the 4-week termite test on all samples. Termite mortality in ponderosa pine sapwood (control) ranged from 0-20% with mass losses >45%, indicating that healthy and aggressive termites were used in this study. The resistance of western juniper to termite attack is comparable to western red cedar, a highly prized commercial species used mainly for outdoor applications such as fencing, decking, roofing and siding (Gonzalez, 2004; Minore, 1983). In fact, Kirker et al. (2013) demonstrated that western juniper had better resistance to degradation against *R. flavipes* than western red cedar.

A similar result has been observed when western juniper heartwood was exposed in an above-ground test to *Coptotermes formosanus* Shiraki, 1909, while the sapwood samples were susceptible (Morrell, 2011). The sapwood used in the current study was highly resistant to termite attack, but *R. flavipes* was less aggressive compared to *C. formosanus*. While sapwood is generally considered as susceptible to decay and termite attack, certain species including western juniper sapwood have displayed high termiticidal activity. Adams et al. (1988) reported complete mortality of *R. flavipes* within the first few days of exposure to sawdust of western juniper sapwood. These

results might be due to the termite species used in the experiment. *C. formosanus* is one of the most aggressive and destructive pests in the United States (Lax and Osbrink, 2003), and may have higher resistance to western juniper extractives although these compounds are both antimicrobial and anti-termitic (Adams et al., 1988; Mun and Prewitt, 2011).

Sapwood is generally considered as non-durable to biodegradation due to lack of toxic extractives and higher starch content (easily available to decay agents) (Zabel and Morrell, 1992). However in the current study, western juniper sapwood displayed excellent resistance to termites but not to fungal attack. It is possible that the sapwood contained extractives that were toxic to termites. Essential oils from the sapwood of several species of juniper from Turkey were reported to contain high concentrations of widdrol (up to 22% of the % total peak area in the GC-MS chromatogram), α-cedrol (up to 19%) and traces of other extractives that may contribute to termite resistance (Uçar and Balaban, 2002).

5.3.4. Relationship between mass loss and extractive content

Relationships between extractive contents and mass losses caused by *G. trabeum*, *R. placenta* and *R. flavipes* were assessed using Pearson's correlation coefficient (Figure

5.2). Negative correlations were exhibited between mass losses and extractives yield, indicating that increasing extractive contents were associated with decreased weight loss (i.e. higher decay resistance). Correlations between mass loss and extractives levels were moderate (0.3 to 0.5) based on Cohen's classification (1988).

Methanol extracts were most closely correlated with mass losses caused by wood decay fungi, especially *G. trabeum* (r = -0.62) (Figure 5.2). Methanol extracts also accounted for 38% and 21% of variability in mass loss to *G. trabeum* and *R. placenta*, respectively (Figure 5.3). Hexane extracts were poorly correlated (r = - 0.2) with mass losses to *G. trabeum* and *R. placenta* (Figure 5.2). Previous studies on eastern red cedar indicated strong inhibitory properties of methanol extracts on *G. trabeum* (Mun and Prewitt, 2011).

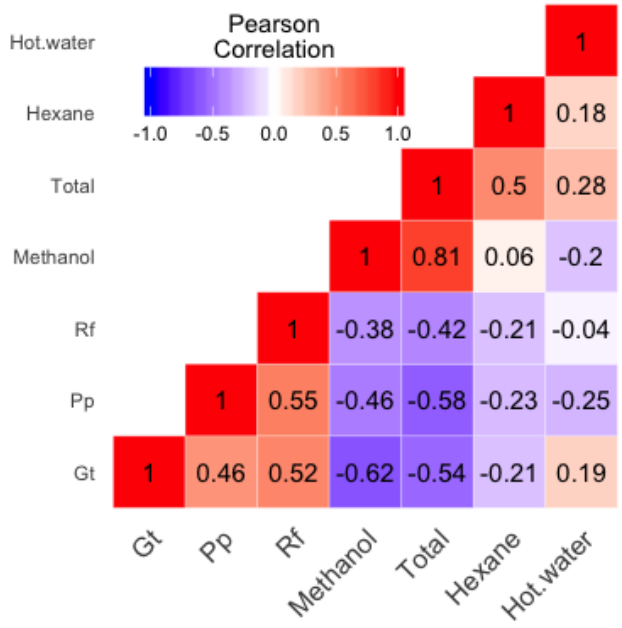


Figure 5.2 Correlation coefficients between hexane, methanol, and hot water extracts and mass loss after exposure to *G. trabeum* (Gt), *R. placenta* (Pp) or *R. flavipes* (Rf).

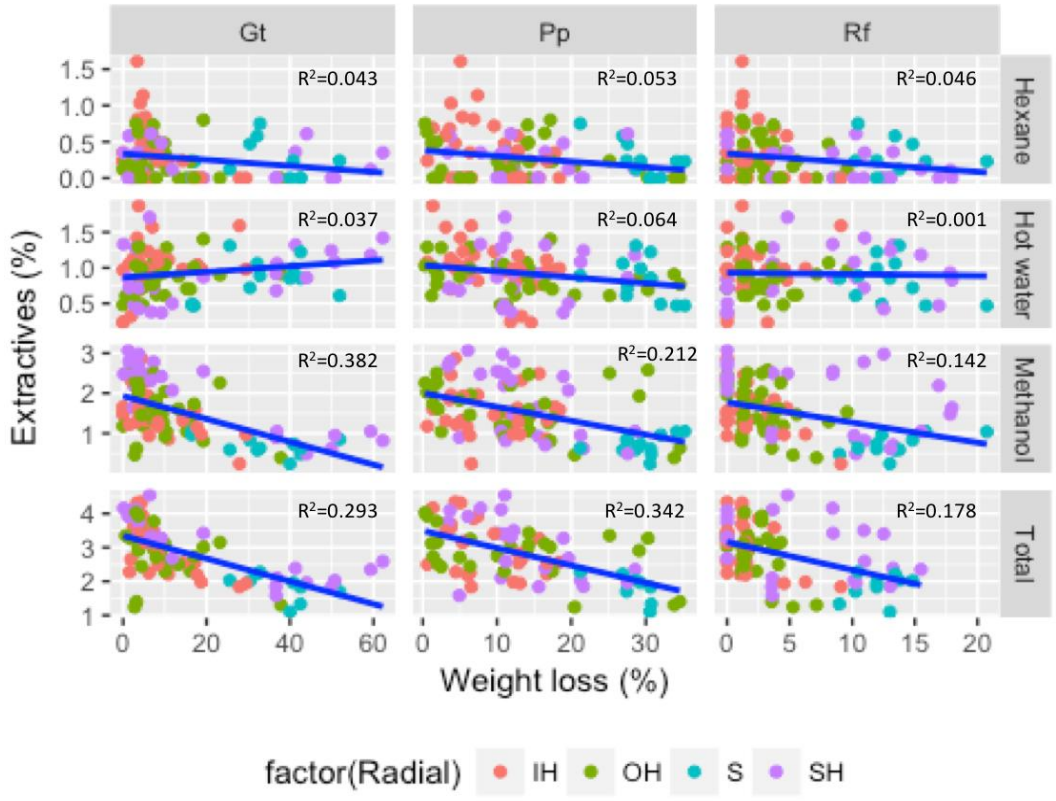


Figure 5.3 Relationship between weight losses (%) caused by exposure to *G. trabeum* (Gt), *R. placenta* (Pp) or *R. flavipes* (Rf) and extractive contents radially from IH = inner heartwood, OH = outer heartwood, SH = sapwood-heartwood, and S = sapwood.

Extractives content and mass losses by termites were poorly correlated, indicating that other factors may contribute to western juniper durability (Figure 5.2). Total extractives accounted for 18% of variability in mass loss to *R. flavipes* (Figure 5.3). Adams et al. (1988) reported that hexane and methanol extracts of western juniper heartwood were associated with high termiticidal activity against *R. flavipes*, with complete mortality observed within several days to one week after exposure.

5.3.5. Extracted and non-extracted spectra

ATR-FTIR spectra of extracted and non-extracted western juniper were divided into two sections: the functional group region between 4000 and 1800 cm^{-1}, and the fingerprint region of 1800 to 650 cm^{-1} (Figure 5.4). Two broad bands were observed at 3339 cm^{-1} and 2897 cm^{-1}. These bands are linked to the stretching vibrations of hydrogen bonds (O-H) and aliphatic C-H, respectively (He et al., 2007; Mattos et al., 2014). The band at 3339 cm^{-1} is characteristic of lignocellulosic materials and has been observed in non-wood materials such as bamboo, jute yarn, and cotton (George et al., 2013; He et al., 2007; Tomak et al., 2013). The FT-IR analyses of extracted and non-extracted samples differed little, although increased intensities were observed at 3339 cm^{-1} and 2897 cm^{-1} after extraction (Figure 5.4A).

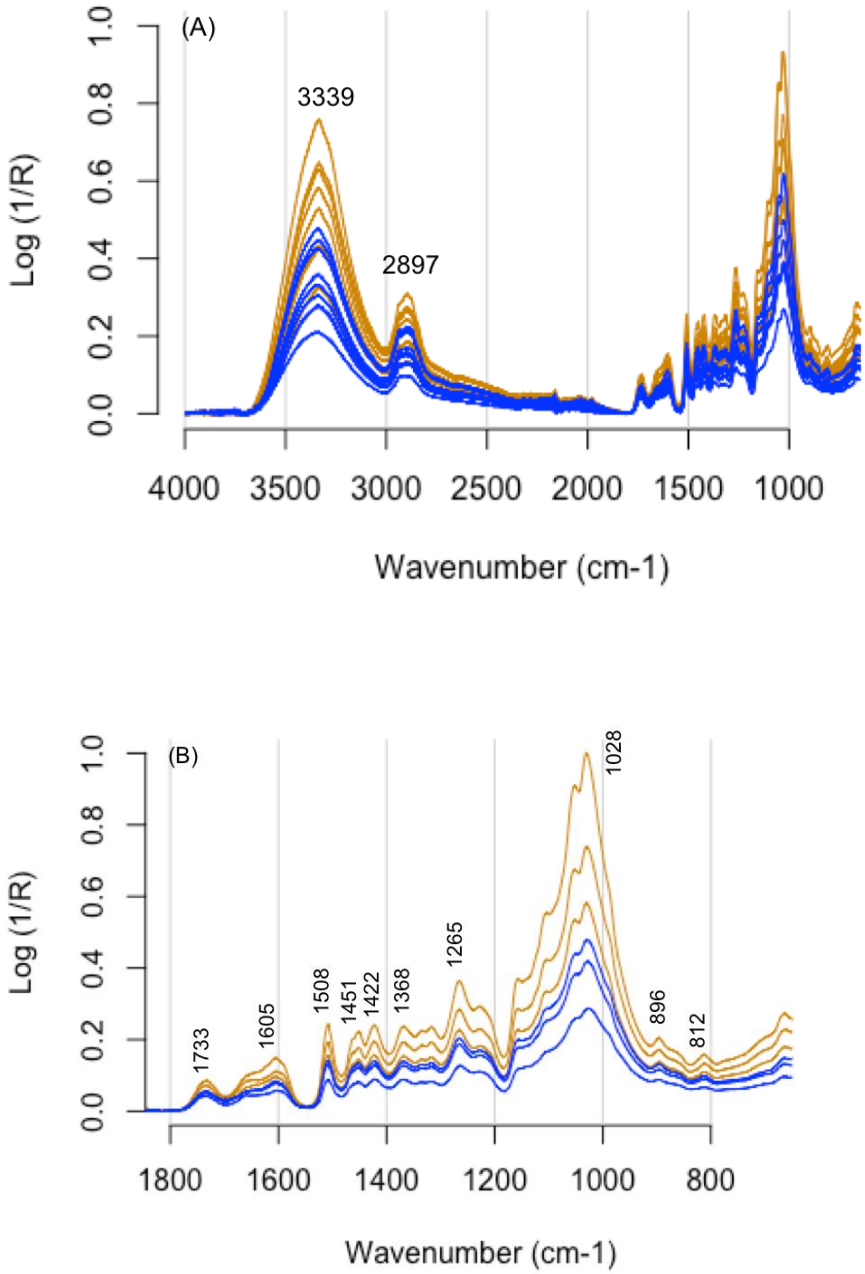


Figure 5.4 Comparisons between ATR-FTIR spectra of non-extracted (blue) and extracted (yellow) western juniper for bands in the range 4000 - 650 cm^{-1} (A) and 1800 - 650 cm^{-1} (B). Each spectrum is the mean of three spectra from different radial locations.

The peaks within the region 1800 to 800 cm^{-1} represent the major wood polymers (cellulose, hemicellulose and lignin) and are considered to be the fingerprint region for wood (Pandey, 1999). No major changes were observed before and after extraction in the main peaks observed in the western juniper spectra (Figure 5.4B). This lack of change might be due to the low extractives levels (1.92 - 3.91%) in the samples (Figure 5.1). The low extractives content may be insufficient to be observed in the extracted spectra. FT-IR could detect carvacrol when added to wood at a high level (5% w/w), but undetectable at lower levels (1-3% w/w). These lower levels were more consistent with actual levels in the wood and suggest that the FT-IR spectrometer employed may lack the sensitivity required to detect subtle changes in extractives content. Previous studies have shown that bands related to wood extractives occur at 1730 cm^{-1}, 1633 cm^{-1}, 1600 cm^{-1}, 1510 cm^{-1} and 1271 cm^{-1} (Colom and Carrillo, 2005; Mattos et al., 2014; Nuopponen et al., 2003; Pandey and Pitman, 2003; Schauwecker et al., 2013; Zhou et al., 2015b) (Table 5.4). These peaks were still present in the extracted spectra, but changes in absorbance at these wavelengths were difficult to detect.

The ATR technique used in this study can be affected by surface properties such as wood powder particle size and density (Stuart, 2004). In order to resolve the problems and produce clearer separations between the spectra, mathematical pretreatments were performed by conversion to second derivatives (Figure 5.5). Derivatives enhanced the apparent resolution and amplified small differences in the IR spectra by

increasing the signal-to-noise ratio coming from random noise, baseline effects or spectral interferences (Siesler et al., 2008). Spectral variations for complex and heterogeneous samples such as wood reflect the interaction of compounds (e.g. intermolecular hydrogen bonds), light scattering from solid samples, poor reproducibility in the measurement process (e.g. through path-length variations), and spectral distortions due to spectrometer hardware (e.g. baseline drift, wavelength shifts). Appropriate pre-processing can improve prediction compared to using the raw spectra (Gierlinger et al., 2002; Wang et al., 2015).

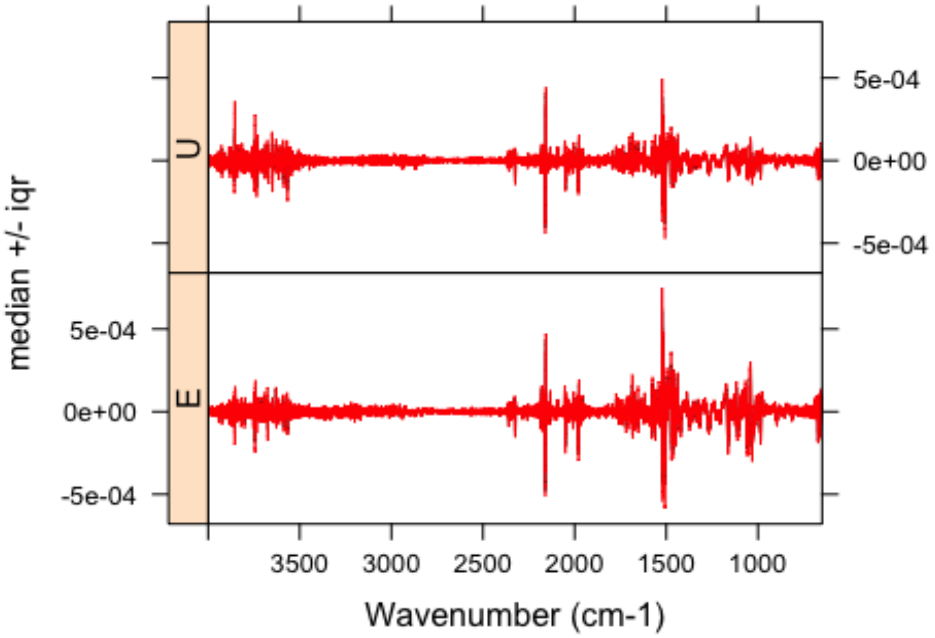


Figure 5.5 The second derivative for the spectra of non-extracted (U) and extracted (E) western juniper.

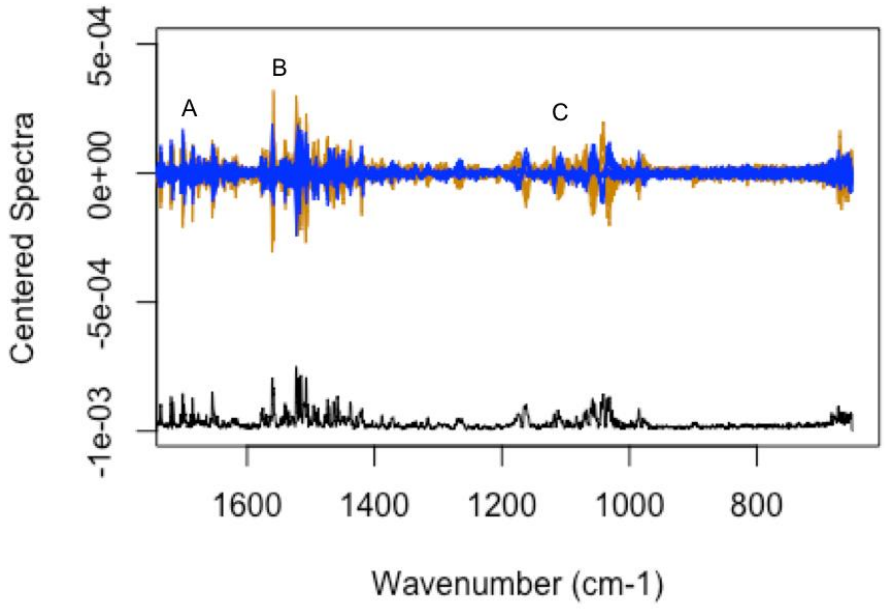


Figure 5.6 Spectral survey emphasizing variations among spectra of the non-extracted (blue) and extracted (yellow) western juniper at the 1800 to 650 cm^{-1}. A: 1600-1500 cm^{-1}, B: 1600-1500 cm^{-1}, and C: 1200-1000 cm^{-1}.

Table 5.4 Possible identities of FT-IR bands found in the western juniper.

Wavenumber (cm^{-1})	Reference wavenumber (cm^{-1})	Band assignment*	Potential compounds	Sources
1733	1740-1720	C=O stretching vibrations produced by ester carbonyl[1-]	Fat, wax or esterified resin acids	Colom et al. 2003
1605	1610-1590	C=C stretching or aromatic ring deformation[2]	Aromatic compounds, phenolic group	Pandey & Pitman 2003
1508	1515-1505	Olefinic double bond[3]	Aromatic compounds	Schwanninger et al. 2004
1265	1271-1268	Deformation vibration within benzene rings[4]	-	Mohebby et al. 2005
896	930-915	Aromatic C-H out-of-plane deformation, pyrene ring vibration[5]	-	Popescu et al. 2007
812	811	Carbon single bonded oxygen[6]	-	Zhou et al. 2015

The use of second derivatives of spectra from extracted samples showed that there were reduced absorption at 1800 - 1750 cm^{-1}, 1600 - 1500 cm^{-1}, and 1200 - 1000 cm^{-1} (Figure 6). The region at 1800 – 1750 cm^{-1} associated with C=O stretching vibrations produced by ester carbonyls (around 1730 cm^{-1}) in fat, wax or esterified resin acids (Zhou et al., 2015b). Meanwhile, the band at 1750 cm^{-1} is associated with conjugated carboxylic aldehydes of lignin and extractives (Colom and Carrillo, 2005; Silverstein and Webster, 2006). The region at 1600 - 1500 cm^{-1} is related to lignin structures, specifically at bands 1592 cm^{-1} and 1504 cm^{-1}, and corresponds to the symmetrical stretching of the C=C aromatic benzene rings (Mattos et al., 2014; Zhou et al., 2015b). C=C aromatic rings are also present as functional group in western juniper extractives that contribute to its durability. The bands at 1200 - 1000 cm^{-1} , specifically 1157 cm^{-1}, correspond to C-O-C asymmetrical stretching in both hemicellulose and cellulose. Peak intensity was lowered which may indicate reduction of these polymers during extraction process.

Chemical structures of the major extractives found in western juniper are shown in Figure 5.7. Many western juniper extractives have antimicrobial and antitermite properties (e.g. cedrol and α-cedrene) (Craig et al., 2004; Liu, 2009; Mun and Prewitt, 2011; Orejuela, 1995). Cedrol has a monoterpene (C_{10}) backbone, and functional group ketone (C=O) which is located at bands ~1700 cm^{-1}.

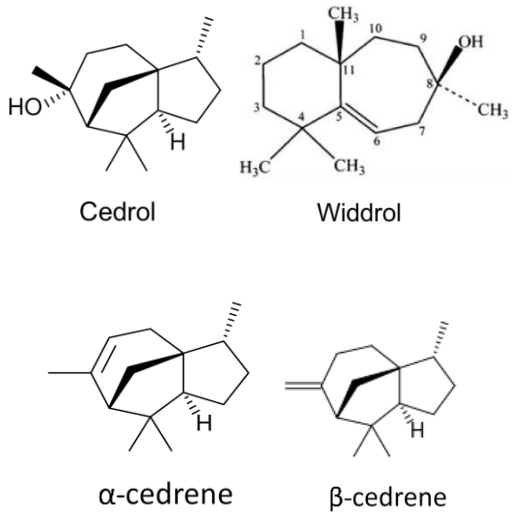
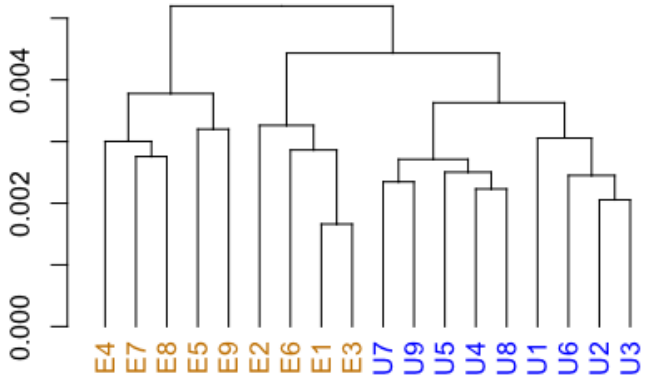


Figure 5.7 Chemical structure of the main compounds found in western juniper extractives (Mun and Prewitt 2011).

Hierarchical cluster analysis (HCA) was performed using the second derivative FT-IR spectra (Figure 5.8). The resultant dendrograms from these analyses allowed visualization of group classifications between extracted and non-extracted western juniper based on the complete linkage method with Euclidean distance as the similarity measurement. The full spectrum (4000 – 650 cm^{-1}) was used to group samples into one block, with the non-extracted spectra, and another with part of the non-extracted and all of the extracted spectra. The majority of variations were in the functional group regions, particularly at 3340 cm^{-1} (Figure 5.4). This region is not particularly useful for extractives analysis but it might be useful for classifying the extracted and non-extracted samples. The fingerprint region (1800 – 650 cm^{-1}) showed

that the classification was less clear with no obvious differences between the two groups.

(A) 4500-650 cm^{-1}



(B) 1800 – 650 cm^{-1}

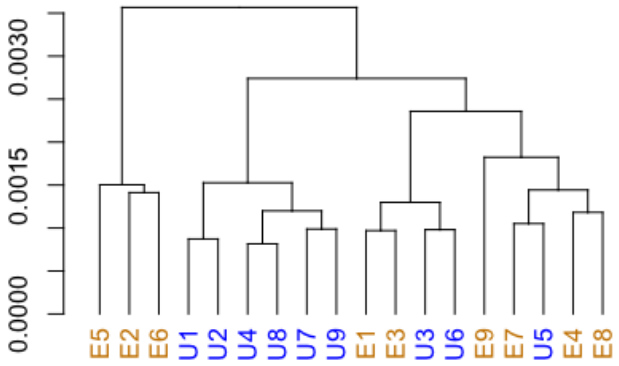


Figure 5.8 Dendogram of hierarchical clustering (with Euclidean distance) of the non-extracted (U, blue) and extracted (E, yellow) western juniper FT-IR spectra using second derivatives at 4500 – 650 cm^{-1} (A) and 1800 – 650 cm^{-1} (B).

Comparisons between spectra of extracted and non-extracted western juniper were used to identify extractives that may contribute to western juniper durability. However, this study showed the difficulties in removing the extractives as seen in Figure 5.1. The soaking extraction technique might not be as effective as soxhlet extraction. However, the conventional soxhlet extraction on western juniper also showed that extractives content on western juniper was between 3 - 4.5% (dry weight basis) (Kirker et al. 2013), which is similar to the present results. Nevertheless, Kirker et al. (2013) showed significantly reduced durability of western juniper heartwood after extraction, although the samples were still classified as 'resistant'. Other confounding factors might have contributed to the resistance such as synergetic effect.

Principal component analysis (PCA) was used for qualitative recognition of different sample groups. The second derivatives (1800-650 cm^{-1}) were subjected to PCA analysis, and the results were examined using the scree plot. The first three principal components (PC) explained 82.7% of the variation in the dataset, with PC1 representing 56%, PC2 5.7% and PC3 5.7% (Figure 5.9 and Figure 5.10). The distribution of samples along PC1 (Figure 5.10A) suggested that there was a slight overlap between the extracted and non-extracted groups. A majority of the non-extracted spectra had positive PC1 scores, while extracted had negative PC1 scores.

Based on the loadings profile (Figure 5.11B), PC1 variation was associated with the regions 1200 to 1000 cm^{-1}, and 1400 to 1600 cm^{-1}. These regions are related to C=C stretching or aromatic ring deformation (1610-1590 cm^{-1}) and deformation vibrations within benzene rings (1271-1268 cm^{-1}). Both these regions are associated with wood extractives compounds (Pandey & Pitman 2003, Mohebby et al. 2005). PC2 variation was related to the region between 1800 to 1400 cm^{-1}. C=O stretching vibrations produced by ester carbonyl bonds (1740 – 1720 cm^{-1}) from fat, wax or esterified resin acids (Colom et al. 2003) are important in this region. While these results suggest that it might be possible to segregate samples with extractives from those that has been removed, there was very little segregate between non-extracted samples. The lack of the differences would make it difficult to use the technique to sort durability differences using spectra. The lack of representation from some of the classifications made this process more difficult. The inclusion of a broader range of durability categories might help improve the comparability.

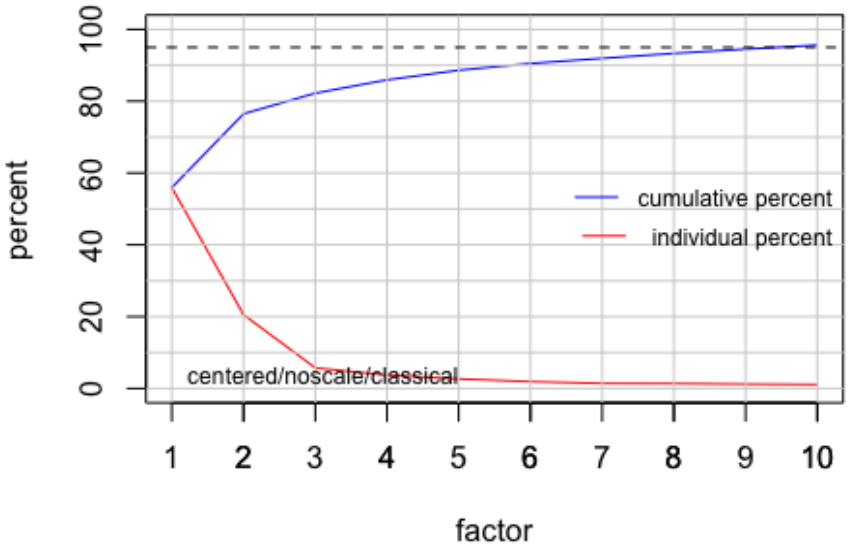


Figure 5.9 Scree plot of the PCA of the FT-IR spectra using second derivatives at 1800 – 650 cm^{-1}.

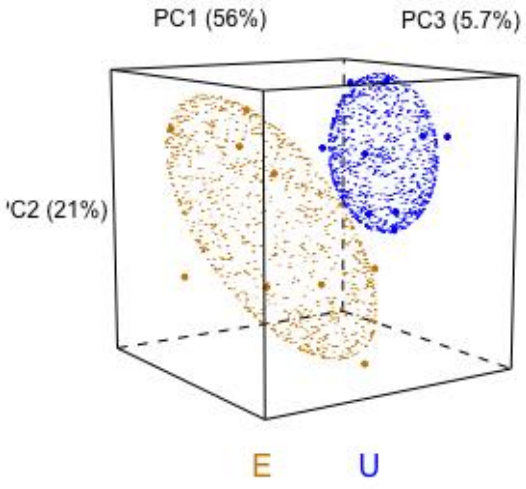
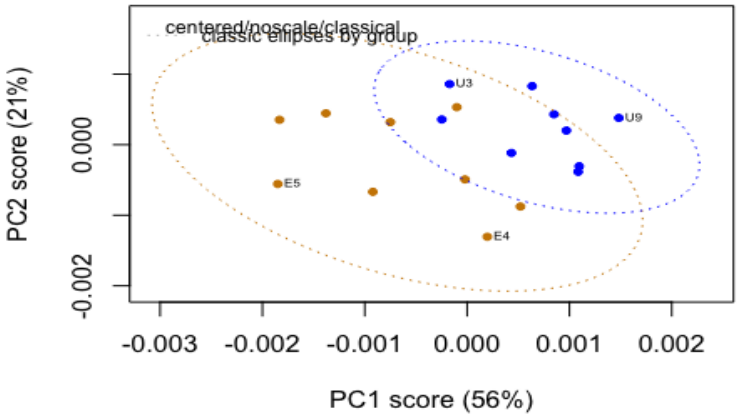


Figure 5.10 Principal component analysis using the classical technique showing 3D representations of the first three principal components (PCs) of second derivative FT-IR spectra of extracted and non-extracted samples.

(A)



(B)

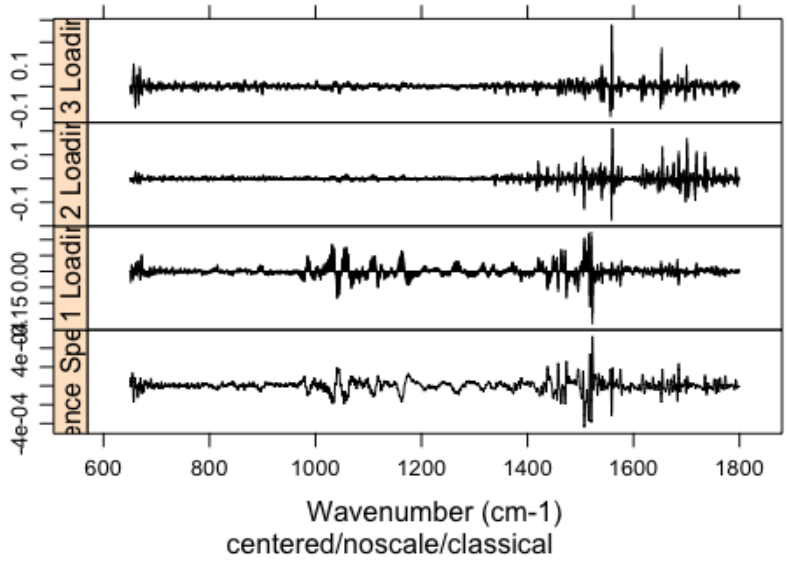


Figure 5.11 PCA analyses with (A) PC1 vs PC2 scores plotted using classical PCA techniques for the non-extracted (blue) and extracted (yellow) western juniper, and

(B) the loadings profile of the first three PCs. Dashed lines on the PC1 vs PC2 plot indicate the 95% confidence interval for each group.

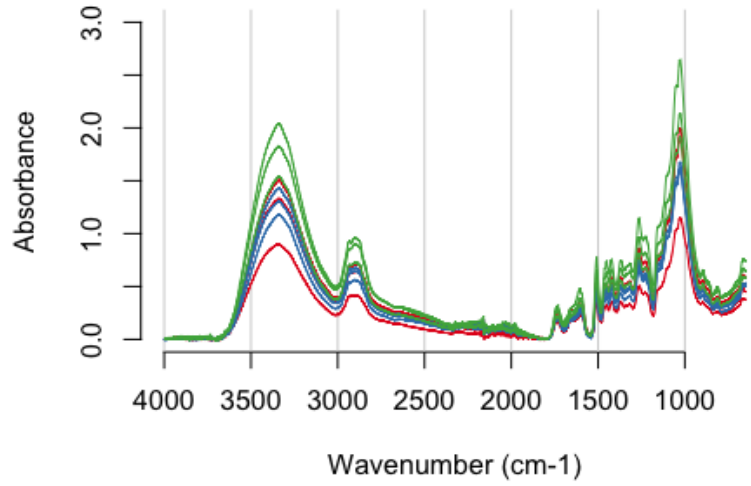
5.3.6. FT-IR for wood durability

Averaged ATR-FTIR spectra (from three spectra / sample) were used to examine the potential for sorting based upon durability classes established by exposure to *G. trabeum* (Figure 5.12). Both 'moderately durable' and 'non-durable' groups (Table 5.3) were classified as 'non-durable' due to lack of representatives of these groups in present study. Only two durability classifications were available for termite resistance, the 'highly resistant' (RR) and 'resistant' (R). Comparisons of averaged spectra between the three different durability classes for *G. trabeum* showed that there were no discernable differences between the highly resistant, resistant and non-resistant western juniper (Figure 5.12). Similar findings were observed in the spectra representing different durability classifications for *R. placenta* and *R. flavipes*.

Hierarchal cluster analysis (HCA) was performed on the second derivative spectra of the fingerprint region (1800-650 cm^{-1}) for different durability classifications established by exposure to *G. trabeum*, *R. placenta* or *R. flavipes*. Dendrograms produced from the HCA on the spectra showed classifications for durability against all the tested organisms is inseparable (Figure 5.13). Western juniper identified as highly resistant, resistant and non-resistant were distributed along the two major blocks, separating the groups in the dendrograms. These results were similar to those for the

averaged FT-IR spectra and suggest that the spectra were not suitable for segregating durability classifications.

(A)



(B)

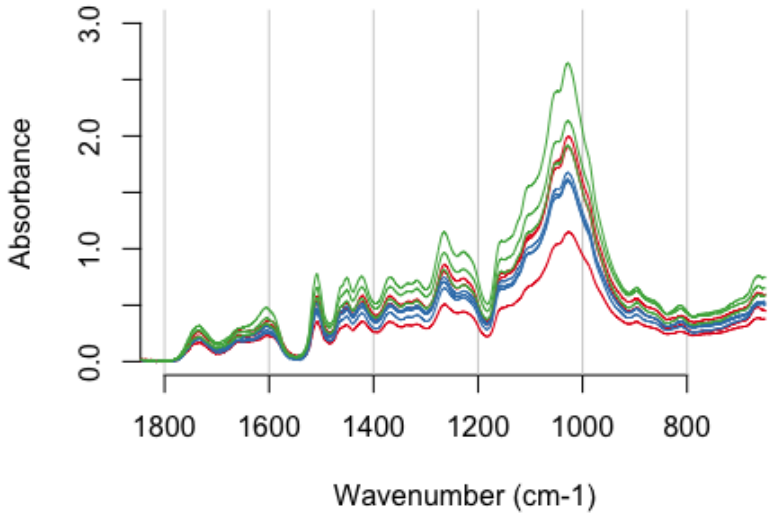
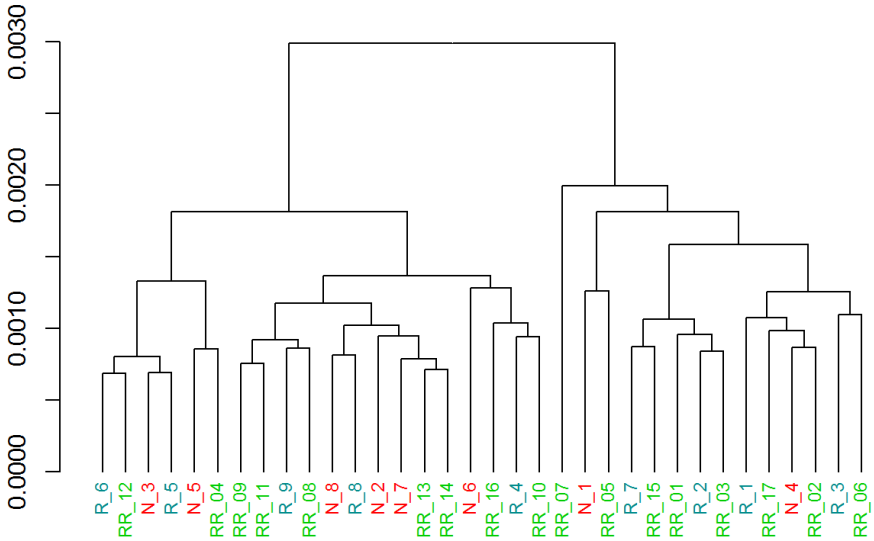


Figure 5.12 Representative FT-IR spectra of western juniper samples according to durability classification established by exposure to *G. trabeum* at wavenumber (A) 4000 – 650 cm^{-1}, and (B) 1800 – 650 cm^{-1}. Green = highly resistant, blue = resistant, red = non-resistant.

(A)



(B)

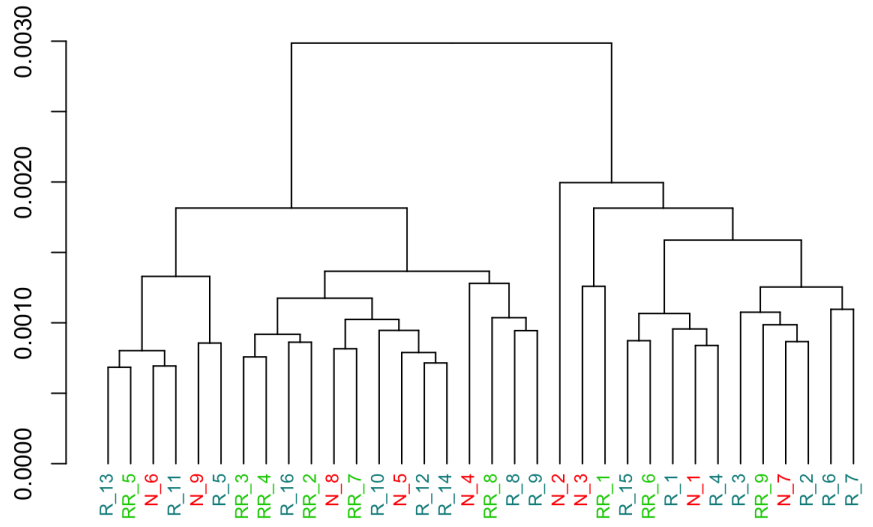


Figure 5.13 Dendrogram plot of hierarchical clustering, with Euclidean distance between western juniper using second derivatives spectra for the 1800-650 cm^{-1} region established by exposure to *G. trabeum* (A), *R. placenta* (B), or *R. flavipes* (C). N = non-resistant, R = resistant, RR = highly resistant.

(C)

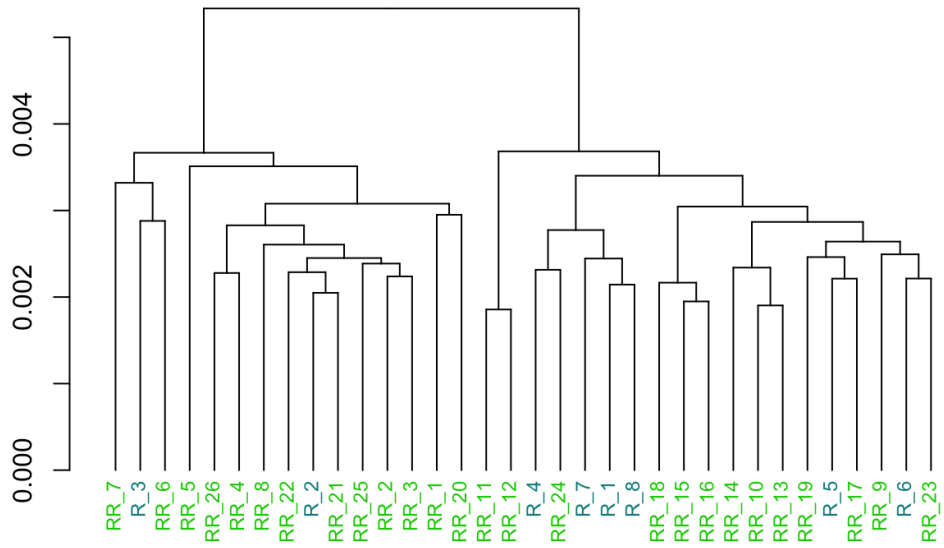


Figure 5.13 (continued) Dendrogram plot of hierarchical clustering, with Euclidean distance between western juniper using second derivatives spectra for the 1800-650 cm^{-1} region established by exposure to *G. trabeum* (A), *R. placenta* (B), or *R. flavipes* (C). N = non-resistant, R = resistant, RR = highly resistant.

Principal component analysis (PCA) was performed on the 1800-650 cm^{-1} region using second derivative spectra to qualitatively discriminate between samples with different durability classifications established by exposure to *G. trabeum*, *R. placenta* or *R. flavipes*. The robust PCA was used as it allowed analysis when outliers were present (Gharibnezhad et al., 2011). Classical PCA is sensitive to outliers and the presence of anomalous data can lead to a first principal component (PC1) that explains the high variance towards the anomalous data (Gharibnezhad et al., 2011). Removal of the outliers can resolve the issues, but a simpler option is using robust PCA that is less

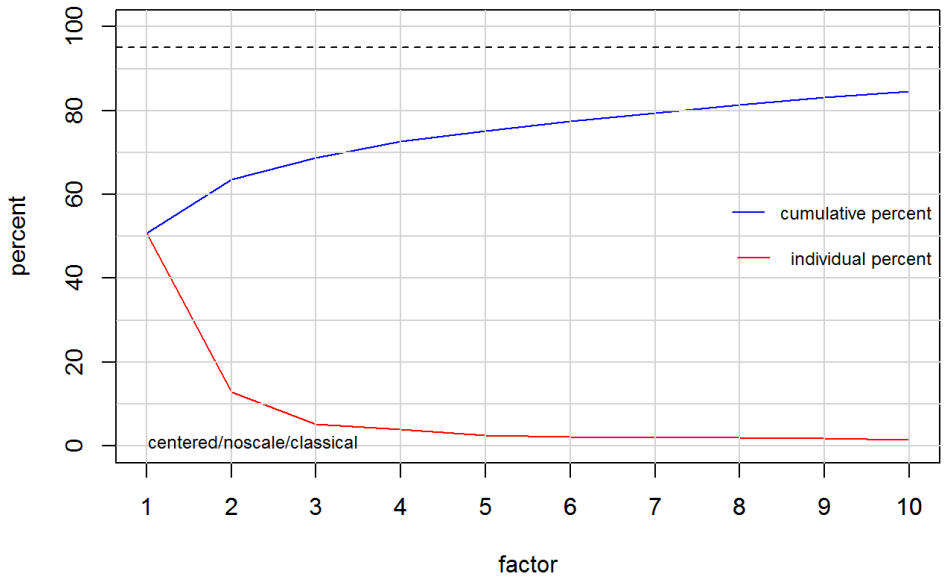
influenced by the outliers. In this analysis, the robust PCA using median absolute deviation was used to measure the spread out of the data instead of using standard deviation (Hanson 2017).

Scree plots were analyzed for spectra on materials exposed to each organism to determine the number of principal components (PC) required to sufficiently explain the variance (Figure 5.14). The number of components was selected by observing the point of the "elbow" in the scree plot (Cattell 1966). The remaining PCs were ignored as the values were relatively small and similar in size. Three PCs were required to categorize durability of western juniper against *G. trabeum* and accounted for 69.1% of variability in the spectral dataset with PC1 representing 51%, PC2 13% and PC3 5.1%. Similarly, the first three PCs were required for resistance against *R. placenta* which explained 74.3% of variability with PC1 representing 42%, PC2 24% and PC3 8.3%. The scree plot for *R. flavipes* resistance required three PCs explaining 74.3% of the variability with PC1 representing 42%, PC2 24% and PC3 8.3%.

Clustering of data using a 3D PCA plots showed overlap between the three different durability classifications to all the tested organisms (Figure 5.15). No obvious groupings were detected using the PCA analysis. Similar results were achieved with the 3D plot using combinations between the first three PCs. These results again illustrate the lack

of separation between the durability classes established by exposure to each organism.

(A)



(B)

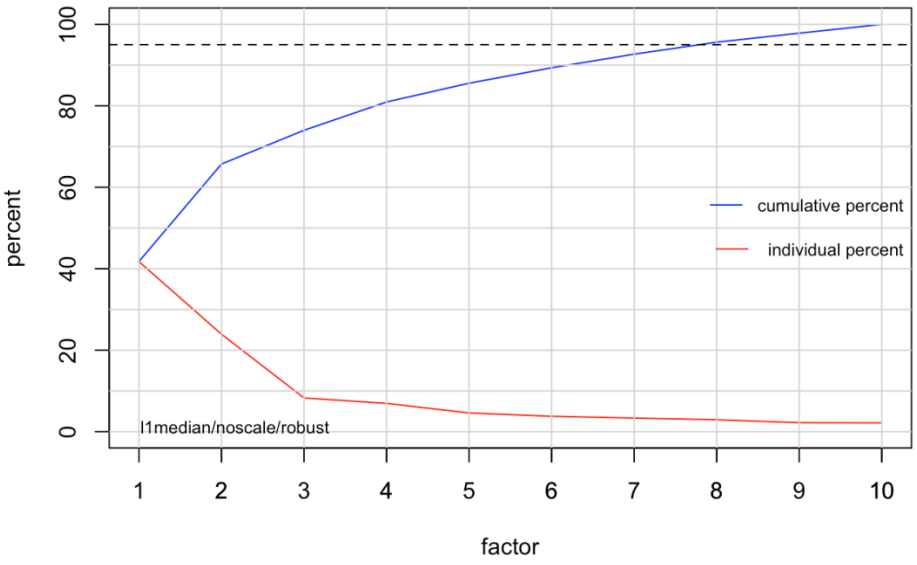


Figure 5.14 Scree plot showing the total variance (%) explained by each principle component (factor) using robust PCA technique for durability established by exposure to (a) *G. trabeum*, (b) *R. placenta* or (c) *R. flavipes*.

(C)

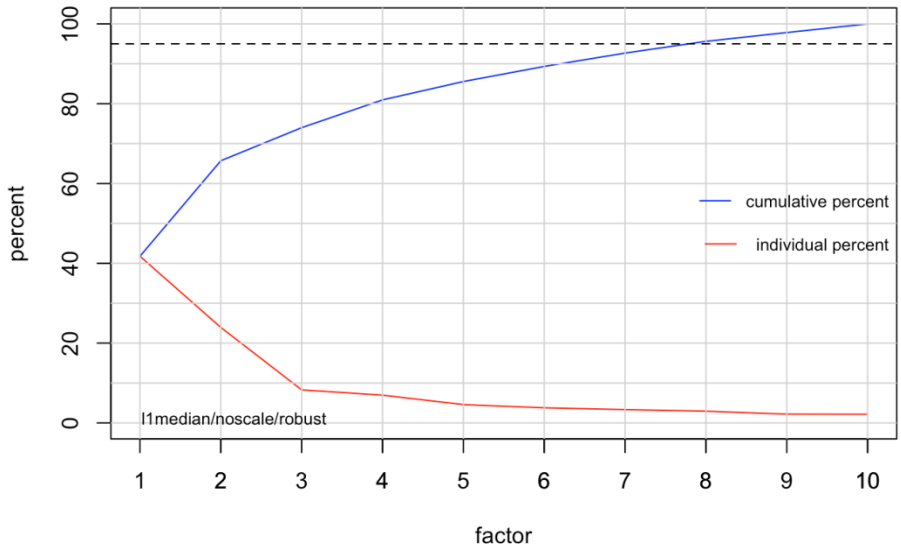
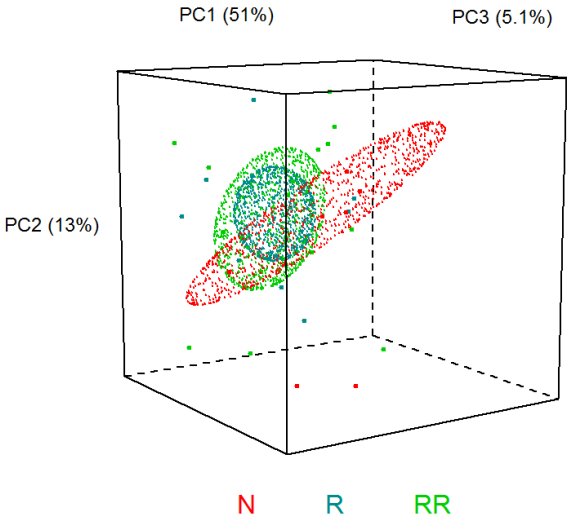


Figure 5.14 (continued) Scree plot showing the total variance (%) explained by each principle component (factor) using robust PCA technique for durability established by exposure to (a) *G. trabeum*, (b) *R. placenta* or (c) *R. flavipes*.

(A)



(B)

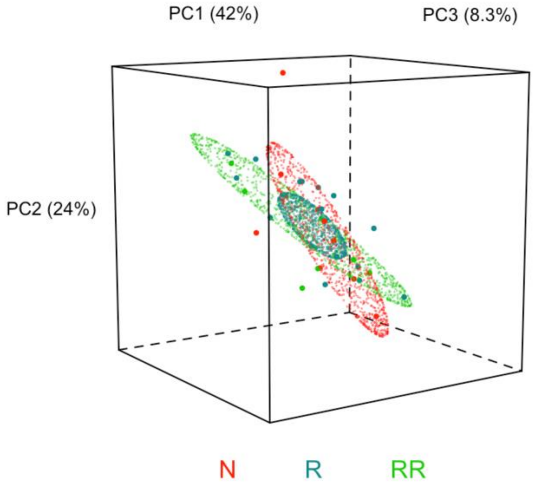


Figure 5.15 PCA analysis showing 3D representations of the first three principal components (PCs) of spectra using second derivatives for durability classification established by exposure to (a) *G. trabeum*, or (b) *R. placenta*. N = non-resistant, R = resistant, RR = highly resistant.

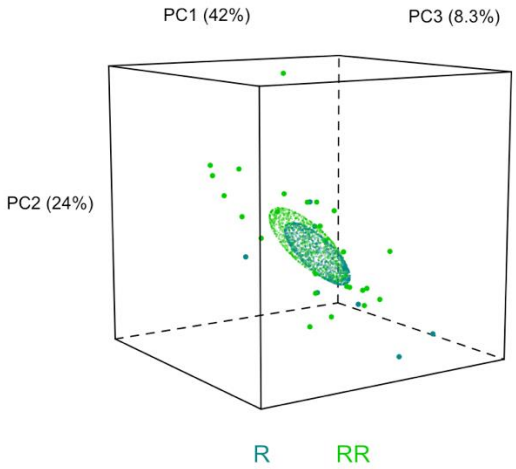


Figure 5.16 PCA analysis showing 3D representations of the first three principal components (PCs) of spectra using second derivatives for durability classification established by exposure to *R. flavipes*. R = resistant, RR = highly resistant.

The analyses clearly showed that extractives content was poorly correlated to durability classifications. There are a number of possible reasons for the separation failure between durability classes:

1. Factors other than extractives that may contribute to the wood durability e.g. synergism between several groups of extractives or other wood compounds.
2. Preprocessing technique – require more detailed modifications on spectra prior to analysis.
3. Lack of representatives from non-resistant and moderately resistant groups. As a result, data were biased towards the more resistant groups.

4. Aggressiveness of test organisms used in the study. While ponderosa pines control samples using suggested conditions were suitable for decay and termite feeding, the organisms used in current study (*G. trabeum*, *R. placenta*) may not be appropriate for the test. Other test organisms might produce better results (more variability in durability).
5. The extraction test method clearly showed extractives distribution along the radius, although values might be lower than those obtained using conventional soxhlet extractions.

5.4. Conclusions

Variations between and within wood in resistance of western juniper to brown-rot fungi and termites were observed in this study. The complexities of the results illustrate the variability in durability with regard to radial distribution within trees and resistance to specific test organisms. Moderate to weak relationships were found between extractives content and durability indicating that other factors might contribute to durability, including synergism between different extractives or the presence of antioxidants. The results might be improved by removing interfering peaks (e.g. H_2O and CO_2 peak), and excluding uninformative regions from the spectra to improve the baseline. For example, the 2500 – 1800 cm^{-1} region typically only contain

the adsorbed CO_2 (C=C and C=O) from the surrounding and is unrelated to the tested sample constituents. Ignoring these spectra components might improve relationship between spectra intensities with durability classification.

Future studies should also examine the extracted samples to determine if they are less durable than non-extracted samples. Many studies have shown that extractives-free wood has lower resistance to biodegradation. Specific extractive compounds toxic and / or repellant to fungi and insects. On the other hand, no differences were observed in durability of heartwood and sapwood, especially among highly durable species. This indicates that reasons other than extractives that might contribute to high durability. These factors should be further explored using infrared spectroscopy or other non-destructive techniques. Despite the lack of predictive ability, infrared spectroscopy could still be applied to observe the chemical compounds that are important to wood durability. This study showed that interpreting infrared spectra is complicated due to the complex nature of individual wood components (cellulose, hemicellulose, lignin, and extractives). In addition, discerning extractives from other major wood components, including those contributing to wood durability, is difficult as two or more components may contribute to the same absorption band.

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Chapter 6. Conclusions, Limitations and Future Work

6.1. Conclusions

The present study investigated the possibility of using infrared spectroscopy - specifically the ATR-FTIR and NIR - in combination with PCA and HCA as a rapid, non-destructive technique for sorting wood durability. The two wood species selected for this study, Alaska yellow cedar and western juniper, are chosen due to their high resistance to biodegradation, mainly due to the biocidal properties of their extractive compounds.

The process for classifying wood durability is time-consuming, laborious, destructive, and may not be representative for the whole durability of the population. Extractives, which play major roles in wood protection against biodegradation, can be a better predictor for their durability. Extractives occur at low concentrations in wood, proving the compounds existence in heterogenous materials (like wood) challenging.

Increased utilization of young-growth trees with generally inferior quality and resistance against biodegradation organisms, requires a rapid, non-destructive method that is able to sort wood in large numbers according to their durability. ATR-FTIR and NIR is a possible solution, as portable models are currently in use for other field tests. Infrared spectroscopy used in the present study showed sensitivity for detecting a biocide compound (carvacrol) as low as 3.5% in wood. But this technique was not

successful to identify extractives in Alaska yellow cedar and western juniper. It might be more effective on species with high extractives content, such as tropical hardwood species.

Alaska yellow cedar and western juniper showed high durability and low correlations with their extractives content, making it difficult to obtain a prediction model based on the extractives. Alternatively, looking at other non-extractives compounds, such as the lignin content, might improve the prediction of wood durability.

Choosing the appropriate pre-processing and chemometrics analysis for the spectral data also proved to be challenging. Pre-processing prior to analysis helped reduce background noise and improve model fit. Selection of software and statistical analysis for big data was crucial in ensuring all the data variations were measured and identified. The study used second derivatives as the pre-processing technique, and although this showed some improvement in differentiating the spectral differences, it did not help improving the chemometrics analysis. Additionally, principal component analysis (PCA) and hierarchal cluster analysis (HCA) performed on the spectral data were able to differentiate carvacrol with varying concentrations, but not the differences in durability classifications in Alaska yellow cedar and western juniper. The low predictions might be due to the low correlations between extractives and durability. Furthermore, variability in durability was also low, with majority of the

Alaska yellow cedar and western juniper samples consist of highly resistant and resistant to decay fungi or termites attack. This can also influence the predictive ability for accurately classifying wood durability in Alaska yellow cedar and western juniper.

6.2. Limitations and future work

Wood is a heterogeneous, orthotropic material and can vary greatly in their anatomical, chemical and physical attributes between matched samples. Although, these characteristics are what make wood an interesting material to work with, they are also a major drawback when performing a durability study. Variations in resistance to decay fungi can be seen within matched samples used in this study. These might come from several factors, including the presence of included sapwood that are more susceptible to degradation. Such variations need to be addressed and noted as one of the possible factors that might affect spectral analysis.

The other half of this study focused on the application of ATR-FTIR and NIR spectrometry. For the success of this section, several precautions had to be taken to ensure the readings were valid and representative of the samples. FT-IR is sensitive to moisture, and wood varies in moisture content depending upon its environment. It is important to ensure dry wood is used and to take precautions to keep the moisture content constant throughout the experiments.

Another disadvantage observed during the experimental stage was the use of wood powders. This ensured homogeneity on the tested materials, but might not be practical for industrial or field applications. For the practical use of this assessment method, standardized techniques are needed that can be reproduced and are comparable by using solid wood samples instead of wood powders. A reference spectral library that is indicative of the important peaks and their absorbance level for different durability classes also need to be developed. For this, species with more variability in durability, and a moderate to high correlation with their extractive contents, will be more useful to allow the distinction among the limit and range of each durability classes.

Portable FT-IR and NIR spectrometers are available, and there are possibilities of using this growing technology for field applications and installation at the mills for in-situ, non-destructive and rapid assessment of wood durability. However, the application of these portable systems require a lot of work and the sensitivity of these miniature equipment for accurately detecting extractives at different concentrations are yet to be explored.

Future work should emphasize on using wood with high extractives content, such as tropical species. Wood in the tropics are adapted to the attack and degradation of many aggressive decay organisms. Thus wood species have adapted by producing high

quantities of extractives and other chemicals (as protective mechanisms) that contribute to durability. Focus will be on plantations and less-utilized species that are slowly dominating the tropical timber markets. Planted species, such as teak, showed decrease in durability as a result of reduced heartwood extractives produced by the younger trees. The ability to sort lumber based on their durability classification may help in proper utilization and applications suitable to their service life. Whilst also sustainable and financially feasible, as it reduces the possibility of replacing the wood due to early failure on wood section that is susceptible to degradations.

Durability testing based on field applications should also be explored in addition to the laboratory testing. Despite the long duration, destructive and laborious work required for field tests, it is crucial to established durability based on actual applications. Durability data of both field and laboratory (as used in present study) using matched samples might be more practical in predicting wood durability. Once a baseline is established along with their spectral information, it can be used as reference for future studies based on the application of infrared spectroscopy for the classification of wood durability.

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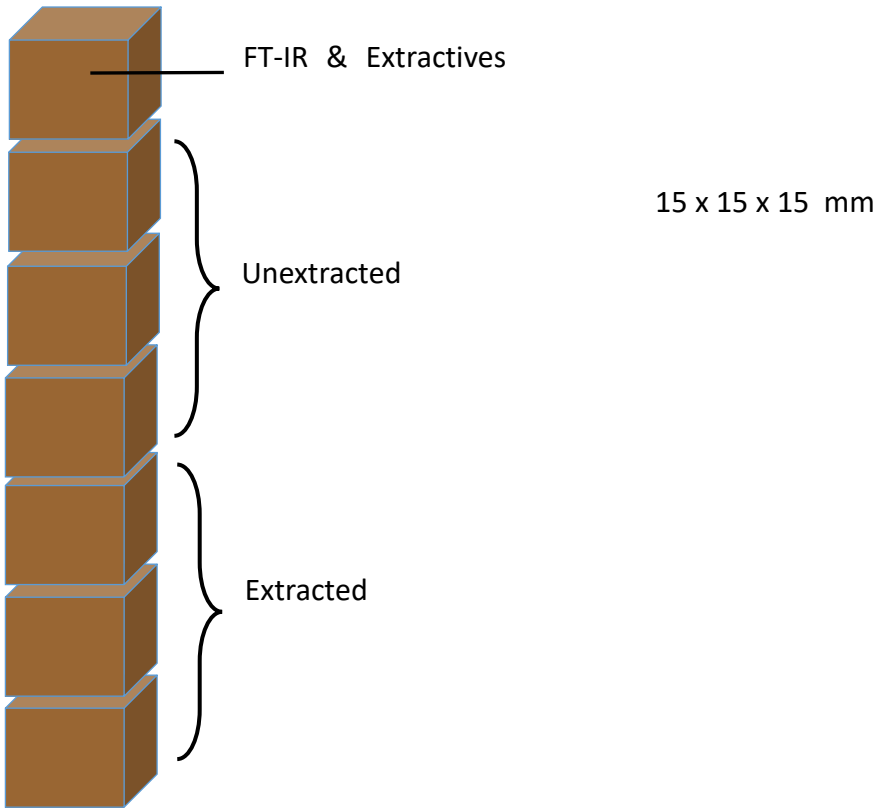
Appendix

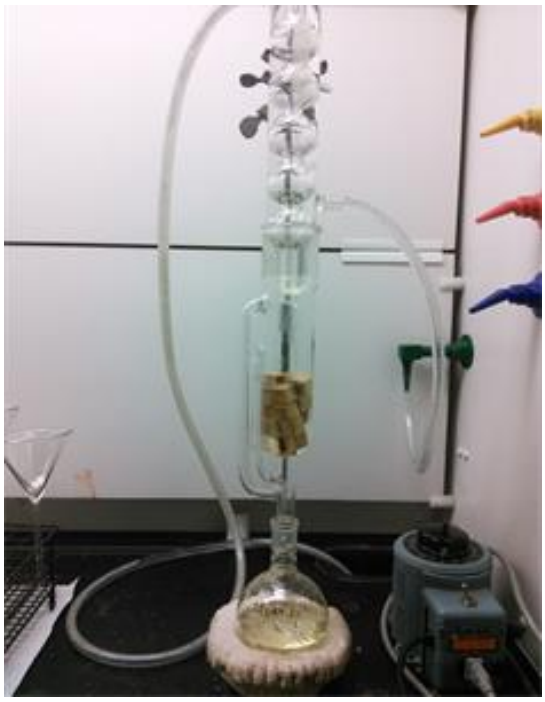
Appendix A

Appendix 1. Alaska yellow cedar boards and sample preparations

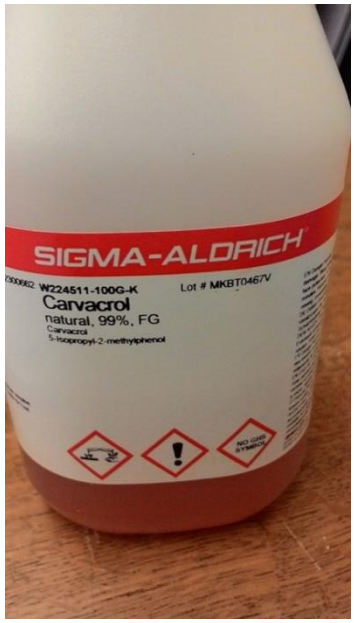
Sample cutting for Alaska yellow cedar treated with carvacrol



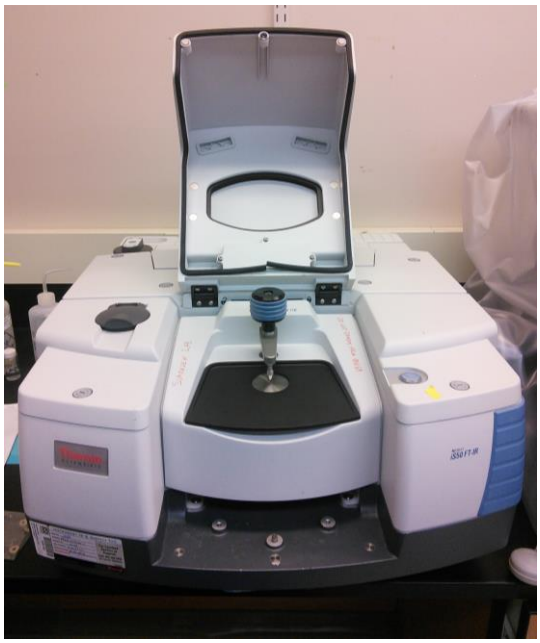
Appendix 2. Soxhlet extraction of wood blocks



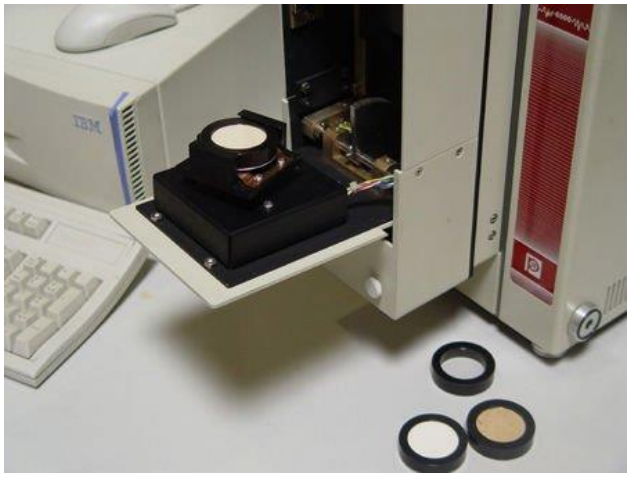
Carvacrol treatment



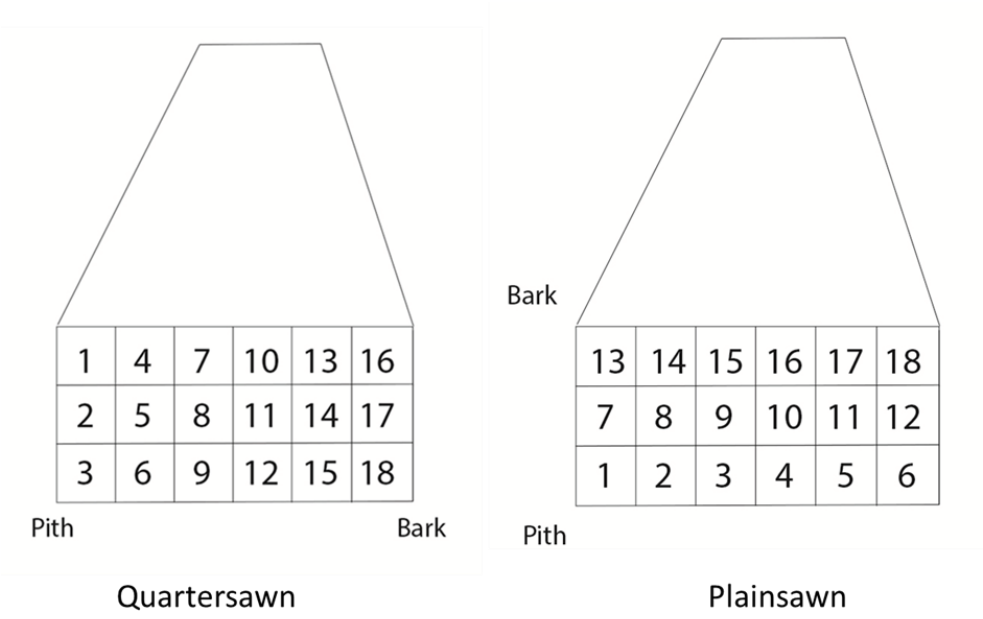
Appendix 3. FT-IR spectrometer equipped with ATR

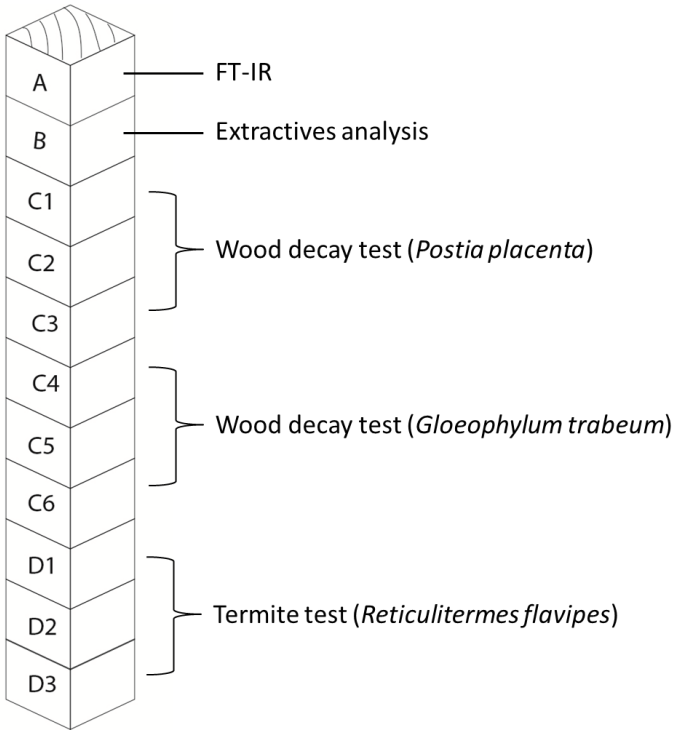


NIR with ring cups and insert



Appendix 4. Diagram of the Alaska yellow cedar sample preparation and cutting

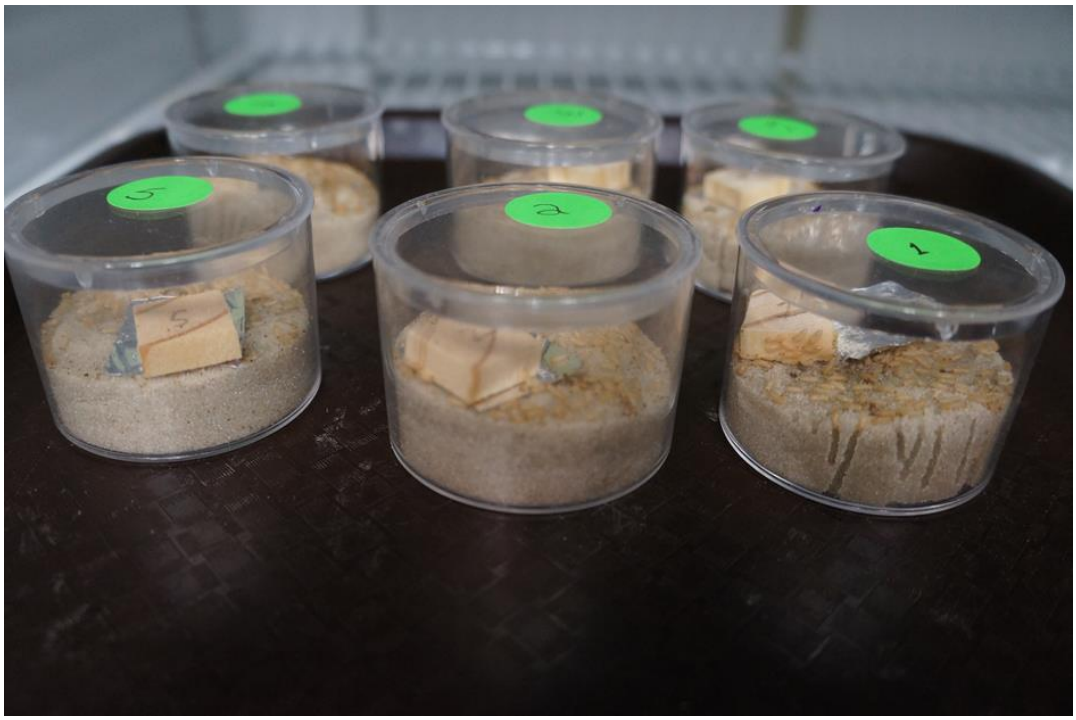




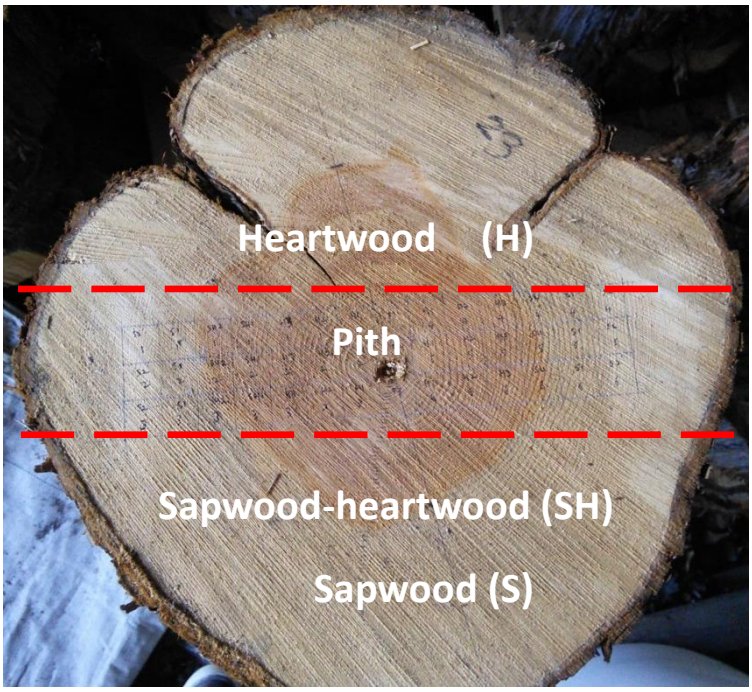
Wood decay fungi test based on AWPA Standard E10 (AWPA, 2017)

Appendix 5. Durability test using brown-rot fungi and eastern subterranean termites

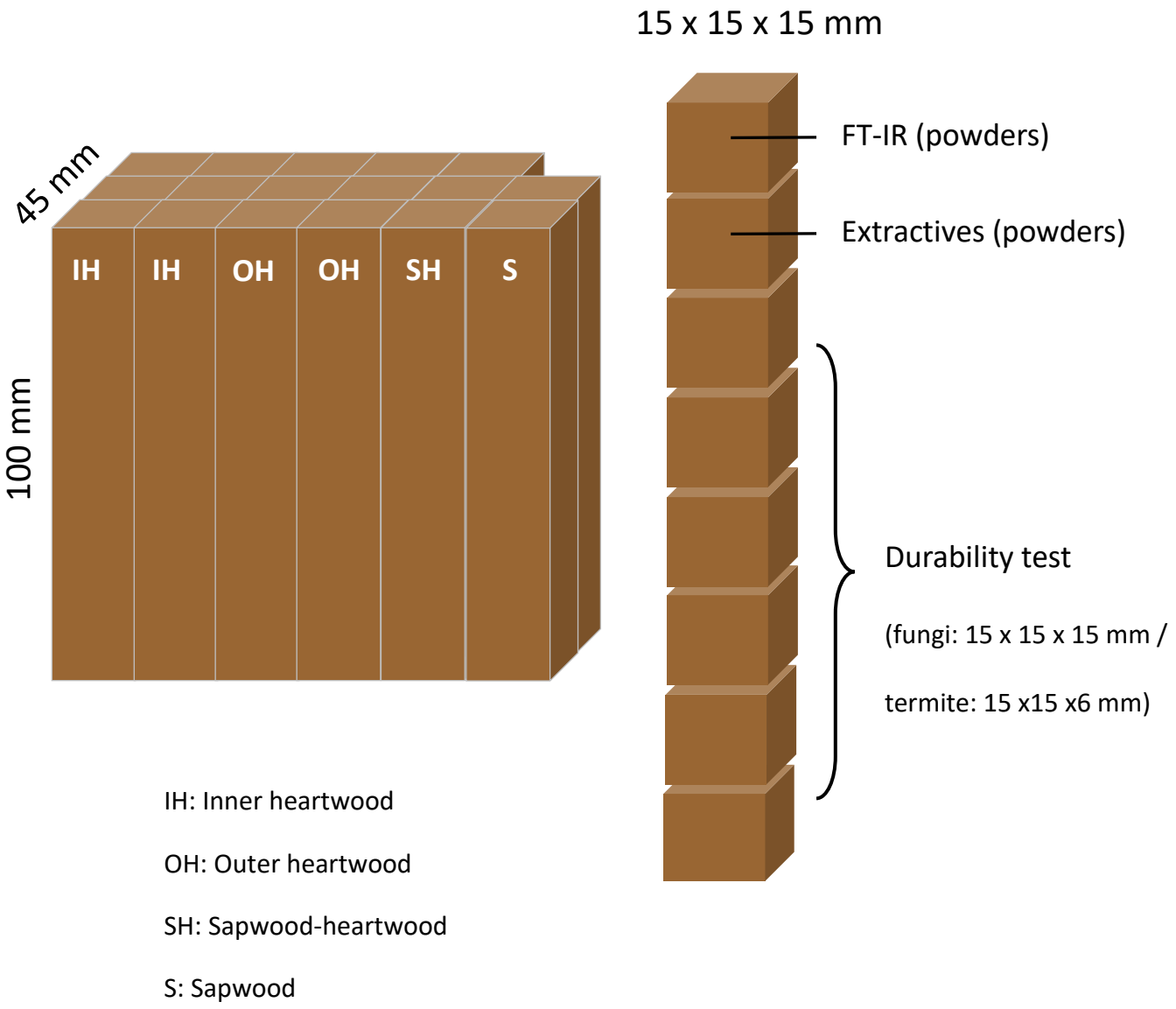
Termite test based on AWPA Standard E1 (AWPA, 2016)



Appendix 6. Western juniper disks used in this study



Sample cutting for western juniper



IH: Inner heartwood

OH: Outer heartwood

SH: Sapwood-heartwood

S: Sapwood