Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Extractives of Naturally Durable Wood

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ABSTRACT

A preliminary study to evaluate naturally durable wood species in an above ground field trial using Gas Chromatography-Mass Spectrometry (GC-MS) detected differences in fatty acid extractives between species and within the same species over time. Fatty acids were extracted with chloroform: methanol mixture then methylated with sodium methoxide and fractionated using solid phase extraction columns. Fatty acids were identified, quantified with known standards, and compared between wood species. Future analysis of exposed specimens will determine longevity of extractives and correlate changes to the initial extractive content.

INTRODUCTION

Naturally durable wood species have been documented and in use since the early 1800's. The main classes of chemical compounds implicated in the durability are typically terpenoids, alkaloids, stilbenes, and tannins. However, these vary greatly between wood species and are often absent in species that still exhibit degrees of decay resistance (1). A greater understanding of all of the facets of natural durability is needed before implementation of technologies based on naturally durable wood. Fatty acids are often overlooked as components of naturally durable wood, but become apparent when performing chemical analyses of extractives. Fatty acids have been determined to possess varying levels of antibacterial and antifungal properties. Kabara, et al. (2) evaluated 30 straight chain fatty acids and their derivatives against 8 gram positive and 12 gram negative bacteria and found that lauric acid (c10) showed the highest degree of antimicrobial activity. Several *in vitro* studies have found fatty acids to be effective against fungi. Bergsson, et al. (3) found that capric (c6) was effective at killing 3 strains of *Candida albicans*, and that lauric acid (c10) was the most effective at lower concentrations and after a longer incubation time. Walters, et al. (4), tested linoleic, linolenic, erucic, and oleic acids against 4 plant pathogenic fungi and found significant reductions in fungal biomass by all the fatty acids in liquid culture; linoleic and linolenic yielded the highest reductions in fungal growth on solid media. Coleman, et al. (5) has shown activity of C6-C10 fatty acid formulations against plant pathogens, sapstain fungi, molds, and wood decay fungi. The objective of this study is to observe changes in fatty acid composition of "naturally durable wood" species over time in above ground field trials.

METHODS

Preparation and analysis of reference materials

The methylated fatty acids were identified using a Restek Food Industry FAME mix (cat#35077). A chromatogram of 37 components of the test mix is presented in Figure 1. In order to minimize co-eluting peaks and better resolve peaks on our stationary phase column, the standards were fractionated through a Discovery Ag-Ion SPE column (Supelco cat#54255-U) which separated individual FAMES by their degree of unsaturation. The separated standards were used to create 3 method files with calibration curves.

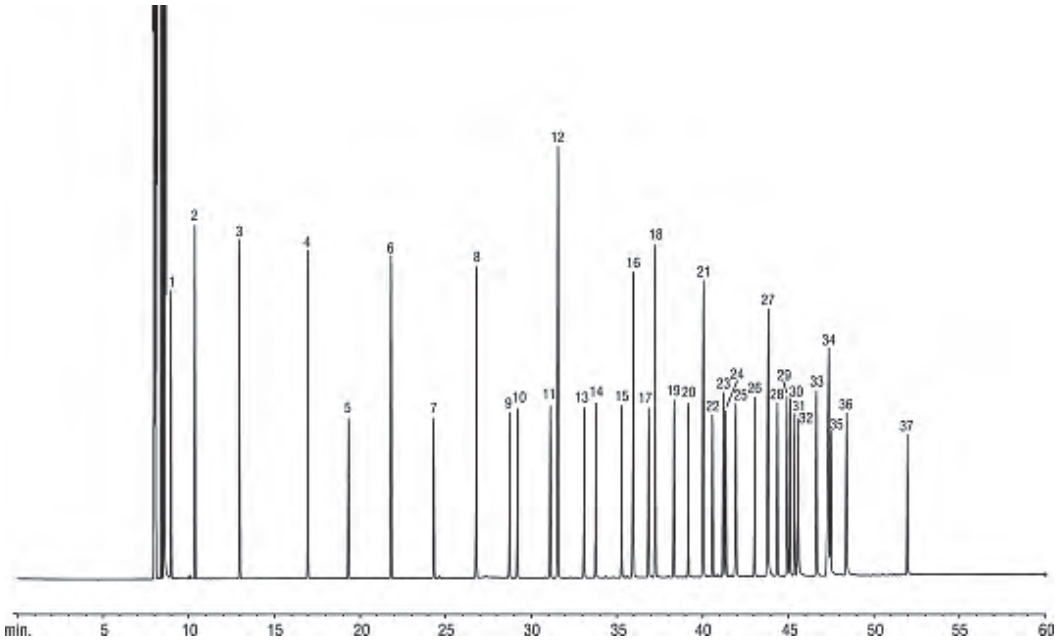

Figure 1: Chromatogram of 37 compounds of Restek Food Industry FAME Mix on Rtx 2560 (polar phase) column, note this research is currently using a Rtx 5 HT (stationary phase column), hence the need to fractionate the standards for better resolution of co-eluting peaks.

Preparation of wood samples for FAME analysis

Two grams of sawdust from each of 8 naturally durable wood species and a non-durable species (southern yellow pine) as a negative control were extracted in a soxhlet extractor at a rate of 6 fluxes/hour in 150 ml (2:1) chloroform: methanol for 6 hours. Extractives were concentrated on a rotary evaporator. Concentrated extractives in 1 ml of toluene were mixed with 3 ml of methanolic sodium methoxide to derivatize the fatty acids into methyl esters. The organic upper layer was concentrated in a rotary evaporator and re-suspended in hexane. The hexane/FAME mixture was eluted on an Ag-Ion (Supelco, Saint Louis, MO) solid phase extraction column with acetone and hexane following manufacturer's specifications. The collected fractions were aliquoted into screw top autosampler vials.

GC-MS Analysis

Fractions from standards and samples were analyzed on a Shimadzu QP-2010A equipped with an OAC-1a autosampler. The GC was fitted with a RX1-5HT column (30m (x) 0.25mmID (x) 0.25uM df) and temperature was initially 60°C and ramped 10°C/min to a final temp of 225°C and hold for 10 min at 225°C. Helium was used as the carrier gas at a flow rate of 1ml/min. The Mass Spectrometer was set to record ranges of spectra from 35 to 400 m/v at a scan speed of 6000 scans/sec.

RESULTS AND DISCUSSION

Eleven fatty acids in the 9 wood samples were identified and quantified using the standard curves. Eastern Red Cedar had the highest amount of total fatty acids as well as the highest diversity of fatty acids (7). Palmitic acid occurred in all of the wood species. Fatty acids that only occurred in a single species included lauric acid (only in ERC), lignoceric (only in AYC), methyl myristoleate (only in ERC), and oleic (only in SYP). A listing of fatty acids detected and their patterns of occurrence are presented in Table 1. Corresponding to the table 1, general proportions of the fatty acids that were identified using the FAME standard is shown in Figure 2.

	PAW	SYP	CAT	HM	BL	WCJ	AYC	WRC	ERC
Lauric acid									+
Myrisitic acid	+	+		+				+	+
Pentadecanoic acid	+	+		+					
Palmitic Acid	+	+	+	+	+	+	+	+	+
Margaric acid	+	+		+				+	+
Stearic acid	+	+	+	+	+		+	+	+
Behenic acid					+		+		
Lignoceric acid							+		
Methyl myristoleate									+
Oleic acid		+							
Linoleic acid	+	+		+	+			+	+

Table 1: Fatty acids detected in candidate naturally durable species and their patterns of occurrence. PAW=Paulownia, SYP=Southern Yellow Pine, CAT=Catalpa, HM=Honey Mesquite, BL=Black Locust, WCJ=West Coast Juniper, AYC=Alaskan Yellow Cedar, WRC=Western Red Cedar, ERC=Eastern Red Cedar.

The baseline concentrations obtained in this study will be used to track the progressive changes in fatty acid presence and concentration in the field specimens. Without a thorough understanding of the variability of fatty acids in the different wood species, it is difficult to make assumptions on their roles. Future samplings will be taken yearly from the exposed above ground specimens and the degradation of fatty acids will be tracked and correlated to the field performance. In addition to the fatty acid analysis, separate analyses of terpenoids and alkaloids will be conducted from field samples from both WI and MS test sites. This study will be sampled annually and is scheduled for completion in 2013.

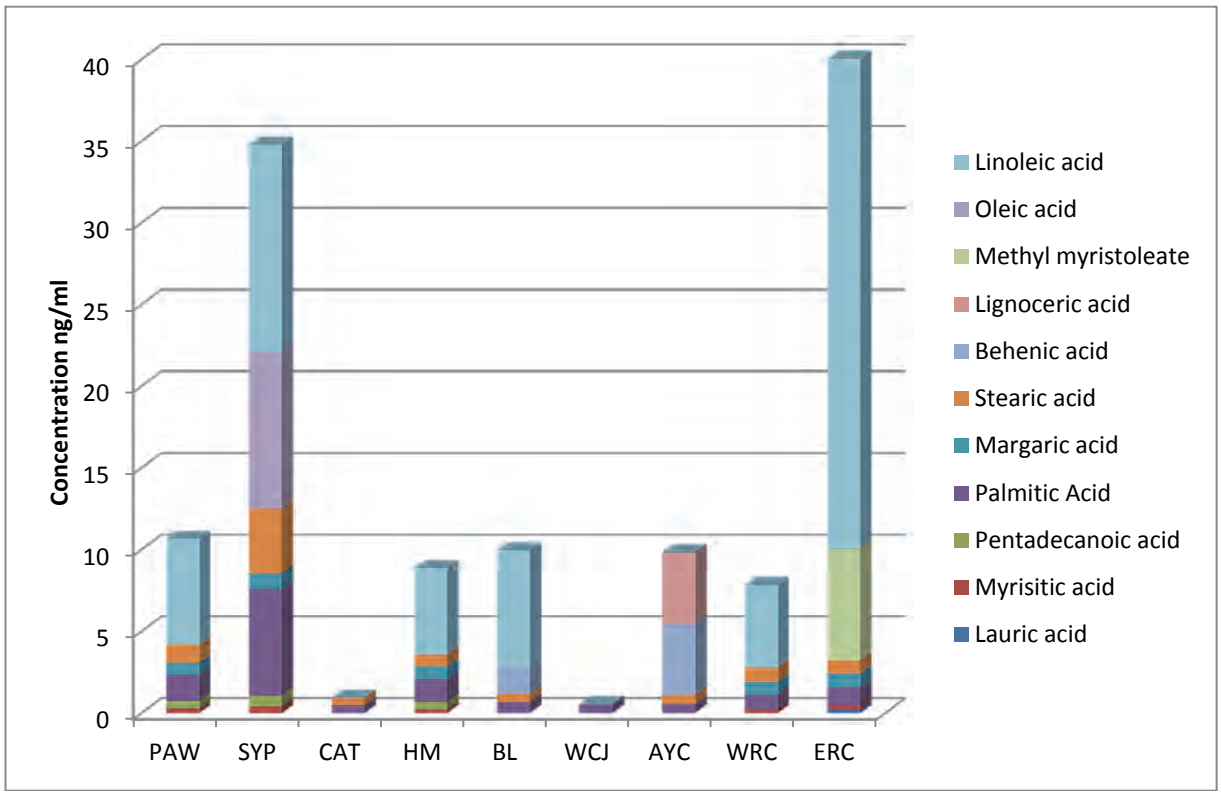

Figure 2: Proportions of identified fatty acids in each of the individual wood species. PAW=Paulownia, SYP=Southern Yellow Pine, CAT=Catalpa, HM=Honey Mesquite, BL=Black Locust, WCJ=West Coast Juniper, AYC=Alaskan Yellow Cedar, WRC=Western Red Cedar, ERC=Eastern Red Cedar.

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