To the Graduate Council:

I am submitting herewith a thesis written by John Powers Wolff IV entitled "Determination of Virgin Wood Remaining in a Cask after Maturation and Predictive Modeling for Spirit Specific Compounds." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemical Engineering.

Nicole Labbé, Major Professor

We have read this thesis
And recommend its acceptance:

Simioan Petrovan

Timothy Rials

David Harper

Accepted for the Council:

Carolyn R. Hodges

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Determination of Virgin Wood Remaining in a Cask after Maturation and Predictive Modeling for Spirit Specific Compounds

A Thesis presented for the Master of Science Degree

The University of Tennessee, Knoxville

John Powers Wolff, IV

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Abstract

The stipulations from the government of the United States require whisky distillers to use a new, charred oak barrel for maturation of the spirit. The amount of money spent on new oak accounts for almost 60% of the price for the finished spirit. Determining the amount of virgin wood, wood not affected by the maturation process, which remains in the stave, could reduce the costs for the production of certain spirits.

Thermal treatment to the wood before maturation creates some of the compounds attributing to the flavor and color of the spirit. Utilizing pressure, temperature, and humidity differences from season to season allows the distiller to create a spirit that reflects the standards of the final product. However, these differences also cause to increase the variability observed in the samples

Multiple methods were tested to determine the amount of virgin wood remaining in the stave. Analysis was performed on the surface of the stave using near infrared (NIR) spectroscopy and on the extract with high performance liquid chromatography (HPLC) and multivariate analysis (MVA). The NIR approah did not yield an amount of virgin wood, but instead showed the drastic modification of the stave from charring and charring process.

A univariate and multivariate approach were used on the HPLC extract. Analysis using nonparametric tests coupled with a univariate approach, a consistent amount of virgin wood could not be determined.

A multivariate approach was implored to find the limit of virgin wood remaining in the stave. By analysis of the HPLC chromatograms using principal component

analysis coupled with the Wilcoxon Rank-Sum test, the amount of virgin wood remaining after maturation could be calculated.

Another issue facing spirit distillers is the natural variation of compounds specific to the spirit in the wood. Determining the amount of "spirit specific" compounds naturally present in the wood and available for maturation will allow for distillers to engineer casks to certain standards. Analysis of near infrared spectra obtained on the milled staves before extraction and correlation with quantified results from the HPLC chromatograms using a partial least squares regression (PLSR), predictive models were generated for certain spirit specific compounds.

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1. Justification and Objectives

In the United States, distillers of whisky are required by law to use a new charred barrel, or cask, for maturation of the spirit (27 C.F.R sec 5.22(b)(1)(i)). The cask costs make up a significant share of the total costs for the production of whisky. About 60% of the manufacturing costs are allotted to the cask. The intensified demand for casks in the whisky industry is additionally bringing cask prices in an upward motion (Doussot 2000). In addition to the demand for casks, with the new trend relating to bio-energy there will be a global demand for hardwoods, including oaks. Without the option to reuse these barrels, the cost of maturing the spirit becomes significant.

For distillers abiding by these laws, the ability to determine if there is any virgin wood, wood not affected by the maturation process, remaining after maturation of the distillate is of great importance. Wood remaining unaffected could be used in a second generation cask. However, with the variability naturally present in wood (Doussot 2002), coupled with the variability of the coopering of casks, there has not been an effective technique in determining this amount.

The use of spectroscopic techniques, such as near infrared spectroscopy (NIR) and high performance liquid chromatography (HPLC), coupled with multivariate analysis (MVA), can give a limit on the amount of virgin wood remaining in the cask after maturation. Analysis of this issue facing distillers can also lead to solutions for other aspects of the spirit production business.

The production of spirits matured in oak casks is a large business in the United States, with sales in the billions of dollars. Most of the flavor of the maturing spirit is due to changes in the composition and concentration of compounds extracted from the oak

casks. These compounds affect the flavor, aroma and color of the distilled spirit. Many of these compounds derive from the thermal degradation of wood macromolecules, such as cellulose, hemicelluloses, and lignin, in the wood during the fabrication of the cask and from other molecules that are naturally present.

During the production of the cask, the wood is thermally treated at different steps in order to modify the cask to a specific type. Toasting and charring steps are applied to casks used in the manufacturing of spirits, including bourbon whisky. Determining the amount of sensory compounds that are present in the wood will allow distillers to engineer their barrels in accordance with final product specifications. This can be accomplished by coupling NIR spectra and quantified HPLC results by partial least squares regression (PLSR) to create a predictive model for the concentrations of certain compounds. The models would allow distillers to determine the amount of these compounds present in the staves. A benefit of this knowledge could be to group similar staves together for more intense properties.

2. Introduction

A. Wood Chemistry

Trees belong to the family of seed-bearing plants, Spermatophytae, which are subdivided into the genera Gymnospermae and Angiospermae (Sjöström 1993). Hardwoods, which include oaks, belong to the angiosperm genus. The chemistry and orientation of fibers and cells in trees are substantially different between hardwoods and softwoods. This variation is also observed inter- and intra-species in hardwoods (Masson et al. 1995, Mosedale et al. 1996, Doussot et al. 2002).

Hardwoods contain several different cell types, specialized for specific functions including water transport and structure support. The xylem or wood is organized in concentric growth rings and contains rays in horizontal files. The rays can extend from the outer bark to either the pith (primary rays) or to an annual ring (secondary rays) and consist almost exclusively of parenchyma cells (Sjöström 1993). At a certain age, the inner wood of the stem of most trees begins to change to dead heartwood. The dying parenchyma cells produce organic deposits such as resin, phenolic substances, and pigments (Core et al. 1979). In some hardwoods, including Q. Alba, the vessels are closed by tyloses (Figure 1), which enter the vessel from neighboring ray cells. The tyloses cause the wood to be impermeable to liquids and thus, an excellent material for the manufacturing of barrels (Sjöström, 1993).

Wood cells consist mainly of cellulose, hemicelluloses, and lignin (Sjöström 1993). The composition of the cell in relation to these macromolecules depends on the species being investigated (Table 1). The main constituent of hardwood is cellulose, which accounts

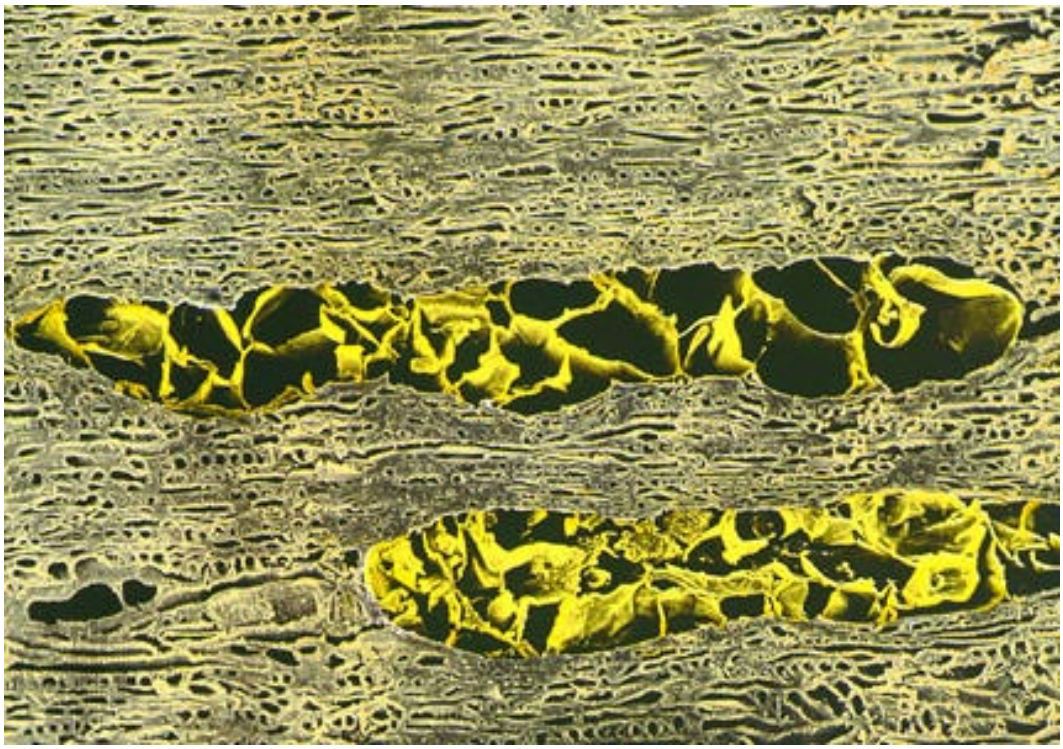


Figure 1: Tyloses blocking large vessel members, shown in a false-colour scanning electron micrograph of a section of oak heartwood
http://www.britannica.com/eb/art-55374

Table 1: Approximate composition of American and European oaks

Species	% Cellulose	% hemicelluloses	% lignin	% extractives	% ash	Reference
Q. robur[a]	39-42	19-26	25-34	3.8-6.1	0.3	(Wagenführ, 1980)
Q. petraea[b]	22-50	17-30	17-30	10-Feb	-	(Puech, 1978)
Q. alba[c]	44	24	24	5.4	1	(Petterson, 1984)
Q. alba[d]	42	28	25	5.3	0.2	(Petterson, 1984)

a – English oak or Limousin oak
b – Sessile oak
c – American white oak from swampy land in Georgia
d – American white oak from dry uplands in Tennessee

for approximately 40-45% of the dry substance of wood and is mainly in the secondary cell wall (Takeshi et al. 2005).

The smallest element of the cellulose skeleton is considered to be an elementary fibril consisting of 36 parallel cellulose molecules which are held together by hydrogen bonds. A cellulose molecule is a homopolysaccharide composed of ß-D-glucopyranose units which are linked together by (1? 4)-glycosidic bonds. They are linear and have a strong tendency to form intra- and intermolecular hydrogen bonds (Sjöström 1993).

Hemicelluloses and lignin compose a majority of the remainder of the xylem. Hemicelluloses in various hardwood species differ quantitatively, but have a common major component, O-acetyl-4-O-methylglucurono-ß-D-xylan. This compound is commonly referred to as glucuronoxylan, with most xylose-based hemicelluloses termed xylans (Sjöström 1993). The chemistry of lignin is not completely understood, with questions to the specific structural features located in various morphological regions of the woody xylem. The known structure suggests that lignin is a polymer of the phenylpropanoids *p*-hydroxyphenyl, guaiacyl, and syringyl structural units (Sjöström 1993). The concentration of each with respect to the composition of lignin varies from species to species.

Natural extractives and ash compose the remaining composition for wood. At a certain age the inner wood of the stem of most trees begin to change to dead heartwood and its proportion of the stem becomes successively larger as the tree grows. The dying parenchyma cells produce organic deposits such as resin, phenolic substances, pigments, etc. (Sjöström 1993).

B. Cask Fabrication for Spirit Maturation

One of the most important parts of the maturation process is the interaction of the distillate with the cask. White oak casks are constructed to influence the maturation process for certain spirits, such as whisky, wine, sherry, etc. (Yoshizawa et al. 1981, Spillman et al. 2004). The casks contain about 33 staves, which are boards of wood that have been seasoned to modify the chemical composition of the staves. After seasoning, the boards must be dried to fewer than 10% rest moisture. The boards are then cut so that none of the radial vessels of the wood penetrate the cask wall and the annual rings stand vertically. If the vessels are exposed, too much alcohol can evaporate or the cask will begin to leak. Different types of cuts are available to the cooper (star, rift, mirror) which can accomplish this, but the yield of these cuts is smaller, resulting in a more expensive cask than with using normal lumber (Mosedale et al. 1998, Twede 2005).

After the staves have been cut to the right specifications, they are placed into a hoop. The hoop is a metal ring which surrounds the cask and helps it to initially hold form. Once the cask has been formed, steam is applied to help the wood bend into shape (Mosedale et al. 1998, Twede 2005). After steaming different stages of thermal treatments are applied to the cask during its fabrication.

The cask is then subjected to a toasting step which typically lasts for 30 minutes at 200°C – 280°C (unpublished results). There are different levels of toasting (light, medium, medium-plus, and heavy) which can be applied to the cask in accordance with the specifications of the spirit being matured (Swan et al. 1996). The toasting stage begins to break down the cellulose and hemicelluloses in the wood which releases furfural and 5-hydroxymethylfurfural (HMF), resulting in a marzipan type aroma (Lee

2001). After toasting, the casks are charred on the interior for 3 to 5 minutes. Charring the interior of the barrel creates a natural filter for the maturing spirit, by removing some of the unpleasant and undesired compounds present in the spirit after distillation. The thermal treatment stages do not only help the cask hold its shape, but begins the release of flavorful compounds and the degradation of tannins (Doussot et al. 2002).

C. Effect of Thermal Treatment on Oak Components

During the coopering process, the staves are subjected to multiple thermal treatments. These treatments are aimed at solidifying the structure of the cask and generating compounds that contribute to the flavor, color, and aroma of the spirit. The effect of the thermal treatment varies with the temperature utilized and the time allowed.

Cellulose, hemicelluloses, and lignin degrade at different temperatures and influence the spirit in various ways. Cellulose is initially degraded in the temperature range of 240-350°C. This partial decomposition results in pairs of glucose molecules being released from the wood, leaving cellulose units and cellobiose (Sjöström 1993).

Hemicelluloses begin to break down at around 140°C (Ramiah 1970), with the majority of degradation occurring in the range of 200-260°C (Sjöström 1993). The decomposition causes the initial release of simple sugars; glucose, xylose, mannose, rhamnose, arabinose, and galactose. These sugars then rapidly break down further into "caramelization" products (Ramiah 1970). Toasting yields furfural, HMF, and other sugar condensation products which will result in the caramel color of the spirit (Chatonnet et al. 1989). These compounds also result in a marzipan, sugary and almond, flavor and odor characteristics (Lee et al. 2001).

Lignin degrades at the higher temperature range of the three with initial degradation occurring at 280°C and continuing until 500°C (Nishimura et al. 1983, Sjöström 1993). For oak lignin, hardwood lignin, this treatment forms *guaiacyl* and *syringyl* structures (Sjöström 1993). Coniferaldehyde and vanillin are two of the most influential compounds to spirit maturation from the guaiacyl structures while syringaldehyde and syringic acid represent the syringyl structures studied (Figure 2).

Thermal treatment to the wood before maturation of the spirit also affects the taste by decreasing the amount of oak tannins, ellagitannins, naturally present in the staves. These ellagitannins are originally formed when glucose combines with ellagic acid and gallic acid to result in bitter and astringent compounds (Vivas et al. 2004). Seasoning, toasting, and charring break down the tannins and render them more palatable in the finished spirit.

D. Chemical Mechanisms in Whisky Maturation

Over the past seventy years there has been much research performed on the chemistry that occurs during the maturation process. Studies have demonstrated that color, acids, esters, furfural, solids, and tannins all increase during the aging process (Liebmann et al. 1943, Liebmann et al. 1945, Valaer et al. 1936). The distillate not only extracts compounds from the interior face of the cask, but also interacts with the cask on a chemical basis.

During the toasting and charring stage, the cellulose, hemicelluloses, and lignin are all degraded. Aromatic aldehydes are formed during the breakdown of lignin in the wood (Spillman 2004). Examples are aromatic aldehydes studied in this method include

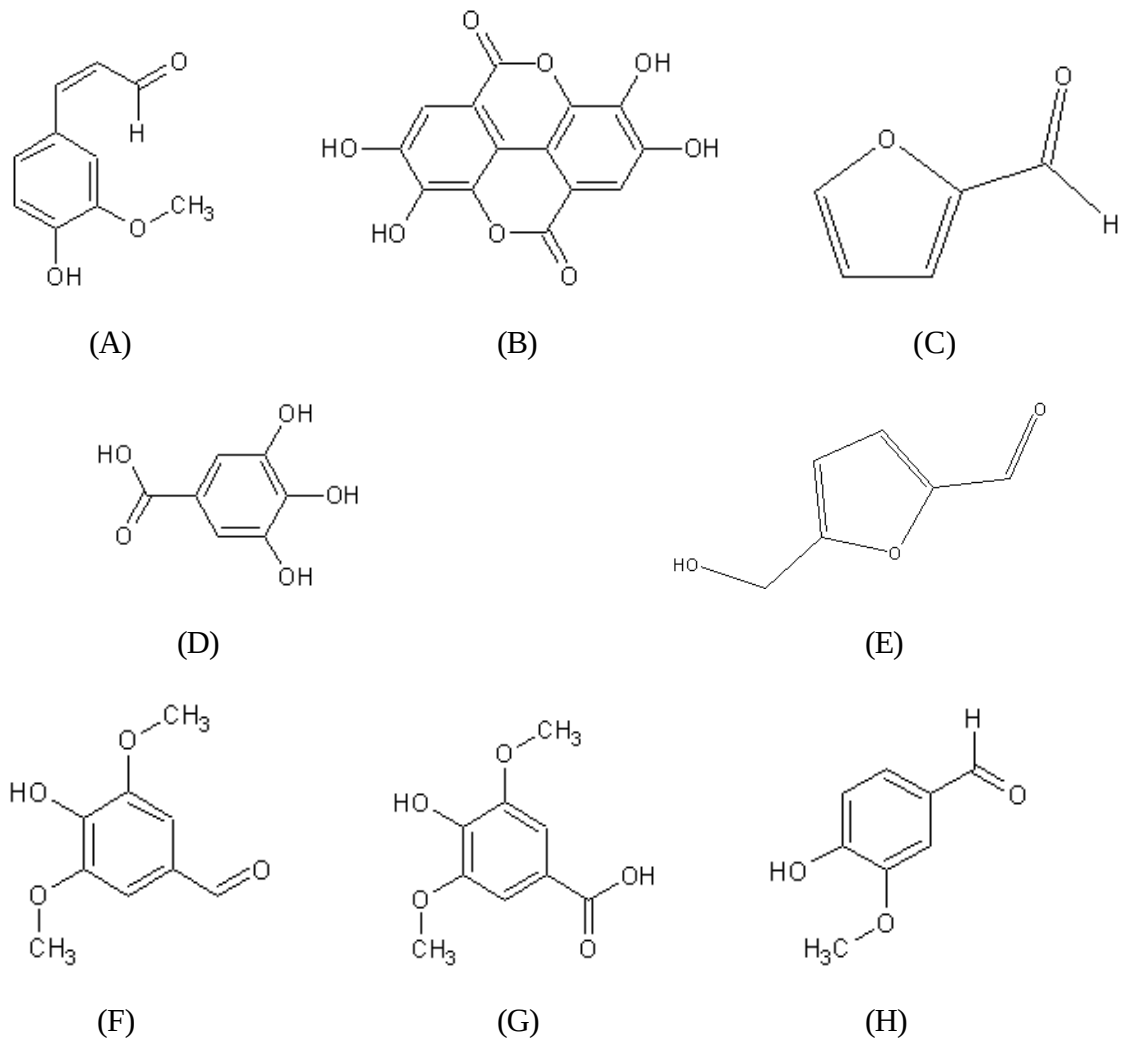


Figure 2: Chemical structures of spirit-specific compounds. (A) coniferaldehyde, (B) ellagic acid, (C) furfural, (D) gallic acid, (E) HMF, (F) syringaldehyde, (G) syringic acid, (H) vanillin

vanillin, syringaldehyde, and syringic acid. Different proposals have been made about the mechanisms involved with the production of these compounds including a theory that an ethanol lignin compound acts as a possible intermediate. Table 2 (Lee et al. 2001, Reazin 1981) shows the proposed mechanisms for the compounds investigated in this method.

E. Near Infrared Spectroscopy

Near-infrared (NIR) spectroscopy is a chemical detection technique used for the routine quantitative determination of species, such as water, proteins, low-molecular-weight hydrocarbons, and fats in products of the agricultural, food, petroleum, and chemical industries (Skoog et al. 1998).

The NIR region of the spectrum extends from the upper wavelength end of the visible region at about 770 nm to 2500 nm (Burns et al. 2001). The NIR spectrometer works by irradiating the sample with one or more narrow bands of radiation ranging from 1000 to 2500 nm. This irradiation causes the vibration and stretching of chemical bonds mostly associated with C—H, N—H, and O—H bonds. These modifications to the bonds results in a reflectance of the sample which is interpreted by a detector similar to that for UV/VIS absorption spectroscopy (Skoog et al. 1998).

F. High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is a useful chemical separation tool available for the quantification of samples. The instrument is widely used because of its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability

Table 2: Proposed mechanisms for aromatic aldehyde formation

1a. Wood-lignin$_x$ + ethanol ? wood-lignin$_{(x-n)}$ + n ethanol lignin
1b. Wood-lignin$_x$ + ethanol ? coniferyl alcohol + sinapic alcohol
2. Ethanol lignin ? ethanol + coniferyl alcohol + sinapic alcohol
3. Sinapic alcohol + O_2 ? sinapaldehyde
4. Sinapaldehyde + O_2 ? syringaldehyde
5. Syringaldehyde + O_2 ? syringic acid
6. Coniferyl alcohol + O_2 ? coniferaldehyde
7. Coniferaldehyde + O_2 ? vanillin

for separating nonvolatile species or thermally fragile ones, and its widespread applicability to substances that are of prime interest to industry, to many fields of science, and to the public (Skoog et al. 1998).

The principles of HPLC are to separate the chemical species in the sample by an elution method determined by the user. The system consists of a pump system, mobile phase, column or stationary phase, and detector (Figure 3). Depending on the type of sample to be separated, these should be chosen in accordance.

The most common type of pumping system for HPLC is the reciprocating pump, in which the solvent is pumped by the forward and reverse motion of a motor-driven piston. The flow of the solvent is controlled by two ball check valves which open and close alternately. Solvent flow can be categorized as isocratic, single solvent of a constant composition, or gradient, two or three solvent systems that differ significantly in polarity. The stationary phase consists of porous micro-particles having diameters ranging from 3 to 10 µm and usually consisting of a nonpolar substance (Skoog 1998). By utilizing the polarity differences in gradient elution coupled with the generally nonpolar stationary phase, it is possible to achieve separation of similar chemical species.

There are different types of detectors used for HPLC analysis depending on the type of compounds being analyzed and the sensitivity and response desired for the chosen method. Examples of the different types of detectors are the photodiode array (PDA) and refractive index (RI) detectors. The PDA detector is an ultraviolet/visible light (UV/VIS) detector with an operating wavelength range from 190 nm to 800 nm (Waters 2001). A grating disperses the light into bands of wavelengths and focuses those bands onto the plane of the PDA. The detector then measures the amount of light striking the PDA to

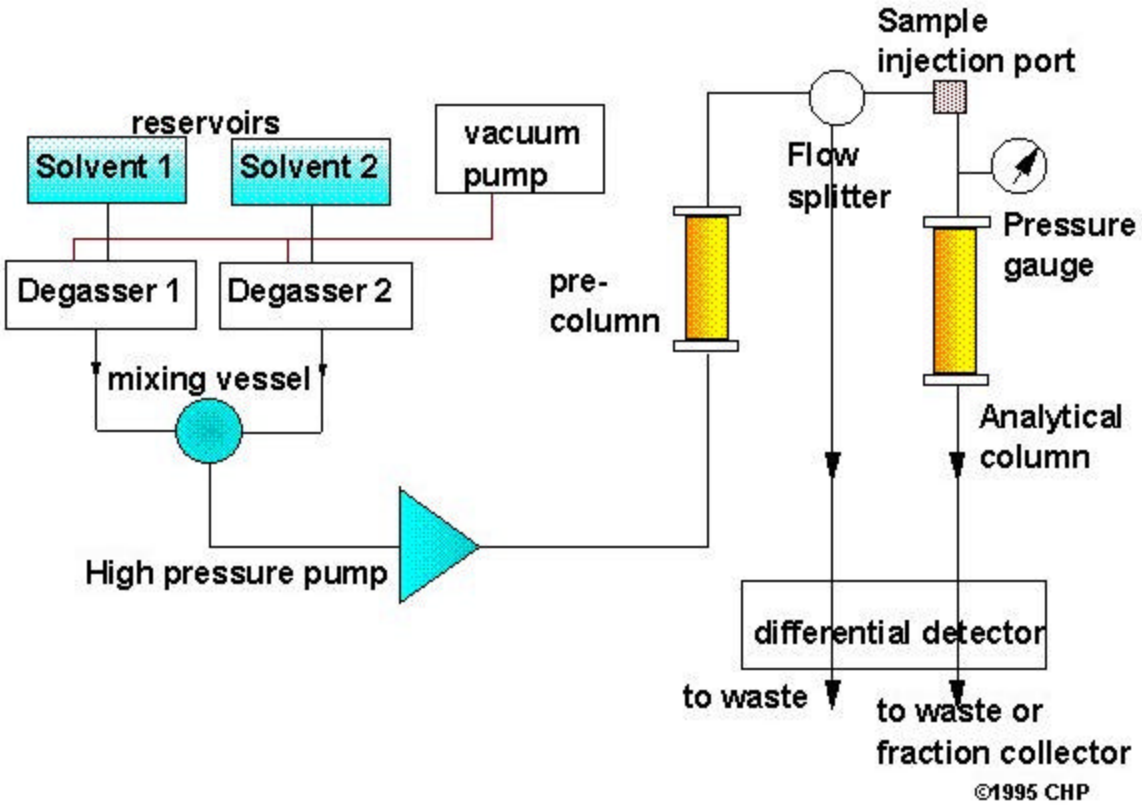


Figure 3: Schematic of HPLC
http://elchem.kaist.ac.kr/vt/chem-ed/sep/lc/graphics/hplc-sch.gif

determine the absorbance of the sample in the flow cell. The light striking the photodiode discharges the diode and the amount of current required to recharge the photodiode is measured by the detector (Waters 2001).

G. Statistical Concepts

i. Principal Component Analysis

The following section is based on information contained in three works (Beebe et al. 1998, Burns et al. 2001, Martens et al. 1989).

Data extracted from a chromatogram or spectra can be represented as a matrix with a particular number of rows and columns. The **X**-variables, wavelengths, in a matrix of this type are expected to be collinear because the number of samples is less than the number of **X**-variables. This co-linearity allows the matrix to have some dominant types of variability, allowing for noise and redundancy to be filtered out.

The goal of principal component analysis (PCA) is to represent the variation present in many variables by using a small number of factors, or principal components (PCs). This is accomplished by an eigenvector decomposition analysis to extract important eigenvalues. The eigenvectors are represented as new PC axes with the eigenvalues showing how much each factor removes from the **X**-variables. The total variation for each eigenvector, or PC, can be calculated by dividing the largest eigenvalue with the remaining eigenvalues of that eigenvector.

Once the pertinent eigenvalues are extracted, new axes using the PC are constructed. The PCs must be uncorrelated and orthogonal to each other in order to

maximize the amount of variation each component contains and minimize the number of PCs needed to represent the variability.

A new row space is constructed to plot the samples by creating new axes using PCs rather than the original sample values. The samples are given new coordinates in relation to each of the PCs, called scores. Samples that are close to each other on a score plot are similar with respect to the original variables as long as the plot displays a sufficient amount of the total variation.

Loadings are used in analysis of the results in order to determine for what variation each PC is accounting. The loadings are normalized vectors that maximize the variance of the X-variables for its respective PC. The loadings can be calculated as the cosine of the angle between the PC axes and the original variables and describes how the original variables are related to each PC. As the loadings approach 1 or -1, this indicates the PC is completely aligned with the original sample, meaning the variable contributes a great amount of the variation to the PC.

ii. Wilcoxon Rank-Sum Test

The Wilcoxon Rank-Sum test is a nonparametric statistical test used to determine similarities and differences between two population sets. Let X and Y be continuous random variables with random sample sizes of m and n, respectively. The null hypothesis of this test is to determine if the X and Y populations are equal.

For the test, the $m + n$ observations are pooled to form a single sample with the group identity of each observation retained. The observations are then ordered smallest

to largest and ranked from 1 to $N = m + n$. The test statistic, W_m, is the sum of the ranks associated with the observations that constitute the smaller sample.

Using this test statistic, if the X population is located below the Y population, then the smaller ranks will be associated with the X values. This will result in a small W_m, while the reverse will result in large values for W_m. Using tables with upper and lower critical points for selected values of m, n, and a will show whether the calculated test statistic means the populations are different or equal (Mendenhall 2005).

iii. Partial Least Squares Regression

The following section is based on information contained in three works (Beebe et al. 1998, Burns et al. 2001, Martens et al. 1989).

Partial least squares regression (PLSR) is a multivariate technique which allows for the calibration of pertinent components while implicitly modeling other sources of variation. This bilinear modeling can yield reliable predictors by projecting many variables onto a few variables. The term bilinear model stems from the fact that **X** and **y** are linear functions of both the scores and loadings. The **X** values correspond to variables of the investigated spectra and **y** corresponds to sample input.

In PLSR, the spectral information in all the relevant variables is compressed into a small subset of artificial variables, called regression factors, $\mathbf{t_a}$. These factors describe as much of the covariance between **X** and **y** after the previous factor has been estimated and subtracted. Estimates of these factors are used as regressors for **y**. For each new factor, the scores are estimated as linear combinations of the **X** residuals and the loadings are

defined as the normalized covariance matrix between the centered **y** variable and the residual variability remaining after the previous a-1 factors.

During the bilinear decomposition of **X**, the **y**-variables are actively used. By using both the **X**- and **y**- variables, the impact of irrelevant **X**-variations in the calibration model is reduced.

There are two approaches available for generating a validation set for estimating the prediction error: internal validation (cross-validation) and external validation. Cross-validation divides the original calibration data set into prediction and calibration subsets. The calibration subset is used to construct the model, which is then applied to the prediction data subset. This process continues until all of the data in the original data set has been omitted from the calibration subset once. After construction of the model, root mean square errors can be calculated for the prediction, calibration, and cross-validation. These errors show the reliability and accuracy of the generated models.

3. Experimental Methods

A. Determination of Virgin Wood by Analysis of Stave Surface

i. Sample Preparation

The used casks, casks which have been used for the maturation of a spirit, were obtained from the Jack Daniel's© Whisky division of Brown-Forman© and were broken down upon arrival. 10 staves from 5 casks were chosen for analysis in the preceding method. The staves were labeled with barrel and stave number along with a designation of middle or edge. The new wood stacks, non-processed wood, were supplied by Brown-Forman© and differed in original location and seasoning time. A total of 10 staves from 5 new wood stacks were chosen for analysis. The different provinces from which the barrels were provided were Jackson, Lebanon, and Yake with seasoning times ranging from 2 to 8 years.

The staves from each barrel were collected with an effort to capture the variances seen in the physical dimensions of the staves. The staves were cut in half, in the lateral direction, with the internal face of the stave designated as the charcoal face (Figure 4). From the half cuts of the stave, samples were cut off on the two outside edges and one sample from the middle of the stave. The dimensions for these samples are 12.7 X 6.4 X 23.4 mm with the 23.4 mm height of sample corresponding to stave thickness, which varied for each stave.

ii. Near Infrared Spectroscopy

The samples were placed on a Plexiglas© platform that was connected to a motor. The NIR spectra were taken every 100 µm using a Labspec Pro©, LSP350-2500P

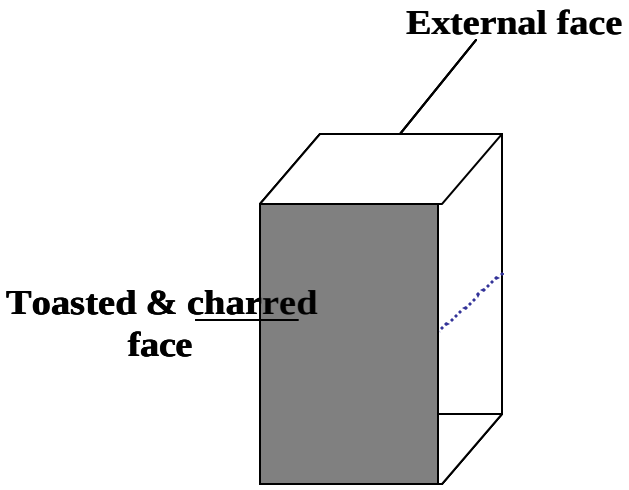


Figure 4: Representation of solid sample used for determination of virgin wood

spectrometer with a fiber optic cable attached to a microscope (Figure 5). Labspec Pro© software was used to control the spectrometer and the motorized platform with an additional program. The spectra were then imported into Unscrambler© for data pretreatment.

Using the software, the spectra were transformed from reflectance to absorbance and the X-variables, wavelengths, were reduced by 4, providing a spectral resolution of 4 nm. The data were then normalized using a mean normalization method (Appendix 1) and a multiplicative scatter correction (MSC) (Appendix 1). The MSC was used in order to minimize the variation seen due to uneven surface area for the samples.

The range of the spectrum used for analysis was 1000 to 1800 nm. Variations due to the visible range were not used for characterization and due to interference in the spectrum caused by the microscope, the range 1801 to 2500 nm was also removed.

iii. Multivariate Analysis and Statistical Tests for Intra-stave Variation

The PCA performed on the NIR spectra taken for the determination of virgin wood was done using The Unscrambler© Version 9.0 software. A maximum rank of 6 and a full cross-validation (Appendix 1) were chosen for this method. The portion of the IR spectrum used for analysis was the range from 1000 nm to 1800 nm in order to exclude differences due to color and eliminate noise observed in the higher end because of the microscope.

After the PCA, the scores of PC 1 were extracted for the data set with each score corresponding to a position on the stave sample. Control charts were then implemented

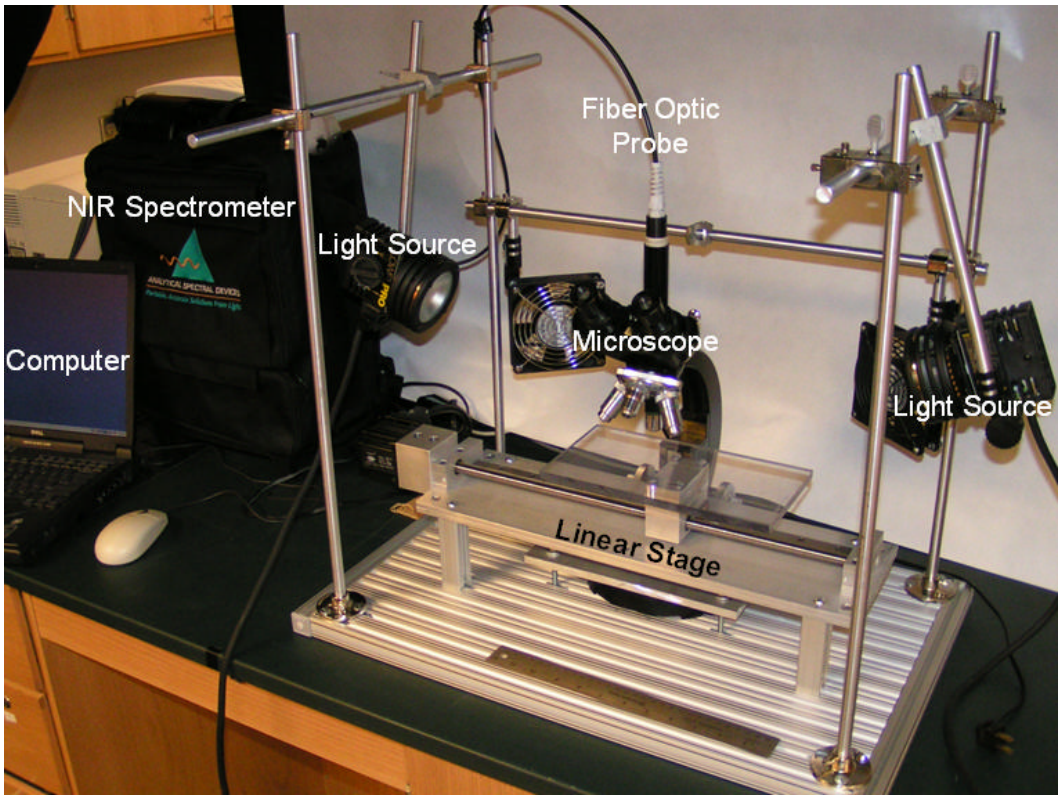


Figure 5: NIR setup for determination of virgin wood using solid sample

on the scores of PC1 with the Gilbert formula (Appendix 1) which uses a moving range (Appendix 1) with an autocorrelation coefficient (Appendix 1). The autocorrelation function was chosen because of the close proximity of the data points to each other. The calculations for the control charts were performed using a MATLAB© file created for this method (Appendix 2).

iv. Multivariate Analysis for Comparison of Matured Staves to New Wood

The Y-variables, number of samples, were averaged into 19 points for each stave to assist in handling the data. The spectra for all the new staves at each position were then averaged into one sample leaving 19 spectra corresponding to each position on the stave. The same process was performed on the 10 staves collected for matured cask 1.

The averaged new wood spectra were subtracted from the spectra of the average matured stave sample with respect to each position. After subtraction, one reduced sample remained with 19 spectra corresponding to each position on the matured stave. The reduced matured stave spectra were imported into Unscrambler© for a PCA following the procedure mentioned in the previous section. The control charts implemented on the resulting scores for PC1 were performed in JMP©. The upper and lower limits were calculated by addition and subtraction of 3s to the average.

B. Determination of Virgin Wood by Analysis of Extract

i. Sample Preparation

The unused staves were obtained from Bluegrass Cooperage© in Louisville, KY. A total of 10 used casks and 10 unused staves were available for research. Out of the 10

used casks, 12 staves were chosen for analysis under the proceeding method. Only 12 staves were chosen due to the time involved in sample preparation, extraction process, and HPLC analysis.

The staves from the casks were selected at random, with consideration only for inter-cask variation. The staves were then cut in half in the lateral direction and moved to the GRIZZLY© G8689 Mini Milling Machine. Starting on the exterior, non-charred, side of the stave, layers of 1.700 mm were milled off and collected. The error of the milling machine is ±0.025 mm, with the error for each layer being cumulative. The milling was continued until the charcoal layer was reached and the process was repeated for all staves being investigated. Once the layers had been coarsely milled, the samples were again milled using a THOMAS® WILEY® MINI-MILL with a 20-mesh screen.

For extraction of the milled powder samples, protocol given by Brown Forman© was used. One gram of each milled powder sample was weighed in a 50 mL beaker. To the beaker, 25 mL of 50% ethanol/water was added and covered with Parafilm© for 4 hours. After extraction, the samples were filtered with fluted filter paper to remove the solids. These filtered solutions, extracts, were then placed in sample jars with Teflon© caps and placed in a -15°C freezer to prevent oxidation of the compounds and evaporation of ethanol.

ii. High Performance Liquid Chromatography

The HPLC method was obtained from Brown-Forman© and used for direct comparison with results from the industry. A gradient elution was employed with a dual solvent system (Table 3). 1.5 mL of extract was filtered with a 0.45 µm Millipore© disc

Table 3: Gradient method for HPLC analysis

Time (min)	% CH_3COOH	% CH_3OH
0	95	5
17	26	74
19	95	5
31	95	5

filter into a 2 mL HPLC sample vial and placed in a Waters© 710 Autosampler. The HPLC method was carried using a Waters© 2695 Separations Module and Empower© software. The analysis on the separation was done on a Waters© 2996 Photodiode Array Detector at 280 nm.

iii. Multivariate Analysis and Statistical Tests

In order to determine the amount of virgin wood remaining in the staves, two statistical approaches were tested on the HPLC chromatograms of the extracts.

In the univariate approach for determining the amount of virgin wood remaining in the staves, concentrations of certain spirit specific compounds for each sample were calculated using calibration curves generated by HPLC. These concentrations were imported into a JMP© spread sheet and grouped into layer and type of stave, un-matured (U) or matured (M). Using the JMP© software, a one-way ANOVA was performed for each layer followed by the nonparametric Wilcoxon Rank-Sum test.

In the multivariate approach for determination of virgin wood, the HPLC chromatograms were extracted at 280 nm and exported to an Excel© file for further analysis. The matrix was then transposed in order to have time as the X-axis and absorbance as the Y-axis. The transposed matrix was exported into Unscrambler© for further analysis using PCA. No pretreatment was implemented for the data because the chromatograms do not contain the same type of information as NIR spectra.

Using the software, a PCA was performed on the chromatograms of all samples including matured and un-matured staves. Scores of PC2 resulting from the PCA on the staves were exported into JMP© for statistical analysis. The samples were grouped into

layer and type of stave, un-matured (U) and matured (M). A one way ANOVA was performed on each layer followed by the nonparametric Wilcoxon Rank-Sum test to statistically determine the amount of virgin wood present in the matured staves.

C. Predictive Modeling for Spirit Specific Compounds

In addition to the understanding of the effect of the maturation process on the wood, the ability to predict the amount of spirit specific compounds in the wood could be valuable. Knowing the amount of compounds available in the wood enables the engineering of casks to produce spirits with particular characteristics.

i. Sample Preparation

Samples used for this method are the same samples used for determination of virgin wood by analysis of the extract. (3.B.i)

ii. Near Infrared Spectroscopy and HPLC

For NIR, the milled powder samples were placed in caps with a diameter of 22.16 mm and smoothed to create an even surface on top. The caps were set on a rotating apparatus under the Labspec Pro, LSP350-2500P spectrometer and spun at 45 rpm. Five spectra were taken of the rotating sample to ensure any variation in the surface would be negligible.

The resulting spectra were imported into Unscrambler© for data pretreatment. The spectra were converted from reflectance to absorbance. A PCA was performed on the 5 spectra for each sample to determine if any disturbances were captured. After

observing no outlying spectra, they were averaged by 5 to leave one spectrum for each sample. The X-variables, spectral wavelengths, were also averaged by 4 providing a spectral resolution of 4 nm.

Further pretreatment on the data included a mean normalization and MSC. The non-homogeneity of the particle sizes in the samples and the possible differences in surface characteristics from the method setup required the use of MSC.

For analysis, 1000 to 2500 nm were investigated in order to observe changes based on the chemistry of the stave and not visible variations.

The HPLC data in this method was obtained in the HPLC section for the determination of virgin wood by analysis of the extract. (3.B.ii)

iii. Multivariate Analysis and Statistical Tests

The PLSR for each investigated compound was performed using the PLS1 function in Unscrambler©. The cross-validation in this method is a full cross-validation which leaves one sample out of the calibrated model at a time. The maximum rank allowed for the regression was chosen to be 6. Samples containing quantified values lower than the limit of detection used in the calibration curve were removed prior to the PLSR for all compounds except HMF. There were not enough samples containing HMF above the limit of detection established by the HPLC for analysis using this method. Further discussion on this topic can be found in the conclusions section for predictive modeling of spirit specific compounds.

4. Results and Discussion

A. Determination of Virgin Wood by Analysis of Stave Surface

i. Intra-Stave Variation of Matured staves

The intra-stave approach investigates whether an amount of virgin wood remaining in the stave after maturation can be calculated by examining the internal and external surface of the staves. NIR spectroscopy was chosen for this method because of its rapid data collection and minimal sample preparation. Figure 6 shows a representation of the entire NIR spectra for B4S7-E1 (Barrel 4, Stave 7, Edge 1). The collected NIR spectra on the stave samples are similar to other spectral data performed on solid wood (Kelley et al. 2004). This shows the collection procedure used in this method is applicable to wood samples.

After pretreatment on the data, a PCA was performed on the samples for each stave to examine the inherent variability present. The scores of the PCA for B4S7-E1 (Figure 7) shows the majority of the data is similar with the end of the data set trailing off. This end corresponds to the charred end of the sample. Each point corresponds to a spectrum taken on the stave at a 0.1 mm interval.

The scores for PC 1 (80%) were further analyzed in order to determine if an amount of virgin wood could be determined. The scores were extracted using Unscrambler© and plotted against distance. Examination of these scores (Figure 8) shows a change between the samples toward the interior face of the stave. Each point in the figure corresponds to a spectrum taken on each distance of the stave sample.

A PCA was performed on all the samples of each stave and the scores for PC 1 were obtained for further statistical analysis. A control chart was implemented on the

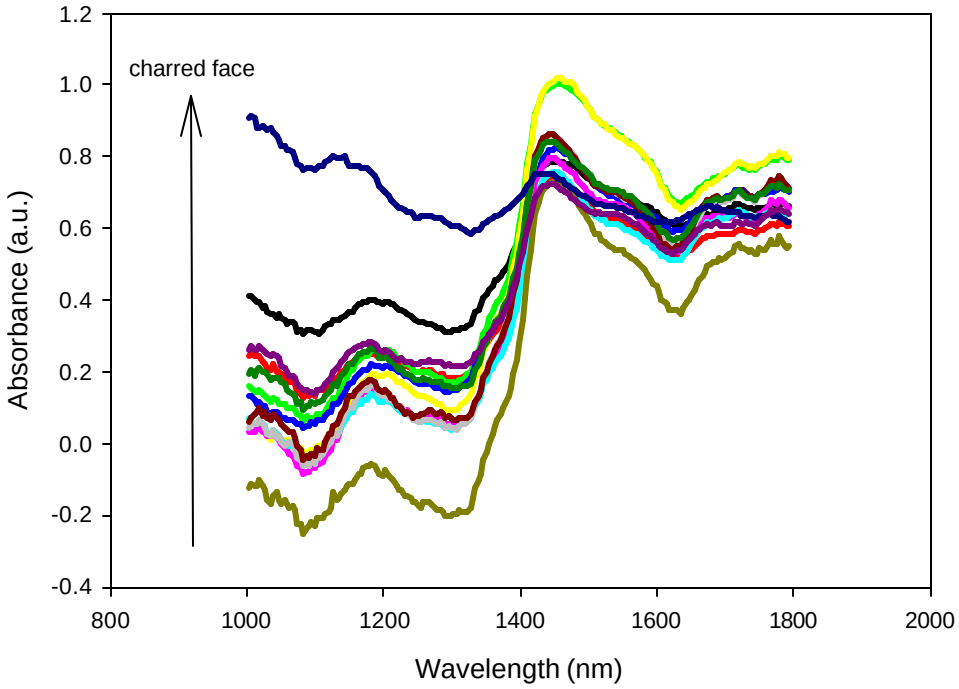


Figure 6: NIR spectra for selected samples of B4S7-E1

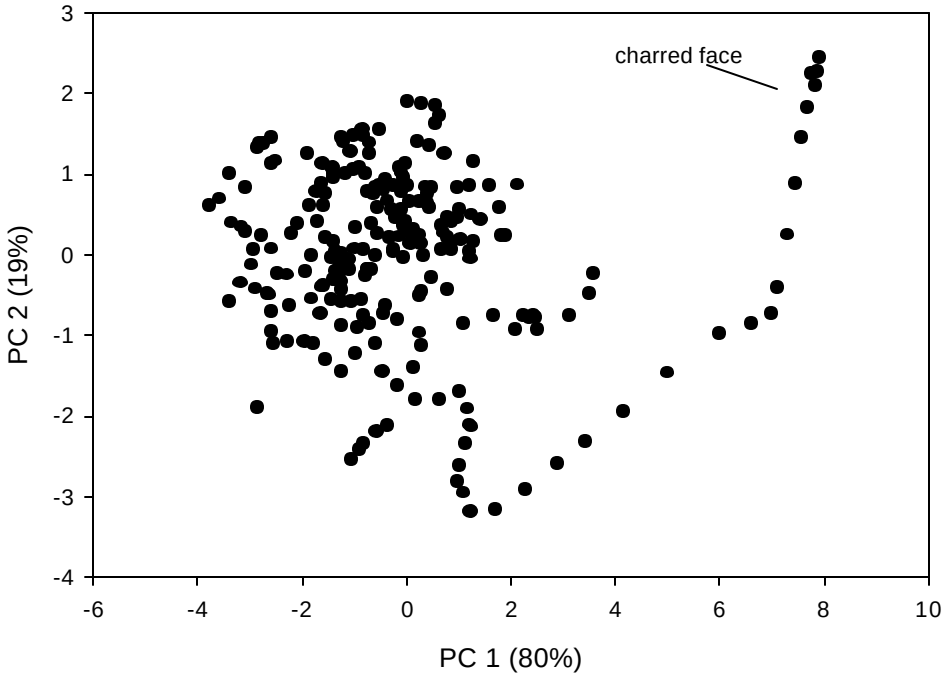


Figure 7: PC 1 (80%) vs. PC 2 (19%) for sample B4S7-E1

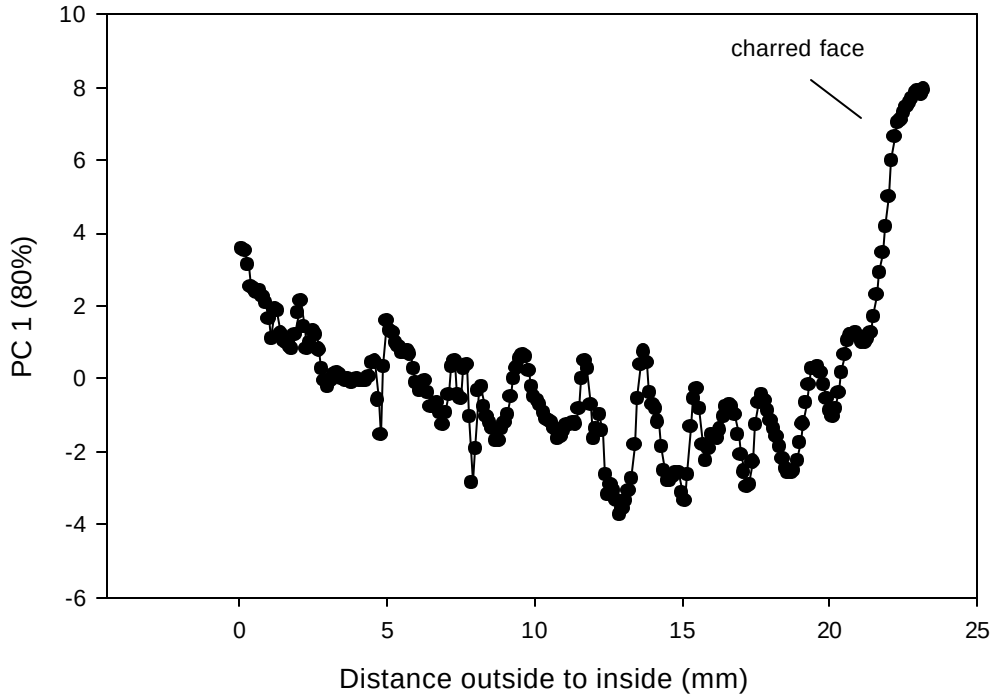


Figure 8: Scores of PC 1 (80%) for sample B4S7-E1

scores to determine where the significant change occurs on the stave. The control limits were calculated using the Gilbert formula with an autocorrelation coefficient. Examination of the control chart of the PC1 scores for B4S7-E1 (Figure 9) shows that a limit of 22.4 (±0.02 mm) can be found using this approach.

The drastic differences in chemistry observed in the charred face in relation to the rest of the stave could be the main source of variation calculated by the PCA. To determine if this is the case, the portion out of control was removed and another PCA was performed on the remaining data.

Figure 10 shows the results of the modified PCA for B4S7-E1. Examination of the scores for PC 1 shows there is not a significant difference across the stave. A control chart was implemented on the data set and concluded that no points were out of control for this sample. Although no points were considered statistically out of control a visual trend is present in the graph. There is variation across the stave with the outside, non-charred face, and the more internal points on the stave being similar. During toasting, the entire cask is placed in an oven. This would result in the modification seen on the exterior face which makes it statistically similar according to the information used to construct the PC.

Table 4 shows the amounts of virgin wood remaining in the cask after maturation by analysis of all the matured stave surface using NIR. There was only a difference of 0.1 mm between the amounts of virgin wood calculated for the edges and middle of the staves using this technique. This shows that using NIR, a difference between the edges and middles can not be determined. However, the maximum and minimum for the result set corresponds to the edges of the sample. This could be due to the fact that the edges

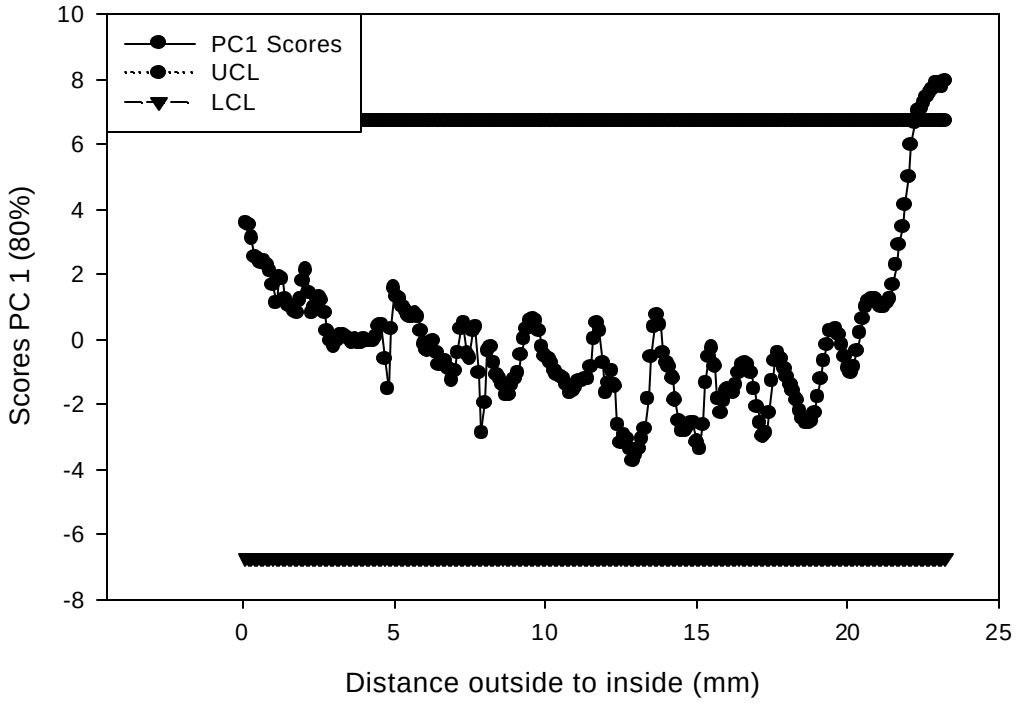


Figure 9: Control chart for scores PC 1 of B4S7-E1

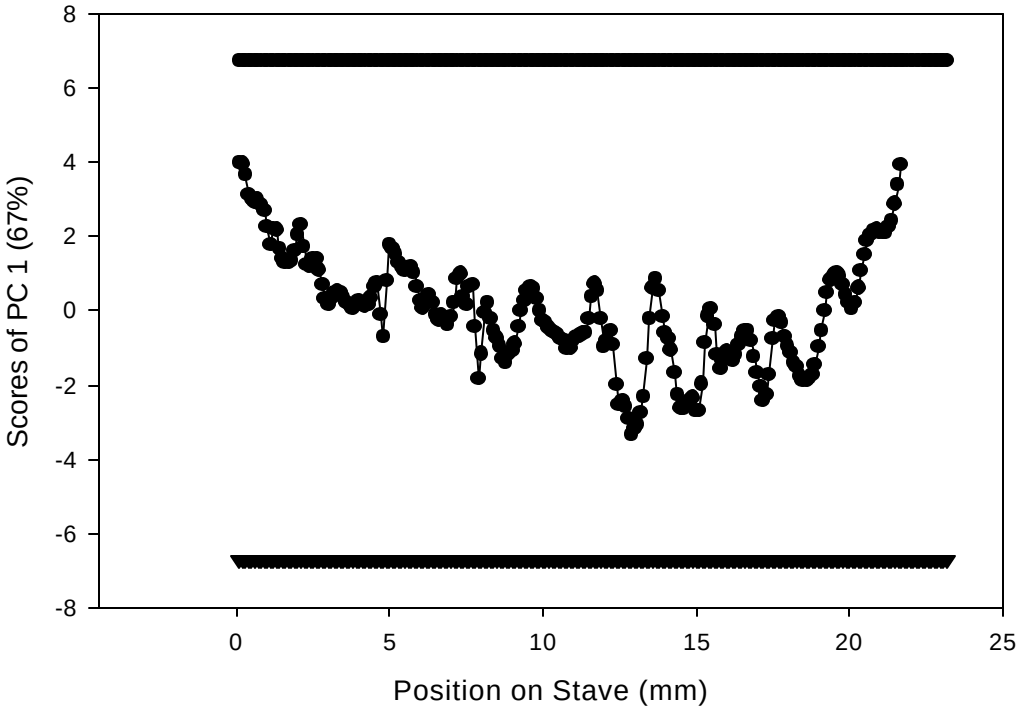


Figure 10: Scores of PC 1 (67%) for sample B4S7-E1 without points out of control

Table 4: Results for NIR approach to determination of virgin wood by analysis of stave surface

Sample	N		Amount of Virgin Wood (mm)	
-	-	Max	Min	Mean
Edge	100	24.4	17.1	21.2
Middle	50	24.3	18.8	21.2
All	150			21.3

are set corresponds to the edges of the sample. This could be due to the fact that the edges are more exposed to the diffusion of the spirit, both in aqueous and gaseous form. This can result in more drastic modification to the stave at that location by creation of the "red line," which is a line on the edge of the stave which might correspond to one of the two types of diffusion of the spirit.

The point on the stave that is out of control using this technique is around the charred region of the stave. The drastic thermal treatment subjected on the casks during fabrication accounts for the chemical transformation in the staves seen by this technique. NIR observes mainly chemical vibrations and molecular overtones of the sample. These observations limit the amount of information that can be used in determining how much virgin wood remains in the stave. Also, the visible portion of the spectrum was removed prior to analysis which would have included color changes, but might have contained other useful information. Using NIR to observe the intra-stave variation of the matured staves, an amount of virgin wood was able to be calculated. However, by visual observation of the red line, it seems that modification to the stave by the spirit occurs deeper in the stave than 21.2 mm.

ii. Statistical Comparison of New Wood to Matured Staves

After analysis of only the matured stave with themselves, a new approach was developed which could compare the matured staves to new, unprocessed wood. NIR spectroscopy was again used in this method because of minimal sample preparation time and high throughput.

The spectra for the matured staves and new wood were obtained according to the experimental methods section (3.A). Figure 11 is a representation of the collected spectra for new wood stack 1 stave 5 (N1S5). There is some variation between the samples, but not a significant amount. The variation seen could be attributed to the natural variability in the wood.

Using the Unscrambler© software, the spectra for the averaged new wood stave was subtracted from the spectra of the averaged matured stave. Using this technique, the new wood will act as a baseline with any deviation from that baseline constituting a change between the two sample types. Figure 12 shows the spectra for the average matured barrel 1 sample with the baseline subtraction. The matured barrel 1 sample is the middle and edges for all 10 staves of this barrel averaged into one sample.

The figure shows that although the spectra for the new wood stave was removed, there are still observable bands. Due to this observation, further analysis into the remaining spectra can lead to an amount of virgin wood using MVA.

The remaining spectra were imported into Unscrambler© for PCA to determine if any variation captured by the analysis can lead to an amount of virgin wood. The charred layer was removed prior to this analysis, leaving a 19 mm sample with one layer per mm. This portion was omitted because of the drastic difference between that layer and new, unprocessed wood. The charring of the wood will be the greatest source of variation and removing this can lead to investigation of other more inherent sources of variation.

PCA was performed and the scores for PC1 were extracted using the software. Visual analysis of the scores (Figure 13) shows that there seems to be a significant difference across the stave. The more interior layers on the stave deviate from the rest of

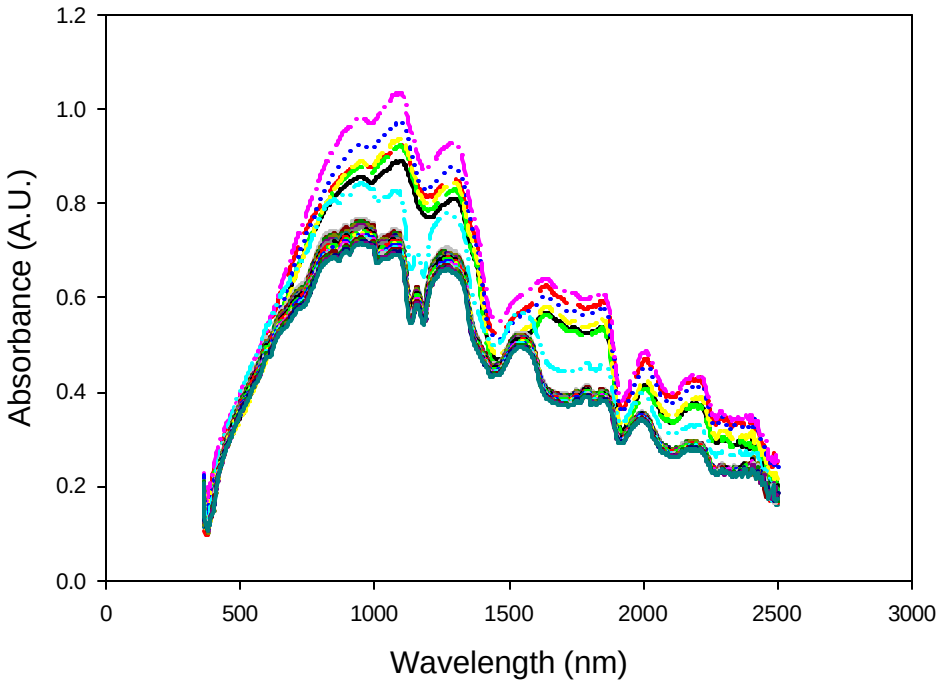


Figure 11: Collected spectra for N1S5

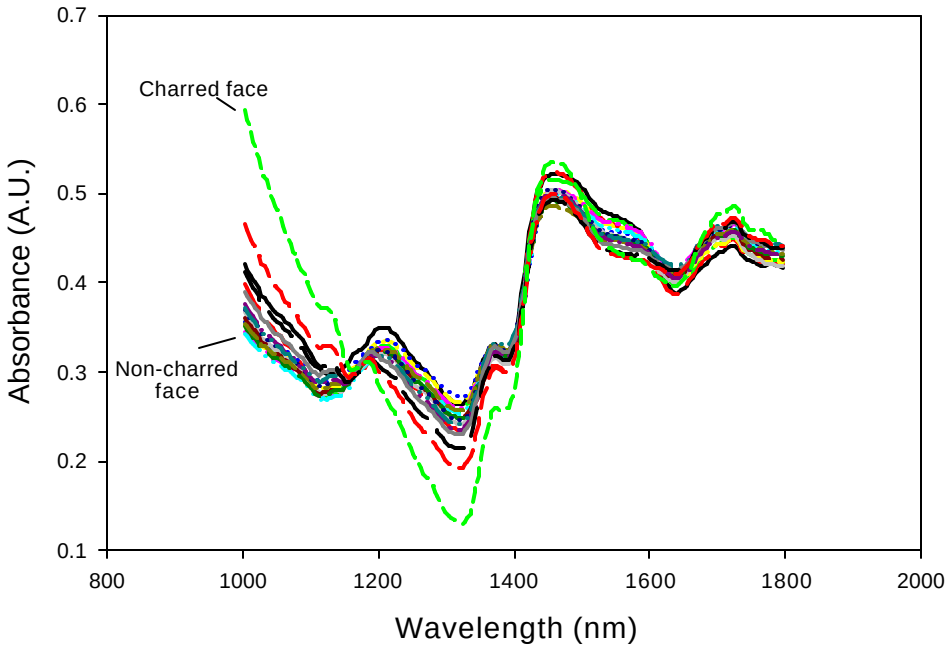


Figure 12: Corrected spectra for averaged mature barrel 1

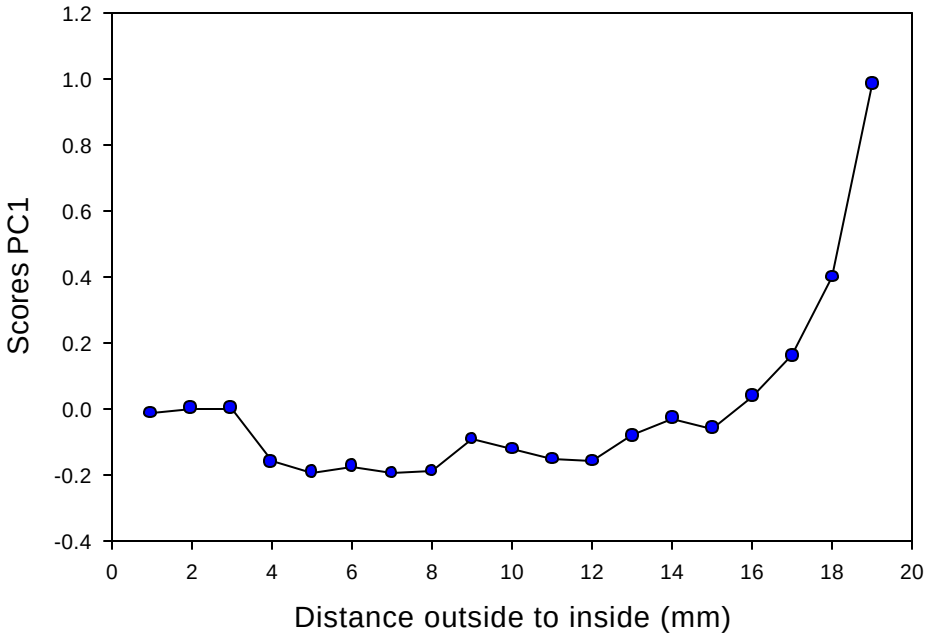


Figure 13: Scores for PC1 of corrected spectra

the layers. This could still be due to the effect caused by toasting and charring the interior face of the barrel prior to maturation.

To analyze the scores and determine where the statistical change on the stave occurs, a Shewhart control chart was implemented (Figure 14). The upper and lower limits were calculated using a k value of 3. The graph shows that using these limits a statistical difference could be calculated. This method yields an amount of 17.50 mm (±0.02) virgin wood remaining in the stave after maturation.

The amount calculated shows that using NIR spectroscopy a difference between the two types of staves, matured and new wood, could be determined. The difference seems to be due to the effect of the thermal treatment applied to the stave with the remaining portion of the stave being similar to new, unprocessed wood.

NIR can only determine certain chemical characteristics of the samples, but takes all of the chemistry of the stave into account. Due to this fact, other methods must be investigated in order to determine if the amount of virgin wood calculated by NIR can be replicated or improved.

B. Determination of Virgin Wood by Analysis of the Extract

The following method is done on analysis of the HPLC chromatograms obtained on the extract from the staves.

i. Univariate Approach

The univariate approach focuses on the investigation of individual compounds for determining the limit of virgin wood remaining in a cask after maturation. The

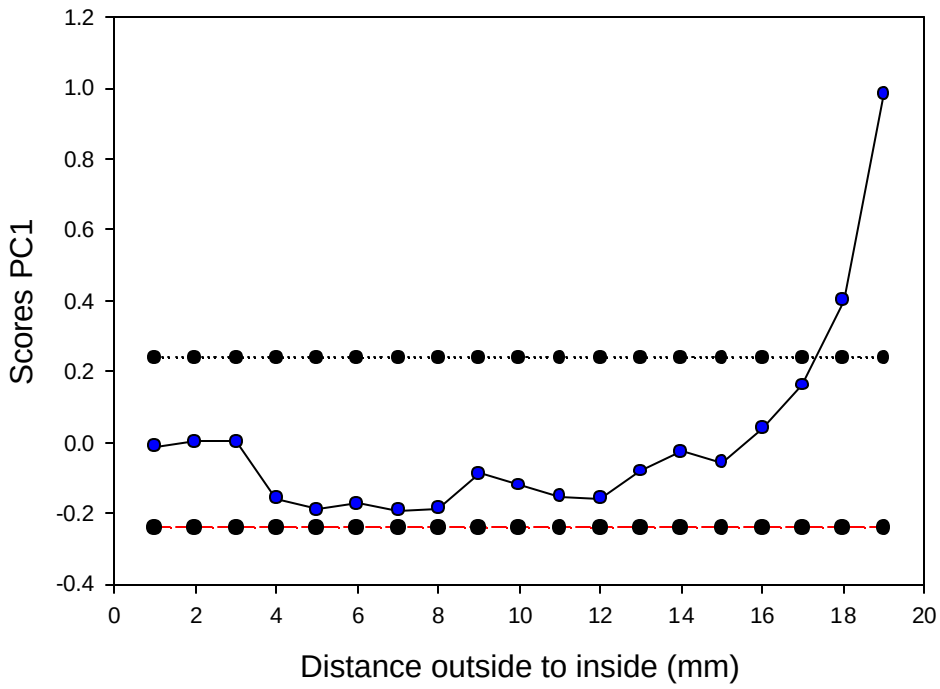


Figure 14: Shewart control chart for scores of PC1

compounds investigated for this approach were gallic acid, 5-hydroxymethylfurfural (HMF), 2-furfuraldehyde (furfural), syringic acid, syringaldehyde, and vanillin. These compounds were chosen because of their important contribution to the final product of the spirit and all of these compounds have maximum absorption around 280 nm when extracted using ethanol.

In order to quantify the results obtained from the HPLC chromatograms on the matured and un-matured staves, calibration curves were generated for these compounds. Standards were created with a range of concentration of 10-30 ppm. The calibration curve for gallic acid (Figure 15) shows the sensitivity of the detector and the HPLC method is applicable in this concentration range. By analysis of the regression coefficients and percent error (Table 5), the calibration equations will allow for the accurate quantification of the compounds.

The HPLC chromatograms for the Un-Matured (Figure 16) and the Matured staves (Figure 17) were analyzed using the calibration equations. The resulting concentrations of each compound in the samples were separated into matured and un-matured results, depending on the origin of the sample. A concentration profile across the stave was constructed for all six compounds. Due to the high variation in the wood before modification and the variation of the thermal treatment, standard deviations were large. Including more samples might not lower the standard deviation because of the inherent variability in wood mention in the wood chemistry section (1.A).

Figures 18 and 19 show the variations in concentration for furfural present in the first five un-matured and matured staves, respectively. The graphs show two distinct trends for the un-matured and matured staves. The concentration of furfural across the

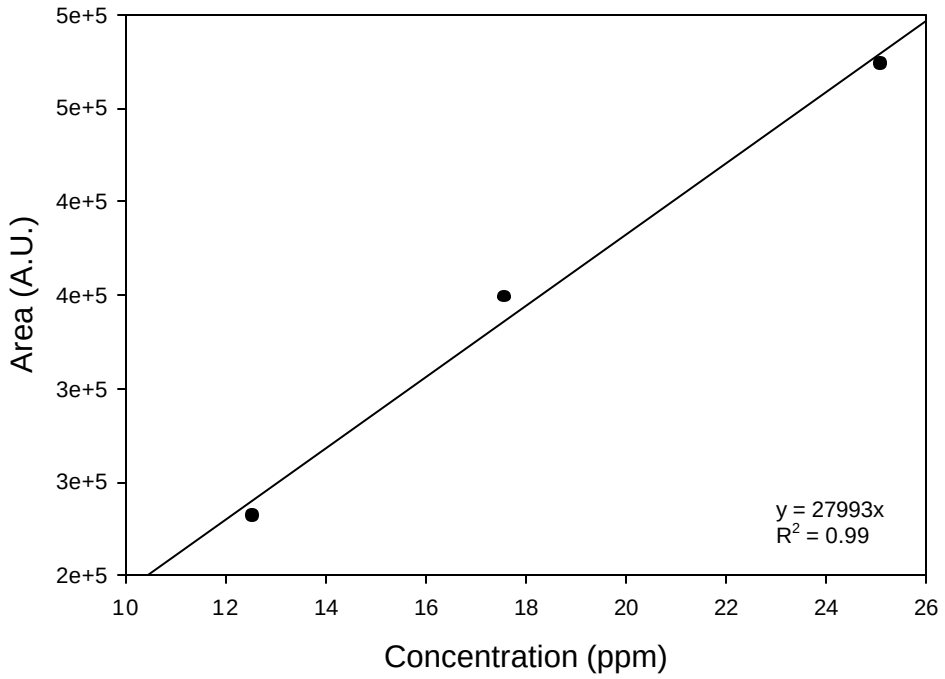


Figure 15: Calibration curve for gallic acid

Table 5: Regression coefficients of calibration curves for HPLC

Compound	R	% Error
gallic acid	0.99	0.14
HMF	0.99	0.12
furfural	0.97	-2.48
syringic acid	0.99	-0.66
vanillin	0.99	0.01
syringaldehyde	0.99	-0.61

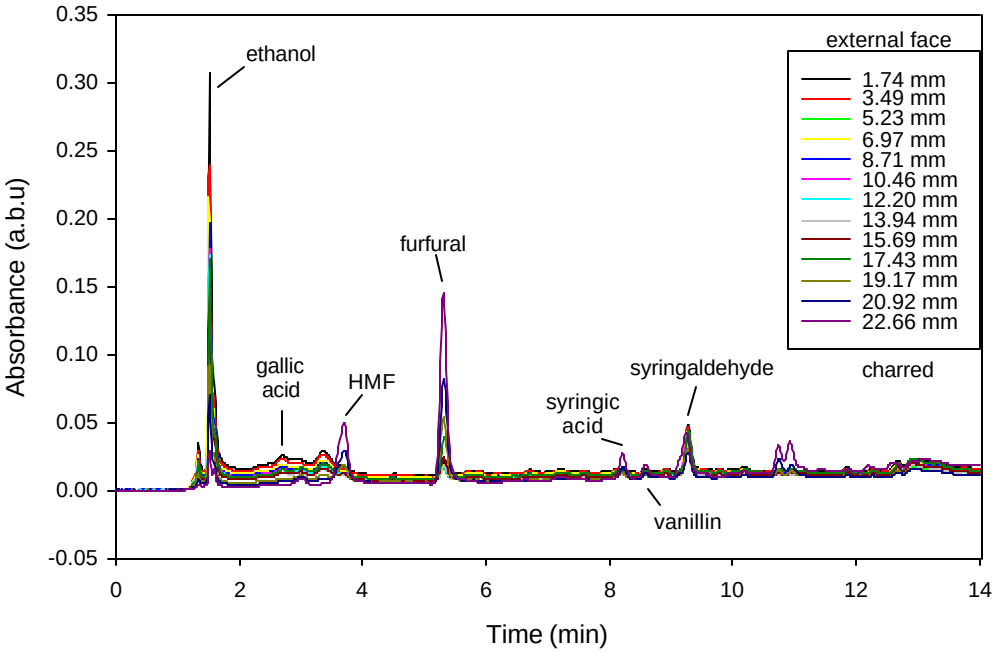


Figure 16: HPLC chromatogram profile for un-matured stave 4 (U4)

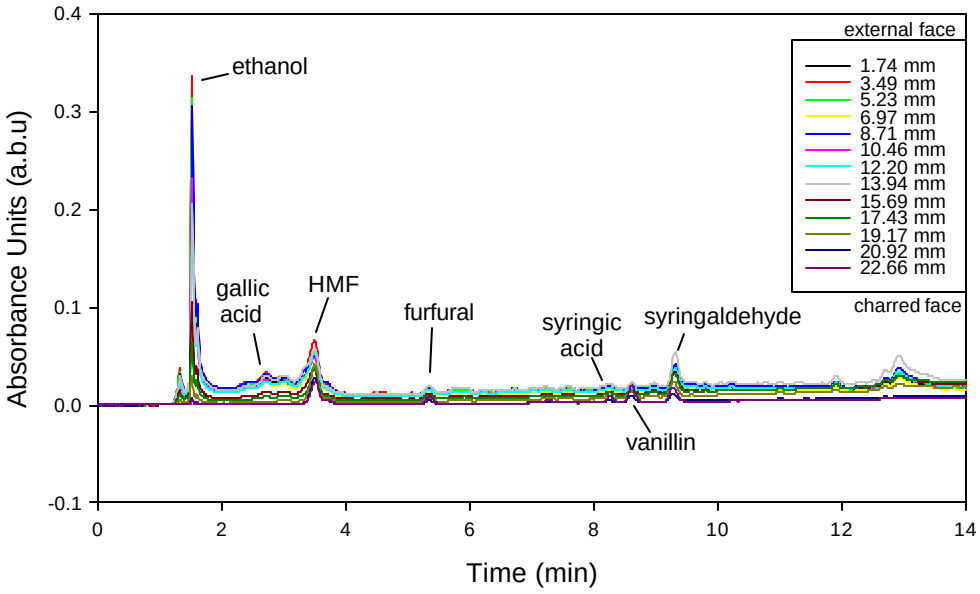


Figure 17: HPLC chromatogram profile of matured stave 6 (M6)

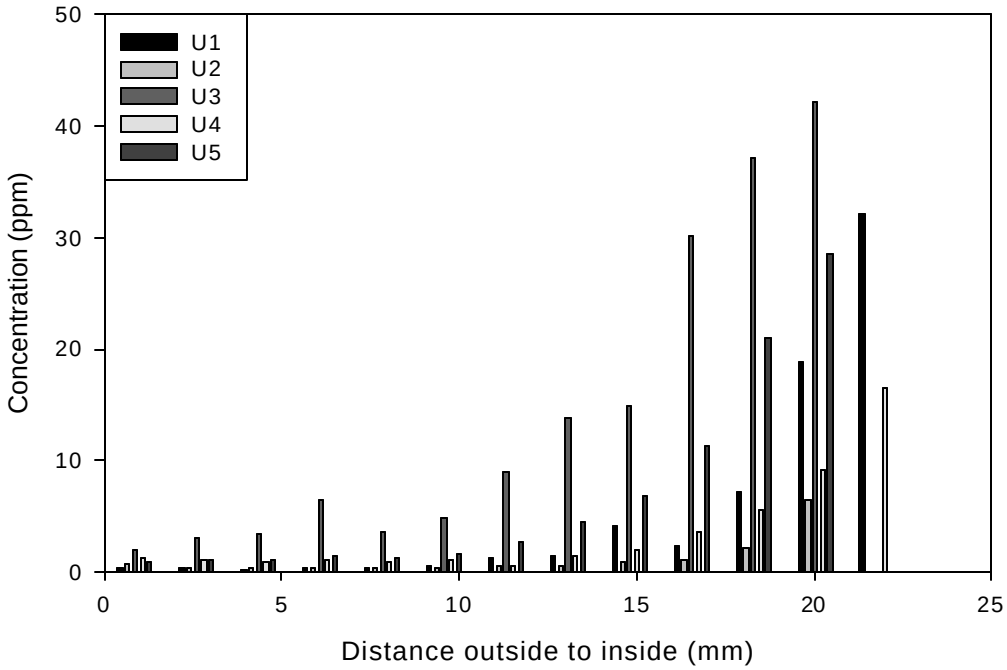


Figure 18: Concentration profile of furfural for un-matured (U) staves 1-5

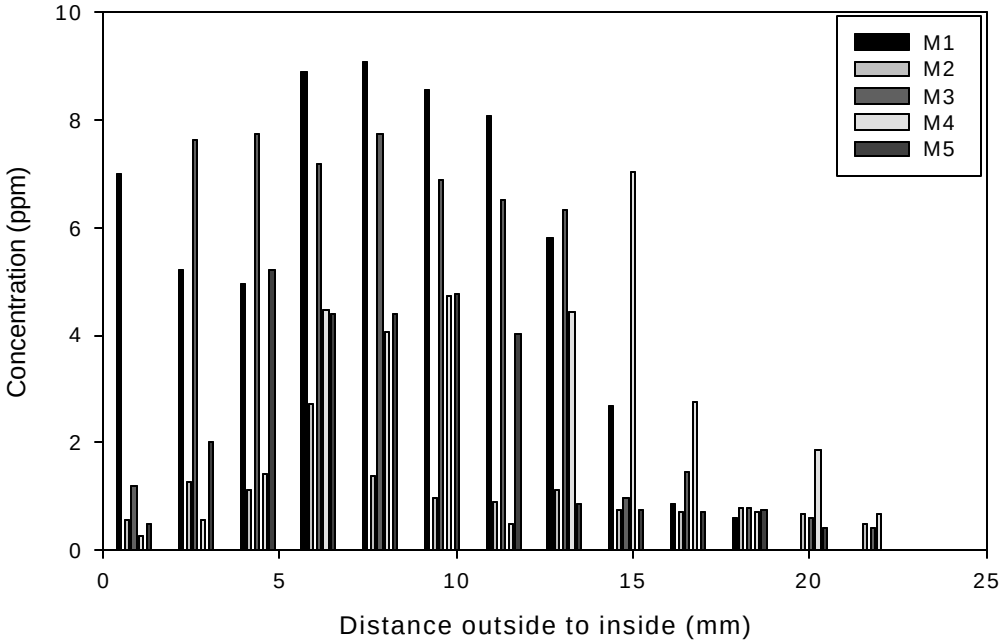


Figure 19: Concentration profile of furfural for matured (M) staves 1-5

un-matured stave increases as the layers approach the charred face. This increase in furfural can be attributed to hemicellulose degradation during the toasting and charring phase of cask fabrication. The profile for the matured stave resembles a bell curve with the maximum occurring around 7.8 mm. The decrease is caused by extraction of the furfural by the maturing spirit.

A graph comparing the two types of staves with standard deviations for furfural concentration was generated by averaging the results for each layer (Figure 20). The averaged concentration profile shows the trends observed for the un-matured and matured staves. The large standard deviation associated with each layer does not allow for any parametrical analysis. The concentration profiles for the remaining spirit specific compounds all exhibit large standard deviation, but different curves and trends (Table 6) (Appendix 3).

Observation of the graph shows that there seems to be a point at which the two curves begin to deviate from each other. Further investigation of the data with nonparametric tests will allow for an amount of virgin wood to be calculated.

A one-way ANOVA was performed on each layer for all compounds followed by a Wilcoxon Rank-Sum test. Figure 21 shows the one-way ANOVA for furfural at layer 1 (0.87 mm). The concentrations at this layer are ranked and centered for each group, un-matured (U) and matured (M). After centering the Wilcoxon Rank-Sum test determines if the groups are equal or different. Using this nonparametric approach, the amount of virgin wood was calculated for each compound. Table 7 summarizes the results for all compounds and shows that the virgin wood remaining in the stave is not equal for all compounds and for most extends near the charred face of the cask.

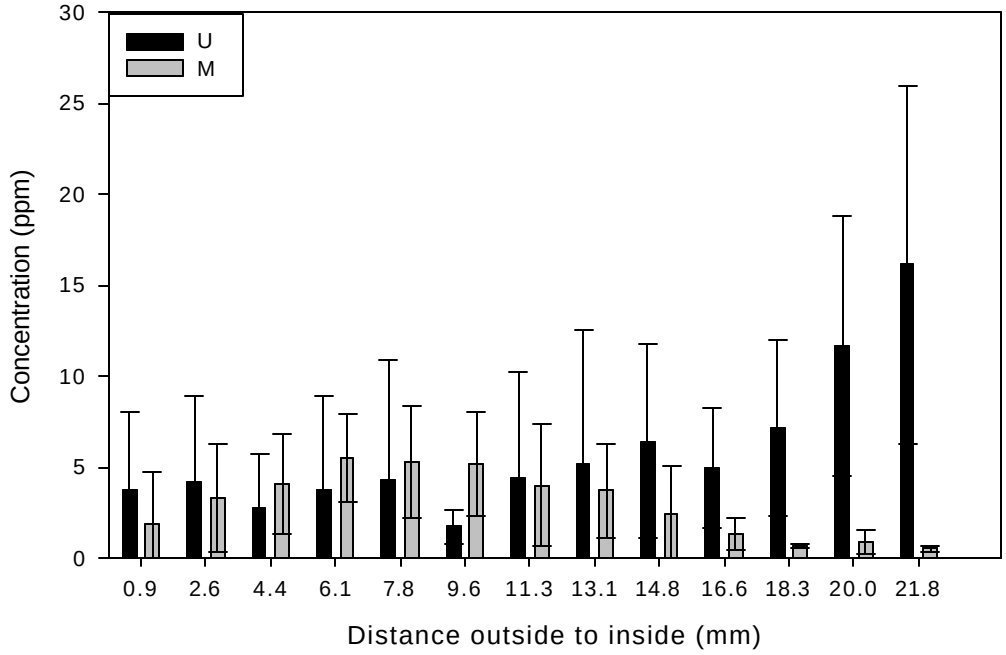


Figure 20: Concentration profile of furfural for matured (M) and un-matured (U) staves

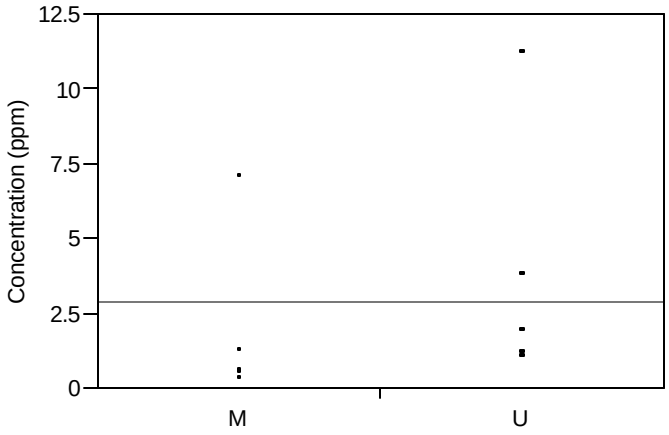


Figure 21: One way ANOVA of layer 1 (0.87 mm) for furfural

Table 6: Concentrations for investigated compounds at each layer for un-matured (U) and matured (M) staves

	Compound Concentrations, ppm											
	Gallic Acid Avg *Stdev*		HMF Avg *Stdev*		Furfural Avg *Stdev*		Syringic Acid Avg *Stdev*		Vanillin Avg *Stdev*		Syringaldehyde Avg *Stdev*	
Distance outside to inside (mm)	U	M	U	M	U	M	U	M	U	M	U	M
0.87	76.84 *82.62*	45.60 *63.89*	0.12 *0.26*	1.50 *3.36*	3.78 *4.29*	1.89 *2.87*	4.97 *4.46*	4.31 *3.50*	3.31 *3.52*	3.19 *1.21*	22.59 *10.70*	18.11 *9.03*
2.61	74.34 *80.23*	59.79 *50.62*	0.26 *0.59*	2.09 *2.95*	4.15 *4.83*	3.34 *2.99*	5.92 *5.34*	5.55 *4.10*	4.04 *4.15*	3.67 *2.18*	24.50 *13.05*	14.53 *7.87*
4.36	64.19 *66.12*	67.29 *52.68*	0.13 *0.28*	2.07 *2.88*	2.77 *3.00*	4.10 *2.80*	3.29 *1.76*	6.92 *3.44*	2.11 *1.41*	5.27 *2.49*	19.15 *3.26*	24.48 *8.28*
6.10	73.28 *84.91*	75.82 *59.95*	0.18 *0.24*	2.65 *2.12*	3.80 *5.08*	5.53 *2.47*	4.75 *5.47*	9.71 *5.35*	3.30 *4.32*	7.81 *3.31*	21.84 *12.78*	30.03 *14.05*
7.84	68.93 *86.90*	71.58 *61.37*	0.13 *0.28*	2.43 *2.43*	4.35 *6.51*	5.33 *3.08*	5.14 *5.36*	10.02 *5.84*	3.54 *4.25*	6.64 *2.66*	22.49 *13.03*	30.36 *14.68*
9.58	57.05 *63.00*	77.18 *62.67*	0.21 *0.32*	2.42 *2.26*	1.76 *0.94*	5.18 *2.84*	2.46 *1.21*	9.95 *5.73*	1.59 *0.76*	7.92 *3.10*	17.09 *5.52*	31.48 *14.27*
11.33	76.61 *84.26*	68.71 *52.25*	0.44 *0.41*	2.38 *2.32*	4.40 *5.85*	4.00 *3.35*	4.53 *4.51*	9.30 *6.09*	2.98 *3.57*	8.46 *4.98*	21.77 *10.46*	32.31 *19.46*
13.07	78.96 *94.67*	53.63 *33.54*	0.49 *0.47*	1.93 *1.72*	5.20 *7.31*	3.71 *2.60*	5.26 *6.50*	7.73 *5.13*	3.70 *5.25*	6.78 *4.36*	23.27 *15.13*	29.46 *16.36*
14.81	79.94 *83.18*	37.99 *21.58*	4.40 *4.11*	1.61 *1.76*	6.42 *5.34*	2.44 *2.68*	5.81 *4.83*	5.79 *5.14*	4.04 *3.35*	5.68 *5.20*	24.36 *9.96*	26.03 *14.99*
16.55	58.33 *58.86*	27.48 *14.86*	2.86 *2.94*	0.84 *0.84*	4.93 *3.30*	1.30 *0.88*	3.55 *1.55*	3.25 *1.10*	2.44 *1.23*	3.46 *1.18*	18.82 *3.81*	18.41 *6.08*
18.29	60.23 *63.44*	15.30 *3.94*	3.80 *3.64*	0.48 *0.32*	7.18 *4.83*	0.71 *0.08*	5.62 *4.13*	2.15 *0.76*	3.73 *2.88*	2.71 *1.65*	21.26 *8.32*	13.02 *3.11*
20.04	58.28 *58.92*	13.53 *10.04*	6.11 *4.45*	0.74 *0.64*	11.68 *7.16*	0.89 *0.66*	8.18 *5.04*	1.66 *0.20*	5.51 *4.05*	2.36 *0.40*	28.41 *10.64*	10.15 *3.27*
21.78	47.79 *44.52*	3.84 *2.35*	8.01 *3.63*	0.43 *0.24*	16.13 *9.85*	0.53 *0.13*	9.64 *7.03*	1.99 *0.25*	3.98 *3.03*	4.33 *0.47*	22.74 *9.06*	10.61 *1.09*

Table 7: Amount of virgin wood remaining in stave after maturation using univariate approach

Compound	Amount of Virgin Wood (mm)
Gallic Acid	18.29 (±0.28)
HMF	18.29 (±0.28)
Furfural	16.55 (±0.25)
Syringic Acid	18.29 (±0.28)
Vanillin	4.36 (±0.08) 9.58 (±0.15)
Syringaldehyde	18.29 (±0.28)

The statistical differences between the un-matured and matured staves for vanillin were found to occur at the layers corresponding to 4.36 and 9.58 mm. The results from the Wilcoxon Rank-Sum test (Table 8) show that although these layers were different, there was no statistical difference between the other layers. Analysis of the concentration profile across the stave for vanillin (Figure 22) also exhibits a drastic difference in the range at layer 6, but not layer 3. The deviations for layer 3 overlap but the mean is not centered statistically enough to consider the groups similar.

The results for gallic acid, HMF, syringic acid, and syringaldehyde all showed the amount of virgin wood to be 18.29 mm (±0.28). This amount seems to be large considering the results from the NIR analysis, but might exhibit some type of diffusion through the stave during maturation. The amount of virgin wood calculated using furfural, 16.55 mm (±0.25), which is less than that calculated by comparing the matured staves to new, seasoned wood. Also, the remaining layers extending towards the charred face of the cask were also statistically different. This might show that furfural is an important compound not only for influencing maturation, but also as a tag for determining the amount of virgin wood.

Using this approach amount of virgin wood remaining in the stave after maturation varied depending on which compound was being analyzed. Also, in the case for vanillin, only certain layers were statistically different and not a continuous section of the stave. This non-biased approach does not effectively give a consistent amount of virgin wood remaining in the stave. Other tests on the chemistry of the stave using the entire HPLC chromatogram, as opposed to individual compounds, must be performed in order to get an accurate consistent amount.

Table 8: Results from Wilcoxon Rank-Sum test for vanillin

Layer	Distance	Chi Square	Prob>ChiSq
1	0.87	0.273	0.602
2	2.61	0.535	0.465
3	4.36	3.938	0.047*
4	6.10	3.153	0.076
5	7.84	2.455	0.117
6	9.58	6.818	0.009*
7	11.33	3.153	0.076
8	13.07	2.455	0.117
9	14.81	1.320	0.251
10	16.55	2.455	0.117
11	18.29	0.011	0.917
12	20.04	2.940	0.086
13	21.78	0.200	0.655

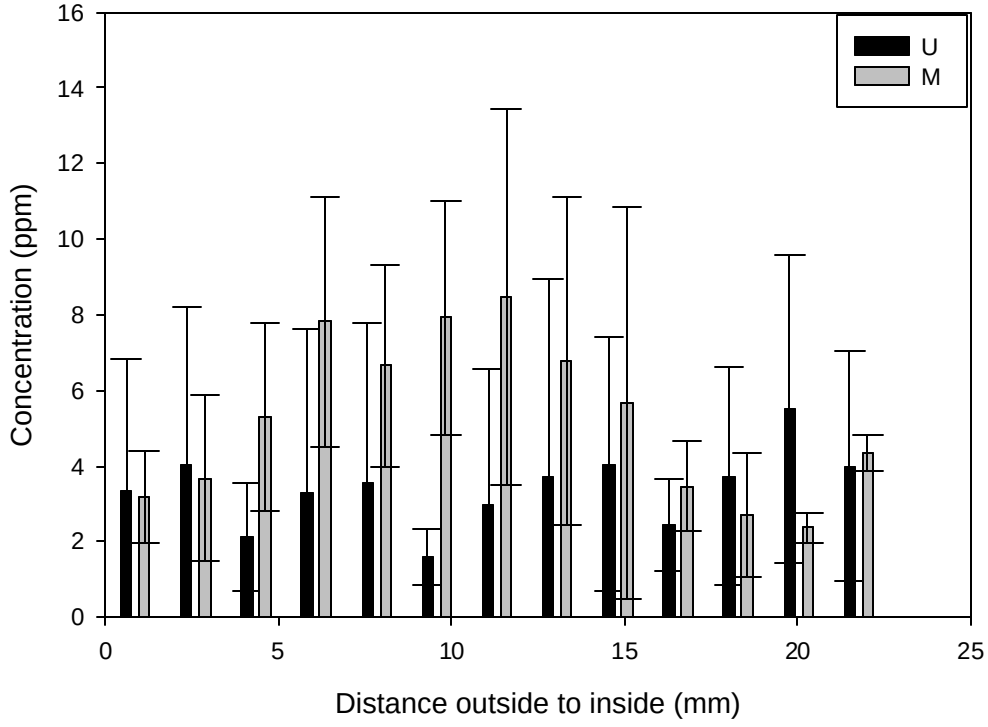


Figure 22: Concentration profile of vanillin for matured (M) and un-matured (U) staves

ii. Multivariate Approach for Determination of Virgin Wood

A multivariate approach was investigated to determine the amount of virgin wood remaining in a stave after maturation of the spirit. This approach focuses on a non-biased, direct comparison of the un-matured and matured staves while taking all of the chemistry of the stave into consideration.

A principal component analysis was performed on the entire HPLC chromatograms of the extracted samples in order to determine sources of variation in the data set (Figure 23). Including both types of staves in the PCA allows for direct comparison using the scores and loadings on the same scale for matured and un-matured results. This analysis shows the first two PCs account for 82% of the variation with the other PCs accounting for redundancy and noise.

The graph shows that the majority of the data aligns with PC1 which accounts for the maximum amount of variation (52%). PC2 exhibits a variation between some samples of the un-matured stave set and the rest of the samples. Further investigation of the scores and loadings for each of the PCs is needed to determine the relevancy of each.

The loadings of PC1 (Figure 24) show a significant peak at 1.85 minutes which corresponds to ethanol. 100 proof ethanol was used in the extraction procedure for each sample and should be present. The variation caused by ethanol might be significant, so the scores should be investigated to determine if the variation modeled shows any indication to the amount of virgin wood.

The scores for PC1 (Figure 25) shows there is variation between the un-matured and matured staves. If this variation was due to the diffusion of ethanol during the maturation process then there would be non variation in the un-matured staves. The vari-

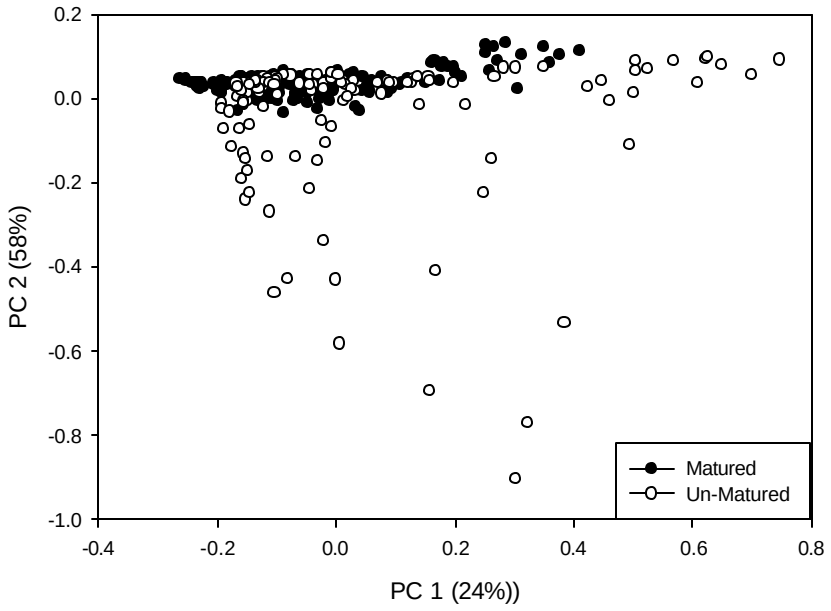


Figure 23: PC1 (58%) vs. PC2 (24%) for matured and un-matured staves

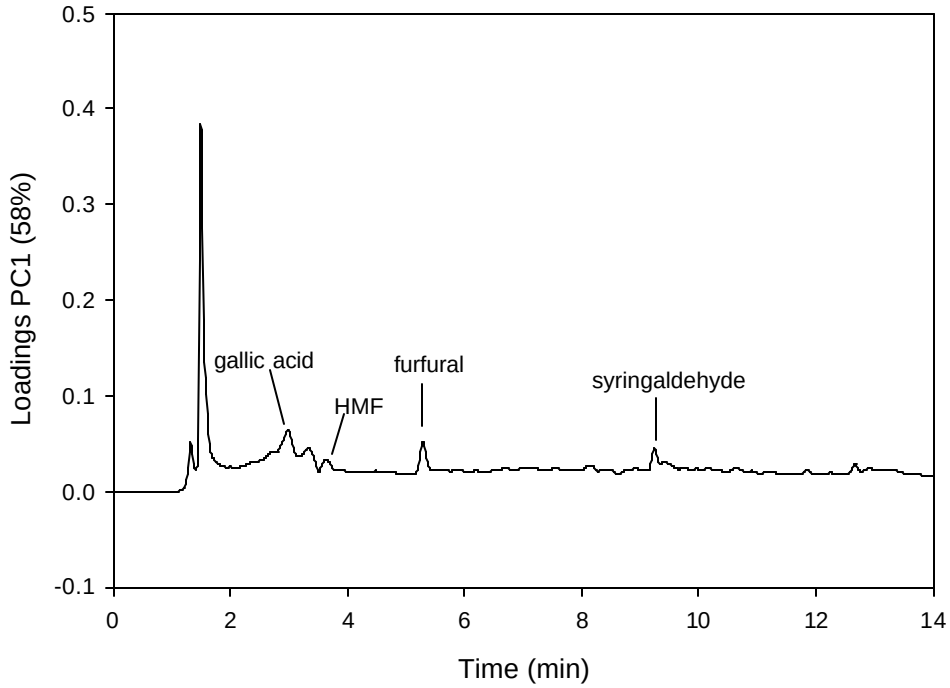


Figure 24: Loadings of PC1 (58%) for un-matured and matured staves

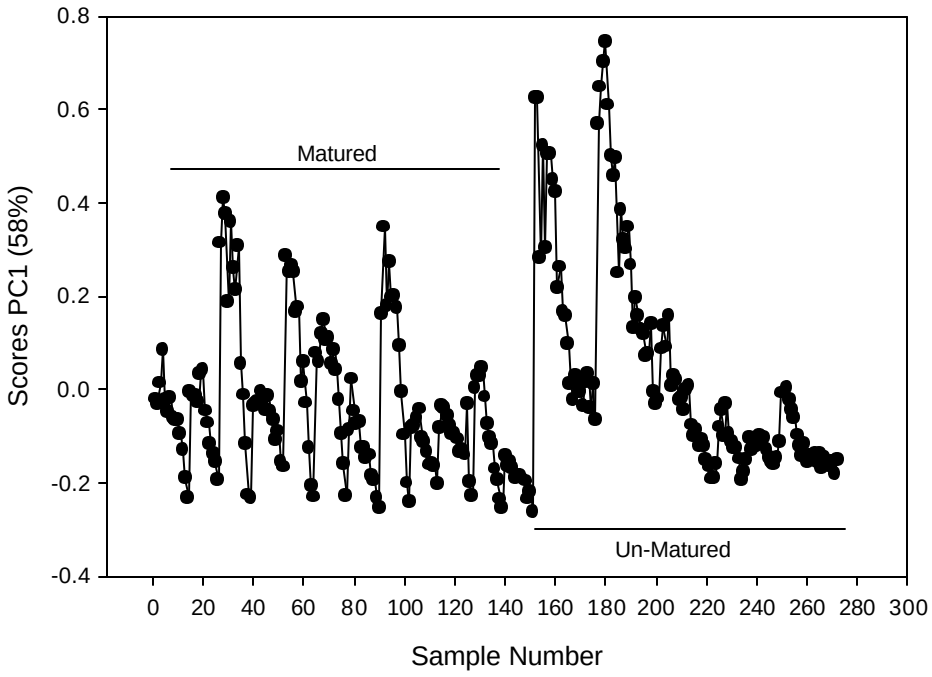


Figure 25: Scores of PC1 (58%) for un-matured and matured staves

-ation observed in those staves and ethanol as the most influential peak in this PC, shows the variation seen for all staves does not indicate an amount of virgin wood. This requires further investigation into the PCA results to determine if any relevant variation was extracted.

In the PCA, PC 2 accounts for 24% of the variation seen by analysis of the data set. The scores for the PC (Figure 26) show a trend for the matured and un-matured staves. Each point on the graph corresponds to a layer of the labeled stave. Towards the charred face of the cask, there is significant variation for the un-matured staves. Using this PC, the matured staves act as a baseline with deviation by the un-matured staves. To determine if the variation observed in PC2 is based on relevant chemistry of the stave, the loading must be investigated.

The loadings (Figure 27) can be used to determine if the variation is a result of chemistry of the stave or interference caused by the detector. Observation of the loadings shows that the main peak contributing to the variation is furfural. The peak for ethanol is present in this PC as well, but the magnitude in relation to other peaks is lower. The variation caused by ethanol in PC1 was removed by the analysis prior to construction of PC2. This shows that the ethanol present in this variation might be due to a type of diffusion of the spirit.

The loadings also show that coniferaldehyde and ellagic acid are influential to this variation. These compounds quickly oxidize and generation of calibration curves for these were not consistent. Also, the samples were placed in an autosampler prior to injection into the HPLC. Although Teflon© caps were placed over the sample vials, oxidation could still possibly occur which could result in the inconsistency observed.

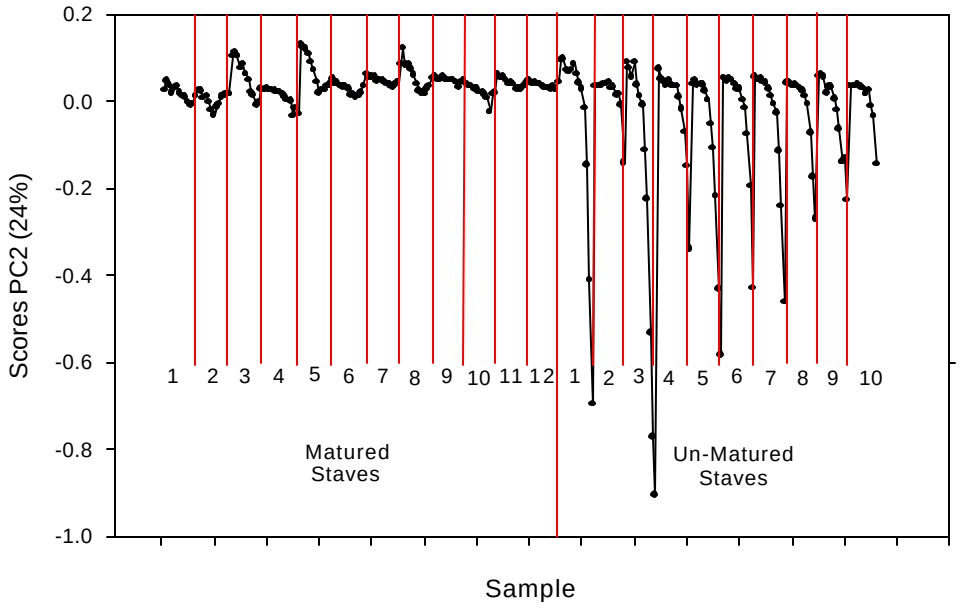


Figure 26: PC 2 (24%) for matured and un-matured staves

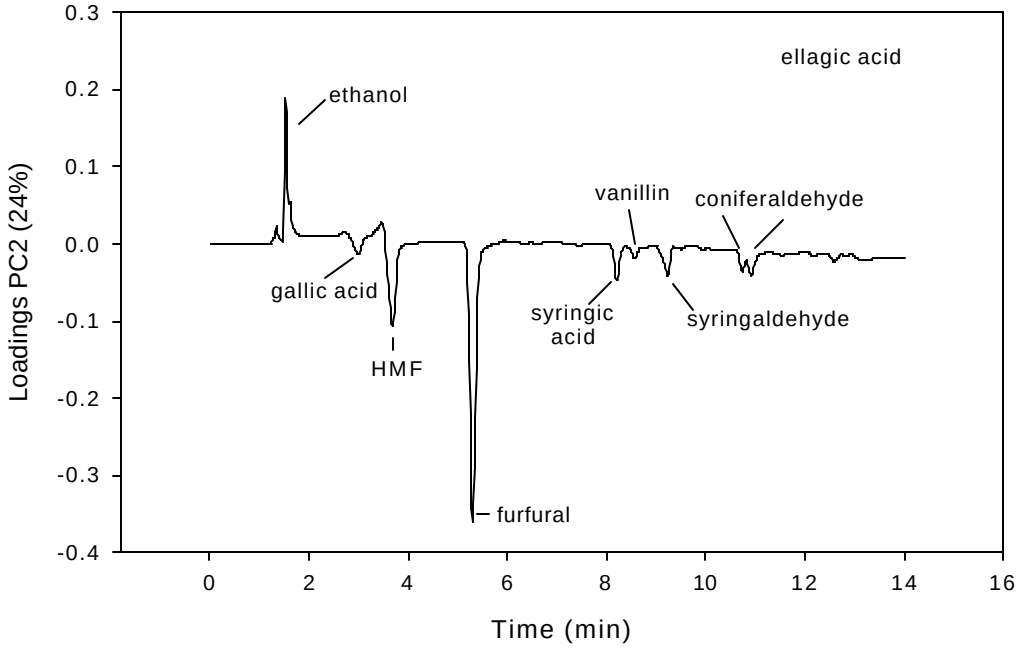


Figure 27: Loadings of PC 2 (24%) for un-matured and matured staves

By determining the relevance of PC 2, statistical tests can be used with the scores in order to calculate the amount of virgin wood remaining in the stave. The scores were separated into the original respective staves and put on the same X-axis, distance from outside to inside of stave, in order to determine the position of the variance between the two types of staves (Figure 28). Control charts and other parametric tests could not be used due to a population size of <30.

The data were organized into Un-Matured and Matured data sets with layer one being on the external face of the cask. A one-way ANOVA was performed on the layer one to determine how the scores are distributed across the two groups (Figure 29). This test can be performed because the scores are on the same scale which resulted from including all the staves in the PCA.

The nonparametric Wilcoxon Rank-Sum test was applied to the results of the one-way test to determine a statistical difference between the un-matured and matured staves. The test estimates a chi square value, test statistic, for the data set. A 95% confidence limit is used to determine the probability of obtaining a chi-square value higher than the one calculated by chance alone. If the probability is less than 0.5, the distributions across the two factor levels are not centered at the same location and are thus, unequal.

This process was repeated for the scores of all layers starting in a stepwise manner with layer one and continuing for all layers of the stave. Table 9 shows the layers with their respective chi square values and probability values. An asterisk denotes a statistical difference between the un-matured and matured staves.

The one-way ANOVA with the Wilcoxon Rank-Sum test at layer 9 (Figure 30) shows there is a statistical difference between the un-matured and matured staves. This

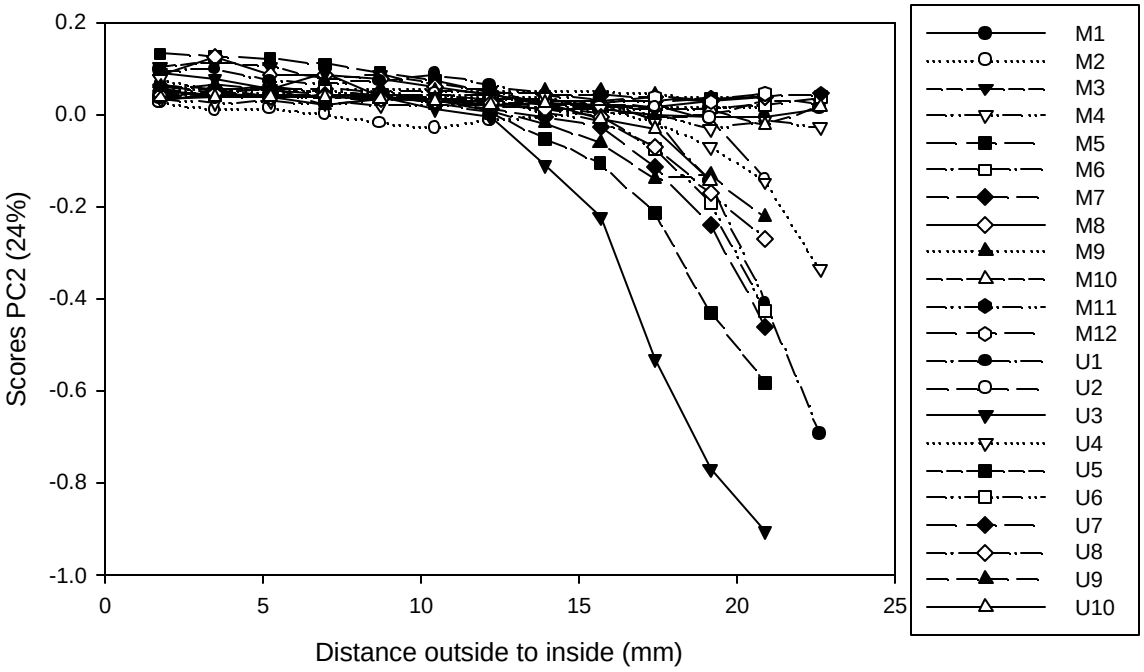


Figure 28: Distribution of scores from PC 2 (24%) for all staves

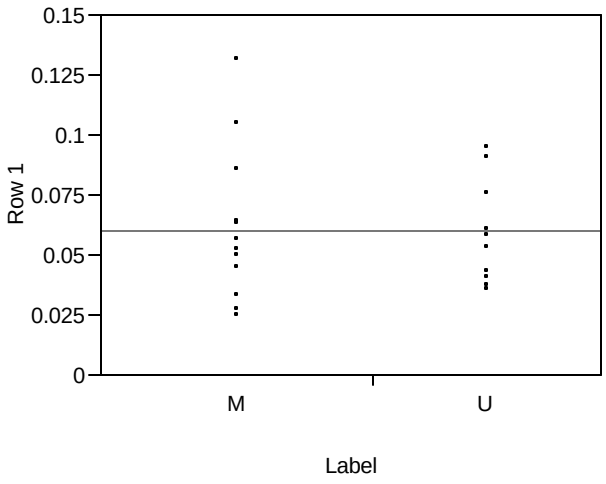


Figure 29: One way ANOVA for layer one of un-matured (U) and matured (M) staves

Table 9: Results from Wilcoxon Rank-Sum test for multivariate approach

Layer	Position on Stave (mm)	Chi Square	Prob>ChiSq
1	1.74	0.004	0.947
2	3.48	0.017	0.895
3	5.23	0.157	0.692
4	6.97	0.010	0.921
5	8.71	0.017	0.895
6	10.45	1.409	0.235
7	12.20	2.402	0.121
8	13.94	1.409	0.235
9	**15.68**	**8.422**	**0.0037***
10	**17.42**	**12.682**	**0.0004***
11	**19.17**	**15.135**	**0.0001***
12	**20.91**	**14.143**	**0.0002***
13	**22.65**	**4.000**	**0.0455***

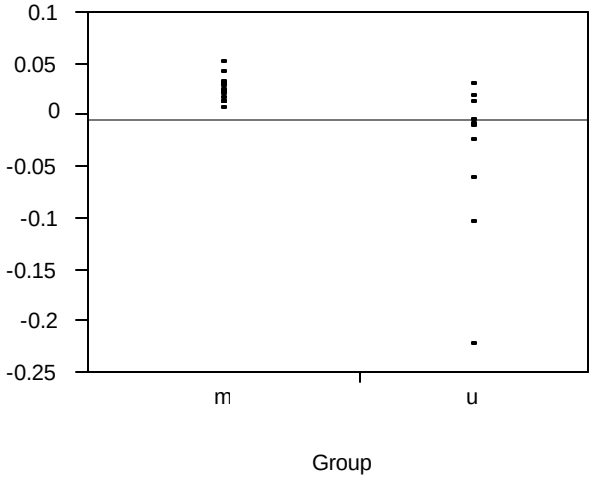


Figure 30: One way ANOVA for un-matured (U) and matured (M) staves at layer 9

layer corresponds to 13.94 (±0.23 mm) into the stave, with the beginning as the external, non-charred face of the stave. The variance for this layer was calculated as the error of the milling machine (±0.025 mm) times the layer number. The remaining layers were also analyzed using this method and were calculated as significantly different.

Including ethanol in the analysis showed that it was an influential compound in determining the variation between un-matured and matured staves. All of the samples are extracted with ethanol, so to be sure that the variation was not influenced by minor ethanol concentrations the analysis was performed omitting the ethanol peak.

After removal of this peak, a PCA was performed on both the matured and un-matured staves. The loadings for PC2 (Figure 31) show that furfural is the main source of variation for this data with the other compounds investigated also contributing. One-way ANOVAs were performed on each of the layers as was performed previously in this section. The results from the Wilcoxon rank-sum test (Table 10) show that removal of the ethanol peak does not influence the amount of virgin wood remaining in the stave. Due to this, inclusion of the ethanol peak might show a type of diffusion present during the maturation process.

The amount of virgin wood remaining in the stave after maturation could be calculated using this method. The amount is based on known chemistry influential to the maturation process of the spirit. Using other approaches, consistent limits based on all the chemistry for the stave could not be determined. The multivariate approach on the HPLC chromatograms of the extract allowed for a non-biased method which yielded an amount of virgin wood as 13.94 mm (±0.23).

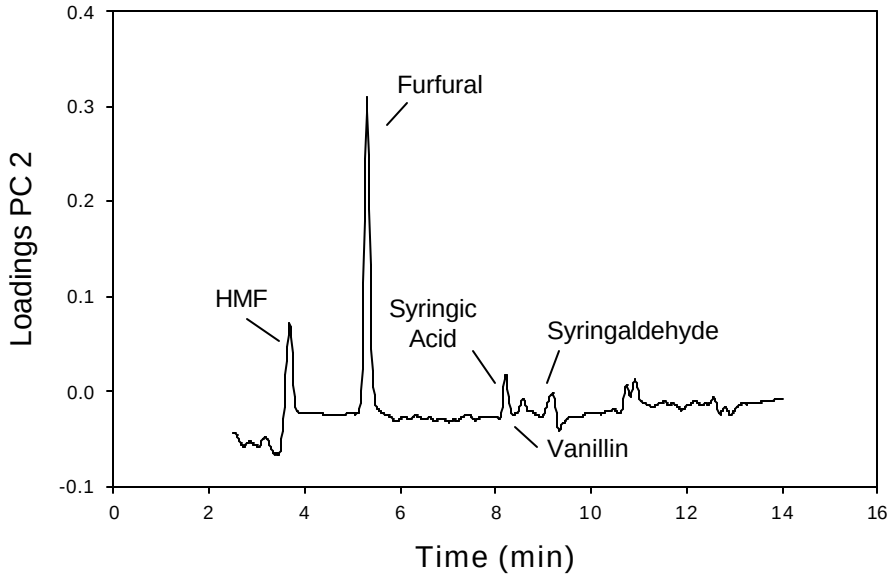


Figure 31: Loadings of PC2 analyzed without ethanol peak

Table 10: Results from Wilcoxon Rank-Sum test analyzed without ethanol peak

Layer	Position on Stave	Chi Square	Prob>ChiSq
	(mm)		
1	1.74	0.28	0.6
2	3.48	0.85	0.36
3	5.23	1.74	0.19
4	6.97	1.41	0.23
5	8.71	1.57	0.21
6	10.45	0.98	0.32
7	12.2	2.11	0.15
8	13.94	3.17	0.08
9	**15.68**	**4.73**	**0.03***
10	**17.42**	**4.45**	**0.04***
11	**19.17**	**6.96**	**0.01***
12	**20.91**	**14.1**	**2E-04***
13	**22.65**	**4**	**0.04***

The analysis performed on the extract was done with the PDA detector set at 280 nm. The compounds investigated have maximum absorption at this wavelength when extracted with ethanol. If the analysis had been done at a different wavelength, other compounds would have appeared in the results. The amount of virgin wood remaining in the stave could vary due to this fact, but the information contained in this project shows that the amount should not vary by much.

Also, due to the population size used to develop this method, nonparametric tests had to be implemented. Including more samples in the method would allow for parametric statistical tests. However, the amount of virgin wood remaining in the stave after maturation should remain near the layer concluded using the nonparametric tests.

C. Predictive Models for Spirit Specific Compounds

The ability to predict the chemical characteristics naturally present in a stave would enable distillers to engineer casks with specific chemical compositions. By correlating the quantified results from the HPLC with spectra taken using the NIR spectrometer, models can be generated to predict the amount of spirit specific compounds available for maturation. The concentrations for the spirit specific compounds used in this method were obtained during the univariate approach for the determination of virgin wood.

After pretreating the NIR spectra for the samples, as mentioned in the methods section (3.C.ii), the quantified HPLC concentrations from the extract were placed into the

Unscrambler© worksheet. The compounds studied using the following method are gallic acid, HMF, furfural, syringic acid, and syringaldehyde.

A partial least squares regression 1 (PLSR1) was performed on the NIR spectral data with the HPLC quantified results for each compound. A predictive model was generated for each compound using a full cross-validation. Once the original model was generated, outlier tests were performed using the **X** and **y** residual sample variance plots (Figure 32 and 33) to show both calibrated and validated sample variance. Points that have a high residual variance, are not explained well by the model and should be considered outliers. Using these graphs, statistically outlying points were removed for the calibrated and predictive models.

The results in Table 11 show the root mean-square error for calibration and validation as well as the correlation coefficients for each model. By removing outliers and samples under the limit of detection, statistically sound models were generated for each compound.

The regression coefficients can be used to determine if the model is based on significant peaks or random noise. Investigation into the regression coefficients for gallic acid (Figure 34) shows that the important peaks are located in the range for stretching and bending of O-H, C-H bonds. Also the presence of an aromatic C-H and an aldehyde are in the regression coefficients. All of these bonds are contained in the structure of gallic acid which shows that this model is based on actual bands and not random peaks and noise.

Further investigation of the regression coefficients for furfural can provide evidence that the model is valid. Furfural is the only compound investigated in this

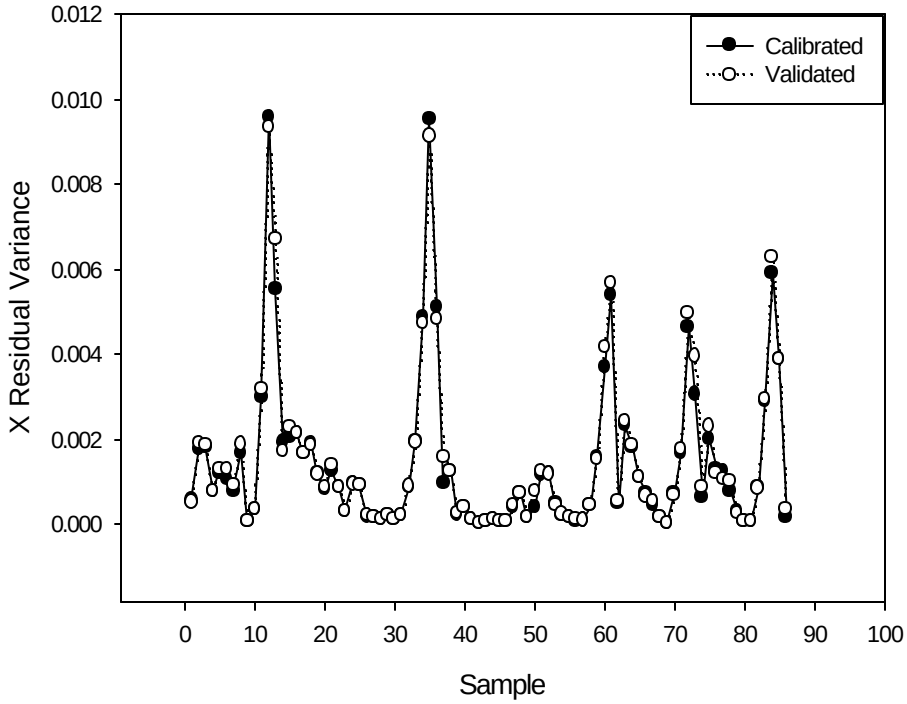


Figure 32: X residual variance plot for gallic acid

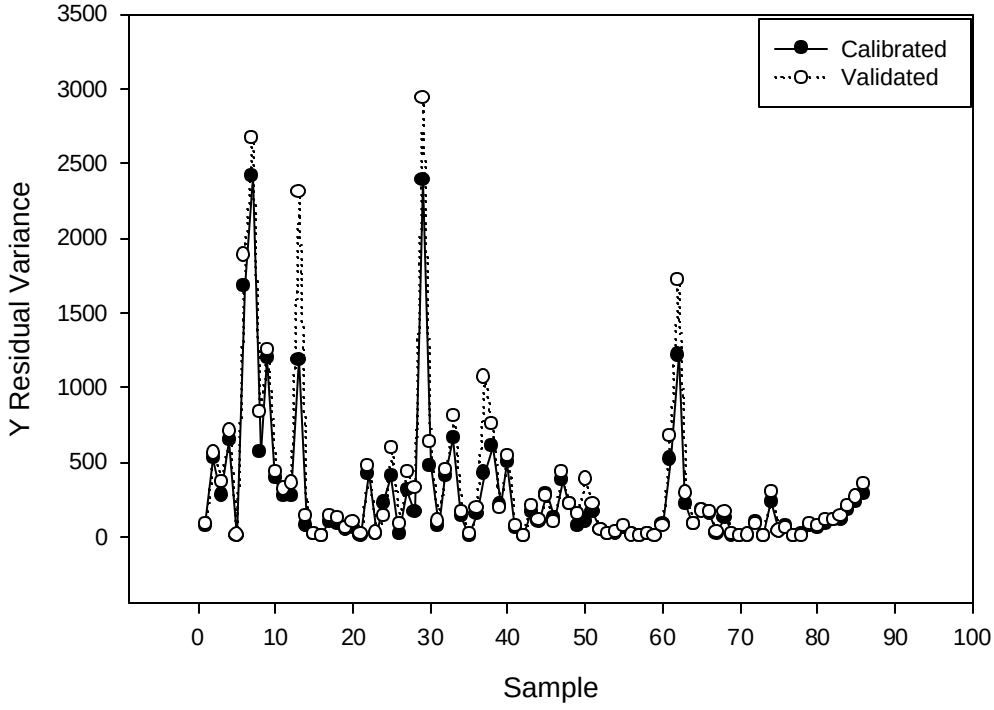


Figure 33: Y residual variance plot for gallic acid

Table 11: PLSR1 results for investigated compounds

Compound	R cal	R val	RMSEC	RMSEV	# of samples	PC
gallic acid	0.939	0.899	7.16	12.65	55	5
HMF	0.917	0.863	1.29	1.61	13	2
furfural	0.899	0.808	3.06	4.8	12	2
syringic acid	0.98	0.868	0.567	3.54	13	4
vanillin	0.997	0.791	0.0634	4.61	11	5
syringaldehyde	0.912	0.831	5.07	8.44	40	4

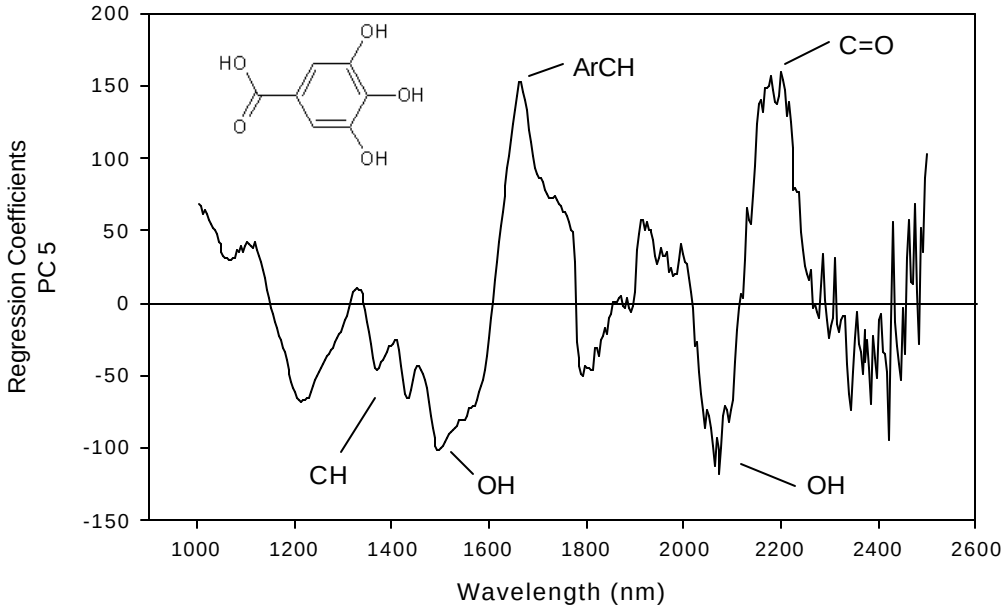


Figure 34: Regression coefficients for gallic acid using PC 5

method which does not contain an O-H bond. The regression coefficients for PC 2 (Figure 35) show that the model constructed is based on C-H and C=O bonds. Although the magnitude of these bands in the regression coefficients is not large, the relative size of these known bands to noise is large. Although the number of samples used to construct the model was not large, there seems to be correlation between the concentrations of furfural and the NIR spectra

The average concentration of samples used to construct the model for HMF was below the limit established by the calibration curve. Due to the fact that HMF is mainly produced in the charred region of the stave, there were not many samples available for analysis. The model generated is statistically applicable although the resulting predictions might not be accurate in the range used for construction.

The root mean square error of calibration and validation for the models can be examined in order to determine if the values are too large in comparison with the quantified HPLC results. Table 12 shows the percent error associated with the RMSE for both the calibration and validation models for all compounds. The percent error of the RMSEV associated with vanillin was the largest of the group. Previous investigation into predictive modeling for vanillin (Doussot 2002) has also showed large RMSEV values with respect to original sample values.

The most statistically sound model concluded from investigation into the RMSEs and regression coefficients would be gallic acid (Figure 36). The correlation for the validation of this model and the number of samples available for the data set were highest with this compound. As a naturally occurring extractive that contributes an unwanted

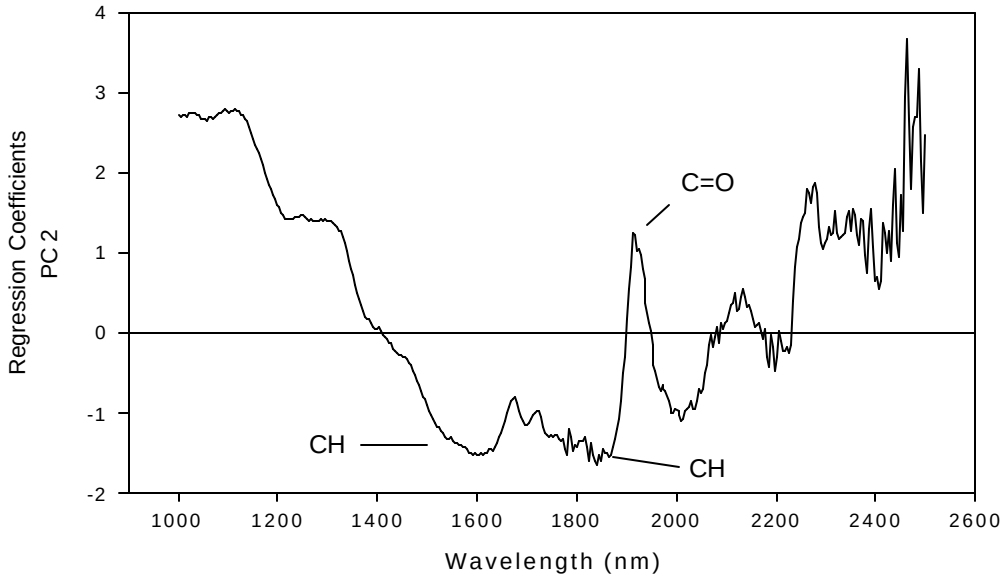


Figure 35: Regression coefficients for furfural using PC 2

Table 12: Percent error of RMSEC and RMSEV for investigated compounds

Compound	Average Concentration (ppm)	% Error Calibration	% Error Validation
gallic acid	66.10	10.83	19.14
HMF	8.43	15.30	19.10
furfural	16.12	18.99	29.78
syringic acid	13.67	4.15	25.90
vanillin	11.64	0.55	39.60
syringaldehyde	24.42	20.77	34.57

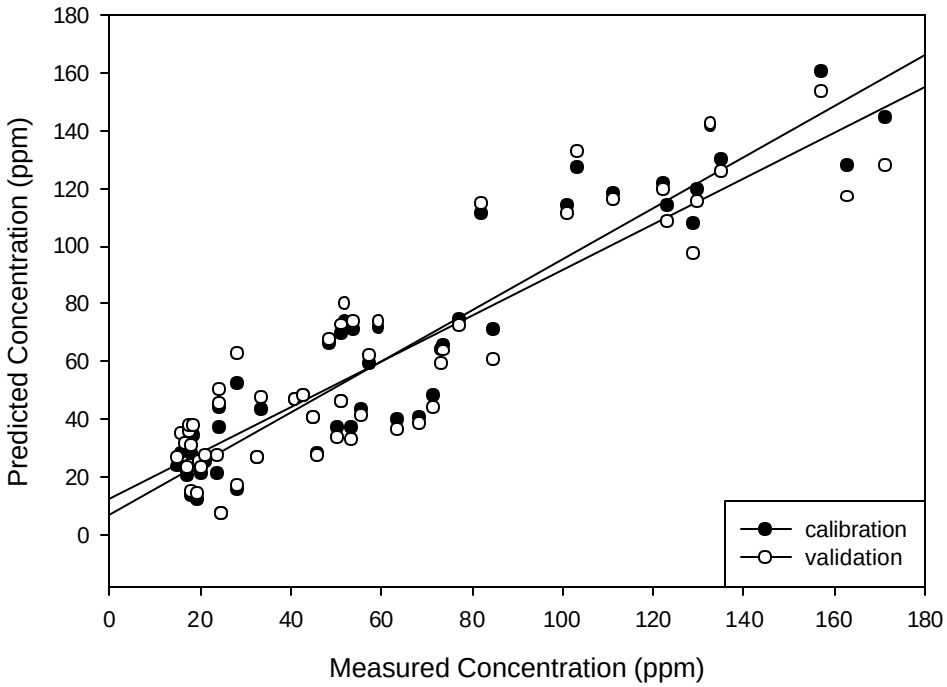


Figure 36: Regression model for gallic acid

aromatic and bitter taste to the spirit, forehand knowledge of concentrations of gallic acid present in the stave would be useful.

The method shows that although these models cannot be used to predict the amount of these compounds present in solid wood, they do show the possibility of correlation between two types of data. More samples must be tested in order to generate models which are applicable to casks used for the maturation of spirits.

5. Conclusions

The maturation process not only filters the spirit of residual compounds from the distillation process, but also allows for extraction of compounds from the wood. The extraction of these compounds modifies the wood by decreasing the availability of these compounds present. The wood that remains unaffected by this process can be reused for a second generation cask or other industrial applications.

Investigation into the intra-stave variability using NIR spectroscopy showed that the amount of virgin wood remaining was 21.2 mm (±0.02). The drastic effect to the wood by charring the internal face of the barrel seems to be the main source of this variation. A comparison of the matured staves with new, unprocessed wood using the same technique yielded an amount of virgin wood to be 17.50 mm (±0.02). This amount was calculated omitting the charred layers of the stave in order to determine any variation seen without this influence. The effect of the thermal treatment to the stave could be the cause of this variation, but because NIR identifies the biggest chemical variation across the staves, it is difficult to specify.

The next technique focuses on two methods applied to the HPLC analysis of the extract. The univariate approach to determining the amount of virgin wood by analysis of the extract yielded multiple amounts. The range of virgin wood was 3.48-17.42 mm depending on which compound was analyzed. Also, for vanillin there were not continuous points out of control, but instead only two of the thirteen layers. This technique can not consistently yield an unbiased amount of virgin wood remaining in the stave, but instead models the flux of individual compounds during the maturation process.

Examination of the extract using multivariate analysis allowed for investigation into all of the chemistry of the stave. The amount of virgin wood was determined to be 13.94 (±0.23 mm). Removal of the ethanol contribution to the variation from the analysis did not change the amount calculated. This shows that the analysis might reveal the position of the stave affected by the diffusion of ethanol, either in aqueous or gaseous form. Also, the amount of virgin wood was calculated using two different data sets (with and without ethanol). The information obtained is reliable and based on the factors that influence spirit maturation.

The amount of virgin wood remaining in the stave is not the only issue facing distillers. The ability to know the concentrations of certain compounds, influential to the maturation process, that are naturally present in the stave will allow for the construction of casks of a specific standard.

Analysis of the regression coefficients shows that although the population size is small, there seems to be correlation between the NIR spectra and quantified HPLC concentrations using known wavelengths.

6. Future Recommendations

Modifications to the method for this project can be made in order to eliminate some experimental and instrumental error. Using a more accurate technique for the removal of layers from the stave should help to narrow the point at which the staves are different. Also, investigation into the staves was begun starting on the external, non-charred, face of the stave for stability purposes with using the milling machine. The variation in the thickness of the staves causes a range for the number of layers in each stave. By beginning the milling of the layers process on the interior, charred, face of the stave would allow for a greater examination into the modifications in the stave due to spirit diffusion. However, the curvature of the stave limited the way the samples could be prepared with the available machinery.

In analysis and quantification of the extracted powder samples, revisions to the method can be made to yield more accurate results. The compounds investigated are not in a steady form. Oxidation occurs which causes the modification of important compounds into non-spirit specific compounds. Examples of this include the oxidation of furfurals into lactic acid and an oxidation of syringaldehyde into syringic acid. By performing the analysis on the HPLC immediately after extraction should allow for more accurate quantified results.

The nonparametric Wilcoxon Rank-Sum test was used because of the population size of the samples. By increasing the number of samples should allow for the use of more advanced statistical models, such as control charts. Also, sample outliers could be removed if a normal distribution is seen in the scores for each layer.

There are two types of diffusion present in the maturation of the spirit with multiple causes for each. Differences in pressure, temperature, and humidity subjected on the cask during maturation causes expansion and contraction of the staves. Aqueous and gaseous diffusion of the spirit and compounds associated with the wood occurs during this time period. A greater understanding of how the process works and a more real time limit to the changes occurring during this process should be investigated using multiple techniques.

Radioactively tagged ethanol could be used for maturation instead of the distilled ethanol. The tagging of ethanol would allow for spectroscopic identification for the amount of wood penetrated by aqueous ethanol. Another possible solution to this problem could be to create a model of a cask using a membrane in place of the oak staves. By scaling the model down, gaseous and liquid diffusion could be determined in a much shorter time span than the two to five years required for spirit maturation.

Future modifications to the predictive modeling for spirit specific compounds could also be made to create more accurate models. A PLSR1 was used in this project because the number of samples pooled with concentrations above the limit of detection was small. The direct correlation between some of the compounds, i.e. syringic acid and syringaldehyde, would be better predicted using a PLSR2 model. Although this model would increase the ability to predict concentrations in samples, the problem of oxidation of samples would still need to be investigated.

Other instrumentation can also be implemented to yield a more accurate determination for the amount virgin wood remaining in the stave. A gas chromatograph with mass spectrometer would allow for the identification of unknown peaks seen in the

HPLC chromatograms and loadings used with the PCA. This identification can show if there are certain compounds specific to ethanol-wood interactions. Observation of any compound not native to the natural wood chemistry and the thermal degradation would show a point at which spirit maturation affects the stave.

References

References

ASTM (2005) Designation: E 1655—97 Standard Practices for Infrared Multivariate Quantitative Analysis, Annual Book of ASTM Standards **14**, 844-869

Bednar, H.; Fengel, D. (1974) Physical, chemical, and structural properties of recent and subfossil oak wood. Holz als Roh- und Werkstoff **32**, 99-107

Beebe, K.; Pell, R.; Seasholtz, M. *Chemometrics: A Practical Guide*. New York, NY: John Wiley & Sons, 1998

Burns, D.; Ciurczak, E. *Handbook of Near-Infrared Analysis*. 2nd ed. New York, NY: Marcel Dekker, Inc., 2001

Chatonnet, P.; Pons, M. (1989) Incidence du traitement thermique du bois de chêne sur sa composition chimique, 2e partie: evolution de certains composes en function de l'intesité de brûlage. Connaissance de la Vigne et du Vin **23**, 223-250

Core, H.; Côté, W.; Day, A. "Wood: Structure and Identification," 2nd ed. Syracuse NY: Syracuse Univ. Press 1979

Doussot, F.; De Jeso, B.; Quideau, S.; Pardon, P. (2002) Extractives content in cooperage oak wood during natural seasoning and toasting; influence of tree species, geographic location, and single-tree effects. Journal of Agriculture and Food Chemistry **50**, 5955-5961

Doussot, F.; Pardon, P.; Dedier, J.; De Jeso, B. (2000) Individual, species and geographic influence on cooperage oak extractible content. Analusis **28**, 960-965

Husson, H.; Capdevielle, P. (2000) Extension of the French barrel-industry and capacity of private oak forest. Revue des Oenologues **94**, 1-6

Kelley, S.; Rials, T.; Snell, R.; Groom, L.; Sluiter, A. (2004) Use of near infrared spectroscopy to measure chemical and mechanical properties of solid wood. Wood Science and Technology **38**, 257-276

Lee, M.; Paterson, A.; Piggott, J. (2001) Origins of Flavour in Whiskies and a Revised Flavour Wheel: a Review. Journal of the Institute of Brewing **107**, 287-313

Liebmann, A.; Rosenblatt, M. (1943) Changes in whiskey while maturing. Industrial and Engineering Chemistry **35**, 994-1002

Liebmann, A.; Scherl, B. (1945) Changes in whiskey while maturing. Industrial and Engineering Chemistry **41**, 534-543

Martens, H.; Naes, T. *Multivariate Calibration.* New York, NY: John Wiley & Sons 1989

Masson, G.; Puech, J-L.; Moutounet, M. (1995) Ellagaitannins content of oak wood as a function of species and of sampling position in the tree. American Journal of Enology and Viticulture **46**, 262-268

Mendenhall, R.; Beaver, R.; Beaver, B. *Introduction to Probability and Statistics.* 12th ed. Belmont, CA: Thomson Brooks/Cole, 2005

Mosedale, J.R.; Ford, A. (1996) Variation of the flavour and extractives of European oak wood from two French forests. Journal of the Science of Food and Agriculture **70**, 273-287

Mosedale, J. R.; Puech, J. - L. (1998) Wood maturation of distilled beverages. Trends in Food Science & Technology **9**, 95-101.

Nishimura, K.; Matsuyama R. "Maturation and Maturation Chemistry." *The Science and Technology of Whiskies.* London, England: Longman Scientific and Technical, **1989**, 253-363

Pettersen, R.C. (1984) The chemical composition of wood**.** Advances in Chemistry Series **207**, 57-126

Puech, J. L.; Jouret, C.; Deibner, L. (1978) Phenolic substances in Armagnac spirits Industries Alimentaires et Agricoles **95**, 13-22.

Ramiah, M. (1970) Thermogravimetric and differential thermal analysis of cellulose, hemicellulose, and lignin. Journal of Applied Polymer Science **14**, 1323

Reazin, G. (1981) Chemical mechanisms of whiskey maturation. American Journal of Enology and Viticulture **32**, 283-289

Skoog, D.; Holler, F.; Nieman, T. *Principles of Instrumental Analysis*. 5th ed. Belmont, Ca: Thomson Learning. 1998.

Sjöström, E.; *Wood Chemistry: Fundamentals and Applications*. 2nd ed. San Diego, Ca; Academic Press. 1993.

Spillman, P.; Sefton, M.; Gawel, R. (2004) The contribution of volatile compounds derived during oak barrel maturation to the aroma of a Chardonnay and Cabernet Sauvignon wine. Australian Journal of Grape and Wine Research **10**, 227-235

Swan, J.; Buchanan, B.; Jones, P. (1996) The use of electro-heat techniques for the treatment and forming of whiskey casks. International Congress on Electricity Applications, Birmingham, UK. June 16-20

Takeshi, S.; Hisao, K.; Shingo, N.; Takao, I. (2005) Emission of Volatile Organic Compounds from Wood-based Materials. Reports of the Mie Prefectural Science and Technology Promotion Center Industrial Research Division **29**, 82-84

Twede, D. (2005) The Cask Age: the Technology and History of Wooden Barrels. Packaging Technology and Science **18**, 253-264

Valaer, P.; Frazier, W. (1936) Changes in whiskey stored four years. Industrial and Engineering Chemistry **28**, 92-105

Vivas, N.; Nonier, M.; De Gaulejac, N.; Boissel, I. (2004) Occurence and partial characterization of polymeric ellagitannins in Quercus petraea Liebl. and Q. robur L. wood. Comptes Rendus Chimie ***7***, 945-954

Wagenführ, R. "Anatomie des Holzes unter besonderer Berücksichtigung der Holztechnik," 2nd ed. VEB Fachbuchverlag, Leipzig. 1980

Waters Corporation. "Waters 2996 PDA Detector: Operator's Guide." Milford, MA: Waters Corporation, 2001

Yoshizawa, Kiyoshi; Momose, Hiroo; Hasuo, Tetsuo. (1981) Studies on aging of whiskey. American Journal of Enology and Viticulture **55**, 1063-8

Appendices

Appendix 1: List of equations

Mean Normalization:

$$X_{(i,k)} = \frac{X_{(i,k)}}{\left|\overline{X}_{(i,*)}\right|}$$

Mulitplicative Scatter Correction:

$$X_{(i,k)} = \frac{X_{(i,k)} - a}{b}$$

a – intercept of regression line
b – slope of regression line

Gilbert Formula:

$$C.L. = \overline{X} \pm 3\frac{\overline{(mR)}}{2\sqrt{(1-r_1)/p}}$$

Moving Range:

$$\overline{mR} = \frac{1}{n-1}\sum_{t=1}^{n-1}\left|x_{t+1} - x_t\right|$$

Autocorrelation Coefficient:

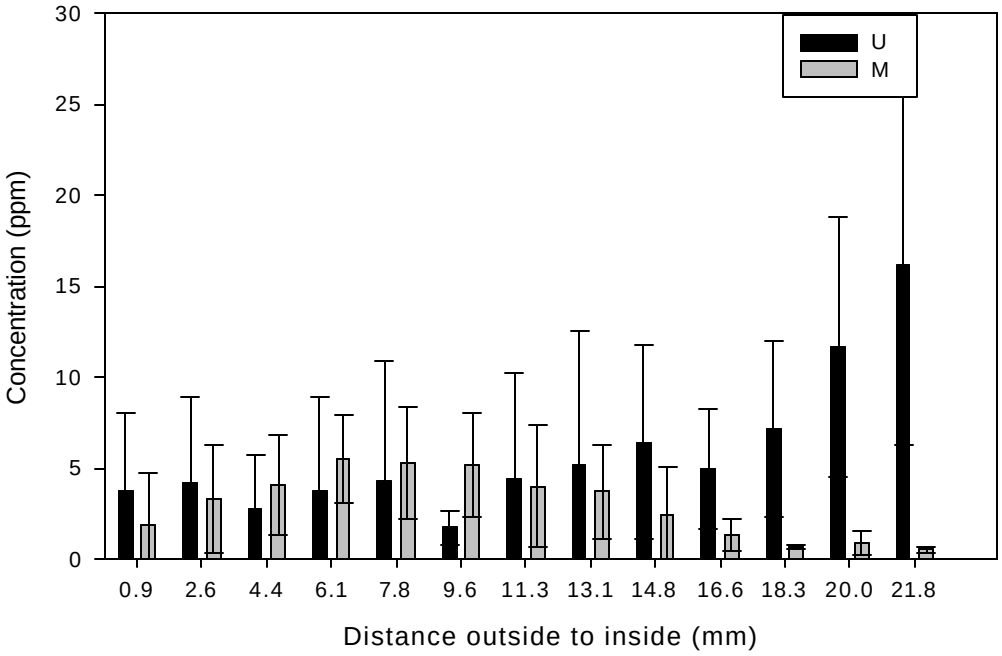
$$r_1 = \frac{\sum_{t=1}^{n-1}(x_t - \overline{x})(x_{t+1} - \overline{x})}{\sum_{t=1}^{n}(x_t - \overline{x})^2}$$

Appendix 2: MATLAB© file of control chart for determination of virgin wood by analysis of stave surface

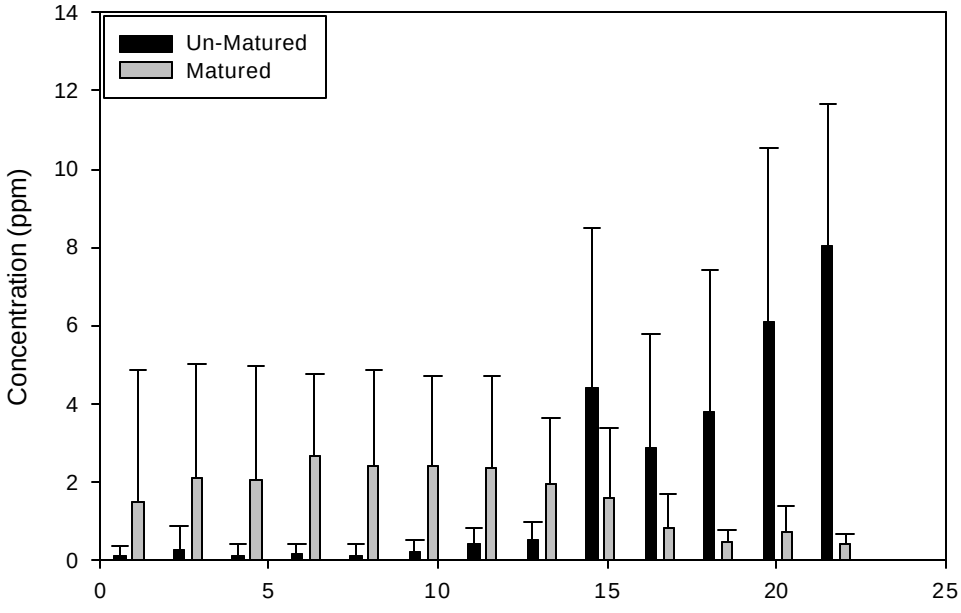
```
[namey]=uigetfile('*.txt','Select a text file');
stringloady=strcat('y=load('',namey,'');');
eval(stringloady);
%%%
c0=0;
c1=0;
mr=0;
outliers=[];
[m,n]=size(y);
for i=1:m-1
%c1(i)=(y(i)-mean(y))*(y(i+1)-mean(y));
%c0(i)=(y(i)-mean(y))^2;
%mr(i)= y(i+1)-y(i);
c1=c1+(y(i)-mean(y))*(y(i+1)-mean(y));
mr=mr+abs(y(i+1)-y(i));
end
for j=1:m
c0=c0+(y(j)-mean(y))^2;
end

%c0=sum(c0)/m;
%c1=sum(c1)/m;
%mr=sum(mr)/m;
%c0=c0/m;
%c1=c1/m;
mr=mr/(m-1);

r1=c1/c0
%LCL-UCL- Gilbert et al.
for i=1:1
lcl=mean(y)-3*mr/(2*sqrt((1-r1)/pi));
ucl=mean(y)+3*mr/(2*sqrt((1-r1)/pi));
end
x=1:1:m;
x=x/10;
lcl=ones(m,1)*lcl;
ucl=ones(m,1)*ucl;

figure,plot(x,y,x,lcl,x,ucl)
axis tight
for i=1:m
   if y(i)>ucl(i) | y(i)<lcl(i)
      outli=cat(2,y(i),i);
      outliers=cat(1,outliers,outli);
   end
end
if isempty(outliers)==1
disp('NOTHING IS OUT OF CONTROL')
else
outliers1=outliers(:,2)/10;
outliers=cat(2,outliers(:,1),outliers1);
```

```
lcl=lcl(1)
ucl=ucl(1)
outliers=outliers
end
```

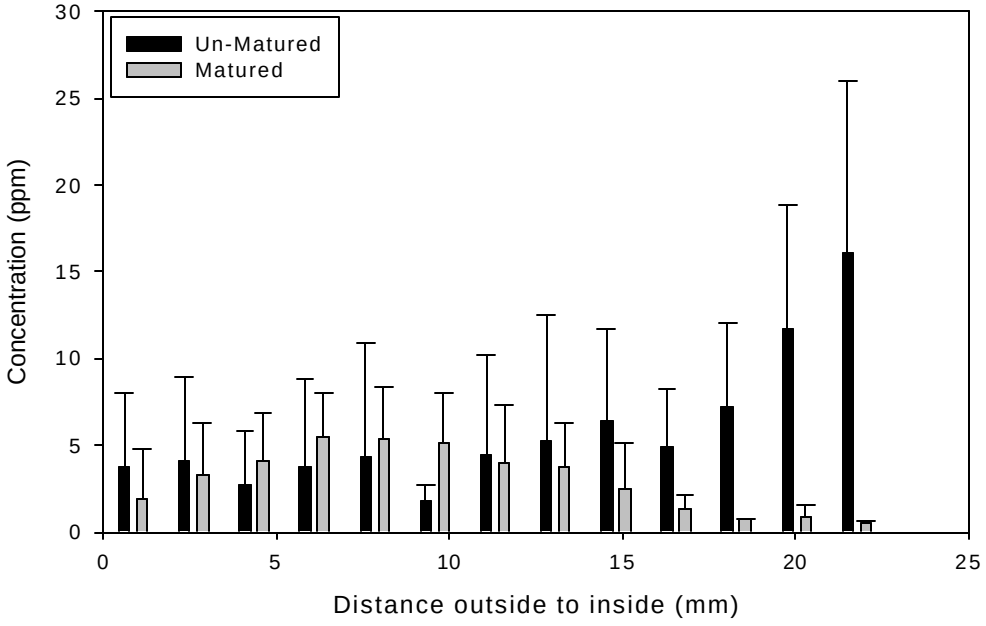
Appendix 3: Average concentration profiles across the stave for all compounds



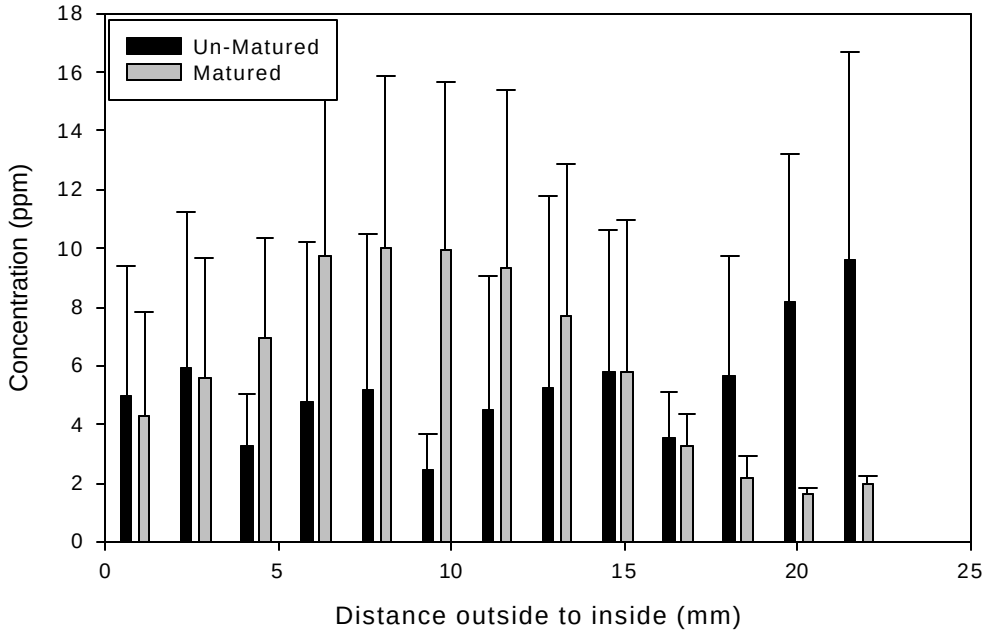
Gallic Acid



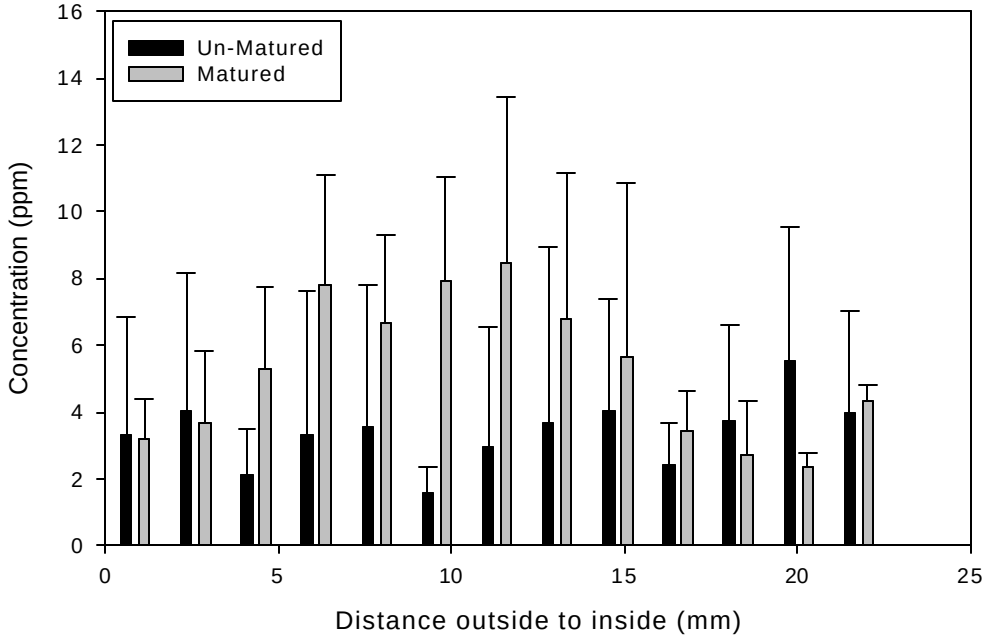
HMF



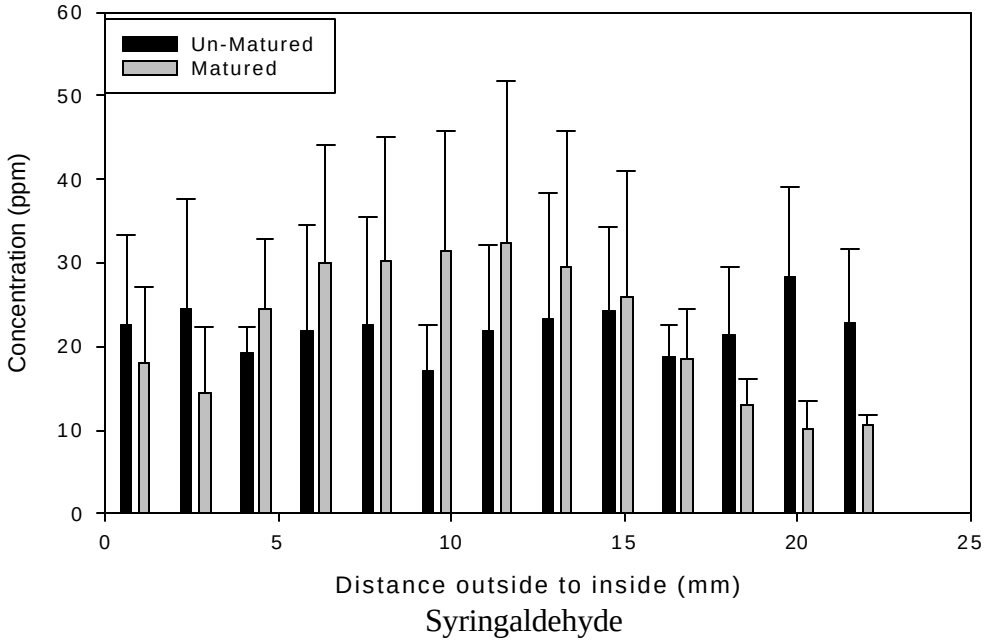
Furfural



Syringic Acid

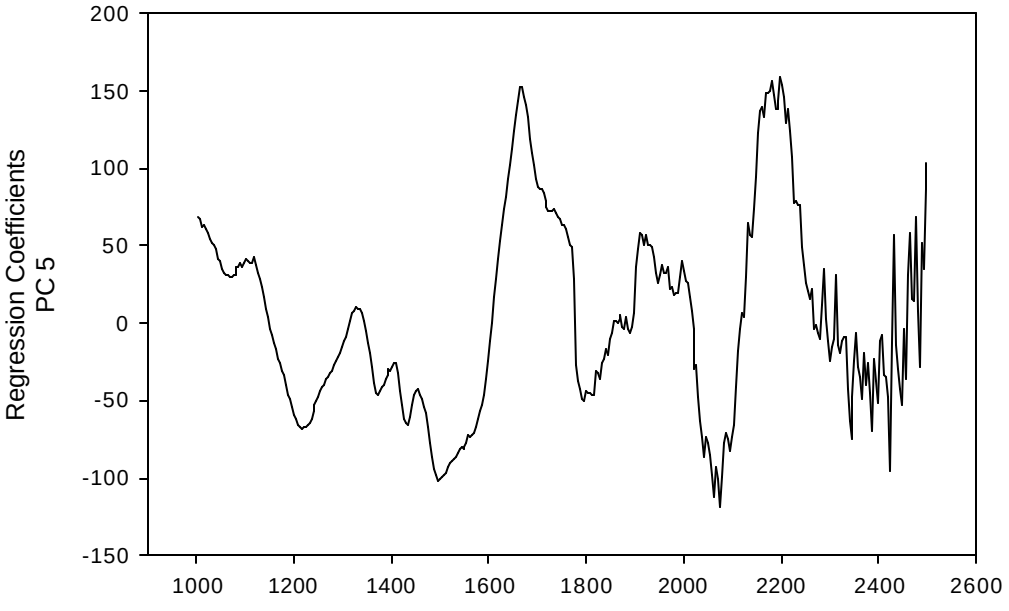


Vanillin



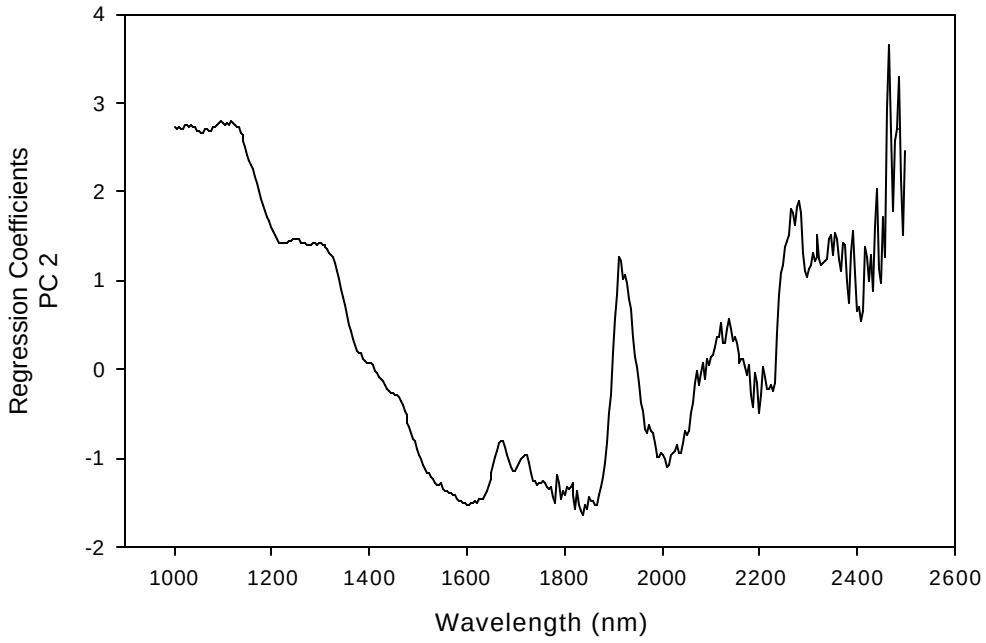
Syringaldehyde

Appendix 4: Regression coefficients for predictive models

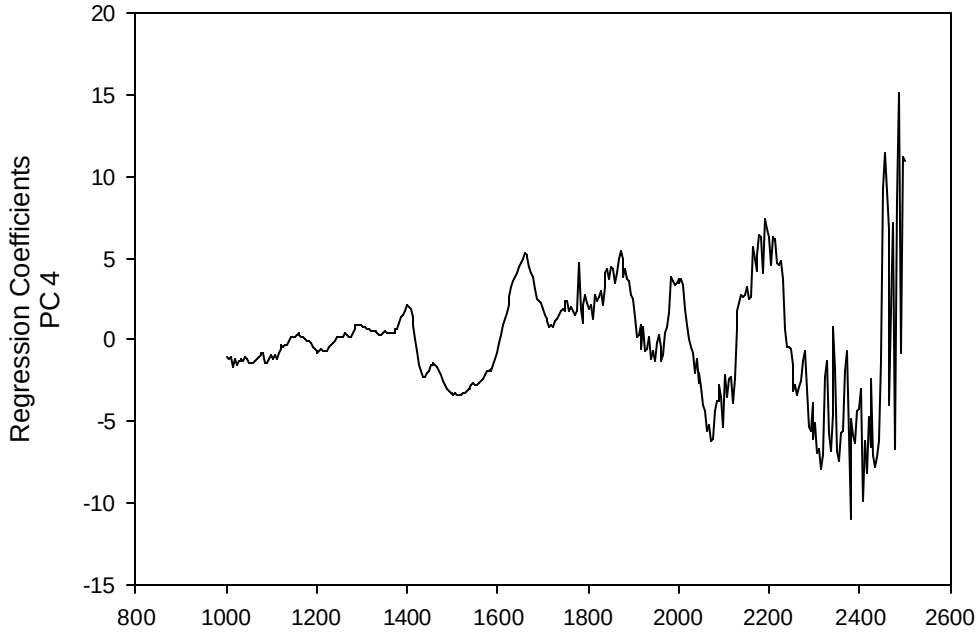


Gallic Acid

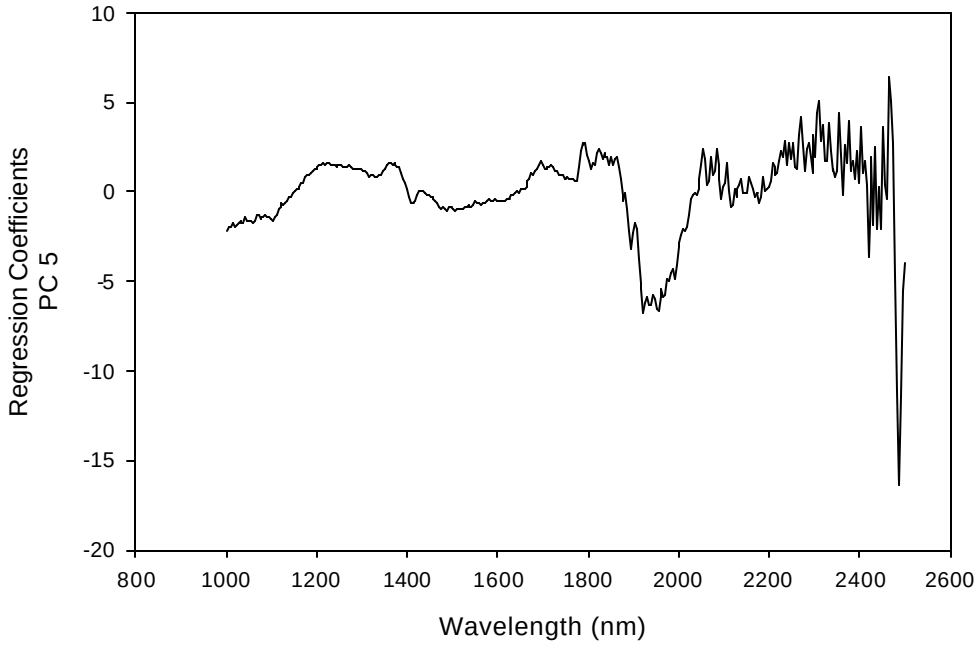
HMF



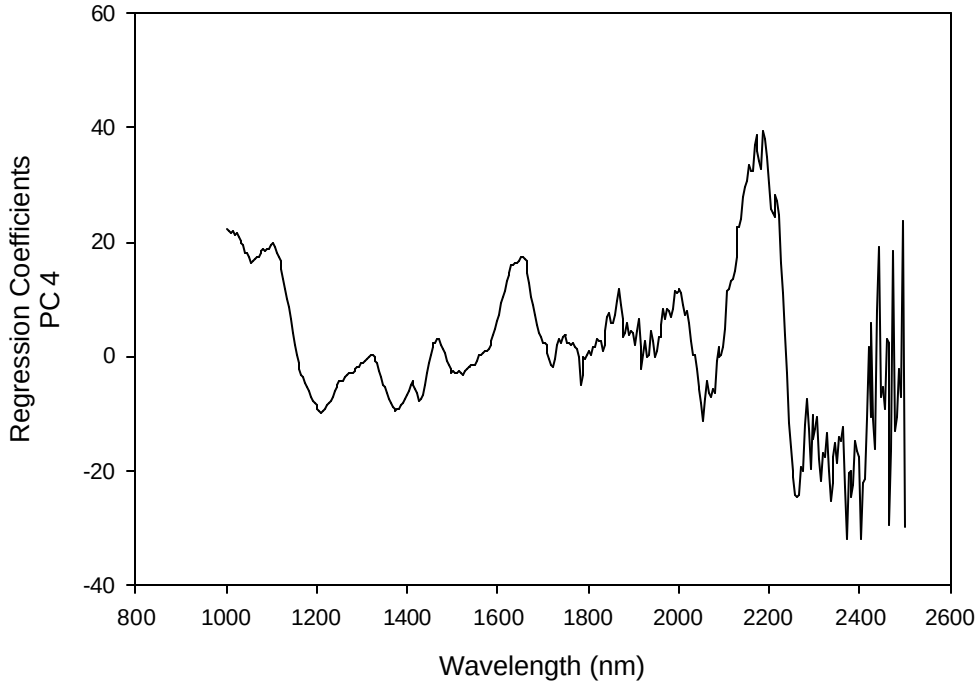
Furfural



Syringic Acid


Regression Coefficients
PC 5
10
5
0
-5
-10
-15
-20
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
Wavelength (nm)

Vanillin


Regression Coefficients
PC 4
60
40
20
0
-20
-40
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
Wavelength (nm)

Vita

John Powers Wolff, IV, was born on the bayou in Baton Rouge, LA, on July 31, 1982. He graduated from high school in 2000 and received a bachelor's degree in chemistry from Southeastern Louisiana University in 2006. Driven by the glorified life of a Master's student, Powers moved to Knoxville to continue his studies at the University of Tennessee. Upon moving, he realized that graduate school wasn't quite the fame and fortune he had hoped. In May 2008, with years of hard work under his belt, Powers earned a Master of Science degree in Chemical Engineering.