Salzburg University of Applied Sciences

Wood extractives and their biological, chemical and physical impact

Internship

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Directory

A Mold testing: Effect of extractives from wood species resistant to decay fungi

A.1 Introduction

The purpose of this study was to determine the effect on mold of extractives from wood species naturally resistant to decay fungi. There have been many studies with the soil-block procedure and decay fungi (Duncan and Richards 1951, Duncan 1953a, L. R. Snoke 1956).This report is about tests with a mold fungus on malt extract agar plates, in order to develop a fast method to discover the effect of wood extractives on mold. There are studies with agar plates and sapwood blocks (Morrell 1987), but these analysis were made only with the extractive solutions. The test was made with the fungus *Aspergillus niger*. This fungus grows rapidly and one can obtain results very quickly.

A.2 Materials

A.2.1 Fungi

Aspergillus niger is a mold fungus, which appears in the soil all over the world. The cause of the name of this fungus is that it has dark, nearly black, spores. The fungus can grow on all organic materials, and even on glass. The best conditions for this fungus are about a temperature of 35 to 37°C, but it will grow between 6 and 47°C. The pH-interval is about 1.5 and 9.8. It produces mycotoxins called kojic acid and oxalic acid and this species of *Aspergillus* can cause health damage in sensitive individuals.

A.2.2 Malt extract agar plate

Malt extract agar (MEA) is a nutrient solution to cultivate fungi, especially for *Aspergillus*. It is composed of 20g malt extract (powdered), 20g glucose, 1g peptone, 20g agar and 1L distilled water. It is sterilized by autoclaving at 121°C for 15 minutes. MEA is a medium with relatively high moisture content. It simulates humid substrates, like in the bath or outdoors.

A.2.3 Solvent

Two solvents were used to remove the wood extractives from the species cherry, walnut, southern pine, eastern white cedar, Atlantic white cedar, Alaska yellow cedar, white oak and western red cedar.

A.2.3.1 Water extracts

Originally, 25g wood was extracted with 400g water. The solvents were more than a half year old and the flasks were not completely locked; therefore it is likely that some of the water evaporated. For this study, the flasks were refilled to the same level to bring every solution to an equal volume.

A.2.3.2 ETT extract

Originally, 25g wood was extracted with 500g ETT. ETT is a mixture of 1 part ethanol and 2 parts toluene. The problem with this solvent mixture is that it evaporates very easily and much evaporation occurred after the extraction process. For this study, each flask was refilled to 190ml.

A.2.4 Filter paper

Filter paper from the company Whatman was used for the examination. The sheets are circles with a diameter of 90mm and are ashless.

A.3 Preliminary tests

Filter papers were treated with 2ml of each one of the wood preservative solvents and also with distilled water and one with the ethanol-toluol mixture for reference. After they had dried, they were cut into small pieces about 1 by 1 cm and sterilized at 115°C for one hour.
Three plates of malt extract agar were contaminated with the fungus *Aspergillus niger* under nearly sterile conditions in the "LABOCONCO Purifier Class II Bio safety Cabinet". These three plates were put into a conditioning cabinet with a temperature of approximately 30°C for one week.
Three pieces of the wood extractives treated filter papers, one of the distilled water and one of the ETT treated filter papers were placed with a plug of the fungus *Aspergillus niger* in one malt extract agar plate under the "LABOCONCO Purifier Class II Bio safety Cabinet". Four replicate plates were made for each of the wood species. Two water solvents and ETT solvents controls were included. The plates were stored in an incubator at 30°C for two days and compared with each other over time.

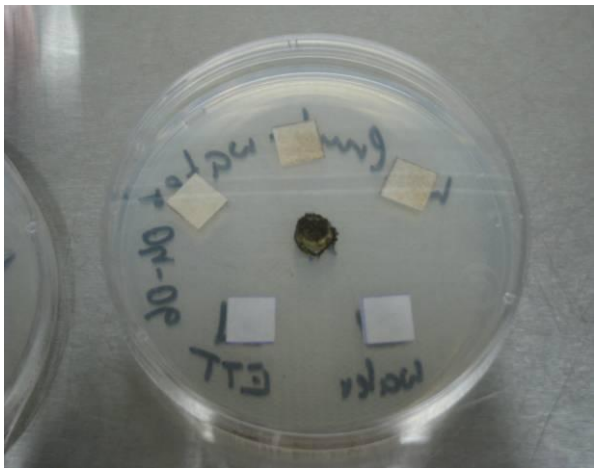


Figure 1: Prepared MEA plate

The fungus grew consistently over the malt extract agar and over the treated filter papers, regardless of the extractives treatments. As a consequence, the extracts were concentrated in a further effort to determine the effect of the extractives on the mold fungus.

A.4 Increasing the concentration

A rotary evaporator was used to increase the concentration of the wood extractive solutions. Each solvent was put into a round bottom flask and placed on the rotary evaporator with a clip and rotated at level 8. The water bath had a temperature of 50°C. It took 15 minutes to dry the ETT solvents and between 30 and 180 minutes for the water solvents. The procedure with the water solvents was more difficult, especially with the white oak solvent. As a result of the high viscosity, the solvent bumped a lot. Therefore the temperature and the rotation were decreased. Final solution volumes of solvent about 160ml (water) and 70ml (ETT solvent) were obtained. After this increasing the test procedure was restarted.

A.4.1 Result

Here is an example of the Alaska yellow cedar. This species has high natural durability in contact with decay fungi and insects. But even after the increase of the extractive solution concentration, the wood extractives had little apparent impact on the fungus. After one week the whole plate was covered with the fungus.

Figure 2: AYC after increase of the concentration

A.5 Prepare wood extractives agar plates

As a next step, some agar plates containing water-soluble extractives in the medium were prepared. This was made only with the water solvents, because it is impossible with the ETT solvent: toluene melts the plastic plates.

100 ml from the wood extractives water solvents were mixed with 4 g of the malt extract agar powder and afterwards sterilized by autoclaving at 121°C for 15 minutes. Three plates were made from each wood species. After cool down, *Aspergillus niger* was placed on each plate and incubated at 30°C for one week. The plates were checked every day by photographing. The areas of the fungus and the whole plate were measured using the software Photoshop. The mean of the proportion from each wood species were compared.

A.5.1 Result

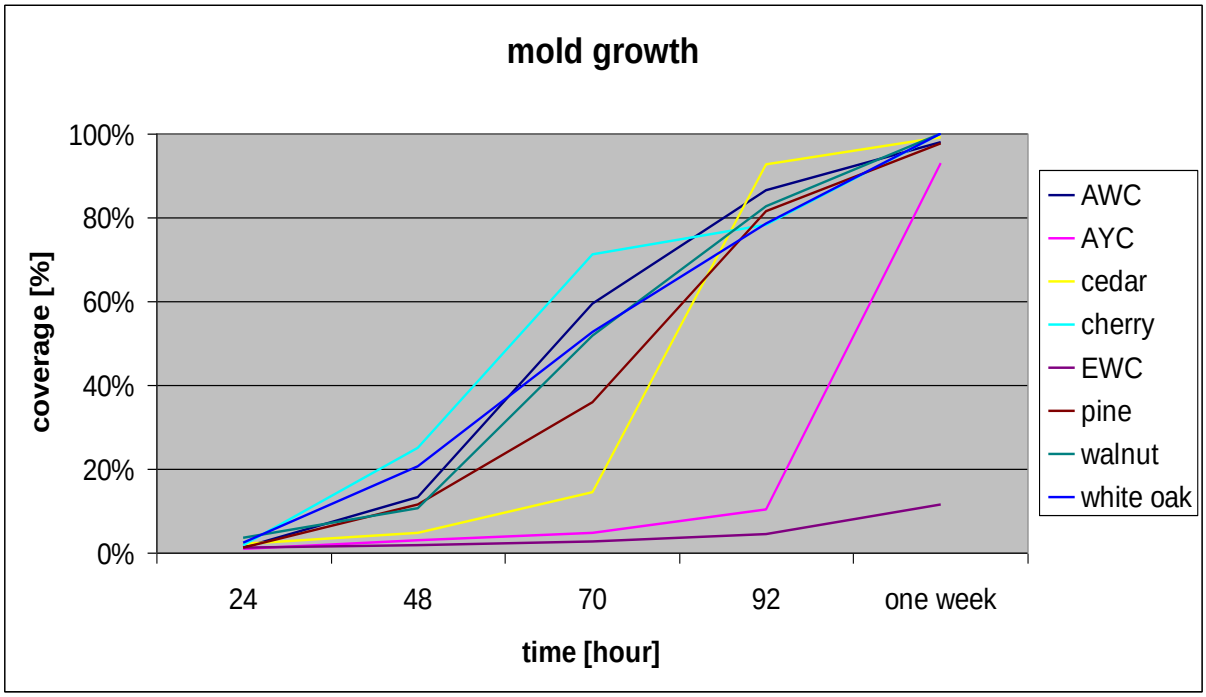


Figure 3: Proportion of the fungus area to the whole plate (water-soluble extractives)

After four days, the agar plates made with wood extractives from Alaska yellow cedar and eastern white cedar had considerably lower fungal growth than the rest of the wood species. After one week only eastern white cedar had reduced fungal growth. All other plates were almost completely covered with the fungus.

A.6 Test of the ETT solvents

A.6.1 Preliminary test

The ETT solvent solution from eastern white cedar was evaporated with the rotovap, but it was not possible to remove all the solvent and the extractive substance adhered strongly to the round bottom flask. Thus the extractives were redissolved into ethanol (it was not soluble in water).

A.6.1.1 Control samples

Filter papers with either water, ethanol or ethanol-toluene mixture were placed in a Petri disk and evaporated under the hood until dry. The filter papers were placed on malt extract agar plates with three circles of *Aspergillus niger*. After four days in the incubator at 30°C the whole plate was covered with the fungus.

A.6.2 Method

Because the fungus grew very well on the ethanol-toluene control filter papers, as described above, the ETT solution of each wood species was placed in a Petri disk with a filter paper and evaporated under the hood until completely dry. Each treated filter paper was then placed on a malt extract agar plate with three circles of *Aspergillus niger* und put into the incubator at 30°C for one week.

A.6.3 Result

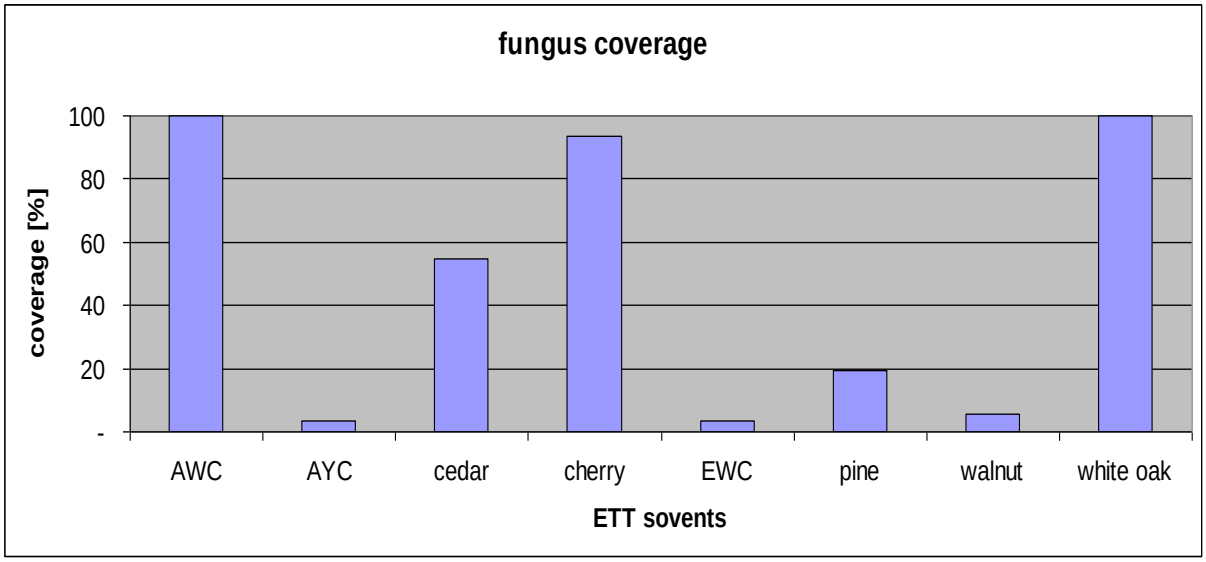


Figure 4: ETT solvent - fungus coverage after one week

The plates with filter papers treated with extractives from white oak and cherry were covered with fungus after one week in the incubator. There was also considerable growth on the pine, walnut and cedar plates. Although the whole Atlantic white cedar plate was covered, the fungus looked different: it was white in color. On the Alaska yellow cedar und also on the eastern white cedar no fungus growth was distinguishable, even after two weeks in the incubator.

From the broad leaved trees, only the walnut ETT solvent reduced growth. It could be that the filter papers were overloaded with the extractives and the filter paper partitioned the fungus from the nutrient. Additionally, the plate from the walnut sample was covered after one day with a high amount of condensation water. Consequently the humidity may have been too high for the fungus.

A.7 Conclusion

The possibility that some wood species have an antibacterial effect has been known for a long time. These tests show that some types of cedar are more resistant than all of the broad-leaved trees that were tested. It could be possible that the cedars contain extractives that are not present in broad-leaved trees. The tests with the water and ETT solvents from white oak, cherry and walnut showed a healthy growth of the fungus *Aspergillus niger*.

Because of their apparent anti-mold activity in these tests, it may be useful to analyze the wood extractives from eastern white cedar using HPLC or NIR.

B Effect of herbicide injections on heartwood formation in hardwood trees

B.1 Introduction

Heartwood has properties that can significantly influence its usefulness to the end user of wood products. (Taylor et al. 2002). As a result of the presence of distinct extractive compounds, and perhaps lower permeability, heartwood often has increased decay resistance. Important functions of the heartwood are structural support, optimizing sapwood volumes and conserving resources. For wood products, the heartwood is sometimes the more valuable portion of the stem because of its distinct durability, color or odor. Examples of wood species with valuable heartwood include walnut, cherry and white oak.

The sapwood of gymnosperms usually has relatively high moisture content, because it provides the foliage with water from the ground. Furthermore the sapwood may contain starch, few toxic extractives and may have a high permeability; therefore it is susceptible to decay. Due to the fact that the foliage is provided with the water through the sapwood, the amount of the sapwood can be influenced by decreasing the amount of foliage.

There are studies with pine trees that have shown that heartwood-like wood can be stimulated by applying herbicide (paraquat) to the living tree. (Roberts and Outcalt 1983). This report describes attempts to induce heartwood formation using herbicides (paraquat and MAT 28) in the hardwoods yellow poplar (*Liriodendron tulipifera)* and black cherry (*Prunus serotina)*.

B.2 Materials

B.2.1 Trees

Fifteen yellow poplar and four black cherry trees with a DBH (diameter at breast height) between 5 ½ and 11 ½ inches were chosen at random from a young plantation (poplar) or from naturally occurring trees scattered within a forest (cherry). The trees contained heartwood at the time of treatment.

B.2.2 Herbicides

Two herbicides were tested: Paraquat and Aminocyclopyrachlor (MAT 28). Each of them was used with a concentration of 0.5 and 2%. Paraquat was used for the treatment of pine in prior studies (Roberts and Outcalt 1983) and affects ethylene production, which is thought to be

associated with heartwood formation and other plant tissue senescence processes. MAT28 is a new herbicide formulation that also affects ethylene production.

B.2.2.1 Paraquat

Paraquat is the trade name for the herbicide called *N*,*N*'-dimethyl-4,4'-bipyridinium dichloride and it is one of the most widely used herbicides of the world. Paraquat is absorbed into the plant quickly, especially in humid conditions which is available in tree tissues. Electrons are transfused in the chloroplasts on the paraquat-cation during the photosynthesis and the outcome of this is a paraquat-radical. The radical transfused his spare electron to an oxygen molecule and generates super oxides. These have a high chemical reactivity und destroy unsaturated fatty acids in the chloroplasts and in cell membranes. Due to the fact that the cation is always reduced to a radical, this procedure is consecutive until the photosystem is destroyed. The cell membranes become porous which is lead to water loss (Babbs et al. 1989). In some countries such as in the European Union and Switzerland the usage of paraquat has been forbidden.

B.2.2.2 Aminocyclopyrachlor

Aminocyclopyrachlor or MAT 28 is a new herbicide which is currently under development for use in non-crop markets by the company DuPont. It is a novel pyrimidine carboxylic acid herbicide which provides postemergent and soil residual activity in controlling many annual and perennial broadleaf weeds and brush species. Aminocyclopyrachlor is less toxic than paraquat. One important function is that it up-regulates the production of ethylene (Nanita et al. 2009).

B.2.3 Injection System

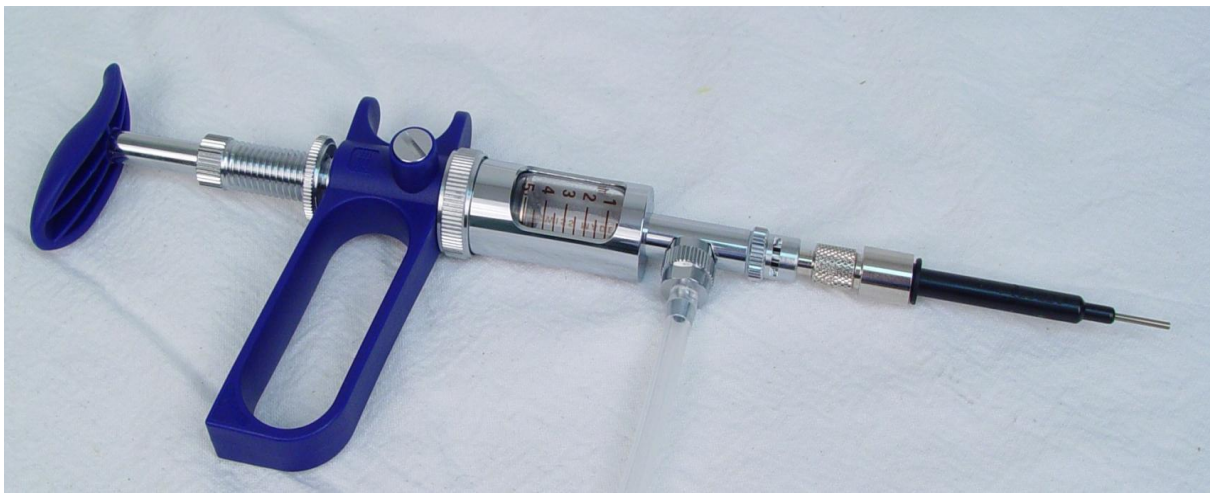


Figure 5: ARBORJET injection system#

The ARBORJET injection system was used to induce the herbicide solutions into the trees. It is an easy handling system which is effective and fast. The adjustable dose from 1 to 5ml makes the work efficient for every DBH.

B.3 Method

Increment cores were taken from each tree to measure the width of the heartwood and of the sapwood, and to calculate the moisture content prior to treatment with the herbicide. Therefore the change in the width of the sapwood can be compared over the different concentrations and with the different herbicide treatments. The DBH was also measured for each tree.

Three holes were drilled into each tree about 30cm above the ground and equally spaced from each other around the circumference. The holes were approximately 4 mm wide and 20 mm deep, which equates to the plugs. Plugs provided with the injection tool were set into the trees using a set tool and a hammer. The correct position of the plugs is shown in the image to the right. The barbs on the plug should make a seal between the xylem and the inner bark.

Figure 6: Plug setting

The herbicide was filled in the white bottle, which was connected to the injection gun. The first step was to pump the solution through the pathways until all air bubble was gone. The injection gun was adjusted to 1ml and afterwards the herbicide was induced into the trees through the inserted plugs. An amount of 1ml was filled into each plug-hole of the herbicides. Additionally, three trees of yellow poplar were treated with water as controls. Each tree was marked with a red flagging and numbered with red spray paint.

B.3.1 Numbering of the trees

Table 1: Numbering of the trees

solutions	yellow poplar			cherry
0,5% MAT 28	1	2	3	16
2% MAT 28	4	5	6	17
0,5% paraquat	7	8	9	18
2% paraquat	10	11	12	19
water	13	14	15	

B.4 Results

After two and four weeks the trees were inspected. The MAT 28-treated cherry trees lost their foliage entirely. After two weeks the MAT 28-treated yellow poplar trees had curly leaves and after four weeks some leaves had a brown discoloration as shown in the pictures below. The paraquat-treated cherry and yellow poplar showed no visible effects. There was no visible effect on the water-treated controls.

Figure 7: Result - curly leaves

Figure 8: Result - brown discoloration

An increment core was taken from one yellow poplar and one cherry tree to measure the differences between the moisture content and also the width of the sapwood. But, as with the controls, there was no apparent effect from the treatments.

B.5 Conclusion

It may be that, to form heartwood, a tree needs time and therefore the MAT treatment on the cherry trees worked too fast and the trees did not have enough time to decrease the sapwood and form heartwood. The MAT treatment on the yellow poplars shows that the area of the

foliage decreased slightly, but apparently heartwood formation has not been significantly affected.

Due to the fact that there is no apparent effect on the paraquat-treated trees, and only minimal change in the MAT 28-treated trees, the injected trees should be further observed and perhaps reinjected in the fall of 2009.

C Determination the differences of extractive compounds from the pith to the bark and correlation to the shrinkage of white oak

C.1 Introduction

American white oak or *Quercus alba* is a hardwood common in the forests of the eastern United States. The ring porous wood has tyloses and is not permeable to liquids and thus it is used for whiskey and wine barrels. During maturation, several wood compounds merge from the white oak into the spirits, which is important for the flavor of the finished product. One important component is vanillin.

A main problem for the producers of whiskey and wine is that the barrels often leak. Wood is an anisotropic material, which means that wood shrinks and swells in the axial, radial and tangential direction in different extents. The proportion is about 1 to 10 to 20 parts. Furthermore there is a hysteresis between the water absorption and desorption. That means that the moisture content at desorption is 1 to 2% higher than at absorption (Niemz 1993).

This report is about the relationship between the extractive content, shrinkage and other wood properties like basic density to optimize barrel production. The analysis should ensure if there are differences from which location in a tree a stave is from. In addition the prediction of extractives content by using near infrared and mid infrared spectroscopy was determined.

C.2 Materials

C.2.1 White oak samples

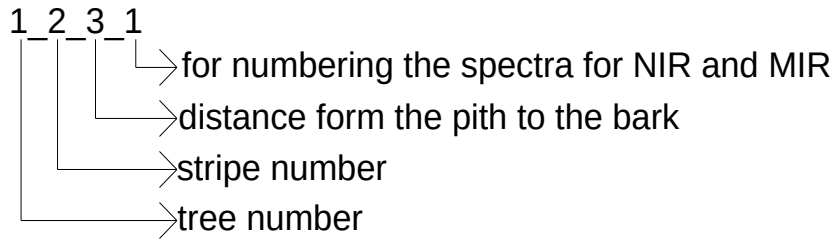
The white oak trees were from a hardwood sawmill in Oak Ridge, TN USA and were harvested in a region close to that sawmill. The logs were designated for the stave production for whiskey barrels. Five trees were used with the ages of:

Table 2: Ages of the white oak trees

tree	years
tree # 1	78
tree # 2	69
tree # 3	76
tree # 4	77
tree # 5	155

A disk was cut from each tree at a height apporximately 30 cm from the ground. Two radial stripes with a width and a height of 20 mm were cut out from the center of the wood disks (Appendix 1). One strip was designated for milling into wood powder and used for the total extractive content, the near infrared, the mid infrared measuring and the analysis with the high pressure liquid chromatography; the other strip was used for the shrinkage, density and the growth ring measurements. Both strips were separated with a chisel into samples with a radial length of 1 cm. Each sample was marked with a code of numbers:

Table 3: Numbering structure of the samples



C.2.1.1 Solid wood pieces

The strips with the number one were used for the density, shrinkage and growth ring width measurements. The number of growth rings and the growth ring width were measured with a ruler. The mean of the growth ring width has been calculated by dividing the growth ring width with the growth rings.

Density was measured by using a scale and a glass of water. With the assumption that water has the density of 1 g/cm³ the green density was calculated by weighting the water displacement of each sample by dipping into the water. The samples were placed into an oven at 105°C until each the samples had a constant weight. This was determined by measuring the weight twice with a time distance of 24 hours. The tangential and radial dimensions of each sample were measured with a caliper.

The (dry) weight and the density were measured by using the water displacement method described before. Afterwards the samples were placed into water for approximately fourteen days until the samples had a constant weight and the tangential and radial dimensions were measured again. With these values the tangential and radial shrinkages were calculated. Also the weight and the density were measured again therefore it was possible to calculate the total shrinkage.

C.2.1.2 Powder production

Each sample was dried into an oven with 60°C for one day. As a pre-preparation for the milling process the samples were separated with a chisel into small pieces. Each sample was milled with the Retsch Mixer Mill MM 400 for 90 seconds and with a frequency of 30 hertz. A consistent wood powder resulted.

A paper cup was numbered for each sample so that the powder was not mixed with different samples. The wood powder was stored for further analysis in a room with a temperature of 23°C and a low humidity.

C.2.1.3 Determining the total extractive content

To determine the total extractive content, 2g of wood powder were measured into teabags which were numbered with pencil. The teabags were produced with a polyester filter-cloth and a plastic-welding-equipment as it is shown in the picture below. The cloth had a mesh size of 25 microns. In subsequent processing the powder stayed in the teabag and only the extractives were washed out. The teabags were put into an oven for approximately 24 hours at 105°C until a constant weight. Every teabag was weighted before the extraction.

All teabags were placed in a Soxhlet apparatus and extracted according the standards ASTM D1105-96. These standards are used to prepare extractive-free wood.

The first step was to extract the wood powder with an ethanol-toluene mixture of one part ethanol and two parts toluene for 4 hours until the liquid in the flask was almost colorless. After the extraction the teabags were dried under the hood overnight. The second step was to extract the teabags again, but only with ethanol and also for 4 hours until the liquid in the flask was colorless again. The ethanol in the teabags was evaporating under the hood overnight. The third step was to cook the teabags into distilled water for one hour. After the extraction the teabags were oven-dried at 105°C again until the weight was constant. The weight difference (before and after extraction) was calculated as the total extractive content.

C.2.1.4 High performance liquid chromatography (HPLC)

HPLC is a technique that can be used to analyze different extractives which are relevant for the typical flavor of whiskey and wine. It is possible to determine not only the existence of some compounds but also to quantify the concentrations of the substances. This chemical separation tool is primarily used in the pharmaceutical and food industry. The machine consist a pump system, a mobile phase, a column, a stationary phase and a detector.

Table 4: Gradient method for HPLC analysis

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

The HPLC method was supported from Brown-Forman© which was already used earlier studies. A gradient elution was employed with a dual solvent system (Table 3).

Wood powder samples (1 g) were extracted with 30 ml of an ethanol/water mixture (7/3) for 4 hours at 23°C in darkness (Doussot et al. 2000). The stirred substance was filtered through a filter-paper, filled in a vial and frozen at -18°C. 1.5 ml of extract was filtered with a 0.45 µm Millipore© disc filter into a 2 ml HPLC vial and placed in a Waters© 710 Autosampler. The HPLC method was carried using Waters© 2695 Separations Module and Empower software. The analysis on the separation was done on a Waters© 2996 Photodiode Array Detector at 280 nm. Two samples were spiked with six different chemicals which are already determined as important to whiskey flavor to identify the different peaks. These compounds were gallic acid, hydroxymethylfurfural (HMF), furfural, vanillin, syringic acid and syringaldehyde. Additionally, a standard solution was prepared with the known concentrations of the chemicals. This solution was diluted four times so that there were five standard solutions with the known concentrations of the compounds.

Each sample was run through the HPLC for 25 minutes. The generated peaks of the spectra were identified and the height was measured. With these values and the concentrations of the standard solutions the extractives vanillin, syringic acid and syringaldehyde were quantified. Gallic acid, HMF and furfural were only in very low concentration available so that the peaks were not measurable.

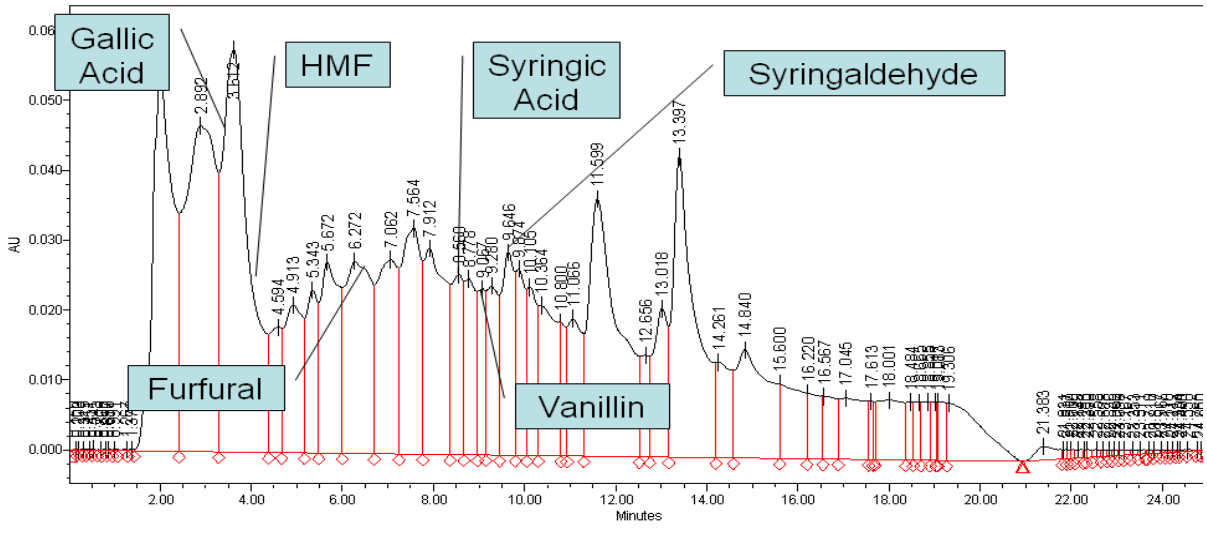


Figure 9: HPLC sepctrum

C.2.1.5 Near infrared spectroscopy (NIR)

NIR is a chemical technique used for the rapid, nondestructive quantitative determination of many material properties such as the content of 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 result in a reflectance of the sample which is interpreted by a detector similar to that for UV/VIS absorptions spectroscopy" (Skoog et al. 1998).

For taking the spectra the milled powder samples were placed in a plastic cap with a diameter of approximately 2 cm. The surface of the samples was smoothed and the whole cap was placed on a rotating apparatus under the Labspec Pro, LSP350-2500P spectrometer and spun at 45 rpm. Five spectra were taken from each sample and subjected to for multivariate data analysis using a computer software package (The Unscrambler©). The spectra were converted from reflectance to absorbance and 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. The data were also mean normalized and MSC treated because of the non-uniform samples.

C.2.1.6 Mid infrared spectroscopy (MIR)

The spectra were collected using a Thermo-Nicolet Nexus 670 FTIR with a Golden Gate MKII single reflection ATR system. Spectra were recorded from wood powder in absorbance mode from 4000 to 650 cm^{-1} with 8 scans per spectrum. Five spectra were taken from each sample and also one background spectrum in the air. The spectra were ATR corrected with 0.5 and imported into the software The Unscrambler©. The spectra were averaged by 5 so that one spectrum was left for each sample. Also the X-variables were averaged by 4 and the data were mean normalized and MSC treated as for the NIR statistics (Urban 1996).

C.3 Results

The correlation between shrinkage, total extractive content and distance from the pith to the bark was very low. The shrinkage decreases a little bit from the pith to the bark as it is shown in the graph below.

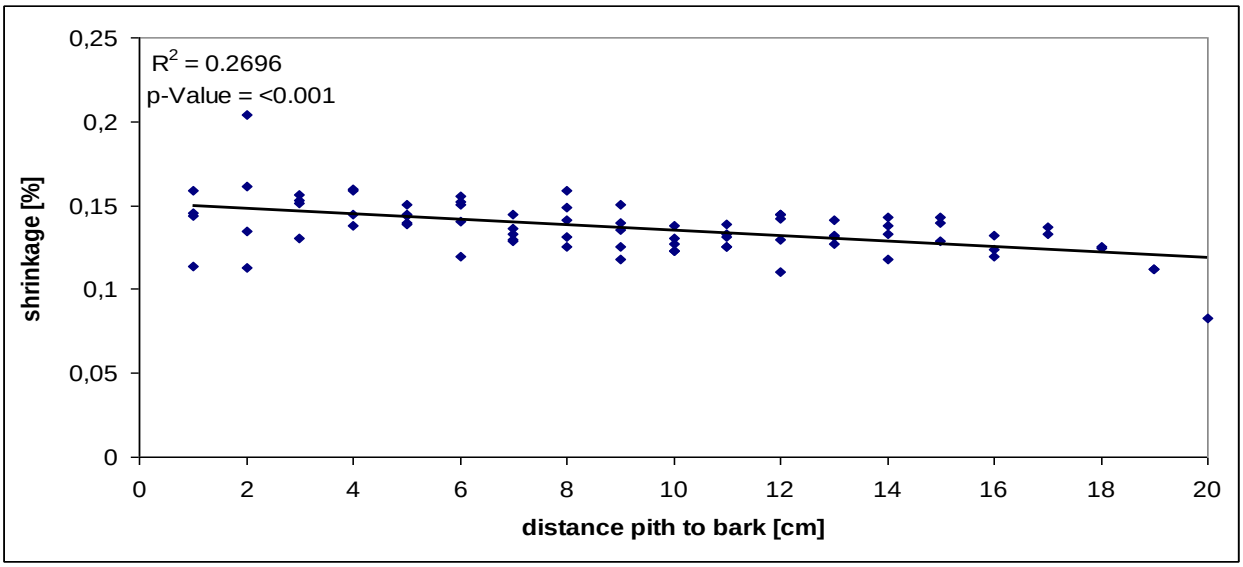


Figure 10: Correlation between shrinkage and distance from the pith to the bark

The coefficient of determination is very low (R^2 = 0.2696) but the p-value less than 0.001 suggests that there is a statistical correlation. Also the correlations between density and shrinkage and the radial and radial and the tangential ratio and the distance from the pith to the bark have very low correlations about 0.16 and 0.12, but there are also p-values of less than 0.001 (Appendix 2 and 3).

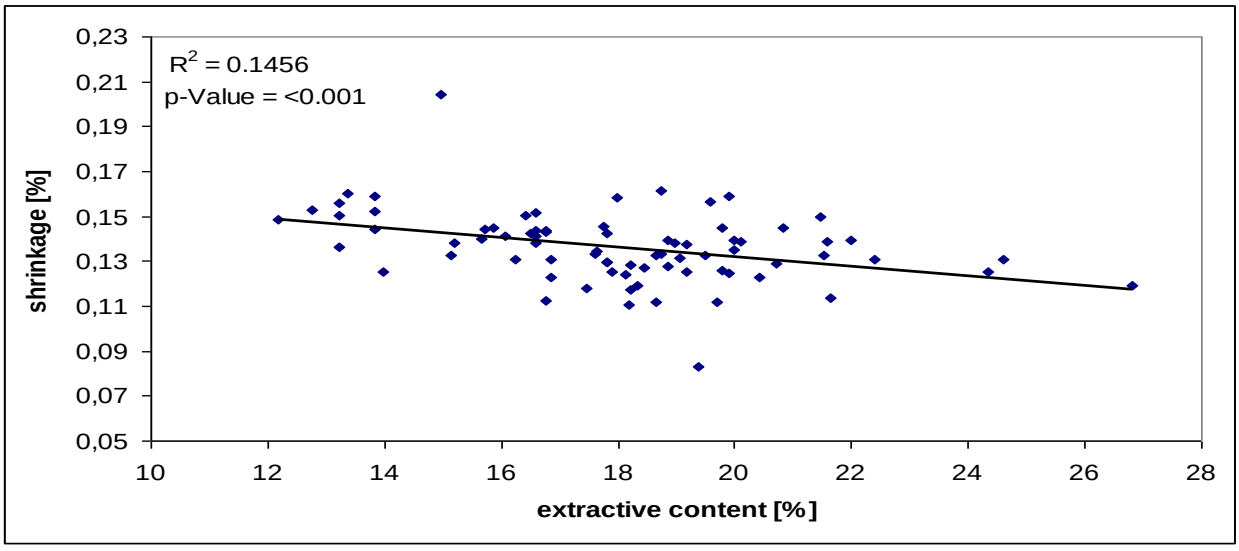


Figure 11: Correlation between shrinkage and total extractive content

Although R^2 is with 0.1456 very low, the p-value indicates that there is a statistically significant correlation.

Furthermore there was no strong evidence of a correlation between the total extractive content and the distance from the pith to the bark (p-value about 0.072). The coefficient of determination is about 0.0426. Also between the density and the total extractive content was no correlation. The R^2 is about 0.015 and the p-value about 0.285.

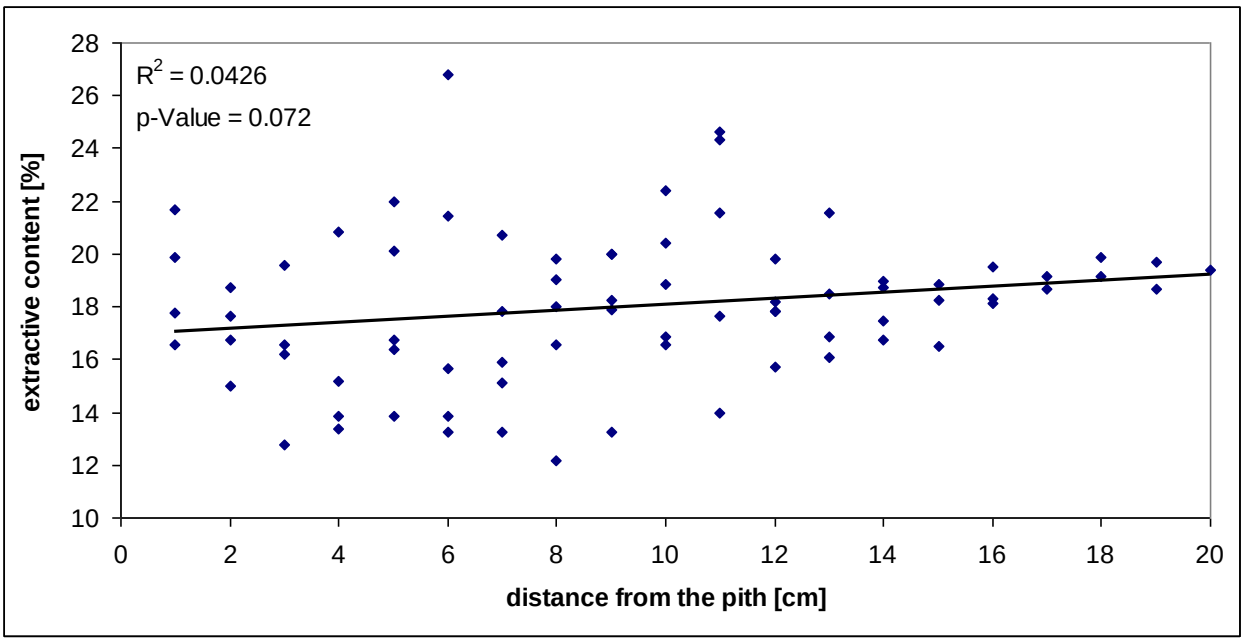


Figure 12: Correlation between TEC and the distance from the pith to the bark

The result of the concentration of vanillin, syringic acid and syringaldehyde shows the same profile. The concentration is very low close to the pith and in the sapwood but it shows an increase from the pith to the boundary from the heartwood to the sapwood in all five trees.

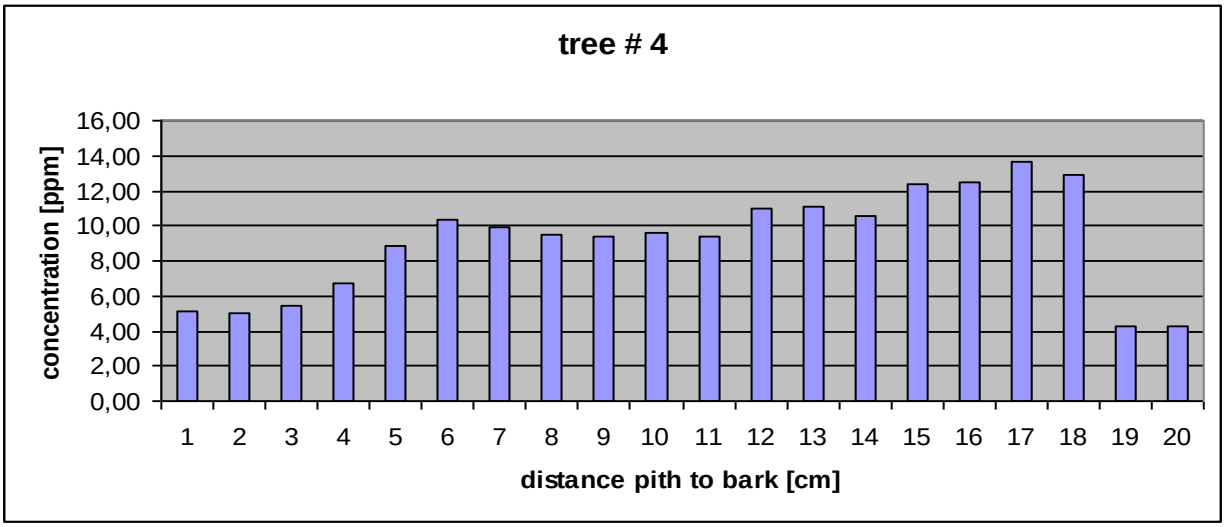


Figure 13: Typical profiles of vanillin, syringic acid and syringaldehyde

The predictions of these compounds with NIR show correlations about 90% as it is displayed in the graph below. To ensure that the prediction is based on the right chemicals of the spectrum, the highest peaks were identified by using different literature (Burns et al. 2001, Siesler et al. 2002) (Appendix 4).

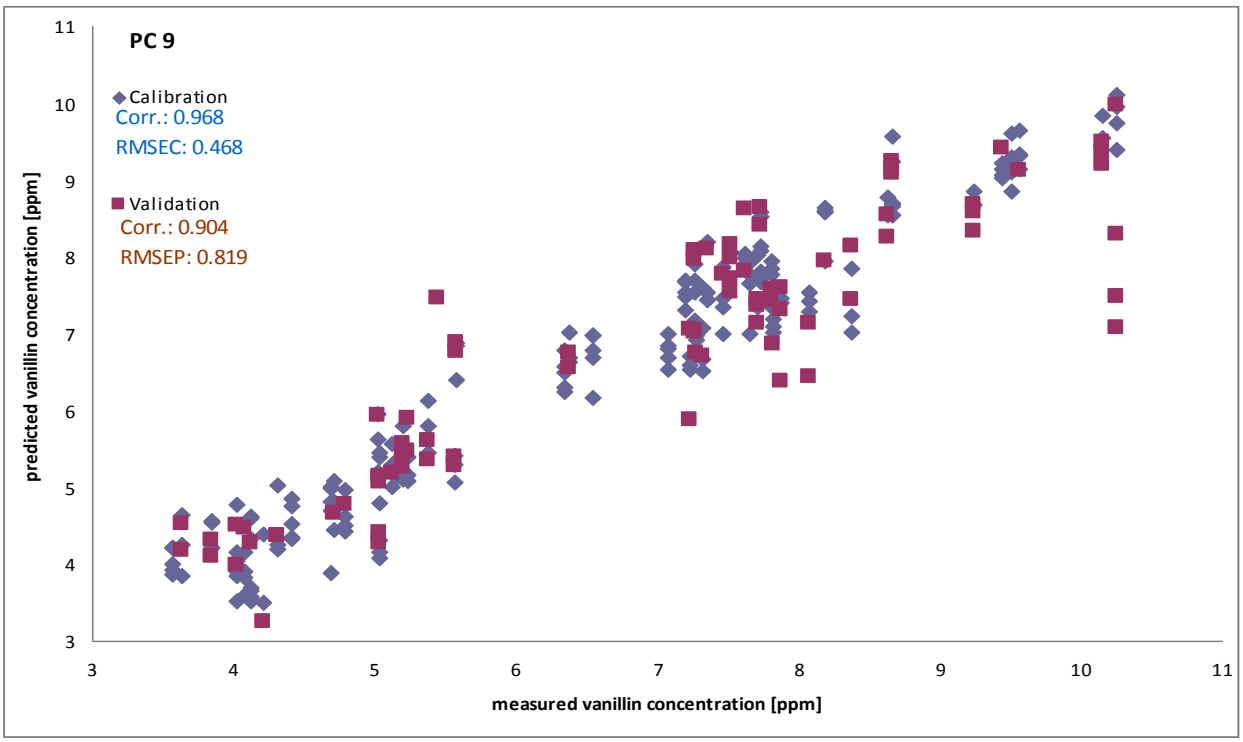


Figure 14: Prediction of vanillin with NIR

The prediction of total extractive content with MIR shows a good correlation of about 71%. The spectrum was also analyzed by comparing it with the literature (Shukry 2008, Gruyter 1991).

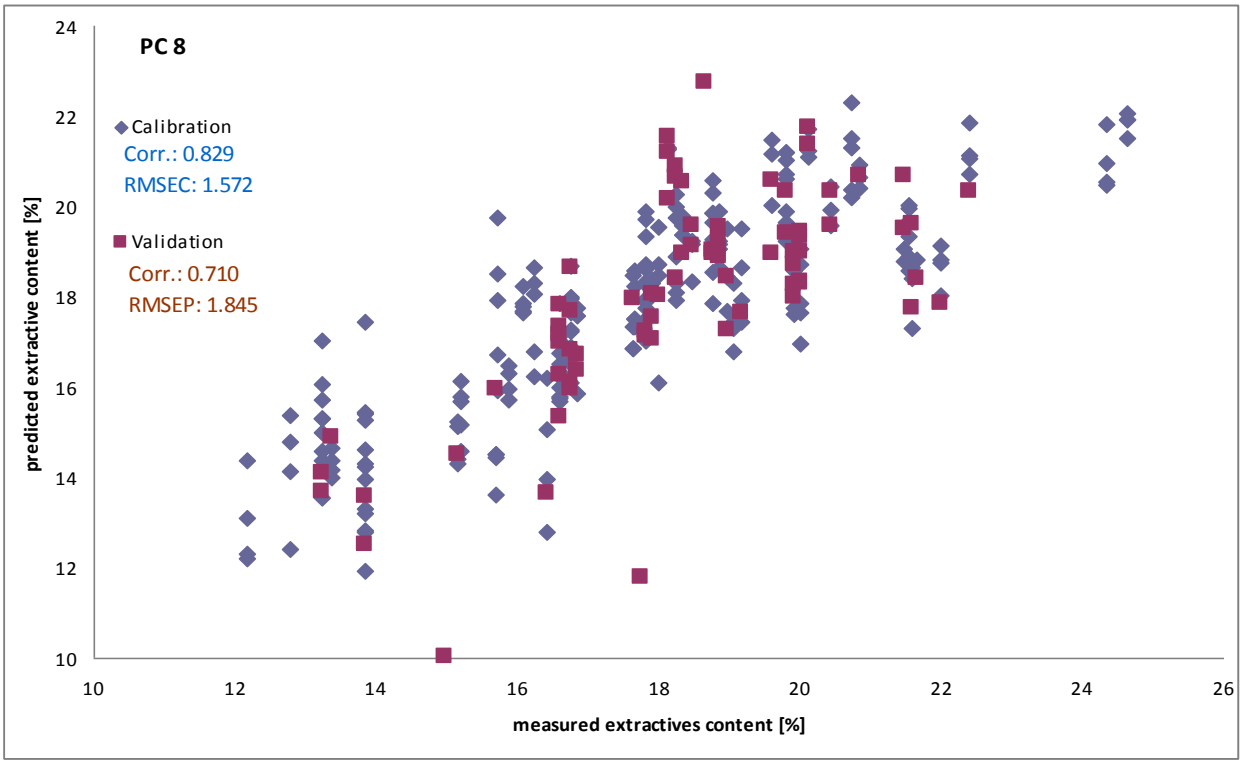


Figure 15: Prediction of total extractive content with MIR

C.4 Conclusion

The radial differences of white oak against shrinkage, density and total extractive content exist but the changes are very low therefore for the production of whiskey barrels is not important from which location the stave is cut. Radial patterns varied among the trees. It is important to analyze more trees to get more data.

The infrared spectroscopy-based predictions for vanillin, syringic acid and syringaldehyde are almost 90%. NIR and also MIR are good techniques to predict these compounds which are very important for the flavor of whiskey. Therefore it may be better for the barrel production to use staves which are located close to the boundary from the heartwood to the sapwood, because of the higher concentrations of these extractives in that location.

D References

DUNCAN, C. G., AND RICHARDS, C. A. 1951 **Evaluating wood preservatives by soil-block tests: 4. Creosotes.** Proc. Am. Wood-Preservers' Assoc., 47, 275-287.

DUNCAN, C. G. 1953a Soil-block versus agar block techniques for evaluation of oil-type preservatives: Creosote, copper naphthenate and pentachlorophenol. Division of Forest Pathology, Bureau of Plant Industry, Forest Pathology Special Release No. 37.

SNOKE, L. R. 1956/4 Specific Studies on the Soil-Block Procedure for Bioassay of Wood Preservatives. Appl. Environ. Microbiol., 21-31

MORRELL, J. J. 1987 Bioassaying wood preservatives with *Aspergillus niger*. Wood and Fiber Sience, 388-391

TAYLOR et al. 2002 **Heartwood formation and natural durability.** Wood and Fiber Science, 34(4), 587-611

ROBERTS, D. R., AND OUTCALT K. W. 1983 **Optimum paraquat treatments for inducing resin-soaking in slash and loblolly pines.** Southern Journal of Applied Forestry, 7(1) 31-33

BABBS et al. 1989 Lethal Hydroxyl Radical Production in Paraquat-Treated Plants. Plant Physiol., 90, 1276-1270

NANITA et al. 2009 Mass Spectrometric Assessment and Analytical Methods for Quantitation of the New Herbicide Aminocyclopyrachlor and Its Methyl Analogue in Soil and Water. Analytical Chemistry, 81(2) 797-808

BURNS D., CIURCZAK E., 2001. **Handbook of Near-Infrared Analysis.** 2nd ed. New York, NY Marcel Dekker, Inc.

DE GRUYTER W., 1991. Classification of Lignin from Different Botanical Origins by FT-IR Spectroscopy. Holzforschung. 45: 21-27

DISCRIPTION MILL;
http://www.retsch-us.com/us/products/milling/ball-mills/mm-400/function-features/

DOUSSOT F., PARDON P., DEDIER J., DE JESO B., 2000 Individual, species and geographic origin influence on cooperage oak extractible content (Quercus robur L. and Quercus petraea Liebl.). Analusis. 28: 960-965

LU JOHN Z., NEGULESCU IOAN I., AND WU QINGLIN. 2004. **Thermal and Dynamic-mechanical Properties of Wood-PVC Composites.** Wood and Fiber Science. 36(4): 500-510

NIEMZ, P., 1993 **Physik des Holzes und der Holzwerkstoffe.** DRW-Verlag, Leinfelden-Echterdingen.

SHUKRY, 2008. **Some Physical Properties.** Journal of Applied Polymer Science. 109: 434

SIESLER H. W., OZAKI Y., KAWATA S., HEISE H. M., 2002. Near-Infrared-Spectroscopy: Principles, Instruments, Applications. Wiley-VCH

SKOOG D., HOLLER F., NIEMAN T., 1998. **Principles of Instrumental Analysis.** 5th ed. Belmont, Ca: Thomson Learning

URBAN M. W., 1996. Attenuated Total Reflectance Spectroscopy of Polymers: Theory and Practice (Polymer surfaces and Interfaces Series). The American chemical society

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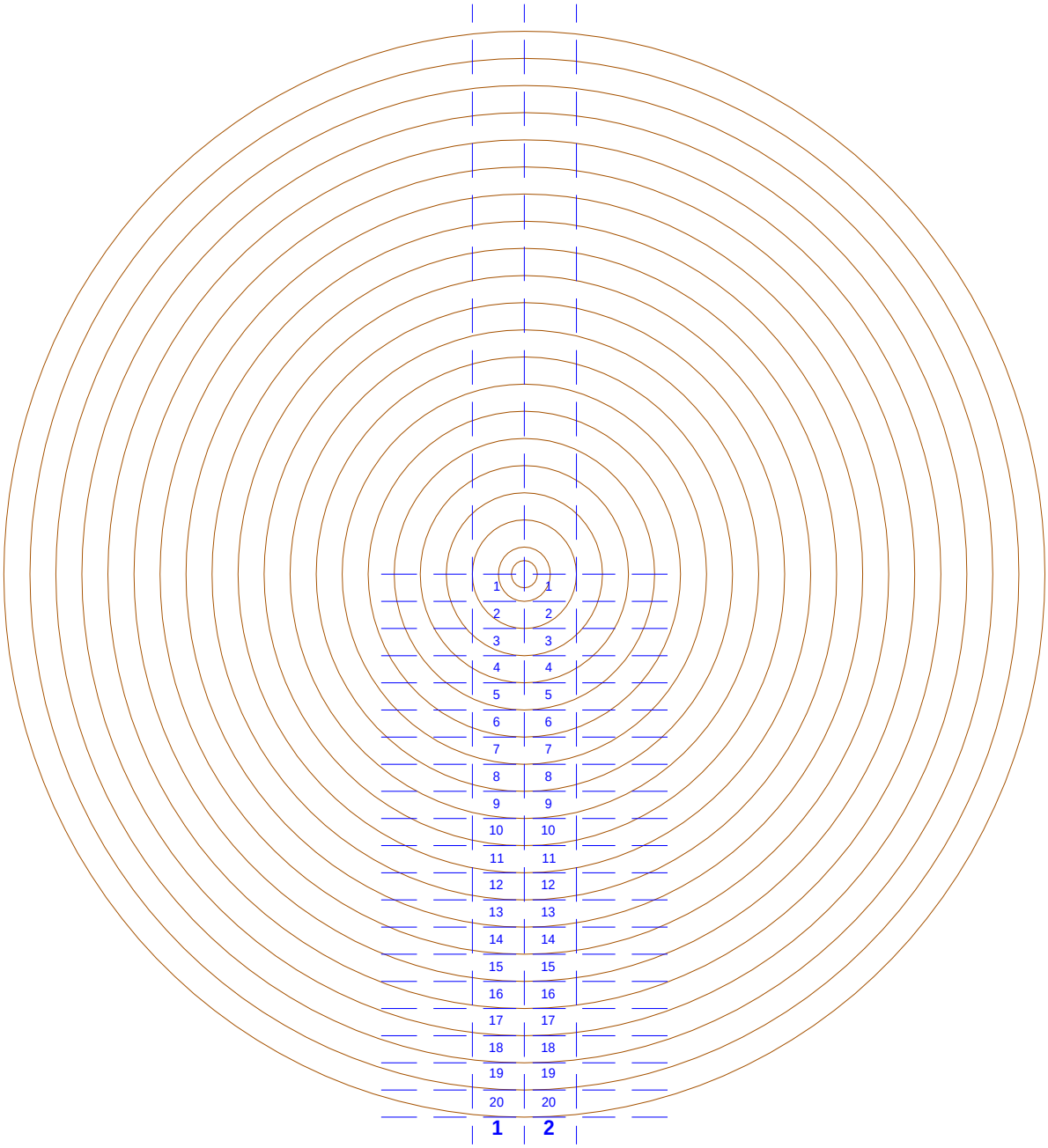
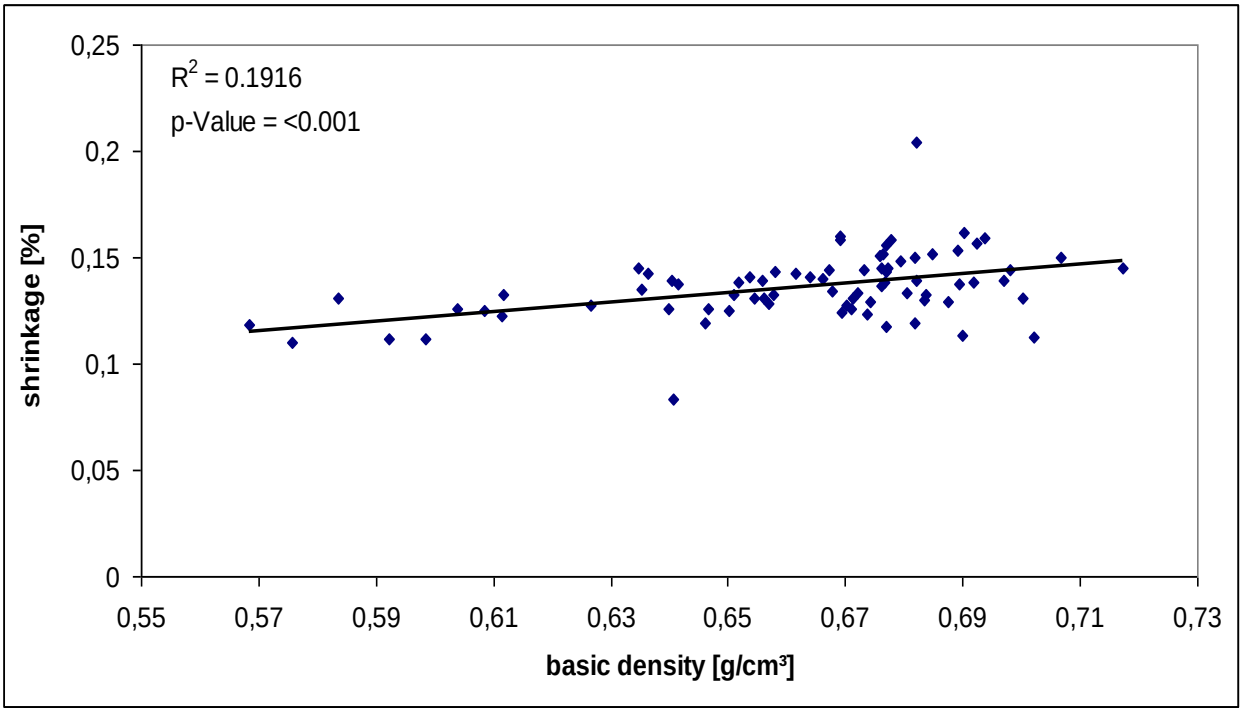
Appendix

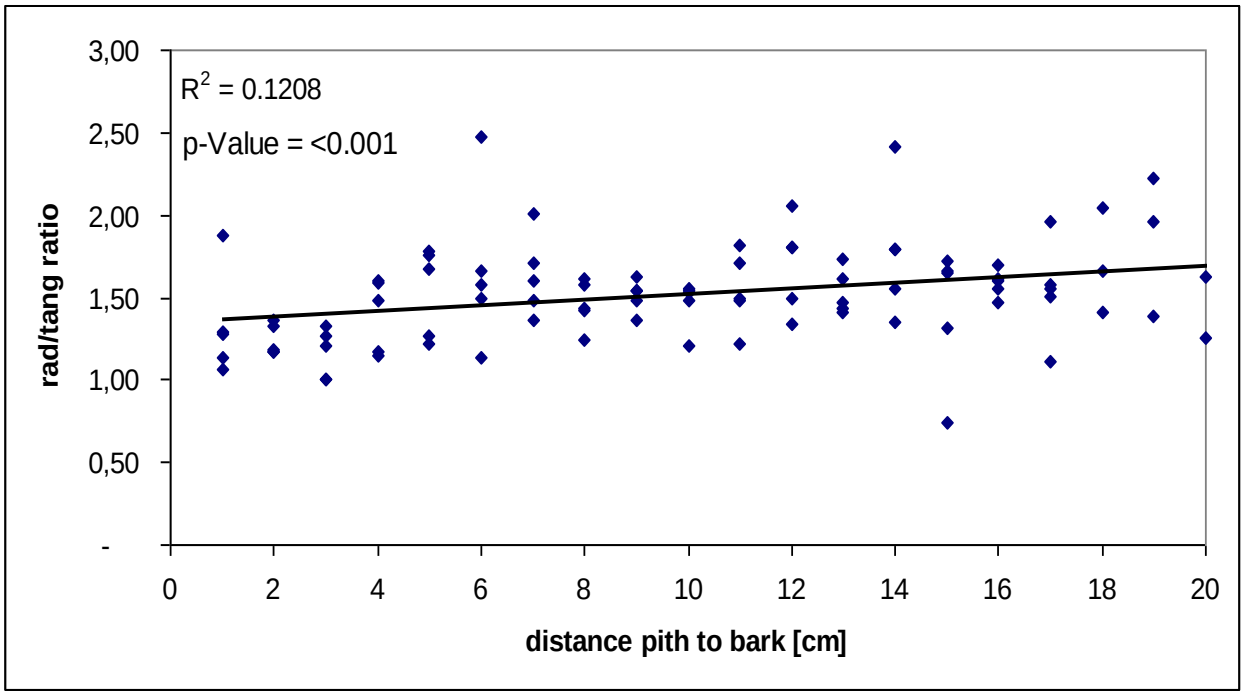
Appendix 1: Location plan of the white oak samples



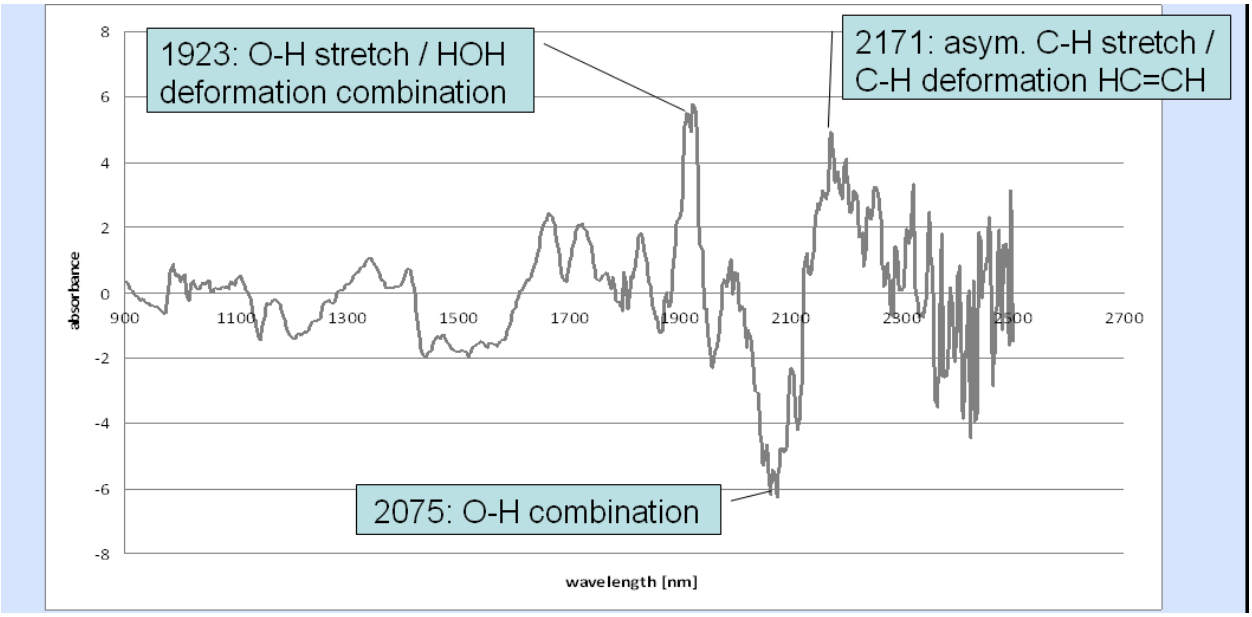
Appendix 2: Correlation between density and shrinkage



Appendix 3: Correlation between the radial and tangential ratio of the shrinkage and the distance from the pith to the bark



Appendix 4: Spectrum of the NIR prediction of vanillin



Appendix 5: Spectrum of the MIR prediction ot the total extractive content