UNIVERSIDAD DE LA REPÚBLICA

FACULTAD DE INGENIERÍA

Tesis para optar al Título de

Magíster en Ingeniería Química

WATER-BASED COATINGS FOR THE PREVENTION OF DISCOLOURATION BY KNOT EXTRACTIVES ON PINE WOOD

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Montevideo, Uruguay

2019

UNIVERSIDAD DE LA REPÚBLICA

FACULTY OF ENGINEERING

Thesis submitted in partial fulfilment of the requirements for the Degree of

Master of Chemical Engineering

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ISSN 1688-2806

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Tesis de Maestría en Ingeniería Química

Facultad de Ingeniería

Universidad de la República

Montevideo, Uruguay, 2019.

It is our choices that show what we truly are, far more than our abilities.

J.K. Rowling

Abstract

In the last decades there has been an increasing development of water-based wood coatings following the environmental concerns to lower the use of toxic solvents and to reduce the emissions of Volatile Organic Compounds. The opaque paints of this nature used in pine wood suffer from undesired discoloration, related to the presence of knots which have an outstanding high concentration of extractives, mainly resin acids, stilbenes and lignans, which migrate through the polymeric structure. This phenomenon is known as Knot Bleeding.

Within the framework of this thesis, the physicochemical nature of the extractives present on knots of *Pinus cembra* was studied and the substances responsible for the yellowing of white opaque paints were identified. Two stilbenes and five lignans were detected in the polar knot extracts and their presence was confirmed in the discolored coating over the knots.

The influence of the ageing conditions on the diffusion of knot extractives through a reference coating was studied. Both temperature and humidity have a key role in the migration of colored and non-colored compounds. The latter get degraded to colored substances after the exposure to sunlight radiation.

The Knot Bleeding resistance was tested according to the Standard Procedure for Knot Staining CEN/TC 139/WG 2 and the color of the coating over the knot was measured using CIELab coordinates

From the work presented in this thesis a deep understanding of the causes of the discoloration of water-based wood coatings is provided.

Keywords: Knot Bleeding, Pine, extractives, knots, discoloration, Water-based wood coatings.

Resumen

En las últimas décadas se ha producido un creciente desarrollo de recubrimientos de madera a base de agua a partir de la preocupación medioambiental por reducir el uso de disolventes tóxicos y las emisiones de compuestos orgánicos volátiles. Las pinturas opacas de esta naturaleza utilizadas en madera de pino sufren decoloraciones indeseadas, relacionadas con la presencia de nudos que tienen una alta concentración de extractivos, principalmente ácidos resínicos, estilbenos y lignanos, que migran a través de la estructura polimérica. Este fenómeno se conoce como *Knot Bleeding*.

En el marco de esta tesis se estudió la naturaleza fisicoquímica de los extractivos presentes en los nudos de *Pinus cembra* y se identificaron las sustancias responsables del amarillamiento de las pinturas. Se detectaron dos estilbenos y cinco lignanos en los extractos polares de nudos, y se confirmó su presencia en la pintura decolorada.

Se estudió la influencia de las condiciones de exposición en la difusión de los extractivos a través de un recubrimiento de referencia. Tanto la temperatura como la humedad juegan un papel clave en la migración de compuestos coloreados y no coloreados. Estos últimos se degradan a sustancias coloreadas después de la exposición a la radiación solar.

La resistencia al amarillamiento fue probada de acuerdo con el Procedimiento Estándar CEN/TC 139/WG 2 y la decoloración fue medida mediante el sistema de coordenadas CIELab.

A partir del trabajo presentado en esta tesis se proporciona un profundo entendimiento de las causas de la decoloración de los recubrimientos de madera a base de agua.

Palabras clave: Knot Bleeding, Pino, extractivos, nudos, decoloración, Recubrimientos de Madera.

Preface

The work presented in this thesis was conducted by Rodrigo Coniglio and supervised by Dr. Andrés Dieste, Associate Professor from the Forest Process Engineering Group of the Faculty of Engineering, Universidad de la República who helped in the design of the methods and procedures as well as studying the results. Furthermore, he contributed to the revision of reports and drafts.

The research that gives rise to the results presented in this publication received funding from the National Research and Innovation Agency under the code **POS_NAC_2018_1_151192.**

Prefacio

El trabajo presentado en esta tesis fue realizado por Rodrigo Coniglio y supervisado por el Dr. Andrés Dieste, Profesor Adjunto del Grupo de Ingeniería de Procesos Forestales de la Facultad de Ingeniería de la Universidad de la República, quien ayudó en el diseño de los métodos y procedimientos, así como en el estudio de los resultados. Además, contribuyó a la revisión de informes.

La investigación que da origen a los resultados presentados en la presente publicación recibió fondos de la Agencia Nacional de Investigación e Innovación bajo el código **POS_NAC_2018_1_151192.**

Acknowledgements

To my thesis director, Prof. Andrés Dieste, for trusting that I could be a good candidate to make this Master´s thesis. For his continuous encouragement and support I am truly grateful.

I want to express my very profound gratitude to my supervisor at BASF, Dr. Wolfgang Gaschler, for the opportunity to work on his team. I feel grateful for the trust he put in me to make this thesis. He consistently allowed this project to be my own work but steered me in the right the direction whenever he thought I needed it.

To my mentors, Prof. Leonardo Clavijo and Prof. María Noel Cabrera, from the Forest Process Engineering group, for the opportunity of being part of a group in which is easy to feel at ease. For their trust and guidance, I feel very grateful.

To my colleagues at the Institute of Chemical Engineering, and especially to all my group mates at the Forest Process Engineering Group, with whom I have been lucky to establish a relationship of friendship that makes daily work enjoyable.

To the staff of BASF and in particular to Mrs. Andrea Schmalenbeck and Mrs. Ellen Schmidt, for their help with all the experimental part of this thesis. I found nothing but willingness whenever I made the most unusual requests.

To Anika Gehrke, for being a true friend for us during our stay in Germany. I want to thank her for all the company and support, for showing us the best of German culture and inviting us to have Christmas with her lovely family. She has been a very good friend and our favorite German teacher.

To all my friends, for always supporting me no matter what I decide to undertake. For their company and understanding I shall be always in debt.

To my beloved family, for providing me with unfailing support and continuous encouragement. For their acceptance and everlasting guidance, I will be forever grateful.

And to Mark, for his unconditional love and wholehearted support. For always bringing calm into my life when things seem to go wrong. For believing in me, much more than I do. For making me feel at home, no matter where in the world.

Agradecimientos

A mi director de tesis, Prof. Andrés Dieste, por confiar en que podía ser un buen candidato para realizar esta tesis de maestría. Por su continuo aliento y apoyo, estoy verdaderamente agradecido.

Quiero expresar mi profunda gratitud a mi supervisor en BASF, Dr. Wolfgang Gaschler, por la oportunidad de trabajar en su grupo. Me siento agradecido por la confianza que depositó en mí para realizar esta tesis. Consistentemente permitió que este proyecto fuera mi propio trabajo, pero me dirigió en la dirección correcta siempre que creyó que yo lo necesitaba.

A mis mentores, Prof. Leonardo Clavijo y Prof. María Noel Cabrera, del grupo de Ingeniería de Procesos Forestales, por la oportunidad de formar parte de un grupo en el cual es fácil sentirse a gusto. Por su confianza y su guía, me siento muy agradecido.

A mis colegas del Instituto de Ingeniería Química, y especialmente a todos mis compañeros del Grupo de Ingeniería de Procesos Forestales, con los que he tenido la suerte de establecer una relación de amistad que hace agradable el trabajo diario.

Al personal de BASF y en particular a la Sra. Andrea Schmalenbeck y a la Sra. Ellen Schmidt, por su ayuda con toda la parte experimental de esta tesis. No encontré nada más que buena voluntad cada vez que hice los pedidos más inusuales.

A Anika Gehrke, por ser una verdadera amiga para nosotros durante nuestra estancia en Alemania. Quiero agradecerle por toda la compañía y el apoyo, por mostrarnos lo mejor de la cultura alemana e invitarnos a pasar la Navidad con su encantadora familia. Ha sido una muy buena amiga y nuestra profesora de alemán favorita.

A todos mis amigos, por apoyarme siempre sin importar lo que decida hacer. Por su compañía y comprensión estaré siempre en deuda.

A mi amada familia, por brindarme su apoyo infalible y su continuo aliento. Por su aceptación y guía constante, estaré eternamente agradecido.

Y a Mark, por su amor y apoyo incondicionales. Por traer siempre calma a mi vida cuando las cosas parecen ir mal. Por creer en mí, mucho más de lo que yo creo. Por hacerme sentir en casa, no importa en qué parte del mundo.

Danksagungen

An meinen Betreuer, Prof. Andrés Dieste, für das Vertrauen, dass ich ein guter Kandidat für diese Masterarbeit sein könnte. Für seine anhaltende Ermutigung und Unterstützung bin ich ihm wirklich dankbar.

Meinem Vorgesetzten bei der BASF, Dr. Wolfgang Gaschler, möchte ich für die Möglichkeit, in seinem Team zu arbeiten, sehr herzlich danken. Ich bin dankbar für das Vertrauen, das er mir bei der Erstellung dieser Arbeit entgegengebracht hat. Er ließ dieses Projekt konsequent zu meiner eigenen Arbeit werden, aber er führte mich in die richtige Richtung, wann immer er dachte, dass ich es brauche.

An meine Mentoren, Prof. Leonardo Clavijo und Prof. María Noel Cabrera von der Forest Process Engineering Group, für die Möglichkeit, Teil einer Gruppe zu sein, in der man sich leicht wohlfühlen kann. Für ihr Vertrauen und ihre Führung bin ich sehr dankbar.

Meinen Kollegen am Institut für chemische Ingenieurwissenschaft und insbesondere an alle meine Kollegen in der Forest Process Engineering Group, mit denen ich das Glück hatte, eine freundschaftliche Beziehung aufzubauen, die die tägliche Arbeit angenehm macht.

An die Kollegen bei der BASF und insbesondere an Frau Andrea Schmalenbeck und Frau Ellen Schmidt für ihre Unterstützung beim gesamten experimentellen Teil dieser Arbeit. Ich habe jedes Mal, wenn ich die ungewöhnlichsten Bitten gemacht hatte, nichts als guten Willen gefunden.

An Anika Gehrke, dass sie uns während unseres Aufenthalts in Deutschland eine wirkliche Freundin war. Ich möchte mich für die Begleitung und Unterstützung bedanken, dafür, dass sie uns das Beste der deutschen Kultur gezeigt und uns eingeladen hat, Weihnachten mit ihrer lieben Familie zu verbringen. Sie ist eine gute Freundin und unsere Lieblings-Deutschlehrerin.

An alle meine Freunde, dass sie mich immer unterstützt haben, egal was ich beschließe zu tun. Für ihre Gesellschaft und ihr Verständnis werde ich immer dankbar sein. An meine geliebte Familie für ihre unermüdliche Unterstützung und anhaltende Ermutigung. Für ihre ständige Führung und Annahme werde ich ihr ewig dankbar sein.

Und an Mark, für seine bedingungslose Liebe und Unterstützung. Weil er immer die Ruhe in mein Leben bringt, wenn etwas schief zu laufen scheint. Weil er an mich glaubt, viel mehr als ich. Weil ich mich bei ihm wie zu Hause fühle, egal wo auf der Welt.

Contents

Chapter 1. Introduction

Wood has always been chosen as a construction and decoration material for its resistance, durability and its natural beauty, which is even sometimes imitated in synthetic materials. To protect the wood and enhance its aesthetical appearance, coatings have been used for centuries in wood timber for exterior and interior applications, such as furniture, joinery and wood panels (Scrinzi, et al., 2011; Bulian & Graystone, 2009).

Durability and biological resistance are given naturally to the plant by some compounds that, although a minor fraction in wood, comprise several types and can differ from each other in its chemical nature, behavior and functions towards the plant development. The extractives, as they are named, contribute to the wood color and odor and confer resistance to fungi or insect attack (Donegan, et al., 1999; Parham & Gray, 1984; Sjöström, 1993).

The knots, which are generated by the normal addition of wood tissue around a branch base, constitute a disturbance of wood appearance and clear boards have always been chosen for applications where wood is used as a finishing material. However, the increasing demand of wood and the harvesting of ever smaller and younger trees, make clear boards more expensive and less available, which make the processing of knotwood ever more frequent (Savidge, 2003).

In the last decades, there has been an exponential development of water-based wood coatings, as a way to reduce the hazardous components in paint formulations, as well as to diminish the emissions of volatile organic compounds. Waterborne opaque coatings have shown to suffer from discoloration when applied to knotted wood, especially from pine wood. This phenomenon is known as *Knot Bleeding* and it is related to the high concentration of extractives present in the knots (Donegan, et al., 1999; Wiedenhoeft, et al., 2010; Nussbaum, 2004; Suttie & Ekstedt, 2004). It is of major concern for the wood coatings industry, and especially for furniture and joinery producers which use them.

Discoloration of paints or stains can be caused by the diffusion of the hydrophilic extractives through the coating due to the penetration of the film by water, either caused by liquid water, such as rain drops, condensed vapor or air moisture, or by the water contained in the coating itself (Donegan, et al., 1999). This phenomenon becomes more complex, as wood extractives include both hydrophilic and hydrophobic compounds. In fact, resins and pitch may be brought to the surface by an increase in temperature. These compounds melt as temperature rises and can be mobilized through natural gaps of the wood, reaching the coating. This problem has a greater incidence in the case of wood with knots, since these usually contain cracks in their structure, which facilitates the transport of the resins (Bulian & Graystone, 2009; Donegan, et al., 1999).

Not all extractives are colored. In fact, some substances that migrate and reach the coating surface are colorless. However, it has been proved that UV radiation oxidize some of these compounds and turn them into colored deposits after the exposition to sunlight. (Bulian & Graystone, 2009).

In the present work, the Knot Bleeding phenomenon was investigated in detail and the chemical nature of the responsible substances was studied. The performance of water-based acrylic coatings was tested using a reproducible alternative method as well as the standard test for knot staining assessment. Furthermore, the influence of the ageing conditions on the discoloration was evaluated.

Chapter 2. Literature Review

1. Introduction

The objective of this chapter is to present the state of the art of the phenomenon of Knot Bleeding as well as to provide information on the chemical nature of the compounds responsible for the event. Particular attention is given to the presence and structure of knots and the most common extractives that can be found in the hardwood of conifer species, and especially pine. The main compounds identified as responsible for the discoloration of opaque coatings are studied. The wood structure, the formation of heartwood tissue and the main causes for extractive synthesis are addressed, as being a key aspect to understand the composition of knotwood and the fundamentals of the staining of wood coatings.

The main influencing factors on the migration of extractives through water-based coatings are described, and the phenomenon of discoloration of paints on hardwoods due to the diffusion of tannins is shortly explained, given the fact that it is a common staining problem over water-based coatings.

2. Wood Structure

Wood is the structural tissue found in all woody plants, forming the interior fraction of every stem, branch and root. It is constituted by different types of cells which play different roles in the living tree such as support, minerals and water transport from the root to the crown of the tree, storing substances as well as metabolizing particular compounds required by the tree for special functions. (Kretschmann, et al., 2007; Bulian & Graystone, 2009).

The formation of wood is a complex process, which depends on different factors such as environmental parameters, temperature and water potential (Schmitt, et al., 2014). Therefore, even though all woody plants can be recognized as similar, different samples of wood even from the same tree are only similar within broad limits (Parham & Gray, 1984; Savidge, 2003).

The tissue responsible for the formation of new wood cells is the *cambium*, a group of living active cells between bark and wood (Figure 1). By dividing and multiplying its cells, the cambium generates *xylem* inwards (constituting tissue of wood) and *phloem* outwards (responsible for bark formation). This growth, called *secondary growth,* in opposition to the vertical grow of trees, leads to a radial thickening of the stem, branches and roots (Schmitt, et al., 2014; Parham & Gray, 1984).

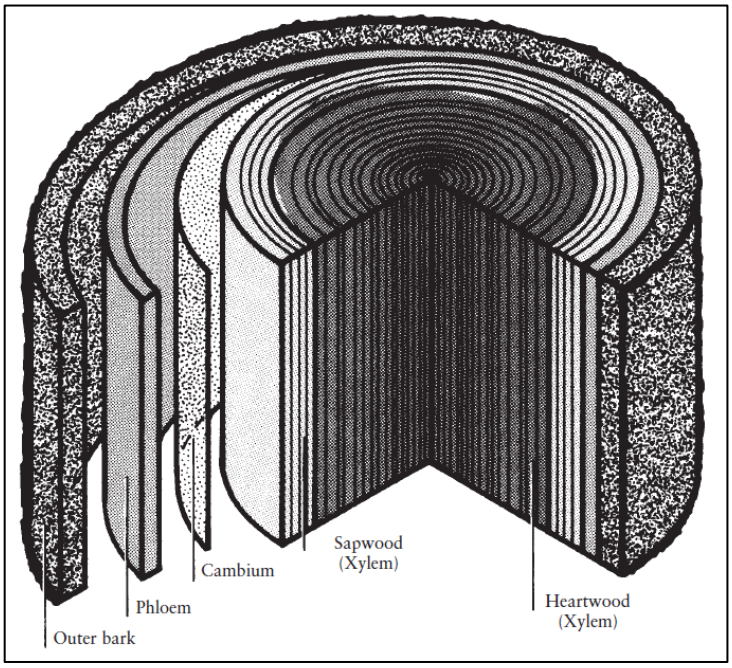

Figure 1. Main parts of a mature tree stem involved in the radial growing (Shmulsky & Jones, 2011).

The basic matrix of wood is cellulose, a polysaccharide chain composed of cellulose monomers. In softwoods, it constitutes around 40-44% of the dry wood. When it comes to hardwoods, this number could be even higher, reaching 50% of the dry wood on some species. Its high degree of polymerization makes it the main structural component of wood. The ability of the hydroxyl groups to bond between parallel chains makes it possible to form fibrils and give the tree structural resistance (Schmitt, et al., 2014).

Table 1. Chemical composition of some wood species (% of dry wood weight). (Sjöström , 1993).

Species	*Extractives*	*Lignin*	*Cellulose*	*Hemicelluloses*
		Softwoods		
Pinus radiata	1,8	27,2	37,4	33,2
Pinus sylvestris	3,5	27,7	40,0	28,5
Picea abies	1,7	27,4	41,7	28,3
Picea glauca	2,1	27,5	39,5	29,6

Hardwoods				
Eucalyptus camaldulensis	2,8	31,3	45,0	19,2
Eucalyptus globulus	1,3	21,9	51,3	25,2

Polysaccharides of different monomers can be also found in wood, combining pentoses like xylose and arabinose, as well as hexoses like mannose and galactose. They are known as *hemicelluloses.* They have lower molecular masses, and by creating links between cellulose and lignin, the hemicellulose confers strength to the cell wall (Schmitt, et al., 2014). Even if they have similar total content of hemicelluloses (20-30% of the dry wood weight), softwoods and hardwoods differ on its composition. The conifer trees have higher amounts of glucomannans while hardwoods' hemicelluloses are mainly composed by xylans.

The third main component of wood is *lignin,* an amorphous polymer of high molecular weight. It is formed by a complex polyphenolic structure which acts as cementing material that bind the wood fibers together. It comprises around 20-30% of the dry wood, mainly concentrated in the wood cell and between fibers (Sjöström , 1993). As it happens to hemicelluloses, some differences can be found between the lignin of conifer and broadleaves species, especially in terms of reactive groups, which is translated into differences on pulping processes for softwoods and hardwoods (Sixta, 2006).

Besides the structural constituents, wood has several other components that usually represent a minor fraction but that can differ from each other in its chemical nature, behavior and functions towards the plant development. They are called *extractives*, since they can be extracted by solvents like water, ethanol, acetone or other organic substances (Sjöström, 1993; Parham & Gray, 1984; Hillis, 1987). Regarding the variability that can be found between two samples of wood, the extractives play a significant role. Their chemical composition can be considerably different in different zones of the same tree and even in different tissues of the same area (Hillis, 1987). Because of the relevance of their chemical nature and behavior for the present work, extractives will be particularly studied.

3. Heartwood formation

While the tree is young, the conduction of water and nutrients in the xylem occurs through the entire section of the tree. The tissue is known as *sapwood* since it is responsible for the transportation of sap, name given to the aqueous solution that contains the main nutrients to the plant. It stores triglycerides and fats as reserve for the winter and it is the main zone of the tree where water is transported from the roots to the crown (Nisula, 2018).

After consecutive secondary growing, the central cells of wood initiate a process of degeneration: by conversion of starch, sugars, and organic compounds present in the sapwood, the cells produce resins, phenolic substances and other organic deposits, which change the appearance and functions of the resulting tissue. Eventually, all the entire area, the *heartwood*, is composed by dead and hollow cells and as the tree grows, its portion in the stem increases. When the tree is mature, the wood is composed essentially of cell walls and voids (Parham & Gray, 1984).

Following the formation of extractives at the cell, the contents then proceed to infiltrate cell membranes and usually confer the central region of the stem a distinctive darker color. However, in some species the compounds present in these tissues have not color of their own, which makes it more

difficult to distinguish heartwood from sapwood with a naked eye (Parham & Gray, 1984).

By this change in the chemical structure, known as *duraminization*, and following a process of lignification, the central wood of the tree acquires more support ability and thanks to its high concentration of extractives it has greater decay resistance when compared to sapwood (Parham & Gray, 1984). The toxic properties of extractives and their formation in response to biological attack are discussed with greater detail in the following sections.

4. Extractives

The extractives comprise compounds of both lipophilic and hydrophilic nature. Even among closely related species, extractives can differ enormously, and they are not distributed equally throughout the wood: as it was already pointed out, the extractives are mainly located in the heartwood. They are substances which are necessary to fulfil some functions of the tree. While some fats constitute an energy source for the tree, phenolic compounds and resin acids protect the wood against microbiological damage (Sjöström, 1993). They give color, smell and taste to the wood, work as reserve nutrients, plant hormones or catalysts for biosynthesis (Nisula, 2018).

The formation of extractives is mainly related to the occurrence of injured areas and reaction wood. It has been observed that after a fungal or bacterial attack, the tissue surrounding the affected area rapidly initiates a process of chemical changes and phenolic compounds, such as lignans and stilbenes, grow in concentration (Hillis, 1972). Therefore, the extractive concentration can be significantly different between trees of the same species.

The formation of extractives can also occur as a way to prevent an infection. When a branch breaks, the damaged tissue of the main stem becomes vulnerable to biological attack. The response to the possible

infection is the rapid formation of phenolic extractives which create a barrier enough to prevent biological attack. (Hillis, 1987). As it was explained, this branch gets eventually embedded into the stem and becomes a knot in the wood.

It is a well-known fact the close relation between the extractive content of wood and its durability. Naturally durable woods have higher concentration of extractives, and they lose their resistance to fungi and termite attack if the extractives get removed (Kirker, et al., 2013).

The effects of environmental conditions have direct influence on wood extractives content. It was observed, for example, that the amount of Pinosylvin in the heartwood of Scots Pine (*Pinus sylvestris*) varied from south to north in Sweden (Erdtman, et al., 1951).

Furthermore, the content of extractives and the amount of each type varies within the same tree, depending on the tissue: in the sapwood, little amounts of extractives are present (around 2-5% in *Pinus sylvestris*) and the most part constitute triglycerides as an energy source. When considering heartwood, the quantity of these compounds gets increased to around 15% and can be as high as 20% of the dry wood weight. In this case, the main compounds are resin acids and phenolic compounds (Figure 2).

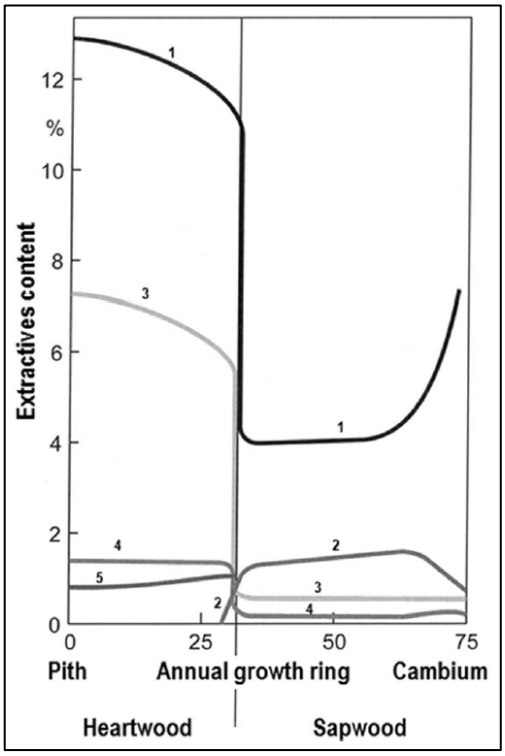

Figure 2. Amount and composition of extractives across the stem of Scots Pine (Pinus sylvestris) 1) Total Resin Content, 2) Fats, 3) Resin acids, 4) Free fatty acids, 5) Pinosylvins. (Roffael, 2016).

Extractives have been considered responsible for the color alongside with lignin, during the weathering of wood surfaces. Different studies suggest that it is the absorption of light, particularly the UV-light portion of sunlight, that it is responsible for the primary photooxidative degradation of wood (Feist & Hon, 1984; Grekin, et al., 2005). In fact, wood is a good light absorber, given that UV light penetrates the wood up to 75 μm and the visible light does it up to 200 μm (Pandey, 2005).

The effect of light on lignin has been studied and it is reported that it leads to the formation of free radicals, which react with oxygen to produce chromophore groups such as carbonyls, carboxyls, quinones, peroxides and

hydroperoxides (Timar, et al., 2016). It is likely that extractives also suffer from similar processes, given the fact that they have the ability to absorb light between 300 and 400 nm and therefore undergo photochemical reactions leading to discoloration (Feist & Hon, 1984). Given the fact that this is a surface phenomenon, normally the discoloration takes place on the first stages of the exposure to sunlight. Pandey (2005) found out that unextracted wood surfaces show a rapid color change at the initial period of exposure because the presence of extractives causes an increase on the rate of photo-discoloration. It must be taken into account, that the phenolic groups that form the lignin and which commonly generate chromophore groups are usually present in many wood extractives. Therefore, generally all woods change toward a yellow to brown color due to the photooxidation of lignin and extractives (Feist & Hon, 1984).

It seems that the ability of phenolic extractives to absorb light and degrade into colored compounds, is in fact a protective property of the extractives to preserve lignin from UV light. In fact, it has been reported that wood extractives, by absorbing light and scavenge free radicals, retard lignin oxidation during wood photodegradation (Chang, et al., 2010).

Extractives are usually classified according to the solvent that allows them to be removed from the wood. For this reason, it is common to find in reports of chemical characterization of wood that the extractives are grouped

under "Water Extractives" or "Ethanol Extractives", among other solvents. This clarification is necessary, since it is not possible to affirm that all extractives have been removed from wood with a single solvent.

According to their chemical nature, the main extractives can be divided into:

1. Terpenes and terpenoids
2. Aliphatic compounds
3. Aromatic phenolic compounds

Nisula (2018) made a complete chemical characterization of the extractives of 14 species of pine, among other conifers. Except the volatile terpenes, all other main extractives were determined by GC and GC-MS. Throughout this revision on the chemical composition and occurrence of wood extractives, this work will be addressed several times.

4.1 Terpenes and terpenoids

This group comprise a huge class of extractives which can be found largely in softwoods. They derive from isoprene and can therefore be classified according to the number of units linked. The simple terpenes, which are formed by the bond of isoprene elements in head-to-tail way, can be monoterpenes, diterpenes or triterpenes if they have 2, 4 or 6 isoprene units respectively (Figure 3).

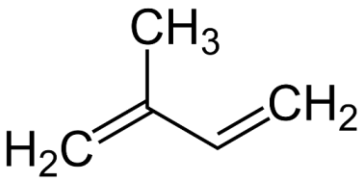

Figure 3. Chemical structure of the isoprene, precursor for terpenes and terpenoids.

However, these compounds react to form substances with oxygen-containing functional groups, such as hydroxyl, carbonyl, and carboxylic acid groups. The resulting compounds are known as *terpenoids* since they are not pure hydrocarbons. The large number of possible pathways and the high reactivity of isoprene make the number of compounds that can be formed, depending on the conditions, considerably high. In fact, more than 7500 structures have been identified (Sjöström, 1993).

Although different, all terpenes and terpenoids share some properties: they are light-colored or colorless, insoluble in water and most of them are fragrant (Hillis, 1987).

In softwoods, they are mainly present in the *oleoresin,* found in resin canals, particularly developed in pine wood (Joye & Lawrence, 1967). These canals keep oleoresin under pressure (up to 50 kPa) to favor transport to damaged areas (Hillis, 1972; Sjöström, 1993; Nisula, 2018).

Monoterpenes can be found in the volatile fraction of many species. In pine, among the most important constituents are *α*- and *β-pinene,* which contribute to pinewood characteristic odor (Figure 4) (Yokouchi & Ambe,

1984). The main function of volatile terpenes is as a solvent for other terpenes and terpenoids. In fact, the oleoresin is basically a solution of diterpenic acids (60-80%) dissolved in monoterpenes (20-40%) (Nisula, 2018). In this way, the plant can seal wounds by transporting the resin to the damaged tissue. Upon evaporation of the volatile fraction, the diterpenoids solidify into a thermoplastic vitreous material which can fill gaps and wounds (Savidge, 2003). This material acts as a waterproof coating which protects the tree from dehydration and microbial attack. This protection is needed temporally, while the tree closes the wound by annual growth (Holmbom, et al., 2008). On some species of conifers, the wound gets eventually sealed by a special resin, often called *callus resin*, which is associated to mainly three types of wounds: traumatic open wounds, where the cambium has been damaged; wounds located arbitrarily on the stem; and wounds closing around dead branches, which are the forerunners to knots (Holmbom, et al., 2008).

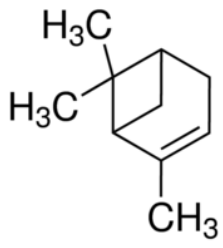

Figure 4. Chemical structure of the α - pinene.

The monoterpenes have not been thoroughly studied, given the fact that they are the most volatile compounds of the wood and, therefore, they volatilize when the raw material for chemical characterization gets dried

(Nisula, 2018). However, some studies have been made regarding their properties as biological defense for the plant. Zhang et al. tested 41 species of monoterpenes for the toxicity against wood white-rot fungi. Even if some oxygenated monoterpenes can have good properties against fungi, the ones typically present in pinewood, such as α-pinene, β-pinene or limonene, show no antifungal properties (Zhang, et al., 2016). This conclusion supports the hypothesis that the main function of monoterpenes is as volatile solvent for resin acids.

A mixture of monoterpenes is industrially obtained by the distillation of pine resin and is worldly known as *turpentine*. It is sold mainly as a solvent and as a source of materials for organic synthesis.

The main diterpenoids present in the oleoresin of softwoods are the resin acids. Even if many representatives can be found, the most common are those classified into *abietane* and *pimarane* types. In the first group it can be found the *abietic acid* (Figure 5), *palustric acid, neoabietic acid* and *dehydroabietic acid.* Among the pimarane-type resin acids the *pimaric acid* is the predominant. The typical average amount of resin acids is 0,2-0,8% of the wood weight. However, their presence in knotwood is much higher, especially on conifer species.

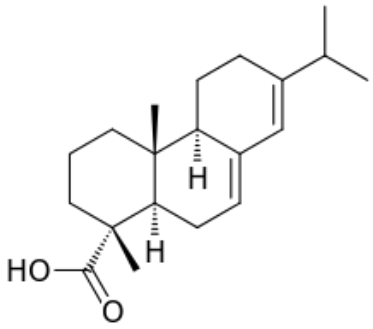

Figure 5. Chemical structure of the abietic acid, the most common resin acid in pine wood.

The resin acids have a hydrophilic carboxyl group and a hydrophobic skeleton that allows them to solubilize agents in soap form (Nisula, 2018).

In fact, during the Kraft pulping of softwoods, the resin and fatty acids present in the wood suffer from saponification reactions which catch many insoluble lipophilic compounds which eventually start to float in the storage tank and are skimmed off and collected (Silvestre & Gandini, 2008). After acidification, the resulting liquor is known as *tall oil* and has 26-42% of rosin, which is essentially a mixture of resin acids (Huibers, 2000). Both tall oil and pine rosin are product with high market value.

While only small amounts can be found in sapwood of pine, from 0,1 to 0,9% of the dry wood, the heartwood can have considerably higher number of resin acids: 0,2-4,4%. However, the knots are the most concentrated part of the tree, reaching as high as 21,5% of the dry knotwood (Nisula, 2018). Due to its functions for wound sealing, the presence on knots can be several times higher than even the heartwood. Nisula (2018) found that in *Pinus sibirica*

the dead knots have a resin acid content more than 30 times higher than the amount present in the normal heartwood.

Willför et al. (2003) studied 7 trees of *Pinus sylvestris* which were different in age, number of knots and place of growing. In all cases, the content of total diterpenoids was almost insignificant in sapwood (0,1 to 0,4%) while the knots contain almost 30% of resin acids. In all knot samples, the abietic acid was the most abundant.

4.2 Aliphatic Compounds

The main aliphatic compounds in wood are fats formed by the esterification of fatty acids as triglycerides. Even though more than 30 fatty acids have been identified, the most commonly present triglycerides in conifer species derive from the *oleic, linoleic* and *pinoleic* acids. They act as an energy source for the plant and are located almost exclusively in the sapwood (Nuopponen, et al., 2004; Willför , et al., 2003; Nisula, 2018). Sapwood can contain more than 10 times the amount which is present in heartwood and knots. This is mainly because of the energy-saving function on sapwood.

The highest number of free fatty acids can be found in heartwood, since the triglycerides are hydrolyzed when heartwood is formed. Apparently, the presence of free fatty acids in heartwood is due to its function as a forerunner

for the formation of phenolic compounds. In fact, it is likely that some fatty acids metabolize to pinosylvin and other aromatic compounds when these substances are needed in the heartwood.

Nisula (2018), concluded that the heartwood of *Pinus* species contains from 0,91 to 16 mg of free fatty acids in 100 g of dry wood. The knots can have a similar number, which is also supported by Willför et al. (2003): while the sapwood has only traces of free fatty acids, the heartwood and knots of Scots Pine, can have around 0,5% of the dry wood weight.

4.3 Aromatic Phenolic Compounds

This group of extractives is the most widespread of the secondary wood components, thousands of them having been identified (Hillis, 1987). They provide to heartwood its particular dark color and although very different in nature among each other, the most of them are present in wood thanks to their toxic or repellent properties for biotic attackers (Savidge, 2003).

The phenolic extractives are mainly located in heartwood and bark of both softwoods and hardwoods, and only traces can be found in sapwood. As it happens to other extractives, their biosynthesis is controlled genetically. Therefore, each species and each tree according to the environmental conditions, produce specific substances as a response (Sjöström, 1993).

For many years, all phenolic extractives in wood were considered *tannins* and no further classification was given. However, true tannins share some properties such as water solubility, molecular weight between 500 and 3000, and the ability to precipitate alkaloids, gelatins or other proteins (Hillis, 1987). Given the fact that there are other phenolic extractives with very different properties, they can be divided as follows:

1. Stilbenes
2. Lignans
3. Flavonoids
4. Tannins

4.3.1 Stilbenes

The stilbenes are characterized by having a conjugated double bond and it is proven that they inhibit the growth of fungi. In fact, after an insect or biological attack, stilbenes and stilbenoids are formed as a response (Hart & Shrimpton, 1979). Many studies have been done related to the biological activity of stilbenes. It has been reported that some of them are toxic to bacteria (Välimaa, et al., 2007) and have water-repellent properties.

The most typical stilbene present in pine is *Pinosylvin*. Together with some derivatives, such as *Pinosylvin monomethyl ether* (Figure 6), is highly present in injured wood and knots, and can be directly responsible for the discoloration of wood coatings due to their migration through the lacquer.

Besides, they can be oxidized in the presence of light, decomposing into colored substances. (Nisula, 2018; Morgan & Orsler, 1968)

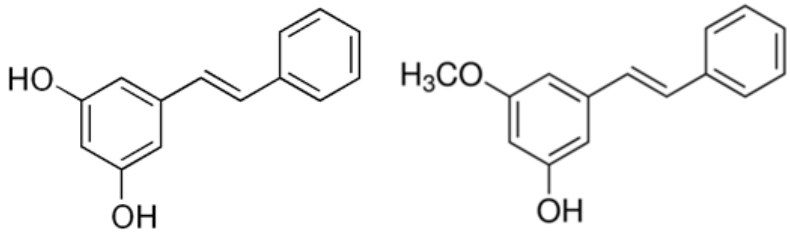

Figure 6. Chemical structure of pinosylvin (left) and the pinosylvin monomethyl ether (right).

The relation between stilbenes and color has been extensively reported. During the acid-sulphite process of cellulose pulping, stilbenes can interfere with the bleaching of the pulp (Fengel & Wegener, 1984). Moreover, it has been observed that discoloration of mechanical pulp and paper can occur due to heating of phenolic extractives which may be stilbenes (Polcin & Rapson, 1971).

Furthermore, it has been reported that wood containing high concentrations of extractives, darkens when exposed to sunlight (Hillis, 1972). Morgan et al. (1968) concluded that light induces the darkening of species containing hydroxystilbenes and the discoloration of the wood surface gets reduced when the wood is previously extracted. It has also been reported that the stilbenes are related to the yellowing of newsprint in *Pinus radiata* thermomechanical pulps.

Some research projects done on the influence of Pinosylvin and other stilbenes on the decay resistance and durability of wood, have shown that these compounds bind to lignin in the cell wall (Belt, et al., 2017; Nisula, 2018). This theory gets supported by the higher concentration of stilbenes usually found on living knots compared to dead knots of the same tree (Willför , et al., 2003; Nisula, 2018). Other phenolic compounds such as lignans, on the other hand, show no significant differences on their concentration on living and dead knots. Living knots are formed by the growing of the main stem around a living branch and, to form a continuous tissue, stilbenes seem to fulfil a fundamental role on bonding with lignin.

The close relation between the decay resistance of heartwood and its concentration of stilbenes has been extensively reported (Harju, et al., 2003; Venäläinen, et al., 2004). While decay-resistant and decay-susceptible wood do not differ from each other in the relative proportion of cellulose, hemicellulose and total lignin, the number of extractives is much higher in the resistant trees, especially for Pinosylvin as well as its mono- and demethylated derivatives.

Stilbenes have been considered to possess fungicide and bactericide properties, but also act as antioxidants (Pietarinen, et al., 2006). It seems that the characteristic property of stilbenes, which set them apart from other extractives, is as radical scavenger (Kebbi-Benkeder, et al., 2015; Kebbi-

Benkeder, et al., 2017; Schultz & Nicholas, 2000). The protection against damaging radicals is particularly necessary on the knots since the motion of the branches generates free radicals in the branch base (Kebbi-Benkeder, et al., 2017).

Moreover, it has been suggested that white-rot and brown-rot fungi use radicals to disrupt cell walls (Backa, et al., 1993). Lignocellulolytic enzymes are not able to penetrate into undecayed wood cell walls, reason why they generate radical intermediates which attack lignin (Valette, et al., 2017). It has been reported that phenolic extractives have high radical scavenging capacities against superoxide anion hydroxyl radical, peroxyl radical, hypochlorite ion, hydrogen peroxide and nitric oxide (Royer, et al., 2011). Willför et al. (2003) compared the antioxidative potency and radical scavenging capacity of knotwood extracts to the commercial antioxidants Trolox and butylated hydroxyanisole (BHA). The studied extracts show good antioxidant properties similar to those of the reference, but the pure compounds perform worse, indicating a possible synergistic effect, which agrees with previous works (Schultz & Nicholas, 2000; Schultz & Nicholas, 2002; Hart & Shrimpton, 1979). Regarding the radical scavenging capacity, the stilbenes extracted from *Pinus cembra* were even more effective than both the Trolox and BHA. The synergistic effect could not be seen but on the contrary, pure compounds containing stilbene-type compounds were the most effective radical scavengers.

Stilbenes are characteristic of pine, being absent in other conifer groups (Nisula, 2018; Hovelstad, et al., 2006). Pietarinen et al. (2006) made a characterization of 14 conifer species, in order to evaluate the antioxidant properties of the hydrophilic knotwood extracts. From all the softwoods evaluated, only the pine species present stilbenes among their water-soluble extractives. In the case of *Pinus resinosa* and *Pinus sylvestris,* the pinosylvins constitute the 38% of the total hydrophilic compounds.

They can be found mainly in the heartwood and knots while their presence in sapwood is marginal (Nisula, 2018). The knots contain much higher number of stilbenes, specially the living knots: in *Pinus sylvestris,* for example, the living knots have 6% against the 2,5% present in the dead knots. The results of Willför et al. (2003) are consistent with the characterization done by Nisula (2018): the number of stilbenes can be as high as 7% and the higher amount is always found on living knots. This may be because of the capacity of pinosylvins to bond with lignin: as it was explained, the living knots are generated when the branch is still living, and the main stem grows around it, which enables the bonding of stilbenes and the surrounding tissue. However, when dead knots are formed the pinosylvins are mainly present as a biological defense.

The stilbenes have hybrid polarity, which varies for each compound. Willför et al. (2003) extracted knotwood from seven Scots pine trees, first

with hexane and then with an acetone:water (95:5 v/v) mixture. While 70-100% of the Pinosylvin present on the knots was extracted into the hydrophilic phase, the Pinosylvin dimethyl ether was only present in the hexane extract (Willför , et al., 2003).

4.3.2 Lignans

Lignans derive from the condensation of two phenylpropane units. They can be found in considerable quantities in softwood species such as pine. The most important members of this group are *Pinoresinol* (Figure 7), *Nortrachelogenin* and in Norway Spruce (*Picea abis*), is abundant the *Hydroxymatairesinol.* In contrast to stilbenes, lignans are highly polar and therefore can be extracted by water or polar solvents. Willför et al. (2003) found that the selectivity for lignans to the acetone extract was higher than 99%.

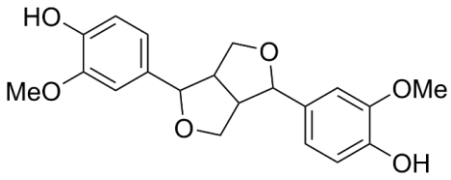

Figure 7. Chemical structure of the pinoresinol.

Significant work has been done on the identification and localization of lignans in conifers. The work done by Willför et al. concluded that the knots are the richest source of lignans in nature. In fact, when comparing to sapwood and heartwood of the same tree, the number of lignans in knots can be more than 50 times higher (Nisula, 2018). While almost absent in

sapwood, the lignans in knots of pine can be as high as 5% of the dry weight of the wood. This concentration of lignans is enhanced by the deposit of callus resin, which is essentially composed by lignans, lignan esters and hydroxycinnamic acid derivatives (Holmbom, et al., 2008). On the characterization of Scots pine knots done by Willför et al. (2003), Nortrachelogenin was the most abundant lignan in all knots.

Lignans have been identified as lignin precursors, therefore their presence on knots might be related to the lignification of compression wood in response to mechanical stress (Kebbi-Benkeder, et al., 2015). It was found that compression wood in Norway spruce knots contained less lignans than the normal wood of the same knot, which may be because of the conversion of lignans into lignin in order to strengthen the tissue subjected to physical stress (Holmbom, et al., 2003). It was reported that knots of Spruce trees in Northern Finland contain more extractives (mainly lignans) that knots from trees grown in the south, attributed to the severe growth conditions and higher requirements (Willför, et al., 2003; Piispanen , et al., 2008). However, this could not be confirmed for Scots Pine knots (Willför , et al., 2003). This could explain why lignans can only be found on conifer trees, which keep their leaves during winter and therefore are more susceptible to the wind.

Furthermore, the antioxidant properties of lignans have been widely reported (Kebbi-Benkeder, et al., 2015; Schultz & Nicholas, 2000; Valette, et

al., 2017; Willför, et al., 2003; Neacsu, et al., 2007; Kirker, et al., 2013; Smeds, et al., 2012). These compounds can scavenge reactive oxygen species that are required for the enzymatic or oxidative process of wood decay.

4.3.3 Flavonoids

The colors found in the heartwood of different species, are related to the *flavonoids*. They have a typical tricyclic carbon skeleton and they differentiate from each other by substitution of hydroxyl and methoxyl groups in the two aromatic rings (Savidge, 2003). Common members are *chrysin*, present in pine, or *catechin* (Figure 8).

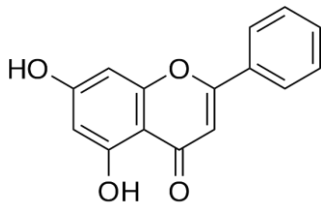

Figure 8. Chemical structure of chrysin, a common flavonoid present in pine wood.

The flavonoids also attract insects for their importance to pollination and act as nectar guides. It has been said that they also protect needles and seedlings from harmful radicals formed by UV radiation (Nisula, 2018). Flavonoids are present in high concentrations in knotwood of some hardwood species, while lignans and stilbenes are predominant in softwood knots (Neacsu, et al., 2007; Pietarinen, et al., 2006). Kebbi-Benkeder et al. (2015) also suggest that the flavonoids may have a significant role on the ability of some species to self-prune. They found that some bad-pruning

species such as alder, cherry and black locust contain high quantities of flavonoids on their knots. On the other hand, species which show ability to self-prune easily have small traces of flavonoids. This may be because these compounds slow down the dynamics of biotic and abiotic agents involved in the degradation of dead branches.

While lignans and stilbenes are the main compounds responsible for defense against fungi and bacteria on softwoods, it has been reported that flavonoids retard the progression of wood decay fungi on hardwoods such as cherry or oak (Neacsu, et al., 2007; Aloui, et al., 2004). This fact could explain why some hardwoods are very resistant to biological attack even if their content of stilbenes and lignans is low (Willför, et al., 2003). Neacsu et al. (2007) studied the antioxidant effect of six flavonoids isolated from European Aspen (*Populus tremula*) knotwood and all of them showed antioxidant properties, based on their capacity to inhibit lipid peroxidation and scavenge peroxyl radicals, similar to the commercial antioxidant Trolox. Pietarinen et al. (2006) also found similar results when comparing the hydrophilic extracts of bark and pure flavonoids.

Regarding their distribution, in conifer species hardly any flavonoids can be found in the sapwood. The number in heartwood and knots is similar and can be around 3% of the dry knotwood weight (Nisula, 2018). For pine, the concentration of flavonoids is usually lower, and for Scots pine it is below

0,02% of the dry wood weight (Willför , et al., 2003). On hardwood knots, however, they can account more than 50% of the total hydrophilic extracts (Pietarinen, et al., 2006). This may be explained by the fact that the compounds responsible for biological defense on softwoods, stilbenes and lignans, are absent on hardwood knots. On broadleaves species, these functions are fulfilled by the flavonoids and tannins (Tascioglu, et al., 2013; Pohjamo, et al., 2003).

4.3.4 Tannins

When flavonoids polymerize, they generate what it is known as *condensed tannins*. While these are usually resistant to hydrolysis, some tannins give gallic or ellagic acid as products of hydrolysis and are therefore known as *hydrolysable tannins* (Sieniawska & Baj, 2017; Amarowicz & Janiak, 2019). Even though tannins are typically found in large quantities in hardwoods of some tropical species (Hillis, 1987), other non-tropical hardwoods, such as oak, are highly tannin-concentrated.

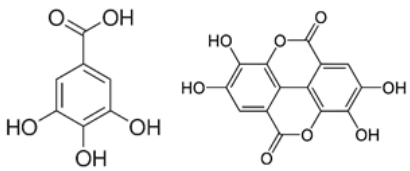

Figure 9. Chemical structure of the gallic acid (left) and ellagic acid (right).

"Tannins" refer to all water-soluble phenolic compounds having molecular weights between 500 and 3000 and having the ability to

precipitate alkaloids, gelatin and other proteins (Ky, et al., 2016; Bate-Smith & Swain, 1962). The number of tannins is countless given the immense number of possible polymerizations between polyphenolic units. For this reason, tannins are usually treated as a group, considering that specific compounds are dependent on even each sampled tree. On the Figure 10 the structure of a well-known tannin, the tannic acid, is presented.

Given the fact that they are water extractable, they constitute a major problem of staining in water-borne coatings in hardwood species, event known as *Tannin Bleeding*. This event will be further studied later in this chapter, as being also a staining problem of water-based coatings due to migration of extractives.

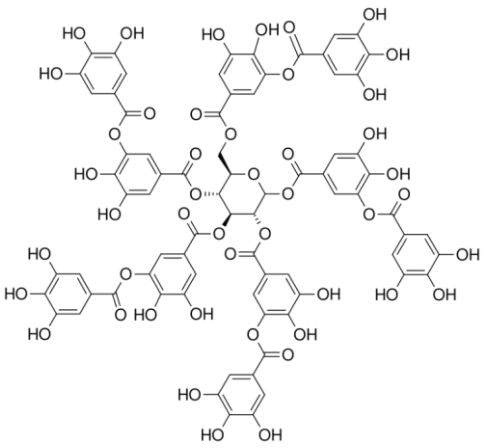

Figure 10. Chemical structure of tannic acid.

Even if the presence of tannins in conifer woods is very low, they can be found in its bark and depending on the species, tannins can constitute around the 10% of the bark dry weight (Pietarinen, et al., 2006; Kemppainen,

et al., 2014; Bianchi, et al., 2015; Chupin, et al., 2013). Among conifers, pine bark, however, is known to have small amount of tannins (Bianchi, et al., 2015; Miranda, et al., 2012).

When a dead knot has encapsulated the former branch bark, some tannins can be also present in the knot. After it has been coated with a waterborne white paint, the bark ring commonly found on these dead knots discolors the surface of the lacquer, mainly because of the tannins being dissolved by the water of the paint.

Some tannins of hardwood species such as Eucalyptus have been reported to cause discoloration problems on pulping. For instance, the insoluble ellagic acid can form very insoluble green-yellow complexes with some inorganic ions such as magnesium, calcium, sodium or aluminum. These substances may remain in the cellulose pulp, therefore increasing bleach requirements (Hillis, 1971).

It is a well-known fact that hardwoods contain more hydrophilic extractives than conifers, which usually contain higher amounts of extractives of lower polarity (Kebbi-Benkeder, et al., 2015). This may be because for hardwoods tannins fulfil fungicide and bactericide functions, reason why sugar derivatives and gallic acid can be found in the polar extracts of hardwoods.

Different studies suggest that tannins have defense functions which act mainly through a metal chelation mechanism. In fact, these molecules chelate trace materials present in enzymes of fungi and bacteria, which are essential for the oxygen metabolism (Pietarinen, et al., 2006; Scalbert, 1991). These molecules drastically reduce the activity of haem-containing class II peroxidases by chelating iron ions and copper-dependent laccases (Valette, et al., 2017; Scalbert, 1991).

As it was discussed, the tannins and flavonoids present on broadleaves trees are responsible for the defense against fungi, bacteria and chemical oxidation. Therefore, flavonoids are usually found on the knotwood of hardwood species and some of them, like Eucalyptus, contain also high concentration of tannins in its knots. Tannins are typically found as well in bark of conifers species (Pietarinen, et al., 2006). However, aqueous pine bark extracts have shown less antifungal activity compared to hardwood extracts such as mimosa and quebracho. In some studies, wood treated with pine bark extractives showed even higher mass losses when compared to the untreated specimens (Tascioglu, et al., 2013). The existing literature regarding this effect, however, differs on the antifungal effect depending on the solvent of extraction and the fungi studied. Some studies suggest that the bark extracts have antifungal activity, particularly the methanol-soluble but much less than hardwood extracts (Kokalis-Burelle & Rodríguez-Kábana, 1994; Alfredsen, et al., 2008). On the other hand, an investigation about the

fungi growth over a substrate made from Pinus sylvestris bark showed that pine bark supports enzyme production and increases the fungi development (Valentín, et al., 2010). It becomes clear then that the extractives responsible for defense against biological factors are specific for softwood and hardwood species, either by toxic, antioxidant, radical scavenging or metal chelation properties.

5. Knots

Knots are branch bases that become embedded into the tree trunk after the addition of successive increments of woody tissue (Hillis, 1987; Parham & Gray, 1984; Zink-Sharp, 2003). Because the growth of the branches is simultaneous to the growth of the main stem in a radial direction, any tangential sawing of the main trunk results in knots on the boards (Shmulsky & Jones, 2011) (Figure 11).

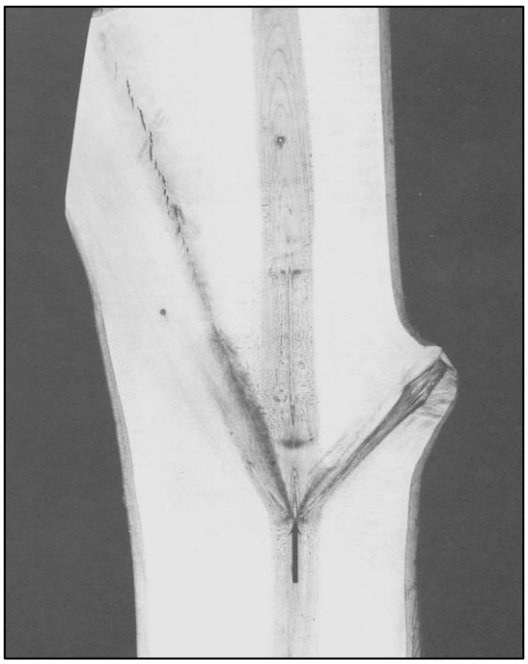

Figure 11. Longitudinal section of a tree stem showing two branches and their union with the main trunk. (Shigo, 1983).

If the branch is still living while the main trunk grows around it, the generated knot is known as *living* (or *intergrown*), since the cambium of the branch and trunk are continuous. In fact, the lignin of the main stem bonds with phenolic compounds present in the branch, such as *Pinosylvin,*

generating a homogenous tissue with no gaps around the knot (Belt, et al., 2017). The living knots do not cause inconvenient when siding and drying the wood, due to its strong bonds to the trunk. Its color is clearly darker than the surrounding tissue, because of its higher concentration of extractives.

During the tree growth, a natural pruning takes place on the lower tree bole of the plant (Parham & Gray, 1984). Once the branch dies, the cambium degenerates and stops diameter growth. The cambial layer of the main stem continues to grow, encasing the dead branch in the process (Shmulsky & Jones, 2011). The resulting knot is known as *dead* (or *loose*). These are generally darker than the living knots because they have a higher concentration of extractives (Bonura, et al., 2010; Kebbi-Benkeder, et al., 2017). Since the plant is not able to create bonds between the dead branch and the surrounding wood, the tree generates resins in the vulnerable area to fill the gaps and produces polyphenolic extractives to create a boundary to prevent the infection from microorganisms. This can be seen as a darker color in the sapwood surrounding the knot (Figure 12). This boundary layer contains large amounts of phenolic deposits conferring this area a greater decay resistance. Sometimes, this tissue who suffered environmentally initiated changes is known as “false heartwood”, due to their darker color in comparison to normal sapwood (Schmitt, et al., 2014). Furthermore, if the branch does not lose its bark before getting embedded by the trunk, it will become a ring surrounding the surface of the knot.

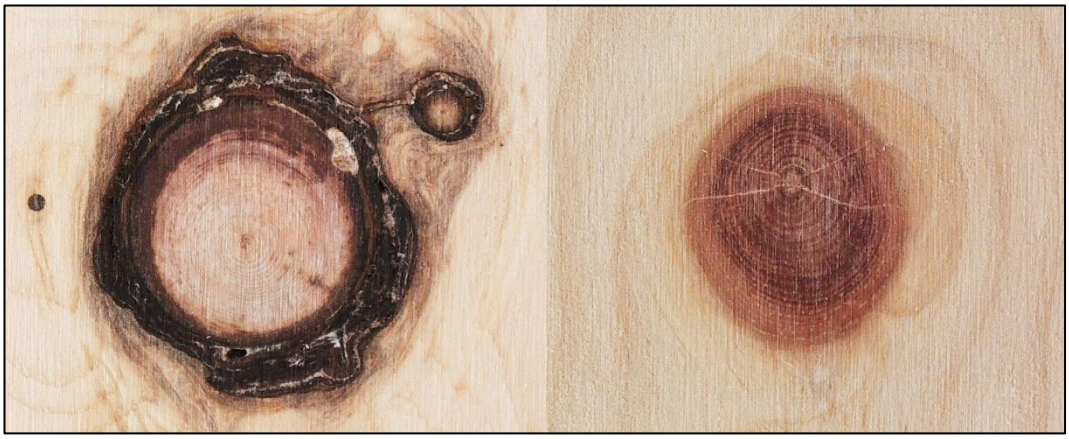

Figure 12. Comparison between a dead knot (left) and a living knot (right). The dead knot shows bark surrounding the embedded branch as well as some gaps in its structure. The wood around has a darker color showing the "false heartwood". Pictures taken from boards used for this project.

All dead branches have an intergrown section originally formed when they were still alive. Depending on the sawing section, it is therefore possible to have a knot which has both loose and intergrown sections (Figure 13). This type of knot is known as *spike knot* (Brundin & Fröbel , 2016).

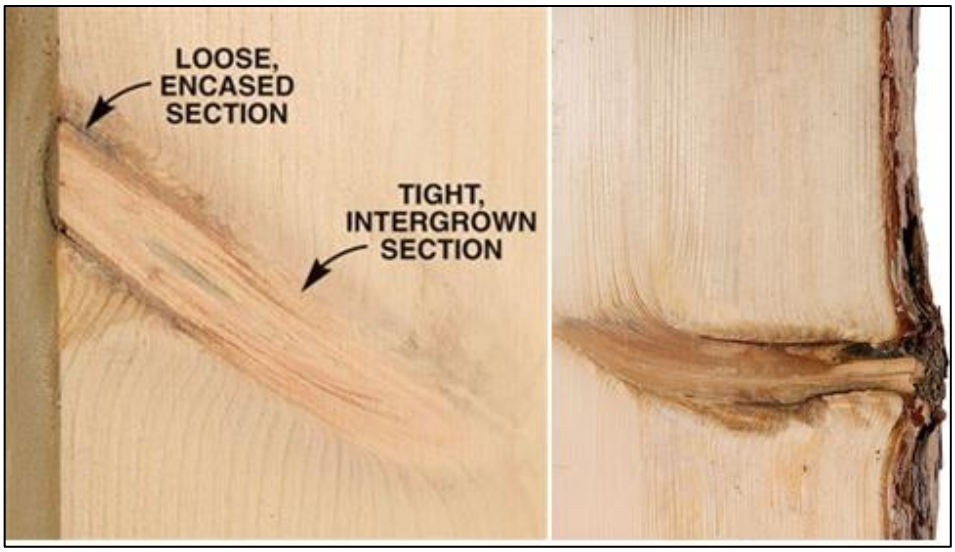

Figure 13. Longitudinal section of dead branches embedded into the main tree trunk. Left: detail of the encased and intergrown sections of the branch. Right: dead branch completely enclosed by the main stem. On the right end of the branch, is it possible to see the ultimate sealing by the wood bark (Caspar, 2004).

Knots have always been considered the main defect in stem wood. Their presence and type are usually the first judgment done to classify the timber not only for furniture but also for structural applications. It is widely known that knots reduce the strength of lumber. In some cases, the knot may even have a greater negative effect than a drilled hole because of the distortion in the grain that happens around it (Figure 14) (Shmulsky & Jones, 2011).

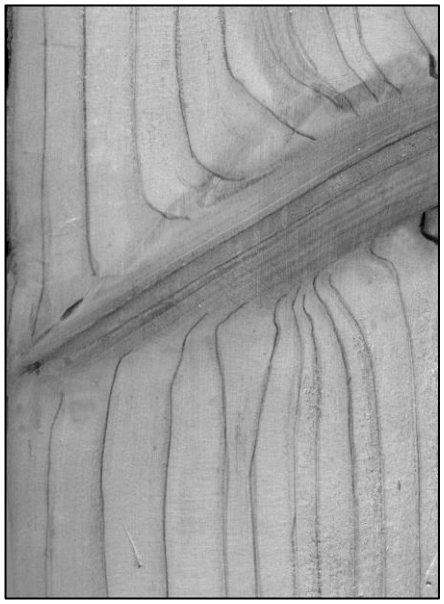

Figure 14. Detail of ring width deformations around a knot. (Duchateau, et al., 2015).

Loose knots are even more detrimental to wood strength and can generate more problems when the board gets dried. As there are not strong bonds between the knot and the wood, the resin that fills the gaps and keeps the knot in its position can melt during drying or become brittle after the evaporation of volatile compounds and therefore it is possible that the knot gets separated from the wood. This generates what is known as a *knothole* and reduces the strength of the board and drastically changes its appearance

(Zink-Sharp, 2003). Even though there are ways to stick the knot to the wood, knot-free wood is widely preferred, and many strategies have been carried out on the plantations to reduce the proportion of knotwood in the trunk. The most effective is the artificial pruning (Parham & Gray, 1984). The branches should be trimmed as close to the bole as possible, to ensure that the sheath of new growth covers the stub and the wood produced thereafter is knot-free (Shmulsky & Jones, 2011). Furthermore, artificial pruning reduces the probability of having dead knots, since the branches are cut before they can naturally die.

Knot-free wood has traditionally been chosen as a raw material for joinery and furniture. The decrease in wood strength due to the loose knots and the staining problems caused by the extractives have led to the preference to discard wood with knots and look for clean wood in applications in which it is used as a finishing. However, as trees are harvested younger, a smaller portion of the stem underwent the process of natural pruning, which increases the fraction of wood with embedded branches (Savidge, 2003).

Softwood knots are known to be very extractive-rich, particularly in the case of pinewood. Some species have knots with more than 40% of extractives making them the highest extractive-concentrated wood structure (Belt, et al., 2017; Kebbi-Benkeder, et al., 2015). On hardwoods, even if the

total amount of extractives on the knots is lower than in softwoods, their concentration is still higher if compared to its sapwood or heartwood (Kebbi-Benkeder, et al., 2015).

The high concentration of extractives on the knots have been interpreted as a response to the physical stress and chemical vulnerability that characterizes this wood. In fact, knots develop reaction wood to protect the tree from mechanical loads such as wind. This is especially true for softwoods, since they do not lose their leaves during winter, and therefore are more vulnerable to high winds or snow (Kebbi-Benkeder, et al., 2015). To give more resistance to the branches of softwoods, some extractives act as lignin precursors. This is supported by the high degree of lignification of compression wood developed in softwoods. Hardwoods, however, present no lignans on their knots. The tension wood developed in those trees, contains higher number of polysaccharides and high sugar derivatives. It has been proposed that these saccharides reinforce the wood subjected to mechanical loads, such as the junctions between the main stem and branches. The high number of saccharides present in hardwood knots could be in accordance to this theory (Kebbi-Benkeder, et al., 2015).

On the other hand, extractives are also present to chemically protect the wood from biological agents as well as chemical oxidation. This is needed, as changes in the fiber orientation surrounding the knot and the self-pruning

of branches create easy spots for pathogens and oxygen to penetrate the stem. As it will be deepened on the next section, the extractives prevent the infestation of fungi and bacteria, thanks to their toxic properties. Moreover, the extractives protect the wood using other mechanisms such as free radical scavenging, antioxidation and metal chelation (Schultz & Nicholas, 2000; Valette, et al., 2017; Pietarinen, et al., 2006). In fact, Schultz et al. (2000 and 2002) found out that some wood extractives with no preservative effect showed an increase in efficacy when combined with a commercial biocide compared to the organic biocide alone. This could be explained by a synergistic effect of wood extractives against biological agents.

The concentration of extractives on the knots is very variable, given the fact that they are formed as a response to biological attack and environmental conditions (Kebbi-Benkeder, 2015). Moreover, it has been seen that knots from the highest branches of trees have smaller extractive concentrations (Kebbi-Benkeder, et al., 2017). This could be explained by three different reasons: first, the upper branches of the trees are the youngest, and therefore the biosynthesis of extractives may not have started yet or it may have extended only to some level. The non-structural carbohydrates found in the youngest knots in different studies support this hypothesis, because they are considered precursors for the formation of extractives (Kebbi-Benkeder, et al., 2017).

Second, the upper branches of trees show higher flexibility, reason why it is less probable that they get injured due to motion. Therefore, the highest branches form fewer traumatic resin ducts and their concentration of resins is lower than the knots of the crown-base knots (Kebbi-Benkeder, et al., 2017).

Last, the knots from the base of the tree have more compression wood given the fact that they are subjected to more physical stress than the upper trees (Kebbi-Benkeder, et al., 2017). As it was said, some extractives act as lignin precursor to add strength to the wood and therefore the compression wood has higher concentration of extractives.

Furthermore, knots are also undesired for cellulose pulp production. Their fibers are almost horizontal and have higher density and therefore high energy is needed to defibrate knotwood during mechanical pulping and higher amount of chemicals are required on chemical pulping (Kebbi-Benkeder, et al., 2015). Moreover, extra energy is required on wood chipping (Pietarinen, et al., 2006). Knots often remain unpulped after chemical pulping and decrease the screened pulp yield (Biermann, 1996). If they get to the bleaching process, they will consume more chemicals given the higher amount phenolic compounds. Moreover, they can cause the failure of downstream equipment in the pulp (Sixta, 2006). For this reason, the knots

are screened from the pulp and normally sent back to the digester and re-cooked (Holmbom, et al., 2003).

6. Extractive Bleeding

Knots are very prone to exudate resins and phenolic extractives because, as it was previously explained and detailed, they have the highest concentration of extractives. Water-based opaque coatings typically suffer from discoloration when applied over knots, because these extractives migrate through the lacquer and get to the surface, adding the effect of the UV radiation on oxidizing some of these components. The result is typically a yellow or brown stain over the knot (Figure 15).

Figure 15. Pine knot on a board used for this project (left) and the corresponding discoloration seen on a commercial water-based coating after artificial weathering (right).

Knots are end-grain in plain-sawn boards and thus they are efficiently positioned to bleed extractives. Moreover, knots commonly crack during drying or even only at room temperature which opens routes for rapid

movement of water, vapor or other substances that can mobilize extractives (Bonura, et al., 2010).

Knot Bleeding constitutes a problem not only because of the aesthetic appearance of the discolored paint but also because the painting can become brittle, crack or peel (Donegan, et al., 1999).

The easiest way to group the wood extractives to study the phenomenon of knot bleeding is between Water-Soluble Extractives (or Hydrophilic), and Solvent-Soluble Extractives (or Lipophilic).

Problems related to hydrophilic extractives are caused either by liquid water or by the exposure to humidity. The increase in water content which causes the diffusion of Water-Soluble Extractives may occur because of the own wood moisture content when the timber is not fully dry, from the water present in the coating itself which solubilizes extractives from the wood or moisture content coming from the environment (Bonura, et al., 2010; Cassens & Feist, 1986).

The most common water-soluble extractives which cause discoloration of opaque coatings are the tannins. This event, known as *Tannin Bleeding*, has been very studied given the fact that it is a typical problem for hardwoods such as Oak, Cedar or Merbau. However, as conifer knots typically do not

have tannins, this phenomenon is not related to the Knot Bleeding. The Tannin Bleeding of hardwoods will be shortly addressed in the next section.

Nevertheless, there are some knot extractives which are hydrophilic and can cause severe discoloration of water-based coatings. As it was already mentioned, lignans such as pinoresinol are polar and can be solubilized by water, together with some stilbenes. As these compounds are present in high concentrations in conifer knots, they represent a serious problem for knot bleeding.

After painting the wood, water can penetrate the wood from the unfinished sides of the board. Interior conditions include high moisture and poor ventilation, which facilitates the condensation of water vapor and causes a run-down type of extractive bleed. In the case of exterior applications, leaks in the walls can allow rain water to penetrate behind the siding and wetting the wood, which facilitates the diffusion of extractives to the surface of the coating (Bonura, et al., 2010; Bulian & Graystone, 2009).

The moisture of the wood itself, can contribute to the mobilization of hydrophilic compounds. If, at the time of painting, the wood has a moisture content higher that its equilibrium humidity, the excess water mobilizes to the surface of the coating and evaporates, carrying hydrophilic extractives which remain as a discoloration on the top of the lacquer.

To prevent the migration of water-soluble extractives to the surface of the coating, practices to reduce the moisture content of wood are encouraged. Keeping wood dry during handling and coating wood only if the moisture content is below 15% are recommended measures (Bonura, et al., 2010; Donegan, et al., 1999).

However, unlike the Tannin Bleeding of hardwoods, usually neither the moisture of the wood nor the water of the coating itself generate the migration of knot hydrophilic extractives to the topcoat, but the ambient humidity, water exposure and environmental conditions. Therefore, the discoloration over the knots is not seen right after the application of the coating, but it usually takes some months to show up. For this reason, the natural weathering procedure for knot staining assessment implies the exposure behind a glass for 120 days (Suttie & Ekstedt, 2004).

The migration of solvent-soluble extractives occurs via two different mechanisms. The first, even though the less common, is analogous to the diffusion of hydrophilic extractives due to the presence of water in the coating formulation. When using solvent-borne coatings, the solvent gets into the wood and dissolve lipophilic extractives. By volatilizing, extractives can be deposited on the coating surface.

However, the most common mechanism of migration of lipophilic extractives from knots it also happens when using water-based coatings and

it is likely to occur due to a combination of temperature, humidity and UV radiation (Bonura, et al., 2010; Williams & Feist, 1999; Feist & Hon, 1984).

The transport phenomena that are behind this event, are more complex than a simple diffusion. In fact, it has been seen that high humidity environments and the presence of liquid water enhance the migration of lipophilic extractives to the surface of the coating, even if the resins and pitch are not soluble in water (Suttie & Ekstedt, 2004). The reason behind this may reside in the ability of resin acids to solubilize lipophilic extractives in soap form, as it was earlier discussed (Nisula, 2018; Silvestre & Gandini, 2008; Huibers, 2000).

Sunlight, especially its UV fraction, enhances the discoloration caused by the migration of extractives through the paint. This problem is therefore particularly important for furniture and flooring but also for window manufacturers. In Scandinavia, for instance, most of the wooden windows are purchased in white, so knot staining and means to avoid it are of great interest (Vetter & Ekstedt, 2001). Moreover, some knot extractives are actually colorless, but upon oxidation due to UV radiation they can degrade into colored compounds which appear as a yellow spot on the coating surface (Morgan & Orsler, 1968).

The interaction between the monoterpenes and temperature seems to a fundamental driving force to resin bleed. It affects the viscosity of the pitch,

getting more fluid when the temperature increases. For this reason, in the Northern Hemisphere it is more common to have problems of resin bleed on wood exposed to the south and west (Bonura, et al., 2010). On the other hand, the temperature also affects the vapor pressure of the monoterpenes which act as solvents for the resin acid. In this regard, the relationship of α-pinene emission rate and temperature is similar to the one between its vapor pressure and temperature (Yokouchi & Ambe, 1984).

However, the influence of humidity and temperature on the migration of resinous extractives must be further studied, particularly the role of the moisture and its incidence on the mobilization of lipophilic compounds.

To prevent the resin to bleed, different strategies have been tried, without significant success. On the one hand, the removal of the monoterpenes by kiln drying have been suggested in order to fix the pitch and discourage its fluidity. However, even if the resin is completely solid, it can soften if the painted wood gets warm, and the bleeding may occur (Bonura, et al., 2010). Moreover, in some cases drying the wood before the application of the paint may be even detrimental to prevent the knot bleeding. Nussbaum (2004) studied the knot yellowing of different wood samples exposed outdoor for five months, previously dried at 50, 60, 70 and 105°C. They observed that increasing the temperature of drying generates an increase on the discoloration of the paint. Only after drying at 105°C a

decrease on the yellowing can be seen. This could also be explained by the loss of water from the wood and not only by the fixing of the resins. Furthermore, kiln drying at such a high temperature is not a definite solution, given the high costs of energy and time to dry the wood before applying the coating.

Regarding the development of wood coatings with blocking properties against knot bleeding, not many solutions are available, especially for water-based anionic coatings. This type of coatings comprises the most common polymers whose polymerization precursor is an anionic molecule. If a cationic molecule is the initiator of the reaction, the polymer is said to be cationic.

Given the importance of water and humidity for the migration of the extractives, water repellents such as paraffin have been used as a way to prevent the water to getting in contact with the wood surface (Williams & Feist, 1999). However, not all paints are compatible over water repellents, and adhesion or penetration problems can result (Donegan, et al., 1999). Moreover, in many cases the water repellence is short-lived, and the formulation of the repellent is solvent-based which makes it incompatible with a waterborne coating.

A two-component binder system was developed for preparation of a coating with anti-bleeding properties which contains tertiary amine

functional acrylic dispersion in combination with an epoxy functional acrylic binder (Bohorquez & Mestach, 2017). This product, however, has proven to be not fully effective.

Cationic binders have also been suggested as good products for the prevention of knot bleeding. It has been reported that a waterborne cationic dispersion has the best anti-knot bleeding performance when evaluated against one and two- component waterborne and solventborne primers (Scheerder, et al., 2011). However, the waterborne binders commonly used for industrial wood coatings are anionic. This implies that using a cationic binder as a primer against knot bleeding requires specific equipment and additives, which cannot be used on the other products. Therefore, the cationic dispersions are not preferred by wood industries and other solutions must be developed.

6.1 Tannin Bleeding

A very common discoloration caused by hydrophilic extractives on highly tannin-concentrated hardwoods occurs in the first cycles of wetting after the application of the paint causing a diffuse discoloration. The main cause of this migration is the water present in the waterborne coatings, which quickly penetrates the wood and acts as a solvent for this particular type of hydrophilic extractives. The result is therefore a rather heavy discoloration of the coat, diffused over the entire applied area in opposition to Knot Bleeding,

which is a localized discoloration. This is explained by the fact that tannins are not localized on a single spot, but all over the wood (Figure 16)

Figure 16. Diffuse discoloration due to tannin bleeding (left) over a wengé board compared to the performance of a paint with tannin-blocking properties (right). The center of the board was left unpainted to show the original color of the wood.

The discoloration can be eventually worsened by the environmental humidity, the moisture of the wood itself or by the exposure to water. However, this is a staining phenomenon that can be seen right after the application and which can be assessed on an easier way when compared to Knot Bleeding.

Water and humidity can also penetrate the coating through gaps and damaged areas created by the use of nails and fasteners on painted wood. In these cases, the discoloration can be typically seen as a localized brown stain around the nail. However, this phenomenon is not directly related to the blueish stain that can also be seen around nails. The last is an iron stain

caused by the reaction between the iron leaching from low-quality fasteners and tannins present in wood (Bonura, et al., 2010).

Traditionally, solvent-borne primers have been used to prevent this kind of extractive bleeding. The use of oil-alkyd primers or water repellents before the application of the waterborne topcoat can prevent the diffusion of hydrophilic extractives (Bonura, et al., 2010; Donegan, et al., 1999). To block these extractives, a coating must contain a hydrophobic region that acts as a barrier to water-soluble molecules (Kimerling & Bhatia , 2004).

However, with the increasing development of full waterborne systems for wood coatings, the use of solvent-borne primers is discouraged, and alternative solutions have been developed. The main idea is to use prime coats which typically get discolored, but chemically block the extractives on this layer, preventing the diffusion to the topcoats (Donegan, et al., 1999).

The most used mechanism to block the tannins in the primer, is by the metal chelation of the phenolic molecules. By the addition of soluble metal cations, such as Zn^{2+}, Al^{3+} or Zr^{4+}, some complexes with the anionic groups of the soluble tannins are formed. The results are insoluble complexes which are no longer carried by the water present in the following coat (Figure 17).

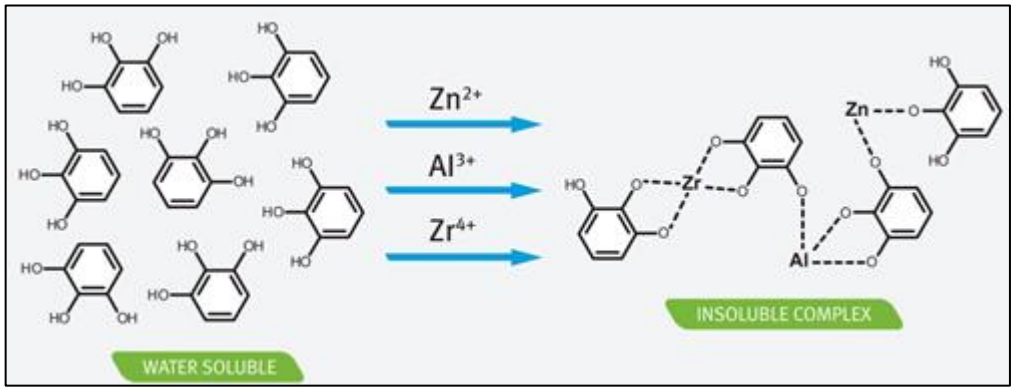

Figure 17. Diagram of the prevention of tannin staining by the formation of insoluble complexes (ICL\ Advanced Additives, 2018).

Therefore, even if the tannins are still colored, they remain as a stain only in the first layer of primer, and the topcoat does not suffer from discoloration. Commercially, this can be achieved by using additives which are basically salts of the metals needed to chelate the tannins (ICL\ Advanced Additives, 2018).

To prevent Tannin Bleeding, the polymer itself can be modified to produce the chelation of the tannins, without the need of the additives. BASF has developed a polymer dispersion, Joncryl ® 8227, which is used in water-based anti-bleeding primers on tropical hardwoods, preventing the discoloration of the topcoat (BASF, 2013).

7. Wood Coatings

According to the European Standard EN 971-1 (2006) a coating is a *"Product that, when applied to a substrate, forms a film possessing protective, decorative and/or other specific properties".*

In this regard, this definition comprises very different types of coatings, applied on very different kind of substrates such as glass, metal, plastic or wood. The information reviewed in this section refers to the technologies related to wood coatings, even if many of these aspects apply to all kinds of substrates.

The main components that may be present in a typical paint are listed on the Table 2. The main properties of a coating are determined by the component that forms the continuous film. This component, usually known as *binders,* are organic polymers which can be either of natural origin (like linseed oil) or completely synthetic (such as acrylics) (Bulian & Graystone, 2009). The characteristics and main properties of the binders relevant for this project are further studied on the next section.

The coating material is applied to the substrate before forming a film and therefore this can be done only if it is in a liquid form. The binders are carried in a volatile component either in solution or dispersion form, which finally evaporates during and after the application.

Table 2. Typical composition of paints. (Lambourne & Strivens, 1999).

	Components	Typical function
Vehicle (continuous phase)	Binder	Provides the basis of continuous film and the properties of the final finish.
	Solvent	The means by which the paint may be applied.
Pigment (discontinuous phase)	Pigment	Provides opacity, color and optical effects.
	Additives	Minor components with different functions such as rheology modifiers, wetting agents, defoamers, etc.

Coatings are classified into *solvent-borne* (or solvent-based) and *water-borne* (or water-based), depending if the volatile component is a non-aqueous solvent or water. Before about 1950, almost all coatings were solvent-borne, and they were second only to the automobile complex as a source of Volatile Organic Compounds (VOC) pollutants (Wicks, et al., 2007). These compounds have effect on the concentration of ozone and therefore in the UV radiation penetration of the earth´s atmosphere. Moreover, the well-known "greenhouse effect" is also exacerbated by some VOCs (Lambourne & Strivens, 1999).

In the second half of the twentieth century, the introduction of latex architectural paints was the first step taken to the increasing development of

the water-borne coatings industry. On these initial stages, the main reasons for stepping away from solvent-borne coatings were better performance, easier clean up and to reduce fire hazards (Wicks, et al., 2007). In the last decades, the need to reduce the emissions of VOC has given further importance on research efforts to reduce and eventually eliminate the need for solvents (Cailleux & Charron, 2016). After the implementation of the European Directive on the limitation of emissions of volatile organic compounds in 2004, the development of water-borne finishes has been growing rapidly (European Parliament and the Council of the European Union , 2004).

Given the fact that almost all water-based coatings contain some solvents, mainly as additives to improve their performance, a major drive continues to be the need to reduce the use of volatile compounds, by making the coatings more highly concentrated or by eliminating solvents altogether (Bulian & Fragassa, 2016). Nowadays, only powder coatings, some special waterborne coatings and some radiation-curable coatings are totally solvent-free. On the Table 3 the solid and VOC contents of some coatings are shown. However, they should be considered as indicative only, given the fact that these characteristics are dependent on the formulation used for each application (Bulian & Graystone, 2009).

Table 3. Solid and VOC contents of some wood coatings (Bulian & Graystone, 2009).

Coating Material	Solid content (%)	VOC (%)
Solvent-based stains	1-10	90-99
Water-based stains	1-20	3-10
Cellulose nitrate coatings	15-25	75-85
Water-based coatings	30-40	3-10
UV Polyesters	60-95	0-40
UV Acrylic Coatings	60-99	0-40
Powder Coatings	99-100	0-1

Pigments are added to a coating formulation to modify the optical properties of the film, particularly the opacity, and to provide color to the finish (Bulian & Graystone, 2009). However, they can have substantial effects on application and film properties.

Pigments, in contrast to dyes which are soluble in the coating vehicle, are finely divided insoluble particles which get dispersed in the vehicle and therefore remain suspended in the binder after film formation (Wicks, et al., 2007; Lambourne & Strivens, 1999; Bulian & Graystone, 2009).

White pigments include zinc oxide, zinc sulphide, and basic lead carbonate. However, the dominant white colorant in the coating industry is the titanium dioxide (TiO_2) due to its high opacity and color stability (Bulian & Graystone, 2009; Lambourne & Strivens, 1999).

Some coatings contain no pigments, called *clear coats*, because it is not desired to add color to the finish. In the wood sector these coatings have a big market (Bulian & Graystone, 2009).

Finally, almost all coatings formulations include *additives* which are added in small quantities in order to modify some properties of the lacquer (Wicks, et al., 2007; Bulian & Graystone, 2009). The number of different existing additives is endless, and they can be specific for each application. Some of them can be used to improve the properties of the wet film, such as wetting agents, dispersants, defoamers or rheology modifiers. Others may modify the film formation during the application, such as the use of some solvents as coalescent agents to ensure a proper film. The appearance and performance of the dry film might also be changed by the addition of some substances like matting agents, UV absorbers of flame retardants.

Research and development on additives are of high interest, given the fact that sometimes these components are the only source of volatile organic compounds and, as it was mentioned, the need to reduce the emission of VOC is a major continuing drive.

Given all the different components that are present, coatings are complex mixtures. An essential part of any paint application is the elaboration of a good formulation that may be different for each case. From the binder to the additives, the possible combinations for making a paint formulation are limitless (Wicks, et al., 2007). It is therefore a fundamental step in any product development or in any coating investigation to study the different possibilities regarding the formulation of the final paint.

Even if the additives total content in the formulation is normally way below 10%, they are necessary for the proper application and normal development of the final film. The viscosity, for example, is a fundamental property to be considered when formulating a coating. In fact, it is controlled during the mixing of the coating components because it is necessary to ensure a proper stirring. Moreover, during the storage of the paint, the viscosity must be high enough to prevent the sedimentation of the pigment particles. But the attention to viscosity is particularly important because many application properties depend on the rheology of the final paint, and it is fundamental to ensure a proper film thickness (Goldschmidt & Streitberger, 2018).

7.1 Binders: Latex coatings

Binders can be classified according to their molecular weight. Some polymers have low molecular weight and require further chemical reaction to form solid films. In this group is it possible to find Alkyds, Polyurethanes, Amino resins, Phenolic resins or Epoxide resins. Other polymers, such as nitrocellulose, polyvinyl acetate, acrylic, styrene or butadiene have high molecular weight and normally form useful films without further chemical reaction (Lambourne & Strivens, 1999).

Polyvinyl Acetate, Acrylic, and styrene-based coatings are part of a group of commonly called *latex coatings* or *colloidal dispersions.* A latex is

basically a dispersion of polymer particles in water produced by the free radical initiated polymerization of vinyl, acrylate or methacrylate monomers. Since the monomers are emulsified in water at the start, this process is called *emulsion polymerization* (Wicks, et al., 2007).

One of the main advantages of having the polymer dispersed in water, is the possibility of having high molecular weights without affecting the viscosity of the latex. In fact, the viscosity is mainly governed by the continuous phase, so it is possible to have high solid contents with low viscosity, something that would be impossible if the high-molecular-weight polymer was dissolved (Wicks, et al., 2007). This is the main reason why the latex paints have been one of the fastest-growing sectors of the paint market (Lambourne & Strivens, 1999). Water-based dispersions get translated into less VOC emissions, faster drying, easy cleanup, and the possibility to use high-molecular-weight polymers on barrier coatings. For this reason, a growing part of special-purpose coatings market is latex based.

The latexes, being dispersions, form films by *coalescence* of the polymer particles (Bulian & Graystone, 2009; Wicks, et al., 2007; Eckersley & Rudin, 1994; Lambourne & Strivens, 1999). The main concept behind this event is that after the evaporation of water the dispersed particles must fuse together to form a cohesive film. The process of getting a film after the application of the latex implies four stages: first, the water evaporates until the particles

start to become in close contact and form a dense particle packing (Figure 18). Once almost all the water has been lost, particles start to deform from their initial shape facilitating the following coalescence. The lowest temperature at which this process can occur is the *Minimum Film-Forming Temperature* (MFFT), which is affected by the *Glass Transition Temperature* (T_g) of the polymers. This temperature is the minimum at which the polymer chains have greater mobility and are able to re-orient.

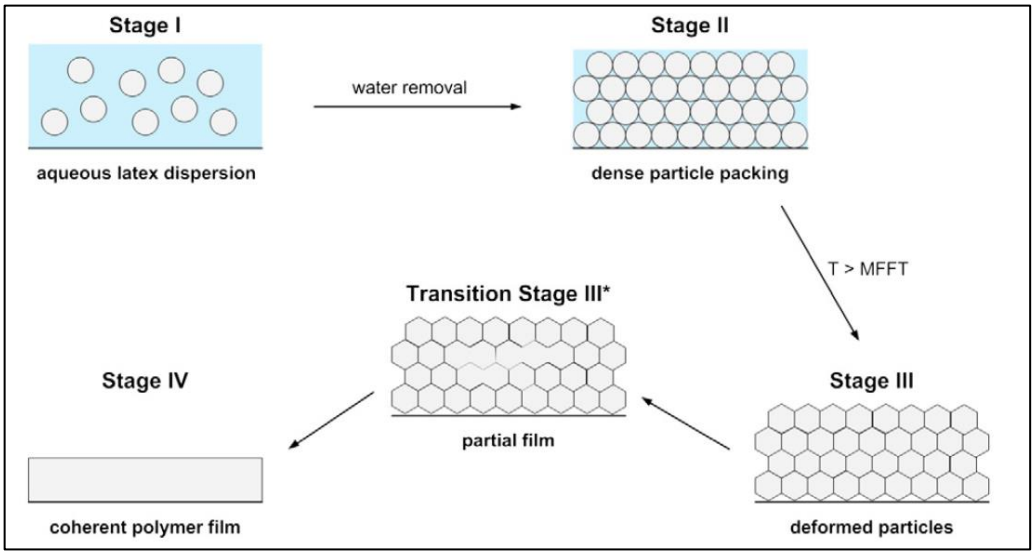

Figure 18. Schematic stages of the film formation of latexes. (Baueregger, et al., 2014)

The deformation of the close packed particles increases the area of contact between them and promotes the coalescence which is the final stage for the formation of the film. It is a relatively slow process in which the polymer particles interdiffuse across the particle boundaries and entangle.

The main property of the polymer that affects the coalescence is the T_g, given the fact that it is the lowest temperature at which segments of polymers

can move with some facility relative to neighboring segments (Wicks, et al., 2007). This implies that a good film formation can only occur if the temperature is higher than the T_g of the polymer particles.

However, there is a conflict between the need for a low T_g to ensure film formation, and a higher T_g to enable good mechanical properties (Bulian & Graystone, 2009; Wicks, et al., 2007; Lambourne & Strivens, 1999). The common way to overcome this situation is to use *coalescing agents* in the formulation, which dissolve some polymer particles and allow the film formation to occur at lower temperatures. After the film forms, the solvent evaporates. However, environmental regulations on VOC limit the addition of coalescent agents on coating formulations and strategies to produce latexes with low T_g yet without sacrificing film properties are being constantly developed.

The knowledge of the Glass Transition Temperature of monomers is very useful to design polymers (Table 4). Almost all dispersions are copolymeric, meaning that a basic monomer chosen for some particular properties is copolymerized with another monomer to achieve the overall balance of properties required.

Table 4. Glass transition temperature and associated hardness of some monomers used in latexes (Bulian & Graystone, 2009).

Monomer	T_g of the homopolymer (°C)	Hardness
Methyl methacrylate	+107	Brittle
Styrene	+100	Brittle
Vinyl acetate	+30	Hard
Dibutyl maleate	-10	Soft
Ethyl acrylate	-24	Very Soft
Butyl acrylate	-55	Very Soft
Ethylene	-125	Very Soft

The two major classes of latexes used in coatings are based on acrylic and methacrylic esters and on vinyl esters.

7.1.1 Acrylic latexes

Acrylic latexes are widely used for exterior coatings, given the fact that they possess higher resistance to photo-degradation, hydrolysis and saponification than vinyl acetate latexes. When selecting the monomers to produce the copolymer, a T_g low enough to permit coalescence of the latex at the application temperature, yet high enough to assure the adequate film hardness. This is particularly important to exteriors coatings, which may be applied at temperatures as low as 2°C. As it was mentioned, the MFFT is actually the minimum temperature at which the film is actually formed, and it is directly related to T_g, but it is also influenced by other factors, so it tends to be actually a little lower than T_g.

The high T_g of Methyl methacrylate makes it possible to have excellent exterior durability, so it usually substitutes partially styrene on polymer

production. To achieve an optimal MFFT acrylic esters are usually used as low T_g monomers and ethyl acrylate and butyl acrylate can be used (Table 4). The selection of the monomers is done by having in consideration the cost of each of them, and other characteristics such as durability or resistance to hydrolysis. The addition of methacrylic acid (MAA) and acrylic acid (AA) improve colloidal stability and affect flow properties (Wicks, et al., 2007).

7.1.2 Vinyl ester latexes

The vinyl resins derive from the polymerization of vinyl monomers, such as vinylidene chloride, vinyl acetate and polyvinyl alcohol derivatives (Bulian & Graystone, 2009). Polymers made from the vinyl acetate (VAc) monomer have been used as binders for decorative paints since the second half of the twentieth century (Lambourne & Strivens, 1999). VAc is cheaper than acrylic ester monomers but have lower photochemical stability and resistance to hydrolysis, reason why these latexes are mainly used in interior coatings.

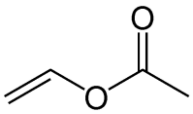

Figure 19. Chemical structure of the vinyl acetate monomer.

The T_g of the vinyl acetate is 32°C, which is usually too high to ensure a film formation under ambient conditions (Goldschmidt & Streitberger, 2018). For this reason, VAc is often copolymerized with other

monomers to reduce the glass transition temperature. Comonomers that are used are butyl acrylate, ethylene and di-butyl maleate. Some longer chain vinyl esters have also been used in order to get latexes with superior hydrolytic stability and exterior durability (Wicks, et al., 2007).

From all the vinyl acetate copolymers, maybe the most well-known are the VAE latexes, formed upon the polymerization with ethylene. Thanks to the softness of the ethylene (Table 4), it is possible to get a copolymer whose T_g can range from -35°C to 30°C depending on the ethylene content. Moreover, since ethylene is a non-polar monomer (Figure 20), it reduces the high polarity of the vinyl acetate and the resulting ratio between cost and performance is very good: the water permeability is increased, and the scrub resistance is superior to vinyl acetate/butyl acrylate copolymers. In fact, it has been reported that the copolymerization with a hydrophobic comonomer enhances latexes water, block and scrub resistance (Wicks, et al., 2007).

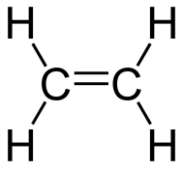

Figure 20. Chemical structure of ethylene.

Given the high T_g of the vinyl acetate, its hard homopolymer, the poly (vinyl acetate) has little use as binder for coatings and paints (Figure 21). It is

mainly used as an adhesive for porous surfaces like wood, paper or cardboard (Cordeiro & Petrocelli, 2004). The very well-known white glue or carpenter´s glue is actually a poly (vinyl acetate) dispersion. Its good stability and easiness of application have made this glue a very popular adhesive, especially for wood furniture and joinery.

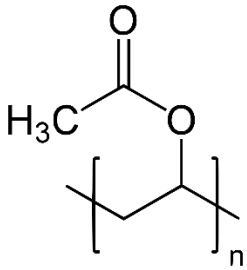

Figure 21. Schematic representation of the poly (vinyl acetate) homopolymer.

Chapter 3. Materials and Methods

1. Introduction

The purpose of this project was to study the migration of extractives present in the knotwood of pine species, which cause discoloration of the coating surface. For this purpose, the barrier properties of water-based wood paints were analyzed. Furthermore, the diffusion of the compounds was studied and the influence of humidity, temperature and solar radiation on the yellowing of a common water-based coating for furniture and flooring was evaluated.

A commercial furniture dispersion was chosen as a reference of a failing water-based dispersion to serve as a comparison paint for the yellowing of the coatings over the knots. This binder, was selected because:

- It has long been in the market and there is a lot of information regarding the formulation and application;
- It is a water-based dispersion for wood furniture and flooring, so it is framed within the market where the Knot Bleeding phenomenon constitutes a major concern;
- It is a self-crosslinking dispersion which shows good barrier properties;

- The dispersion has good water and chemical resistance;
- The results are more consistent, and the discoloration is less dependent on the variability of extractive concentration on knots (see Results and Discussion).

On a first stage of the project, the discoloration of the reference coating was studied, by the artificial weathering of knotted wood samples, following the standard procedure for Knot Staining described under the Norm CEN/TC 139/WG 2. The migrated compounds through the coating were sampled and chemically analyzed.

The influence of humidity, temperature and solar radiation on the discoloration of the water-based reference coating applied over knotted wood was evaluated. The studied variable was the color difference between the paint over the knot and the surrounding coating. The color was measured using CIELAB coordinates.

Thereafter, wood knots were extracted through Soxhlet extraction with non-polar and polar solvents and the extracts were chemically identified. Using the extractive solutions, an alternative test on glass substrates was performed, in order to screen different water-based acrylic systems.

2. Experimental material

2.1 Substrate

In order to study the Knot Bleeding phenomenon wooden boards with at least one knot were used to perform staining tests derived from the standard procedure for knot bleeding assessment, as well as ageing experiments on a climate chamber with controlled temperature and humidity.

The chosen wood for this work was a particular species of pine, the Swiss Stone pine (*Pinus cembra),* known in German as *Zirbelkiefer*. This wood was selected as it has an unusual high number of knots. The tree is commonly under 25 m of height, and its growth is very slow, keeping the lowest near-to-ground branches while growing (Ulber, et al., 2004). Given the fact that the plant does not prune naturally its lower branches, they remain embedded inside the stem providing multiple knots when the wood is sided. The most part of them are living knots since they are not generated after the death of the branch but constitute the living base of the branch inside the main trunk. Because of this, Swiss Stone Pine wood has always been used as a raw material despite of the knots: the wood can be worked without problems and the knots do not fall, since they are linked to the surrounding wood by bonds between the extractives and lignin (see *Chapter 2. Literature Review*). Furthermore,

this wood is appreciated for its rustic appearance and it is rarely coated with pigmented paints.

This exceptional number of knots provides the Swiss Stone Pine wood with a remarkable quantity of extractives. The wood has a very appreciated aroma and the scent, given by the aromatic compounds, can remain for long time (Ulber, et al., 2004). In fact, its extractive content, in particular of Pinosylvin, has been used as a marketing strategy to promote its use in furniture and flooring. The extractives are said to have properties of reducing the heart rate during the physical training (in a room made of Swiss Stone Pine wood) and to improve the quality of sleep. (Joanneum Research, n.d.; Dormiente, 2018; Fantin Falegnameria, 2018).

Regarding the extractive composition, some studies have been done which show the high extractive content of its knots, particularly of stilbenes such as pinosylvins (Willför, et al., 2003). Nisula (2018) made a complete chemical characterization of *Pinus Sibirica,* which is considered a subspecies of *Pinus cembra* (Rogachev & Salakhutdinov, 2015; Nisula, 2018)(Table 5). The results show that this tree has similar contents of extractives in its knots compared to other pine species.

Table 5. Extractives present in the knots of Pinus sibrica (Nisula, 2018).

	Concentration on living knots (mg/g $_{dry\ wood}$)	**Concentration on dead knots (mg/g $_{dry\ wood}$)**
Stilbenes	120	88
Lignans	50	33
Resin acids	9	90

Considering the importance of the resin acids, stilbenes and lignans for the discoloration of waterborne coatings, the results of this project carried out on *Pinus cembra* wood can be reproducible and translated into other Pine woods.

The wood was obtained from a local joinery and cut into boards of 150 x 65 x 20 mm, each of them containing at least one knot (Figure 22). During all the project, the boards were kept in a fridge (-18 °C) in order to avoid the evaporation of volatile extractives (such as terpenes) and to reduce the probability of any migration of extractives due to a temperature increase (Suttie & Ekstedt, 2004).

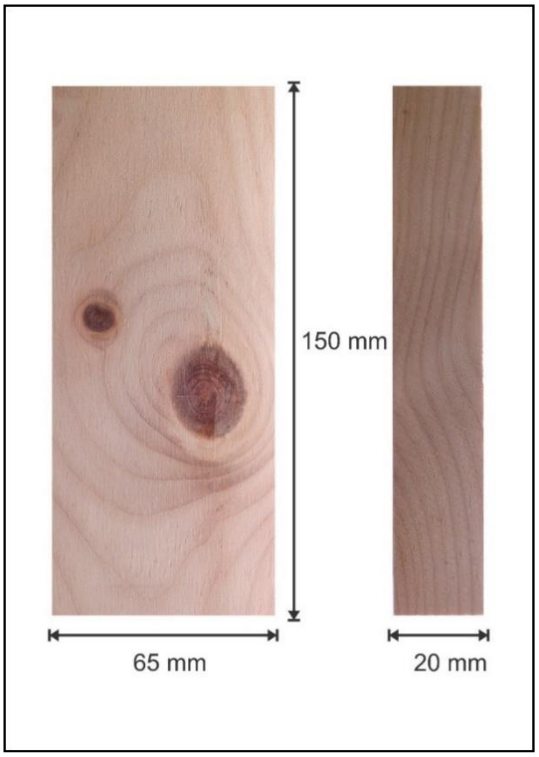

Figure 22. Front and side view of a Pine board used for this project.

2.2 Pigments and additives

For all topcoats, white opaque formulations were prepared as being the most common coatings on which the Knot Bleeding phenomenon is seen. In all cases, titanium dioxide (TiO_2) was used as white pigment. For the products tested as primers, either clear formulations or the pure binder was used.

Several additives such as defoamers, wetting agents, coalescent agents, thickeners or plasticizers were used, according to known. In the cases were no formulation was available, additives were used to ensure good properties

and proper application, such as viscosity, film formation or adhesion to the substrate.

Given the scope of this project and the fact that formulation of coatings requires a study of its own, the use of additives and the development of recipes will not be addressed in this work.

3. Staining tests on Wood substrates

3.1 Wood preparation

The wood was stored in a climate room for 4 weeks after thawing. Afterwards, the boards were halved and sanded with 80 grit sandpaper. The edges were rounded to avoid straight borders, which could be easier paths for water to get beneath the coating surface. Finally, the boards were sanded on all sides again with 180 grit sandpaper.

After this procedure, twin panels are produced which often perform similarly in the test, given the fact that the knot present in each of them was generated from the same branch (Figure 23).

Figure 23. Pair of test samples for Knot Staining produced after cutting the board in half, sanding and bordering the edges. The resulting knots usually perform similarly in the bleeding tests.

The boards were conditioned for two weeks at 20°C and 65% RH, to prevent a more pronounced discoloration due to the freshly machined surface.

3.2 Coating

Each coating or system was applied by brush with a total 4 layers, with a drying time between layers of at least 1 hour at room temperature. After the first layer was completely dry, the surface was sanded with a 400-grit sandpaper to smooth the fibers that commonly rise after the first layer of the lacquer wets the wood. For the systems comprising a primer and a topcoat, two layers of each of them was applied.

Following the painting of the fourth layer, the boards were left to dry for 7 days on a climate room at 20°C and 65% RH.

3.3 Accelerated ageing

The standard procedure for the assessment of knot staining resistance of wood coatings is described on the CEN/TC 139/WG 2 Norm by the European Committee for Standardization. The discoloration is assessed by colorimetry and the result is stated as the color difference between the surface on the knot and the coated surface beside the knot.

The boards were mounted in an exposure cabinet, with the coated surfaces towards the xenon-arc lamps and exposed for 72 hours according to

EN ISO 4892-2:2006, Method A, Cycle No. 10. This procedure establishes 2-hour-cycles which alternates 102 minutes dry and 18 minutes of water spray under a constant irradiation with a narrowband of (0,51±0,02) W/(m².nm) at 340 nm (Table 6).

Table 6. Exposure parameters according to EN ISO 4892-2:2006.

Exposure Period	**Broadband 300nm to 400nm (W/m²)**	**Narrowband 340nm (W/(m².nm))**	**Black-panel temperature (°C)**
102 min dry	60 ± 2	0,51 ± 0,02	65 ± 3
18 min water spray	60 ± 2	0,51 ± 0,02	Not controlled

4. Knotwood extraction

To isolate the knotwood extractives, a knot was cut from a wooden board, and then triturated using a nut grater. This could have also been done using a knife-mill until the entire sample passes through a 2-mm screen (10 mesh) (Hames, et al., 2008).

Approximately 4 g of sample were added to an extraction thimble and put into a Soxhlet tube. 200 mL of hexane were added to a receiving flask and placed on the Soxhlet apparatus. The device was completed with a water condenser and a heating plaque. Once boiling, the solvent was refluxed for a minimum of 6 hours (Scandinavian Pulp, Paper and Board Testing Committee, 2003).

After cooling to room temperature, the extraction thimble was removed and allowed to air-dry in a beaker inside a fume-hood until all the remaining hexane was evaporated. The lipophilic-extractives-free sample was put again into the Soxhlet tube and a new receiving flask was filled with 200 mL of a mixture of acetone and water 95:5 (v/v). The extraction was carried out in an analogous way, with a minimum of 6 hours of reflux. (Willför , et al., 2003).

The separated solutions were then concentrated by evaporating the solvents until getting 50 mL of solution.

For the chemical identification of hydrophilic extractives, an intermediate additional extraction using ethanol was performed. It was done in an analogous way, by extracting with ethanol the hexane-extracted sample before the last treatment with acetone and water.

5. Influence of ageing conditions on the discoloration

It is reported that the discoloration of water-based coatings applied over knotted wood, is affected by moisture, temperature and UV radiation (Wiedenhoeft, et al., 2010; Donegan, et al., 1999). In fact, the Knot Bleeding phenomenon is typically seen on furniture and flooring used in high-humid environments such as bathrooms or under sunlight exposure such as window frames. Moreover, paints applied on wood for exterior use, are known to suffer from discoloration over knots (Donegan, et al., 1999).

To evaluate the influence of humidity, temperature and UV radiation, wood boards like to the ones used for the knot staining procedure test were painted using the reference coating.

To fix the conditions of exposure, a Weiss® climate chamber was used, which allows to set the temperature and the relative humidity (RH).

Exposure tests were designed in order to evaluate the influence of temperature keeping the absolute humidity as a constant and to evaluate the influence of absolute humidity keeping the temperature unchanged.

The influence of temperature was evaluated working with two different humidity values, in order to study if the effect of temperature is different in a low-humidity environment than in a climate with a higher moisture.

For each condition, six boards with different knots were painted using the same procedure as the one used for the standard knot staining test (see Staining tests on Wood substrates). After 1 week of drying at room temperature, the samples were placed on the climate chamber with fixed temperature and relative humidity for 24 hours.

After the exposure, the samples were removed from the chamber and the color difference between the coating surface over the knot and the non-discolored coating over clear timber was measured using the CIELab system.

5.1 Variation of absolute humidity

On the first group, the objective was to work with three different humidity values at the same temperature. As a reference for a low absolute humidity, the standard conditions of 25°C and 65% RH were considered. The absolute humidity for these conditions is 0,0132 $kg_{water}/kg_{dry\ air}$ (Perry & Green, 2008). Given the fact that the climate chamber used for these tests does not have the possibility to fix the absolute humidity, a higher temperature than 25°C was chosen, in order to allow a broader humidity range. The selected temperature was 50°C and the variation of humidity was done from the lowest RH that the equipment allowed (23%).

The resulting parameters for this group of tests is detailed in the Table 7. In all cases, the absolute humidity was obtained from a psychrometric diagram using the temperature and relative humidity (Perry & Green, 2008).

Table 7. Parameters for the evaluation of the influence of humidity at a constant temperature on the discoloration over knots.

Test	Temperature (°C)	Relative Humidity (%)	Absolute humidity $\left(kg_{water}/kg_{dry\ air}\right)$
1	50	23	0,0179
2	50	60	0,0491
3	50	95	0,0815

5.2 Variation of temperature with a low absolute humidity

The low absolute humidity to evaluate the effect of temperature on the discoloration of water-based coatings was chosen to be the same as the lowest humidity of the previous group of tests.

The temperatures selected were 35°C, 45°C and 55°C. The RH values were calculated using a psychrometric diagram (Perry & Green, 2008). The resulting parameters for this group of tests are detailed in the Table 8.

Table 8. Parameters for the evaluation of the influence of temperature at a constant low humidity.

Test	Temperature (°C)	Relative Humidity (%)	Absolute humidity $\left(kg_{water}/kg_{dry\ air}\right)$
4	35	50	0,0179
5	45	30	0,0179
6	55	18	0,0179

5.3 Variation of temperature with a higher absolute humidity

Finally, the effect of temperature was evaluated using a higher humidity than the previous test. The selection was done in order to have a significant high relative humidity for the first temperature (90%). The resulting parameters for this group of tests are detailed in the Table 9.

Table 9. Parameters for the evaluation of the influence of temperature at a constant higher humidity.

Test	Temperature (°C)	Relative Humidity (%)	Absolute humidity $\left(kg_{water}/kg_{dry\ air}\right)$
7	35	90	0,0327
8	45	52	0,0327
9	55	32	0,0327

5.4 Evaluation of the influence of solar radiation

After the 24 hours of exposure in the climate chamber, the boards were placed under solar radiation on an ATLAS Suntest XLS+ device (Figure 24) for 2 hours.

Figure 24. ATLAS Suntest XLS+ device used for the exposure to sunlight of the coated boards. Source: ATLAS Suntest Brochure.

The conditions of irradiation were equal to the conditions established by the Standard Procedure for Knot Staining and are detailed on the Table 10.

Table 10. Irradiation parameters for the evaluation of sunlight influence on the discoloration of coated knotted wood panels.

Parameter	Value
Wavelength (nm)	300-400
Irradiation band (W/m^2)	60
Black panel temperature	65

After the irradiation exposure, the color difference was immediately measured, and the boards were left again for 2 hours under solar radiation. Finally, the color difference after the 4 hours of radiation was measured.

6. Discoloration measurement

To measure the discoloration the CIE Lab color system was chosen. It involves three variables which, together, are characteristic of every color (Schanda, 2007). *L** represents the lightness and therefore it gives information on the whiteness of the sample: it goes from 0 for black samples to 100 for a white color. The variables *a** and *b** indicate color directions: the negative scale of *a** is the green direction, while the positive values are indicative for red. The *b** value goes from yellow to blue between positive and negative values (Figure 25).

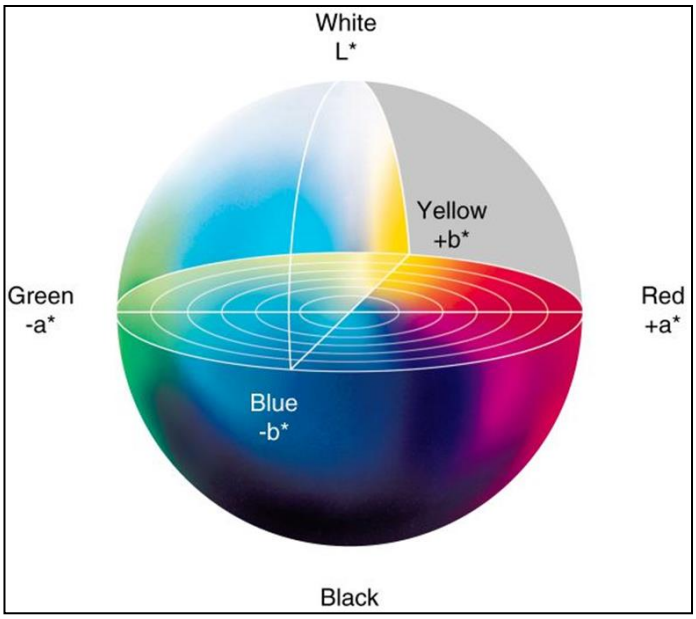

Figure 25. Diagram of the CIE Lab color values (MacAdam, 1985).

Given two different samples it is possible to calculate the differences between their L*, a* and b* values, to determine which color is contributing to the difference between the two samples.

The absolute color difference (ΔE^*) can be calculated using the following equation (MacAdam, 1985):

$$\Delta E^* = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

Given the fact that the discoloration occurring on water-based coatings due to Knot Bleeding is usually seen as a yellowish stain, it could be assessed by evaluating the Δb^* between the paint over the knot and a reference considered as the non-discolored coating over the same board.

$$\Delta b^* = {b^*}_{reference} - {b^*}_{knot}$$

Generally, the resulting measurement is a positive Δb^* since the stain over the knot has a higher yellow value than the white paint. In many cases this value is the one that significantly contributes to the absolute color difference.

However, sometimes the discoloration has a quite intense brown color, and the L* and a* acquire importance for the absolute color difference. For this reason, the ΔE^* is the recommended parameter to assess the knot staining (Suttie & Ekstedt, 2004; CEN/TS, 2011).

For all the tests performed on this project, the discoloration was calculated as the color difference between the coating over the knot and the coating over clear timber. The device used was a BYK Gardner portable

spectrophotometer which measures three repetitions of reference and three of the sample (Figure 26), giving the average values of Δa^*, Δb^* and ΔE^*.

Figure 26. Measuring points for determining the difference in color between the discolored surface (in red) and the reference (in black) on a painted board used for this project.

7. Chemical analysis

7.1 Identification of yellowing extractives

To identify the compounds in the discolored coating over the knots, samples were taken by scraping the paint, trying not to damage the wood underneath (Figure 27). Also, non-yellowed coating samples were taken for reference.

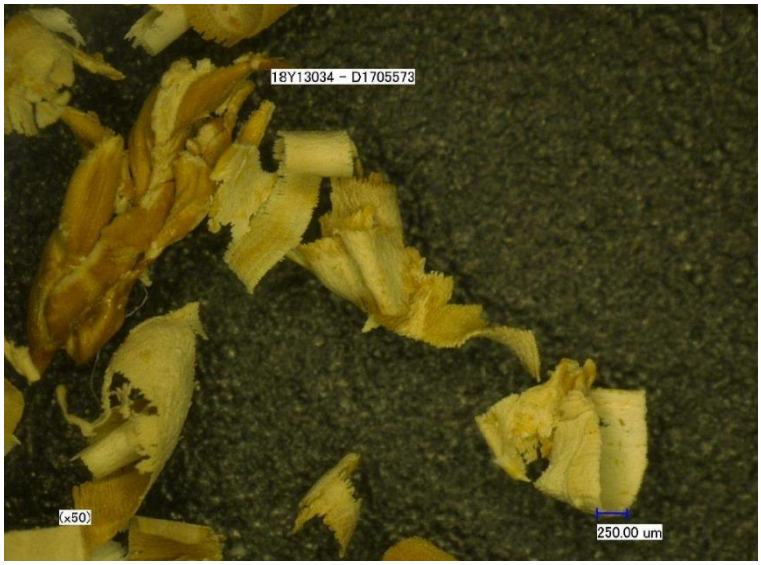

Figure 27. Microscopic photograph of a scraped discolored coating sample used for the identification of the responsible substances.

The analytical determinations were done by the Competence Center Analytics of BASF.

On the one hand, samples were sent to the Laboratory of Optical Spectroscopy and Thermal Analysis, to measure the IR spectrum. The scraped

coating samples were treated with 1-2 drops of acetone and measured with the IR microscope in transmitted light.

On the other hand, approximately 1.6 mg of the sample were extracted with 5 mL of water or ethanol in an ultrasonic bath for one hour. The supernatant was pipetted into a vial and directly injected to separate the compounds using High Pressure Liquid Chromatography (HPLC), with a column Ascentis Express C18 2,7 µm (50x4,6 mm). The mobile phase was Acetonitrile (0,1% formic acid) and the flow rate was 1,2 mL/min, with a temperature of 40°C. The compounds were afterwards identified by Mass Spectroscopy.

7.2 Characterization of knotwood extracts

To identify the hydrophilic extractives, a particular Soxhlet extraction was done using first hexane, secondly ethanol and lastly an acetone:water mixture as already described. The knotwood extracts of ethanol and water were sent to the Mass Spectroscopy Laboratory in order to compare the hydrophilic substances involved in the discoloration of the coatings and those present in the knots. Moreover, it was of great importance to determine the affinity of the extractives to the different solvents, to assess the polarity of each compound to test barrier coatings.

The compounds were separated using High Pressure Liquid Chromatography (HPLC), and the column used was an Ascentis Express C18 2,7 µm (50x4,6 mm). The mobile phase was Acetonitrile (0,1% formic acid) and the flow rate was 1,2 mL/min, with a temperature of 40°C. The compounds were afterwards identified by Mass Spectroscopy.

Chapter 4. Results and Discussion

1. Introduction

This chapter presents the experimental results of the present study of the phenomenon of Knot Bleeding, comprising the identification of the main extractives responsible for the discoloration of the surface of waterborne coatings and their relationship with some compounds present on the knotwood of *Pinus cembra*.

The results of the staining standard procedure performed on the reference coating are presented as well as the study to determine the effect of temperature, air humidity and sunlight radiation on the color change of this reference paint over the knots.

To evaluate the ability of a coating to block the knot extractives discoloration (ΔE*) was measured using CIELab coordinates. For the purposes of studying the blocking properties of the polymers, a visual analysis is enough, given the fact that the knot bleeding is an aesthetic problem. However, given the nature of the present project, the discoloration was measured and a limit ΔE* was defined. The discoloration threshold depends on many factors including the physical properties of the substrate, the observer's capabilities and the experience acquired from observation of

similar objects (Mokrzycki & Tatol, 2012). In this regard, the limit at which the difference of color is perceivable by the human eye varies, depends on the literature considered and it goes from 1 to 3,5 (Mokrzycki & Tatol, 2012; Witzel, et al., 1973). It is though widely accepted that a ΔE* with a value less than 1 is not noticed by any observer. Therefore, a conservative value of $\Delta E^* = 1$ as a limit to accept the performance of the coatings was established.

2. Identification of knot extractives

The water and ethanol extracts obtained after the Soxhlet extraction of the knots were analyzed by HPLC-MS.

Five different lignans were identified in the aqueous extraction, while none of them was present in the ethanol phase. Regarding the presence of stilbenes, Pinosylvin was found in both water and ethanol extracts while a hydrogenated pinosylvin was only present in the aqueous extract. In addition, 2 flavonoids were detected in both extracts. The molecular masses of the detected compounds as well as the possible compound structures are detailed in the Table 11. The chromatograms of the compounds separated by HPLC can be seen in the corresponding appendix.

Table 11. Detected compounds in the water and ethanol extracts by Mass Spectroscopy.

Compound	Extractive type	Molecular Mass	Possible compound	Extract detected	
				Water	Ethanol
1	Lignans	272	Unknown	X	
2		328	Pinoresinol - CH_2O	X	
3		360	Pinoresinol $+H_2$	X	
4		362	Pinoresinol + $2xH_2$	X	
5		330	Pinoresinol - CO	X	
6	Stilbenes	212	Pinosylvin	X	X
7		214	Pinosylvin + H_2	X	
8	Flavonoids	254	Chrysin	X	X
9		256	Pinocembrin	X	X

Even if not all the chemical structures were identified, some important results can be obtained. First, the structure of the pure pinosylvin was detected, and as it was already discussed, it is widely known as being one of the most important extractives responsible for the discoloration of water-based coatings. A second stilbene was detected, which according to its molecular mass it is likely to be a hydrogenated derivative.

Furthermore, four derivatives from pinoresinol were detected as well as another compound which is likely to be a lignan as well. Given the importance of lignans to the yellowing of the coatings it is very important to be able to identify these compounds in the knots.

Finally, two other compounds were detected which according to their molecular masses, are likely to be chrysin and pinocembrin, two flavonoids which are present in pine wood.

3. Knot Bleeding Test on the reference coating

The knot staining standard test was performed on 20 different boards painted with the reference coating, and all of them showed very distinct discolorations over the knots (Figure 28).

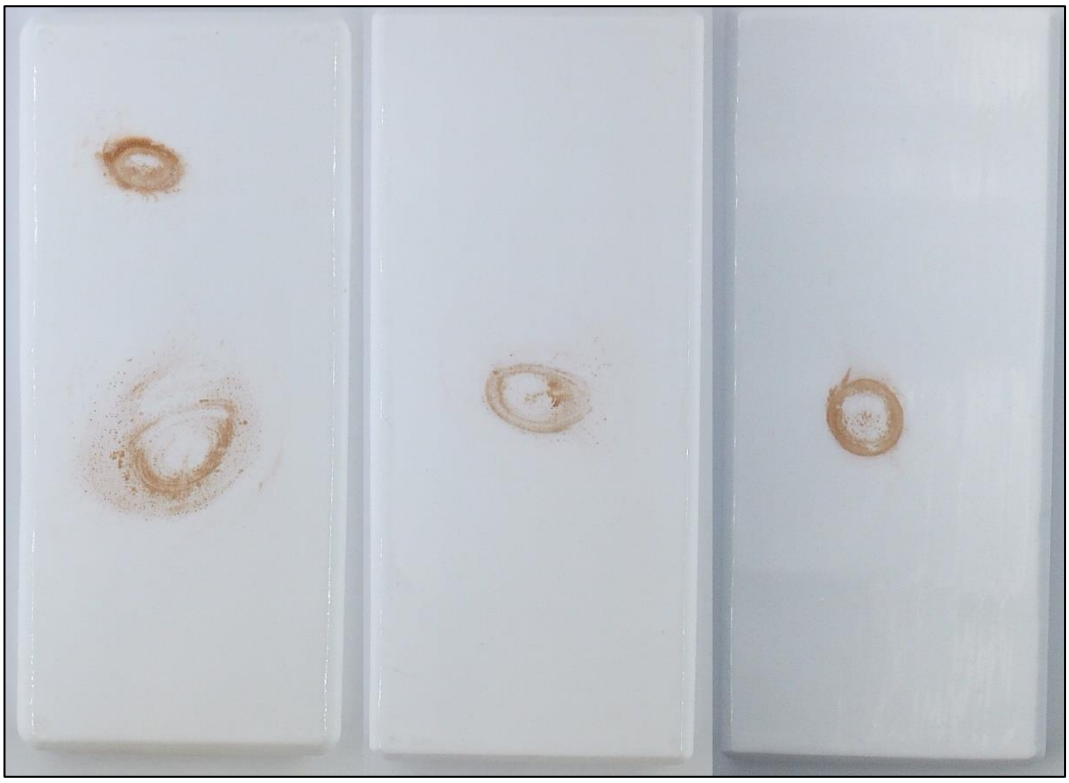

Figure 28. Pictures of three boards painted with the reference coating which show severe discoloration over the knots.

The results of the discoloration measurement are displayed in the Figure 29 and in the Table 12.

Table 12. Discoloration results of the Standard Test on 20 boards painted with the reference coating.

*Average ΔE**	20,3
Standard deviation	6,2

The average ΔE* is much higher than the established limit for the discoloration and the standard deviation of the results is also big, which is expected due to the variability of extractive concentration on wood knots. The high deviation of the discoloration over knots is reported and it gets increased with the ageing time. In fact, the natural weathering tests, which usually take 4 months, show the highest deviation of results which turn the test difficult to be reproduced (Suttie & Ekstedt, 2004).

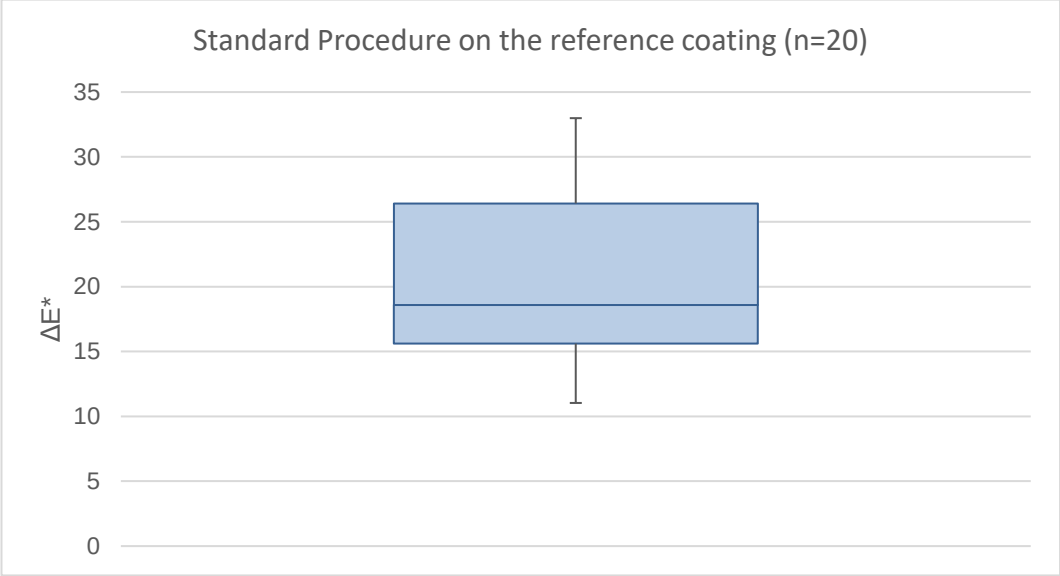

Figure 29. Results of the discoloration between the coating over the knots and the surrounding paint for 20 different boards coated with the reference coating.

The performance of this dispersion is, however, consistent given the fact that even the minimum value of discoloration is corresponding to a heavy discoloration over the knot and which can be easily noticed by any observer ($\Delta E^*_{min} = 11{,}0$). As it was explained before, this dispersion constitutes a good example of a product which fails in the discoloration test and it is therefore considered as a comparison reference for this project.

3.1 Identification of yellowing extractives

The Figure 30 shows an IR-spectrum of the dissolved discolored coating over a knot. When the spectrum is compared to the one obtained from a non-discolored coating sample used as a reference (Figure 31), additional absorptions can be observed on the range of (1598-1600) cm^{-1} and (1155-1158) cm^{-1}. These absorptions were detected for all samples and some additional peaks were also seen at around 1018 cm^{-1}. The complete spectra can be seen in the corresponding appendix.

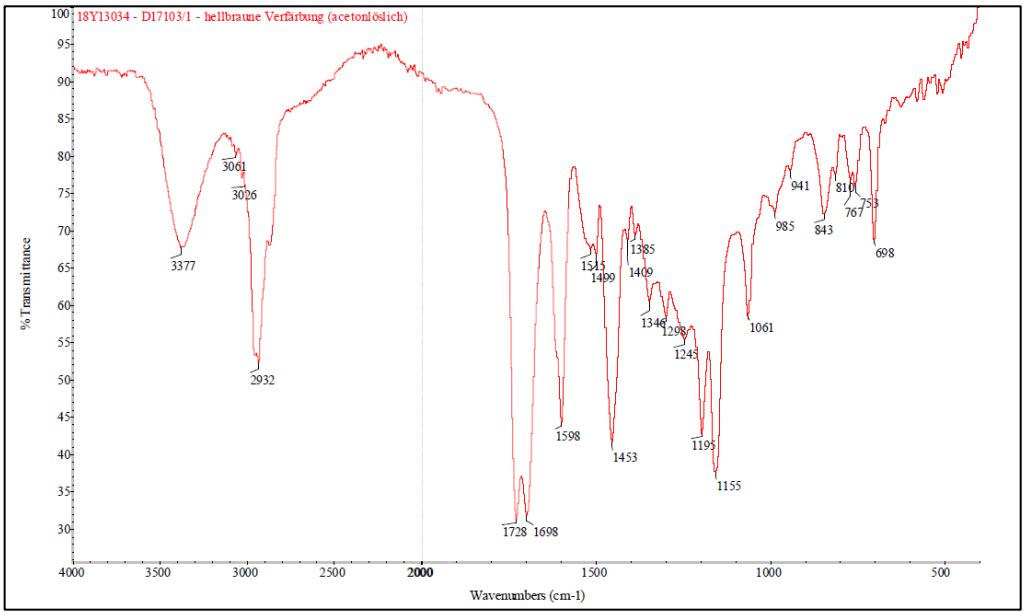

Figure 30. IR Spectrum of a discolored coating sample over knotted wood.

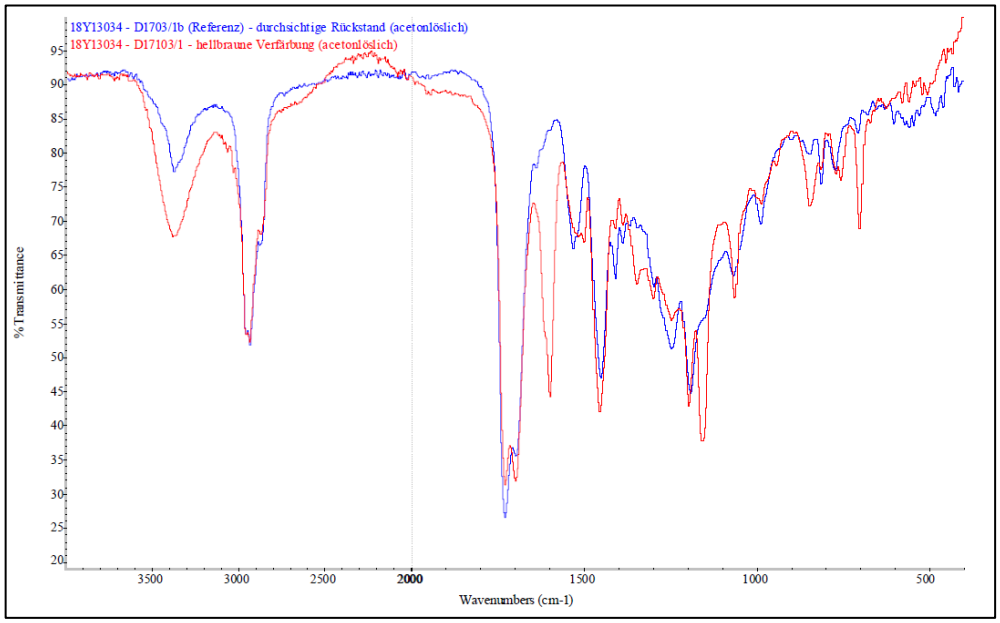

Figure 31. Compared IR spectra of the discolored sample (red) and the reference being the same coating without discoloration (blue).

The additional absorptions observed for the samples are attributed to the extractives responsible for the discoloration, which have been discussed in the Chapter 2. The stilbenes and in particular the pinosylvin absorb around (1596-1598) cm^{-1} (Figure 32), due to the symmetric aromatic ring stretching (Belt, et al., 2017). The absorption at 994 cm^{-1} is usually much lower and it is related to the vibration of the 1,3,5-substituted aromatic ring. This peak was only seen in one sample and it may be either due to overlapping of bands or because of the degradation of the stilbenes, giving products with different groups on the aromatic ring.

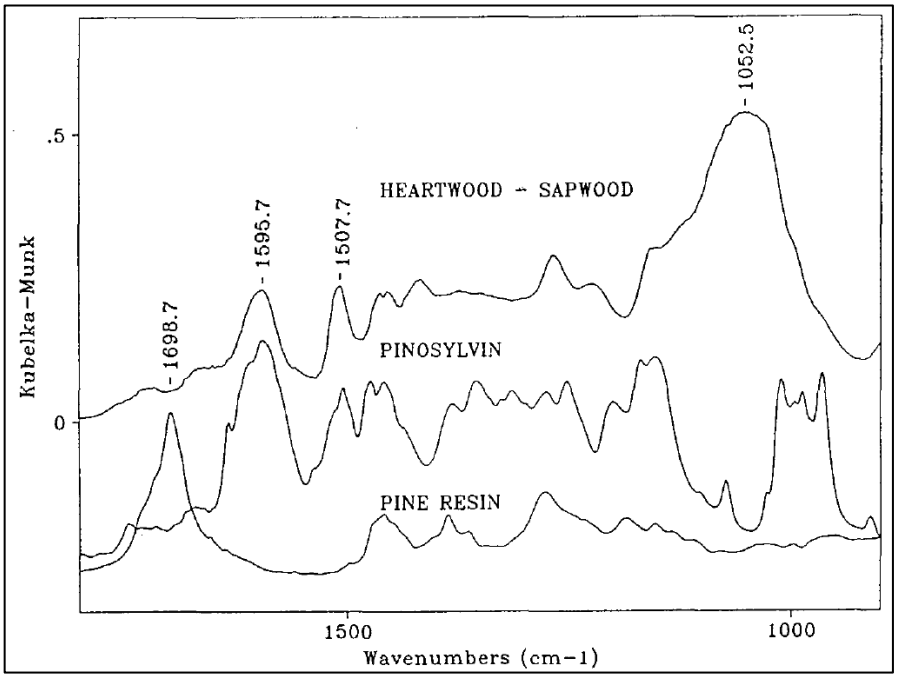

Figure 32. Fourier transform infrared spectra for heartwood, pinosylvins (pinosylvin and pinosylvin monomethylether) and resin extracted from Scots pine. (Holmgren, et al., 1999).

The big signal observed at (1598-1600) cm^{-1} could also contain other phenolic compounds such as flavonoids or lignans which show the same absorption due to the stretching of the aromatic ring (Nuopponen, et al., 2004).

The absorption range of (1155-1158) cm^{-1} is related to the presence of -OH groups in a phenolic ring and are therefore indicating the presence of stilbenes, lignans and other phenolic groups (Nuopponen, et al., 2004). Thus, this absorption is detected on both heartwood and sapwood spectra (Figure 33).

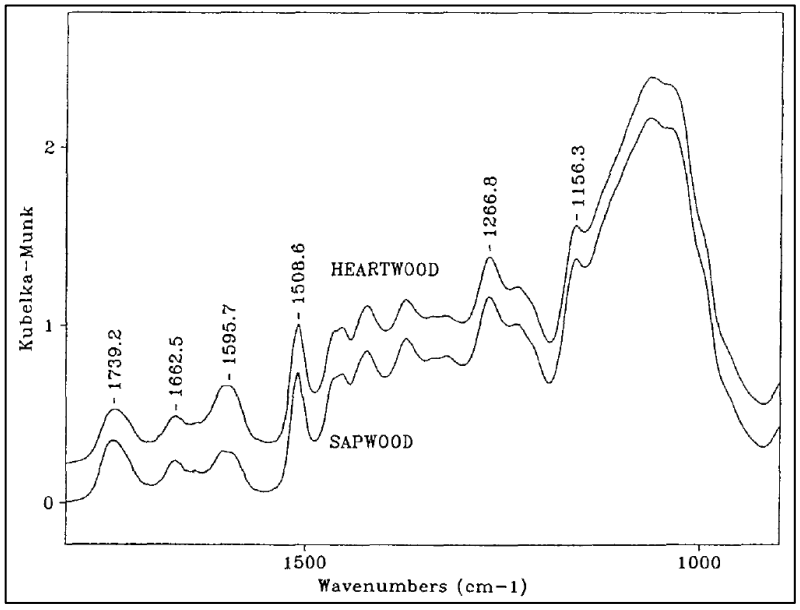

Figure 33. Fourier transform infrared spectra of heartwood and sapwood from Scots pine (Holmgren, et al., 1999).

Regarding the identification of resin acids, a strong band at 1699 cm^{-1} is the signal for the Carbon-Oxygen double bond present in all resin acids. The spectrum obtained for pine resins has thus a strong peak at this wavelength (Figure 32). An absorption at 1698 cm^{-1} was observed for most of the samples. However, the reference coating shows also smaller absorptions at similar wavelengths and a big peak at around 1730 cm^{-1} which makes it difficult to ensure the proper identification of resin acids only by this analysis. The absence of a clear signal contrasting to the reference absorption may be explained by the saponification or esterification of the resin acids (Holmgren, et al., 1999). The detection of abietic acid and other terpenoids is reported to be difficult by IR or Raman spectroscopy, mainly because of the weakness of the absorptions compared to other compounds (Talian, et al., 2010).

Furthermore, the discolored scraped coating over a knot was extracted with water and ethanol and analyzed by HPLC-MS.

7 compounds already detected in the knot extracts were identified in the discolored coating samples as well as 7 new extractives. Pinosylvin and three other stilbenes were detected, which is in agreement to the results of the IR Spectrum.

Three lignans with similar chemical structure to pinoresinol were present in the samples as well as the two compounds detected in the knot extracts, which may be some common flavonoids.

No resin acid was detected in the samples, which is expected given the fact that the coating samples were extracted with polar solvents.

The molecular masses of the detected compounds as well as the possible compound structures are detailed in the Table 14. The complete chromatograms can be seen in the corresponding appendix.

Table 13. Detected compounds in the discolored coating samples by Mass Spectroscopy.

Compound	Extractive type	Molecular Mass	Possible compound	Detected in the knots
1	Lignans	360	Pinoresinol +H_2	Yes
2		362	Pinoresinol + 2xH_2	Yes
3		330	Pinoresinol - CO	Yes
4	Stilbenes	212	Pinosylvin	Yes
5		272	Pinosylvin + CO_3	No

6		214	Pinosylvin + H_2	Yes
7		226	Pinosylvin + CH_2	No
8	Flavonoids	254	Chrysin	Yes
9		256	Pinocembrin	Yes
10	Unknown	482	Unknown	No
11		310	Unknown	No
12		354	Unknown	No
13		398	Unknown	No
14		294	Unknown	No

5 compounds were detected, the structures of which cannot be determined from HPLC/MS data.

The high number of extractives detected in the stained coating proves the complexity of the phenomenon of Knot Bleeding and how broad the group of extractives involved it may be.

4. Study on the influence of ageing conditions

The results of the discoloration measured over the reference water-based coating after exposure to different ageing conditions are presented, and the influence of each variable on the yellowing is discussed.

4.1 Variation of absolute humidity and sunlight exposure

The influence of absolute humidity was evaluated at a constant temperature of 50°C. The results of the average discoloration measured over knots and the corresponding standard deviation are shown in the Table 14, where the effect of sunlight exposure is also detailed.

Table 14. Discoloration results for the variation of absolute humidity and sunlight exposure at a constant temperature of 50°C.

Temperature (°C)	**Absolute Humidity** $\left(kg_{water}/kg_{dry\ air}\right)$	**No sunlight exposure**		**2 Hours sunlight exposure**		**4 Hours sunlight exposure**	
		ΔE^*	**Sd**	ΔE^*	**Sd**	ΔE^*	**Sd**
50	0,0179	2,5	0,5	7,7	0,5	12,8	1,1
	0,0491	2,4	0,2	7,4	0,5	13,3	1,7
	0,0815	5,1	1,1	8,9	1,0	13,5	1,2

The results are graphically presented in the Figure 34. First, it can be observed that for all cases the discoloration gets significantly enhanced after sunlight exposure, even for the lowest humidity.

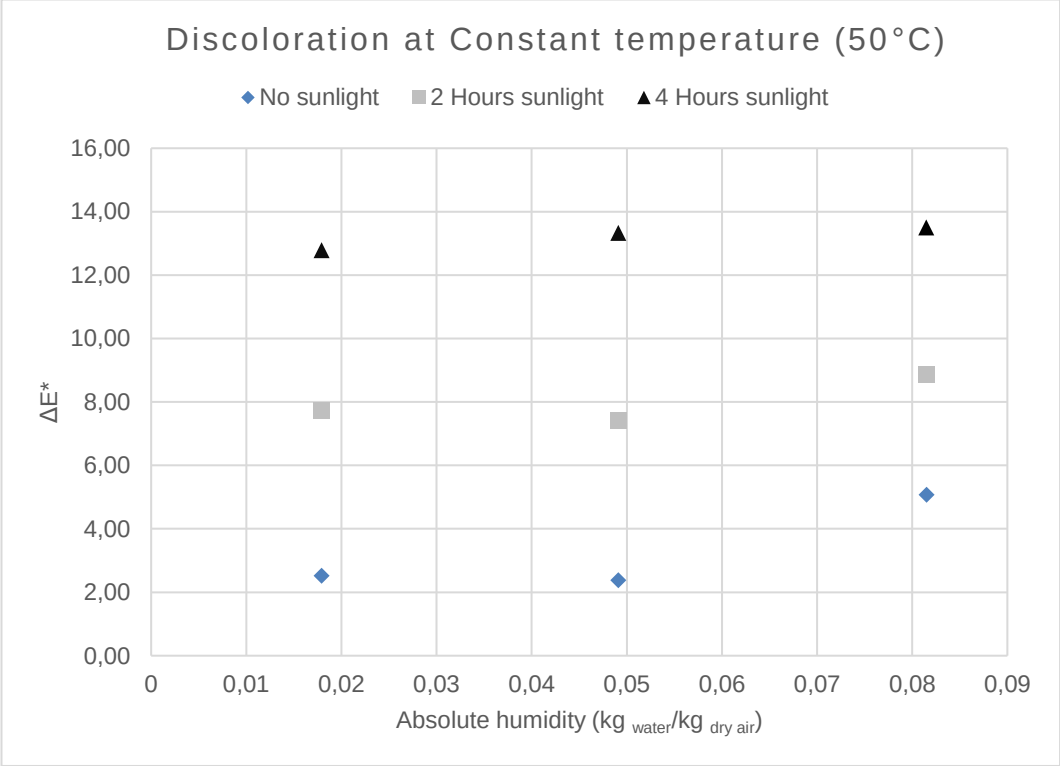

Figure 34. Discoloration results for the variation of absolute humidity and sunlight exposure at a constant temperature of 50°C.

However, the discoloration is practically unchanged at an intermediate moisture level, for all levels of sunlight exposure. Nevertheless, when the highest humidity is considered, the yellowing gets enhanced even without sunlight exposure. It is interesting to observe that after 4 hours of exposure in the UV-Sunlight chamber, all humidity conditions end up with a similar discoloration.

4.2 Variation of temperature and UV with a low humidity.

The influence of temperature was first evaluated at a constant low humidity chosen as the lowest condition of the previous test. The results of the average discoloration measured over knots and the corresponding

standard deviation are shown in the Table 15, where the effect of sunlight exposure is also detailed.

Table 15. Discoloration results for the variation of temperature and sunlight exposure at a constant low absolute humidity.

Absolute Humidity $\left(kg_{water}/kg_{dry\ air}\right)$	**Temperature (°C)**	**No sunlight exposure**		**2 Hours sunlight exposure**		**4 Hours sunlight exposure**	
		ΔE^*	**Sd**	ΔE^*	**Sd**	ΔE^*	**Sd**
0,0179	35	1,0	0,2	4,1	1,2	6,3	1,7
	45	1,1	0,3	5,3	1,1	9,1	2,4
	55	2,7	0,2	8,2	1,1	13,3	0,3

The results are graphically presented in the Figure 35. The first observation that can be easily obtained is that the change in this case by the modification of the temperature of exposure is much more evident that for the previous test. It must be noticed, however, that the discoloration values for 35°C and 45°C are considerably lower even after 4 hours of sunlight exposure, if they are compared to the values obtained after exposure at 50°C at low and medium humidity levels.

For the highest temperature, however, the discoloration is comparable to the results of the previous test. As a matter of fact, the condition of 55°C and 0,0179 $kg_{water}/kg_{dry\ air}$ is a condition with the same humidity but higher temperature than the first point of the previous test. The discoloration is, therefore, quite similar.

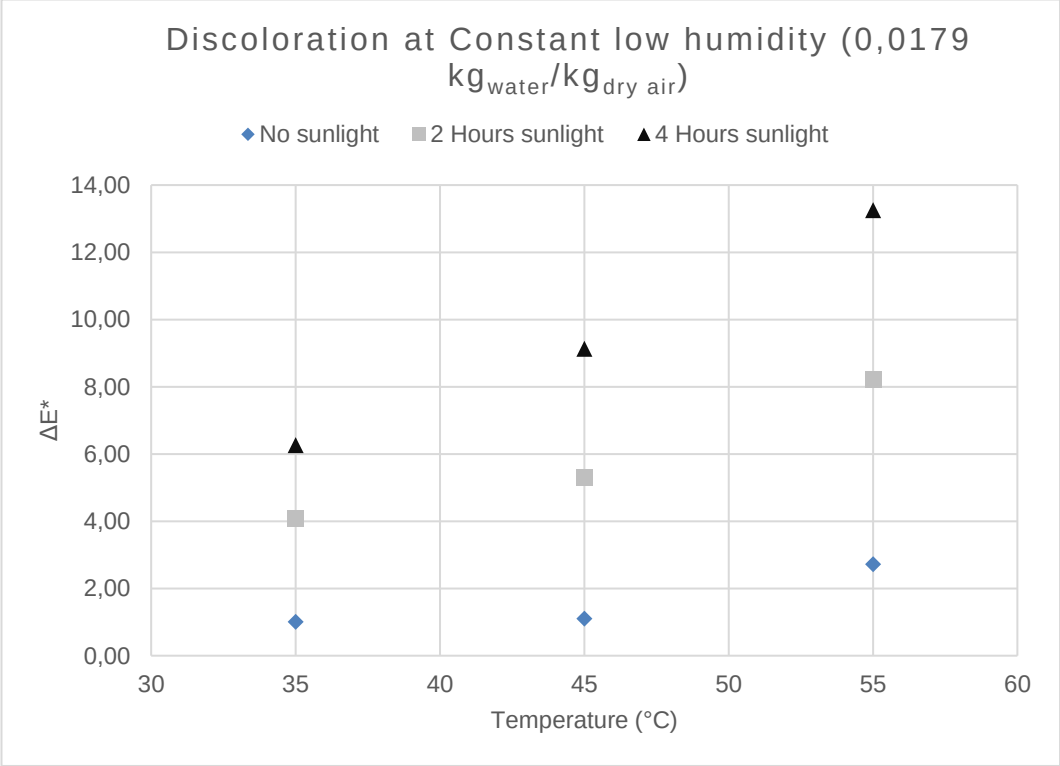

Figure 35. Discoloration results for the variation of temperature and sunlight exposure at a constant low absolute humidity.

4.3 Variation of temperature and UV with a high humidity.

The influence of temperature was afterwards evaluated at a constant higher humidity. The results of the average discoloration measured over knots and the corresponding standard deviation are shown in the Table 16, where the effect of sunlight exposure is also detailed.

Table 16. Discoloration results for the variation of temperature and sunlight exposure at a constant high absolute humidity.

Absolute Humidity $\left(kg_{water}/kg_{dry\ air}\right)$	**Temperature (°C)**	**No sunlight exposure**		**2 Hours sunlight exposure**		**4 Hours sunlight exposure**	
		ΔE^*	**Sd**	**ΔE^***	**Sd**	**ΔE^***	**Sd**
0,0327	35	1,7	1,1	5,4	1,6	10,3	2,0
	45	1,6	0,4	5,6	1,4	10,6	3,3

55	2,2	0,4	7,3	0,3	12,8	1,1

The results are graphically presented in the Figure 36.

When the effect of temperature is studied at a constant high humidity, a similar effect is observed as the tests done varying the humidity at 50°C. The discoloration measured for the first two temperatures is similar for the test without sunlight exposure as well as with two hours of radiation. For the case with 45°C and 4 hours of exposure, if the variation is considered, the results are also very similar to the exposure at 35°C.

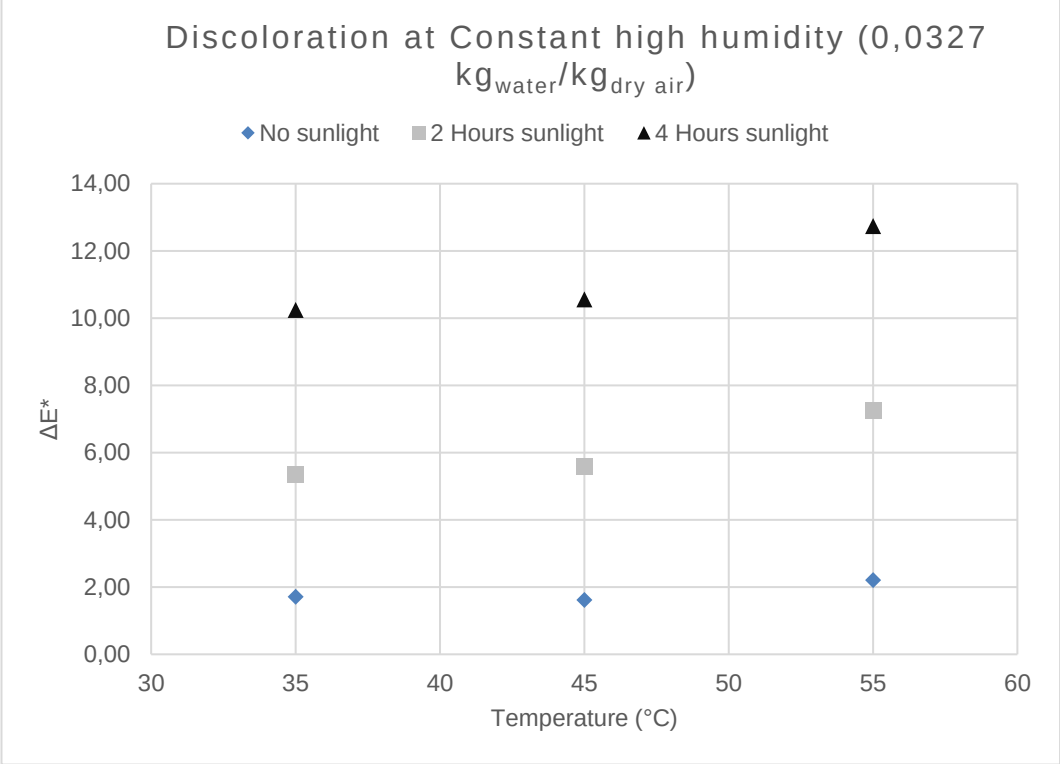

Figure 36. Discoloration results for the variation of temperature and sunlight exposure at a constant low absolute humidity.

A significant higher discoloration, however, can be seen for the maximum temperature tested which is quite similar to the yellowing of the extreme conditions of the two previous tests.

4.4 Discussion on the influence of each variable

From the results of the different exposure tests varying the ageing variables neither the temperature nor the humidity has a more pronounced effect on the discoloration than the other. It seems reasonable to say that a combination of temperature, humidity and sunlight radiation are responsible for the migration of knot extractives through the water-based coating and the yellowing of the paint.

If the exposure at 50°C is evaluated (Figure 34), only at the highest humidity tested, a significant difference in the discoloration can be observed for the same time of sunlight exposure. This may be explained by the fact that, given the high temperature, at the lowest humidity values it is the temperature to dictate the yellowing. However, when a very high humidity is tested, the discoloration gests significantly increased, showing that moisture is also important for the staining. An analogous observation can be made if the sunlight exposure is studied: 2 hours of radiation show a difference for the highest humidity, but 4 hours result in a similar discoloration for all humidity levels.

Similar conclusions can be obtained if the discoloration at a constant high humidity is analyzed (Figure 36). In this case, as the exposure is done at a high humidity value, the discoloration for the first two temperatures is similar when the same time of sunlight exposure is studied. When the temperature is high enough (55°C) the discoloration shows a significant difference. It is consistent to the previous test that the discoloration of the last point after 4 hours of sunlight exposure is higher than the 35°C and 45°C values. In fact, if the conditions are compared, for the case of a humidity of 0,0179 $kg_{water}/kg_{dry\ air}$, 50°C and 4 hours of sunlight exposure the discoloration is similar.

Finally, the study of the influence of temperature at a lower humidity also gives information to say that it is a combination of the different ageing conditions to determine the discoloration over the knots. Given the fact that the effect of humidity is lower, it is possible to see more clearly the effect of temperature and more differences can be seen at the same time of sunlight exposure. However, it may be wrong to affirm that the temperature is therefore more decisive to the yellowing, because the absolute values of discoloration are lower than the obtained for the first points on the previous tests. If the first temperature is studied, it can be seen that after 4 hours of radiation, the discoloration ($\Delta E^* = 6,3$) is quite lower that the values obtained for the first conditions of the previous tests: for 50°C and 0,0179 $kg_{water}/kg_{dry\ air}$ the resulting ΔE^* is 12,8 and for 35°C and 0,0327 $kg_{water}/kg_{dry\ air}$

the resulting ΔE^* is 10,3. This could be explained by the fact that for these two cases, there are two ageing variables with high values. For the first case it is a high temperature (50°C) and 4 hours of radiation. For the second it is a high humidity as well as the 4 hours of sunlight exposure. For the discoloration at constant low humidity, however, at 35°C and 4 hours of radiation, there is only the sunlight variable which is high, and it may be not enough to get a hard discoloration.

It seems evident, however, that the sunlight radiation plays a significant role on the yellowing of the coating. In fact, the highest discoloration values were obtained only after 4 hours of exposure. Nevertheless, it is not sufficient to get a significant discoloration as it is seen on the results for low temperature and low humidity, which even after 4 hours of sunlight radiation results in a difference of color similar to the one obtained for boards exposed at high humidity and temperature but without sunlight exposure. This may be explained by the fact that as it was studied on the Literature Review, UV and sunlight radiation may have a role on oxidizing and degrading some extractives to colored compounds. Therefore, at low temperature and humidity, the migration of extractives to the surface may be not yet sufficient to produce a significant discoloration after the radiation.

Moreover, it must be noticed that the discoloration on boards exposed to high temperatures and humidity values already showed very visible

discolorations, even before the sunlight radiation. The condition of 50°C and 0,0815 $kg_{water}/kg_{dry\ air}$ shows a discoloration of 5,1 which is already far higher than the limit when the stain becomes visible ($\Delta E^* > 1$). This may be explained by the migration of already colored compounds such as lignans or resin acids. In fact, in some boards exposed to the highest temperature, a significant distortion in the film and blisters were seen which are attributed to resin exudation (Figure 37) (Suttie & Ekstedt, 2004).

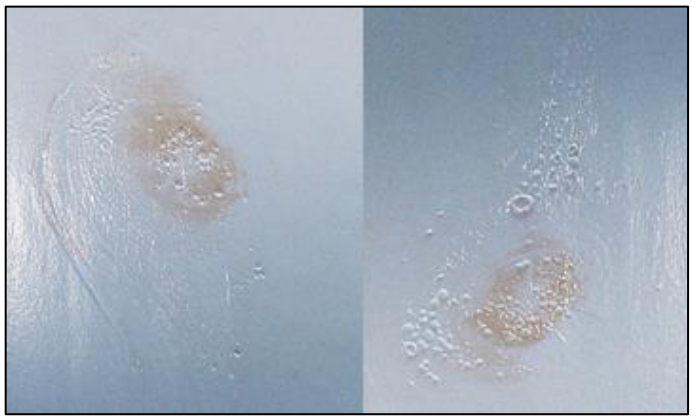

Figure 37. Appearance of boards exposed at 55°C which show the film distortion and blisters typically attributed to resin exudation.

The effect of temperature on the mobilization of resins it is related mainly to two factors. On the one hand, the increase of temperature reduces the viscosity of the pitch, making it more fluid. Therefore, the normal diffusion which may happen even at lower temperature, gets severely enhanced (Wiedenhoeft, et al., 2010). On the other hand, the temperature increases the emission rate of monoterpenes which is related to their vapor pressure (Yokouchi & Ambe, 1984). These compounds are the solvents which carry the resin acids to the wounded areas of the tree. An increase in the

monoterpene emission results in a higher flow of resins. Some previous studies also suggest that the sunlight increases the rate of emission (Yokouchi & Ambe, 1984), which may be the reason why even at a low humidity and high temperature (Figure 35) after 4 hours of radiation the discoloration is similar to the extreme conditions of the other tests.

The evident importance of all the variables studied in the discoloration, suggests a possible synergistic effect between them which result in distinct stains over the knots.

Chapter 5. Conclusions

Water-based coatings suffer from severe discolorations when applied over knotted pine wood due to the migration of extractives. The main compounds present in the yellowish stains are resin acids (abietic acid), stilbenes (pinosylvin and pinosylvin monomethylether) and lignans (pinoresinol and other derivatives).

The migration of the extractives through the paint is due to a combination of weathering conditions, namely humidity and water, temperature and sunlight radiation. No significant predominance of a variable over the others was found that would suggest a predominant effect on the discoloration. The sunlight and UV radiation, however, play a significant role in the degradation of migrated compounds to colored substances and it therefore cause a yellowing enhancement.

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Glossary

Broadleaves: see *Hardwoods*.

Callus Resin: Resin exuded from the callus tissue formed to as a wound is closed by annual growth. It is typically composed by lignans, lignan esters and hydroxycinnamic acid derivatives.

Cambium: growth layer of trees, which produces xylem cells to the inside and phloem cells to the outside to generate wood and bark.

Conifers: see *Softwoods*.

Dead Knot: knot generated after the tree stem grows around a dead branch. At the relevant surface is intergrown with the surrounding wood less than 75% of its circumference. It may present a bark ring. Also known as loose knot.

Duraminization: Ultimate process of differentiation of wood cells, which confer more support ability and greater durability. The resulting tissue is known as heartwood or duramen.

Extractives: group of non-structural components of wood, usually the smallest fraction, which can be obtained by treatment with solvents.

Exudates: group of substances secreted by the tree after biological damage. It comprises gums, oils and resins.

Flavonoids: Group of extractives usually containing a tricyclic carbon skeleton, and which are typically found in heartwood of conifer species.

Glass transition temperature: lowest temperature at which segments of polymer can move with some facility relative to neighboring segments.

Hansch parameter: value representing the hydrophobicity of a compound and which is related

to the coefficient of partition between water and octanol. Higher values indicate greater hydrophobicity.

Hardwoods: group of trees characterized by having flat leaves and to keep their seed inside fruits. They have vessels elements throughout the wood. Examples include eucalyptus, beech, maple and oak. Also known as broadleaves.

Heartwood: area of the secondary xylem, formed by dead cells and voids, with high concentration of extractives, which fulfil mainly structural functions. Generally darker than the surrounding sapwood. Also known as duramen.

Intergrown Knot: see *Living Knot*.

Lignans: group of polar extractives derived from the condensation of phenylpropane units and which are highly concentrated in conifer knots.

Living Knot: knot generated after the tree stem grows around a living branch. At the relevant surface is intergrown with the surrounding wood more than 75% of its circumference. Also known as intergrown knot.

Loose Knot: see *Dead Knot*.

Minimum Film-Forming Temperature (MFFT): Lowest temperature at which a polymer coalesces and forms a film over a substrate.

Oleoresin: mixture of oils and resins present in conifer trees.

Phloem: Vascular tissue in plants that functions primarily in transporting organic food materials from the leaves to other parts of the plant. In trees, the phloem is the innermost layer of the bark, next to the wood.

Pinosylvins: group of stilbenes which share the basic chemical structure of pinosylvin and are

differentiated by the substituents of the aromatic ring.

Primer: undercoat applied to the unpainted substrate to improve some properties of the topcoat such as adhesion, penetration or staining resistance.

Resin: exudate of conifer trees, with high viscosity and composed of resin acids dissolved in volatile terpenes.

Rosin: solid form of conifer resin, formed after the evaporation of the volatile terpenes.

Sapwood: tissue of the secondary xylem, formed by living cells during the growing phase and which principal functions are to conduct water and nutrients to the crown and leaves.

Softwoods: group of trees which usually have needles and cones. Instead of vessels, they have rays and tracheids to transport water. Examples include pine, cedar and spruce. Also known as conifers.

Stilbenes: group of phenolic extractives exclusively present in pine wood, particularly on knots. They are both polar and non-polar constituents.

Tannins: phenolic compounds soluble in water under colloids which have typically molecular weights between 500 and 3000 and can precipitate alkaloids, gelatins and other proteins.

Terpenes: group of extractives derived from isoprene and largely found in softwoods. The monoterpenes are highly volatile and are natural solvents for resin acids.

Terpenoids: group of extractives formed upon the oxidation of terpenes. The most important ones for softwoods are the resin acids, largely found in the oleoresin.

Topcoat: final paint applied over the primer and other layers, which normally possess the main properties and appearance wanted in the final dry film.

Xylem: Vascular tissue in plants primarily involved in transporting water and nutrient from the roots to the crown and providing structural support. In trees, the secondary xylem is the constituent of wood tissue.

Appendix A. Chromatograms of the polar knot extracts

The chromatograms of the individual compounds separated by HPLC from the extracted knots with water and ethanol are presented. As it was discussed on the Results and Discussion Chapter, nine different extractives were detected in the water extracts. Four of these were also present in the ethanol extracts, as it can be seen in the chromatogram (Figure 39).

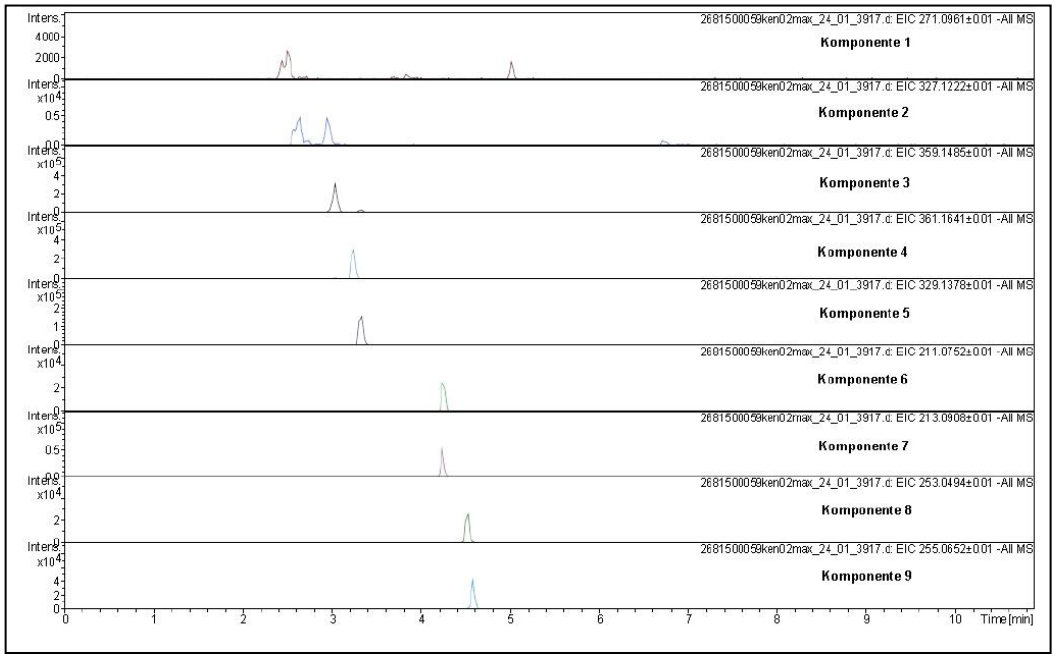


Figure 38. Chromatogram of the water knot extract.

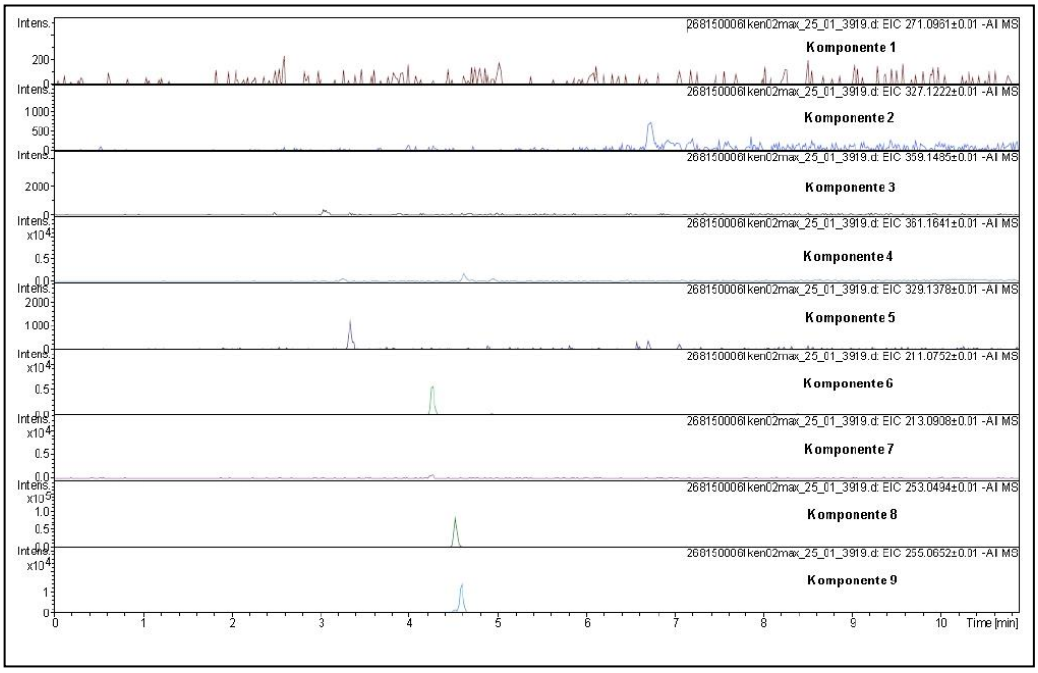
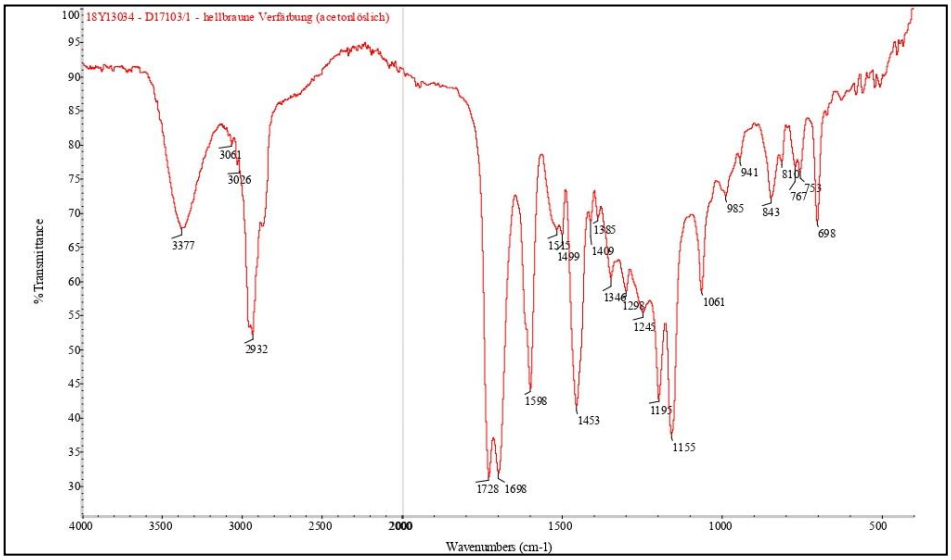


Figure 39. Chromatogram of the ethanol knot extract.

Appendix B. IR Spectra of the discolored coating samples

The IR Spectra of three samples of discolored coatings over knots are presented as well as the comparison with the sampled coating on a non-discolored surface.

In all the spectra additional absorptions can be observed on the range of (1598-1600) cm^{-1} and (1155-1158) cm^{-1} in comparison to the spectrum of the reference non-discolored coating.



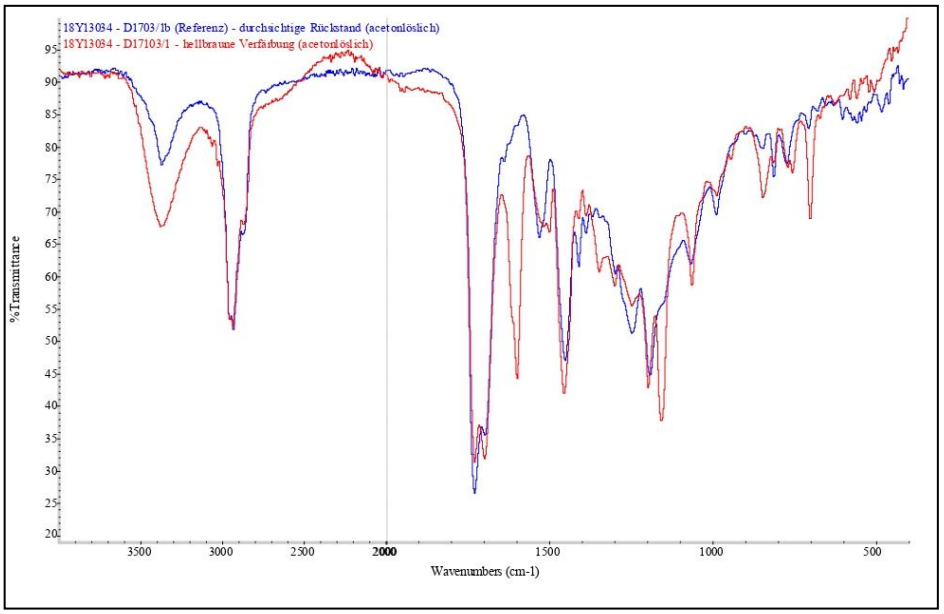


Figure 40. Up: IR Spectrum of the first discolored coating sample. Low: IR Spectrum of the first discolored coating sample (red) compared to the IR Spectrum of the non-discolored coating (blue).

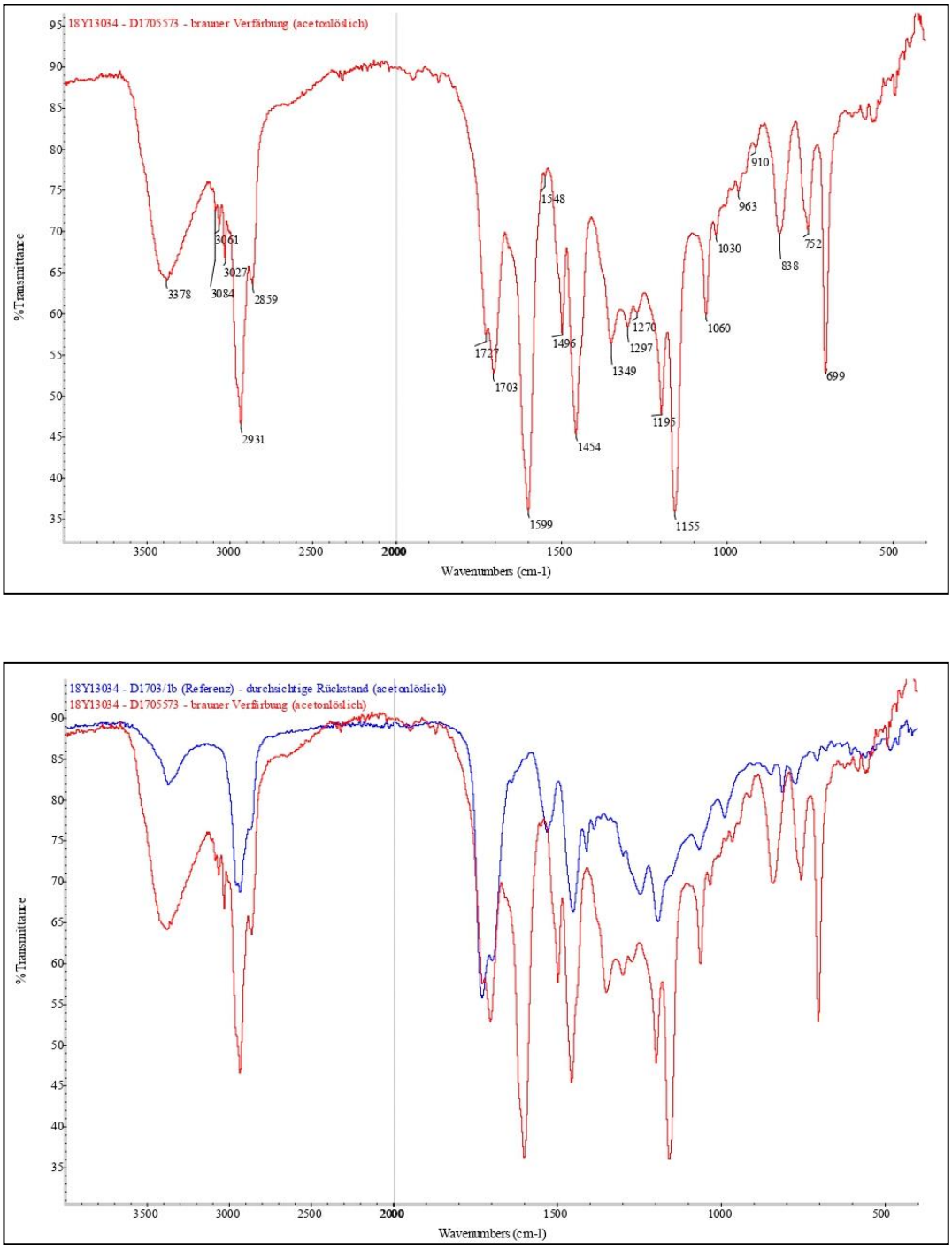


Figure 41. Up: IR Spectrum of the second discolored coating sample. Low: IR Spectrum of the second discolored coating sample (red) compared to the IR Spectrum of the non-discolored coating (blue).

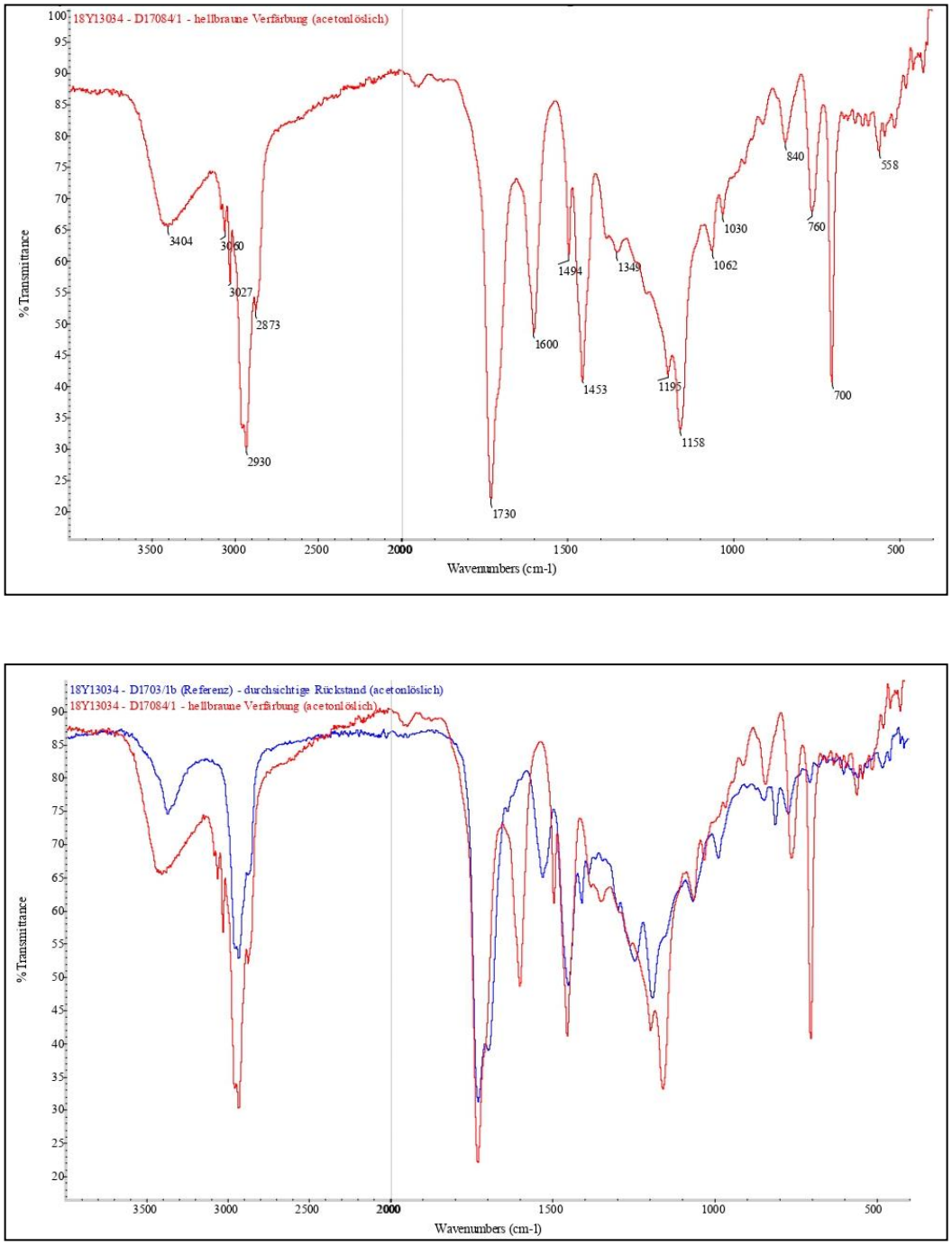


Figure 42. Up: IR Spectrum of the third discolored coating sample. Low: IR Spectrum of the third discolored coating sample (red) compared to the IR Spectrum of the non-discolored coating (blue).

Appendix C. Chromatograms of the discolored coating samples

The discolored coating samples were extracted separately with water and ethanol. All compounds (with the exception of the Compound 10, for which no chemical structure was given) were detected in both water and ethanol extracts.

The chromatograms of the individual compounds separated by HPLC are presented.

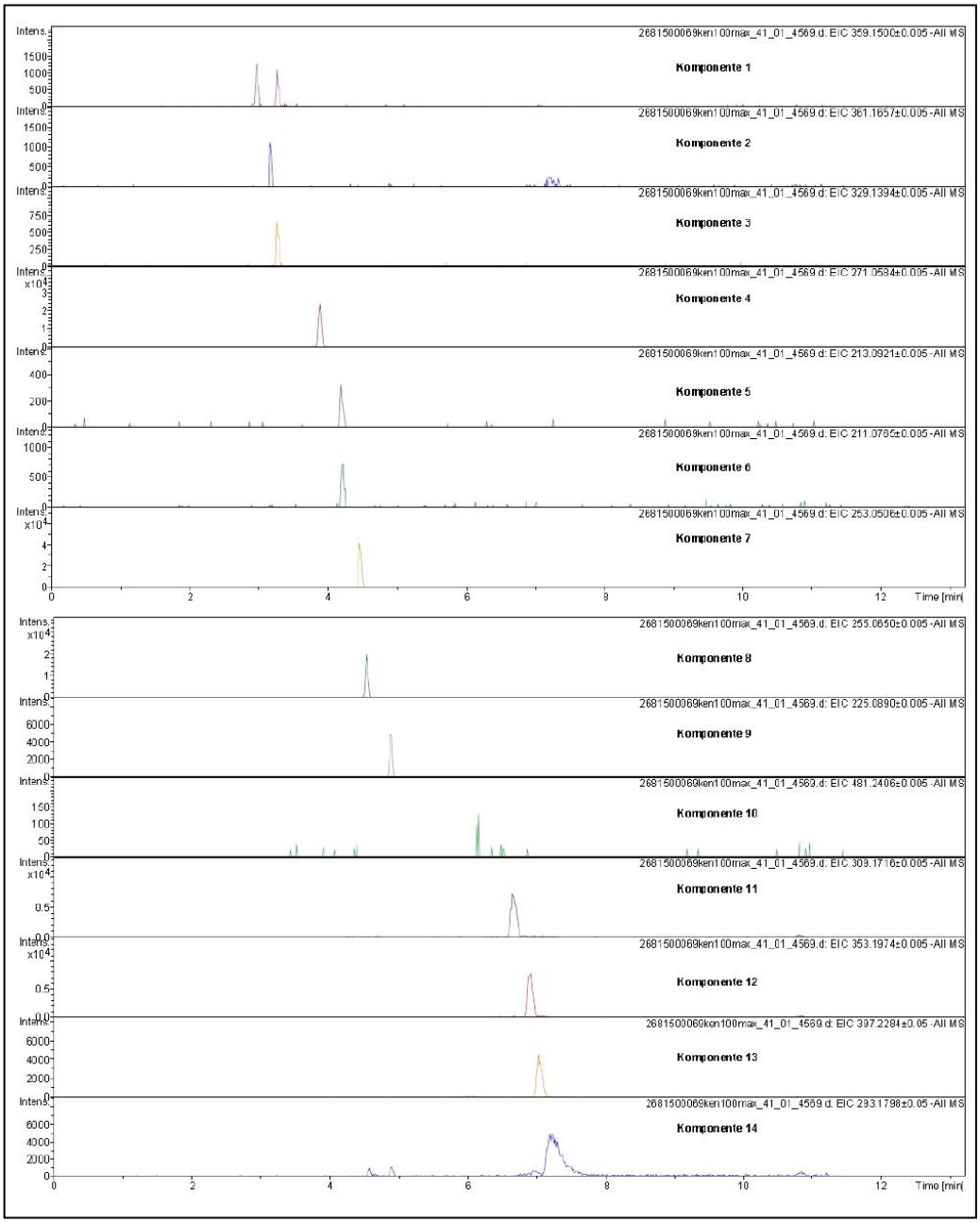


Figure 43. Chromatogram of the water extraction of the discolored coating sample.

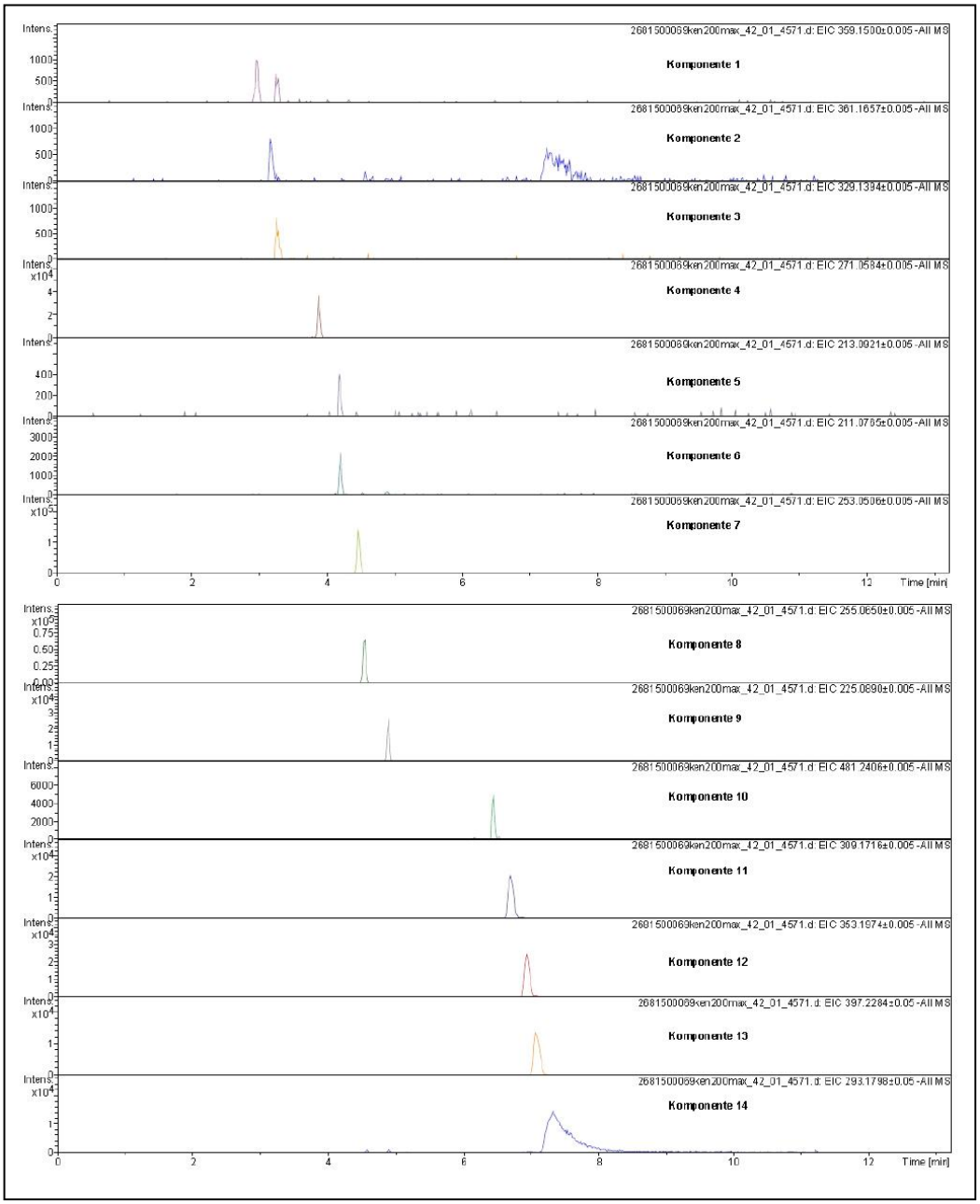


Figure 44. Chromatogram of the ethanol extraction of the discolored coating sample