

Colour development in *Pinus radiata* D. Don.
under kiln-drying conditions.

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Andrés Dieste

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ABSTRACT

This study quantifies discolouration on the surface of *Pinus radiata* boards during kiln drying, particularly kiln brown stain (KBS), and models it as a function of chemical compounds present in the wood closest to the surface. The discolouration was investigated with two experimental factors: drying time, which consisted in drying at 70/120 °C for 0, 8, 16 and 24 hours; and leaching, done at three levels, no-leaching, mild and severe, to reduce the soluble compounds present in wood suspected of developing coloured compounds. The colour change was quantified using a reflectance photometer (colour system CIE Yxy, brightness) and by the analysis of digital photographs (colour system CIE Lab). The chemical analysis of the wood closest to the surface of the boards determined fructose, glucose, sucrose (HPLC), total sugar (sum of fructose, glucose and sucrose), total nitrogen (combustion gas analysis), and phenols discriminated by molecular weight (Folin-Ciocalteu method). In the cause-effect analysis, colour was the dependent variable, and drying time and the determinations of chemical compounds were independent variables. After statistical analysis (ANOVA and MANOVA) the dependent variables to be included in the models were luminance factor (Y), brightness ($R457$ and the blue-to-yellow scale of CIE Lab (b)); and the independent variables were drying time, nitrogen, total sugar, and high-molecular-weight phenols. Linear (multivariate regression) and non-linear models (Neural Networks) showed that discolouration during kiln drying was best predicted when the luminance factor (Y) was used to quantify colour change as a function of the content of nitrogen-containing compounds and drying time. Furthermore, the data were fitted into an empirical model based on simple reaction kinetics that considered the rate of discolouration as a function of nitrogen concentration. The results suggest that nitrogen could act as a limiting reactant in Maillard-type reactions that produce colour during kiln drying.

Keywords: kiln brown stain; *Pinus radiata*; reflectance photometer; CIE Yxy; CIE Lab; fructose; glucose; sucrose; nitrogen; phenols; multivariate linear regression; Neural Networks.

Chapter 1

INTRODUCTION

Wood, scientifically termed secondary xylem, is a tissue developed in shrubs and trees for support of the crown and transportation of water from the roots to the leaves. From an early stage humanity discovered the uses of wood as a material for construction of dwellings and utensils (Hon 2001). Later on, those first uses developed into sophisticated buildings and furniture, with higher requirements for the original material. To meet those requirements, wood was worked and adapted to the new uses, which was done first by craftspeople and then industrially; likewise, the techniques developed to work timber have evolved from an art into a combination of art and science. The drying of wood in kilns under controlled conditions is an example of both an industrial process, which transforms wood into a more useful material, and also a scientific field that has received considerable attention (Keey 2000). The improvements made in wood drying are illustrated in the following example: it takes on average approximately 60 days to air dry a stack of 25 mm thick *Pinus sp.*, depending on the season, while in a modern high-temperature kiln the same stack of wood can be dried in 14 hours, less than 1% of the time required to air dry it in open air (Walker 1993).

Wood that has just been sawn from the tree has a high water content. When wood is used as a material, is necessary to dry it for at least the following reasons (Walker 1993):

- Once the wood is in contact with the atmosphere, it will dry until it reaches equilibrium with the moisture content of the atmosphere. The main objective of drying wood is to control this phenomenon.
- Drying the wood improves its stability under service. When wood is dried below fibre saturation, it shrinks; therefore it is best to stabilize the dimensions of the material before using it.
- It diminishes the mass of wood, with evident advantages for construction (lighter buildings; lighter furniture) and transportation (lighter loads).

- It reduces the susceptibility of wood to the attack of insects and microorganisms. Life needs water to thrive, and where there is less water, there is less life.
- It improves the capacity of the wood to be machined, as well as allowing for the application of paints and glues.

Drying enhances wood as a material, but it can also degrade it. Wood has evolved as a specialized tissue of certain plants, with a different functionality from that required for wood as a material, defined in properties such as anisotropy, density and stiffness (Keey 2000). The interactions of those properties with the shrinkage of wood during drying might produce drying degradation, such as collapse, warping, or surface checks (Simpson 1991; Keey 2000). In addition, there are delicate relationships between molecules and compounds present in wood that are altered when the content of water is drastically reduced (Terziev 1996). Modern drying processes impose higher stress on the wood, and therefore tend to exacerbate the drying defects (Sundqvist 2002).

The discolouration of wood during kiln drying is a well-known process (Stenudd 2001). It involves an overall darkening of the wood, which depends on the species, the temperature and the humidity. The oxidation of phenolic compounds and the hydrolysis of hemicellulose have been identified as reactions that produce dark materials on the surface of boards during drying (Hon and Minemura 2001). In addition, water-soluble compounds present in the sapwood are considered responsible for discolouration reactions that develop dark brown stains, termed kiln brown stain (Kreber and Haslett 1997). *Pinus radiata*, as well as some of the United States Southern pines, such as *Pinus taeda* and *Pinus elliottii*, has a characteristic light colour, which is one of its more desirable features as a market product; therefore, discolouration during drying is particularly undesirable.

Consumers generally prefer a light colour in wood, because it is easier and cheaper to change it into any other colour of their choice. To change the colour of darker wood requires a treatment to reduce the darkness, and only then can the colour be changed. A similar situation occurs with other wood products, such as paper or

medium density fibreboard (MDF) (Smith 1989). One of the distinguishable features of New Zealand's MDF is its light colour (Long 1989), suitable to be covered with white wallpaper without further modification. Therefore, there is interest in keeping the colour of wood after drying as light as possible (Kreber and Haslett 1997). For countries that base their wood industry on exports, such as New Zealand or Uruguay, this point acquires further relevance. Most of the wood that is offered in international markets can be divided according to two purposes: structural and appearance grades. Structural wood is used for building construction; and appearance wood is used in services where the wood is exposed, such as wall panels, mouldings, and furniture. Wood that does not meet the standards to be sold as appearance wood would have to be downgraded as structural wood. The structural grade of wood can be determined according to its stiffness (Tsehaye *et al.* 2000; 2000); several international standards specify the requirements for wood to be used in structural purposes. Appearance wood is exposed on service, and colour is one of its crucial features. Pine-wood, including *Pinus radiata*, is preferred light, so there is little demand for dark or stained pine-wood (Kreber and Haslett 1997). Hence, for wood that is not destined for structural grade, it is necessary to control discolouration during drying to avoid losing the appearance wood market.

Due to its strategic marketing importance, wood discolouration during kiln drying, and particularly kiln brown stain, has been extensively studied. Kiln brown stain (KBS) is a brown discolouration that occurs just below the surface of boards during kiln drying, and subsequent planing exposes the stain, which is not desired by the international market (Kreber and Haslett 1997). To understand KBS, at least two different processes have to be identified: the transport of KBS precursors to the surface of the boards during drying and the reactions that those precursors undergo, developing KBS. There is considerable evidence that during kiln drying, water-soluble compounds are redistributed and concentrated from the centre to the surface of the boards (Theander *et al.* 1993; Terziev 1995; Kreber and Haslett 1997). Below the surface, KBS occurs due to non-enzymatic chemical reactions between low-molecular-weight sugars and amino acids, which are part of the water-soluble constituents of *Pinus radiata* sapwood (Kreber *et al.* 1998). The interactions between low-molecular-weight sugars and nitrogen during drying, yielding yellow-brownish substances, have been described as caused by a Maillard reaction (Theander *et al.*

1993; Terziev 1996; McDonald 1997; Kreber *et al.* 1998). There is evidence that phenols and lignin derivatives could be related to KBS development (McDonald 1997; McDonald *et al.* 2000). In addition, there are no reports of the connection of KBS to the colour changes that occur in pine wood when exposed to sunlight. The ultraviolet component of light decomposes wood extractives such as stilbenes, producing coloured products that discolour wood (Morgan and Orsler 1968).

The previous research in discolouration during kiln drying can be divided into two aspects: the study of the reactions that produce coloured compounds that change the colour of the surface of the boards; and the measurement of that discolouration. However, both approaches have not been combined in a single study for *Pinus radiata* wood. That is the contribution of this study.

1.1. Objective and aims.

The objective of this research is to study whether the discolouration on the surface of *Pinus radiata* boards during kiln drying can be described as a function of non-structural sugars, nitrogen compounds and phenolic compounds. To accomplish that main objective, the following aims were defined:

- Quantify the colour on the surface of the boards using colorimetric methods, particularly colour systems, CIE Yxy and CIE Lab.
- Quantify the compounds present in *Pinus radiata* wood closest to the surface of the boards liable to produce colour during kiln drying. The compounds to be determined would be non-structural sugars (fructose, glucose and sucrose), nitrogen compounds, and phenolic compounds (high and low-molecular-weight phenols).
- Produce statistical models, namely multivariate linear regression and Neural Network models, to investigate the cause-effect relationship between compounds present in the wood closest to the surface and discolouration on the surface. These models would be constructed to identify a hierarchical order of importance of the chemical compounds related to their ability to produce discolouration on the surface.

- Produce an empirical equation based on simple reaction kinetics to model the discolouration of the surface as a function of chemical compounds present in the wood closest to the surface.

Chapter 2

LITERATURE REVIEW.

2.1. Introduction.

This literature review is intended to present the current knowledge on kiln brown stain. It is concerned with describing the Maillard reaction, which is reported to be the cause of the discolouration and staining of wood. In addition, it explores the possibility of discolouration during drying associated with lignin derivatives, particularly phenolic compounds, as has been reported for other species. Finally, it presents a brief summary of the research done on the control of discolouration during kiln drying, where the work of the New Zealand Forest Research is predominantly discussed.

2.2. Maillard-type reaction and the colour development in kiln brown stain.

The Maillard reaction involves any chemical reaction of amino acids, peptides, and proteins with sugars (Ellis, 1959, quoted by Tomasik 1989). A more recent definition describes this reaction as any chemical reaction between an amine group and a carbonyl group:

“In a series of reversible reactions, the carbonyl moiety of the sugar molecule forms a Schiff base with a biological amine, typically an amino acid or the lysine residue of a protein. The apparently simple reaction leads to a huge variety of products in varying yields. Each of these compounds is itself reactive and will participate in a vast array of possible reactions. Substantial evidence points towards the formation of polymeric material during the later stages of the Maillard reaction, often termed melanoidins (Fayle and Gerrard 2002).”

The Maillard reaction is represented by thousands of individual non-enzymatic reactions, each of them dependent on factors such as temperature, composition of the system, water activity, pH and sulphur dioxide (Finot *et al.* 1990; quoted by Cioroi 1999). The reactions result in the formation of phenols, furans,

nitrogen heterocyclic compounds and yellow and brown polymers, known as melanoidins (Theander *et al.* 1993). The reaction between an amino group and a carbonyl group in a Maillard-type reaction could lead to the covalent crosslinking of proteins, which has been used to probe the occurrence of Maillard-type chemistry (Fayle *et al.* 2000). Even though the Maillard reaction has been extensively studied in the fields of Food Chemistry and Medicine, among others, its molecular mechanisms have not yet been completely elucidated (Fayle and Gerrard 2002).

Theander *et al.* (1993) studied the migration of carbohydrates and nitrogen compounds during the drying of *Pinus sylvestris* and *Picea abies*. They took samples at different depths in the plank, and determined the content of free sugars and nitrogen. The sugars found were glucose, fructose and sucrose, and after acid hydrolysis, and in smaller concentrations, galactose, mannose, arabinose and xylose. The investigators estimated the content of proteins, peptides and free amino acids by measuring the amount of total nitrogen. It was shown that there was a clear migration to the surface of both sugars and amino acid compounds, along with a yellow colour in the surface. Theander and his colleagues suggested that a Maillard-type reaction was responsible for the colour development in the studied wood.

The influence of drying schedules on the redistribution of low-molecular sugars and nitrogenous compounds has been quantitatively studied (Terziev *et al.* 1993; Terziev 1995; 1996; Kreber *et al.* 1998). Boards obtained from *Pinus sylvestris* were dried using two different schedules, one with faster wet-bulb depression than the other, and both with a constant dry-bulb temperature of 80°C. It was observed that after drying with the faster schedule, the surface of the boards had a darker yellow-brownish colour while the boards dried with the slower schedule have a bright yellow discoloured surface. High Pressure Liquid Chromatography (HPLC) analysis showed that the sugars found were fructose and glucose; also, after the slower drying schedule it was possible to measure more glucose than fructose, while there was not such a difference after the faster drying schedule. It was observed that the faster drying schedule, gave higher concentrations of sugars at the surface. Both schedules produced high concentrations of sugars and nitrogenous compounds at the surface of the boards, even below the fibre saturation point.

Kreber and Haslett (1997) from the New Zealand Forest Research found that KBS in *Pinus radiata* increases with both high kiln temperature and with low kiln humidity; in short, the intensity of KBS is proportionally related to the drying exigency. However, even at low drying temperatures KBS is not entirely eliminated, which is consistent with Terziev's work (1995). Clearly, KBS starts in the first stages of drying, even with a moisture content as high as 100%. KBS seems to be a continuous process, depending on the concentration of the KBS precursors, which during drying are known to concentrate close to the surface (Terziev 1995; Kreber and Haslett 1997).

Kreber *et al.* (1998) monitored the translocation and accumulation of KBS precursors during the drying of *Pinus radiata* sapwood. Drying temperature and humidity are reported to have an effect on the migration of the compounds suspected to produce discolouration. Hence, the researchers investigated that effect by testing two drying schedules, one mild schedule with a dry bulb temperature of 90 °C, and a wet-bulb temperature of 60 °C; and another severe schedule with a dry-bulb temperature of 120 °C, and a wet-bulb temperature of 70 °C. Kreber *et al.* (1998) traced the concentration of extractives, namely polysaccharides, oligosaccharides, monosaccharides, cyclitol and nitrogen, from the centre to the surface of *Pinus radiata* planks at different times. The results clearly showed that during kiln drying water-soluble compounds migrate from the centre to the surface of the board where they accumulate. Besides, this experiment provided evidence that there were enough reducing sugars and nitrogen compounds near the surface of the boards to undergo Maillard-type reactions. Figure 1 illustrates the marked change in concentration of extractives in the centre and on the surface of the boards, suggesting the migration and accumulation described by Kreber *et al.* 1998. In addition, Figure 1 shows that the drying schedule with higher temperature and lower humidity increases the rate of change of concentration of the extractives both in the centre and on the surface of the boards: also, lower and higher levels of extractives in the centre and on the surface of the boards, respectively, are observed with the 120/70 °C drying schedule.

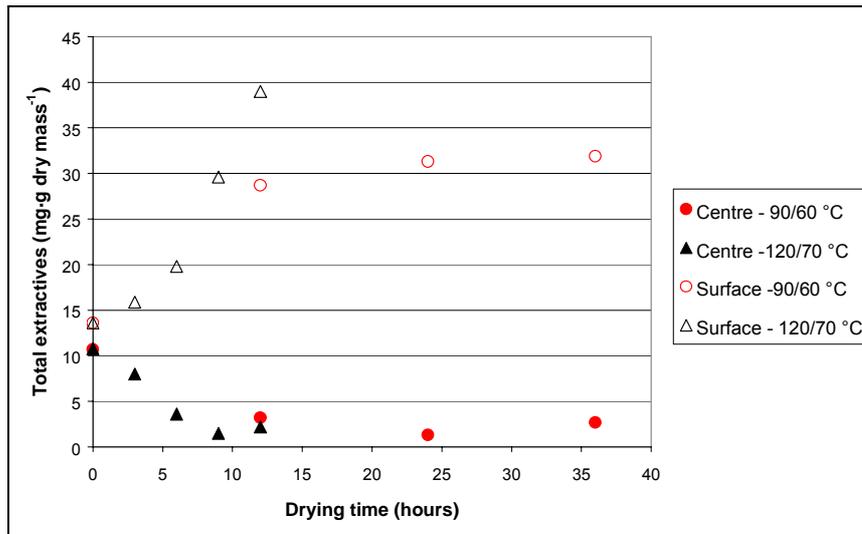


Figure 1 Migration of extractives from the centre to the surface of *Pinus radiata* boards during drying ($\text{mg}\cdot(\text{g dry mass})^{-1}$). Modified from Kreber *et al.* 1998.

McDonald *et al.* (2000) thoroughly investigated the chemical constituents that develop KBS in *Pinus radiata* lumber. To obtain the KBS reactants that are present in the wood they forced cold water through samples, following the method of Edwards and Booker (1984). The solution thus obtained was a sap displacement and was named sap-A1. A sap-displaced wood sample was kept in distilled water for 7 days and then heated (90°C for 3.5 hours) to destroy the membrane of the ray-parenchyma cells. After this process, hot water (70°C) was forced through it, thus obtaining extract-A3. Both solutions sap-A1 and extract-A3 were concentrated and freeze dried. The concentrated extract-A3 formed a precipitate, which was centrifuged to obtain a water-soluble fraction and a water-insoluble fraction. A series of analyses was conducted on the concentrated solutions, and it was observed that the extract-A3 had more solids than the sap-A1. The different composition of sap-A1 and extract-A3 was thought to be due to preheating and use of hot water in sap displacement that destroyed the ray parenchyma cells in the sample allowing its contents to be collected. The ray parenchyma is a living tissue that, among other functions, enables communication between the phloem and the sapwood, and therefore is a reservoir of many compounds, such as starch, malto-oligosaccharides, silica, lipids, proteins and extractives softwoods (Hillis 1987; Higuchi 1997).

The carbohydrate composition of the sap-A1 and extract-A3, consisted of sugars, cyclitols, and organic acids, presented in Table 1, which is consistent with the data reported in a previous work on *Pinus radiata* wood (Cranswick *et al.* 1987). Typical sapwood sugars, such as D-glucose, D-fructose, sucrose, raffinose and stachyose, were found in both the sap and the hot water extract. As expected, hexoses such as glucose and fructose were abundantly present in the sap solution. On the other hand, the analysis detected organic acids such as shikimic acid and quinic acid, both involved in the biosynthesis of aromatic compounds from sugars.

Table 1. Saccharides and organic acid content composition of sap-A1 and extract-A3 samples (modified from McDonald 2000).

Organic compound	Denomination	Sap-A1 % of extract dry mass	extract-A3 % of extract dry mass
Saccharides	Fructose	21.0	1.2
	Glucose	7.0	0.9
	Sucrose	2.1	4.9
	Raffinose	0.3	1.0
	Stachyose	0.2	1.0
	Arabinose		1.5
	Maltose		0.4
	Malto-oligosaccharides		0.2
Organic acids	Quinic acid	0.8	0.2
	Shikimic acid	0.5	0.1

The protein content of sap-A1 was 5.7%, estimated from a nitrogen determination of 0.9%, while the protein fraction of the extract-A3 was lower, 5.1%, estimated from a nitrogen determination of 0.8%. Proteins were present in both solutions, with a molecular weight ranging between 6.5 to 52 kDa. The total amino acid composition was determined; the investigators analysed the free amino acids present in the solutions, shown in Table 2, and, after hydrolysis, determined the amino acids that constituted the proteins. The most abundant amino acids in both sap-A1 and extract-A3 were glutamic acid and aspartic acid. The amino acids found are consistent with previous work done on *Pinus radiata* xylem (Scurfield and Nicholls 1970).

Table 2. Free amino acid composition of sap-A1 and extract-A3 samples (modified from McDonald *et al.*, 2000).

Amino acid	sap-A1	extract-A3
	mole %	
Glutamic acid	81.0	16.0
Aspartic acid	7.0	6.0
Valine	4.0	13.0
Alanine	4.0	38.0
Phenylalanine	2.0	6.0
Proline	2.0	11.0
Tyrosine	1.0	3.0
Arginine		1.0
Lysine		6.0

McDonald and his colleagues showed that the lumber that has been only sap-displaced developed KBS after drying, while the lumber that was sap-displaced, heated and then hot-water extracted did not. Therefore, it was observed that KBS not only depends on the quantity of the compounds that are available for translocation but also on the composition of compounds, which perhaps are released only at higher temperatures. McDonald *et al.* (2000) concluded that the high level of water-soluble compounds, such as the carbohydrates, organic acids, and amino acids presented in Table 1 and Table 2, all of them capable of being displaced with water, supported the hypothesis that KBS occurs due to Maillard-type reactions.

To further investigate the hypothesis that KBS is a result of Maillard-type reactions, New Zealand Forest Research scientists used UV-VIS spectroscopy to monitor and to compare the chromophores¹ developed in heated solutions of sap and hot water extracts, with heated aqueous model systems of reducing sugars and amino acids. Even though the use of model systems of sugars and amino acids was a new approach to study chromophore formation in KBS, it is a standard method to investigate Maillard-type reactions in other fields (Cioroi 1999; Carabasa Giribet 2000). The sap and the hot water extract solutions were obtained by the same

¹ Chromophores: limited number of functional groups capable of absorbing longer-wavelength and visible radiation (modified from Skoog and Leary 1992).

mechanism proposed in the earlier experiment. The reducing sugars were glucose, fructose and sucrose, known constituents of *Pinus radiata* sapwood, and the amino acid used was glutamic acid, which is the predominant amino acid found in sap and hot-water extract. It was observed that in both groups of solutions studied, namely the solutions extracted from sapwood and the aqueous model systems, chromophores appeared at 220 and 280 nm; furthermore, the intensity of the absorbance increased with prolonged heating. The results obtained supported the hypothesis that the colour of KBS is derived from Maillard-type reactions (McDonald 1997).

2.3. Lignin derivatives and wood extractives: their importance in colour development during kiln drying.

New Zealand Forest Research has reported the possible implication of lignin derivatives and wood extractives in the colour development of KBS. The investigators found that as much as 5% of a hot-water extract of *Pinus radiata* wood was composed of phenolic material. Besides, differences in the UV spectrum, indicating changes in chromophores, were observed when lignin was heated, even at such a low temperature as 50°C for one hour. Therefore, there is evidence that the transportation and deposition of soluble phenolic compounds, following the water paths during kiln drying, could be responsible for some of the colour developed by the lumber (McDonald 1997; McDonald *et al.* 2000).

On the other hand, some extracts, such as hydrolysable and condensed tannins, are known to be involved in colour development in wood upon drying (Zavarin and Smith 1962; Charrier *et al.* 1992; Charrier *et al.* 1995). The hydrolysable tannins are only present in the tissues of certain families of angiosperms, such as *Aceraceae*, *Fagaceae*, *Rosaceae*, and therefore are not present in the order *Coniferae* (Haslam 1989). The condensed tannins are distributed both in angiosperms and gymnosperms (Haslam 1989; Porter and Hemingway 1989). In the order *Coniferae*, the higher concentrations are in the bark, needles, flowers and cones (Porter and Hemingway 1989). However, there is evidence of the presence of phenols and condensed tannins in the wood of *Pinus sylvestris* (Scalbert *et al.* 1989).

The hydrolysable tannins are esters of gallic acid and its dimers (digallic, ellagic acid) with monosaccharides, mainly glucose (Fengel 1984). Therefore, upon hydrolysis, the gallotannins and the ellagitannins yield their respective acids, gallic and ellagic acid (Jurd 1962, quoted by Fengel 1984). The hydrolysable tannins are less common in wood than condensed tannins (Fengel 1984), and do not occur in gymnosperms (Haslam 1989). The wood content of water-soluble phenols with galloyl groups, such as tannins, is a known cause for discolouration problems during industrial processes that involve wood and water. The water-soluble compounds are transported to the surface of the wood by the water, leaving stains upon evaporation (Yoshimoto 1989). Hydrolysable tannins, such as gallic acid and ellagic acid, are related with undesirable colouration developed during kiln drying of oak wood (Wassipaul *et al.* 1987; Wegener *et al.*, 1988; Bauch *et al.*, 1990; quoted by Charrier *et al.*, 1992). What is more, Charrier *et al.* observed that the amount of hydrolysable tannins increased in the wood from drying at a low temperature drying (45 °C) to drying at a higher temperature (120 °C) (Charrier *et al.* 1992).

Condensed tannins, such as catechin and epicatechin, are highly concentrated constituents of conifers and angiosperms, particularly in the bark and fruits (Porter and Hemingway 1989). Zavarin and Smith (1962) studied *in vitro* the darkening of redwood (*Sequoia sempervirens*) upon kiln drying, particularly the influence of the tannin concentration, partial oxygen pressure, pH and temperature on the rate of darkening of the redwood tannin. They quoted the work of Freudenberg (1954) and Hathway (1958) to relate the chemical darkening of the tannin with the condensation of the phenolic monomers of the catechin type, involving oxidation and oxidative polymerisation. Similar processes were suggested to contribute to “hemlock brownstain”, a discolouration that affects Western hemlock (Hrutfiord *et al.*, 1985).

Other extractives, such as pinosylvin, contribute to the colour of wood by oxidising to dark-coloured products. Pinosylvin is a known constituent of *Pinus radiata* wood (Norin 1989). There is evidence that hydroxystilbenes decompose to give coloured products under light (Morgan and Orsler 1968). The stilbenes could be related to the yellowing of newsprint in *Pinus radiata* thermomechanical pulps (Suckling 1995, quoted by McDonald 1997).

2.4. Research on KBS control: a brief summary.

To control KBS several techniques have been proposed based both in physical and on chemical treatments.

Drying *Pinus radiata* at low temperatures (45/ 35 °C) avoided KBS, but the drying times were four times longer than when drying at 90/60 °C (from 230 hours to 50-60 hours, respectively). The use of high air velocity to accelerate the low temperature drying process did not reduce the drying time to a commercially acceptable level (Kreber *et al.* 1998).

Vacuum drying was tested to assess the incidence of oxygen in KBS development in *Pinus radiata*. Even though the intensity of KBS was reduced, the improvement was not sufficient to recommend the installation of commercial vacuum kilns, which have higher investment costs than standard kilns. However, vacuum drying has other benefits that would demand further investigation, such as reduction of drying time, drying stress and energy consumption (Wastney *et al.* 1997).

Compression rolling was an alternative physical treatment to prevent the migration of water-soluble compounds to the surface of the boards, by disrupting the water-movement mechanisms. However, compression rolling did not eliminate KBS, and increased the drying times and the thickness shrinkage (Kreber and Haslett 1997).

Kreber *et al.* (1999) designed a dewatering process, which involved the cyclic application and release of compressed air to the wood to drive out free water by expanding internal air bubbles. The objective was to reduce the moisture content of the wood down to 40%, and consequently reduce the mass water flow that transports the water-soluble compounds to the surface. The procedure was successful in eliminating KBS, but unfortunately, pressure cycles of more than 10000 kPa were required. Such pressures would not be commercially viable.

Chemical treatments were also explored to reduce KBS. Sodium dithionite, a reducing agent that has proven to be successful in controlling stain in other species, was tested in *Pinus radiata*. The chemical was applied to the lumber by dipping,

accelerated diffusion, and pressure treatment. It was observed that sodium dithionite could control KBS only if the lumber was completely penetrated by the chemical. Regrettably, such condition extended the drying times (Kreber *et al.* 1999).

Biological control of KBS could be an alternative, under the hypothesis that in a pre-kiln drying treatment, biological entities would consume KBS precursor. Kreber *et al.* (1999) have reported a study in fungi to control sapstain, and it was suggested that this technique could have potential to reduce KBS.

Kreber *et al.*, unpublished (1999) reported that there is no evidence that KBS is related to genetic clones. Studies by Cranswick *et al.* (1987) and Donaldson *et al.* (1997) have given evidence of the unlikely success of a selection programme on the basis of altering KBS sugar precursors: the genotypic differences in the monosaccharides and sucrose composition of *Pinus radiata* are not significant and, in addition, glucose has low repeatability. Therefore, there is enough evidence to indicate that the concentration in *Pinus radiata* wood of Maillard reaction reagents has low heritability, and that this characteristic could not be exploited in a selection program.

Chapter 3

EQUIPMENT AND METHODS.

3.1. Introduction.

The purpose of this project is to determine how compounds present in sapwood of *Pinus radiata* D. Don, such as non-structural sugars, nitrogen compounds and phenols, contribute to discolouration and stains on the surface of kiln-dried boards. The hypothesis was that the chemical compounds present in wood are the reactants of a complex discolouration reaction, and that the colour on the surface of the boards is the physical property by which the discolouration reaction could be assessed. The possible reactions considered in this study were Maillard-type reactions; oxidation and polymerisation of phenolic compounds; or a combination of both. Therefore, the final output of this experiment was an equation based in simple reaction kinetics that modelled discolouration on the surface of *Pinus radiata* boards during drying.

Different pieces of equipment and methods were used to prepare the samples and to make the determinations according to the experimental design. Some equipment used was devised and constructed for the purposes of this research. Figure 2 is a diagram of the experimental design, summarized in the next paragraph and later described in detail in this chapter.

Fresh sawn *Pinus radiata* boards were collected from a local sawmill, and stored under water. The two experimental factors in this study were leaching and drying time. Section 3.3 describes the leaching of the soluble compounds present in wood, done at three levels, namely control, mild leaching and severe leaching. Next, as presented in section 3.4, the wood samples were dried using the same drying schedule for every sample, which consisted of an air temperature of 120 °C, a wet bulb temperature of 70 °C, which corresponds to 14.3% relative humidity, and air velocity of approximately 5 m·s⁻¹. The experimental factor drying time had four levels: 0, 8, 16, and 24 hours. After each drying, colour on the surface of the boards was assessed using a reflectance photometer, as described in section 3.5.1, and also by image analysis of digital photographs, using commercially available software,

according to the details given in section 3.5.2. Different methods to measure colour were used to compare their ability to measure discolouration of pine-wood during kiln drying. The concentration of non-structural sugars, nitrogen compounds and phenols were determined for every sample, as to be explained in section 3.6. The variables to be included in a cause-effect analysis to investigate the relationship between colour on the surface and the chemical compounds determined in the wood closest to the surface were selected through statistical methods described in section 3.7.1. Multivariate linear regression and Neural Networks were used to construct models that had colour variables as dependent variables and chemical variables as independent variables, along with drying time, as explained in sections 3.7.2 and 3.7.3. On the basis of the statistical cause-effect analysis, the discolouration of pine-wood boards during drying was expressed in an empirical kinetic equation presented in section 3.7.4.

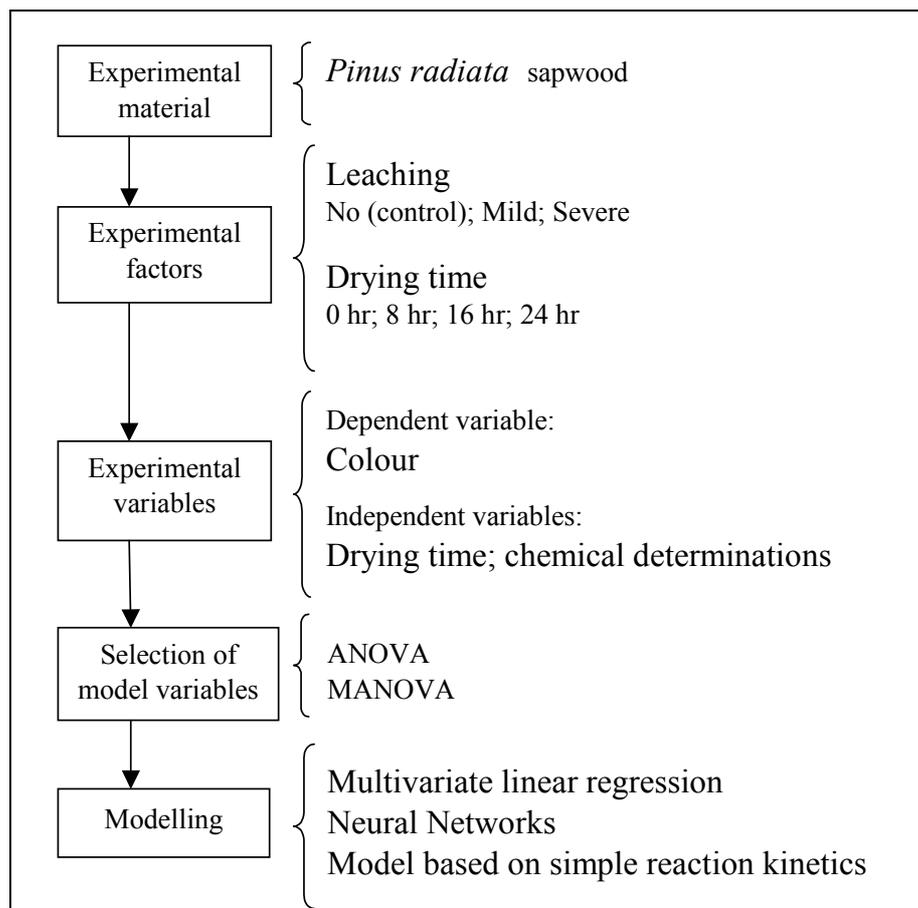


Figure 2. Diagram of this study's experimental design.

3.2. Experimental material: *Pinus radiata* sapwood.

Three boards of 50x100x4900 mm fresh-sawn *Pinus radiata* sapwood were obtained in a local sawmill from trees felled the same day. Neither environmental nor genetic variability was considered in this study. The wood was treated with a solution of carbendazim and oxine copper to prevent mould discolouration. The flat face of the board toward the cambium side was surfaced to expose kiln brown stain. The control boards were sawn from clear wood, free of knots or defects, into 50x100x250 mm blocks, wrapped in plastic sheeting and kept at 5 °C. The boards to be leached were sawn to 50x95x250 mm and were kept under distilled water at 5 °C. The difference in width was necessary to fit the samples into the wood-leaching apparatus, and it was not considered necessary to saw the control samples to the same dimensions.

3.3. Wood-leaching apparatus.

There is evidence that the colour change in the boards of *Pinus radiata* is due to the concentration of soluble compounds in the surface. McDonald (1997) and McDonald *et al.* (2000) proposed the use of an apparatus to displace the solution present in a sample of wood with distilled water. When softwood is saturated in water, the concentration of water-soluble compounds is reduced by diffusion into the aqueous medium (Hon 2001). Among those water-soluble compounds are non-structural carbohydrates, proteins and amino acids, mainly present in the ray parenchyma of the ray tissue of the xylem. The replacement of sap with water produces a reduction in the discolouration during subsequent drying (McDonald *et al.* 2000).

The displacement of the wood sap with water-soluble preservative solutions is a well-known method to preserve wood, called the Boucherie process. One of the ends of a sample of fresh wood is attached to an inflow port, which is connected to an elevated tank containing the solution. Due to hydrostatic pressure, and if there is no air embolism, the solution displaces the sap present in the wood (Walker 1993). To test the discolouration effect of different levels of reduction of soluble compounds, a leaching apparatus was designed to reduce the amount of soluble compounds present

in wood. This new design was based on previous work on the principle of the Boucherie preservation process, but mainly in the work of Edwards and Booker (1984).

The apparatus was built to force water through the longitudinal axis of a sample of wood using a maximum pressure difference of 115 kPa. The sample was bolted between an inflow and outflow port, with silicone applied to the perimeter of the sample to improve the sealing against the ports. The inflow port was connected to a 30-litre tank elevated 3 m, which represented a head of water of approximately 30 kPa. The water was pumped to the elevated tank from a 70-litre reservoir tank, using a centrifugal pump. The outflow port was connected to a vacuum line of maximum pressure 22 kPa absolute. The water extracted from the sample was collected in a 5-litre glass flask. A diagram of this apparatus is presented in Figure 3.

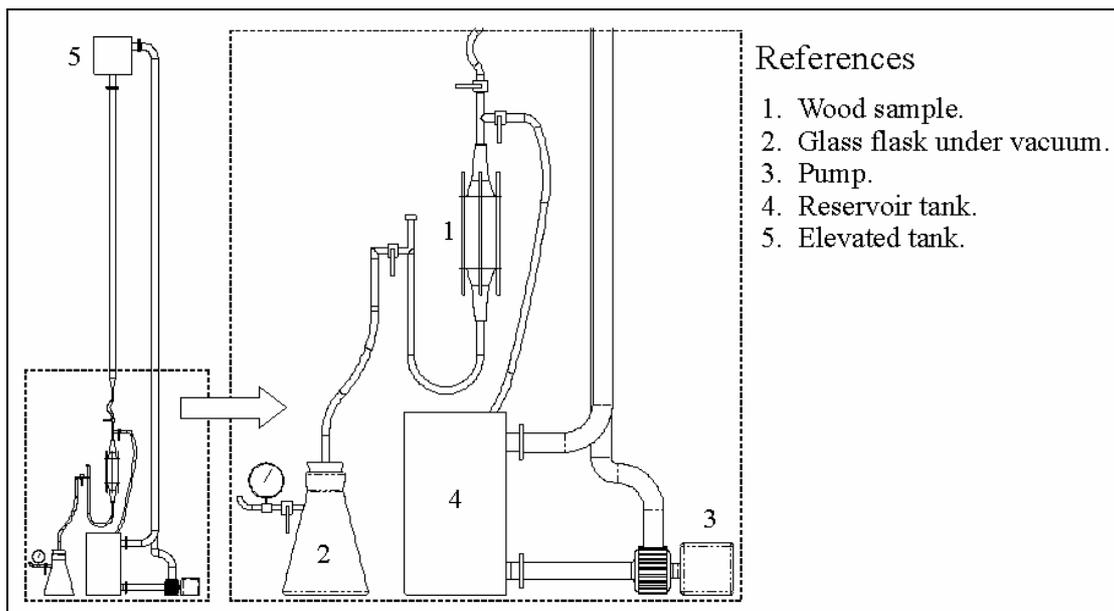


Figure 3. Sketch of the wood leaching apparatus.

The leaching of the samples was performed in two levels, mild and severe. Prior to leaching the samples were immersed in distilled water at ambient pressure and under vacuum to diminish the content of air in the wood, and therefore avoid air embolism. The samples were stored in distilled water at approximately 5 °C for at

least 3 weeks, to prevent sap stain development. Just before leaching the samples were immersed for 3 hours in distilled water under vacuum (22 kPa absolute).

The mild leaching treatment consisted of forcing through the wood sample 4.5 litre of water at ambient temperature with a pressure difference of approximately 110 kPa. After the treatment the samples were kept under water until needed for drying, to avoid colour change due to microorganisms' activity. The severe leaching treatment differed from the mild treatment in that it included the heating of the samples before leaching, and the use of larger volumes of water at a higher temperature. The 50x95x250 mm-wood samples were heated at 90 °C for 5 hours. Approximately 250 ml in excess of 10 litre of water at 50 ± 3 °C was forced through the samples with a pressure difference of approximately 110 kPa. The objective was to force at least 10 litre of water through the sample, although generally it was approximately 250 ml more because after the objective volume was reached, and the flow stopped, more water had already gone through the sample.

Heating elements were installed in the reservoir tank to control the temperature of the water. The heating system consisted of two heating elements with a total output power of 4.5 kW. The apparatus's design allowed the continuous circulation of the water to maintain the desired temperature.

3.4. Drying tunnel.

The samples were dried in a laboratory-scale drying tunnel, designed for wood drying research at the Department of Chemical and Process Engineering of the University of Canterbury. The drying tunnel dries wood at high temperatures and high humidity conditions, with a maximum operational temperature of 140 °C, a maximum relative humidity of 100%, and a maximum airspeed of $20 \text{ m}\cdot\text{s}^{-1}$. The drying tunnel is controlled by Advantech ® Genie™ software. The software also monitors and records temperature, humidity and sample mass during the drying process (McCurdy 2000).

The aim of this experiment was to assess colour change in the tangential face of the boards. Therefore, the ends and sides of the samples were painted with Altex

Coating ® to reduce evaporation from those surfaces. The boards were dried at 120 °C dry bulb and 70 °C wet-bulb (relative humidity of 14.3%), with an air speed of approximately 5 m·s⁻¹. The drying time treatment had four levels: 0, 8, 16, and 24 hours. The drying tunnel had the capacity to dry two samples simultaneously.

The samples were dried in the following sequence: two samples were placed in the kiln; after 8 hours, one sample was taken out, and another fresh sample was placed in the drying tunnel; after another 16 hours, both samples were removed from the drying tunnel, having had 16 and 24 hours of drying, respectively. This resulted in three samples having had 8, 16, and 24 hours of drying respectively, and the same procedure was repeated 12 times. Each sample was considered to be an independent experiment; hence this study involved 36 drying experiments.

The dry-basis moisture content was calculated using the following equation (Walker 1993):

$$\text{Eq. 1} \quad \text{MC}\% = \frac{m - dm}{dm} \times 100\%$$

where MC% is moisture content in percentage; m is original mass of the sample; and dm is the mass of the sample after drying to constant mass at 103 ± 2 °C.

Immediately after drying, a sample of approximately 5x60x150 mm was sawn from the flat face of the board towards the cambium as shown in Figure 4. The depth of the colour layer was within 1 mm and the kiln brown stain was exposed at the surface. The sample was then wrapped with paper and nylon, and placed in a sealed plastic bag at approximately -30 °C.

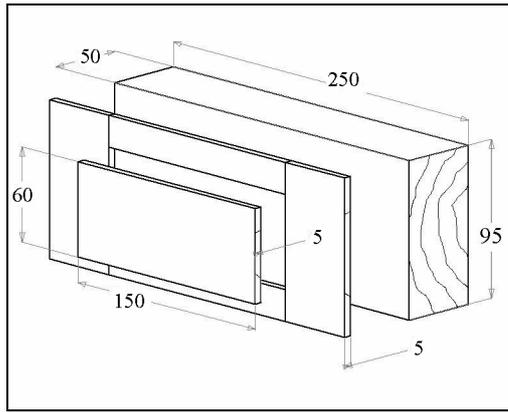


Figure 4. Dimensions of the sample used for colour determination and chemical analyses.

3.5. Colour determination.

This research intended to find the relationship between the discolouration of the surface of the boards during drying and certain compounds present in the xylem. Therefore, the measurement of the colour of the wood samples was a crucial aspect of this work.

Colour can be determined in three ways: by comparison with colour samples, as in the Munsell chart; by specifying the spectral energy distribution of the colour and of the illuminant (this method is ambiguous, because different spectral energy distributions could generate the same visual sensation); and by determining the chromaticity and the luminance, or luminous reflectance, of the colour (Stimson 1974).

Luminance, the photometric brightness, describes “the light being radiated per unit area of a surface” (Wright 1964). The units of luminance are candelas per unit area. The luminance factor (β) of a sample is the ratio between its luminance and that of a perfect white diffuser. The luminance factor can be determined by varying certain conditions, such as illuminants, direction of illumination, and direction of viewing (Wright 1964). In relative terms based on a scale from 0 to 100, the luminance factor of absolute black is 0, and that of a perfect white is 100 (MacAdam 1985).

Several methods have been developed to measure colour. The *Commission International de l'éclairage* (CIE, or International Commission on Illumination) has proposed different colour systems, or colour spaces, based on the determination of chromaticity and luminance. Two of them are the CIE Yxy colour space and the CIE $L^*a^*b^*$ colour space, or CIE Lab (MINOLTA 1998), as shown in Figure 5.

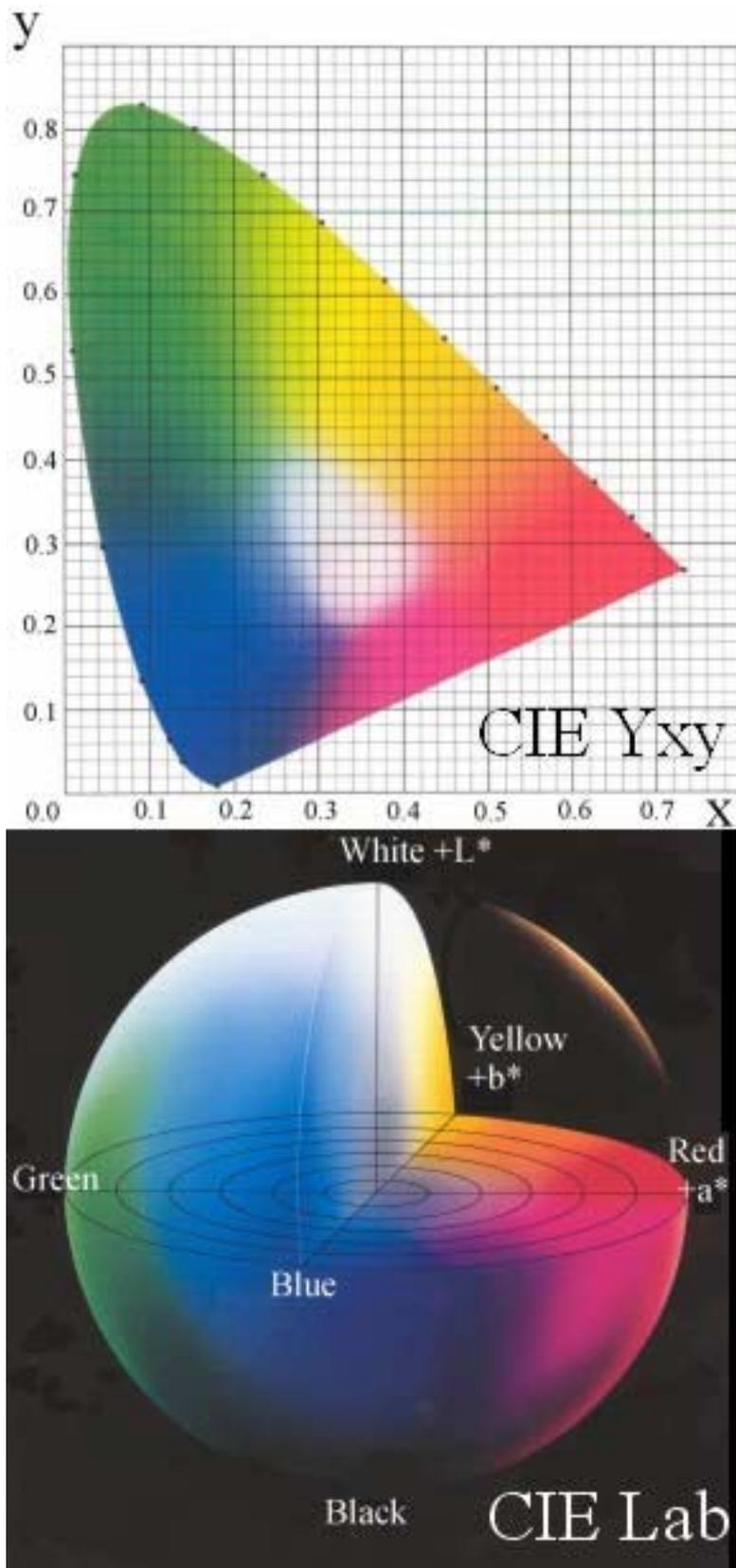


Figure 5. Colour systems CIE Yxy and CIE Lab (modified from MINOLTA 1998).

To standardise an observer's response to colour, the CIE has defined a *standard observer* using three parameters: the test field size, the brightness of the field, and the spectral sensitivity of the sensor. The response of a CIE-defined Standard Observer to the ideal primary colours red (R), green (G), and blue (B) are the CIE colour-matching functions, the x_λ , y_λ , and z_λ functions. Those functions represent “the amount of each of the CIE primaries that is required to match the colour of one watt of radiant power of the indicated wavelength” (MacAdam 1985). The colour-matching functions are illustrated in Figure 6.

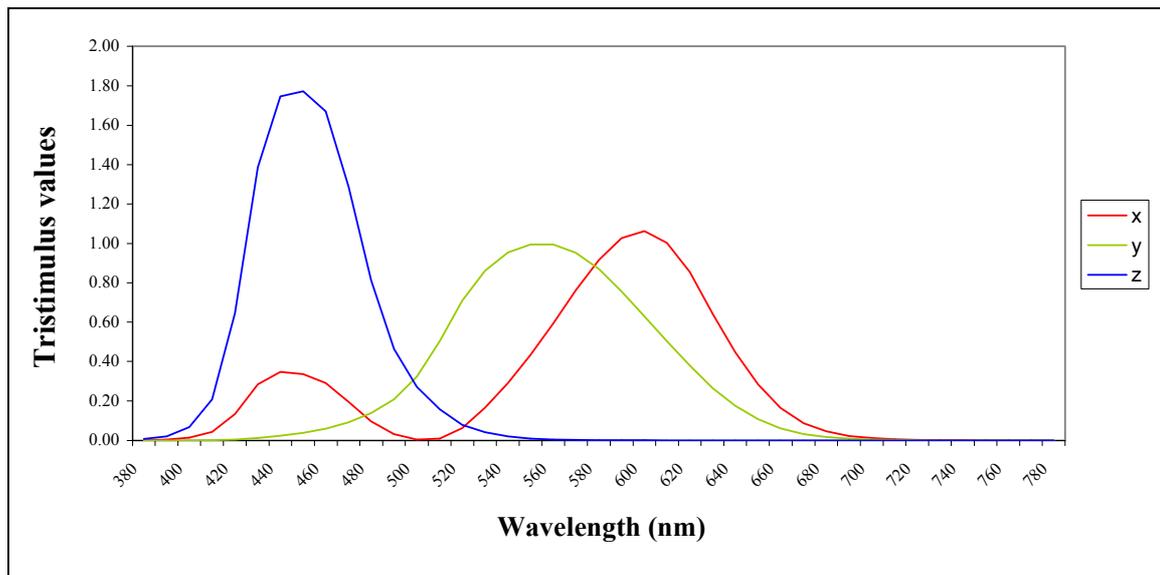


Figure 6. Tristimulus values of equal energy spectrum as adopted by CIE in 1931 for the colorimetric Standard Observer (from Stimson 1974).

The tristimulus values are algebraically expressed by the following equations (Stimson 1974):

Eqs. 2 $X = \int E_e \bar{x} d\lambda$

$$Y = \int E_e \bar{y} d\lambda$$

$$Z = \int E_e \bar{z} d\lambda$$

where E_e is spectral energy of the illuminant; $\bar{x}, \bar{y}, \bar{z}$ are the CIE colour-matching functions; and λ is the wavelength spectrum where the integration is done, ranging from 360 to 830 nm (Wyszecki and Stiles 1982). Using the tristimulus values, the chromatic information can be expressed in three dimensions (x, y and z) by the following equations (Stimson 1974):

Eqs. 3
$$x = \frac{X}{X + Y + Z}$$

$$y = \frac{Y}{X + Y + Z}$$

$$z = \frac{Z}{X + Y + Z}$$

The dimensions x and y are used to plot the information in two axes. They are termed the chromaticity coordinates. According to the CIE, a colour is unambiguously defined by the chromaticity coordinates x and y, and the tristimulus value Y (Hunter 1942; Stimson 1974). The tristimulus value Y represents the luminance factor (MacAdam 1985).

The colour system CIE Lab was proposed in 1976 as an attempt to improve the uniformity of the CIE Yxy colour space, and to provide an estimation of colour difference (Wyszecki and Stiles 1982). This system “involves non-linear, cubic transformations of the tristimulus values Y, X and Z” (MacAdam 1985). The variable L^* represents lightness, an attribute of a visual sensation which is a relative comparison between the light emitted by a sample and the light emitted by a white standard (Wyszecki and Stiles 1982). The scales a^* and b^* are dependent on lightness, and therefore are not chromaticity coordinates (Wyszecki and Stiles 1982), but can be plotted to indicate colour directions: a^* is the red to green direction, and b^* is the yellow to blue direction (MINOLTA 1998).

The new variables L^* , a^* and b^* are defined by the following equations:

$$\begin{aligned} \text{Eqs. 4} \quad L^* &= 116 \cdot \left(\frac{Y}{Y_n} \right)^{1/3} - 16 \\ a^* &= 500 \cdot \left[\left(\frac{X}{X_n} \right)^{1/3} - \left(\frac{Y}{Y_n} \right)^{1/3} \right] \\ b^* &= 200 \cdot \left[\left(\frac{X}{X_n} \right)^{1/3} - \left(\frac{Z}{Z_n} \right)^{1/3} \right] \end{aligned}$$

with the constraints that $\frac{X}{X_n}$, $\frac{Y}{Y_n}$, $\frac{Z}{Z_n} > 0.01$; where X, Y and Z are the tristimulus values of the sample illuminated by the same source; and X_n , Y_n and Z_n are the tristimulus values of the white standard illuminated by the same source (Wyszecki and Stiles 1982; MacAdam 1985).

The absolute colour difference (ΔE) between two colours, given in terms of $L^*a^*b^*$ is calculated using the following equation (Wyszecki and Stiles 1982; MacAdam 1985):

$$\text{Eq. 5} \quad \Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

where ΔE is the absolute colour difference; L^*_1 , a^*_1 , b^*_1 are the CIE Lab values of colour 1; and L^*_2 , a^*_2 , b^*_2 are the CIE Lab values of colour 2.

Colour has been extensively measured in wood. It has been reported that tristimulus colorimeters are useful equipment to distinguish colours in wood (Loos and Coppock 1964; Salamon 1972; Phelps *et al.* 1983; McGinnes and Rosen 1984; Charrier *et al.* 1995; Smith and Herdman 1996). The development of software for digital analysis of images has opened a new field in the identification of colour (Andersson and Walter 1995; Krutz *et al.* 2000).

The colour assessment of the pine-wood samples was done on the cambium surface of the samples after drying. The samples were divided into three equal sections of approximately 50 x 60 mm. This division was done to leave open the opportunity of repeating observations if necessary. The colour determination was done on section **i**, as shown in Figure 7.

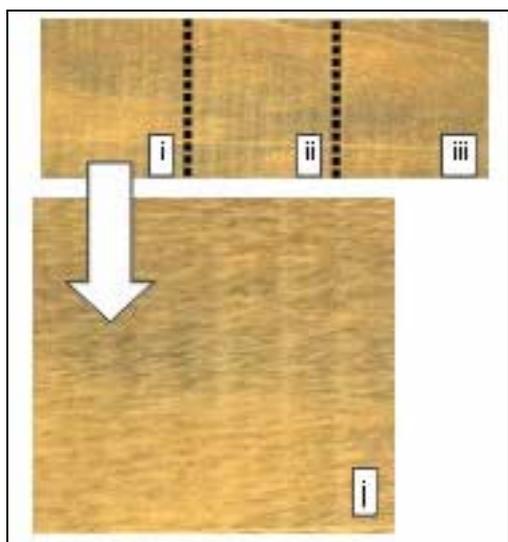


Figure 7. Division of the samples in approximately three equal sections.

Two methodologies were used to determine the colour of the samples, namely reflectance measurements, using a photoelectric reflectance photometer, and digital image analysis, described in sections 3.5.1 and 3.5.2, respectively.

3.5.1. Photoelectric reflectance photometer (ELREPHO).

The photoelectric reflectance photometer used was a Carl Zeiss ELREPHO, designed to measure diffuse reflectance according to German standard DIN 5033. Diffuse reflectance is “the ratio of the radiant or luminous flux of a specimen to that of a perfectly reflecting diffuser, each being irradiated hemispherically and viewed identically” (standard method T 534 pm-92, TAPPI 1994). Reference standards, such as barium sulphate, magnesium oxide or clay, are calibrated in terms of absolute reflectance, because there is not a perfectly reflecting or diffuse substance. ELREPHO consists of an integrating sphere that provides diffuse illumination over

the sample, generating a current in a photocell. The light sources used were two incandescent lamps projecting into the sphere from right and left. The voltage of the lamp defines its temperature and therefore its illuminance. The German Standard DIN 5033 defines illuminant A and C. Illuminant A is comparable to the light of a tungsten filament lamp, and it is produced with the ELREPHO lamps operating at 10 volts at a temperature of 2854 °K. Illuminant C is an approximation of daylight without the UV component, obtained with ELREPHO lamps operating at 12 volts at a temperature of 2980 °K. For the purpose of this experiment only illuminant C was used, because it was intended to reproduce conditions of daylight. The sample is perpendicularly observed through an aperture with a diameter of 34 mm, and its reflectance is compared against an internal standard.

ELREPHO is equipped with a number of filters to measure reflectance at different wavelengths. The light filter R457, with a wavelength of 457 nm \pm 0.5 nm, was used to measure “the reflectance of blue light corresponding to a specific spectral distribution with an effective wavelength of 457 nm by a perfect diffusing surface” (standard method T 534 pm-92, TAPPI 1994). This method was designed to measure brightness in pulp, paper and paperboard. “Brightness is the attribute of a visual sensation according to which the area in which the visual stimulus is presented appears to emit more or less light” (Wyszecki and Stiles 1982). The use of the light filter R457 to determine brightness is a standard test method of both the International Organization for Standardization (ISO) and the American Society for Testing and Materials (ASTM). In addition, brightness has been used before to discriminate wood: Salamon, (1972), used the determination of reflectance at 457 nm to distinguish between fresh and dried samples; and Hrutfiord *et al.*, (1985), measured brightness in mechanical pulp to study its correlation with oxidized d-catechin.

ELREPHO also has filters that modify the spectral response of the photocell to match the CIE x_λ , y_λ , and z_λ functions. The tristimulus values can be determined by measuring the directional reflectance of the sample using three special filters, tristimulus filters R_x , R_y , and R_z , red, green and blue, respectively. The primary standard is barium sulphate. The tristimulus values for illuminant C are calculated using the following equations:

Eq. 6

$$X = 0.782 \cdot R_x + 0.198 \cdot R_z$$
$$Y = R_y$$
$$Z = 1.181 \cdot R_z$$

The determinations of X, Y and Z of ELREPHO are obtained through a selection of wavelengths given by the filters, and therefore are an approximation of the actual X, Y and Z values given in Eqs. 2. A spectrophotometer is an instrument that records the response of a photocell to the entire spectrum, and not just the response to a selection of wavelength given by the filters, giving an accurate measurement of X, Y and Z. A photoelectric detector with a set of filters provides “abridged spectrophotometric data”, although it can be used for certain purposes (MacAdam 1985).

The tristimulus value Y of the CIE Yxy colour space and the brightness value obtained with filter R457 are related because they are estimations of the luminance of colour by measuring diffuse reflectance against the barium sulphate standard. Two filters are used, namely R_y and R457, and they determine the different wavelengths to be reflected, and hence the basic difference between the determinations. Basically, both determinations are an estimation of luminance factor, because they are relative measurements compared to a standard. Nevertheless, to avoid further confusion, in this work luminance factor is the quantity determined with filter R_y, and brightness is the quantity determined with filter R457.

According to the ELREPHO Operating Instructions, “the systematic errors of colorimetry result from the limited accuracy when adapting the filter transmittance curves to the spectral distribution curves, from the deviation of the spectral sensitivity of the photocells from the nominal values, and from the deviation of the spectral energy distribution of the sample illumination from the normal values”. To reduce the error, the manufacturer recommends diminishing the colour difference between sample and standard.

The internal standard must be calibrated with a tablet of barium sulphate for the white standard according to DIN 5033. The directional reflectance of the barium

sulphate depends on the batch code. The tablets should be produced in a powder press that is part of the ELREPHO equipment. However, the ELREPHO equipment used did not have a complete powder press, and a new powder press was designed to produce barium sulphate tablets. Refer to the Appendix A for a detailed description of the press.

The ELREPHO user's manual indicates that 13 g of barium sulphate are required to produce a tablet with a diameter of 45 mm. TAPPI standard method T-534 pm-92 describes how to produce tablets of clay and other mineral pigments for the determination of diffuse brightness of pulp. The measurement of brightness is performed using equipment similar to ELREPHO. The pressure applied to the powder was 210 kPa for 5 sec. The ELREPHO has a powder tablet reservoir of 45 mm in diameter, which represents a cross-sectional area of 159.04 mm². For a pressure of 210 kPa the mass needed for that area is equivalent to 34.05 kg. That pressure was tested, but the tablet produced was too fragile, with imperfections on the surface. Therefore, the tablets were produced by hydraulic compression at a much higher pressure, approximately 1350 kPa for 5 seconds. The tablet produced was free of defects and showed no glossy surface. This tablet was used to calibrate the internal standard of the ELREPHO.

After the calibration of the equipment, the colour of the surface of the wood samples was determined by comparison against an internal standard. Four filters were measured, namely R457, R_x , R_y and R_z . The chromaticity co-ordinates x and y were calculated from R_x , R_y and R_z as given in Eqs. 3.

3.5.2. Image analysis to measure colour.

Digital image analysis of colour has been used to classify compression wood (Andersson and Walter 1995). The researchers used the RGB colour system, based on the primary colours red, green and blue, and developed software able to distinguish between mild and severe levels of compression wood. The RGB system is commonly used in colour TV and computer monitors.

In this work, digital images were analysed to identify the colour of a determined area. The digital images were taken using a digital camera Nikon Coolpix 900, under controlled conditions in a light box especially designed for the camera as shown in Appendix A. The inside of the light box was painted white, and it had a diffused source of light. The distance from the camera to the sample was 400 mm. The software used to split the image in the colour system was Corel Photo-Paint® Version 9.337. The digital photograph included the whole sample, but the analysis was done on section  of the sample as shown in Figure 7.

The system used to measure colour was CIE Lab. The quantities L^* , a^* and b^* were obtained directly from the software after the analysis of the digital photograph. There were no calculations involved. The software used considered the L^* quantity from white (-127) to black (128); the a^* scale from green (-127) to red (128); and the b^* scale from blue (-127) to yellow (128). The CIE Lab quantities were used to determine absolute colour difference (ΔE). The colour reference used to calculate absolute colour difference was the average of the three repetitions subjected to no drying and no leaching (average values: $L^*=56.01$; $a^*=9.65$; $b^*=50.40$).

A dark pixels index was also designed to measure stains in the surface of the samples. The dark pixels index consisted of counting the number of pixels darker than a certain limit in a lightness scale ranging from black (0) to 256 (white). The limit that allowed the distinction between the natural shades of wood and the stains produced during kiln drying was set at 150. Hence, the Dark Pixels Index (DPI) was obtained by counting and adding the pixels darker than 150. The DPI could be calculated using the Histogram Tool of Corel Photo-Paint, or by running a MATLAB® 5.3 program written for that purpose. For details on the program see Appendix A. An example of two images measured by the index is presented in Figure 8. This figure shows that the darker image has more pixels darker than 150, and therefore has larger DPI .

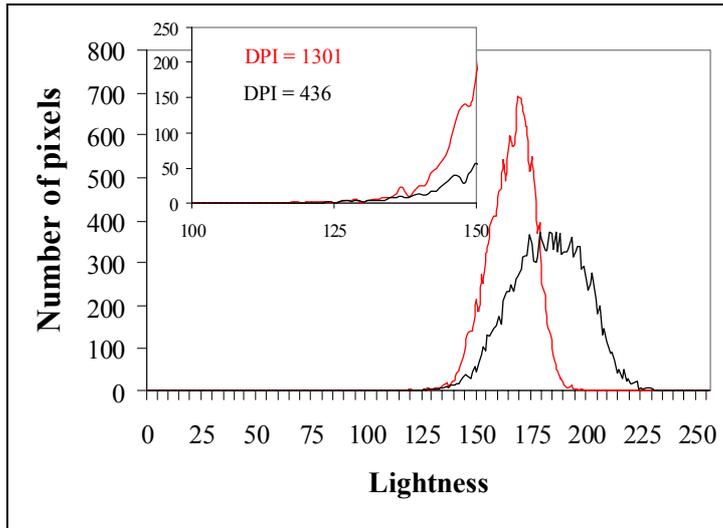


Figure 8. Example of the Dark Pixels Index (*DPI*).

3.6. Chemical determinations.

Previous research has related colour change in wood during drying to the presence of certain compounds in the sapwood, as described in Chapter 1. The evidence presented suggests the non-structural sugars, such as fructose, glucose and sucrose, and the nitrogen compounds are important. Some authors suggested that phenolic compounds could be relevant to explain colour change in pine-wood (McDonald *et al.* 2000), as they are for hardwoods (Hrutfjord *et al.* 1985; Charrier *et al.* 1995). Therefore, this study determined fructose, glucose, sucrose, total nitrogen, low-molecular-weight and high-molecular-weight phenols in the wood closest (<5 mm) to the surface of the boards.

As was mentioned before, this study did not consider either environmental or genetic variability, although it exists. There is considerable evidence that the sugar content in the xylem is variable (Terziev 1996). Basically, the tree produces carbohydrates by photosynthesis, and consumes them by respiration and tissue creation (Cranswick *et al.* 1987). The sapwood of Scots pine (*Pinus sylvestris*) has a higher sugar concentration in the colder season than in summer (Saranpaa and Höll 1989; Fischer and Höll 1992). The variability of the sugar determinations will be large from tree to tree, but it would also be affected by seasonal changes. As Figure 9 shows, there is seasonal variation during the year, with peaks of glucose and

Generally, the determination of carbohydrates is done using chromatographic techniques such as gas-liquid chromatography (Paterson 1975; Cranswick *et al.* 1987; Theander *et al.* 1993) and high-pressure liquid chromatography (HPLC) (Wilson *et al.* 1995; Kreber *et al.* 1998). Due to its speed and efficiency, HPLC is now the accepted technique to determine carbohydrates in wood (Hicks 1988).

Table 3 presents a brief summary of analysis of non-structural sugars in the sapwood of *Pinus radiata* and *Pinus sylvestris*. The levels of non-structural sugars are usually below $2 \text{ mg}\cdot(\text{g dry mass})^{-1}$, with the exception of sucrose determined by Cranswick *et al.* 1987 and Saranpaa and Höll 1989. There are other carbohydrates present in the sapwood apart from fructose, glucose and sucrose, as the investigation done by Kreber *et al.*, 1989, shows.

Table 3. Sugars determined in the stem wood of *P. radiata* and *P. sylvestris*.

Species	Carbohydrates	Quantity $\text{mg}\cdot(\text{g dry mass})^{-1}$	References
<i>Pinus radiata</i>	Fructose	2.25	Kreber <i>et al.</i> 1998 ²
	Glucose	0.42	
	Sucrose	1.21	
	Stachyose	0.98	
	Raffinose	0.57	
	Arabinose	Trace	
<i>Pinus radiata</i> 2-3 years	Fructose	0.60	Cranswick <i>et al.</i> 1987
	Glucose	1.20	
	Sucrose	6.90	
<i>Pinus sylvestris</i>	Sum of fructose and glucose	0.54 - 1.54	Saranpaa and Höll 1989 ³
	Sucrose	2.05 - 4.45	

Generally, wood samples are subject to three processes before carbohydrate extraction, namely drying, size reduction and removal of water-insoluble extractives. Drying is necessary to determine the anhydrous mass of the wood sample. Wood samples are reduced to particles to increase the surface contact with solvents and thereby facilitate the extraction. Water-insoluble extractives should be removed from

² Outer green wood.

³ Original data in $\mu\text{mol}\cdot(\text{g dry mass})^{-1}$.

the sample before or during the extraction because they can eventually interfere with the determination of carbohydrates. A detailed description of the three processes mentioned is presented below.

Researchers have proposed different methods to dry the sample prior to chemical analysis. Samples were dry at 103 °C for 24 hours (Kreber *et al.* 1998), or at 105 °C until constant mass was achieved (Pettersen 1984). Alternatively, it was recommended that vacuum drying should be done at 50 °C for 4 hours (Pettersen 1991) and at 40 °C overnight (Worall and Anderson 1993). Paterson reported air-drying the fresh samples, and after grinding them, drying the wood powder over concentrated sulphuric acid in a desiccator for 48 hours (Paterson 1975). Freeze-drying was another alternative (Cranswick *et al.* 1987). Wilson *et al.* (1995) suggested the lyophilization of wood tissue because it allows a carbohydrate determination that is more similar to fresh tissue than oven-dried tissue (Smith 1981, quoted by Wilson *et al.* 1995).

The sample size should be reduced to pass through a sieve ranging from 40 to 60 mesh (Wright and Wallis 1996; Kleinschmidt *et al.* 1998; Kreber *et al.* 1998).

Extractives can interfere with the determination of non-structural carbohydrates. Different suggestions to solve this problem have been found in the literature. The groundwood samples should be extracted with aqueous acetone followed by extraction with a toluene and ethanol mixture (Pettersen 1984; Pettersen 1991); dichloromethane in a soxhlet extractor for 8 hours (Paterson 1975); or hexane for 5 min in a 42 °C water bath under a fume hood, followed by centrifugation and disposal of supernatants (Hinesley *et al.* 1992).

Water is commonly used as the extraction medium for non-structural sugars. Kreber *et al.* (1998) suggested four extractions with deionised water at 40 to 50 °C for 23 hours. Paterson (1975) used similar temperatures, but an extraction time of 36 hours. Also, he added an aqueous streptomycin sulphate solution to protect the sugars from being consumed by bacteria. In both cases, the aqueous extract was evaporated to dryness and the samples were frozen until analysed. Other researchers proposed aqueous ethanol as extraction medium, three times at 80 °C, for 15 min each time

(Hinesley *et al.* 1992; Theander *et al.* 1993). In a different approach, Wilson *et al.* (1995) suggested an extraction medium of methanol, chloroform and water. The lyophilised and ground samples were extracted overnight. The pigmented chloroform phase was discarded and the aqueous phase was evaporated to dryness.

On the basis of this information, a method for the extraction of non-structural sugars was designed and executed.

Prior to analysis, the samples were weighed and dried to constant weight at 40 °C under vacuum (22 kPa absolute) to determine the moisture content on a dry basis. The sample was first reduced to 2 mm particles using a Willey mill, and later to 0.25 mm particles using a ball mill. A sample of approximately 500 mg of wood powder was extracted twice with 5 ml of acetonitrile for 5 minutes. Acetonitrile was used as extractive because it was also a component of the mobile phase of the HPLC method, namely acetonitrile:water 75:25 (Binder 1980). Therefore, any traces left on the sample will not be detected by the HPLC method. The procedure was done twice to ensure that most of the extractives were removed. Next, the solution was centrifuged and the supernatants were eliminated (Hinesley *et al.* 1992). The remaining acetonitrile was removed by drying the samples in a vacuum oven at 22 kPa absolute and 45°C for 5 hr. The wood pellets were extracted with 20 ml of deionised water in a water bath at 40 °C as proposed by Kreber *et al.* (1998), but only for 8 hours, since 23 hours seemed excessive. After the extraction, the aqueous solution was centrifuged at 4000 rpm for 20 min. An aliquot (5 ml) of the supernatants was collected and freeze dried, and then kept at -30 °C until analysis. Just before analysis, the extracts were resuspended in 200 µl of deionised water and the solution was immersed in a sonic bath (Kreber *et al.* 1998) for 10 minutes to ensure complete solubilization.

The carbohydrates to be determined were fructose, glucose and sucrose. Therefore, calibration samples were prepared using fructose (BDH, GPR), glucose (BDH, GPR) and sucrose (BDH, AnalR). For each sugar, a solution was made with approximately 3 g of sugar in 1 litre of distilled, deionized water, which was later injected into the HPLC in the volumes 5, 10, 20, 50 and 100 µl. The response factors obtained were used to calculate the absolute concentration for each sugar, termed the calibration standard curve. No internal standard was used. The calibration standard

curve for the fructose, glucose and sucrose was calculated at 0.9 ml·min⁻¹ and at 1.0 ml·min⁻¹, as shown in Table 4. Also, the correlation between the experimental results of the injected calibration solutions and the values calculated with the standard curves are presented in Table 4.

Table 4. Sugar calibration standards ran at flow rate 0.9 ml·min⁻¹ and 1.0 ml·min⁻¹.

Flow rate ml·min ⁻¹	Sugars	Retention time		Calibration range ng	Correlation
		<i>n</i>	min		
0.9	Fructose	5	7.86 ± 0.01	15.01-300.10	0.998
	Glucose	5	8.62 ± 0.02	15.00-300.07	0.998
	Sucrose	5	11.28 ± 0.02	15.00-300.01	0.995
1.0	Fructose	5	7.02 ± 0.01	15.01-300.16	0.999
	Glucose	5	7.87 ± 0.03	15.02-300.43	0.999
	Sucrose	6	11.39 ± 0.03	6.00-299.97	0.999

Note: values for retention time are the mean and the standard error, calculated as presented in Eq. 15.

The HPLC used to determine the non-structural carbohydrates present in the sapwood of the *Pinus radiata* samples was a Hewlett Packard 1100 Series, equipped with a Hewlett Packard 1047a refractive index detector. The column used for the separation was a Phenosphere 5μ NH₂ 80A, internal diameter 4.6 mm, and length 250 mm. A guard column was used to protect this column. The mobile phase was acetonitrile:water 75:25 (Binder 1980). In this study the sugars were detected and quantified using RI, as it is the detector usually used for the determination of simple sugars and oligosaccharides (Hicks 1988), but the elution was also monitored with UV at 191 nm. This was especially useful because, for the low levels of detection needed, the refractive index baseline was difficult to stabilize. Therefore, the detection with UV at 191 nm was used as a backup detection, particularly when the amount of sugars was low and the RI peaks were not clear, as occurred with severely leached samples.

The original flow rate was 1.0 ml·min⁻¹, but it was noticed that due to the viscosity of the sample, after a few runs there was a pressure build up in the column. To work below the pressure limit, namely 80 bar, the flow rate was reduced to 0.9

ml·min⁻¹. A standard calibration curve was calculated for both flow rates as shown in Table 4. The problem due to the viscosity of the samples was tackled in another two ways. First, viscous samples were diluted to 250 µl instead of 200 µl. Second, the injection volume of the viscous samples was reduced according to the viscosity of the samples. The injection volumes used were 10, 25, 50, 75 and 100 µl. There was no evidence that these modifications affected the results.

The retention times for the determinations of non-structural sugars are presented below in Table 5, and illustrated in an example in Figure 10. The RI units of the ordinate axis of Figure 10 represent “the refractive index difference between the mobile phase and the mobile phase plus the sample” (Hewlett-Packard 1994). Figure 10 shows that fructose had usually clear peaks, while glucose and sucrose presented broad-based peaks, sometimes difficult to recognize from the baseline. The first large peak is the solvent used.

Table 5. Retention times for the determination of non-structural sugars at flow rate 0.9 ml·min⁻¹ and 1.0 ml·min⁻¹.

Flow rate ml·min ⁻¹	Sugars	Retention time	
		<i>n</i>	min
0.9	Fructose	35	7.86 ± 0.08
	Glucose	31	9.88 ± 0.10
	Sucrose	35	12.01 ± 0.08
1.0	Fructose	5	7.49 ± 0.08
	Glucose	5	8.69 ± 0.11
	Sucrose	4	11.73 ± 0.18

Note: values for retention time are the mean and the standard error, calculated as presented in Eq. 15.

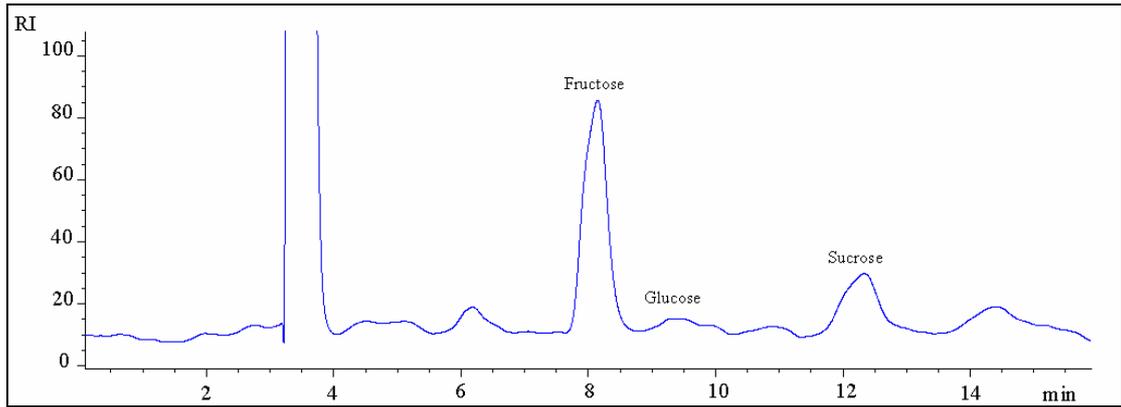


Figure 10. Separation of fructose, glucose and sucrose.

Note: RI detector; flow rate $0.9 \text{ ml}\cdot\text{min}^{-1}$.

3.6.2. Determination of total nitrogen.

The xylem of trees contains nitrogen compounds, mainly in the organic form. Due to the presence of proteins in the living cells of the sapwood, this region is richer in nitrogen compounds than the heartwood; nitrogen would be in the form of cytoplasmic protein (Scurfield and Nicholls 1970). Nitrogen is present in pine sapwood mainly as amino acids and ureides⁴ (Barnes 1963). Glutamic acid, aspartic acid and their amides, asparagine and glutamine, are the most abundant amino acids. The synthesis of glutamine from glutamic acid and ammonia incorporates inorganic nitrogen into the amino acids (Higuchi 1997), shown in the stoichiometric Eq. 7.



Several authors have studied the presence of nitrogen compounds in the sap of pines. A brief selection is presented in Table 6. The levels of nitrogen content are usually below $2.0 \text{ mg}\cdot(\text{g dry mass})^{-1}$ and can be as low as $0.03 \text{ mg}\cdot(\text{g dry mass})^{-1}$.

⁴ Ureides: urea, allantoinic acid ($\text{C}_4\text{H}_8\text{O}_4\text{N}_4$) and allantoin ($\text{C}_4\text{H}_6\text{O}_3\text{N}_4$).

⁵ ATP (adenosine triphosphate) provides the energy for the reaction, and is transformed into Pi (inorganic phosphorous).

There is a significant difference between the nitrogen content of young and mature *Pinus radiata* wood, but this study used only mature wood.

Table 6. Total nitrogen present in the stem wood of *Pinus radiata*, *P. ponderosa* and *P. sylvestris*.

Species	Total nitrogen mg·(g dry mass) ⁻¹	References
<i>Pinus radiata</i>	0.03 – 0.04	Kreber <i>et al.</i> 1998 ⁶
	0.5	Orman and Will, 1960 quoted by Uprichard 1991
<i>Pinus radiata</i> (seedlings)	2.4	Scurfield and Nicholls 1970 ⁷
<i>Pinus ponderosa</i>	1.15-1.86	Sheppard and Thompson 2000
<i>Pinus sylvestris</i>	0.6	Terziev 1995 ⁸

Most of the nitrogen present (up to 99%) in the sapwood of the *Rosaceae* family is in the organic form (Bollard 1958), and this situation can be regarded as being similar for other tree species (Barnes 1963). Therefore, the determinations done in this study can be considered predominantly as measurements of organic nitrogen.

The determination of total nitrogen was done at the Analytical Services of the Soil, Plant and Ecological Sciences Division at Lincoln University, New Zealand, and the equipment used was a Leco CNS-2000 Elemental Analyser. It was the same method used by Kreber *et al.*, (1998), whose results are presented in Table 6. Approximately 200 mg of powdered wood (particle size smaller than 0.25 mm) was burnt at 1250 - 1450 °C in an oxygen atmosphere. The gases were analysed with a thermal conductivity detector to determine N₂. This equipment also determined total carbon as a parallel measurement, but the results were not regarded relevant for this study. The carbon determinations are presented in Appendix B.

⁶ Outer green wood.

⁷ Original data in % of dry weight

⁸ Original data in % of dry weight.

3.6.3. Determination of low-molecular-weight and high-molecular-weight phenols.

The phenols present in wood comprise a wide group of compounds including tannins, phenolic acids, lignans, stilbenes and simple phenols, among others. The tannins are more oligomeric than polymeric, and they are classified into hydrolysable tannins and condensed tannins. The gymnosperms produce only condensed tannins, and these are concentrated in the bark and heartwood (Zavarin and Cool 1991).

The phenols usually identified in gymnosperm wood are low-molecular-weight phenols, such as lignans, stilbenes or flavonoids. Both lignans and stilbenes are common in the heartwood of conifers, but rare in the sapwood. Flavonoids, such as catechin, are abundant in the transitional zone between heartwood and sapwood. Catechin, the structural unit of condensed tannins, is suspected to produce colour change in *Tsuga heterophylla* wood when it polymerises. Phenolic compounds are distributed among many plant families, including *Pinaceae* (Fengel 1984; Scalbert *et al.* 1989; Higuchi 1997). Table 7 presents a summary of determinations of phenolic compounds in some species of softwoods. Significantly, Uprichard, (1991), reported that no phenolic compounds were present in the sapwood of *Pinus radiata*. Scalbert *et al.*, (1989), found that low-molecular-weight phenols were in higher concentration than high-molecular-weight phenols, with the exception of *Tsuga heterophylla*.

Table 7. Total phenolic compounds present in the xylem of some species of the order *Coniferae*.

Species	Total phenols mg·(g dry mass) ⁻¹		References
	High molecular weight	Low molecular weight	
<i>Pinus radiata</i>	0.0	0.0	Uprichard 1991
<i>Pinus sylvestris</i>	0.8	5.9	Scalbert <i>et al.</i> 1989
<i>Cedrus atlantica</i>	2.4	5.8	
<i>Pseudotsuga menziesii</i>	2.0	11.6	
<i>Picea sitchensis</i>	1.6	1.7	
<i>Tsuga heterophylla</i>	2.4	1.9	

The chemical extraction and the determination of total phenols was done following the Folin-Ciocalteu method proposed by Scalbert *et al.*, (1989). As this method was designed to determine the total phenols present in the sample, it did not differentiate between compounds. Two solvent media were used for extraction: aqueous methanol, which included the risk of extracting not only phenolic compounds but also sugars and cyclitols (Uprichard 1991); and di-ethyl ether, intended to separate low-molecular-weight phenols, such as catechin, from the high-molecular-weight phenols, such as condensed tannins, extracted previously with aqueous methanol (Scalbert *et al.* 1989). Therefore, high-molecular-weight phenols are determined by extraction with aqueous methanol, and low-molecular-weight phenols are determined by two consecutive extractions, first with aqueous methanol and second with di-ethyl ether.

The spectrophotometer required for the determination was a Shimadzu Multispec 1500 with 10 mm quartz cell. According to Scalbert *et al.*, (1989), the final spectroscopic absorbance of the samples ought to be below 0.5. Therefore, the water extractions were diluted from 5 to 10 times, and the di-ethyl ether extractions were diluted from 10 to 50 times. Sometimes the samples were turbid and centrifugation was necessary; hence it was done at 4000 rpm for 2 minutes. Senthilmohan (priv. comm. 2000) provided the calibration standard curve for equivalents of gallic acid, as presented in the equations below:

$$\text{Eq. 8} \quad ABS760 = -0.00006[GA]^2 + 0.0146[GA] - 0.0202$$

$$[GA] = \frac{-0.0146 + \sqrt{0.0146^2 - 4 \cdot [-0.00006 \cdot (-ABS760 - 0.0202)]}}{2 \cdot (-0.00006)}$$

where $ABS760$ is the absorbance at 760 nm; and $[GA]$ is the concentration of gallic acid in $\mu\text{g}\cdot\text{ml}^{-1}$. The coefficient of determination between the experimental data and the values predicted with the calibration model was 0.99. Results are obtained in gallic acid equivalents per amount of wood extracted, and were converted to $\text{mg}\cdot(\text{g dry mass})^{-1}$ (Scalbert *et al.* 1989).

3.7. Data analysis.

The aim of this study was to investigate the relationship of discolouration during kiln drying with certain chemical compounds present in wood. The data analysis for this study was divided into four stages: selection of dependent and independent variables for model construction; multivariate linear regression; Neural Network modelling; and modelling based on simple reaction kinetics. The software used to performed the data statistical analysis was SAS System® V8, and the Neural Network Toolbox of MATLAB® 5.3 was used for Neural Network modelling.

3.7.1. Model construction: selection of dependent and independent variables.

One of the objectives of this study was to analyse the causes of discolouration of wood during drying through statistical analysis. Therefore, it was necessary to select the dependent and independent variables from the group of variables determined during experimentation. The dependent variables were identified among the colour variables, as presented in section 3.5. The independent variables were selected from the chemical determinations described in section 3.6. The selection criterion identified the variables, both dependent (colour determination) and independent (chemical determinations), that were affected by the experimental factors or their interaction, as this study intended to investigate the relationship between colour development on the surface during drying with the reduction of soluble compounds (leaching) and drying time. From that point of view, the variables that were not sensitive to leaching or drying time were considered irrelevant to model the surface discolouration of *Pinus radiata* wood during drying.

The effects model used to analyse the variables of this study, dependent or independent, with k number of replications had the form of Eq. 9:

$$\text{Eq. 9} \quad y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \epsilon_{ijk}$$

where y_{ijk} is the estimation; μ is the overall mean effect, τ_i is the effect of the i th level of leaching, β_j is the effect of the j th level of drying time; $(\tau\beta)_{ij}$ is the effect of the

interaction between τ_i and β_j ; and ε_{ijk} is a random error component (Montgomery 2001). Leaching had three levels: control, mild and severe; and drying time had four levels: 0, 8, 16 and 24 hours. Using this model, the following hypotheses were tested for all the variables determined in this study: there were no differences between the levels of the treatments, leaching and drying time, (H_0), or at least one level was different from zero (H_1), as shown in Eq. 10 and Eq. 11; and there was no interaction effect between the combination of levels of leaching and drying time (H_0), or at least one combination of levels of the treatments was different from zero (H_1), as presented in Eq. 12.

$$\text{Eq. 10} \quad \begin{cases} H_0: \tau_{\text{Control}} = \tau_{\text{mild}} = \tau_{\text{severe}} = 0 \\ H_1: \text{at least one } \tau_i \neq 0 \end{cases}$$

$$\text{Eq. 11} \quad \begin{cases} H_0: \beta_0 = \beta_8 = \beta_{16} = \beta_{24} = 0 \\ H_1: \text{at least one } \beta_j \neq 0 \end{cases}$$

$$\text{Eq. 12} \quad \begin{cases} H_0: (\tau\beta)_{ij} = 0 \text{ for all } i, j \\ H_1: \text{at least one } (\tau\beta)_{ij} \neq 0 \end{cases}$$

The method used to test these hypotheses was univariate analysis of variance for two factors (two-factor ANOVA). The null hypothesis was rejected with a P -value of F statistic < 0.05 . The fraction of variability in the data that can be described by the analysis of variance model is termed coefficient of determination (R^2) and is calculated using Eq. 13 (Hair *et al.* 1995; Montgomery 2001):

$$\text{Eq. 13} \quad R^2 = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - \bar{y})^2}$$

where y_i is the experimental value; \hat{y}_i is the value predicted with the model; \bar{y} is the mean of n number of observations, for $n=1,2,\dots,i$.

When one of the null hypotheses H_0 of the analysis of variance presented in Eq. 10, Eq. 11, and Eq. 12 was rejected, giving evidence of the effect of the experimental factors on the overall mean μ , a multiple comparison test was applied to distinguish between treatment means. The test used was the Tukey test, also termed the Honestly Significant Difference Test (HSD test) (Zar 1996). The hypotheses of this test are that there was no difference between the means of group A and group B ($H_0: \mu_A = \mu_B$) and that the means of groups A and B are different ($H_A: \mu_B \neq \mu_A$), where A and B are any possible pair of groups. There are a number of possible different pairwise comparisons depending on the number of groups; the number of different pairwise comparisons is given by the equation presented below:

$$\text{Eq. 14} \quad c = \frac{k \cdot (k - 1)}{2}$$

where c is the number of comparisons; and k is the number of groups. Calculating the following statistics tests for the hypotheses H_0 and H_A given above:

$$\text{Eq. 15} \quad SE = \sqrt{\frac{s^2}{n}}$$

where SE is the standard error; s^2 is the error mean square from the analysis of variance; and n is the number of data in each of groups A and B. The test statistic is therefore:

$$\text{Eq. 16} \quad q = \frac{\bar{X}_B - \bar{X}_A}{SE}$$

where q is a “Studentized range” which is compared with a critical value $q_{\alpha, v, k}$, determined by the significance level α , the error degrees of freedom of the analysis of variance v , and the total number of means being tested k ; \bar{X}_A and \bar{X}_B are the means of groups A and B, respectively; and SE is the standard error defined in Eq. 15. The null hypothesis $H_0: \mu_A = \mu_B$ is rejected if $q \geq q_{\alpha, v, k}$. A common way of presenting the results of the Tukey’s HSD test is to arrange the means in order of increasing magnitude, and then to underline the means that are not significantly different. For details on multiple comparisons refer to Zar (1996).

The exception to the use of the univariate analysis of variance (ANOVA) to identify the variables sensitive to the experimental factors was the analysis of the colour systems CIE Yxy and CIE Lab. Both CIE Yxy and CIE Lab are three-dimensional variables, and therefore the effect of the experimental factors, leaching and drying time, was assessed using the two-factor multivariate analysis of variance (MANOVA). MANOVA simultaneously investigates the variance of the three dimensions (L , a , and b ; and Y , x and y , respectively) as a group response for the experimental factors. The data for these two colour systems were divided in 12 groups (leaching: 3 levels; drying time: 4 levels) with 3 observations per variable per group, and each of these groups defined a vector, as shown in Table 8. The multivariate linear model related a multivariate vector \mathbf{x} with the experimental factors, giving 12 observations. The model is presented in Eq. 17:

$$\text{Eq. 17} \quad \mathbf{x}_{kl} = \boldsymbol{\mu} + \boldsymbol{\alpha}_k + \boldsymbol{\beta}_l + (\boldsymbol{\alpha\beta})_{kl} + \boldsymbol{\epsilon}_{kl}$$

where $\boldsymbol{\mu}$ is the grand mean vector; $\boldsymbol{\alpha}_k$ is the effect vector due to the leaching treatment with $k=3$ levels; $\boldsymbol{\beta}_l$ is the effect vector due to the drying time treatment with $l=4$ levels; $(\boldsymbol{\alpha\beta})_{kl}$ is the interaction effects vector; and $\boldsymbol{\epsilon}$ is the error vector (Jobson 1992).

Table 8. Grouping of the data of colour systems, CIE Yxy and CIE Lab, for two-factor multivariate analysis of variance.

MANOVA groups		Drying time (hours)							
		0	8	16	24	0	8	16	24
Leaching	Control	μ_{L*1}	μ_{L*2}	μ_{L*3}	μ_{L*4}	μ_{Y*1}	μ_{Y*2}	μ_{Y*3}	μ_{Y*4}
		μ_{a*1}	μ_{a*2}	μ_{a*3}	μ_{a*4}	μ_{x*1}	μ_{x*2}	μ_{x*3}	μ_{x*4}
		μ_{b*1}	μ_{b*2}	μ_{b*3}	μ_{b*4}	μ_{y*1}	μ_{y*2}	μ_{y*3}	μ_{y*4}
	Mild	μ_{L*5}	μ_{L*6}	μ_{L*7}	μ_{L*8}	μ_{Y*5}	μ_{Y*6}	μ_{Y*7}	μ_{Y*8}
		μ_{a*5}	μ_{a*6}	μ_{a*7}	μ_{a*8}	μ_{x*5}	μ_{x*6}	μ_{x*7}	μ_{x*8}
		μ_{b*5}	μ_{b*6}	μ_{b*7}	μ_{b*8}	μ_{y*5}	μ_{y*6}	μ_{y*7}	μ_{y*8}
	Severe	μ_{L*9}	μ_{L*10}	μ_{L*11}	μ_{L*12}	μ_{Y*9}	μ_{Y*10}	μ_{Y*11}	μ_{Y*12}
		μ_{a*9}	μ_{a*10}	μ_{a*11}	μ_{a*12}	μ_{x*9}	μ_{x*10}	μ_{x*11}	μ_{x*12}
		μ_{b*9}	μ_{b*10}	μ_{b*11}	μ_{b*12}	μ_{y*9}	μ_{y*10}	μ_{y*11}	μ_{y*12}

Note: μ =group mean.

The null hypothesis (H_0) of the analysis was that all the group mean vectors are equal, tested with Pillai's criterion of significance (Hair *et al.* 1995). This criterion was used because it is more robust than Wilks's lambda, especially when sample size is small, as was the case in this study. The software used converted the Pillai's criterion into a F statistic; hence the null hypothesis was rejected when P -value <0.05 (refer to Hair *et al.* (1995) for details on multivariate analysis of variance).

Drying time was included as a independent variable, because it was a relevant factor in at least two aspects: the compounds present in the wood require time to migrate and accumulate at the surface of the boards (Kreber *et al.* 1998); and the reactions supposedly involved in the process, namely Maillard-type reactions and oxidation and polymerisation of phenolic compounds are time-dependent (Hrutfiord *et al.* 1985; Cioroi 1999). Therefore, drying time was both an experimental factor and an independent variable for modelling the discolouration of the surface of board during drying.

The variables affected by the experimental factors were correlated in pairs to investigate interactions between them. High correlations, such as 0.90 or above, are an indication of collinearity (Hair *et al.* 1995). Two statistical methods were used to model colour development on the surface during drying due to chemical components present in the wood, namely multivariate linear regression and Neural Networks modelling.

3.7.2. Multivariate linear regression.

After the selection of the dependent and independent variables, modelling was first attempted with multivariate linear regression. The models had the form of Eq. 18 (Montgomery 2001):

$$\text{Eq. 18} \quad y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \dots + \beta_kx_k + \epsilon$$

where y represents the colour response, with variables selected as described in section 3.7.1; $\beta_j, j=0,1,\dots,k$ are termed regression coefficients; $x_j, j=0,1,\dots,k$ are the regressors,

drying time and the chemical components of wood selected as described in section 3.7.1; and ϵ is the error. The models considered in this study were restricted to first-order polynomials for simplicity.

The significance of the regression was tested with a null hypothesis that all coefficients were equal to zero ($H_0: \beta_1=\beta_2=\dots\beta_k=0$), and with an alternative hypothesis that at least one coefficient was different from zero ($H_0: \beta_j\neq 0$). This hypothesis was rejected with a P -value for the F statistic <0.05 . The reduction in the variability of the colour variables obtained by modelling with the selected regressors was measured with the multiple coefficient of determination (R^2), where higher values were preferred. The multiple coefficient of determination is calculated using Eq. 13. The mean squared error (MSE), calculated using Eq. 19, estimated the error of the model; lower values of MSE indicated less error (Smith 1996).

Eq. 19
$$MSE = \frac{\frac{1}{2} \sum_{n=1}^N (y_n - t_n)^2}{N}$$

In Eq. 19, y_n is the value predicted by the model; t_n is the corresponding experimental value; and N is the number of observations, for $N=1,2,\dots n$.

The sample size n has a decisive effect on the ability of the model to describe the data. The probability of failing to reject the null hypothesis when it is false, termed type II error and symbolised β , decreases with larger number of observations. The power of a statistical test is defined as $1-\beta$. Therefore, it is useful to determine the number of observations needed to achieve a desired power for each regression model, and compare it with the actual number of observations (36). The equations used for the power analysis are presented below. Eq. 20 gives the population effect size f^2 .

Eq. 20
$$f^2 = \frac{R^2}{1 - R^2}$$

where R^2 is the coefficient of determination as presented in Eq. 13.

The necessary number of cases n^* becomes

Eq. 21
$$n^* = \frac{L}{f^2} + k + 1$$

where L is a table number obtained from a defined significance α (Table E.2 Cohen and Cohen 1975, p. 477), the number of independent variables k , and the desired power. This study used $\alpha=0.05$ and a power of 0.80, as it is conventional (refer to Cohen and Cohen (1975) for details on power analysis for multivariate linear regression.).

The significance of the individual regressors was tested with the null hypothesis that the coefficient for that particular regressor (eg. β_j) was equal to zero ($H_0: \beta_j=0$). The null hypothesis was rejected when the P -value of the t statistic < 0.05 . The regressors were selected by the stepwise estimation, and had to meet the 0.05 significance level to enter the model. The stepwise estimation is a method that selects regressors according to the incremental explanatory power that they incorporate to the regression model (Hair *et al.* 1995). Refer to Hair *et al.* (1995) and Montgomery (2001) for details on multivariate linear regression.

3.7.3. Neural Networks modelling.

Non-linear models were built using the Neural Networks Toolbox of MATLAB® 5.3. Neural Networks are a mathematical method that aims to resemble the cognitive process of the brain. An input layer, a hidden layer and an output layer form the basic neural network, and each layer is composed of a number of *nodes*. The nodes of the input layer represent the value of one independent variable; the nodes of the hidden layer contain a mathematical function that calculates a node output based on weighted inputs; and each of the nodes of the output layer represent a dependent variable. The network is connected with links between the nodes, termed weights. Backpropagation is a neural network technique that allows the hidden nodes to adjust the values of their weights if they perceive error information from the output layer. Using backpropagation, the model can be trained to change the weights to reduce the error (Smith 1996).

In this study the Neural Network model was designed as illustrated in Figure 11. The input layer was formed with k vectors of independent variables, and the output layer was the vector of the colour variable, with input (independent) and output (dependent) variables selected as described in section 3.7.1. The two hidden layers had three neurons with one bias connected to each neuron. The first layer had two neurons with a sigmoid activation function (*tansig*), and the second layer had one neuron with a linear activation function (*purelin*). The subscript in the activation functions indicates the layer. Figure 24 in Appendix A presents a diagram of the activation functions.

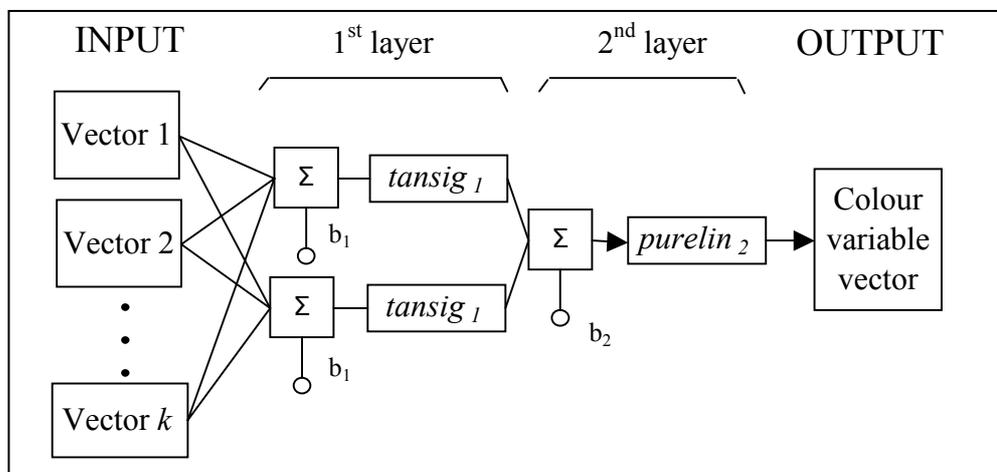


Figure 11. Basic structure of the Neural Network model used in this study.

The Neural Network model was trained with 80% of the data, according to common practice, using the Levenberg-Marquardt and Bayesian regularization (*trainbr*). The Levenberg-Marquardt is a numerical optimisation technique, used in similar problems (Hernández *et al.* 2001). The Bayesian regularization was used to avoid over-fitting of the data, which occurs when the error in the training set is small, but when all the data is used in the model, the error is large (Demuth and Beale 1998). The code written to build the models is presented in Appendix C.

Assuming a linear relationship between the experimental values of colour and the values calculated with the Neural Network model, their interdependence was measured using the simple correlation coefficient, as presented in Eq. 22 (Smith 1996).

$$\text{Eq. 22} \quad r_{12} = \frac{\sum_{j=1}^n (x_{1j} - \bar{x}_1)(x_{2j} - \bar{x}_2)}{\sqrt{\left[\sum_{j=1}^n (x_{1j} - \bar{x}_1)^2 \right] \left[\sum_{j=1}^n (x_{2j} - \bar{x}_2)^2 \right]}}$$

where r is the simple correlation coefficient between variables 1 and 2; n is the number of observations for $n=1,2,\dots,j$; \bar{x} is the mean of n values of x .

The error of the model predictions was estimated by the mean square error (MSE), calculated as shown in Eq. 19.

In addition, the residuals were plotted versus the values predicted by the models to observe the dispersion about zero; models with no clear pattern and randomly distributed were preferred (Hair *et al.* 1995). The residuals were standardized to consider the inequality of the variance and therefore allow the comparison between models, using Eq. 23.

$$\text{Eq. 23} \quad d_i = \frac{e_i}{\sqrt{MSE}}$$

where d_i is the standardized residual for $i=1,2,\dots,n$; e_i is the residual of the i element, namely the difference between the experimental value and the value predicted by the model; and MSE is the mean square error as calculated in Eq. 19. The values of the standardized residuals should lie within ± 3.0 units (Montgomery 2001).

The prediction abilities of the statistical models, multivariate linear regression models and Neural Network models, were compared using the coefficient of correlation and the mean square error, as presented in Eqs. 22 and 19, respectively. The standardized residuals for both linear and Neural Network models, calculated using Eq. 23, were plotted versus predicted results. Refer to Fleming (1993) for a detailed description of a comparison between multivariate linear regression models and Neural Network models performed in this study

3.7.4. Empirical models based on simple reaction kinetics.

Colorimetry has been used to investigate the kinetics of Maillard-type reactions (Ibarz *et al.* 1999; Carabasa Giribet 2000), while kiln brown stain has been studied by reproducing in test tubes the browning that occurs in wood during drying (McDonald 1997). This study proposes the use of empirical models based on simple reaction kinetics to describe the change of a physical property of the discolouration reaction, colour, as a function of the concentration of precursor chemical present in wood. This approach is not an attempt to present a kinetic model that describe the complex reactions that are suspected to contribute to colour development, namely Maillard-type reactions and oxidation and polymerisation of phenolic compounds, as presented in Chapter 1. In contrast, the construction of these empirical models had two objectives:

- To model the experimental data according to simple reaction kinetics, in a first approach in the investigation of the kinetics of discolouration during kiln drying.
- To elucidate the order, m , of these empirical models, which would allow future comparison of the discolouration reaction with reactions that can be reproduced in test tubes, as suggested by McDonald *et al.*, (2000).

Chemical kinetics is the field of chemistry that studies the rate at which the properties of a chemical system change. The rate of the reaction is usually calculated from the change of concentration of reactant or product with time, but it can also be estimated from a change in a physical property of the reaction with time. In this study the rate under investigation was the change in colour with drying time. One of the objectives of this study was to relate the rate of colour change during drying with the concentration of certain chemical components of wood. Therefore, the proposed stoichiometric equation had the form presented in equation Eq. 24:



where C is the measurement of colour on the surface of the boards; A and B are the concentration of two chemical compounds determined in the analysis of the wood

closest the surface (<5 mm); and k is the rate constant. This study was concerned with two main reactions: Maillard-type reactions, involving non-structural sugars and nitrogen compounds; and oxidation and polymerisation of phenolic compounds. As mentioned, both are complex reactions, which would not be completely described by Eq. 24. However this stoichiometric model is the simplest possible relationship and relates the concentration of two detectable chemical compounds with the rate of change of a physical property, colour, as presented by Ibarz *et al.* (1999).

The rate of colour change on the surface of boards as a function of the concentration of certain chemical compounds in the wood can be calculated as presented in Eq. 25:

Eq. 25
$$\frac{dC}{dt} = k \cdot [A_1]^m \cdot [B]^n$$

where C is the measurement of colour on the surface of the boards; t is drying time in hours; $[A]$ and $[B]$ are the concentration of two chemical compounds determined in the analysis of the wood closest the surface (<5 mm); k is rate constant; m and n are the exponents of the equation, which determine the order of the equation $p = m + n$. The chemical compounds to be included in the empirical model were selected based on the results of statistical models, namely multivariate linear regression and Neural Network models.

4.1. Introduction.

This chapter presents the experimental data and the calculations done to investigate the relationships between the colour measured at the surface of the boards and the determinations of chemical compounds closest to the surface of the boards. The data was fitted using linear and non-linear models, with colour as the dependent variable and the chemical determinations as regressors as described in section 3.7. A simple reaction kinetics model of the wood discolouration reaction was then constructed, based on the results of the previous analyses.

4.2. Kiln drying.

Fresh samples had a dry-basis moisture content of 147.0 ± 24.9 %; after 8 hours the mean moisture content was 6.1 ± 2.2 %; after 16 hours the moisture content was 2.5 ± 0.8 %; and finally after 24 hours the moisture content was 1.5 ± 0.5 %. A common target for industrial kiln drying is an average moisture content of $10\% \pm 2\%$ (Walker 1993), although target may differ according to product requirements, but the drying schedule and the drying times used in this study, described in section 3.4, dried the samples well below that limit. This was done to allow a complete migration of compounds from the centre to the surface of the boards, as is described in section 2.2. It was observed that the difference in mean moisture content between 16 and 24 hours of drying was only 1%.

4.3. Colour determination.

The experimental factors, reduction of soluble compounds and drying time produced a variation in colour of the surface of the samples that was detectable by visual assessment; kiln brown stain decreased when the quantity of soluble compounds was reduced, and increased with drying time. Only samples with severe leaching did not produce kiln brown stain. Nevertheless, after drying, the surface of the severe leached samples acquired a greenish colour. Figure 12 is a diagram that

shows how colour changed due to experimental factors. Longer drying time produced darker colour; leaching developed less stains but a greenish colour appeared.

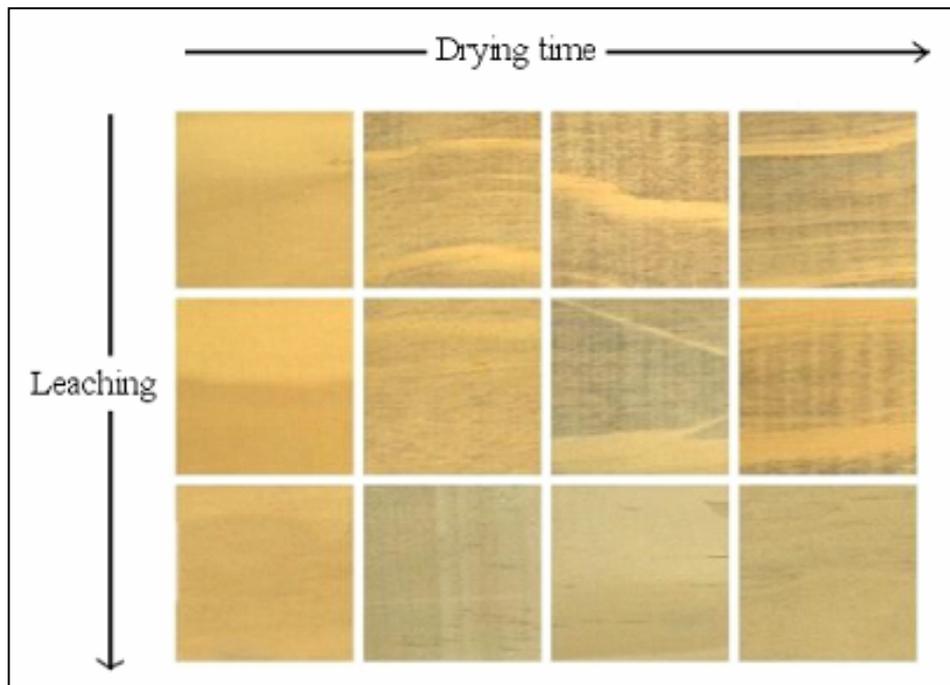


Figure 12. Colour change due to experimental factors.

The experimental data for colour measurement on the surface of *Pinus radiata* boards are presented in Appendix B in Table 16, along with a calculation of the maximum absolute uncertainty for each colour variable, shown in Table 17. Univariate and multivariate analyses of variance, presented in section 3.7.1, showed the effect of leaching and drying time on the colour variables. A summary of results is presented in Table 9. Drying time had a statistically significant effect on eight out of eleven colour variables, and therefore was the most important factor in producing colour change in the samples. A longer drying time produced a darker colour on the surface. Only two colour variables, brightness ($R457$) and the blue-to-yellow scale of CIE Lab (b^*) were sensitive to leaching ($p=0.0190$ and $p=0.0314$, respectively). Only the luminance factor (Y) detected the combined effect of drying time and leaching ($p=0.0096$). The statistical models that tested the variables that were affected by both experimental factors, either directly ($R457$ and b^*) or indirectly (Y), presented the highest coefficient of determination. The results are shaded in Table 9. Among them,

the model that included the luminance factor showed the highest coefficient of determination ($R^2=0.75$), as shown in Table 9.

Table 9. Effect of experimental factors on colour variables.

Colour variable	Leaching	Drying time	Interaction	R^2
	<i>P</i> -values			
<i>Y</i>	<i>ns</i>	<0.0001	0.0096	0.75
<i>x</i>	<i>ns</i>	0.0195	<i>ns</i>	0.50
<i>y</i>	<i>ns</i>	0.0003	<i>ns</i>	0.65
<i>Yxy</i>	<i>ns</i>	<0.0001	<i>ns</i>	
<i>R457</i>	0.0190	0.0020	<i>ns</i>	0.62
<i>L*</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.37
<i>a*</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.33
<i>b*</i>	0.0314	0.0023	<i>ns</i>	0.59
<i>L * a* b*</i>	<i>ns</i>	0.0004	<i>ns</i>	
ΔE	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.40
<i>DPI</i>	<i>ns</i>	0.0065	<i>ns</i>	0.51

Note: P -values>0.05 were considered not significant (*ns*). CIE *Yxy* and CIE Lab MANOVA Test Criteria for the hypothesis of no overall effect was done using Pillai's Trace, as described in section 3.7.1.

On the basis of Eq. 9 the results of Table 9 could be illustrated in the following equations:

$$\text{Eq. 26} \quad \begin{aligned} R457_{ijk} &= \mu + \tau_i + \beta_j + \epsilon_{ijk} \\ b^*_{ijk} &= \mu + \tau_i + \beta_j + \epsilon_{ijk} \\ Y_{ijk} &= \mu + \beta_j + (\tau\beta)_{ij} + \epsilon_{ijk} \end{aligned}$$

where $(R457, b^*, Y)_{ijk}$ are the model estimation of *R457*, *b** and *Y*, respectively; μ is the overall mean effect, τ_i is the effect of the *i*th level of leaching, β_j is the effect of the *j*th level of drying time; $(\tau\beta)_{ij}$ is the effect of the interaction between τ_i and β_j ; and ϵ_{ijk} is a random error component.

The brightness and the blue-to-yellow scale of CIE Lab presented different treatment means, and the luminance factor presented different treatment interaction means, as tested with the ANOVA procedure presented in Table 9. The Tukey's Honestly Significant Difference (HSD) method, as described in section 3.7.1, was used to investigate pair wise mean comparisons within each treatment with $\alpha=0.05$. The results are presented in Table 10. Means underlined with the same line were not significantly different. Severe leaching produced samples that after drying were significantly brighter (higher $R457$) than the mildly leached ones ($p=0.0157$) and the non-leached ones ($p=0.0459$). In contrast, luminance factor and the blue-to-yellow scale of CIE Lab presented no significant difference between the leached samples and those not leached. Drying time produced a significant effect on the colour variables studied, and the difference between 0 and 24 hours was statistically significant for all three of them ($Y p=<0.0001$; $R457 p=0.0039$; $b^* p= 0.0104$). Longer drying times yielded darker colours, measured by luminance factor or brightness, or bluer colour when measured with the blue-to-yellow scale of CIE Lab.

Table 10. Tukey HSD ($\alpha=0.05$) grouping of the means of luminance factor Y , brightness $R457$ and the blue-to-yellow scale of CIE Lab b^* .

	Leaching			Drying time (hours)			
	Control	Mild	Severe	0	8	16	24
Y	42.8	43.3	46.6	52.5	43.1	42.0	39.3
$R457$	31.5	30.6	36.4	36.8	36.1	30.2	28.1
b^*	44.4	44.6	40.2	48.2	43.1	40.1	40.9

Note: Means underlined with the same line are not significantly different.

4.4. Chemical determinations.

The chemical analyses included the determination of fructose, glucose and sucrose; total nitrogen; and total phenols, discriminated by molecular weight, low and high. All the analyses were performed on a dry mass basis, with units $\text{mg}\cdot(\text{g dry mass})^{-1}$. The determinations were replicated three times. The experimental data of the chemical determinations of the wood closest to the surface (<5 mm) of *Pinus radiata* boards is presented in Appendix B in Table 18, along with a calculation of the maximum absolute uncertainty for each chemical variable, shown in Table 19.

The determination of non-structural sugars suggested that the most abundant sugar was fructose, followed by sucrose and glucose. Even though there was considerable variation between samples, the ratio of fructose, sucrose and glucose was approximately 10:2:1, as shown in Table 18. The total sugar, a sum of fructose, glucose and sucrose, was calculated as an estimation of the amount of soluble carbohydrates present in the samples. The average total sugar content is illustrated in Figure 13. On average, severe leaching produced a diminution of 88% of the total sugars. The non-leached samples presented an increasing trend of sugar accumulation, whereas this trend disappeared when there was a reduction of soluble compounds. There was a peak of sugar concentration at 16 hours, followed by a decrease in sugar content at 24 hours.

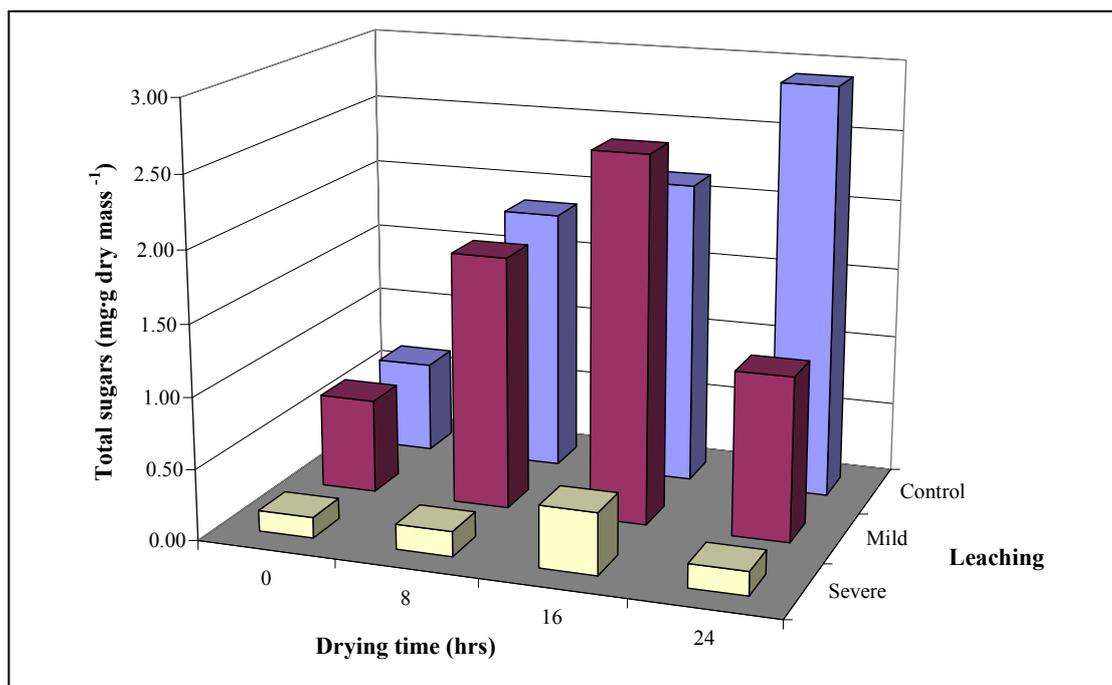


Figure 13. Average of the replications of total sugar, the sum of the average contents of fructose, glucose and sucrose.

As mentioned in section 3.6.2, the determination of total nitrogen done in this study can be considered an estimation of organic nitrogen. On average, the amount of nitrogen present in the sapwood was approximately one half of the total sugar, as shown in Table 18. Between the non-leached and the severely leached samples there was an average nitrogen diminution of 23%. During drying, there was evidence of accumulation of nitrogen compounds in the control samples. That trend was not present in the samples where the soluble compounds were reduced. The results of total nitrogen analysis are presented in Figure 14.

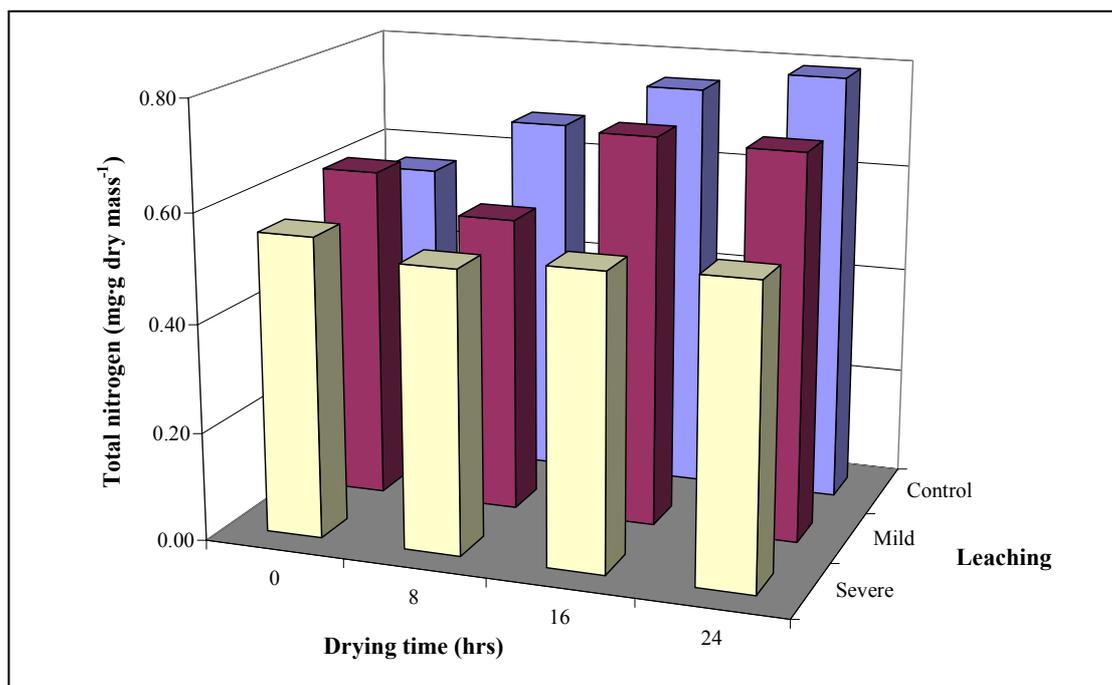


Figure 14. Average content of the replications of total nitrogen.

The phenols data were divided into high (HMWP) and low (LMWP) molecular weight compounds. HMWP, such as condensed tannins, were twice as abundant as LMWP, such as catechin or ellagic acid. A summary of the analysis of phenolic compounds is presented in the Appendix B, Table 18. On average, severe leaching eliminated 28% of the heavier phenolic compounds, where for the lighter phenolic compounds the diminution was not statistically significant. Longer drying times increased the concentration of the HMWP on the surface. Nevertheless, after 24 hours of drying the severely leached samples presented a diminution in HMWP. The LMWP accumulated in the surface only when the samples were not leached. Figure 15 illustrates the results of the analysis of the HMWP.

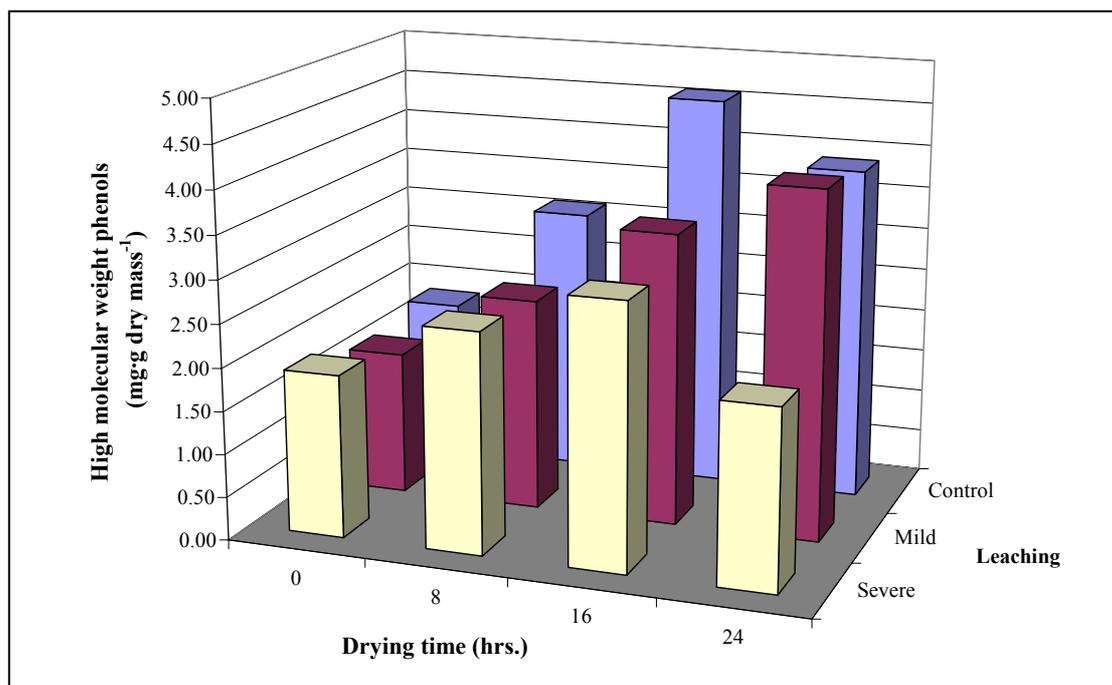


Figure 15. Average content of the replications of high-molecular-weight phenols.

Table 11 summarizes the results of two-factor ANOVA, which tested the effect of the experimental factors on the chemical determinations. Fructose, glucose, sucrose and total sugars were significantly affected by leaching ($p=0.0005$, $p=0.0473$, $p=0.0036$, and $p=0.0003$, respectively). Fructose, glucose and sucrose were not affected by drying time, whereas total sugar was, although just below the not significant probability ($p=0.0485$). There was a significant difference between non-leaching and severe leaching. The data for the sugars did not show indication of interaction between the two experimental factors. Both leaching and drying time had an effect on the nitrogen content of the samples ($p=0.0021$ and $p=0.0421$, respectively). There was no evidence of interaction between leaching and drying time. When the samples were not leached, nitrogen content increased with drying time, as illustrated in Figure 14. The determination of the heavier phenolic compounds was sensitive to leaching ($p=0.0207$) and drying time ($p=0.0002$). In contrast, the lighter phenolic compounds were not affected by the experimental factors or their interaction. Furthermore, the sum of both determinations of phenolic compounds, total phenols, was sensitive to drying time ($p=0.0006$), but neither to leaching nor the interaction between leaching and drying time.

Drying time and leaching had a simultaneous significant effect in three chemical determinations, namely total sugars, total nitrogen and high-molecular-weight phenols. Moreover, the coefficients of determination of these three variables were among the highest, meaning that approximately 60% of their variance about their means was explained by the experimental factors. The results of the statistical analysis of total sugars, total nitrogen and HMWP are shaded in Table 11.

Table 11. Effect of the experimental factors on the chemical determinations

Variables	Leaching	Drying time	Interaction	R^2
	<i>P</i> -values			
Fructose	0.0005	<i>ns</i>	<i>ns</i>	0.61
Glucose	0.0473	<i>ns</i>	<i>ns</i>	0.54
Sucrose	0.0036	<i>ns</i>	<i>ns</i>	0.53
Total sugar	0.0003	0.0485	<i>ns</i>	0.63
Nitrogen	0.0021	0.0421	<i>ns</i>	0.58
HMWP	0.0207	0.0002	<i>ns</i>	0.68
LMWP	<i>ns</i>	<i>ns</i>	<i>ns</i>	0.43
Total phenols	<i>ns</i>	0.0006	<i>ns</i>	0.61

Note: *P*-values>0.05 were considered not significant (*ns*).

4.5. Causal effect analysis: wood extractives and colour development during drying.

Linear regression and neural network models were built to explain colour change in the surface of the boards in relation to the chemical components of the wood. The variables included in the models were those affected by the experimental factors, as determined by the two-factor ANOVA and the two factor MANOVA (section 3.7.1), and presented in Table 9 and Table 11. The response variables tested were luminance factor, brightness and the blue-to-yellow scale of CIE Lab. Before presenting the results of the causal effect analysis the definitions of the colour variables will be restated. Luminance is the light being radiated per surface, and the luminance factor is the ratio between its luminance and that of a perfect white diffuser (Wright 1964). Brightness represents the visual sensation that evaluates the amount of light emitted per surface (Wyszecki and Stiles 1982). Both variables are

determined using the photoelectric photometer: luminance factor with the filter *Ry* and brightness with the filter *R457*. Even though it can be argued that brightness is another estimation of luminance factor, as discussed in section 3.5.1, they have to be distinguished for two reasons: they are obtained from different procedures and they have different magnitudes. The blue-to-yellow component of CIE Lab is self-explained. The variables selected to explain the response were total sugars, total nitrogen and high-molecular-weight phenols.

Table 12 shows the results of a correlation analysis of the variables to be used in the construction of models. Luminance factor and brightness, which were correlated (0.76), were the dependent variables with highest correlation with the regressors; values were higher than -0.50, except for the correlation between HMWP and brightness (-0.39). The correlation between luminance factor and brightness with the chemical variables was always negative. Overall, luminance factor presented a higher correlation with the regressors, except nitrogen, than brightness. On the contrary, the blue-to-yellow scale of CIE Lab presented low correlations with most of the regressors, except drying time. Among the regressors, nitrogen and sugars had higher correlation, an evidence of possible collinearity. A possible explanation for this could be that xylem non-structural sugars and nitrogen compounds are present in the same biological niches, the parenchyma ray tissue (Fischer and Höll 1992). When this tissue was affected by the experimental factors, the concentration of non-structural sugars and nitrogen compounds was modified in a similar way. This point will be further discussed in section 5.4.

Table 12. Correlation between variables.

	<i>Y</i>	<i>R457</i>	<i>b*</i>	Drying time	Nitrogen	Sugar	HMWP
<i>Y</i>	1.00						
<i>R457</i>	0.74	1.00					
<i>B*</i>	0.25	-0.02	1.00				
Drying time	-0.57	-0.54	-0.51	1.00			
Nitrogen	-0.57	-0.60	0.13	0.38	1.00		
Sugar	-0.52	-0.49	0.05	0.30	0.76	1.00	
HMWP	-0.53	-0.39	-0.29	0.56	0.53	0.43	1.00

4.5.1. Linear regression.

The first approach to investigate the relationship between colour on the surface and the concentration of certain chemical compounds in the wood was linear regression. A linear model was built for each one of the response variables, namely luminance factor, brightness and the blue-to-yellow scale of CIE Lab. Drying time, nitrogen, total sugars and high-molecular-weight phenols were included as regressors with a stepwise selection method, as described in section 3.7. The results suggested that the variables that were affected by the experimental factors produced the best models.

Table 13 presents a summary of the linear regression analysis of the models. The three linear models, one for each response variable, showed that the variables that best explained the change in colour were drying time and nitrogen content. The probability (*P*-value) that the nitrogen content, the drying time or the intercept would have a coefficient of zero was below 0.05. The coefficients of the other regressors, high-molecular-weight phenols and total sugar, had a probability greater than 0.05 of being zero, and therefore were not included in the model. The linear regression with higher coefficient of determination had luminance factor as the dependent variable ($R^2=0.52$). Furthermore, this model's coefficients presented the higher probability of being different than zero (highest $p=0.0061$). The power analysis of the models, described in section 3.7.2, resulted in a necessary number of cases n^* smaller than 36. Therefore, the number of observations was sufficient to produce a statistically significant value for the coefficient of determinations of the three linear regression models. The linear regression model that had the luminance factor (*Y*) as dependent variable needed less number of observations to be statistically significant, due to its higher R^2 .

Table 13. Multiple linear regressions for different colour variables with stepwise selection.

Dependent variable	Regressors	Coefficients	<i>P</i> -values	<i>R</i> ²	<i>n</i> *
<i>Y</i>	Intercept	63.31	<.0001	0.52	13
	Nitrogen	-22.95	0.0061		
	Drying time	-0.39	0.0008		
<i>R457</i>	Intercept	51.75	<.0001	0.48	15
	Nitrogen	-25.03	0.0018		
	Drying time	-0.27	0.0112		
<i>b</i> *	Intercept	37.11	<.0001	0.38	22
	Nitrogen	17.16	0.0154		
	Drying time	-0.40	0.0001		

Note: *P*-values>0.05 were considered not significant (*ns*).

The linear regression models are presented in the following equations:

$$\text{Eqs.27} \quad Y = 63.31 - 22.94 \cdot [N] - 0.39 \cdot t$$

$$R457 = 51.75 - 25.03[N] - 0.27t$$

$$b^* = 37.11 - 17.15[N] - 0.40 \cdot t$$

where *Y* is luminance factor; *R457* is brightness; *b** is the blue-to-yellow scale of colour space CIE Lab; [*N*] is the nitrogen content present in the wood closest to the surface (<5 mm), determined as presented in section 3.6.2; and *t* is drying time in hours.

4.5.2. Neural Networks.

The second approach to fit the data to a model was to use Neural Networks to produce a non-linear model for each of the colour variables. To the best of my knowledge Neural Networks has not been used to study discolouration in wood during drying. Nevertheless, it has been used to predict colour in beans as a function of roasting time and bean temperature (Hernández *et al.* 2001). According to the linear model, the desired output was respectively luminance factor, brightness, and the blue-

to-yellow scale of CIE Lab, and the inputs were drying time, nitrogen, total sugars and high-molecular-weight phenols. The non-linear models were tested with four different combinations of regressors to infer their causal effects on colour change. Those combinations had to be consistent with the chemical reactions expected to occur, namely Maillard-type reactions and oxidation and polymerisation of phenolic compounds, as mentioned in Chapter 1. Four Neural Networks models were constructed, as described in section 3.7.3, for each of the three selected colour variables, totalling twelve models. The level of linearity between the experimental data and the data predicted with the model was measured with the coefficient of correlation (r). The error of the estimation was assessed with the mean square error (MSE). The statistics r and MSE are calculated as presented in Eqs. 22 and 19. The Neural Network models had two nodes: it was observed that more nodes did not improve the results, while increasing the risk of overfitting the data. $NN1$ had drying time, nitrogen content and total sugars content as input; $NN2$ included drying time, nitrogen content, total sugars content and HMWP content as input; $NN3$ included drying time and nitrogen content as input; and $NN4$ had drying time and HMWP as input. Refer to Appendix C for a summary of weights and biases for the different models. The diagrams of the models are presented in Figures 16, 17, 18, and 19.

In model $NN1$, shown in Figure 16, the evidence of Maillard-type reaction was investigated with the combination of content of nitrogen, content of total sugars, and drying time. This combination of input variables produced the best results in models that included luminance factor and brightness as dependent variables, as measured by the coefficient of correlation between experimental data and data predicted by the model ($r=0.75$ and 0.69); moreover, both models had similar error in the prediction ($MSE=11.43$ and 11.29). Nevertheless, model $NN1-b^*$ presented a slightly lower coefficient of correlation than $NN1-R457$ ($r=0.68$), although the error of the prediction was lower ($MSE=8.22$) than for models $NN1-Y$ or $NN1-R457$.

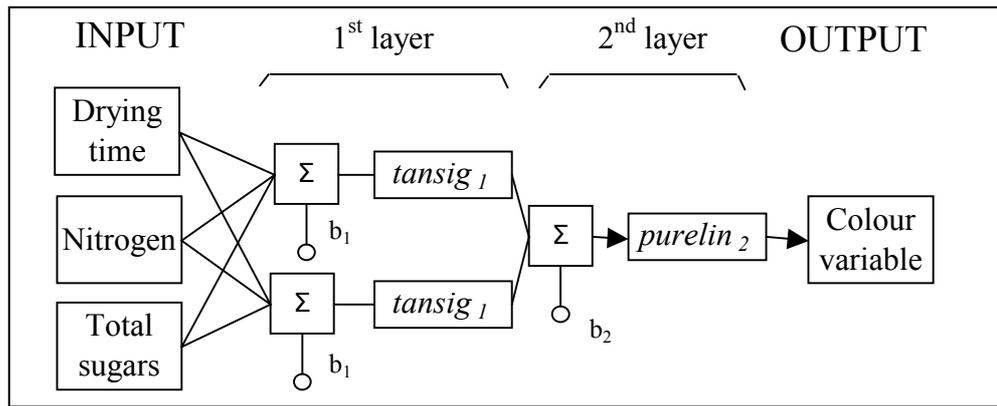


Figure 16. Model *NN1*. Output: Y or $R457$ or b^* . Input: drying time, nitrogen content, and total sugars content.

Model *NN2*, presented in Figure 17, included all the selected regressors to test the effect that their interactions had on the development of colour on the surface of the boards. It produced the largest weight matrix. The correlation between experimental data and data predicted by the model was high when Y was the colour variable ($r=0.75$), but lowest when brightness was the colour variable ($r=0.69$). Among the models that included b^* as the colour variable this model produced the best results ($r=0.70$; $MSE=7.75$), even though the value of the correlation coefficient was lower when compared with the models that have luminance factor as colour variable.

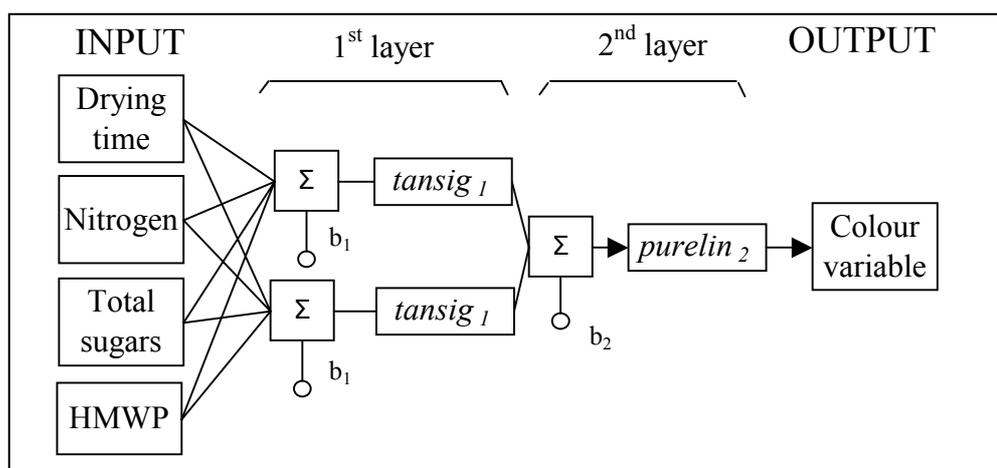


Figure 17. Model *NN2*. Output: Y or $R457$ or b^* . Input: drying time, nitrogen content, total sugars content and HMWP content.

Model *NN3* was a simplified version of model *NN1*. Model *NN3*, shown in Figure 18, included only drying time and nitrogen as inputs. By leaving the total sugar content out to the model, an attempt was made to test its relative importance in colour development on the surface. Consequently, the results would highlight the relevance of nitrogen content in Maillard-type reactions in wood. As measured by the coefficient of correlation, the model's ability to describe the data was very similar to models *NN1* and *NN2* (*NN3-Y* $r=0.74$; *NN3-R457* $r=0.69$; and *NN3-b* $r=0.66$). Likewise, the error of the estimation was also comparable with models *NN1* and *NN2* (*NN3-Y* $MSE=12.08$; *NN3-R457* $MSE=11.26$; and *NN3-b** $MSE=8.62$). Therefore, Neural Network modelling was improved by the input of the total sugar content of sugars, but most of the variation of colour on the surface was explained by nitrogen and drying time.

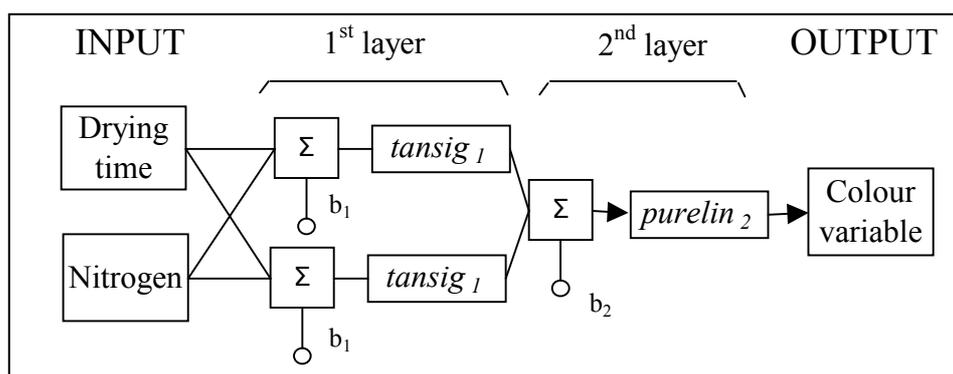


Figure 18. Model *NN3*. Output: *Y* or *R457* or *b**. Input: drying time and nitrogen content.

Model *NN4*, presented in Figure 19, included drying time and HMWP as input variables. High molecular weight phenols were expected to affect colour on the surface by oxidation and polymerisation, both phenomena being connected neither with total sugars nor with nitrogen content. Consequently, this model did not include them. Model *NN4* produced the worst results, regardless of the colour variable used, *Y*, *R457* or *b**. The values of coefficient of correlation were the lowest of each group of models related to one colour variable (*NN4-Y* $r=0.65$; *NN4-R457* $r=0.56$; and *NN4-b** $r=0.51$), and, moreover, the values of error of the prediction were the highest (*NN4-Y* $MSE=15.10$; *NN4-R457* $MSE=15.07$; and *NN-4 b** $MSE=11.25$).

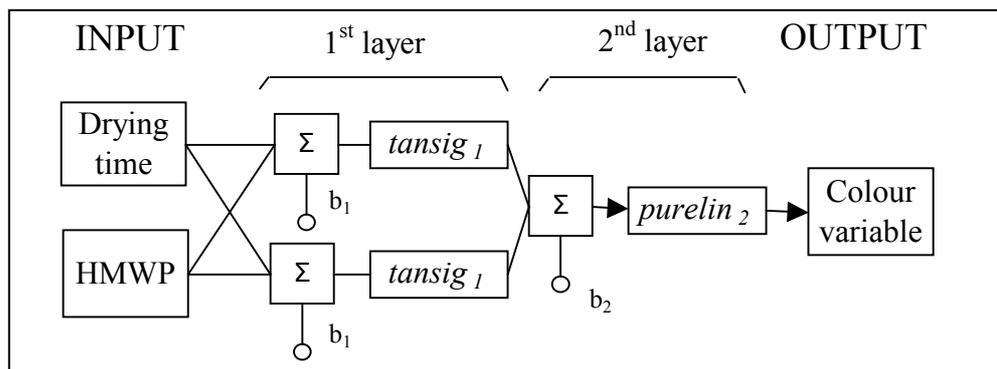


Figure 19. Model *NN4*. Output: or *R457* or b^* . Input: drying time and nitrogen content.

Table 14 presents a summary of the linear and non-linear models built to analyse colour development in the surface of boards as a function of certain chemical compounds present in wood. In agreement with the linear regression, luminance factor was the colour variable that better explained discolouration during drying as a function of drying time and certain compounds present in wood, followed by brightness and finally the blue-to-yellow scale of CIE Lab. However, the models that had b^* as the colour variables presented consistently lower estimation of error in the prediction. Model *NN3*, which included only drying time and content of nitrogen as independent variables, obtained basically the same levels of correlation of experimental data with predicted data than more complex models, such as *NN1* and *NN2*. Furthermore, the error of the estimation was similar between these three models. In addition, model *NN4*, which included high-molecular-weight phenols and drying time as independent variables presented the lowest coefficient of correlation between experimental data and data predicted by the model, regardless of the colour variable used.

The linear model could be compared with Neural Network model *NN3*, since both models included drying time and nitrogen content as independent variables. The experimental data presented a higher correlation with the predicted data with model *NN3* than with the linear model, except when brightness was used as the colour variable, in which case there was no difference. Furthermore, the mean square error

was lower in the Neural Network models, with the exception of *NN3-b**, where the mean square error was higher than in the linear model, as shown in Table 14.

Table 14. Multivariate linear models and Neural Network models ability to describe the experimental data.

Model	Colour variables					
	<i>Y</i>		<i>R457</i>		<i>b*</i>	
	<i>r</i>	<i>MSE</i>	<i>r</i>	<i>MSE</i>	<i>r</i>	<i>MSE</i>
Linear	0.72	12.69	0.69	11.29	0.62	9.34
<i>NN1</i>	0.75	11.43	0.69	11.29	0.68	8.22
<i>NN2</i>	0.75	11.44	0.69	11.27	0.70	7.75
<i>NN3</i>	0.74	12.08	0.69	11.26	0.66	8.62
<i>NN4</i>	0.65	15.10	0.56	15.07	0.51	11.25

To further investigate the relationship between experimental data and data predicted by the models, the residuals were plotted against predicted results. Both linear and non-linear models' residuals were standardized to consider the inequality of the residuals variance, and therefore to make the residuals comparable, as described in section 3.7.3. Most values were found within three standard deviations from zero, as illustrated in Figure 20. Likewise, no specific pattern was found in the plotting of the standardized residuals, although the approximately triangular shape of the models that have luminance factor or brightness as dependent variables could indicate the presence of unequal variances (Hair *et al.* 1995). The plot of the standardized residuals as a function of the experimental results gave evidence that the multivariate linear regression and the Neural Network models were similar in their residuals patterns.

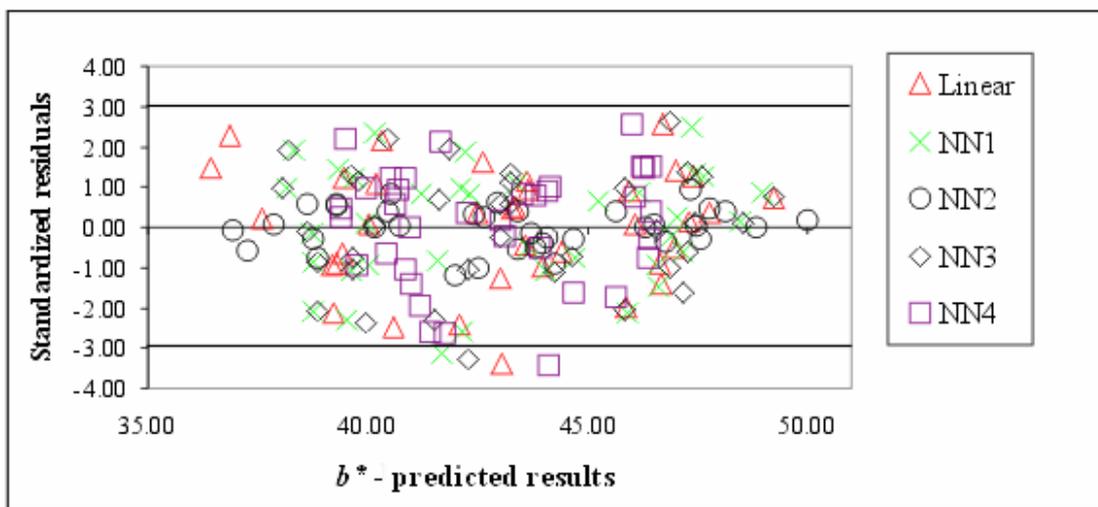
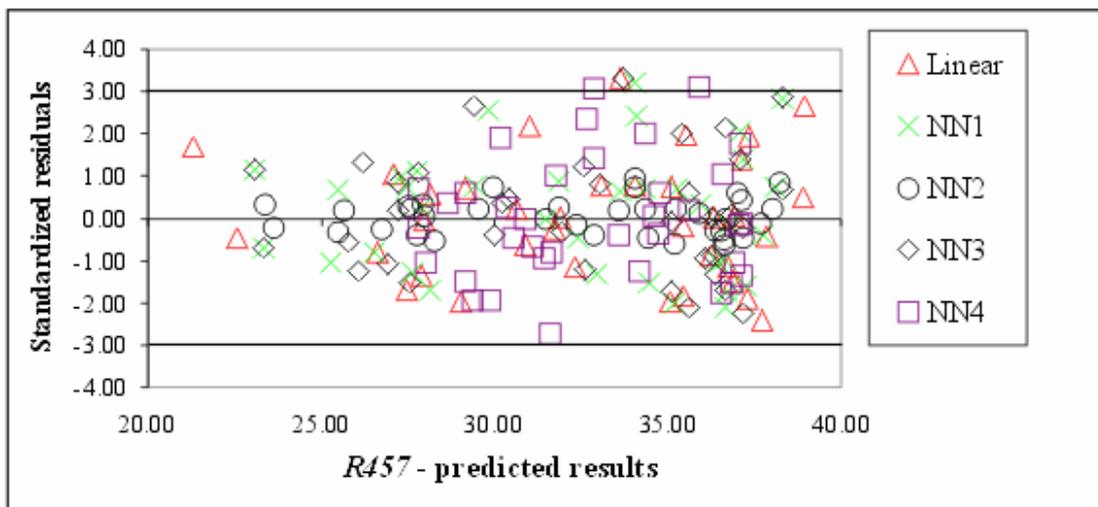
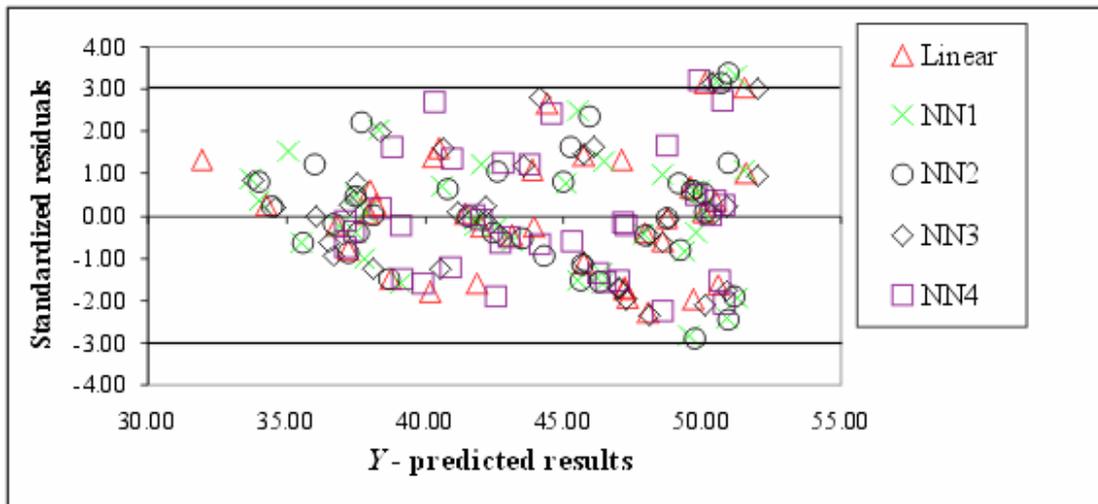


Figure 20. Standardized residuals for the linear and Neural Network models versus predicted results for the three response variables, namely luminance factor, brightness and blue-to-yellow scale of CIE Lab.

The two approaches proposed, linear regression and Neural Network modelling, showed that the variables nitrogen content and drying time could be used to explain the colour change in the surface. Both approaches highlighted that luminance factor as the dependent variable that produced the best results in a regression model that included drying time and nitrogen content. The Neural Network models supported the results of the linear models.

4.5.3. Empirical models based in simple reaction kinetics.

The results obtained by the statistical analysis supported the concept that the discolouration on the surface of the boards was due to Maillard-type reactions. Therefore, an empirical model based on simple reaction kinetics was constructed: it included the rate of colour development as response, and the concentration of nitrogen and total sugars as reactants. The basic stoichiometric equation of the discolouration reaction is presented below:



where N is the molecular nitrogen present in the wood closest to the surface of the boards; S are the molecular non-structural sugars present in the wood closest to the surface; k is the rate constant; and C is the measurement of colour on the surface of the boards.

As the effect of non-structural sugars was not evident in the statistical models, they were not part of the empirical model. Their contribution to the discolouration of the surface was considered to be included in the rate constant k . The empirical model is presented below:

Eq. 29
$$\frac{dC}{dt} = k \cdot [N]^m$$

where C is a measurement of colour on the surface of the boards; t is drying time in hours; $[N]$ is the concentration of nitrogen in the wood closest (<5 mm) to the surface;

k is the rate constant; m is the order of the kinetic equation. The experimental design limited drying time to four observations, and hence the kinetic equations had only four experimental points for fitting. The rate constant k and the order of the equation m were determined experimentally for a model including each colour variable. For details in the calculation of m and k refer to Appendix C. They are presented in Table 15, along with the coefficient of determination between experimental results and results calculated using the empirical model.

Table 15. Empirical equations for the colour variables selected: rate constant, order, and coefficient of determination.

Colour variables	m	k	R^2
Y	0.979	0.826	0.925
$R457$	0.614	0.528	0.924
b^*	0.156	0.342	0.923

5.1. Introduction.

The discussion of the results opens with an overview of the outcome of this research and the main ideas extracted from it. Next, experimental techniques, results, and the methods used to analyse the results are discussed in detail.

5.2. Overview.

The purpose of this work was to determine the effect that compounds present in sapwood have on colour development during kiln drying. The conceptual model regarded colour on the surface of the boards as the dependent variable, and drying time and certain chemical compounds present in the wood closest to the surface (up to 5 mm) as the independent variables. A similar approach has been presented previously to study the relationship between brightness and the oxidation of catechin in western hemlock (Hrutfjord *et al.* 1985); and to investigate the correlation between absolute colour difference (ΔE) and the content of soluble carbohydrates in *Picea abies* and *Pinus sylvestris* (Tarvainen *et al.* 2001).

The experimental factors were leaching and drying time as illustrated in Figure 2. Leaching had three levels: control or non-leaching, mild, and severe. The pine-wood was leached before drying to diminish the components non-structural sugars and nitrogen compounds that are responsible for most of the discolouration during drying. The drying schedule, described in section 3.4, was the same for the whole experiment, and temperature, humidity and air speed remained constant. Drying time was both an experimental factor and a regressor, and it had four levels: 0, 8, 16 and 24 hours. According to the results of luminance factor, brightness, and dark pixels index, it was observed that longer drying times produced a darker colour on the surface. The results suggested that drying time had a clearer impact than leaching on the variables selected for colour measurement, as shown in Table 9.

In addition, leaching the samples had the objective of testing the difference in colour development between low-molecular-weight phenols, which are insoluble in water, and high-molecular-weight phenols, which are water-soluble. Even though the diminution of high-molecular-weight phenols was effective (28% from unleached to severely leached, as described in section 4.4), this reduction did not significantly affect colour change. According to the literature, low-molecular-weight phenols, insoluble in water, were more abundant in pine than high-molecular-weight phenols (Scalbert *et al.* 1989; Uprichard 1991). After leaching, the content of low-molecular-weight phenols was reduced by only 4%. Nevertheless, their relevance in the discolouration of the surface of the boards was negligible, as will be discussed in section 5.5

Due to the difficulty of measuring colour, illustrated in Figure 21, different methods were used to assess colour change during kiln drying. Only two variables detected the effects of both experimental factors: brightness ($R457$) and the blue-to-yellow scale of CIE Lab (b^*): one variable, luminance factor (Y), detected the effect of drying time and the effect of the interaction between leaching and drying time. Those three variables were included in the models as dependent variables, as explained in section 3.7.1

The chemical determinations were the content of: non-structural sugars (fructose, glucose, sucrose and its sum, total sugar); total nitrogen; and phenolic compounds (low and high-molecular-weight phenols, and its sum, total phenols). As determined by the ANOVA, three variables among them were affected by both experimental factors: total sugar content, nitrogen content, and high-molecular-weight phenols. Those, along with drying time, were considered the independent variables to be included in the models for the cause-effect analysis, following the criteria presented in section 3.7.1.

Linear and non-linear statistical models were constructed to fit the data to a mathematical expression. The models that best described the data included luminance factor as their dependent variable. The results suggested that drying time and the content of nitrogen were the variables responsible for most of the discolouration. Hence, an empirical model on the basis of simple reaction kinetics was proposed for

each of the three colour variables stating that the rate of discolouration was a function of the content of nitrogen. This empirical equation connected discolouration on the surface of the boards during kiln drying, measured as the rate of the change of luminance factor, brightness, or the blue-to-yellow scale of CIE Lab, to the content of nitrogen in the wood closest to the surface.

5.3. Colour determination.

One of the aims of this work was to quantify the discolouration of wood during kiln drying, particularly kiln brown stain. Different methods with their variables and combination of variables were tested, as presented in section 3.5, and only a few methods were able to differentiate what was clear to the naked eye. The difficulty of measuring discolouration has been reported in previous work (Andersson and Walter 1995; Nyström and Kline 2000; Ledig and Seyfarth 2001). Figure 21 shows the plotting of the results of the 36 determinations done in this study of the chromaticity coordinates x and y of the colour system CIE Yxy; all the determinations are concentrated in a small portion of the graph. A similar phenomenon occurred with the other colour system used, CIE Lab.

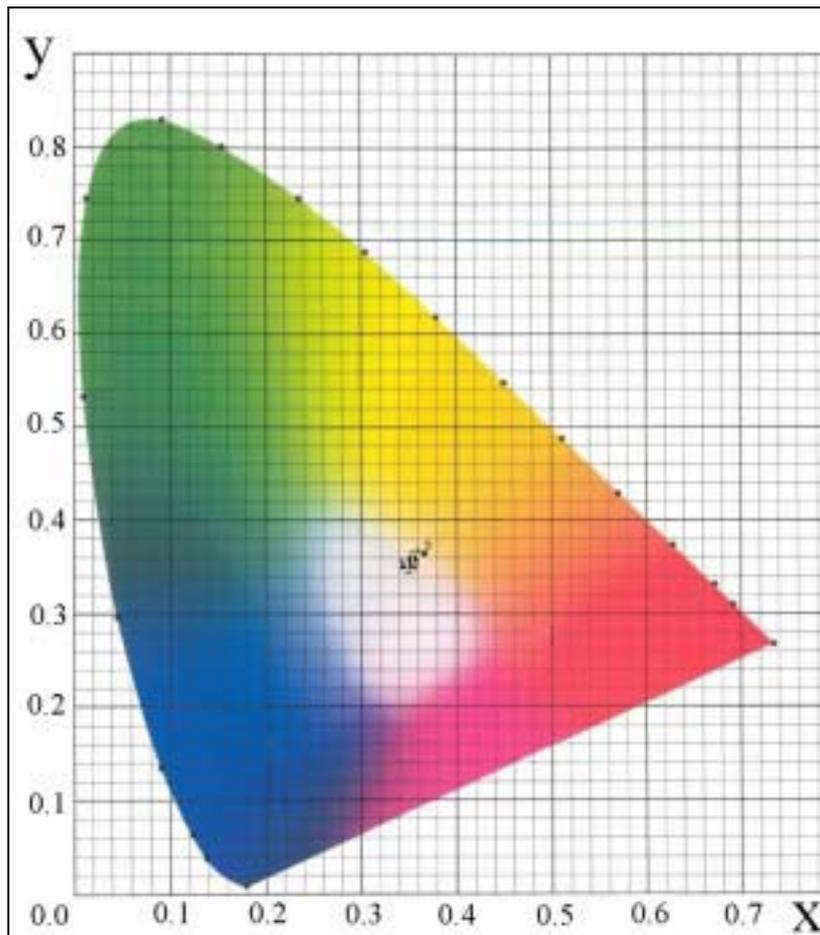


Figure 21. Colour system CIE Yxy: example of colour determination.

Two of the three variables that were useful to simulate colour change in wood were measured using the photoelectric reflectance photometer, namely luminance factor and brightness. The correlation between the two variables was high, as shown in Table 12, because the methods to determine the two variables are very similar, differing only in the filter used, as discussed in section 3.5. The photoelectric reflectance photometer is an instrument based on the same principles as a colorimeter, which could also be an adequate instrument to accurately measure colour development during kiln drying conditions. Both instruments measure the reflectance at certain wavelengths, followed by the calculation of tristimulus values and the determination of colour using colour systems. However, the most complete instrument to measure colour is a spectrophotometer that can determine the spectral reflectance through the entire spectrum, and therefore provides a more accurate measurement.

The manufacturer of the photoelectric reflectance photometer recommends that for colour measurements the colour difference between the standard and the sample should be the lowest possible (Operating Instructions; ELREPHO, Zeiss). This recommendation was not followed. The use of a colour standard adds uncertainty to the measurements because it is difficult to specify. To the best of my knowledge, there are no published colour standards for *Pinus radiata* wood, neither fresh nor dried. Therefore, a conventional white standard, barium sulphate, was used to calibrate the equipment. The barium sulphate tablet used to calibrate the reflectance photometer was produced using a non-standard press, constructed for that purpose. Refer to Appendix A for details on the construction of the barium sulphate tablet press.

The determination of the colour on the surface of the boards using the CIE Lab system was based on previous research at this University (McCurdy *et al.* 2001). The standard instrument to determine CIE Lab quantities, as well as unabridged tristimulus values, is a spectrophotometer. However, the method used allowed the determination of CIE Lab values by analysing the image using standard software that was not especially adapted for this study. One variable, the blue-to-yellow scale of CIE Lab was relevant to describe colour change during kiln drying according to the defined experimental factors presented in Table 9. Longer drying times produced a bluer colour on the surface of the boards, which might be the greenish colour perceived by visual assessment. Nevertheless, the failure of the method to identify colour differences based on the CIE Lab colour system, absolute colour difference (ΔE), or a designed colour index (DPI), suggests that the analysis of digital photographs using non-specific software is not the path to follow in future research.

The absolute colour difference (ΔE) is a standard variable defined by CIE, previously used in other studies to differentiate wood colour (Stenudd 2001; Tarvainen *et al.* 2001). Its determination is also included in colour measuring instruments (MINOLTA 1998). Nevertheless, in this study the variable ΔE did not detect the effect of the experimental factors. That is not unexpected, because ΔE is the difference of the vectors L^* , a^* and b^* for two samples, and of the CIE Lab quantities only b^* was sensitive to the experimental factors.

The digital analysis allowed the studying of the images in detail, pixel by pixel. The Dark Pixels Index (*DPI*) was an attempt to discriminate the images according to the number of dark pixels present. It was intended to be a measurement of the stains on the surface of the boards. *DPI* was successful in detecting the effect of drying time, but not that of leaching (Table 9). The reduction of soluble compounds reduced the amount of stains, but it also changed the lightness of the surfaces of the boards, and that alteration was not detected by *DPI*.

The colour variables were to be the dependent variables of statistical models that attempted to investigate the relationship between colour development on the surface and chemical compounds present in wood. The colour variables to be included in the models were selected after statistical analyses, namely ANOVA and MANOVA, described in section 3.7.1. The variables selected were those sensitive to drying time and leaching, or to the interaction between both experimental factors. They were luminance factor, brightness and the blue-to-yellow scale of CIE Lab, as shown in Table 9.

5.4. Chemical determinations.

The results of the analysis of non-structural sugars presented differences from previous analysis of *Pinus radiata* sapwood. The levels of fructose were similar to the reported ones, but particularly glucose and sucrose were lower. This can be attributed to the HPLC method to determine non-structural sugars: it was difficult to stabilize the baseline using a refractive index detector for the levels of detection needed; therefore the detection of glucose and sucrose were sometimes confounded with the noise of the baseline, as illustrated in Figure 10. Nevertheless, soluble carbohydrates are expected to have large variability in wood due to the combination of genetic and environmental factors, as described in section 3.6.

After mild leaching the concentration of sugars was similar to that of the non-leached samples, except for the determination at 24 hours. On the contrary, severe leaching was effective in diminishing the levels of sugar, as illustrated in Figure 13. A possible explanation is that the heating of the samples and the use of hot water to

displace the sap solution destroyed the parenchyma cells and allowed a significant reduction in the content of sugars (McDonald 1997). This point will be further discussed in this section.

The increase in concentration of soluble sugars close to the surface during drying was clear in the not-leached samples, as has been reported before (Theander *et al.* 1993; Terziev 1995; Kreber *et al.* 1998). Nevertheless, in the mildly leached samples the migration trend was interrupted at 24 hours, and evidence of sugar migration was not present in the severely leached samples, as illustrated in Figure 13. This was unexpected. The heating used to diminish the concentration of soluble compounds for severe leaching might have damaged the tissues where that migration occurs. That phenomenon would contribute to the absence of kiln brown stain in the samples with the severe treatment.

The nitrogen levels determined were in agreement with previous studies. Even though leaching was not as effective to diminish the nitrogen levels as it was with the sugar levels, there was a high correlation between the two variables. Most of the nitrogen measured in the samples could be considered to be in the organic form, as a component of amino acids and proteins (Bollard 1958). Therefore, these results suggested that the nitrogen left after the severe reduction of soluble compounds was part of the structure of the wood, and consequently insoluble in water (Terziev 1995). The control samples presented a gradient increase of nitrogen compounds in the wood close to the surface during drying, but this trend was absent when there was reduction of soluble compounds, as illustrated in Figure 14. A similar phenomenon was observed for sugar accumulation on the surface, but the decrease in sugar content from no leaching to severe leaching was clearer, as shown in Figure 13.

In addition, there was a clear correlation (0.76) between the content of sugars and the nitrogen content, presented in Table 12. It is expected that these compounds would be present in the same tissue, the parenchyma ray-cells of the xylem, in the sapwood. In softwoods, these cells store and transport photosynthesis assimilates in the xylem (Fengel 1984). The non-structural carbohydrates present in the xylem are reported to be located in the parenchyma ray-cells (Fischer and Höll 1992); likewise, the nitrogen compounds present in the xylem are also located there (Terziev 1996).

McDonald *et al.* (1997) suggested that the preheating and use of hot water to leach the wood samples before sap displacement destroyed the parenchyma cells, and released their content. In this experiment, the severe leaching, described in section 3.3, involved a preheating treatment of the samples at 90 °C for five hours, and the use of water at 50 °C. Therefore, there are reasons to believe that the parenchyma cells were destroyed, and the contents of non-structural sugars and nitrogen compounds were reduced simultaneously. This phenomenon would explain the high correlation between the two variables.

In this study, the determination of phenolic compounds presented differences from previous reports for gymnosperm species. Unexpectedly, low-molecular-weight phenols were twice as abundant as high-molecular-weight phenols, as presented in Table 18. Nevertheless, *Pinus radiata* bark is particularly rich in condensed tannins (Markham and Porter 1973), which could explain a higher concentration of high-molecular-weight phenols in the sapwood. One of the objectives of the reduction of the soluble compounds was to enhance the effect of phenols in colour development during drying. The hypothesis for the reduction of soluble compounds was that it would diminish the concentration of Maillard reaction reagents, namely non-structural sugars and nitrogen compounds. Consequently, this would make more evident the effect in colour development of water insoluble phenols, such as catechin. Basically, this experiment was intended to provide evidence of the existence of catechin polymerisation into coloured compounds during drying, as suggested by previous research (Hrutfjord *et al.* 1985; McDonald *et al.* 2000). This study did not find such evidence, as will be further discussed in section 5.5. In addition, the low-molecular-weight phenols were barely affected by the treatments with cold and hot water, but there was no evidence of their responsibility in the colour developed in the surface during drying, as discussed in section 4.5. Low-molecular-weight phenols, such as catechin, could oxidise during drying, which would favour polymerisation and insolubilisation. They could become insoluble by combining with cell-wall components. In that way, they would be undetectable by the determination method used (Lavisici *et al.* 1991; Peng *et al.* 1991).

5.5. Cause-effect analysis: wood extractives and colour development during drying.

The objective of modelling the discolouration of the surface of the boards during kiln drying as a function of both drying time and the content of chemical compounds present in wood was to identify which compounds were more relevant to the development of colour. The models were not intended to predict the discolouration phenomenon beyond 24 hours, or how variations in the content of chemical compounds present in wood would affect colour change. The purpose was to identify hierarchically the reactants of a complex discolouration reaction by studying the relationship between the reactants and a physical property of the reaction, its colour. Throughout the cause-effect analysis the models were constructed with the three colour variables selected as described in section 3.7.1, namely luminance factor, brightness, and the blue-to-yellow scale of the CIE Lab colour system. According to the selection methods, there was no reason to discard any of the three colour variables.

Three different approaches were proposed to model discolouration on the surface of the boards during kiln drying as a function of drying time and the chemical compounds present in the wood closest to the surface: multivariate linear regression, Neural Network, and an empirical model based on simple reaction kinetics. The three approaches were consistent in indicating that discolouration on the surface could be described as a function of drying time and nitrogen content in the wood. The results suggested that the nitrogen content could act as a limiting factor in the reaction, because the levels of sugars were higher than the levels of nitrogen; furthermore, much of the nitrogen appeared to be insoluble, and therefore not available to react with carbonyl groups to form melanoidins that would discolour the surface of the boards.

The multivariate linear regression and the Neural Network models presented a higher coefficient of correlation ($r=0.75$) between the experimental results and the predicted results when the luminance factor was used as the dependent variable, as presented in Table 14. In contrast, the lowest error in the prediction was found in the models constructed with the blue-to-yellow scale of CIE Lab ($MSE \leq 11.25$), but their

highest coefficient of correlation was 0.70. The design of the empirical model on the basis of simple reaction kinetics supported the concept that the Maillard-type reactions are responsible for colour development during kiln drying. The concentration of total sugar was not included in the equation under the assumption that its effect was included in the constant k of Eq. 29.

The models did not provide evidence of a connection between the phenolic compounds present in wood and colour development on the surface of boards during drying. Even though phenolic compounds were determined in relatively high proportions, their participation in colour development could not be found. It is worthwhile to mention that discolouration due to phenolic compounds, particularly tannins, is reported for hardwoods (Hrutfjord *et al.* 1985; Peng *et al.* 1991; Charrier *et al.* 1995).

5.5.1. Models to investigate causes of discolouration during drying.

The independent variables to be used in the multivariate linear regression were selected using a stepwise selection method. The regression models are presented in page 66. They were consistent in including drying time and the nitrogen content regardless of the dependent variable used. Table 13 shows that the model with the higher coefficient of determination had luminance factor as the dependent variable. The next best colour variables were brightness, and finally the blue-to-yellow scale of CIE Lab. The two independent variables, drying time and nitrogen content, were included in the models with a high probability of having coefficients other than zero: the highest P -value among the three models between drying time and nitrogen content was 0.0154 (Table 13). The models that included luminance factor and brightness as colour variables predicted darker colours if both the drying time and the nitrogen content were increased: the model that had the blue-to-yellow scale of CIE Lab as dependent variable predicted bluer colour for similar changes in the independent variables. These results suggested that the discolouration reaction was mainly a Maillard-type reaction, where the nitrogen content could be considered the limiting reactant. Under this hypothesis, there would be sufficient carbonyl groups present in wood for the Maillard-type reactions to occur, whereas the scarcity of the nitrogen

compounds limits the development of the melanoidins, and therefore the discolouration of the surface of the boards.

The subsequent step in modelling was the use of Neural Networks as described in section 3.7.3. Four different models were constructed in an attempt to reflect the reactions that were expected to develop colour on the surface, namely Maillard-type reactions and the oxidation and polymerisation of phenolic compounds. As occurred with the linear models, the colour variable that presented the higher coefficient of correlation for any of the four models was luminance factor. The models that better predicted the experimental results were constructed under the hypothesis that the Maillard-type reactions were responsible for the discolouration of the surface of the boards, namely *NN1*, *NN2* and *NN3*. *NN2* contained all the chemical variables selected by the statistical analysis, included high-molecular-weight phenols, and the results for each colour variable presented a high coefficient of correlation, as shown in Table 14. In contrast, model *NN4* included only high-molecular-weight phenols and drying time as independent variables, and presented a low coefficient of determination for each of the colour variables. Its construction suggested that the good performance of model *NN2* was not due to the inclusion of high molecular phenols as an independent variable. *NN1* and *NN3* differed only in the presence of total sugars as independent variables in *NN1*, and it was observed that for each of the colour variables the improvement in the coefficient of determination or in the error of the prediction was slight. Therefore, this was in agreement with the results of the multivariate linear regression, which identify drying time and content of nitrogen as the most relevant independent variables for the modelling of discolouration on the surface.

The multivariate linear model could be compared with *NN3*, since both have the same independent variables, namely drying time and content of nitrogen. The results suggested that there was no significant difference between modelling using multivariate linear regression or Neural Network, as presented in Table 14. The performance of the model depended on the dependent variable used. A possible explanation for this similarity probably lies in the reduced number of data points: a larger sample size would distinguish between the two methods of modelling, as it does in the discussion presented by Fleming (1993).

Therefore, three main points could be extracted from the statistical modelling of the discolouration of the surface of boards as a function of chemical compounds. The first point is that the results show that the models with higher correlation between experimental values and predicted values, both in multivariate linear regression and in Neural Networks, included luminance factor as the dependent variable. From this study, the luminance factor would be selected to measure colour change due to kiln drying. The second point is that Maillard-type reactions appear to be the relevant chemical interaction that produces colour during drying, with nitrogen compounds and soluble sugars as possible reactants. A relationship between the change in the content of phenolic compounds and discolouration, under the hypothesis that these phenolic compounds could undergo oxidation and polymerisation during drying, was not found in this experiment. The third point is that these results suggested that the critical independent variables were drying time and the nitrogen content in the wood closest to the surface. The inclusion of the content of total sugar in the Neural Networks model represented a slight improvement in the correlation between experimental values and predicted values. Arguably, the nitrogen compounds could be considered the limiting factor in a Maillard-type reaction that includes carbonyl groups, apparently abundantly present in wood.

The construction of an empirical model on the basis of simple reaction kinetics aimed to find empirical mathematical expressions that would describe discolouration on the surface as a function of chemical compounds present in the wood, according to the objectives presented in section 3.7.4. This empirical model expressed colour change on the surface of the boards as a function of the content of nitrogen present in the wood closest to the surface, as presented in Eq. 29. The equation was constructed for each of the colour variables, and the results are shown in Table 15. The results of this empirical model have to be treated with extreme caution, due to the fact that only four data points, one for each drying period, were available for the calculation of the rate constant and the order of the equation. This precaution applied particularly to the correlation calculated between the experimental values and the values predicted by the empirical model equations, presented in Table 15.

From this data it is not possible to state whether this simple reaction kinetics model is described by a zero or first order equation. The equation constructed for

luminance factor and brightness presented an order m close to the unity, which indicated a first order reaction (Y , $m=0.979$, and $R457$, $m=0.614$), whereas the equation that included the blue-to-yellow scale of CIE Lab presented an order m close to zero (b^* , $m=0.156$). Maillard-type reactions of sugars and amino acids in aqueous model systems are described by zero order kinetics (Carabasa Giribet 2000). However, even though the size of the data is too small, the correlation between experimental data and data predicted with the simple reaction equation was highest when Y was used as colour variable (Table 15), which would suggest that the simple reaction kinetics is described by a first order kinetic model.

In addition, the determination of nitrogen included the reactants and the products of the reaction, because the entire sample was analysed and it was not possible to discriminate between reactants and products. This fault on the experimental design could confuse the effect of nitrogen on colour development.

Chapter 6

CONCLUSIONS.

6.1. Introduction.

The conclusions of this study are divided into general and particular conclusions. The general conclusions answer the objective of this work, which was to investigate if discolouration on the surface during drying can be described as a function of the chemical compounds present in the wood closest to the surface. The particular conclusions are specific to aspects of this study and deal with the experimental techniques employed, and the methods used to analyse the results.

6.2. General conclusions.

- The results of this study indicate that discolouration of *Pinus radiata* wood during kiln drying, measured using colorimetric variables (namely luminance factor; brightness; and the blue-to-yellow scale of CIE Lab), can be described as a function of drying time and the concentration of nitrogen compounds in the wood closest to the surface.
- Maillard-type reactions appear to be the relevant chemical interaction that produces colour during drying, with nitrogen compounds and soluble sugars as reactants. The results indicate that the nitrogen compounds that migrate to the surface could act as the limiting reactant in a discolouration Maillard-reaction that includes carbonyl groups, which apparently are abundant in wood.
- This study found no evidence of the incidence of phenolic compounds in the discolouration of *Pinus radiata* wood during kiln drying.

6.3. Particular conclusions.

- Although colorimetric techniques were useful in assessing discolouration of *Pinus radiata* wood during kiln drying, it was difficult to measure colour on the surface of boards with the equipment used. Only three variables detected the changes in colour due to the experimental factors,

leaching and drying time, and therefore were selected as independent variables for model constructions. They were the luminance factor (Y) from the colour system CIE Y_{xy} ; brightness (R_{457}), which is a measurement of relative reflectance at wavelength 457 nm; and the blue-to-yellow scale of CIE Lab (b^*).

- Fructose was more abundant than sucrose and glucose: the relative ratio of fructose, sucrose and glucose was approximately 10:2:1.
- The high-molecular-weight phenols were twice as abundant than the low-molecular-weight phenols.
- The models constructed using both multivariate linear regression and Neural Networks showed that higher correlations between experimental data and data predicted with the models were obtained when the luminance factor was included as the dependent variable, and drying time and the nitrogen content were included as independent variables.
- There was no detectable difference between the two statistical methods of modelling: multivariate linear regression and Neural Networks.
- The discolouration reactions were expressed in empirical models on the basis of simple reaction kinetics that described the rate of discolouration as a function of the content of nitrogen in the wood closest to the surface. Due to the small size of the data, it was not possible to identify the order of the simple kinetic equation. When luminance factor and brightness were included as colour variables, the order of the equation was approximately one. In contrast, when the blue-to-yellow scale of CIE Lab was the colour variable, the order of the equation was approximately zero.

RECOMMENDATIONS.

One of the most critical parts of this study was colour determination on the surface of boards. The results suggest that the analysis of digital photographs using no specific software is not the path to follow in future research. It was observed that the determination of colour is a difficult task, and adequate equipment would render the best results. For future research on colour development in wood I would recommend equipment such as a photoelectric reflectance photometer or a colorimeter, but particularly a spectrophotometer, which determines the colour by analysing the whole spectrum, and therefore renders more detailed information.

In this study it was not possible to find any connection between colour development under kiln drying conditions and the presence of phenolic components in the wood closest to the surface. Therefore, I think that future research in discolouration of *Pinus radiata* during drying should concentrate on the investigation of Maillard-type reactions on the surface of boards.

This work agrees with previous research in emphasizing the importance of Maillard-type reactions for the discolouration of wood during drying. However, the data produced is original in indicating that the nitrogen content could play a significant role on the discolouration reaction, acting as a limiting factor. Carbonyl groups seemed to be in abundance, but that statement needs to be supported with further evidence. The amount of nitrogen ready to react with carbonyl groups should be determined more precisely. This experiment did not discriminate between the amount of nitrogen ready to react with carbonyl groups and the nitrogen present in products of the discolouration reaction. Before the determination of total nitrogen a careful sampling that distinguishes between different levels of discolouration could be a possible approach to tackle this problem.

Due to the low levels of saccharides present in wood the HPLC method used in this study to determine fructose, glucose and sucrose is not recommended. Except for fructose, at those levels it was difficult to obtain a stable refractive index baseline.

Therefore, I would recommend a different HPLC method that includes anion-exchange columns and pulsed amperometric detection (PAD) (Wilson *et al.* 1995).

Dr. Gerrard proposed an interesting experiment to test the importance of different compounds present in wood. Using the wood-leaching apparatus, different chemical compounds could be injected into the wood, and their effect upon drying under kiln conditions assessed. That experiment could give evidence of the importance of certain compounds commonly present in *Pinus radiata*, such as monosaccharides and amino acids, as precursors of Maillard-type reactions (Gerrard 2001).

Raw models of the discolouration of wood as a function of chemical components were constructed in this study. More data is required to verify the results obtained in this study and to improve the prediction ability of the models. It would be particularly interesting to study the same variables under different drying schedules, to investigate the effect of humidity and especially temperature on discolouration. The non-linearity of the Neural Network models make them attractive to be used in future research. In addition, an adjustment of the models on the basis of simple reaction kinetics would be most useful to the industry. The results obtained in this study are encouraging to continue research on those models.

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Appendix A

EQUIPMENT AND METHODS.

A.i. Press to produce barium sulphate tablets.

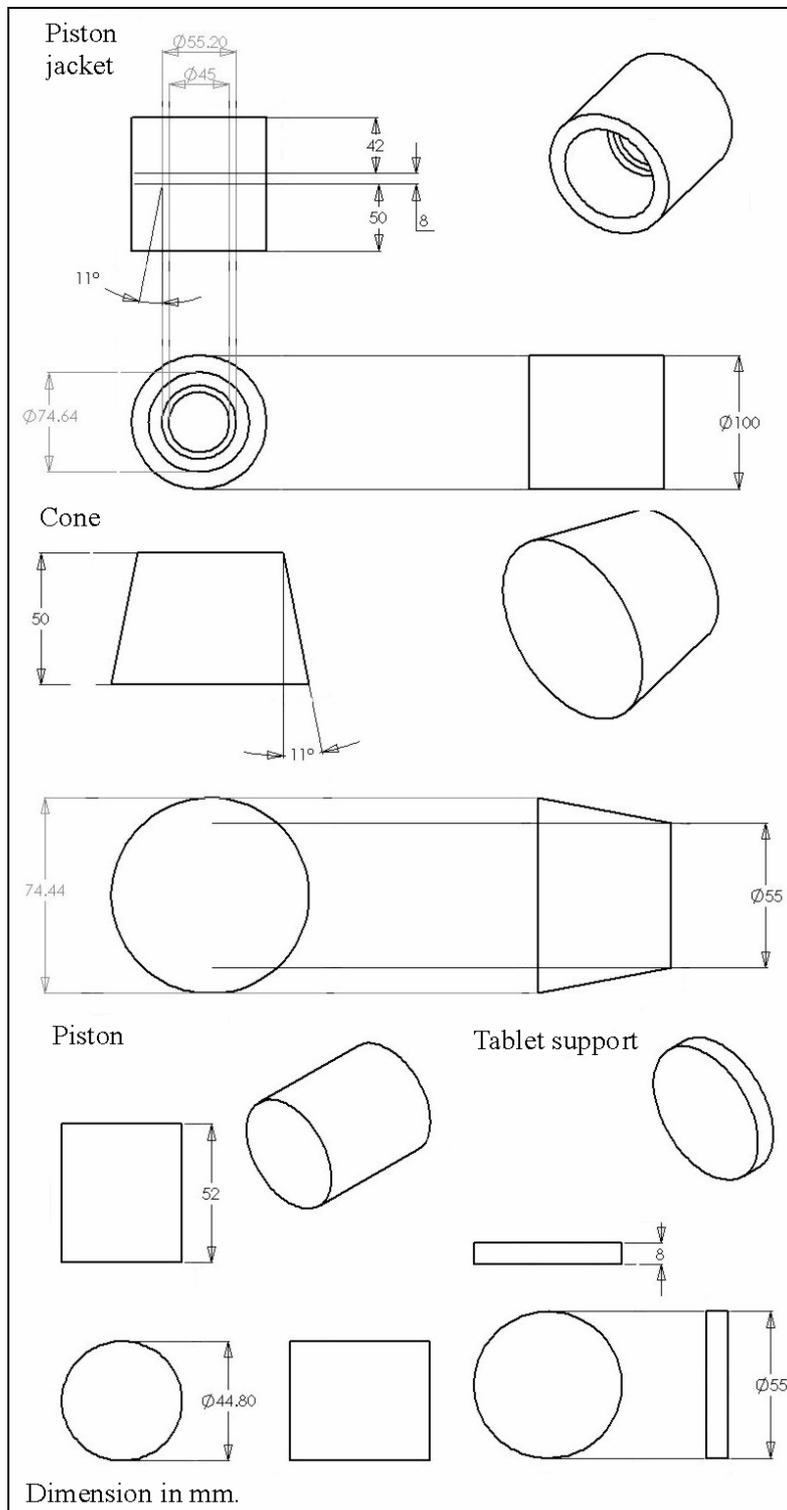


Figure 22. Press to produce barium sulphate tablets (scale 2:1).

A.ii. Digital camera settings.

Digital camera Nikon Coolpix 900. Manual record, exposure +1.0, metering spot, cont. mode single, white bal fluorescent, image adjustment standard and extra function VGA shot. The software used to process the pictures was *ACD See95* ®. The light source was a circular fluorescent light Mirabella ® FLC 22, 230 V. Figure 23 presents a sketch of the light box used to photograph the surface of the boards.

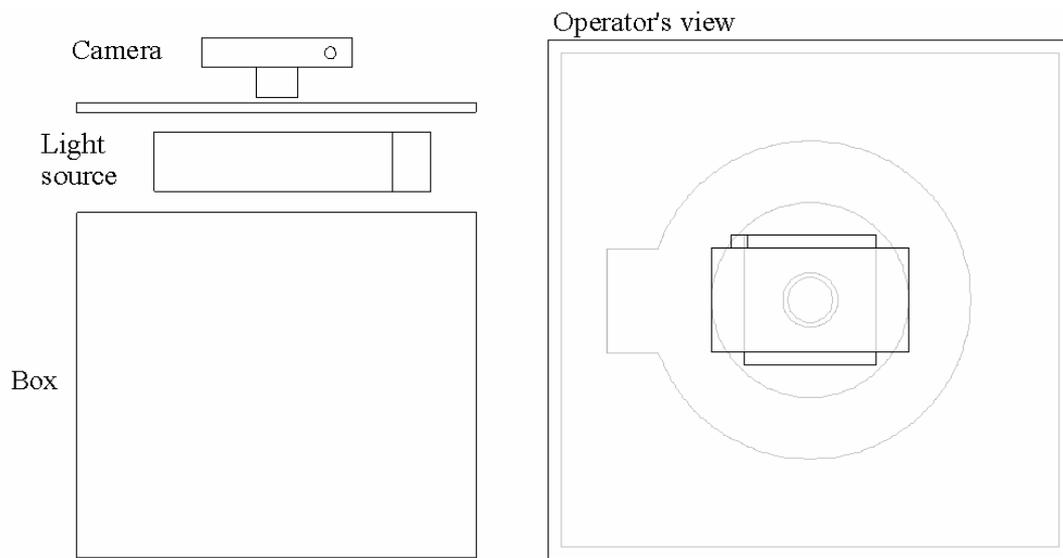


Figure 23. Sketch of the light box used for the assessment of digital images.

A.iii. Dark pixels index: the MATLAB® program used to produce it.

```
%Dark Pixels Index (DPI)
format bank;
photo1=imread('j:\thesis\wood photos\dark pixels index\c9.tif','tif');
%convert from unit8 to double array
photo=double(photo1);
%matrix into vector
data=photo(:);
%x axis from 0 to 256
x1=[0:256];
%histogram
hist(data,x1);
a=hist(data,x1);
%DPI calculation
x2=[0:151];
b=hist(data,x2);
b(152)=0;
index=sum(b)
```

A.iv. Neural Networks activation functions.

The Neural Network Toolbox of MATLAB ® 5.3 does not present details on the activation functions. The following figure is a diagram of the activation functions used, namely *purelin* and *tansig*, where a is the response of the input variable n (modified from Demuth and Beale 1998)

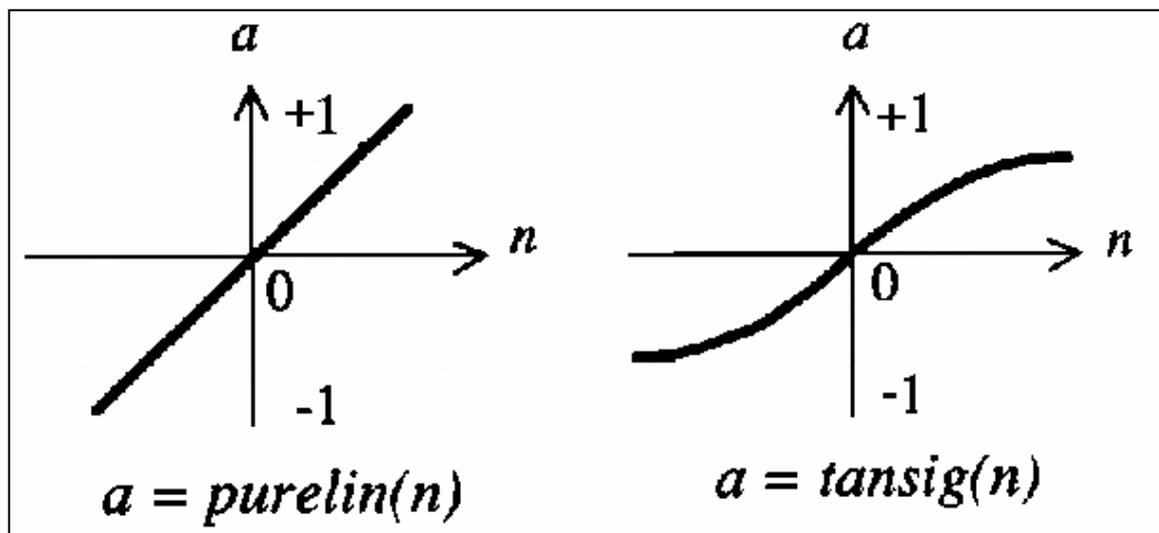


Figure 24. Diagram of the activation functions provided by the Neural Network Toolbox of MATLAB ® 5.3.

Appendix B

EXPERIMENTAL DATA.

The following tables present the experimental data gathered in this experiment. Table 16 is a summary of the colorimetric data obtained in this study; and Table 17 presents the maximum absolute uncertainty of that data. Likewise, Table 18 is a summary of the data obtained by the determination of non-structural sugars, total nitrogen and phenolic compounds in the wood closest to the surface of the boards; and Table 19 shows the maximum absolute uncertainty of that data.

In addition, Table 20 presents the data of the total carbon determination, which was done automatically along with the determination of total nitrogen, as explained in section 3.6.2. The data of total carbon present in the wood closest to the surface of the boards was not considered relevant for this study.

Table 16. Colorimetric data of the surface of kiln-dried *Pinus radiata* boards.

Leaching	Drying time	n	R457		Y		x		y		L*		a*		b*		ΔE		DPI	
	hours		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Control	0	3	34.80	5.12	54.90	6.54	0.37	0.01	0.37	0.00	56.01	12.32	9.65	2.72	50.40	1.53	9.14	6.02	0.00	0.00
	8	3	37.53	5.15	41.63	1.31	0.36	0.00	0.35	0.00	50.49	5.40	8.79	1.06	43.65	2.31	9.38	4.35	1.77	2.14
	16	3	25.23	5.44	37.43	6.73	0.36	0.00	0.35	0.01	52.35	4.27	9.03	0.41	44.05	1.29	7.98	2.40	1.60	1.22
	24	3	28.47	1.89	37.07	2.97	0.35	0.00	0.35	0.00	43.01	7.52	6.29	2.35	39.54	1.90	18.08	4.80	7.40	6.25
Mild	0	3	36.73	7.15	54.93	5.51	0.36	0.01	0.37	0.01	60.26	6.67	9.60	4.00	50.48	4.64	7.11	5.78	0.00	0.00
	8	3	33.73	4.01	42.83	3.09	0.36	0.01	0.35	0.00	52.75	5.53	9.19	0.77	47.08	0.54	6.12	2.87	0.60	0.69
	16	3	26.07	4.39	37.73	3.56	0.35	0.01	0.36	0.01	49.14	10.74	5.96	4.31	38.77	8.77	16.54	9.74	3.22	3.30
	24	3	25.73	3.71	37.77	1.59	0.37	0.01	0.37	0.00	46.12	7.48	7.51	3.97	42.23	5.23	13.89	7.98	5.29	4.00
Severe	0	3	38.73	8.25	47.53	6.71	0.36	0.01	0.36	0.01	49.78	5.82	6.09	4.30	43.83	3.97	11.00	5.35	0.14	0.12
	8	3	37.17	4.30	44.97	4.50	0.35	0.00	0.35	0.01	47.66	9.50	5.18	2.95	38.48	5.48	16.67	7.74	2.20	3.06
	16	3	39.37	4.80	50.80	3.10	0.35	0.01	0.35	0.01	54.35	4.62	4.58	3.30	37.58	5.37	14.33	6.51	0.42	0.54
	24	3	30.23	1.53	43.00	2.79	0.36	0.00	0.37	0.00	45.73	1.70	6.77	2.17	41.02	2.76	14.45	2.25	1.85	1.43

Table 17. Maximum absolute uncertainty of the colour measurements.

Variables	Symbol	Unit	Maximum absolute uncertainty
Brightness	$R457$	$\% \beta_{\text{dif}, 0}$	± 0.1
Luminance factor	Y	$\% \beta_{\text{dif}, 0}$	± 0.1
Chromaticity coordinates	$x; y$	$(\% \beta_{\text{dif}, 0}) \cdot (\% \beta_{\text{dif}, 0})^{-1}$	± 0.1
Lightness	L^*	No units	± 0.01
Green to red scale	a^*		
Blue-to-yellow scale	b^*		
Absolute colour difference	ΔE		
Dark pixels index	DPI	$(\text{darker pixels}) \cdot (\text{total pixels})^{-1}$	± 1

Table 18. Data of non-structural sugars, total nitrogen, and phenolic compounds present in the wood closest (<5 mm) to the surface of the boards.

Leaching	Drying time hours	<i>n</i>	Total nitrogen		Fructose		Glucose		Sucrose		Total sugar		HMWP		LMWP		Total phenols	
			Mean	S. D.	Mean	S. D.	Mean	S.D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
			mg·(g dry mass) ⁻¹															
Control	0	3	0.56	0.04	0.58	0.14	0.01	0.01	0.07	0.04	0.65	0.12	1.83	0.20	0.90	0.09	2.74	0.29
	8	3	0.67	0.10	1.49	0.25	0.10	0.03	0.27	0.19	1.86	0.41	3.10	0.49	1.25	0.65	4.35	1.11
	16	3	0.76	0.04	1.34	0.61	0.25	0.19	0.56	0.20	2.15	0.96	4.58	0.31	1.37	0.29	5.95	0.40
	24	3	0.79	0.14	2.18	1.49	0.22	0.11	0.52	0.61	2.91	1.95	3.88	1.82	2.15	0.82	6.03	2.59
Mild	0	3	0.61	0.08	0.50	0.40	0.06	0.05	0.11	0.07	0.67	0.39	1.67	0.40	0.80	0.40	2.47	0.74
	8	3	0.55	0.03	1.58	1.15	0.15	0.10	0.05	0.04	1.78	1.07	2.47	0.22	2.07	1.40	4.53	1.26
	16	3	0.72	0.25	2.35	1.73	0.08	0.09	0.15	0.11	2.57	1.87	3.39	0.35	1.16	0.52	4.55	0.78
	24	3	0.71	0.03	0.97	0.13	0.02	0.02	0.16	0.16	1.15	0.16	4.03	0.60	1.84	0.38	5.86	0.35
Severe	0	3	0.55	0.04	0.06	0.05	0.04	0.03	0.05	0.06	0.15	0.10	1.89	0.75	0.90	0.25	2.79	0.90
	8	3	0.52	0.02	0.08	0.02	0.03	0.03	0.07	0.06	0.18	0.06	2.56	1.07	0.99	0.42	3.55	0.81
	16	3	0.54	0.04	0.24	0.04	0.10	0.07	0.09	0.04	0.43	0.07	3.06	0.91	2.30	1.46	5.35	2.37
	24	3	0.55	0.03	0.08	0.08	0.07	0.07	0.01	0.02	0.17	0.11	2.08	0.45	1.25	0.33	3.33	0.66

Table 19. Maximum absolute uncertainty on the chemical determinations.

Variables	Unit	Maximum absolute uncertainty
Fructose	$\text{mg}\cdot(\text{g dry mass})^{-1}$	± 0.01
Glucose		± 0.002
Sucrose		± 0.004
Total sugars		± 0.01
Total nitrogen		± 0.02
Low MW phenols		± 0.03
HMWP		± 0.02
Total phenols		± 0.03

Table 20. Determination of total carbon present in the wood closest (<5 mm) to the surface of the boards.

Leaching	Drying time hours	<i>n</i>	Total carbon	
			Mean	S. D.
mg·(g dry mass) ⁻¹				
Control	0	3	506.78	10.37
	8	3	508.52	4.18
	16	3	505.48	7.63
	24	3	503.37	1.50
Mild	0	3	511.50	4.67
	8	3	510.60	5.73
	16	3	507.32	2.44
	24	3	505.78	8.81
Severe	0	3	509.87	9.24
	8	3	509.44	6.46
	16	3	510.02	7.81
	24	3	505.39	7.61

Appendix C

DATA ANALYSIS.

C.i. Neural Networks models: the MATLAB® program used to produce them.

This program would produce a Neural Network model (*NN2*) that would have the luminance factor (*Y*) as dependent variable; and total sugar; nitrogen; high-molecular-weight phenols; and drying time as dependent variables.

```
%Neural Networks - training Bayesian
format bank;
data0=dlmread('j:\thesis\statistics\datamatLab.txt','t');
data=data0.';
%Define variable matrix
drytime=data(1,:);
R457=data(2,:);
Y=data(3,:);
b=data(4,:);
nitrogen=data(5,:);
sugars=data(6,:);
HMWP=data(7,:);
%Define targets and inputs
T=[Y];
P=[sugars;nitrogen;HMWP;drytime];
%Normalice data
[Pn,meanP,stdP,Tn,meanT,stdT] = prestd(P,T);
%Select 80% data for training
[R,Q]=size(Pn);
trnP=[1:2:Q 16:2:Q];
Ptr=Pn(:,trnP);
Ttr=Tn(:,trnP);
%Neural network using Bayesian training
netBay=newff(minmax(Ptr),[2,1],{'tansig' 'purelin'},'trainbr');
net.trainParam.goal=1e-5;
netBay=train(netBay,Ptr,Ttr);
an=sim(netBay,Pn);
a=poststd(an,meanT,stdT);
%Regression results vs. target
[m,b,r]=postreg(a,T);
ylabel('Y - Estimated results');xlabel('Y - Experimental results');
```

C.ii. Neural Networks models: weights and biases.

Table 21. Neural Networks models: weights and biases.

Neural Network model	Input	1 st Layer			2 nd Layer		Output
		IW ^{1,1}		bias ₁	LW ^{2,1}	bias ₂	
		<i>tansig</i> ₁	<i>tansig</i> ₁		<i>purelin</i> ₂		
NN1	Drying time	-0.39	-0.39	-0.03	0.70	-0.07	Y
	Nitrogen	-0.27	-0.26	-0.03	0.70	-0.07	
	Total sugar	-0.31	-0.31				
NN2	Drying time	-0.37	-0.37				
	Nitrogen	-0.24	-0.24	-0.01	0.67	-0.08	
	Total sugar	-0.29	-0.29	-0.01	0.67	-0.08	
	HMWP	-0.10	-0.10				
NN3	Drying time	0.15	0.63	-0.11	-0.83		
	Nitrogen	0.62	0.11	0.43	-0.78	0.09	
NN4	HMWP	-0.36	-0.30	0.02	0.63	-0.00	
	Drying time	0.36	0.30	-0.02	-0.63	-0.00	
NN1	Drying time	0.26	0.26	-0.00	-0.71	-0.12	
	Nitrogen	0.32	0.32	-0.00	-0.71	-0.12	
	Total sugar	0.19	0.19				
NN2	Drying time	0.27	-0.27				
	Nitrogen	0.33	-0.33	-0.00	-0.69	-0.12	
	Total sugar	0.20	-0.20	0.00	0.69	-0.12	
	HMWP	-0.00	0.00				
NN3	Drying time	0.25	-0.25	-0.01	-0.74	-0.12	
	Nitrogen	0.43	-0.43	0.02	0.74	-0.12	
NN4	HMWP	-0.38	0.38	0.06	0.48	-0.05	
	Drying time	-0.27	0.27	-0.06	-0.48	-0.05	
NN1	Drying time	0.94	0.09	0.46	-0.98	0.23	b*
	Nitrogen	-0.22	-0.39	-0.04	-0.44	0.23	
	Total sugar	-0.26	0.04				
NN2	Drying time	0.64	0.16				
	Nitrogen	-0.57	-0.00	-0.03	-0.84	0.09	
	Total sugar	-0.15	-0.24	0.24	-0.82	0.09	
	HMWP	-0.34	0.92				
NN3	Drying time	-0.17	-0.82	0.03	0.39	0.17	
	Nitrogen	0.35	0.43	-0.29	0.95	0.17	
NN4	HMWP	0.42	0.43	0.14	-0.47	0.06	
	Drying time	0.16	0.17	0.15	-0.49	0.06	

Note₁: The subscript in the activation functions indicates the Neural Network layer.

Note₂: After consecutive training weight and biases might vary slightly.

C.iii. Calculation of the order (m) and rate constant (k) of a equation based on simple reaction kinetics.

The colour variables selected to construct the simple reaction kinetics model, namely luminance factor (Y), brightness ($R457$) and the blue-to-yellow scale of CIE Lab (b^*), decrease during drying time. Darker surfaces reflect less light, hence the values of Y and $R457$ were low; likewise, darker surfaces shifted the measurement of the blue-to-yellow scale of CIE Lab to the blue extreme of the scale. To calculate the order (m) and the rate constant (k) of an equation on the basis of simple reaction kinetics, the variables were transformed to represent an increasing rate of discolouration. Y and $R457$ are 0 to 100 scales, so subtracting the experimental values from 100 did the necessary transformation; b^* is a -127 to 128 scale, therefore the experimental values were subtracted from 255. Figure 25 presents the colour variables plotted versus drying time, and the equations used to calculate the discolouration rate for each of the colour variables. Table 22 presents the calculation of the order (m) and rate constant (k) of the equation based on simple reaction kinetics.

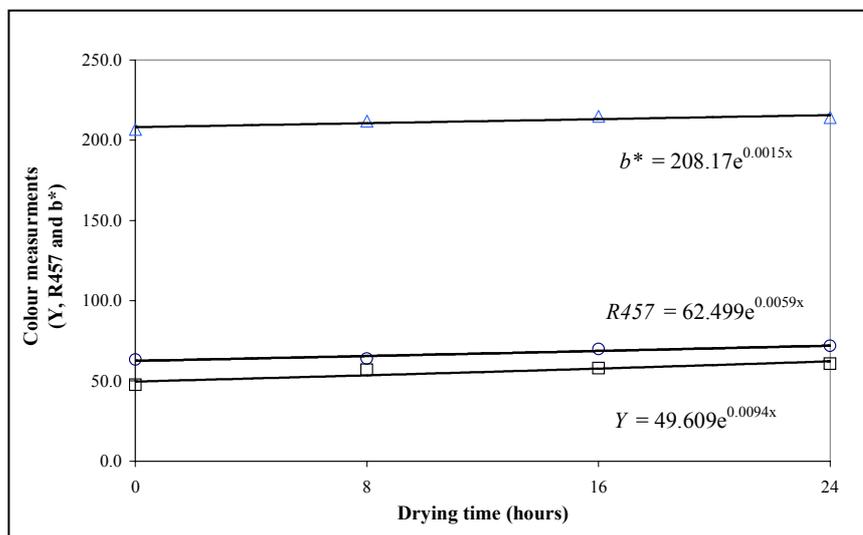


Figure 25. Plot of colour versus drying time: calculation of colour development rate.

Table 22. Calculation of the order (m) and constant (k) of the simple reaction kinetics model.

Drying time hours	Total nitrogen mg·(g dry mass) ⁻¹	Ln (total nitrogen)	(100- Y)	$\frac{d(100 - Y)}{dt}$	$Ln \frac{d(100 - Y)}{dt}$	m	k
0	0.577	-0.549	47.54	0.466	-0.763	0.979	0.826
8	0.579	-0.546	56.86	0.503	-0.688		
16	0.671	-0.399	58.01	0.542	-0.612		
24	0.684	-0.380	60.72	0.584	-0.537		
Drying time hours	Total nitrogen mg·(g dry mass) ⁻¹	Ln (total nitrogen)	(100- R_{457})	$\frac{d(100 - R_{457})}{dt}$	$Ln \frac{d(100 - R_{457})}{dt}$	m	k
0	0.577	-0.549	63.24	0.369	-0.998	0.614	0.528
8	0.579	-0.546	63.86	0.387	-0.950		
16	0.671	-0.399	69.78	0.405	-0.903		
24	0.684	-0.380	71.86	0.425	-0.856		
Drying time hours	Total nitrogen mg·(g dry mass) ⁻¹	Ln (total nitrogen)	(255- b^*)	$\frac{d(255 - b^*)}{dt}$	$Ln \frac{d(255 - b^*)}{dt}$	m	k
0	0.577	-0.549	206.76	0.312	-1.164	0.156	0.342
8	0.579	-0.546	211.93	0.316	-1.152		
16	0.671	-0.399	214.87	0.320	-1.140		
24	0.684	-0.380	214.07	0.324	-1.128		

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