VARIATION OF OAK WOOD PROPERTIES INFLUENCING THE MATURATION OF WHISKY

D. Phil. Thesis

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Foreword

This research was initiated and funded by United Distillers. The general purpose of the research was to examine how oak wood influences the maturation of whisky and whether the origin or type of wood can affect flavour significantly. Precise terms of reference were not set at the beginning of the project and these were developed as a result of a literature review, preliminary studies and by the resources available.

Analytical work was carried out at the Oxford Forestry Institute and the Dyson Perrins Organic Chemistry Laboratory, University of Oxford; International Technological Services, Glenochil; the Qualité des Bois unit at the INRA Centre of Research at Nancy, and l'Ecole Supérieure du Bois, Nantes.

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ABSTRACT

Oak casks are used for the maturation of a wide variety of alcoholic beverages including Scotch whisky. The process of maturation has a profound but variable effect on the colour and flavour of the whisky, with cask wood playing an important role, particularly through the release of extractives to the distillate. This thesis examines variation in European oak wood (*Quercus petraea* Matt. Liebl. and *Q. robur* L.) of ellagitannins, oak lactones and other extractives, and physical wood properties. Investigations particularly sought to establish whether the properties and their effects on flavour can be predicted from either the species, the various forest origins, or identifiable wood or tree characters.

The treatment of wood after felling, through seasoning and particularly the toasting or charring of casks, has a major effect on the levels of many extractives. Heating reduces the concentrations of ellagitannins and increases levels of lignin-derived extractives. However, the effects are not such as to render variation in untreated wood inconsequential. Within trees, the concentration of soluble ellagitannins declines and the composition changes with heartwood age. When heartwood of a similar age is compared concentrations vary by up to ten times between different trees, making up to 14% of the heartwood dry weight. Concentrations of oak lactones appear to increase with heartwood age and are also very variable between trees.

Wood samples were taken from two different forests, corresponding to opposing types of French oak used for cooperage. Over 70% of the total variation of soluble tannins in the wood occurred between the forests. A difference between the two forests in the rate of tree growth and the heartwood age of samples could not explain all of the difference in the amounts of soluble tannins. After heating the wood, the concentrations of other extractives and the flavour and colour imparted to solutions, also varied significantly between the two forests and between trees within each site.

Studies on clonal, progeny and provenance material concluded that the concentration of ellagitannins, oak lactones and many physical properties of heartwood are under strong genetic control. However, a large proportion of this variation is attributable to variation between the two species *Q. robur* and *Q. petraea*. *Q. robur* is characterised by high concentrations of tannins and, after heating, of lignin-derived products, but low or negligible levels of oak lactones. *Q. petraea* has opposing extractive properties and after heating, imparts a more pleasant and complex flavour. Although there is large variation between trees within each species, this difference between the species is proposed as the main factor explaining the different flavour and extractive properties found in European oak wood from different origins.

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Glossary of terms

Because of the inter-disciplinary nature of the research the use of technical terms has been kept to a minimum. However, they have been used occasionally where they provide concise and accurate descriptions. An understanding of the following terms is assumed and they are not explained in the main body of the text.

Anova abbreviation for 'analysis of variance'.

Bole the trunk of the tree from which all quality wood is obtained.

Cambial age the number of annual rings from the pith.

Cask viability the speed and quality of maturation that occurs in a given cask. Declines as a cask is reused.

Coupe or felling coupe. Area of forest designated to be clear-cut in a single year.

Heartwood age the number of annual rings counting towards the pith from the sapwood/heartwood boundary.

Organoleptic (*adj.*) affecting the senses of taste or smell.

Provenance the term is often used as an equivalent to population. More precisely it refers to the place from which a seed lot has been collected. Seed origin refers to where the most remote traceable ancestors came from.

Silviculture the commercial cultivation of trees for the production of timber.

Thinning the felling of some trees growing on a site to assist the subsequent development of remaining trees.

Note on the representation of statistical significance

The probabilities of falsely rejecting the null hypotheses of statistical tests are represented in two ways throughout this thesis. Either actual numeric probabilities are given or symbols are used, which, unless specified otherwise in the table heading, have the following meanings.

*	$P < 0.05$
**	$P < 0.01$
***	$P < 0.001$
ns	$P > 0.05$ (not significant)

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Chapter 1: The effects of oak wood on the maturation of whisky

For whereas the order on which magic reckons is merely an extension, by false analogy, of the order in which ideas present themselves to our minds, the order laid down by science is derived from patient and exact observation of the phenomena themselves.

J.G.Frazer - *The Golden Bough*

1.1. Chapter summary

Oak casks are used for the maturation of a wide range of alcoholic beverages. Focusing on whisky production, this chapter reviews the influence of oak wood properties on the flavour of alcoholic beverages. It examines whether the selection of wood or casks on the basis of their effects on flavour can be justified given our present understanding of the process. The current use of oak casks in whisky manufacture is summarised briefly and the wood properties, both chemical and anatomical, that might influence flavour are described. These characters vary in both the virgin wood and the used casks. Likely factors influencing this variation are identified. The review highlights weaknesses in past studies and outlines the aims of later chapters which investigate how wood and cask properties, which influence flavour, vary and whether they can be identified and selected for.

1.2. Role of oak in the maturation of whisky

1.2.1. The use of oak casks for the maturation of whisky

The whisky industry has traditionally always used oak casks for the maturation of their product and in both the United States and the U.K. there are now legal requirements for their use. In the U.S.A. the bourbon industry is required to store the raw distillate for a year in new, charred oak casks. In Britain, the law demands that Scotch whisky be stored in oak casks for a minimum of three years. Legal constraints in both countries prevent the use of flavour additives and discourage the adoption of new production techniques.

The different legal requirements of the U.K. and the U.S.A. reflect very different traditions in the use of casks. The bourbon industry, prevented from reusing old casks, is the main purchaser of new American oak casks. In contrast, the Scotch industry does not generally purchase new casks, depending instead upon the reuse of casks already used for the maturation of other alcoholic beverages. Traditionally, particularly in the 19th century, old sherry casks were used, but by far the most common source of oak casks presently used by Scotch producers are old bourbon casks. It is estimated that between 700-800,000 used bourbon casks are sold every year and although some are used for rum and brandy production, the Scotch whisky industry is a major purchaser.

Within the Scotch whisky industry it is estimated that approximately 13 million casks are in use at any time.

Oak casks are used for the production of a wide range of alcoholic beverages, including wine, brandy, sherry and rum. These industries use a wider range of oak casks than that used by the whisky industry.

1.2.2. Process of whisky maturation

The effect of maturation on whisky is quite distinct, with the raw, colourless, immature spirit generally having few of the desirable properties sought in whisky taste and aroma (Reazin 1983). Therefore the importance of the maturation process should not be underestimated. The following points are known about the maturation of whisky (Nishimura and Matsuyama 1989):

(i) Satisfactory maturation times may vary from three to more than ten years.

(ii) There is normally a significant flavour difference between matured and unmatured spirits.

(iii) Volume and alcohol content are lost due to the evaporation of water and alcohol through the porous wood of the casks.

(iv) Maturation time and the quality of the matured spirit may vary according to the type of whisky, the size, wood type and prior treatment of the cask and the environment in which the whisky is matured.

The mechanisms by which maturation in oak casks occurs are incompletely understood. Research has been carried out to identify compounds that contribute to the flavour or aroma of whisky, referred to as flavour congeners. Correlations between descriptive flavours and chemical analyses of mature whiskies have identified over 400 flavour congeners (Philp 1989a). The principle ones are esters, carbonyls, sulphur compounds, lactones, phenols, and nitrogenous bases, including compounds with both desirable and undesirable flavours. In some cases the origin and method of synthesis have been further studied and the involvement of the maturation stage confirmed. Changes in taste or aroma will be due to changes in these flavour congeners, with their rate of formation being generally greatest in the first year of maturation (Reazin 1983). The methods by which this may occur, during the maturation of whisky in oak casks, are listed below (Nishimura and Matsuyama 1989).

(i) Direct extraction of wood chemicals.

(ii) Decomposition of wood macromolecules and extraction of these into the distillate.

(iii) Reactions between wood components and constituents of the raw distillate.

(iv) Reactions involving only the wood extractives.

(v) Reactions involving only the distillate components.

(vi) Evaporation of volatile compounds through the cask.

However, as emphasised by Piggott *et al.* (1992) it is the concentration in the 'headspace' (the air space in the cask or container) rather than in the mature spirit that determines the influence on flavour of many volatiles. The concentration of volatiles in the headspace will be influenced by any factors affecting their solubility in the distillate, including the concentrations of involatile compounds.

Canaway (1983) described how the variation of samples from different casks could equal the variation between samples of differing age. Such variation between casks could be due to differences in the raw distillate, the conditions of maturation or the cask wood.

Although both the raw distillate and particularly the conditions of maturation may play an important role in determining the result of maturation, the oak cask in which maturation takes place appears to be of prime importance to the final flavour of whisky (Williams 1983a). The type of cask can affect both the taste, colour and aroma of whisky. The desired effect of maturation will depend upon the nature of the immature whisky. It may sometimes be desired that the oak wood contribute significantly to the flavour, while for other whiskies, perhaps with an already characteristic taste, the desired effect of maturation may be less. The time taken to reach a satisfactory condition is of financial and practical concern for the manufacturer and varies according to the type of cask used.

1.2.3. Cask and oak types

The source of oak wood used for the construction of casks will normally be one of two general types. Most commonly used is American oak, which is predominantly *Quercus alba*, but may include wood from 10 or more other species of American white oak (Singleton 1974). The majority of casks used by both the bourbon and Scotch whisky industries will be of this type. Less often used is European oak, consisting of wood from either *Quercus robur* or *Quercus petraea*. Spanish sherry casks may be manufactured from either American or European oak, and it is possible that a single cask may include both types of wood, particularly after repair or reconstruction work.

The division of wood into American and European oak will encapsulate more than simply the botanical species. The two types are associated with different environments and are generally used by very different cooperage industries. The growth, silviculture and exploitation of oakwoods have a long history, particularly in Europe. In general, classical oak silviculture has been oriented towards producing wood with regular, narrow annual rings through maintaining

high stand densities (Schütz 1994). Such slowly grown oak was considered to produce better quality wood (Weaver and Spiecker 1994). However, attitudes have begun to change as it has been realised that faster grown oak can produce equally high quality wood (Kenk 1994).

There is also a long tradition of using different types of French oak for different purposes (Schahinger 1991), as the effect on flavour is thought to vary according to the forest where the oak is grown. However, there is great uncertainty over what determines the different geographic types. Wood from different locations, even if of the same species, may have grown in different climatic or silvicultural regimes. Furthermore the felling and later selection and treatment of wood may vary, with many oak types associated with particular cooperage methods as well as geographic origins. Therefore it is often difficult to discriminate between wood and cask origins.

Although most of the oak used derives from the USA or Europe, many other sources of oak have been used for the production of whisky and other alcoholic beverages. Table 1.1 lists and describes some past and present sources of oak cask wood. Wood other than oak is occasionally used to store alcoholic beverages, although the cask is normally coated on the inside by paraffin or silicone to prevent leakage and the release of unpleasant odours (Knox pers. comm.). *Robinia pseudoacacia* has been reported as being used for wine casks in Hungary (Lamfalussy 1953; Molnar *et al.* 1985), apparently without any coating. Trials in India on the suitability of 12 native timbers to mature whisky found *Terminalia tomentosa* and *Shorea robusta* to be the best substitutes for imported oak. Other woods that gave tolerable results included *Quercus dilata* and *Q. semecarpifolia* (Anon. 1950).

Table 1.1: Sources of oak wood reported as having been used for the maturation of alcoholic beverages.

Wood origins	Species reported as being used for cooperage	Main cask uses	Comments
America	*Q. alba* and related white oak species (see Singleton 1974).	Bourbon and subsequently Scotch whisky. Wine and sherry.	Low tannic content but high levels of volatiles.
Western Europe (mostly France)	*Q. robur, Q. petraea.*	Wine and brandy.	Varies depending upon precise origins.
Eastern Europe	*Q. robur, Q. petraea, Q. cerris.*	Wine, brandy, beer.	Present state of oak forestry uncertain - but potentially a major source of cask wood.
Japan and Asia	*Q. dentata, Q. crispula, Q. mongolica.*	Whisky and brandy.	*Q. crispula* reported to release a sweet taste (Kanazashi pers. comm.).
Near East	*Q. mirbeckii* and possibly others.		Oak staves imported from Iran and Turkey during 1940-50's (Williams 1983b).
South America	probably *Q. copeyensis* (see Singleton 1974).	Sherry and Whisky casks.	Costa Rican oak reported to have been exported to Spain.

1.2.4. The cooperage industry

The methods and regulations of cooperage differ between America and Europe, with tighter control generally being found in the supply of American oak. In America the cooperage industry

is more automated and operates on a much larger scale than most European cooperages. It accounts for approximately 3% of all the American white oak harvested each year. In the American mid-west 950,000 - 1,200,000 casks per year are made, the majority destined for the bourbon industry (Knox pers. comm.). In comparison the French cooperage industry, which is by far the largest in Europe, produced around 160,000 casks per year in the early 1990's. However, while new American casks may cost between £50-£100, new French casks will normally be over £250 each.

1.2.5. Selection of cask and wood types

The most important factors governing the choice and use of oak casks are practical concerns, such as the ease of supply and economics of use. Therefore, although their previous use is often claimed to contribute to the taste of the mature whisky, the purchase of used casks is due primarily to their low cost. Economic constraints demand the reuse of casks within the industry, despite casks decreasing in viability with each use.

Singleton (1974) claimed that whisky producers have nearly always displayed some preference for the source of oak-casks used, but the preferred wood has often been identified simply by the port of importation. Despite widely made claims and traditions that different types of oak will influence maturation in varying ways, at present the flavour effect of oak does not play a major role in the selection of casks. However, the producers of other alcoholic products, particularly wine and brandy, show more discrimination between different types of casks. This is reflected in the much higher prices that French coopers are able to demand for their products, and it is interesting to observe that despite their own sizeable cooperage industry, the U.S.A purchases around half of all French cask exports (Knox pers. comm.).

1.3. Wood Properties affecting the maturation of whisky

1.3.1. Wood chemistry

Flavourful whisky is thought generally to contain high levels of wood-derived compounds. Therefore particular focus has been placed on the role of oak extractives as either flavour congeners in themselves, or involved in their formation or breakdown.

Extractives are compounds found in oak wood that are soluble in either water or organic solvents. There is no precise definition, with the composition dependent upon the solvent used, method and conditions of extraction. The synthesis of most extractive material is associated with the formation of heartwood, and is thought to be controlled by both genetic and environmental factors

(Sjoström 1981). Those compounds believed to be of importance in the maturation of whisky include the following groups.

Tannins and their derivatives

The most important group of phenolic compounds are the tannins, being a loosely defined group of water soluble plant polyphenols (Haslam 1981). The main characteristic responsible for their biological activity, including their astringency (Hagerman and Butler 1991), is their ability to bind proteins.

The tannins may be further divided into two separate groups. The principle group in oak are the hydrolysable tannins, including gallotannins and ellagitannins, which are described by Hagerman and Butler (1991) as having a polyol (normally D-glucose) as the basic structural unit, of which the hydroxyl groups have been esterified by gallic acid or hexahydroxydiphenic acid (HHDP acid). These tannins are easily hydrolysed either enzymically or in acid or base conditions, to form free gallic or HHDP acid, the latter spontaneously lactonising to give ellagic acid. Their biosynthesis probably occurs at the transition zone boundary, during the transformation of sapwood to heartwood (Hillis 1987), with their precursors thought to derive from the shikimic acid pathway (Haslam 1981; Haslam 1992; Gross 1992). The ellagitannins have been found to make up to 10% of heartwood dry weight (Scalbert *et al.* 1988a). The most common ellagitannins in oak (see figure 1.1) have been identified as vescalagin and castalagin (Mayer *et al.* 1967), with eight water soluble ellagitannins being characterised by Herve du Penhoat *et al.* (1991a and b). The second category are the non-hydrolysable tannins (condensed tannins or proanthocyanidins). These are oligomeric or polymers of flavonoid units, linked by carbon-carbon bonds, that are not susceptible to hydrolysis (Hagerman and Butler 1991). In oak heartwood they are found in much lower concentrations than hydrolysable tannins (Scalbert *et al.* 1988a and 1988b).

The solubility of tannins may vary, according to their type, size and various binding reactions with other compounds. Solubility will generally decline with increasing molecular weight. Therefore polymerisation is likely to lead to a decrease in the level of soluble tannins. This effect of polymerisation has been widely reported for condensed tannins (Hagerman and Butler 1991), but fewer studies have examined hydrolysable tannins. However, Peng *et al.* (1991) studying tannins in the wood of *Castanea sativa* and *Quercus petraea* concluded that insolubilisation of tannins in heartwood probably results from their slow oxidation, leading to polymerisation or copolymerization of both condensed and hydrolysable tannins with cell wall components. Such oxidation reactions could be enzymatic (involving peroxidases), but in heartwood are more likely to be non-enzymatic. The degree of polymerisation would be dependent upon how readily tannins oxidise, with condensed tannins considered more vulnerable to such reactions. Oxidation and polymerisation of tannins will considerably modify their astringency and toxicity (Peng *et al.*

1991). The lower solubility of tannins, due to polymerisation, is thought to cause the loss of astringency as fruit ripens (Hagerman and Butler 1991) and also to explain the reduction in central heartwood durability and resistance to fungal attack (Hart and Hillis 1972; Peng *et al.* 1991; Scalbert 1992b). Partially oxidised and polymerised ellagitannins may also influence heartwood colour (Charrier 1992; Haluk *et al.* 1991; Klumpers *et al.* 1994).

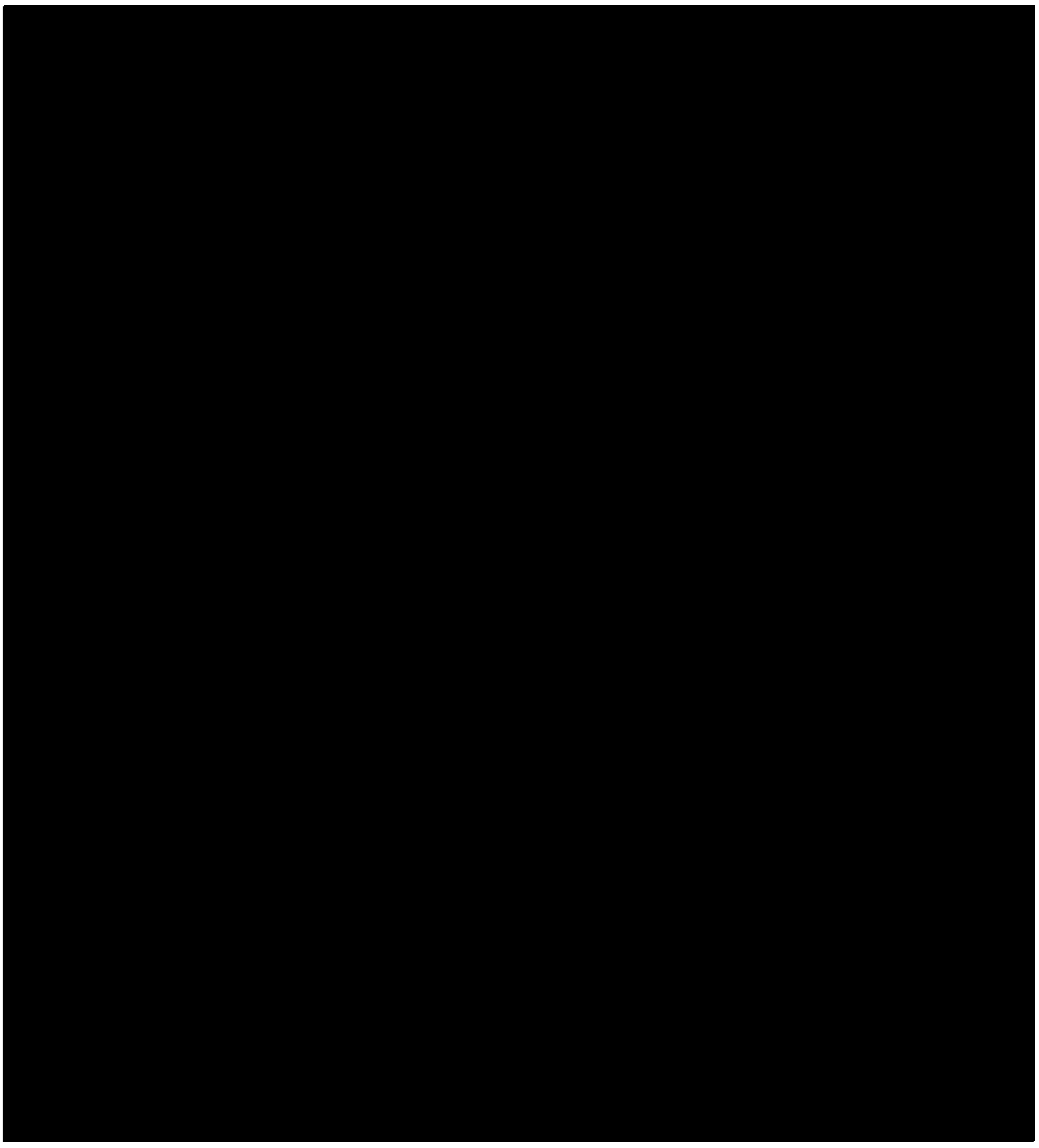


Figure 1.1: Structure of ellagitannins identified in extractions from the heartwood of oak.
Vescalagin (1); Castalagin (2); Grandinin (3); Roburin E (4); Roburins A; B; C; D (5, 6, 7, 8).
L = lyxose; X = xylose.

(from Viriot *et al.* 1993).

Numerous studies have described tannins in whisky as arising through direct extraction from the wood, the concentration in maturing whisky increasing rapidly in the first six months, after which the rate of increase declines (Baldwin *et al.* 1967; Reazin *et al.* 1976; Baldwin and Andreasen 1974). However, the role of tannins in the flavour of whisky and other alcoholic beverages, has not been well established despite the taste often being described in terms of a tannin character. Studies have been further confused by imprecise measurement and use of the term tannins. Most early studies used solely the Folin Denis or Folin Ciocalteau methods of estimating 'total tannins', or more accurately 'total phenolics', which measured both tannins and non-tannin phenolics. Puech *et al.* (1990) showed that the Folin Denis measure of total phenolics gave a good correlation with the tannin content of wood extracts but not with the amounts found in maturing spirits, due to the higher levels of ellagic acid and lignin derived phenolics.

Many recent studies report very low levels or no ellagitannins occurring in many spirits (Puech *et al.* 1990; Puech and Moutounet 1992; Ford and Done 1991). Viriot *et al.* (1993) describe the level of ellagitannins in maturing brandies as increasing over the first 5 years, but then subsequently decreasing with further ageing, probably due to chemical degradation through hydrolysis. In a comparison between wine and an alcohol-water solution, both of which had been stored in oak casks for 12 months, Chatonnet *et al.* (1989) found that the wine contained lower levels of ellagitannins. This is probably due to reactions with wine constituents, involving binding with proteins or oxidation reactions, whether they be direct, coupled or after hydrolysis. Tannins or their products may be important to the maturation process as oxidative catalysts, or in the removal of sulphides (Chatonnet *et al.* 1991). The hydrolysable products of tannins, such as gallic and ellagic acids, have been found in many spirits (Jindra and Gallander 1987; Puech and Moutounet 1992; Wilker and Gallander 1988), suggesting the breakdown of the hydrolysable tannins. The bitterness and astringency of gallic acid has been demonstrated (*eg* Robichaud and Noble 1990) and ellagic acid is likely to have a similar effect on flavour. However, Sefton (1991), describing results of Somers (1990), claimed that in regards to wine maturation the sensory role of oak was not related to total phenolics, while the role of the involatile tannins was unknown. Viriot *et al.* (1992) were of the opinion that ellagitannins do not play an important role in the maturation of cognac or other spirits, while Herve du Penhoat *et al.* (1991b) thought that tannins contribute indirectly to the taste of brandies, through their complexing or reducing properties. Therefore, despite their abundance in the extract of oak wood, the role of tannins in the flavour of whisky remains uncertain.

A variety of other phenolic compounds is also found in the extract of oak wood. The fluorescent coumarin compound scopoletin is used as an indicator of spirits having been matured in wooden casks (Puech and Moutounet 1988). Nabeta *et al.* (1987) reported the lignan lyoniresinol as being the most abundant phenolic compound both in the heartwood of European oak and in cask-aged

brandy. A wide range of low molecular weight phenolic compounds, particularly aromatic aldehydes and acids, derived from lignin are thought important and these are discussed below.

Lignin degradation products

Many compounds found in mature whisky derive from oak lignin. A number of studies have described differences in the composition of these compounds between different spirits (*eg* Lehtonen and Jounela-Eriksson 1983) and used them to indicate genuine ageing in oak casks (Delgado *et al.* 1990). Mechanisms for their formation have been proposed by many authors (Baldwin *et al.* 1967; Puech *et al.* 1977; Reazin 1981; Nishimura *et al.* 1983; Nishimura and Matsuyama 1989; Conner *et al.* 1989; Sarni *et al.* 1990) and the following pathways have been verified (Nishimura and Matsuyama 1989) for the origin of lignin degradation products in matured distillate.

(i) Degradation of lignin to aromatics due to toasting or charring of casks.

(ii) Extraction of monomeric compounds and of lignin from the wood.

(iii) Formation of aromatics by ethanolysis of lignin.

(iv) Further conversion of compounds in the spirit.

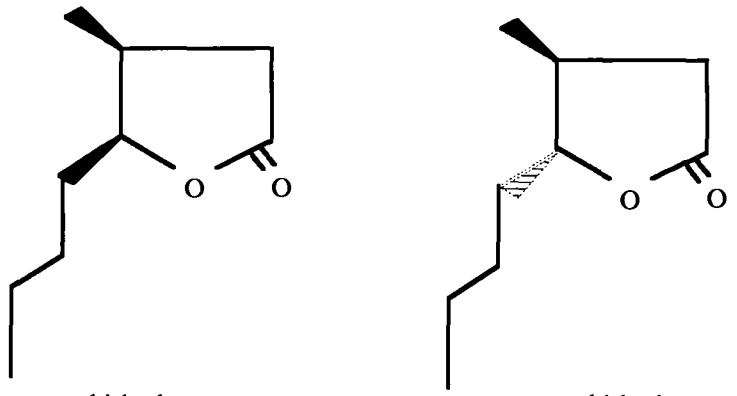
Ethanolysis involves the reaction of the distillate ethanol with lignin, to produce an alcohol-soluble form of lignin. As the solubilization involves the splitting of alkyl aryl ether covalent linkages it is a slow process likely to occur throughout the ageing process (Viriot *et al.* 1993). Studies (Puech and Sarni 1990; Puech *et al.* 1985) have described an easily extracted lignin complex, that amounted to approximately 4% of the total lignin, and have observed that higher levels of tannins appeared to increase the rate of delignification. A variety of phenolic compounds may be produced, which may readily oxidise to give various aromatic aldehydes such as vanillin and syringaldehyde, as well as their respective acids (Baldwin *et al.* 1967). Puech (1981) described how lignin underwent intense oxidation when in contact with spirits and oxygen, forming aromatic aldehydes. Such reactions may also occur by means of heating or charring the wood.

The role of lignin-derived products on flavour is uncertain, with none of the many studies of them providing conclusive evidence of an effect on flavour. Indeed, their levels in matured distillate are often lower than their individual flavour thresholds (the concentration at which they produce a detectable flavour). However, Maga (1985) found that compounds were synergistic, with a mixture of seven congeners giving a very low mutual flavour threshold of about 2 ppm in 40% alcohol. Therefore, despite individually low concentrations, they may nonetheless influence

flavour. Lignin degradation products also highlight the difficulty in defining the extractive content of wood, with many compounds being formed indirectly through subsequent reactions.

Oak lactones

Masuda and Nishimura (1971) identified the two gamma-lactone isomers (see figure 1.2 from Tsukasa, 1988) as being major components of the volatile fraction of oak wood extractives. These lactones, the so called oak or whisky lactones, derive solely from oak and may be formed from the oxidation of lipids (Maga 1989b). Tsukasa (1988) reviewed the chemistry of oak lactones, examining a number of different synthesis pathways. These lactones increase in concentration in the distillate during maturation in oak casks, reaching concentrations of up to 5 ppm (Nishimura and Matsuyama 1989). Otsuka *et al.* (1974) found a direct correlation between oak lactone concentration and assessed quality scores of different whiskies. Studies on their concentration in red wine (Chatonnet 1991) suggest they are beneficial to flavour in low concentrations but detrimental in excess, having an aroma of new oak and coconut. Reazin (1981) claimed that their flavour was modified by the presence of furfural. The precise role of oak lactones in whisky flavour is unknown, although relatively high levels in mature whisky are considered desirable.



cis -whisky lactone *trans* -whisky lactone

Based on Tsukasa (1988)

Figure 1.2: Structure of the two isomers of oak lactone.

Acids

Measurements of volatile and fixed acids have found that both may increase during maturation (Reazin 1983). The amount of acetic acid has been found to increase dramatically during maturation (Franco and Singleton 1984), with studies by Reazin *et al.* (1976) showing that most derives from wood extractives rather than the distillate ethanol. Nykänen (1984) identified dicarboxylic acids as generating aroma compounds and catalysing reactions forming lactones, esters and other compounds.

Fatty acids and other apolar extractives

The apolar fraction of oak extract has received relatively little study, despite it being thought that many compounds are important flavour components. The group includes steroids and triglycerides, as well as palmic, stearic and oleic acids.

Carbohydrates

The levels of many sugars are found to increase in maturing spirits, normally displaying a hyperbolic increase over maturation time (Reazin 1983). Many of the reducing sugars probably derive from the breakdown of hemicellulose and hydrolysable tannins (Wilker and Gallander 1988). Nykänen (1984) found that the most abundant sugars in the maturing spirit were glucose, arabinose, and proto-quercitol, while other studies (Charrier 1992) have found fructose and glucose as the most common sugars in oak extract. The thermal degradation products of cellulose and hemicellulose, such as furan and pyran volatiles, have also been reported frequently in whisky, most recently by Clyne *et al.* (1993). However they are not thought to have a major influence on flavour (Chatonnet *et al.* 1991).

Nitrogenous compounds

Both polyphenoloxidase and peroxidase activity have been found in heartwood of oak (Ebermann and Stich 1992). Amino acids and other nitrogenous compounds, such as pyrazines and pyridines (Maga 1985) have been detected in charred oak extract, with concentrations of 17 and 2 mg respectively per 100g dry weight of wood. Although it is not certain that they influence flavour, such compounds are known to have very low flavour thresholds.

Terpenes

This group of volatile compounds has received relatively little attention, despite being important flavour and colour compounds in spices, perfumes and other aromatic products. Studies by Sefton *et al.* (1990a), Nabeta *et al.*(1986) and Nishimura *et al.* (1983) have identified monoterpenes, sesquiterpenes and various norisoprenoids among the constituents of oak wood. Sefton (1991) described the norisoprenoid group as the most diverse and identified, in oak extract, precursors of compounds patented as flavour additives.

Other compounds

A range of additional compounds found in the extractive content of oak may be of relevance to whisky maturation, but have yet to receive attention. Those of importance include both volatile and non-volatile compounds. Over 200 volatile components of cask wood have been identified

(Sefton *et al.* 1990a), and many others may be present. Non-volatiles, such as tannins, may influence flavour by direct or indirect effects, such as affecting the solubility of volatile compounds (Piggott *et al.* 1992). As already indicated, while whisky may obtain some flavour congeners by direct extraction, many others, particularly aromatic aldehydes and their related acids, will derive from both extraction and further reaction of compounds from the cask.

1.3.2. Wood anatomy

Effects of wood anatomy

The use of oak wood for the manufacture of casks is primarily due to its ability to contain liquids with little leakage. The wooden cask must also meet other cooperage criteria, such as suitable strength and flexibility. Anatomical features could influence the maturation process through two possible mechanisms. Firstly the location of extractive deposits and any factors that influence the permeability of wood, are likely to affect the availability of wood extractives to the maturing distillate. Secondly, the cask wood will influence the maturation conditions and environment. For example, any features that influence the movement of gases through the wood will affect both the rate of evaporation of the distillate and the availability of oxygen.

As well as influencing whisky maturation directly, anatomical features may correlate with other properties that affect maturation. If chemical requirements can be shown to correlate with physical properties the selection of wood for flavour effects could be easier.

Anatomy and properties of oak wood

Oak wood is ring porous. The earlywood is laid down at the start of the growing season and consists mostly of large vessels (see figure 1.3). The latewood has a greater proportion of fibres and only small vessels are present. The rate of growth determines the size of annual rings and the proportion of late to earlywood increases with ring width.

Sapwood, containing living parenchyma cells and frequently starch, eventually transforms into heartwood. This transformation involves cell death, the removal of starch and laying down of extractives. Structurally the woods are very similar, despite the lower permeability, greater durability and darker colour of heartwood. The heartwood periphery may be undulatory, and does not normally correspond with a specific growth ring.

Tyloses are occluding structures found in vessels which develop through pit apertures from adjacent parenchyma cells. The formation of tyloses is generally associated with the conversion of sapwood into heartwood. However they also form in the sapwood of felled timber to an extent depending upon the conditions and duration of storage (Alexander 1972; Bolton pers.comm.).

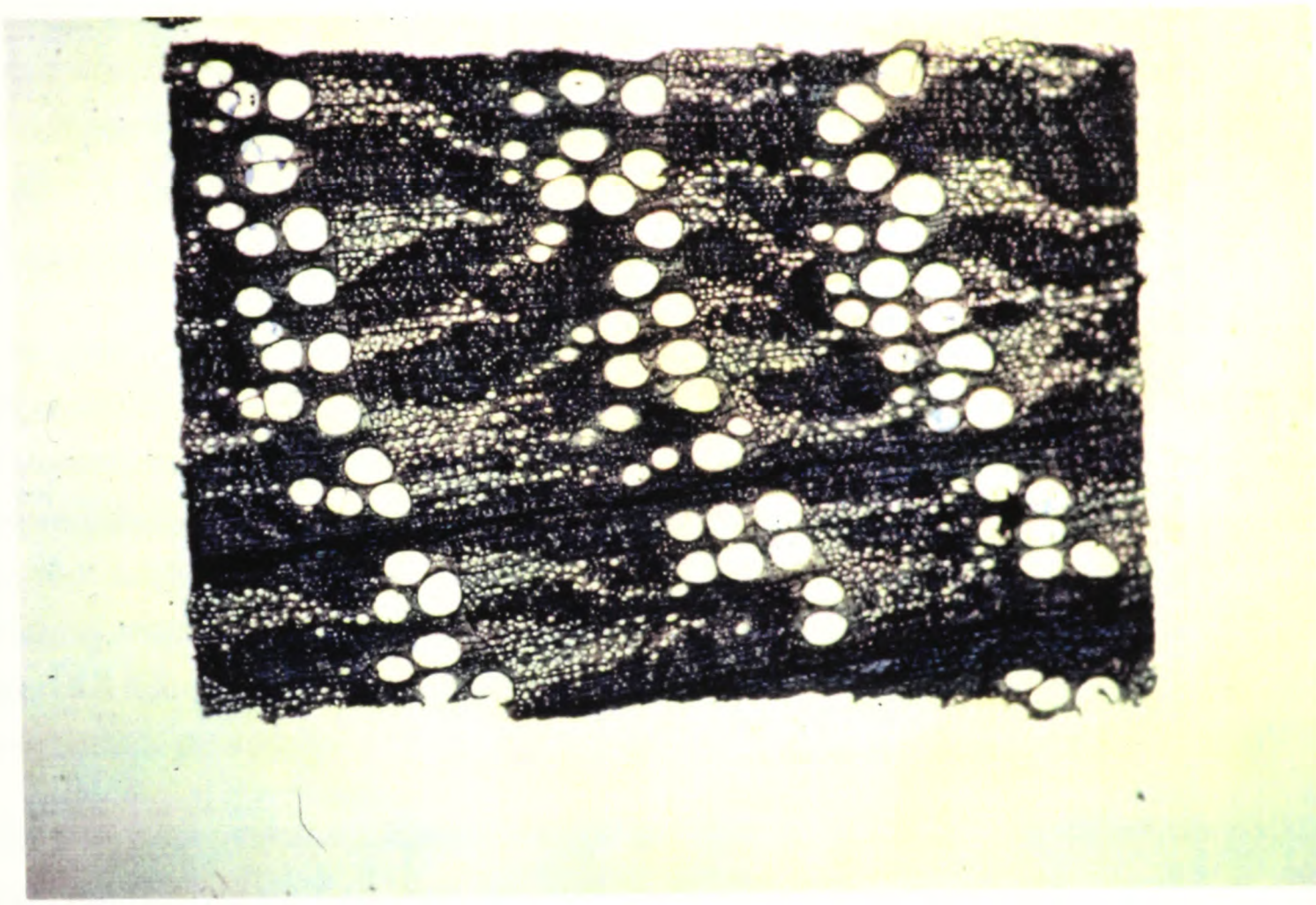


Figure 1.3: Photo-micrograph of oak wood which shows the abrupt transition from earlywood to latewood.

Tyloses may also form as a response to fungal infection or injury. Wheeler and Thomas (1981) reported the findings of Williams (1942) who found that in white oak the latewood vessels rarely have tyloses, in contrast to the larger earlywood vessels. The precise stimulus for their formation remains uncertain, with exposure to air and changes in the concentration of ethylene among the proposed causes (Hillis 1987). Tyloses are absent in the wood of some species of oak, with *Q. rubra* being a notable case, making their wood unsuitable for leak-proof cooperage.

The number and structure of wood rays are thought to influence the radial permeability of wood, possibly to both gases and liquids. Rays may consist of either single (uniseriate) or 5-30 (multiseriate) rows of cells across a transverse section and be hundreds of cells in height. The size of rays was found by Feuillat (1991) to be inversely correlated with their number.

Permeability

Low wood permeability is necessary in order to ensure a tight cask with little leakage. The impermeable nature of oak wood, together with its wide availability, were probably the main reasons for its initial use. Tyloses are generally considered to be the primary cause for heartwood impermeability to both gases and liquids. Lehmann (1988) found that air permeability of *Fagus syvatica* heartwood was closely correlated to tylose formation. Likewise Kuroda *et al.* (1988), in studying twenty hardwood species, found that the presence of tyloses in the wood could be predicted from measuring permeability. The ring porous nature of oak may also be important in restricting its permeability.

The most relevant measure of permeability is the tangential permeability, which corresponds to movement through a stave, from the inside to outside of a cask. The size, abundance and distribution of vessels, degree of closure by tyloses, the number of wood rays and other anatomical factors are all likely to influence tangential permeability. Due to the ring porous nature of oak, the size of annual rings may also be important, as this will determine the relative abundance of large vessels, which are found only in the early wood. The anatomy of oak wood is very variable, even when the ring size is similar.

However, studies examining the permeability of wood in relation to specific anatomical features have rarely found any strong correlations. Kuroda *et al.* (1988) found that neither vessel radius, nor percentage of vessels by volume nor total number of vessels per cross sectional area, gave good agreement with permeability in ring porous woods such as oak, which have high variation in vessel size. Sato *et al.* (1990) studied the penetration depth of cask staves by malt whisky, which correlated with neither ring widths nor the alignment of the wood relative to the radial direction. The dominant role of tyloses in determining the permeability through wood may explain the failure to detect such correlations. In contrast Maga (1989b) stated that it was the preponderance

and spacing of large rays that made it difficult for liquid to pass through oak wood. The importance of rays in liquid or gas movement in wood is uncertain, although the estimated 25% lower tangential compared to radial permeability is thought to be due to rays allowing greater radial flow (Kumar and Kohli 1988). Wheeler and Thomas (1981) suggested that the permeability of rays depends on their width, with single cell uniseriate rays being the most permeable. Other features that are thought to influence permeability include the deposits of extractable material, which may block vessels and cell pits (Kumar and Kohli 1988; Wheeler and Thomas 1981).

The permeability also varies between different liquids. Kiseleva and Zoldners (1986) found that pure water diffused through birch wood four times faster than pure alcohol, when restricted to passing through cell walls, as is likely to be the case when movement through vessels is blocked by tyloses.

Wood density

Zhang *et al.* (1994) described correlations between oak wood density and both ring width and cambial age (the number of rings away from the pith). Ring width influences density predominantly by the ratio of early to latewood which declines with ring width. As the earlywood has a lower density than the latewood one would expect wider rings to have a lower mean density. However, recent studies (*eg* Ackermann 1994) examining oak wood from a variety of forests have found no correlation between wood density and ring width. Keller (1987) described the best cask wood as being less dense and therefore more permeable than wood used for most other applications.

Wood colour

Klumpers *et al.* (1993) found a tentative correlation between wood colour and extractive content, with heartwood colour being loosely correlated with the tannin content. The darker colour of heartwood is frequently claimed to be due to the laying down of phenolic extractives. The colour of the wood certainly correlates with some measure of extractives, as the wood becomes visibly paler after extraction with polar solvents such as acetone. Klumpers *et al.* (1994) suggested that the polymerization, hydrolysis and insolubilization of ellagitannins during heartwood ageing induces the darkening of heartwood. Recent studies have focused on the problem of discolouration during seasoning of the oak wood (*eg* Haluk *et al.* 1988 and 1991; Bauch *et al.* 1991). Charrier (1992) found that brown discolouration was associated with a decrease in the levels of ellagitannins, with a corresponding increase in the concentration of ellagic acid and proposed coloured degradation products.

Wood grain

The empirical term 'grain' is much used among wood traders and coopers to describe cask wood. The grain of the wood is determined by the visual impression produced by the size of wood elements, particularly vessels. Different types of grain may include fine, coarse, tight and loose. Feuillat *et al.*(1992) described how the term has varied in its use over time and how it presently most often refers to the ring width and wood texture. This in turn relates to the porosity of the wood and the abundance and distribution of large vessels. Although the term lacks objective precision, nonetheless its continued use among professionals, particularly those working in French forests, makes it important to understand the properties to which the term relates.

1.3.3. Summary of the role of wood in the maturation process

The properties of the cask wood clearly have a major effect on the maturation process. Of particular importance, and the subject of numerous studies, is the amount and composition of the extractive content. Various flavour congeners are thought to derive directly or indirectly from extractive compounds and a number have been identified as being of likely importance. Anatomical characteristics of the wood may also influence the maturation process, particularly the rates of evaporation, oxidation and extraction. Any factors that cause variation of these properties will thereby influence the maturation of whisky.

One cannot define a set of properties, be they chemical or physical, of the cask wood which determine the effects on whisky maturation. However, a number of characteristics are accepted as influencing maturation. By examining variation in these characteristics one can determine the feasibility of selecting wood for its effects on flavour. The next section examines evidence for variation in relevant properties and likely causes and patterns in this variation.

1.4. Factors determining cask properties

Numerous studies have compared the effect of using different types of cask and many have found apparent differences. However it is often found that numerous factors such as the method of cooperage, age and drying of the wood, in addition to the source of oak used, vary between cask types. Therefore, although many studies have shown that there is variation in cask properties and effects, the source of this variation has not often been identified. An attempt will be made to discriminate between variation in the properties of the virgin wood and that among used casks.

1.4.1. Variation in oak wood properties

Variation between species

Many studies examining variation of the properties influencing maturation fail to identify the species of oak from which the wood derives. Frequently oak wood is referred to as simply American or European. Despite this there is evidence that a range of properties appear to vary between at least some species, although few studies have compared the most relevant species: *Q. alba*, *Q. robur* and *Q. petraea*.

Azizol and Rashid (1981) claimed that there is a common pattern of fluorescent phenolics for most hardwood species and Salagoity-Auguste *et al.* (1986) were able to distinguish between chestnut and oak by the ratio of gallic to ellagic acids. Kishimoto and Kitiamura (1973) used IR and UV absorption spectra of different extracts to classify 13 *Quercus* species into four main groups.

Similarly, Knops and Jensen (1980) found that phenolic variation supported morphological evidence of hybridization in three species of red oaks, while Li and Hsaio (1973) described how the leaf phenolics differentiated between the subgenera of American oaks.

Although some studies have found no flavour differences between American and European casks (for example Aiken and Noble 1984), there are many others, which have controlled other sources of variation, to conclude that there are distinct differences between American white oak species (such as *Q. alba*) and the European species *Q. robur* and *Q. petraea*. Guymon and Crowell (1970) described clear differences in the effects of the two types and their results are shown in Table 1.2.

Table 1.2: Composition of Brandy matured for 72 months in American or European oak casks. Units: mg/100ml; Colour: units relative to standard. From Guymon and Crowell (1970).

	Tannins	Esters	Furfural	Total extract	Colour
New US standard cask	56	72	4	176	11
New French Limousin cask	102	61	1.1	232	20

Rous and Alderson (1983) also reported differences between French and American oak casks, each constructed identically by the same cooper. Both the quantitative differences given in Table 1.3. and qualitative differences in the taste imparted to the wines were found.

Table 1.3: Gain in phenolics (mg Gallic acid equivalents / litre) found in white wine after 13 weeks maturation in either French or American casks. From Rous and Alderson (1983).

Extractives measure	1st fill French cask	1st fill American cask
Total phenolics	231	56
Non flavonoid phenolics	144	49

Quinn and Singleton (1985) found levels of ellagitannins extracted from French oak were greater than those from American oak. Variation in the proportions of different ellagitannins was also found. Singleton (1974) likewise found that the levels of tannins from American oak were less than European oak, as did Puech (1984), who also found the levels of non-tannins were greater in American oak.

Nabeta *et al.* (1986) compared the volatile components of heartwood from the four species *Q. robur*, *Q. mongolica, Q. dentata* and *Q. serrata*, and found that *Q. robur* had the lowest concentrations of oak lactones and furfurals. Guymon and Crowell (1972) suggested that American oak contained higher levels of lactones than European oak. Nabeta *et al.* (1986, 1987) reported that *Q. robur* contained five times the amount of the most commonly occurring phenolic, lyoniresinol, than did American oak. In another comparison between the two types, American oak has been found to contain greater concentrations and variety of volatile norisoprenoids, than three types of French oak (Sefton *et al.* 1990b; Sefton 1991).

Comparisons between the two European species, *Q.petraea* and *Q. robur*, have given less clear results. Chatonnet (1991) reported that the average levels of lactones and eugenol were in the region of four times greater in *Q. petraea* than *Q. robur*, concluding that the wood of *Q. petraea* was more aromatic. Chatonnet (1991) also indicated that *Q. robur* was richer in phenolic extractables, particularly ellagitannins. However, Feuillat (1991) expressed reservations as the study did not adequately differentiate between variation due to species and that due to geographic origins.

A few studies have examined variation in relevant physical properties, such as wood permeability. Comparisons between American and European species claim that American oak has more tyloses and is less permeable (Rickards 1983). Comparisons between *Q. robur* and *Q. petraea,* of various physical properties, have also been made (*eg* Keller 1987). There is much overlap of characteristics and few properties act as a reliable guide to distinguishing between the two species. Indeed, there exists much controversy over the occurrence and abundance of hybrids and intermediate forms (*eg* Rushton 1993; Dupouey *et al.* 1993).

Variation within species

A number of studies have reported geographic differences in the properties of oak wood. Kleinschmit (1993) described how wood properties, including density, relate to flushing time (Nikolov *et al.* 1981; Jevlev 1972a,b), which has been found to vary between populations of both European (Burger 1949; Liepe 1993) and American oaks (Kriebel 1993). Studies on Douglas fir describe how wood permeability declines significantly with altitude of growth (Polge 1973), and it is possible that oak may be similarly affected. McDougal and Parks (1984; 1986) showed that

leaf phenols of *Q. rubra* varied between sites at 75 and 1140m elevation. Variation in growth rates have also frequently been reported from oak provenance trials (for example Jensen 1993; Gracan 1993; Barzdajn 1993). These geographic differences are frequently found to be greater than corresponding species differences. Kalinkov and Shipchanov (1976) in comparing anatomical properties, such as vessels and fibre abundance of *Q. petraea* and three other species of oak, found the greatest variation to occur between moisture loving and drought hardy forms of *Q. petraea*.

However, many authors report greater ecotypic variation between local populations, than geographic or clinal variation. Baranski (1975) found variation in several properties of *Q. alba* to be greatest within regional populations than between them and no evidence of altitudinal ecotypes of this species. Kriebel (1993) also emphasized ecotypic variation among American oaks, while Kleinschmit (1993) and Jensen (1993) stressed a similar pattern of variation in European oak populations.

A number of studies indicate the importance of various environmental factors that may account for this local variation. Feuillat (1991) summarised some of the conclusions of Henry (1886, 1887, 1892, and 1896) which were that, all other factors being equal, oak wood was richer in tannins when the trees grew on calcareous soils or were isolated and more exposed to light. This is in accord with Mallet (1946) who claimed that oaks in full sunlight gave rise to a better quality Armagnac. Keller (1987) examined morphological variation in French oak and identified the rate of growth as the main cause of differences between oak types. He also described some oaks as producing low density wood despite rapid growth, with few fibres being laid down in the late wood: a trait he considered to be genetically determined. Nepveu (1993) also emphasised the genetic control of wood properties and claimed that the large phenotypic variation cannot be explained purely by variation in growth rates or environmental factors. Studies on the heritability of various oak wood properties have been summarised in Savill and Kanowski (1993). Although several studies of anatomical properties have been made, no work on the heritability of extractive content has been published.

Many authors have described very high levels of gene diversity within oak species and populations, with high individual tree variation (Kremer *et al.* 1993; Olson 1975; Muller-Stark *et al.* 1993). However, Zanetto *et al.* (1993) reported that the pattern of genetic variation itself varied between regions of Europe. Studies indicate variation in both chemical and physical wood properties within species, but it is unclear how this reflects differences between geographic races, populations and ecotypes.

Overall, patterns of geographic variation of both phenotypic and genotypic characters have been found to be a combination of clinal and predominantly ecotypic variation. However, the natural pattern of variation, has been confused by provenance transfer, plantation activities, and

subsequent hybridisation. These occurrences have drastically altered the natural range of oak species and prevent one from discriminating between what was natural variation and what is artificial (Kleinschmit 1993; Weaver and Spiecker 1994). Therefore, even the relative importance of environmental and genetic differences remains uncertain.

Studies focusing on possible flavour-influencing properties have frequently given ambiguous results. Despite claims that tannin content is more variable between different provenances than different species and the tradition of identifying French oak according to its geographic origins, recent comparisons between geographic types of cooperage oak have produced conflicting results. Puech (1984) compared extractives of different types of European oak with American oak but, due to low replication, the study failed to show any clear evidence of variation between geographic types of European oak, although it did demonstrate the high level of variation between individual trees. Miller *et al.* (1992), in comparing wood of *Q. alba* and *Q. robur* from two American forests, found significant differences between the sites in total phenols and some phenolic acids. However, site differences were not as important as species differences and only two trees, of each species, were selected from each site.

Puech (1984) found lower levels of coniferaldehyde and sinapaldehyde in French oak than American, Russian, or Bulgarian oaks, while greater concentrations of lactones have been reported in Russian brandy than French brandies. It might be expected that lignin degradation products will vary little between oak origins or species, due to the common source from which they derive. However, Puech and Sarni (1990) suggested that the fraction of easily extractable lignin, from which these compounds derive, could vary between different provenances.

French oak from the Limousin region and from the forests of central France (frequently referred to as Tronçais or Allier oak) are the two types most frequently claimed to cause different flavours. More than referring to oak from a particular geographic region, these two types typify opposing characteristics with regard to the species, silviculture, location and age of exploitation of the forests (Feuillat 1991; Pontallier 1991; Remy 1991; Giraud pers. comm.). Feuillat (1991) reviewed studies on the properties of these two types and his conclusions are summarised in Table 1.4.

In general, there is evidence for variation between both geographic regions and local populations in wood properties affecting flavour. However there is much uncertainty over the pattern, degree and causes of this variation.

Table 1.4: Summary of the properties of Limousin and Allier oak. Derived from Feuillat (1992)

Characteristic	Limousin Oak	Tronçais or Allier Oak
Climate	Moist, high rainfall.	Drier climate & environment.
Silviculture	Both coppice and high forest systems. Greater thinning than Allier.	High Forest - with a greater tree density than Limousin.
Age of Trees Used	90-120 yrs	200-250 yrs
Species	Mostly *Quercus robur*	Mostly *Quercus petraea.*
Physical Properties		
Grain	Coarse/loose	Fine/tight
Ring Width	2.5 - 5 mm	1-2 mm
No. of lines of large vessels	2 +	1-2
Size of large vessels	Larger	Smaller
% Open Vessels	Greater (fewer tyloses)	Fewer (More tyloses)
Ray No. & Size	Equal	Equal
% Fibres	Equal or greater	Equal or fewer
Wood Colour	Yellow	Pink/Rose
Tightness of cask	Less: possibly due to fewer tyloses in large vessels.	Greater
Permeability	Higher	Lower
Extractables	Greater exchange with spirits.	Less
Chemical Pro erties	g p	
Total Extractives	140 mg/g wood	90 mg/g wood
Polyphenols (D280)	30	22
%Tannins	10	6
Ellagitannins	15.5 mg/g wood	7.8 mg/g wood
Colour of Extract	Yellow	Pink
Lactones	17 ug/g wood	77 ug/g wood
Phenolics	Greater	Less.
Aromatics	Less	Greater
Aroma	Less expressive, and not as complex.	Richer, more intense.
Taste	Astringent	Pleasant, Complete
Notes on Use	Used for Cognac & eaux de vie, as tannins released rapidly.	Used for wines, as resulting flavour is released more slowly, and therefore more easily controlled.

Variation within trees

Variation within trees is frequently ignored and yet it is known that this may be significant for many properties. Many authors (*eg* Peng *et al.* 1991) described how the concentrations of soluble and insoluble ellagitannins respectively decreased and increased, from the periphery of the heartwood towards the centre. The results corroborate earlier studies on the distribution of tannins by Schultz (1959) and Henry (1886, 1887, 1892, 1896). It is thought that the tannins slowly oxidise and polymerise as the heartwood ages, thereby becoming less soluble. Similar reactions may also affect the levels of other extractives, but few studies have been undertaken, beyond simple comparisons of sapwood and heartwood, such as Maga (1989a) who reported levels of oak lactone to be higher in heartwood.

The speed of growth varies with age, and therefore properties that are influenced by ring width may vary likewise. The degree of variation will depend upon the regularity of growth and can vary not only between growth rings, but also within a ring if radial growth is asymmetrical. In

addition to noting that any direct relationship between ring width and phenolics will be complicated by the phenolics being laid down in the wood ten to fifteen years after the growth ring is formed, Singleton (1974) described how greater amounts of phenolics were extracted from the sawdust of earlywood than latewood. These results suggest that one would expect slowly grown oak to contain greater concentrations of tannins due to the higher proportion of earlywood. Hillis (1975) proposed that polyphenol formation in heartwood was related to ethylene production that would increase as 'stress' increases. If slow growth is an indicator of greater stress one might again assume that low growth rates will produce heartwood containing high amounts of tannins. One might also expect slow grown oak to be more permeable (and therefore release more extractives) as the density of early wood is much lower, containing as it does the large vessels. Singleton suggested that speed of growth may explain the lower extraction and less leakage of casks made from American oak, as they have a reputation of being grown faster than European oaks. However variation of many wood properties influenced by ring width, such as density and shrinkage, have frequently been found to vary more between individual trees, than within. Zhang *et al.* (1994) describe how the relationship between wood density and ring width can vary between different trees.

1.4.2. Summary of the variation of oak wood properties

There is evidence for variation in properties affecting whisky maturation both between and within species of oak. Less certain are the causes of such variation, and also the significance of the different levels of variation, be they between species, populations or individual trees. However, there is sufficient evidence to conclude that the species of American oak differ in chemical and possibly anatomical properties from the European species, *Q. robur* and *Q. petraea*. Feuillat (1991) in studying a number of properties of French oak concluded that any geographic effect on both physical and chemical factors was due primarily to differences between species. Therefore *Q. robur* from the St. Palais region was more similar to *Q.robur* from the Limousin than it was to *Q. petraea* from the same region. The considerable differences often attributed to Limousin compared to Allier oak could be primarily due to *Q. robur* being most abundant in the Limousin, while *Q. petraea* is dominant in the Allier region. However this in turn is not a very satisfactory conclusion given the difficulty of species identification and the known hybridization that occurs between the two main European species. It is reasonable to conclude that provenance or forest differences exist, but the degree to which these are genetically or environmentally determined remains uncertain. Furthermore it should be remembered that nearly all studies highlight the large degree of variation between trees within the same population.

1.5. Variation in cask wood properties

Among the various factors that may alter the extractive content of wood between its felling and the use of casks, only those considered to be most important are discussed here. Some of the main effects are summarized in Table 1.6

Table 1.6: Summary of the effects of cooperage treatments on wood properties.

Treatment	Treatment Effects			
	Tannins	Oak lactones	Other Extractives	Anatomical Properties
Seasoning	Decrease in soluble tannins during wood ageing widely reported.	Lactones reported to increase during wood ageing.	Conditions of drying reported to influence volatile constituents.	Decrease in moisture content to approximately 14% of dry weight.
Toasting	Decreases levels of ellagitannins through oxidation or hydrolysis reactions.	Heating may increase synthesis, but there will be loss through volatilising from the wood surface	Degradation of lignin leads to the formation of lignin derived compounds available for extraction. Levels of volatiles reported to increase.	At high temperatures physical degradation of the wood may occur. Effects will vary through the depth of the stave.
Charring	Decreases ellagitannins through the same reactions as occur in toasting	Conflicting reports about the effect on lactones.	Large amounts of furan and lignin derived compounds formed. Charred layer may also remove undesirable sulphur compounds.	Break up of wood structure near surface may allow easier penetration of new wood and extraction of compounds.

1.5.1. Selection of wood

The selection of wood for cooperage imposes a number of restrictions on the wood used for whisky maturation. Generally oaks with a diameter at breast height of not less than 35 cm are used (Keller 1987). Only the oak heartwood is used and this is normally explained by the formation of tyloses and the laying down of extractive material, which reduce wood permeability and ensure a tight container. High quality oak wood is required, free of defects such as knots, frost or fungal damage. Trees with spiral grain are also considered less suitable, as the vessels may not run longitudinally along the resulting staves. Coopers may display other preferences, so as to reduce splitting or checking in the wood. However these vary so widely that Alexander (1972) found no common guidelines among various American coopers. By selecting only high quality oaks with suitable characteristics, limitations are placed on the range and type of variation in properties influencing flavour.

1.5.2. Manufacture of staves and cask

Claims are frequently made, particularly by European coopers, that the initial cleaving of the wood is preferable to sawing. Reasons for this include that sawn wood gives rise to "strange aromas" (Castelli and Peynaud 1990) or that cleaving staves helps prevent leakage from the finished cask by ensuring that the vessels are correctly aligned along the wood (Edlin 1973).

While this latter claim does not seem unreasonable, the American coopers are able to produce tight casks without cleaving. Sawing has the added benefit of producing less waste (Williams 1983b) and is more easily mechanised. Furthermore the fact that Portuguese coopers produce casks from sawn staves of Limousin oak, discounts the possibility that cleaving is a requirement for *Q. robur,* if not *Q. alba.*

Quarter-sawing staves, so that rays run horizontally across and vessels longitudinally along the stave (see figure 1.4), appears a common practice among coopers. This is often claimed to reduce leakage, by restricting movement along the wood rays to going across, rather than through the stave. However, Howard (1964) reported that quarter-sawing also decreased the amount of warping and the effect of any splitting parallel to the rays.

Finally it should be noted that a finished 190 litre cask will normally contain in the region of 31 pieces of wood, including both staves and headings. These are all likely to derive from different trees, or even provenances, and therefore the variation in cask effect may be less than if each cask derived from an individual tree.

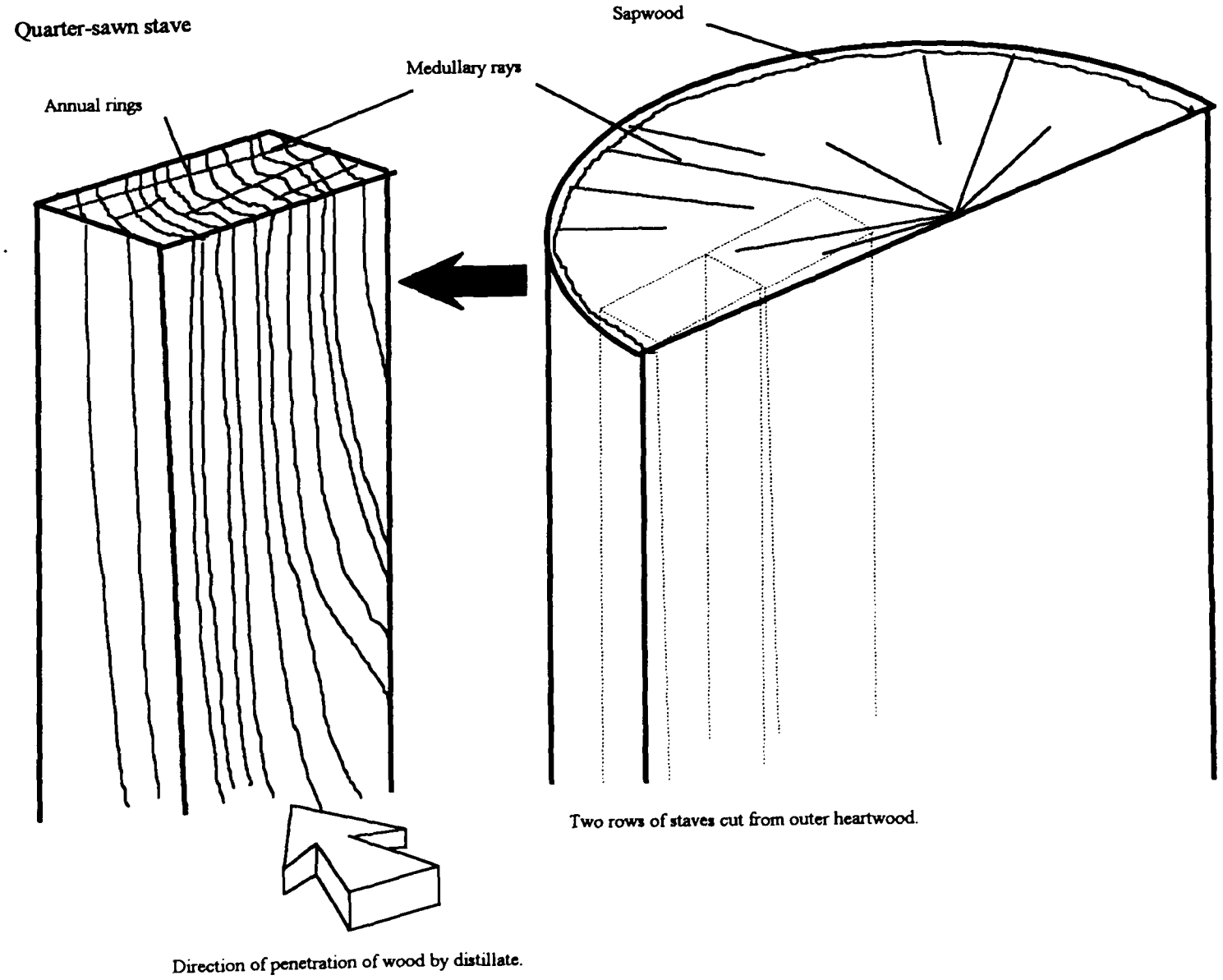
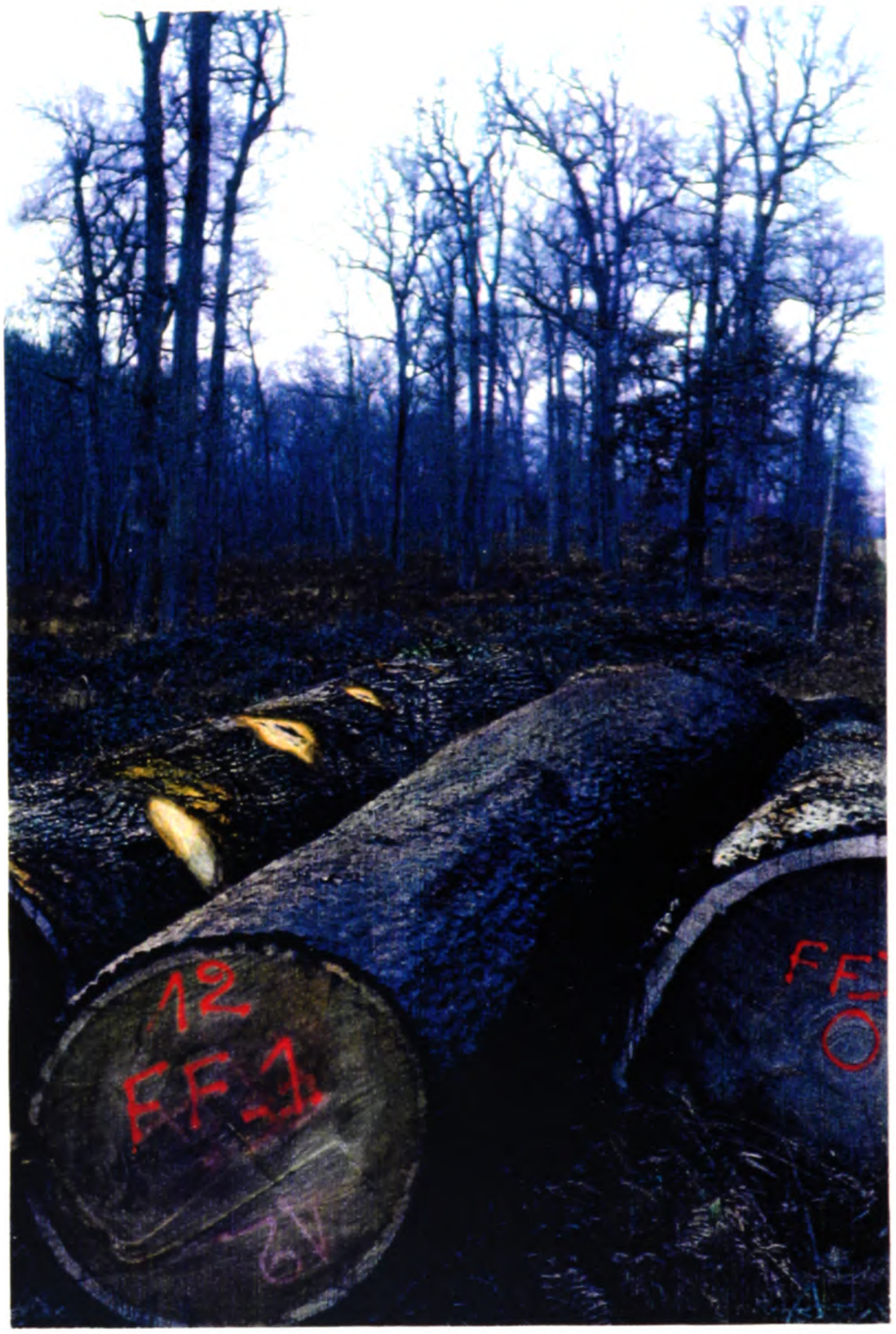
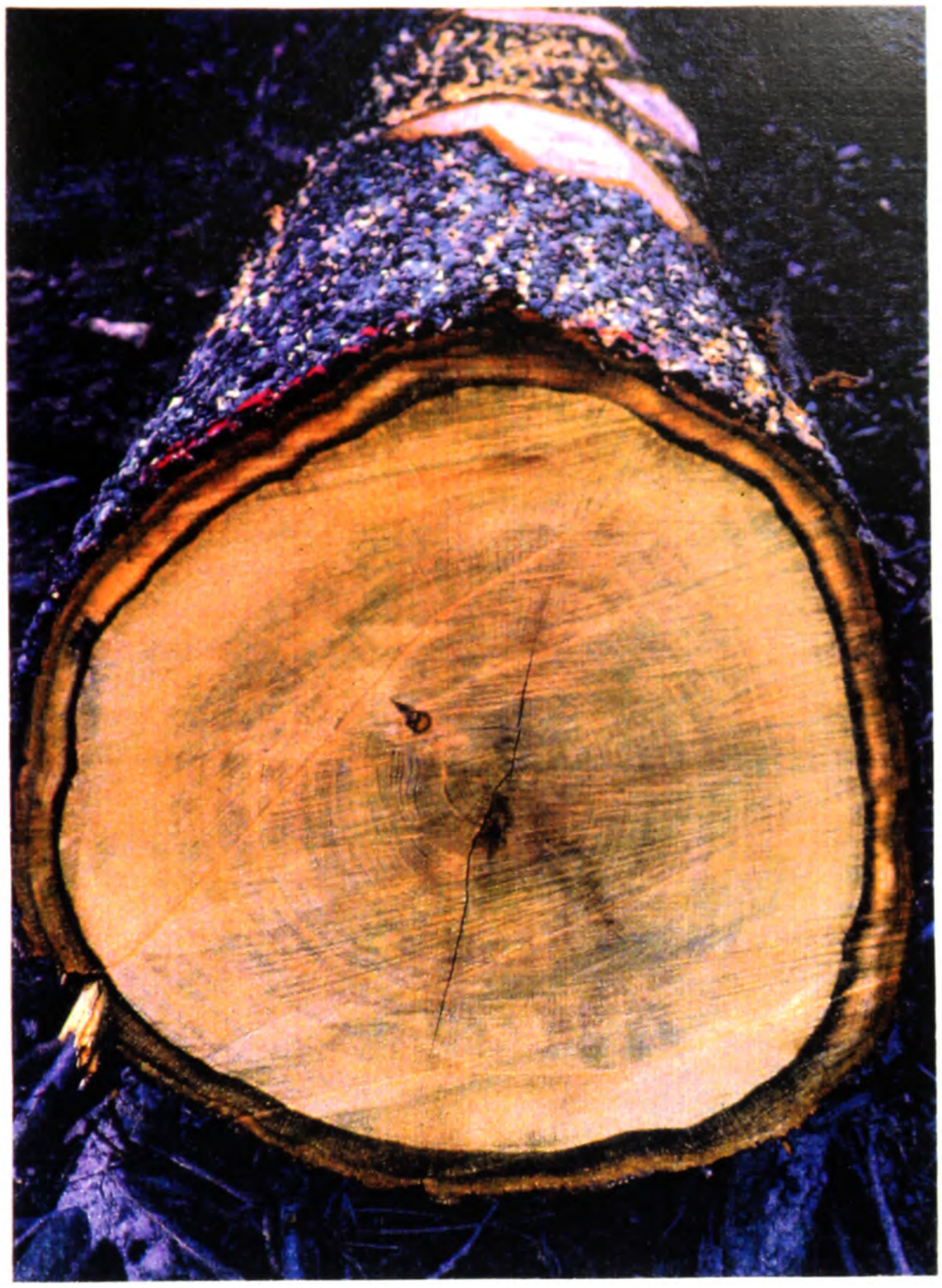


Figure 1.4: Alignment of stave wood.



Figures 1.5.a-b: Oaks from forests near Tronçais, in central France, destined for the manufacture of casks (above and overleaf).



1.5.3. Seasoning

The method of seasoning wood is often said to affect both the structural integrity of the wood and the flavour imparted by the cask. Glaetzer (1991) claimed it was the most critical factor in oak selection for wine maturation. The traditional, European method of seasoning is by air-drying, taking approximately three years to reduce the moisture content of staves to the required level of approximately 15% (Pontallier 1992; Remy 1991; Giraud pers. comm.). The time required varies according to the climate and has been reported as taking only nine months in the U.S.A. Air-drying was adopted because of the problems associated with kiln-drying oak wood. Casks made from wood that has been seasoned too quickly are liable to be brittle or to develop open joints (Panshin *et al.* 1962). Better control of kiln-drying now allows oak to be seasoned successfully in this way, and although structural defects and discolouration can still present problems (Charrier 1992), the speed of kiln-drying has the major advantage of allowing the cooper to meet demand more easily. The process normally involves leaving the staves in a ventilated drying room between 40-60°C (Pontallier 1992).

Many wine producers still express a preference for air-dried wood, it being frequently claimed that the flavour produced is more subtle and complex than that from kiln-dried oak. Studies comparing the results of kiln and air-dried wood give conflicting results. While some have found no significant differences (Wilker and Gallander 1989), other studies claim to have done so. Pontallier *et al.*(1982) found that air dried Allier oak resulted in lower levels of phenolic acids being extracted by wine than occurred in kiln dried oak. Sensory comparisons of oak-aged wines also found that air dried wood gave a stronger "vanillin wood" character than the "green and dusty" kiln dried casks.

Many recent studies have found the effects on flavour of drying depend on the seasoning method and conditions. Sefton *et al.* (1990b) found that the conditions of air-drying were important, reporting that levels of oak lactone decreased in oak seasoned in France, but were unchanged when the same type of oak was seasoned in Australia. Differences in other volatile compounds also appeared to depend on seasoning conditions. They concluded that although the reasons for such variation were unknown, the local climatic conditions such as rainfall and temperature may have a major effect. A number of studies appear to indicate that the age of the wood after felling is the most important seasoning factor influencing extractive content. According to Maga (1989a) significant loss or degradation of particularly unsaturated compounds, fats and fatty acids occurs during ageing. He also reported that the concentration of oak lactone increased five fold during six years of seasoning American oak under cover. Sefton (1991) described how the levels of vanillin in oak seasoned for two years were twice those found in green oak, while the amount of eugenol decreased. A decline in tannins during wood storage and air drying was reported as early as the last century (Jolyet 1892). Theories proposed to explain this phenomenon include enzyme

activity causing condensation and polymerisation of tannins, or that when exposed during air drying rainwater leaches out soluble tannins (Wilker and Gallander 1989). Pontallier (1992) suggested that hydrolysis and oxidation reactions, provoked by the action of enzymes in the wood and those secreted by micro-organisms which develop during air-drying, causes condensation and polymerisation of tannins. Although the role of fungi and other organisms has received little consideration, the decline in soluble tannin content may also be due to non-enzymatic oxidation reactions. These changes will occur to a lesser extent when kiln-drying as the process takes less time. It would explain the lack of significant differences found between air and kiln-dried wood by Wilker and Gallander (1989), as the kiln dried oak was stored for an additional two years, while the air-dried samples were seasoned. These studies suggest air-drying causes a decrease in the soluble tannins, which although perhaps beneficial for many wines, might be considered undesirable for maturing spirits such as whisky.

In summary it appears that the age of wood after felling may be of great significance, with the conditions of seasoning also playing a role in determining flavour effects. Furthermore, different oak wood appears to respond to seasoning in different ways (Sefton 1991). This is in agreement with Skurriken *et al.* (1970) who, on evaluating the quality of Bulgarian and Russian oak wood for brandy ageing, reported that both the origin and seasoning influenced the brandy quality. The wood judged to be most suitable for brandy maturation had been stored for 17 years before being made into barrels.

1.5.4. Charring and toasting of casks

Charring is carried out on new casks by American coopers for the bourbon whisky industry and also as a means of rejuvenating used whisky casks. Toasting occurs during the heating process of bending the staves (see figure 1.6), but is frequently continued beyond the level required for this purpose. John (1991) claimed that slow constant heating was preferred for the manufacture of barrels for wine storage, but the duration of heating may range in time from 20-60 minutes, with many coopers offering their customers varying degrees of toasting. However the toasting of casks is usually an entirely empirical process, and therefore there is no objective ranking of toast between different coopers.

The charring of casks dramatically affects the volatile composition of the oak wood and increases the levels of many cask extractives. The flavour derived from charring has long been thought favourable in whisky maturation, as shown by recent studies (Clyne *et al.* 1993). Many studies (Baldwin *et al* 1967; Singleton 1974; Marsal and Sarre 1987; Sarni *et al.* 1990; Clyne *et al.* 1993; Reazin 1983) have found that concentrations of furan and lignin degradation products are higher in spirits matured in charred casks. Maga (1989a) proposed that thermal lipid oxidation would give rise to lactones. According to work by Ford and Done (1991) the charring of wood

also eliminates, or greatly reduces, ellagitannins from the extractive content. Another reported effect (Paterson and Piggott 1989) is that charring forms a layer of active carbon that may remove undesirable flavour congeners. Rejuvenating used casks by re-charring increases the amount of colour, solids, fixed acids, tannins and aromatic aldehydes that can be extracted, increasing the viability of the cask. However, the same levels as those found in a new cask will never be reached and viability will once again decline with reuse.

Similar effects are reported by studies on toasting, although some apparently conflicting results have been obtained. Chatonnet *et al.* (1990) found that while toasting increases the levels of lignin degradation products and furan products, it decreases the levels of polyphenols and lactones. Marsal and Sarre (1987) in addition to reporting an increase in the furan products, furfural and 5-hydroxymethyl furfural, also noted decreased levels of oak lactones extracted from toasted wood. Maarse and van der Berg (1990) described how pre-treating wood by heating decreases tannin concentrations, giving a less bitter taste.

The effect of varying degrees of charring and toasting has also been studied. Nishimura *et al.* (1983) found that although the amount of aromatic aldehydes extracted from heat treated wood increased with temperature, the amounts decreased when charring of the wood had occurred; although levels were still higher than those from untreated wood. Chatonnet *et al.* (1989) also found that the maximum levels of phenolic aldehydes occurred at medium to high levels of toasting, with very high toasting decreasing the aromatic impact of vanillin, presumably due to the conversion of aldehydes to their respective acids. However the effect of toasting varied between different wood types, with greater toasting of Allier oak decreasing the levels of total aromatics, compared to Limousin oak where these increased while the tannic flavour of the wood declined. Sarni *et al.* (1990) found that increased heating led to a decrease in the levels of ellagitannins and a corresponding increase in ellagic acid. Their results, describing the effects on lignin of increasing temperature, suggested the progressive formation of different degradation products. From a temperature of 120°C, initially aldehydes would appear followed by an increasing abundance of acids at temperatures of over 165°C. Thermolysis would occur at higher temperatures according to the monomer units being considered, with firstly guaiacyls and later syringyls, degrading to form guaiacol, dimethoxyphenols, cresols and other phenol type compounds characteristic of burned wood. They noted that spirits such as whisky, matured in casks subjected to direct charring, contained higher levels of syringyl compared to guaicyl units, as well as containing typical pyrolysis products. Levels of the lignan lyoniresinol appeared unaffected by heating.

Figure 1.6: The toasting of a new cask. The fire in the centre of the cask is fuelled by oak wood. Tanoaria J.Dias, Espinho, Portugal.

Figure 1.7: Finished casks at Tanoaria J.Dias, Portugal.

When considering the impact of either toasting or charring, two distinct effects are likely. Firstly there will be the effect of heat on the wood chemistry, the degree of which will vary throughout the depth of the treated staves, thereby ensuring a complex range of effects upon the cask. Secondly, at high toasting levels and during charring, there will be physical degradation of the wood, which may allow increased penetration by the distillate.

In summary, the heating of wood appears to increase levels of lignin degradation products, aldehydes, acids, furan products and many volatiles. At high levels of toasting, or charring, thermolysis products will develop. Phenolics are likely to undergo oxidation or possibly hydrolysis reactions, decreasing the amounts of soluble ellagitannins. The effect on oak lactone appears to be uncertain, for while some studies suggest charring of a new cask increases levels, studies on toasting and rejuvenating casks by charring suggest that the treatment results in little increase. The physical degradation of the wood by charring may be the cause of increased extraction of many compounds, particularly when charring is used to rejuvenate old casks. The layer of charcoal may also remove undesirable compounds from the maturing distillate.

1.5.5. Other cooperage effects

Methods other than toasting barrels are used for the bending of cask staves, particularly in America where the wood is pre-heated by steaming for 10 to 15 minutes (John 1991). This is likely to influence the cask properties in a different way from toasting. Immersing the staves in hot water is practised in Hungary, resulting, it is claimed, in less harsh tannins and a more subtle pickup of flavour by the wine (Degaris 1991). It is probable that the treatment would cause the removal of water soluble extractives, particularly tannins. Similar effects may be caused by the practice of testing the tightness of the finished cask by partially filling under pressure with hot water or by sterilising treatments which are often carried out by cask users.

1.5.6. Previous use and rejuvenation of casks

It is accepted that the levels of cask extractives decline with reuse and that the quality and speed of maturation is strongly influenced by this decline (Puech and Visockis 1986; Puech and Goffinet 1987). Decline in cask viability has led to the adoption of various methods of rejuvenation. The most common one, involving the re-charring of casks, has already been discussed. Re-firing has the disadvantage that it frequently requires cask heads to be refitted after heating (Zimmerman 1991). Another frequently used method is scraping the inside of casks, removing the inner surface, possibly in conjunction with recharring. Examination, by electron microscopy, of the inner and outer surfaces of an oak stave used to mature Armagnac for the previous 80 years (Puech 1984), indicated that the process of maturation did not appear to alter the structure of the wood. Furthermore the levels of extractives had only been decreased in the

wood to a depth of approximately 9 mm. This depth is typical of the distance travelled by the spirit front, easily observable in used staves. It confirms the effectiveness of scraping as a method of rejuvenation, as it would expose new unextracted wood to the distillate. However the treatment may weaken the cask and cannot be repeated frequently .

In apparent contradiction to the detrimental effects of reuse, the previous storage of some alcoholic beverages, such as sherry, is often considered beneficial for the later maturation of whisky. This previous use will change the composition of extractives by removing compounds but may also cause the direct or indirect formation of new compounds in the wood, which may then be available for later extraction during whisky maturation. There is little published evidence for claims that the flavour and colour of the whisky is improved by the previous storage of sherry. Nonetheless the marketing of many whiskies place particular emphasis on the use of casks previously used for storing not only sherry, but other drinks including brandy, port and rum.

Despite the uncertainty over its importance, attempts to simulate previous sherry storage have been made. Wine-treatment of casks involves allowing the wood to absorb a very sweet, dark sherry under pressure. Changes in the analytical composition of casks by this treatment include an increase in total esters, colour and sugars, but this appears to be of little significance relative to flavour (Philp 1989a). Attempts to simulate true sherry shipping cask flavour include processes involving wine, steam and ammonia treatments. The methods include a combined rejuvenation and sherry treatment process, which involves identifying and cleaning European oak casks, followed by adding sherry to the cask interior and allowing it to penetrate the wood (Philp 1989b).

1.5.7. Other wood treatments

A variety of wood treatments have been experimented with, mostly with the aim of increasing the speed of maturation for various alcoholic beverages. Litchev (1989) described how treating wood with pressurised oxygen at high temperature increased the maturation speed of wine. Maga (1989b) also described a number of treatments, including ultrasound, which have been claimed to enhance the release of lignin and amino acids, while heating brandy in the barrel combined with ultrasound apparently reduced maturation time from 3 years to 3 months. Both ultraviolet irradiation and the use of gamma radiation have also been reported as enhancing maturation.

1.6. Conditions of extraction and maturation

Maturation and extraction results may vary under different conditions, with a wide range of factors influencing the process. For this reason one must be cautious in comparing different

studies and in interpreting their relevance to the maturation process. Conditions of maturation can affect both the direct extraction of compounds and subsequent reactions.

1.6.1. Temperature

It has been found (Nykänen 1986; Reazin 1983) that extraction and formation of flavour congeners will generally occur more rapidly at 30 ^{o}C compared to 20 ^{o}C. The greatest increase was in acetaldehyde and fixed acids, while lactones and furfural levels appeared unaffected. Philp (1989a) summarised previous studies that showed how non-volatile components and component groups were significantly influenced by temperature and to a lesser extent by humidity. Maturation of whisky normally occurs at relatively low temperatures, which are used primarily to reduce the rate of evaporation from the cask.

1.6.2. Extraction solvent and proof of distillate.

Most studies use either an ethanol-water mixture or raw distillate to extract wood compounds. Nykänen *et al.* (1985) found that maximum extraction efficiency occurred at about 60% alcohol concentration. However, Maga (1989a) found that maximum lactone extraction was obtained with 40% alcohol, while Puech (1984) found that the optimum extraction levels of tannins and lignin by Armagnac occurred at 55%. It is likely that similar variation may be found for other types of extractives. If the alcohol content rises above approximately 60%, then the rate of extraction for colour, solids, tannins, and volatile acids have all been found to decrease. A number of authors have emphasized the effect that distillate strength can have on maturation speeds (*eg* Baldwin *et al.* 1967, Baldwin and Andreasen 1974, Sharp 1983). The reason for this variation is that while the hydrolytic reactions, such as the breakdown of polymeric material, require water, the solubility of degraded compounds improves with increasing alcohol concentration. Therefore the highest rate of extraction will occur at the concentration when these two processes are optimally balanced.

Maga (1989a) found that the pH of the extraction solution affected the concentrations of oak lactone extracted from wood by an alcohol solution. Peng *et al.* (1991) described how water extractions of ellagitannins were more efficient at higher pH levels. The pH of wines and spirits is influenced by the cask wood extractives, declining during maturation (Maga 1989b; Lehtonen and Jounela-Eriksson 1983) probably as a result of pyrolysed wood (Sarni *et al.* 1990). This change in pH may in turn influence the formation of many compounds.

1.6.3. Oxygen content and availability

Oxidative reactions have been intensively studied in the process of wine fermentation and maturation (see the review by Singleton 1987). In wine maturation slow oxidation is preferred and the varying rates at which oxidation will occur in a wooden cask are deemed beneficial as this leads to a more complex mixture of oxidative products than would a constant rate throughout. Sefton (1991), in discussing the maturation of wine, claimed that controlled oxidation led to less astringency (perhaps due to the oxidation of tannins) and increased colour and stability.

Chatonnet (1991) found that old barrels lose their oxidative properties, possibly due to the loss of hydrolysable tannins that may act as catalysts for many oxidative reactions during maturation. Metal ions, such as copper, have also been identified as oxidation catalysts.

The extraction of oak wood using alcohol-water mixtures with varying oxygen concentrations has shown that a higher oxygen content results in greater concentrations of lignin degradation products, such as vanillin and syringaldehyde, while more sinapaldehyde was produced when extracts were prepared with little oxygen (Maarse and van der Berg 1990). Higher levels of eugenol and furfural were also found in high-oxygen containing extracts. However, despite the lower levels of most lignin degradation products, the extracts obtained under low oxygen concentrations had a more harmonic, cognac-like and less astringent flavour.

An indication of the importance of oxygen in whisky maturation is that if a maturing cask is wrapped in a film impervious to air, no change in flavour will take place (Jago pers. comm.). However, although the exclusion of oxygen is the most likely cause of this result, the treatment will also prevent evaporation and possibly decrease the penetration of the cask by the whisky distillate.

1.6.4. Humidity

This has been claimed to affect the rates of oxidation and particularly evaporation (Castelli and Peynaud 1990). Guymon and Crowell (1970) described how humidity affects the final proof of maturing brandy. Low humidity produces an increase in alcohol content due to the evaporation rate of water being higher than that of alcohol. In high humidity conditions, the proof declines, as more alcohol than water evaporates (alcohol evaporation being independent of humidity). Evaporation may then result in further effects, due to the changes in the proof of the maturing distillate.

1.7. Summary

1.7.1. Methods and limitations of previous research

The difficulty in summarising the effects, and how they vary, of oak wood on the maturation of alcoholic beverages reflects the diverse range of disciplines and subjects that it involves. One of the main aims of this review has been to draw together the various aspects of different research relevant to the subject. An additional problem arises from the various commercial interests involved. Coopers do little to discourage various rumours and claims regarding the benefits of cask treatments or wood types. The producers of the alcoholic products, while wishing to maintain an aura of mystique around the process of maturation, have powerful financial interests in any understanding that would allow better control over flavour or quicker maturation times. There are also many practical limitations and problems arising from the subjective nature of flavour trials and the cost and time involved in research.

It is perhaps not surprising therefore that there is often a notable lack of clarity in many of the studies that have examined the process of whisky maturation. Each study tends to focus on a single factor that may influence the maturation process, while frequently failing to control variation of other factors adequately. Studies on the effect of charring will often compare charred and uncharred casks, but the past use and origin of these casks is frequently uncertain. There is often little attempt to discover even the species involved, let alone more precise origins. Even less consideration is normally given to the wood age, treatment and seasoning.

Another feature that is frequently ignored, but is always a problem when comparing different studies, is the method of extraction. Many studies involve the extraction of compounds from the wood by either the unmatured distillate (which may vary in its composition), or by using water-ethanol mixtures or other solvents. Both the solvent and the conditions under which the extractions occur influence the resulting composition of the extract. Many of the studies that have produced the most interesting and applicable results have compared the amounts of wood derived compounds found in whisky or brandy matured under different conditions or using different types of casks (for example Guymon and Crowell 1970, Baldwin *et al.* 1967). Although the relevance of such studies may easily be recognized, they suffer from a number of limitations. The most obvious restrictions on their use is the length of time and the resources needed to carry out such a comparison. It is also often difficult to control the various factors that may influence the properties of the cask. Lack of replication is also a frequent problem in many studies, particularly when comparing different sources of oak wood, such as in Puech (1984) and Miller *et al.* (1992). Practical limitations frequently make a high degree of replication impossible for the types of study undertaken.

1.7.2. Cask requirements and effects on flavour

A suitable supply of cask wood must meet three separate criteria:

i Economic feasibility - the wood supply must be adequate to meet present and future demand at an economic price.

ii Cooperage criteria - the wood must allow the construction of a tight cask, allowing minimal leakage, and be of suitable strength.

iii Flavour criteria - the wood must have the properties that will produce mature whisky of the desired flavour.

Because of the ability to obtain the required flavour by means of blending and also because flavour properties may to some extent be conferred on a cask by the way it is treated (such as heating), there has been a past emphasis upon the first two criteria. However, advances in the understanding of the maturation process and the role of the cask in producing flavour congeners have emphasised the importance of the wood in affecting the flavour of whisky. It is now accepted that the origin of oak, although modified by cask treatment and age, affects the final flavour of whisky and other alcoholic beverages. The effects of wood vary not only between species (at least with respect to American *vs* European species and probably between *Q. petraea* and *Q. robur*) but may also vary between different geographical regions and forests. The causes of this variation have received little attention, but studies on the variation of wood properties indicate that silvicultural, environmental, and genetic factors are all important.

One would ideally like to characterise mature whisky flavours by means of the levels of the various flavour congeners involved. By study of the synthesis or source of these compounds, one could elucidate properties required in the virgin wood, separately from those that may be induced at a later stage through cask treatments or blending of matured distillates. However, although the understanding of the maturation process has improved greatly over recent years, it is still not possible to define a desirable whisky flavour by means of flavour congener levels, let alone construct an index of properties required of the cask or in the timber from which it is constructed. Furthermore, the role that anatomical features may play in determining the availability of extractives and the cooperage requirements of wood are not well understood. It is also apparent that the method of cooperage may greatly influence the flavour derived from the cask. This may allow certain properties of the wood to be altered after harvesting, according to the required needs.

Any change in American law, allowing Bourbon producers to reuse oak casks, is likely to increase the demand for casks, previously used almost exclusively by Scotch whisky producers. Although the cost of new casks has been considered to be prohibitively high, this must be

contrasted with the potential of much more rapid maturation and distinctive flavours that certain types of new casks may offer. However, to utilise this potential fully requires a better understanding of the way in which wood may influence flavour and how these properties vary between possible sources of cask wood.

1.8. Research aims

The purpose of the studies described in this thesis was to examine variation of European oak wood properties likely to influence the flavour of whisky. In particular it sought to establish whether the properties of wood and their effects on flavour can be predicted from either the species, provenance or identifiable wood properties of individual trees.

It was decided to examine variation in high quality oak and, where possible, sources of wood known to be suitable for cask manufacture. Properties, thought to influence whisky maturation, were chosen that could be measured in either cask or virgin wood. As the heartwood ellagitannins make up such a large proportion of oak extractives these were selected as the main compounds to be examined. Additional extractives, such as oak lactones which are generally accepted as influencing flavour, were also measured in many studies. It was not a primary aim of the research to determine precisely how the methods of study (such as extraction methods) or chosen properties relate to the precise performance of the wood in a used cask.

The initial aims were to see if the properties chosen varied significantly in European cooperage oak and whether the pattern of variation might allow selection of casks or wood for their effects on flavour.

Chapter 2: Methodologies

2.1. Sampling of wood

Wood chips or shavings were obtained by means of a hand held plane or electric router. The latter was used when it was necessary to obtain samples from different depths, as this could be controlled to within approximately ± 0.25 mm. Wood shavings or chips were then granulated by passing through an electric coffee mill (Glen Creston type 14-580) and placed in a desiccator for 3 days, to reduce moisture content uniformly to approximately 4% of dry weight. Samples of 50 mg (unless otherwise stated) were weighed to within 2% accuracy and extracted with 5 ml of the appropriate solvent. After extraction the samples were filtered through 0.45 micron membranes.

Shavings taken from the same stave with a hand held plane and then ground in a coffee mill were compared with samples taken directly by an electric router. Levels of tannins extracted from the wood varied no more between the two sampling methods than variation within a sampling method. Extraction from shavings without grinding gave lower concentrations of tannins.

2.2. Measurement of ellagitannins

2.2.1. Extraction solution

Peng *et al.* (1991) compared the efficiency of different solutions in extracting tannins from oak heartwood. Of the solvents compared aqueous acetone was the most effective, extracting approximately 35% more tannin than water. However using water or a very low percent methanol solution allowed the measurement of ellagitannins by HPLC without additional analytical steps. As the aim was to compare relatively large numbers of samples it was decided that a simple extraction was better, particularly as no internal standard was used initially. The solution used was Methanol/H_2O/H_3PO_4 2/97/1 v/v/v, with 100 mg/l of pyrogallol used as the internal standard in later studies.

2.2.2. Extraction times and conditions

The extraction times of 24 hours without agitation or 2 hours with agitation were chosen as being suitable and convenient times for the extraction of soluble tannins. The amount of tannin extracted from wood samples over different extraction times with or without agitation were measured. In general, the majority of soluble tannins were found to be very rapidly extracted, with a very gradual and often insignificant increase in levels over longer extraction times. Table 2.1 is typical of this pattern when extracted under agitation.

Table 2.1: Mean total soluble ellagitannins (mg/l) from two replicate extractions over varying lengths of time (hours).

Extraction time	1	2	3	4	6	8
Mean	209.6	211.7	210.2	218.2	222.0	229.0
Standard Error	0.403	0.623	0.487	0.195		0.523

Temperature did not affect extraction efficiency between 10-40°C. Higher temperatures were not used as they may cause the degradation of ellagitannins (Charrier 1994).

2.2.3. Sample stability

It has been reported in other studies (*eg* Charrier 1992) that ellagitannins are not stable in solution. However, repeated analyses of the same sample found that even the less stable ellagitannins, such as vescalagin, only showed a measurable decline after 3-4 weeks in solution. Samples were stored at 5°C and generally measured within 1-5 days of extraction.

2.2.4. HPLC conditions

Waters HPLC system: Waters pump 600E; Waters auto sampler 712 Wisp; Waters UV absorption photo diode array 991. **Column:** Waters reverse phase C18; 260x4 mm; Spherisorb packing. **Injection Volume:** 20 µl. **Detection:** at 230 nm (190-400 nm for identification). **Internal standard:** pyrogallol (Aldrich) at 100 mg/l extraction solution. **Gradient:** The following solutions were used: H_2O/H_3PO_4 99/1 v/v (solvent A); $MeOH/H_3PO_4$ 99/1 v/v (solvent B). The best separation of ellagitannins was obtained using a linear gradient from 0 to 9% of solvent B over 40 minutes. Between each sample the gradient was increased to 100% solvent B and then returned to 100% solvent A, for a total sample run time of 90 minutes.

2.2.5. Identification and calibration

Using the criteria suggested by Scalbert *et al.* (1990a), that ellagitannins have near identical absorption spectra with no maxima between 240-400 nm but a shoulder around 280 nm, 12 ellagitannins were identified. Comparison with results described in earlier studies (Scalbert *et al.* 1988a; Scalbert *et al.* 1990b; Viriot *et al.* 1994) allowed the identification of 9 of these 12 ellagitannins: see figure 2.1. Purified samples of vescalagin, castalagin, grandinin and roburin a (obtained from Dr Scalbert, INRA, Paris and Dr Bertrand Charrier, École Supérieure du Bois, Nantes) allowed confirmation of their identification and were used for calibration. Because of the occasional overlap of ellagitannin peaks, heights instead of peak areas were used for calibration in some studies.

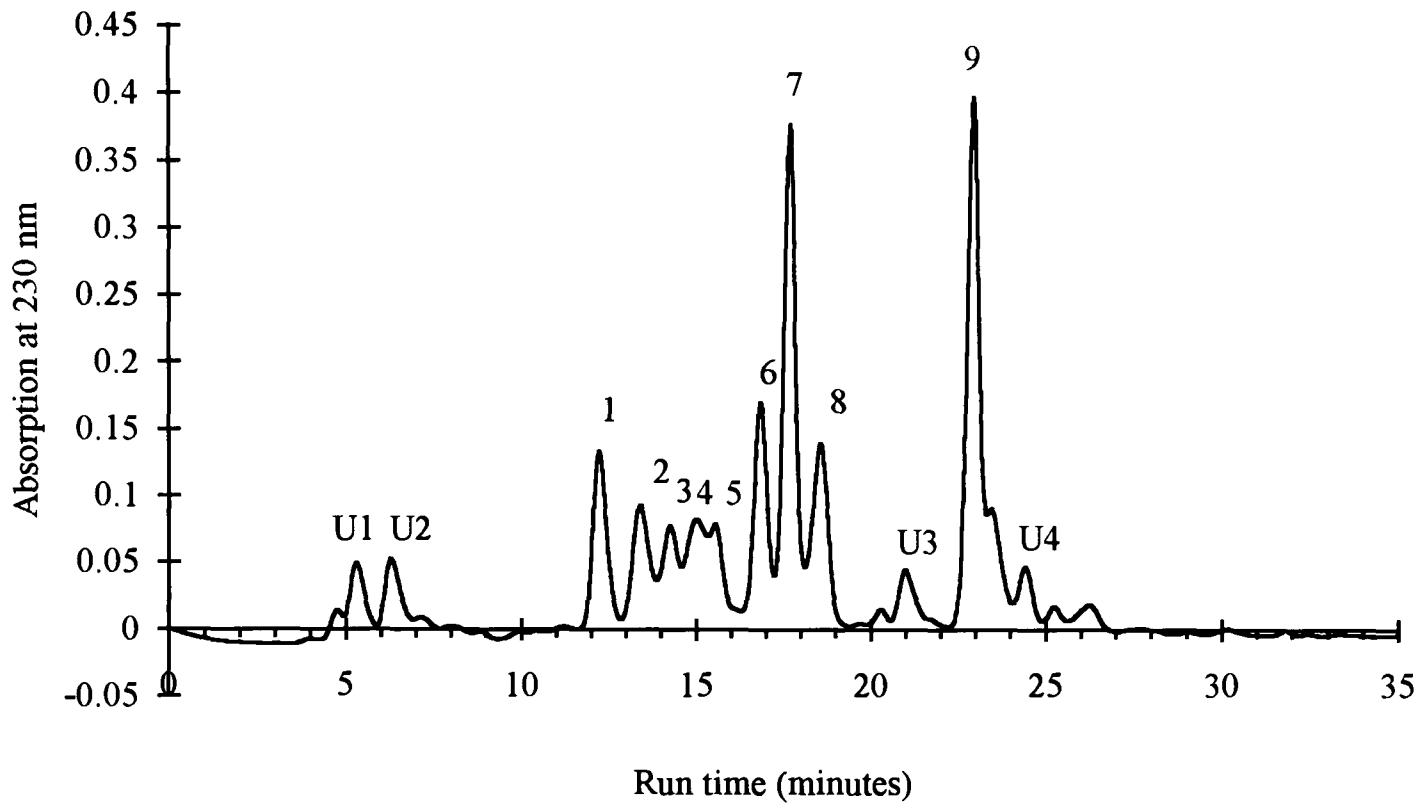


Figure 2.1: Chromatogram of separated ellagitannins from a water extraction of oak heartwood..
Key: U1-U4: Unidentified possible ellagitannins
1: Roburin A (vescalagin-vescalagin)
2: Roburin B (vescalagin-vescalagin-lyxose)
3: Roburin C (vescalagin-vescalagin-xylose)
4: Gallic acid
5: Grandinin (vescalagin-lyxose)
6: Roburin D (vescalagin-castalagin)
7: Vescalagin
8: Roburin E (vescalagin-xylose)
9: Castalagin

Nb: The retention time of gallic acid was found to be highly variable relative to the other peaks, occasionally interfering with roburin a. This behaviour was confirmed by discussion with other researchers (Bates pers. comm.), although no explanation was found. Identity was always checked by examination of spectra and/or co-injection with an authentic standard. The possible tannins U1 and U2 were tentatively identified as vescalin and castalin on the basis of their appearance in standard solutions of castalagin and vescalagin that had been stored over several months.

From published studies (*eg* Scalbert *et al.* 1990a and b) no mention is made of an internal standard when measuring ellagitannins by HPLC. On the suggestion of Dr Charrier pyrogallol was chosen as such for the later studies in this thesis. Having a suitable retention time and not interfering with any ellagitannin peaks, repeated HPLC analyses indicated there was no degradation of pyrogallol over a period of two weeks when the extraction solution was stored at 5°C. From replicate extractions and measurements it was found that accuracy was not increased with the adoption of pyrogallol as an internal standard. Despite this later studies were carried out using pyrogallol as an internal standard to correct for any instrument drift, including injector inaccuracy. Whenever possible, the extraction solution, containing the internal standard, was prepared in adequate quantities to be used for all extractions within a study.

2.2.6. Analytical precision

The precision of analytical methods is a measure of how reproducible they are (Burgard and Kuznicki 1990). The overall precision (or variation) resulting from the sampling, extraction and instrumental analyses steps may be broken down into that of the different steps of the analytical method.

Instrumental precision is estimated by replicate injections of the same sample extract solution. Variation will be caused by differences in sample injection, detection and measurement of detector response (such as peak integration methods). Extraction variation may then be estimated from replicate extractions of wood material from the same sample source, assuming the material is homogenous. Both instrumental and extraction variation were measured for each study. Typical coefficients of variation for instrumental precision were less than 3% and of extraction variation approximately 5%. However the degree of variation was dependent upon the level of tannins, with greater variation at lower concentrations. This trend is illustrated in Table 2.2, which describes the results obtained from ellagitannin analysis of replicated samples from two staves.

Table 2.2: Variation of total and selected ellagitannins (mg/l) measured in three replicate extractions of low (1) and high (2) tannin containing staves without an internal standard. 'R' refers to the roburins, G grandinin, Vesc. and Cast. to vescalagin and castalagin respectively.

Sample		U1	U2	Ra	Rb	Rc	G	Vesc	U3	Cast	U4	Σ
Stave 1	Means	6.08	6.43	10.33	10.25	7.27	21.69	53.48	3.42	57.03	6.07	206.05
	% variation	5.62	6.64	3.51	7.47	8.80	2.29	9.09	18.78	0.41	6.50	10.25
Stave 2	Means	27.14	24.67	37.41	19.84	29.32	61.13	228.77	10.66	218.40	19.52	722.99
	% variation	3.49	6.78	5.56	5.52	2.24	0.49	2.38	19.19	3.27	5.63	4.02

These estimates of instrument and extraction variation are typical to those of early studies (Chapters 3-5). Better repeatability was obtained in later studies when extractions were carried

out under agitation and when there was better separation and integration of ellagitannin peaks. Typical extraction variations from these later studies were between 4% and 6%, irrespective of the levels of tannins.

Method accuracy is a measure of how close the reported value is to the real value. This will depend on instrumental calibration and extraction efficiency. Due to very limited amounts of purified ellagitannins available and their subsequent degradation in solution, calibration was carried out infrequently. Later calibrations were made using castalagin, as this purified ellagitannin was found to be more stable than vescalagin. Caution should be used when comparing absolute concentrations between studies, as separate calibrations were not always possible for each study.

2.3. Measurement of insoluble ellagitannins

Insoluble ellagitannins may be estimated following degradation in alcohol-hydrochloric acid solutions (see figure 2.2) measuring the resulting ellagic acid by HPLC or GC (Puech *et al.* 1990; Peng *et al.* 1991; Scalbert 1992a). Peng *et al.* (1991) used this method for the estimation of insoluble ellagitannins, after initial extraction with alcohol or acetone solutions.

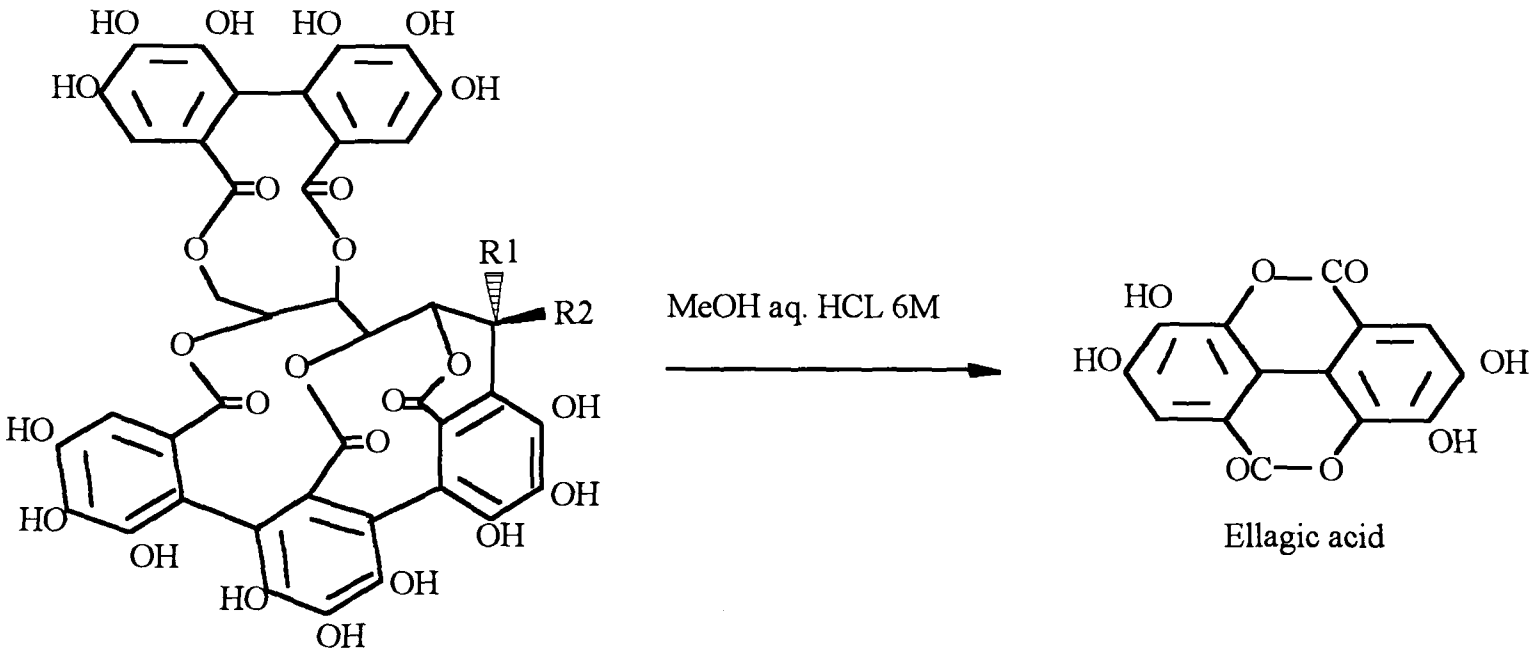


Figure 2.2: Acid degradation of castalagin and vescalagin.

2.3.1. Extraction and degradation

The methodology is derived from Peng *et al.* (1991). 5 ml of MeOH/HCl 6M 9/1 v/v, containing 0.5 mg 1-Napthol (Aldrich) was added to 50 mg of the wood sample in a teflon tube. After heating at 120°C for 160 minutes, the solutions were then filtered and analysed by HPLC to determine quantities of ellagic acid.

2.3.2. HPLC measurement of ellagic acid

Waters HPLC system: Waters pump 600E; Waters autosampler 712 Wisp; Waters UV absorption photo diode array 991. **Column:** Waters reverse phase C18; 260x4 mm; Spherisorb packing. **Injection Volume:** 20 µl. **Detection:** at 280 nm (190-400 nm for identification). **Internal standard:** 1-Napthol (Aldrich).

Gradient: the following solvents were used: H_2O/H_3PO_4 99/1 v/v (solvent A) and $MeOH/H_3PO_4$ 99/1 v/v (solvent B) to run a linear gradient from 0 to 100% solvent B over 30 minutes with a flow rate of 1 ml/min.

2.3.3. Identification and calibration

If one mole of ellagic acid is assumed to form from the degradation of one mole of castalagin, concentrations of ellagic acid may therefore be expressed as castalagin equivalents (Peng *et al.*1990; Viriot *et al.* 1994). The results should be treated as approximate given the relatively crude methodology and the very low solubility of ellagic acid.

2.3.4. Initial extractions of wood

The wood samples may be previously extracted with other solvents to determine the relative proportions of soluble and insoluble tannins. The first extraction sample was allowed to settle before removing the solvent with a syringe fixed with a fine hypodermic needle. The wood samples were then allowed to dry, and were re-weighed, before continuing with the degradation procedure. The total soluble ellagitannins in a solution may also be estimated by acid hydrolysis.

2.4. Measurement of total phenolics

Folin Denis reagent was used to measure total phenolics (AOAC 1984; 1990; Scalbert 1992a). This aqueous solution of sodium tungstate, phosphomolybdic acid and phosphoric acid is reduced by phenolic compounds, in the presence of excess sodium carbonate solution, to form a blue tungstate and molybdenum complex. 1 ml of Folin Denis reagent (Fisons diluted 1:4 with water),

was added to 1ml of the extraction solution followed by 1 ml of a 3% sodium carbonate solution. After agitation, the samples were placed in a water bath at 50 °C for 20 mins. After cooling for 5 minutes, absorbance at 760 nm was measured by spectrophotometer (Phillips). Calibration of the spectrophotometer was performed for each batch of samples using gallic acid (Aldrich) solutions and the results were expressed as gallic acid equivalents (GAE). Extract solutions were suitably diluted, typically 1:5 for a water extraction.

2.5. Measurement of oak lactones

Analysis was carried out at International Technological Services laboratories, Glenochil. A 40% or 63% ethanol solution was used for extraction, corresponding respectively to the strength of bottled and cask strength whisky.

2.5.1. Method 1 (Ford 1991)

(i) To 2 ml of the oak extract is added 0.1 ml of the internal standard, δ-decanolactone (Aldrich) and 1.5 ml of 2:1 diethyl-ether: n-Pentane (Aldrich HPLC grades) solution. After agitating for two minutes and leaving to settle, the clear organic layer is removed. This extraction is repeated 2 further times, the organic layers being bulked in a 10 ml graduated tube.

(ii) The organic solution is blown down to less than 1 ml with nitrogen, while standing in a block heater at 30 °C. It is then brought up to 1 ml with the pentane:ether solution, before being transferred to a 2 ml GC vial and sealed.

GC Conditions

Instruments: Hewlett Packard 5890 Series II gas chromatograph, with HP7673 autosampler. **Column:** DB-Wax 30m, film thickness 0.25 microns (J&W Scientific). **Carrier gas:** helium 1 ml/min column flow. **Sample injection:** 1 microlitre, at injector temperature of 220 °C. **Detector temperature:** 220 °C. **Oven temperature:** initial = 60 °C; ramp rate 10 °C/min, final temperature = 200 °C, after 15 mins. **Run time:** 30 min.

MS Conditions

Instrument: Hewlett Packard 5971A. **Solvent delay:** 7 min. **Ions plotted:** m/e=99.00. **Start time:** 5 min. **Off time:** 20 min.

Identification and calibration

The oak lactone standard from Aldrich contains a mixture of the *cis* and *trans* isomers. Once the proportion of the different isomers has been determined this oak lactone standard was used to calibrate the method.

2.5.2. Method 2

Identical GC-MS conditions are used, but with the 0.5 ml of the 63% ethanol extract solutions being diluted with 0.5 ml of pure ethanol and 0.05 ml of δ-decanolactone before injection. Although less sensitive than the previous method it was found adequate for these studies.

2.6. HPLC measurement of lignin derived extractives, gallic and ellagic acids

Extraction solvent: 40% or 63% ethanol solutions.

2.6.1. Methodology

Two different methods were used. The first (used in chapters 3-4) used a gradient from 5% acetic acid to 12% acetic acid and 12% methanol over either 40 or 20 minutes with a flow rate of 1 ml/min. 20 µl was injected and UV absorbance at 230-280 nm measured. However this method was not found to be satisfactory, especially for measuring the low concentrations of compounds found in untreated oak wood extracts. Therefore future measurements were carried out by International Technological Services using the method outlined below.

2.6.2. HPLC conditions

Column: 260 x 4.6 mm; 5 micron Spherisorb ODS 2 with 30 x 4.6 mm Spherisorb ODS 2 guard column. **Column Temperature:** 40 °C. **Injection Volume:** 10 µl..

Gradient: the linear gradient shown below was used with the following mobile phases: H_3PO_4/MeOH / Acetonitrile / H_2O / 0.5/0.5/1/98 v/v/v (solvent A); H_3PO_4 / MeOH / Acetonitrile / H_2O 0.5/15/35/49.5 v/v/v (solvent B).

Table 2.3: Gradient for the measurement of extractives - see text for solvent composition.

Time (min):	0	30	40	50	55
% solvent B:	15	47	65	80	15
Time (min):	0	25	40		
Detector λ:	280	340	280		

2.7. Measurement of pH

A Jenway 3020 pH meter was used for the determination of pH in aqueous and aqueous alcoholic solutions.

2.8. Extract colour

MacDougall (1989) found that absorption at 520 nm was a suitable single wavelength measure of colour intensity in whisky and brandy, correlating strongly with CIELAB lightness (described later). Absorbance at this wavelength was chosen as a suitable quick measurement of the colour intensity of extract solutions, corresponding well with visual impressions.

2.9. Water content

Because of the sensitivity of ellagitannins to heating most samples for extraction were stored in a desiccator for several days before extraction. Where sufficient material was available the water content of the wood was estimated by weighing samples before and after heating in an oven for 24 hours at 120°C.

2.10. Definition and measurement of wood colour

There are numerous systems used to represent the colour of objects as viewed by the human eye. These systems seek to represent the perception of colour, under standard illumination, within a geometrical space. The system used in this study is the C.I.E.L.A.B. which evolved from the C.I.E. system that was defined by the International Commission of Colour in 1931. This system has been widely used in studies of wood colour (*eg* Klumpers *et al.* 1994; Charrier *et al.* 1995; Janin 1987). The C.I.E. represented colour within a triangle defined by three trivariates: X (red), Y (yellow-green) and Z (blue). The C.I.E.L.A.B. system is a transformation of the C.I.E. scale which aims to increase sensitivity and uniformity by means of three parameters: L*, a* and b*. Figure 2.3 shows the three dimensional colour-space defined by this system. Additional parameters used to describe colour may be derived from these variables. These include the angle of taint, or hue, $h = \arctan(b^* / a^*)$ and the saturation $C^* = \sqrt{a^{*2} + b^{*2}}$.

Measurements were made at INRA, Nancy with a HUNTERLAB ColorQuest spectrocolourimeter using standard illuminant D65 and an observation angle of 10°, coupled to an IBM PC. The percentage of reflected light at 32 wavelengths, distributed at 10 nm intervals between 400 and 710 nm, is measured. For further details see Hunter (1975) and Janin (1987).

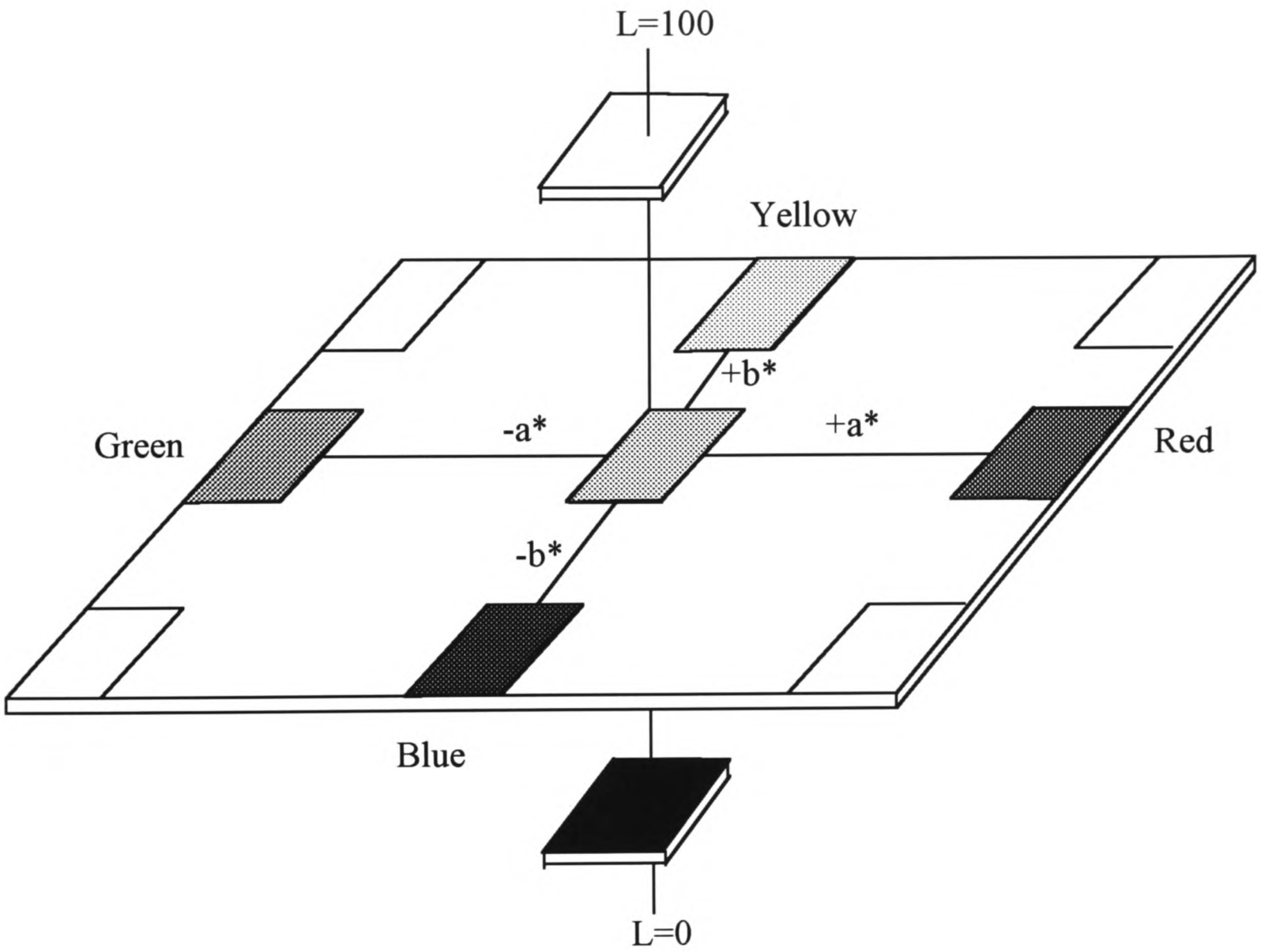


Figure 2.3: CIELAB colour space defined by the variables L, a*, b*. Oak wood colour values fall into the far right quarter, with positive values for all variables.

2.11. Ring widths

The widths of annual rings were measured by a binocular microscope mounted on a travelling stage. The stage was connected to an electronic digitiser sensitive to its movement. This allowed measurements to be made to within an accuracy of 2 μm.

2.12. Microdensitometry

All measurements were carried out at INRA, Nancy, on 5 mm wood cores by means of direct radiographs and then scanning the negatives with a digital microdensitometer (see Polge 1966). Cores were cut to approximately 2 mm depth and arranged over photographic paper, which was then exposed to X-rays for 2 hours at 10,000 mV and 10,000 mA. Each core image was measured by three traverses of its length using an image widow of 500 x 12 μm. The three traverses were adjacent to one another as the cores often had only a narrow central region that was suitable for measurement.

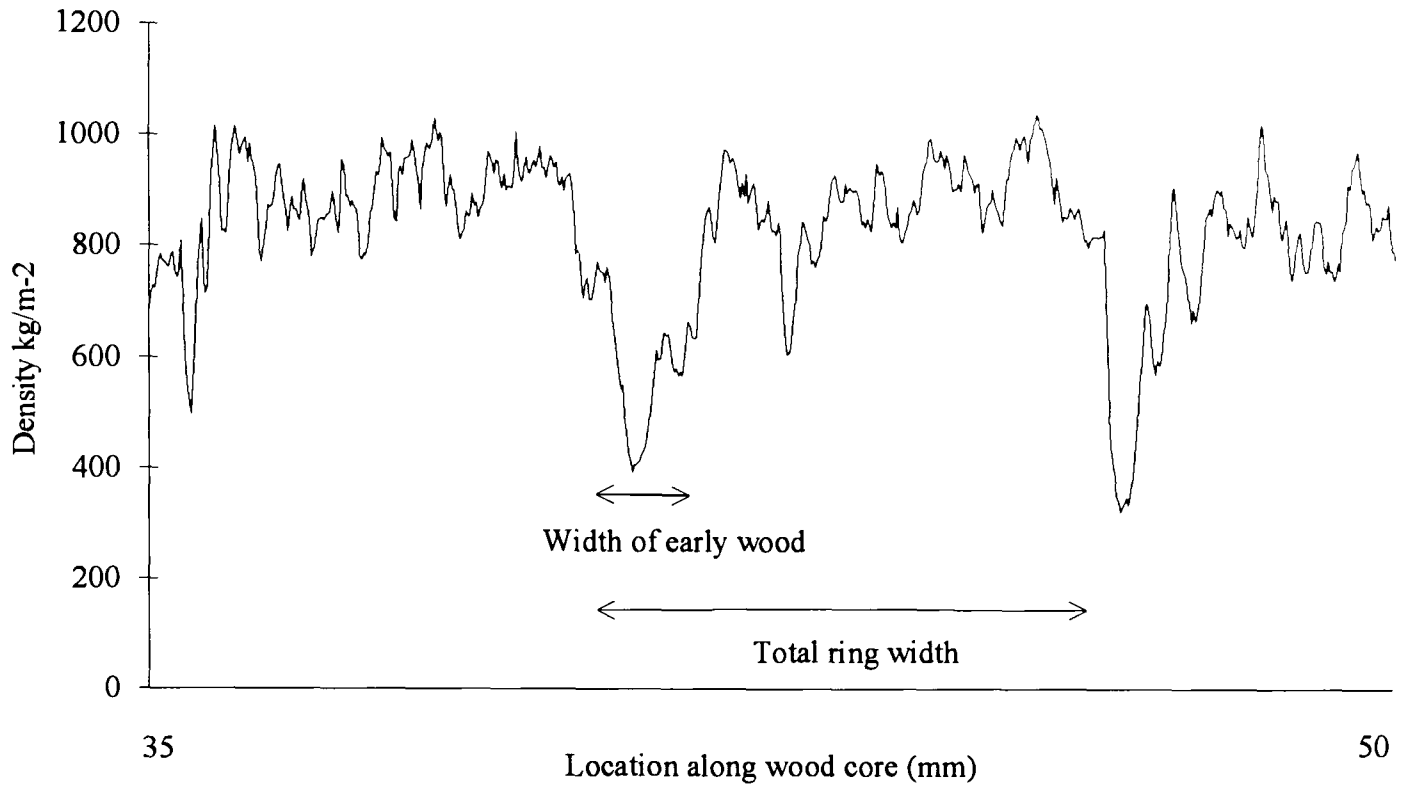
The resulting data were then analysed using the CERD program developed at INRA, Nancy, to determine earlywood, latewood and total ring widths and densities for each annual ring (see figure 2.4). Each annual ring bears an equal weight in subsequent calculations of core, wood zone or tree means which therefore equate to the mean annual ring properties of the wood. Actual mean core densities will be influenced more by the larger annual rings which make up a greater proportion of the wood area. The calculated mean core densities were compared with control measurements of core weight and volume.

2.13. Sensory assessment

Sensory analysis is used to characterise and measure sensory attributes (ITS 1993). The methods used here were either descriptive and/or difference tests and were carried out at International Technological Services.

2.13.1. Triangle tests

Triangle tests are used to test whether unspecified differences exist between two products. Assessors are presented with three samples and informed that two of the samples are identical. The samples may be presented in six possible orders, with each order ideally being used an equal number of times. Each assessor must select the sample they consider different. The statistical significance of the number of correct judgements vs the total number of judgements may subsequently be determined.



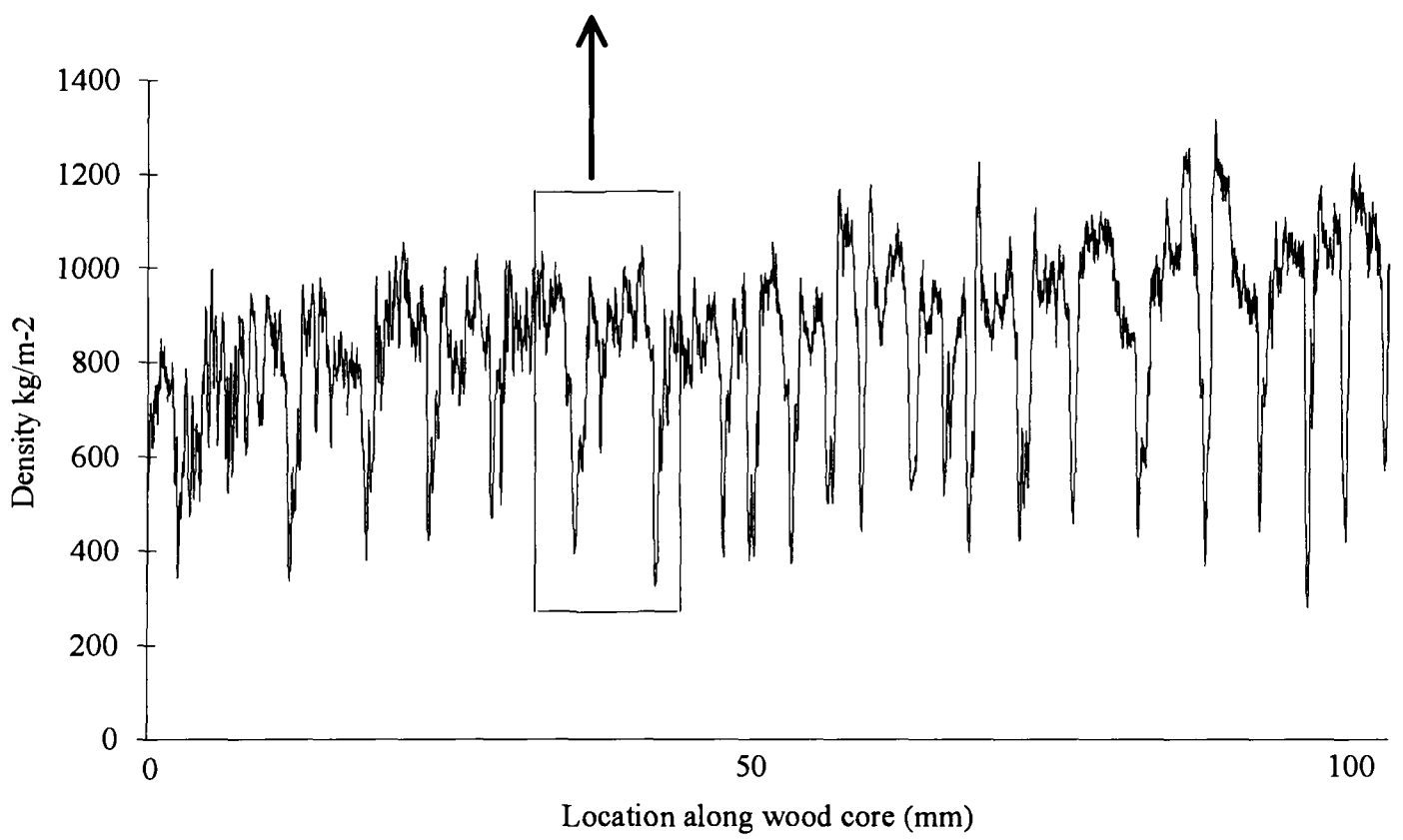


Figure 2.4: Data from microdensitometric scans of the negatives of wood core radiographs displayed by the program CERD.

2.13.2. Descriptive techniques

These are primarily used to describe the sensory characteristics of a product, to produce product descriptions that may subsequently be compared. The method used was a simplified method of free choice profiling (ITS 1993; Williams *et al.* 1984). Samples are presented to a panel of between 8-12 assessors who each describe the sample in their own words. Any number of terms may be used to describe a sample. Ideally samples are presented to each assessor in a different order. The results may be analysed by a number of multivariate techniques, which aim to reduce the variation of samples due to the various flavour descriptors to variation on a greatly reduced number of derived variables with minimal loss of information.

The total number of possible times that a term may be used to describe a set of samples, is equal to the total number of assessments made (n = judges x samples). This may then be used to calculate the number of times a term has or has not been used to describe samples within each group. The *expected* number of assessments in which the flavour term was or was not used can then be calculated for each group assuming the null hypothesis that frequency of use is independent of group. Expected and observed frequencies can be compared through the calculation of a test statistic, such as X^2 which has an approximately chi-square distribution. For these studies X^2 corresponded very closely to the G statistic with Williams' correction for continuity, which is recommended by Sokal and Rohlf (1981) to compare frequency data. The G statistic was not used as it is not suitable if an observed frequency is zero.

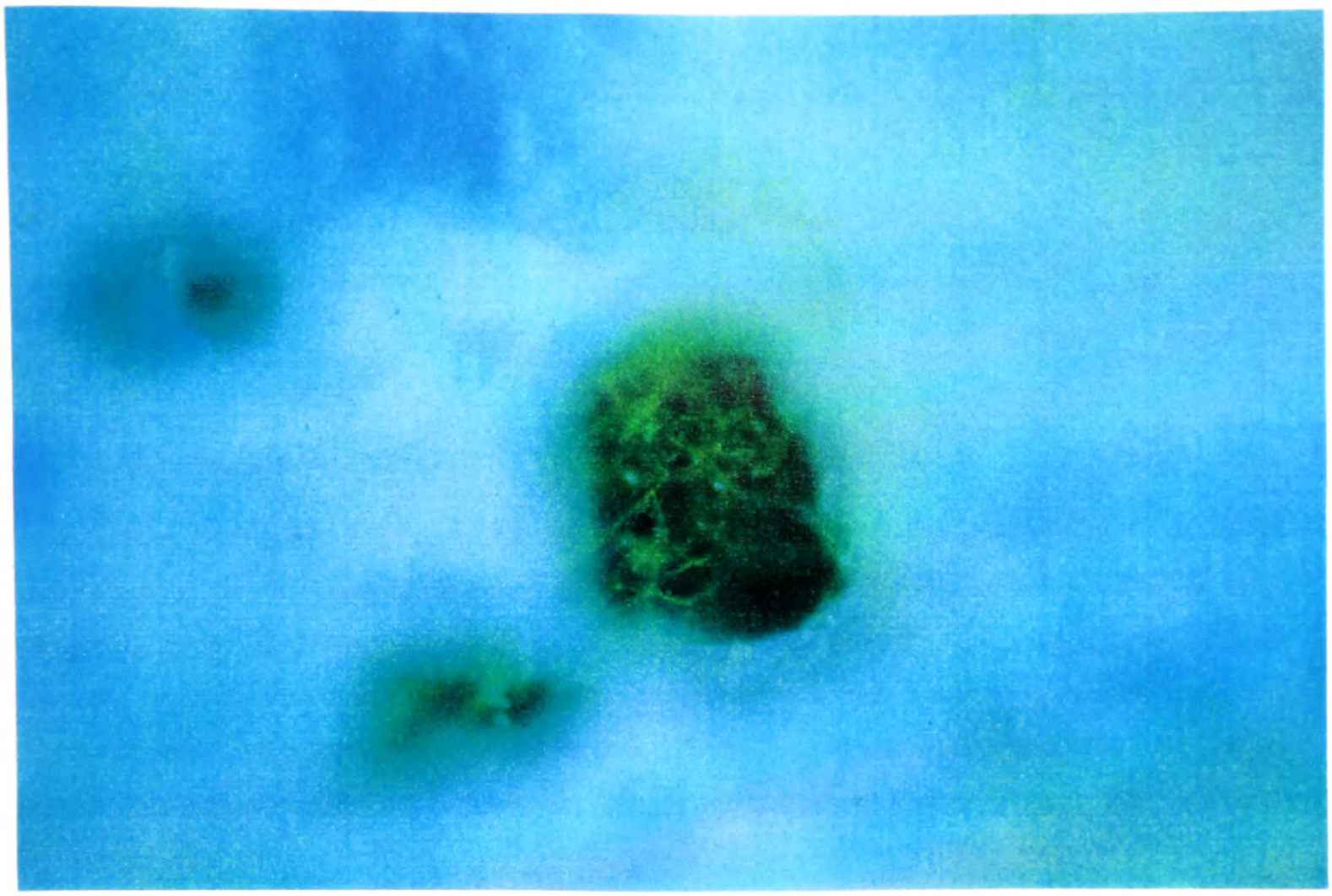
Confidence intervals for percentage use may also be calculated as: $p \pm (50/n + 1.96 \times \sqrt{(\times (100-p)/n)})$. Where p is the percentage of times the term is used and n the possible number of times it could have been used (*eg* as in ITS 1993).

2.14. Microscopy

Both light IR fluorescence and confocal microscopy were experimented with, to determine their suitability in examining wood structure and investigating the location of extractive deposits. Sections of wood approximately 0.5 mm to 1 mm thick were used and a variety of dyes experimented with. No differences between wood sections extracted in ethanol or acetone and non-extracted sections were observed using these methods. However, two different occluding structures (almost certainly tyloses) were found in vessels, which while appearing structurally similar, exhibited different colours under UV light (see figure 2.5). It is possible that the difference in colour may be due to extractive deposits covering some tyloses.

To examine further the location of extractives would require the cutting of ultra-thin sections but this frequently requires softening of the wood by, for example, prolonged soaking in 30% hydrofluoric acid (Barnett 1988). This will remove most wood extractives from parenchyma cells

and vessel segments where they are normally concentrated (McGinnes and Rosen 1984). However, had time allowed, further studies, using methods described by Wheeler and Thomas (1981) or McGinnes and Rosen (1984) could have been attempted to compare sections of wood before and after extraction.



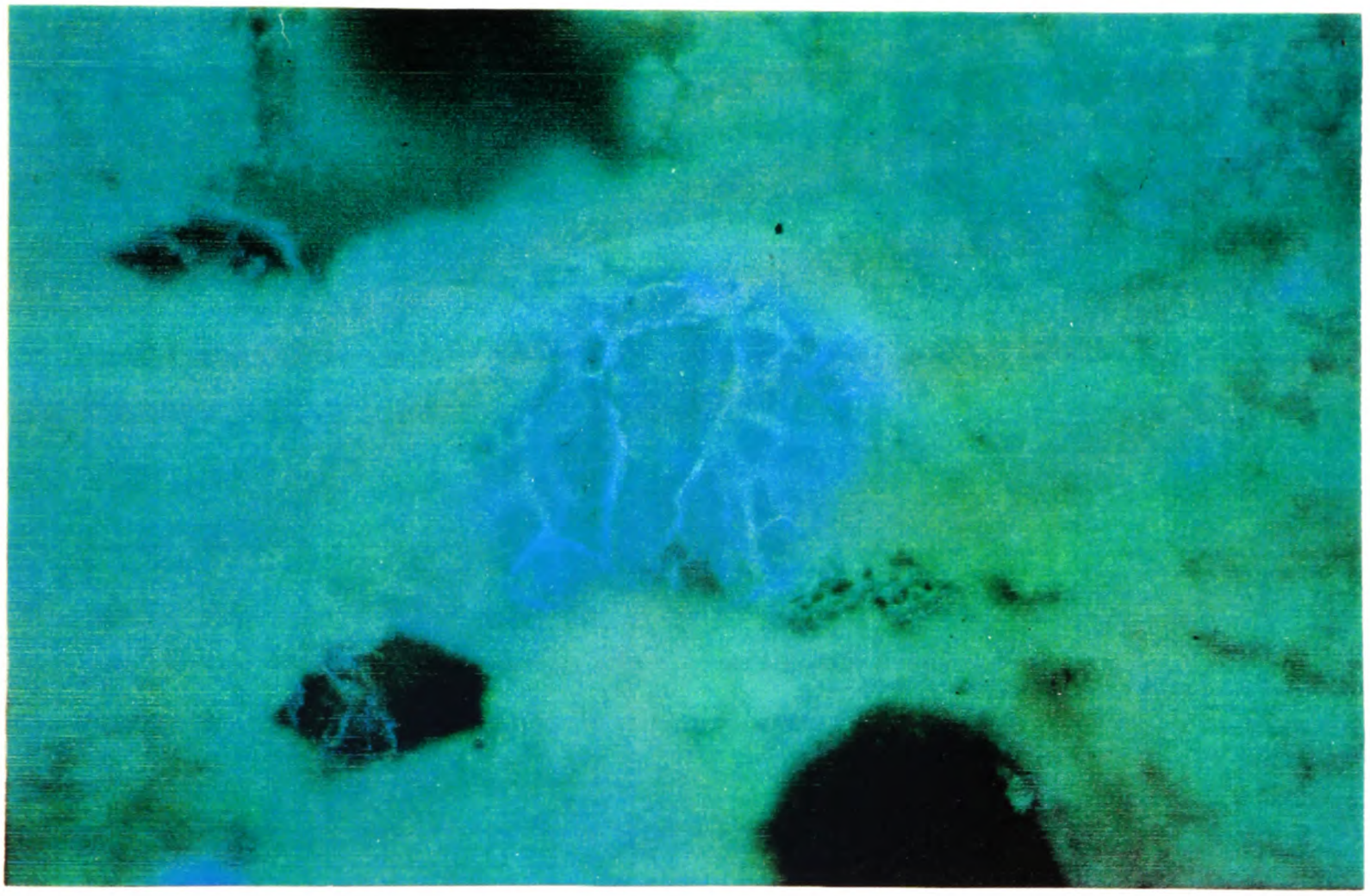


Figure 2.5.a-b: Two contrasting tyloses found in vessels of oak heartwood using UV light microscopy.

Chapter 3: Preliminary studies on the variation of extractives between and within cask staves

There are barrels without taste and then others that give cognac like gold. After several years one knows which barrels are which.

R. Kapuscinski - *Imperium*

3.1. Chapter summary

This chapter describes two studies examining the variation of oak extractives between and within cask staves. In the first, pairs of green and air-dried staves from four different forest origins were compared. Both the concentrations of ellagitannins and oak lactones appeared to vary greatly between the different wood origins. Tannin concentrations were highest in staves from the Limousin and Chateauroux regions, while oak lactone was more abundant in the low tannin staves from Allier and Nevers. Despite the lack of replication the results suggest that seasoning has a predictable if lesser effect; causing a decline in soluble tannins. The results lend support to claims that cooperage wood may vary with regards to its effect on the flavour of alcoholic beverages. The second study compared variation in oak extractives between and within European and American oak staves subjected to different intensities of toasting or charring. Levels of many extractives were found to be dependent upon the exposure of the wood to heating. However, the effects of toasting and charring were generally limited to the outermost 2 to 8 mm of wood, depending upon the intensity of the treatment. Furthermore the variation of some extractives, particularly the oak lactones, was evidently independent of any effect due to heating. The study confirmed differences between American and European cask wood with the former having much lower levels of tannins, but greater amounts of oak lactones.

3.2. Introduction

These studies were carried out to gain a better understanding of the variation of the selected extractives in cask wood. The primary aim was to confirm that significant variation was found between staves and if possible to estimate the degree of variation due to stave origins, seasoning and cask manufacture. It was intended to briefly examine whether the claims of different flavour effects, that had been attributed to different types of oak wood, were in any way reflected by different extractive properties. For these purposes samples that characterised the range of variation possible in cask wood were chosen, rather than numerous samples typifying any one source of wood or cask treatment.

3.3. Comparison of seasoned and unseasoned staves with different forest origins

The tradition of using wood from different forest regions for specific purposes had been emphasised in discussions with Tonnellerie Taransaud, Cognac. It was claimed that wood from the Limousin and near Chateauroux was typically favoured for cognac and brandy casks as the extractives would be released more quickly and give rise to a bitter and tannic taste. In contrast wood typical of the forests of Nevers and Allier was most suited to the maturation of red wine as the flavour imparted was more subtle and developed more slowly.

The differences in flavour effect are most often attributed to a difference in wood grain, which is at least partly correlated with ring width. Limousin wood was characterised by rapid growth and large ring widths. From further discussion it became apparent that the term 'Limousin' wood might be used to describe wood from other regions that had characters typical of Limousin oak. This was supported by discussions with other people involved in the cooperage trade.

Cask wood is frequently claimed by coopers to be entirely air-dried, with various flavour enhancing effects being attributed to this process. Traditionally such seasoning occurs over three years in exposed drying stacks. However, from various discussions it became clear that the majority of wood used is actually kiln-dried as it is impossible to forecast, three years in advance, the amount of wood required for cask manufacture. Nonetheless, some coopers continue to use some air-dried wood, often charging a premium for such casks.

The aims of the first short study were to examine the variation of extractives found in staves from different forests and the influence of seasoning by air drying.

3.4. Methods and materials

3.4.1. Sampling

With the co-operation of Tonnellerie Taransaud in Cognac, four pairs of staves were selected, each consisting of one stave that was unseasoned (green) and another that had undergone three years of air-drying. The stave pairs were from four different French forest regions: the Limousin, Allier, Nevers, and Chateauroux. Staves were taken from the centre of drying stacks, but no details of their precise origins were known. From each stave, the top 1-2 mm of wood was removed with a plane, the surface frequently being either discoloured or covered with a black fungal layer. Samples of wood were then taken at three different points along the length of each stave, using an electric router.

Two separate extractions were made on wood from each of the three stave samples. The first was a water extraction to measure amounts of ellagitannins (according to the method described in 2.2) and the second an extraction with 63% ethanol for the subsequent determination of ellagic and

gallic acids and scopoletin using the method described in section 2.6.1. This method was not very satisfactory, with large background noise and variable retention times. A third extraction with 63% ethanol was carried out on a single sample from each stave to determine the concentration of oak lactones using the method described in section 2.5.1. The mean ring widths of each stave were also measured.

3.5. Results

3.5.1. Ellagitannin concentration

Because of poor separation, roburin d was not measured and it is likely that this peak merged with that of vescalagin.

From the three replicates, variances for each extractive were calculated and tested for homogeneity using Cochran's test (Sokal and Rohlf 1981). With the exception of some of the unidentified ellagitannins, none of the within stave variances were significantly heterogeneous. The overall means and variances of each ellagitannin were calculated and are shown in table 3.1. Total ellagitannins were calculated from the sum of the identified ellagitannins. Means were calculated for each stave, for each origin and for green and seasoned wood. The results are shown in table 3.2.

A 2-way analysis of variance of total ellagitannins confirmed that there were significant effects due to both stave origins and seasoning for concentrations of most soluble ellagitannins (see table 3.3). Significance tests on means of individually identified ellagitannins were not carried out due to the lack of replication and the exploratory nature of the study. However, figure 3.1 shows how the variation in total ellagitannins between forest origins is the same within both green and seasoned staves, while the seasoned stave of each pair has lower levels.

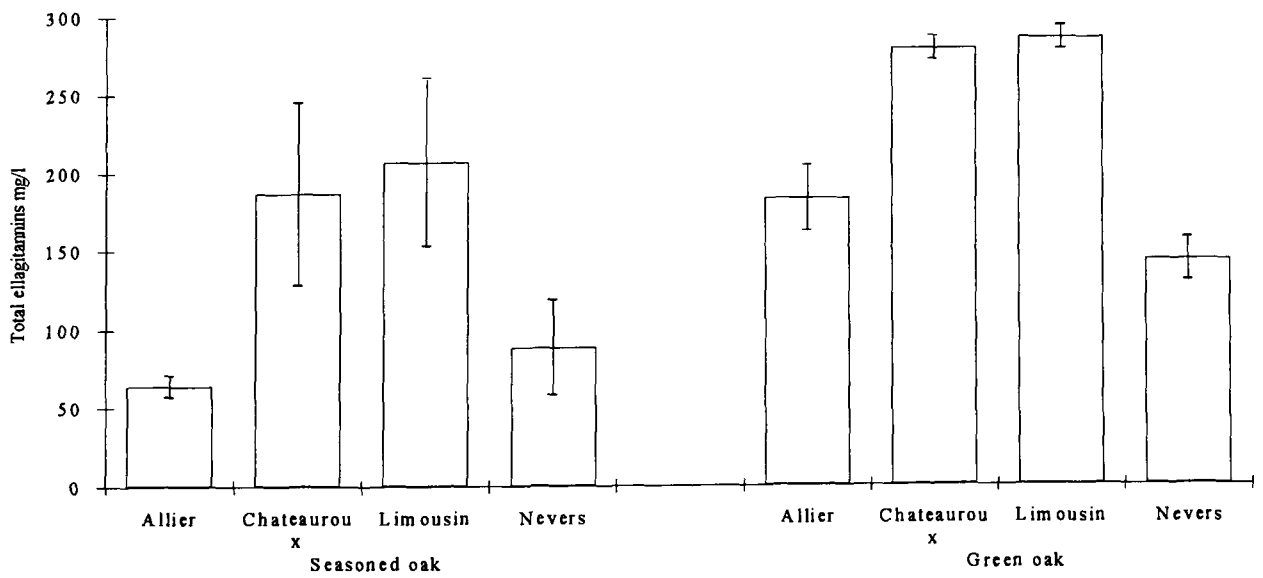


Figure 3.1: Variation in mean concentrations of total ellagitannins between seasoned and unseasoned French oak staves from four different origins.

Table 3.1: Mean concentrations and coefficients of variation of ellagitannins in three extractions from each of eight staves. The staves were air-dried or green oak from four different regions of France.

Ellagitannin	Mean (mg/l)	Coeff. of (%) variation
U1	7.7	83
U2	7.3	35
Roburin a	10.9	55
Roburin b	11.9	44
Roburin c	13.3	41
Grandinin	33.1	44
Vescalagin	55.2	64
Roburin e	14.1	77
U3	2.6	56
Castalagin	41.1	44
U4	1.3	61

Table 3.2: Mean extractive concentrations (mg/l), mean ring widths (mm) and their standard errors (SE). Ellagic acid and tannins measured from three extractions, lactones from a single extraction, from each of 8 French oak staves of different origins and seasoning.

Forest origin	Seasoning	Sum of ellagitannins		Ellagic acid		Trans-lactone		Cis-lactone		Ring width	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	Seasoned	63.87	*3.45*	9.83	*0.14*	0.51		0.12		1.23	
Allier	Green	182.77	*10.80*	9.15	*0.43*	0.35		0.36		1.77	
	Mean	**123.3**	*27.07*	**9.49**	*0.23*	**0.43**	*0.08*	**0.24**	*0.12*	**1.5**	*0.27*
	Seasoned	186.77	*30.02*	17.63	*1.79*	0.01		0.01		2.22	
Chateauroux	Green	279.32	*3.91*	9.98	*0.70*	0.01		0.01		1.98	
	Mean	**233.0**	*24.73*	**13.80**	*1.85*	**0.01**	*0.0*	**0.01**	*0.0*	**2.1**	*0.12*
	Seasoned	206.29	*27.60*	14.11	*1.20*	0.01		0.01		3.71	
Limousin	Green	286.34	*3.92*	10.30	*0.42*	0.01		0.01		3.56	
	Mean	**246.3**	*21.81*	**12.20**	*0.95*	**0.01**	*0.0*	**0.01**	*0.0*	**3.64**	*0.08*
	Seasoned	87.77	*15.36*	23.09	*2.20*	0.05		0.05		2.88	
Nevers	Green	143.38	*7.09*	11.65	*0.29*	0.02		0.09		1.98	
	Mean	**87.8**	*14.55*	**17.37**	*2.67*	**0.035**	*0.015*	**0.07**	*0.02*	**2.43**	*0.45*
All	Seasoned	**136.18**	*20.71*	**16.16**	*2.36*	**0.118**	*0.083*	**0.098**	*0.0892*	**2.51**	*0.52*
	Green	**222.95**	*18.79*	**10.27**	*0.32*	**0.048**	*0.0259*	**0.145**	*0.122*	**2.32**	*0.42*

Table 3.3: Analysis of variance of total ellagitannins (mg/l.) between staves. Both wood origins and seasoning were treated as fixed effects. df = degrees of freedom.

Source	df	Sums of Squares	Mean Squares	F value	P > F
Wood origin	3	87443	29148	36.86	0.0001
Seasoning	1	45181	45181	57.13	0.0001
Origin x Seasoning	3	3123	1041	1.32	n.s.
Error	16	12652	791		

3.5.2. Ellagitannin composition

The percentage of the total ellagitannin concentration, made up by each individual ellagitannin, was calculated for each sample and mean values are shown in table 3.5. A chi-square test found significant variation in the ellagitannin composition of staves (see table 3.4).

Table 3.4: Chi-square test rejecting the null hypothesis (H_0) that the percentage composition of total ellagitannins, made up of 7 ellagitannins, in 24 wood samples is independent of the 8 staves, of different origins and seasoning, from which they derive.

	Degrees of freedom	Value	P of accepting H_0
Chi-Square X^2	42	72.6	0.002

The lack of replication made further statistical analysis of limited value, although a two-way anova (using the same structure as that in table 3.3), carried out on untransformed percentage data of each ellagitannin, found significant differences (at a probability of 0.05 or less) between both origins and seasoning for all tannins except castalagin which varied significantly only between origins.

Table 3.5: Means of percentage composition of total identified ellagitannins by each individual tannin, in three water extractions of wood, from each of 8 seasoned and unseasoned staves from different French forest regions.

Forest origin	Seasoning	Roburin a		Roburin b		Roburin c		Grandinin		Vescalagin		Roburin e		Castalagin	
		Mean	*SE*	Mean	*SE*	Mean	*SE*	Mean	SE	Mean	*SE*	Mean	*SE*	Mean	*SE*
	Green	4.26	*0.30*	9.66	*0.94*	12.04	*0.76*	21.65	*0.57*	14.13	*1.55*	18.05	*1.04*	20.22	*2.09*
Nevers	Seasoned	4.55	*0.32*	6.08	*0.47*	7.29	*0.82*	19.24	*0.78*	28.99	*5.88*	11.02	*7.39*	22.83	*1.39*
	Mean	**4.41**	*0.13*	**7.87**	*0.85*	**9.66**	*1.10*	**20.44**	*0.59*	**21.56**	*3.67*	**14.54**	*2.49*	**21.52**	*0.87*
	Green	6.21	*0.11*	6.25	*0.19*	6.44	*0.58*	17.65	*0.11*	37.40	*6.88*	4.96	*6.91*	21.08	*0.19*
Limousin	Seasoned	8.84	*0.43*	6.06	*0.87*	6.68	*0.42*	15.19	*0.67*	40.20	*2.46*	0.73	*0.84*	22.30	*0.25*
	Mean	**7.53**	*0.60*	**6.16**	*0.23*	**6.56**	*0.19*	**16.42**	*0.58*	**38.80**	*1.99*	**2.85**	*2.03*	**21.69**	*0.29*
Chateau-	Green	5.63	*0.06*	6.11	*0.91*	6.37	*1.53*	18.21	*1.02*	30.84	*3.56*	9.52	*6.59*	23.32	*0.14*
roux	Seasoned	6.81	*0.90*	5.19	*1.20*	5.80	*0.82*	17.01	*1.77*	36.91	*8.67*	4.39	*5.92*	23.89	*1.91*
	Mean	**6.22**	*0.35*	**5.65**	*0.44*	**6.08**	*0.47*	**17.61**	*0.59*	**33.88**	*2.78*	**6.96**	*2.56*	**23.60**	*0.51*
	Green	5.05	*0.26*	8.48	*0.79*	9.72	*0.95*	23.20	*0.85*	13.26	*0.50*	14.21	*0.98*	26.09	*2.16*
Allier	Seasoned	5.99	*0.25*	6.36	*0.07*	7.49	*0.79*	16.02	*0.99*	38.44	*0.56*	1.76	*0.56*	23.96	*0.84*
	Mean	**5.52**	*0.23*	**7.42**	*0.52*	**8.60**	*0.59*	**19.61**	*1.64*	**25.85**	*5.63*	**7.98**	*2.80*	**25.02**	*0.76*
All	Green	**5.29**	*0.22*	**7.63**	*0.49*	**8.64**	*0.76*	**20.18**	*0.72*	**23.91**	*3.31*	**11.69**	*1.9*	**22.67**	*0.78*
n=12	Seasoned	**6.55**	*0.48*	**5.92**	*0.23*	**6.81**	*0.27*	**16.86**	*0.54*	**36.14**	*1.85*	**4.47**	*1.68*	**23.24**	*0.38*

In general the variation between origins of tannin composition can be seen from table 3.5 to be similar to that observed for the total amount of tannins, in that Allier and Nevers staves have a contrasting composition to the Limousin and Chateauroux staves. Two star diagrams of the mean ellagitannin composition of unseasoned Limousin and Nevers staves are shown in figure 3.2.

These illustrate that the latter is characterised by lower relative amounts of vescalagin but high amounts of the roburins b, c and e and grandinin. A similar pattern of variation is seen between the seasoned and unseasoned staves, the latter having higher amounts of the same ellagitannins which all incorporate a pentose residue.

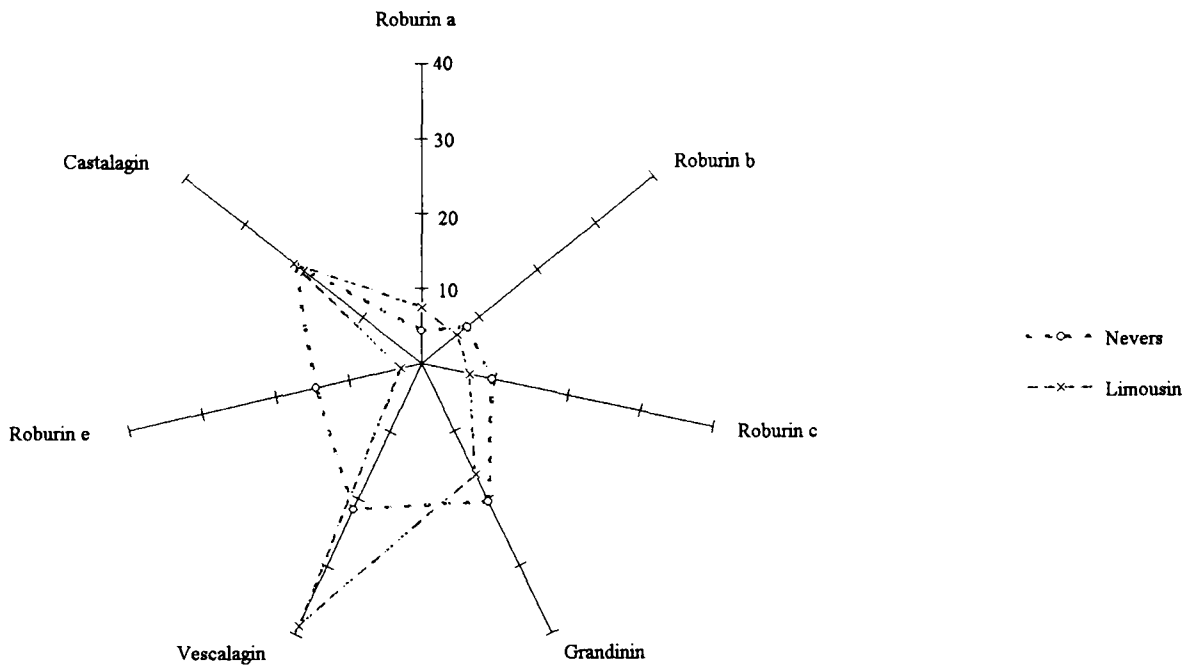


Figure 3.2: Mean proportions of total ellagitannins of seven individual tannins in three water extractions from an unseasoned Limousin and Nevers oak stave.

3.5.3. Other extractives

Only a single extraction to measure oak lactones was made for each stave, although two later replicate extractions of one of the staves gave identical concentrations. As with the tannin variation the results, shown in table 3.2, emphasise the differences between wood origins, with only two of the four (Nevers and Allier) having measurable quantities of lactones. However, within these pairs, the level of the *trans* isomer is higher and that of the *cis* isomer is lower in seasoned staves than in green oak.

The level of ellagic acid was found to be higher in the seasoned staves, but with no clear pattern observable among the different wood origins, as shown in figure 3.3. Poor separation of peaks prevented the measurement of gallic acid, while no effect of wood origins or seasoning was apparent in the levels of scopoletin, which varied little between staves.

Although the two Limousin staves did posses the largest annual growth rings, the study does not allow one to adequately test for correlations with extractives. From table 3.2 no simple relationship can be observed.

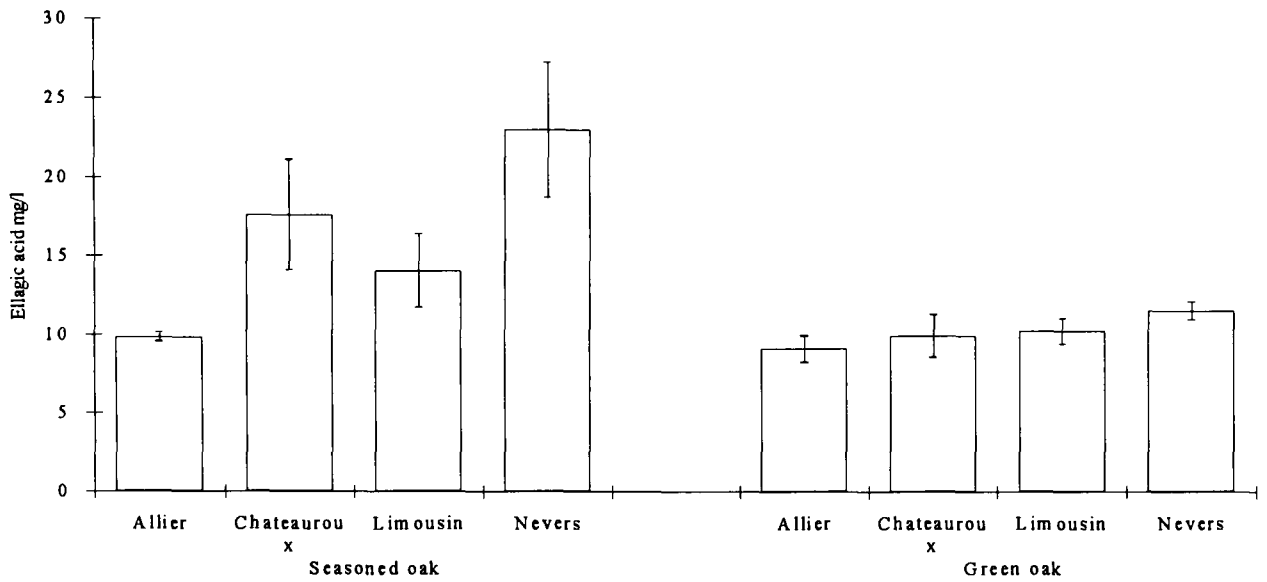


Figure 3.3: Variation in ellagic acid between seasoned and unseasoned staves from four different French forest origins. 2 x standard error bars are shown.

3.6. Discussion

The results suggest that significant differences in the amounts of wood extractives do exist between staves from different wood origins and that air-drying has also an influence on both composition and quantities. The lack of replication in the study prevents any firm conclusions being reached, as the level of variation between staves of the same type cannot be determined. Ford (1992) found significant differences between staves from the same American oak barrel, although this was after charring and previous storage of bourbon. Nonetheless, the pattern of variation between the forest origins was the same for both seasoned and green staves in both the levels of tannins and lactones. Similarly, all four pairs of staves displayed similar differences between the seasoned and green wood. The lack of any significant interaction term in the analysis of variance of total tannins further suggests that the differences between staves are explained largely by origin and seasoning.

The lower levels of extracted ellagitannins in the air-dried staves supports the observations of other studies, from Jolyet (1892) to Pontallier (1992). This difference may be caused by hydrolysis or polymerisation reactions during seasoning, similar to those reported during ageing of heartwood (Pontallier 1992; Peng *et al.* 1991; Klumpers *et al.* 1994; Viriot *et al.* 1994). The higher levels of ellagic acid found in the seasoned wood are most likely the result of ellagitannin hydrolysis. Due to the very low levels of lactones found in half of the staves the results cannot be used to test the claims by Maga (1989a) that the levels of lactone increase with the ageing of wood, but do suggest that the relative proportion of the two isomers may change during air-drying. Sefton *et al.*(1990b) similarly found that the proportion of *trans* lactone may increase after air-drying, depending on the conditions of seasoning and wood origins.

The relative amounts of the different ellagitannins also varied widely between staves, with significant differences between forest origins and seasoning. The differences in composition between origins were similar to those between seasoned and unseasoned staves, perhaps indicating a common cause.

The four stave origins could be divided into two groups that were clearly differentiated from one another by the level of tannins and oak lactones. The staves from the Limousin and Chateauroux had high tannin levels but very low amounts of oak lactones, while the staves from Nevers and Allier displayed opposing characteristics. The results from this study could easily be interpreted as lending support to the descriptions of the flavour properties of the wood types, described earlier.

3.7. A comparison of staves after different intensities of toasting and charring

The treatment of wood during the construction of casks may profoundly influence its extractive properties. Of most importance are the methods which involve the heating of the wood (as discussed in chapter 1). Among French coopers the most frequent method of bending the staves prior to hooping the barrels is to 'toast' them over a fire of oak chips. Although the inside of casks made from toasted staves may be scorched and blackened, even intense toasting does not cause any charring of the wood. This is in contrast to the ex-bourbon casks, made of American oak, which are subjected to more intense temperatures, causing a layer of char to form on the inside of casks. The aims of this short study were to examine the effect of both toasting and charring on the extractives studied. It helps set in context the importance of other factors, such as oak origins, compared to the cooperage processes most likely to influence wood flavour.

3.8. Method

Two sets of staves were obtained for the study. The first set of five Limousin (*Q. robur* or *Q. petraea*) staves, toasted to differing degrees, were obtained from Tonnellerie Taransaud, Cognac. The second set was of three new American oak (*Quercus alba*) staves charred at three different intensities and was obtained through United Distillers. The precise origins of the American staves were not known.

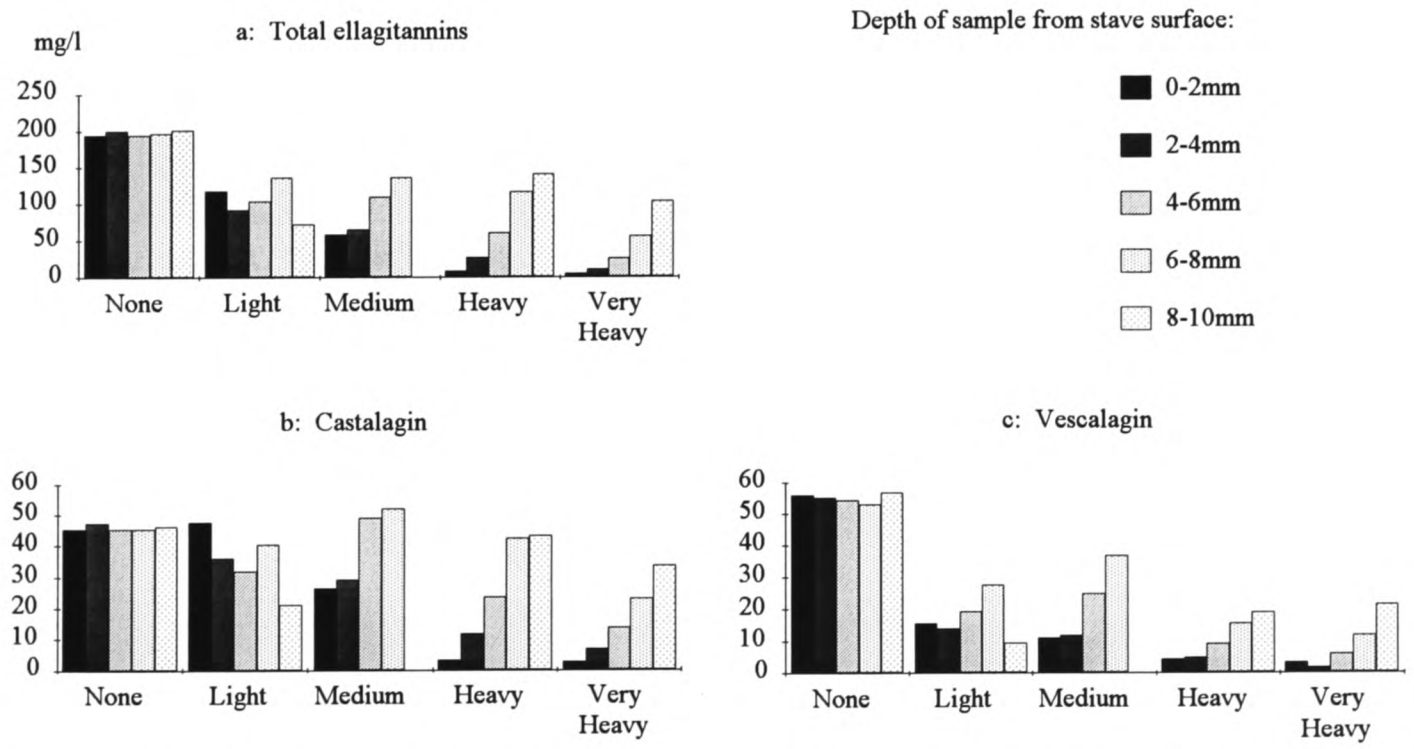
Samples were taken from the central part of each stave at depths of 2 mm intervals from the heat exposed surface, using an electric router. A water extraction was used to measure ellagitannins (see section 2.2) and an extraction with 63% ethanol was used to determine the concentrations of ellagic and gallic acids, lactones and certain lignin derived extractives (see sections 2.5.1 and 2.6.1).

3.9. Results

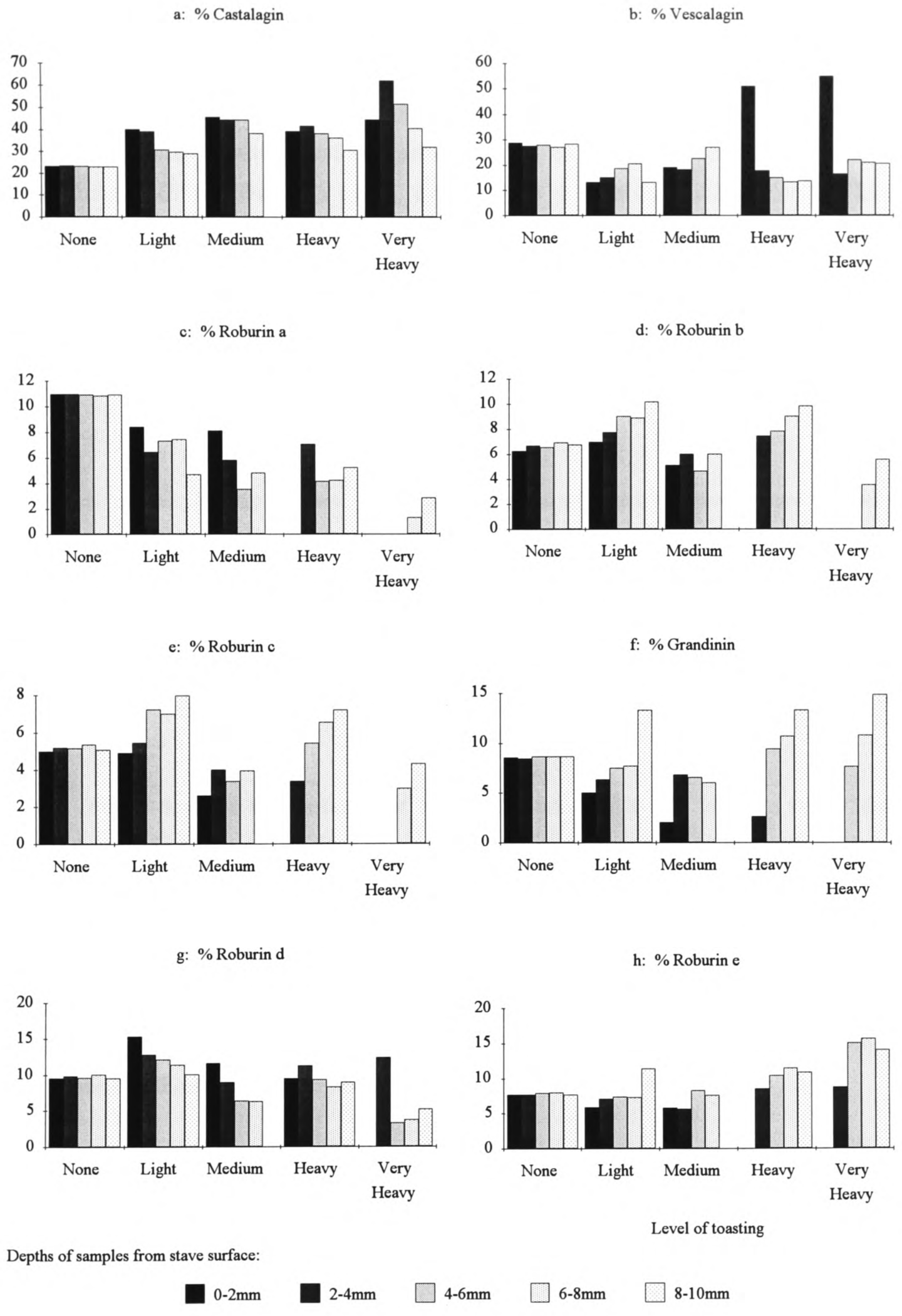
3.9.1. Toasted staves

Ellagitannin content

The concentrations of individual ellagitannins and their sum declined towards the surface of toasted staves and between staves as the level of toasting increased. It is clear that the general effect of heating is to reduce the levels of tannins in the wood. This is in agreement with other studies (such as Charrier 1992; Ford and Done 1991), with the tannins most likely forming ellagic acid and other degradation products. Figure 3.4.a shows this effect of heating on the total amount of ellagitannins. Variation in the levels of individual ellagitannins was also examined to determine whether they responded differently or displayed different levels of stability on heating. Plots of the absolute amounts indicate that tannins respond differently, figures 3.4.b-c suggesting that castalagin is degraded less by heating than vescalagin.



Figures 3.4 a-c: Concentrations in castalagin, vescalagin and total ellagitannins in samples taken at different depths from the surface of five staves of French Limousin oak toasted at different intensities.



Figures 3.5 a-h: Concentrations of individual ellagitannins expressed as the percentage of total ellagitannin concentration. Measured in samples taken at different depths from five staves of French Limousin oak, toasted to different intensities.

The percentages of the total tannins made up by each individual ellagitannin were calculated for each sample and figures 3.5.a-h show the results. These confirm that castalagin increases in relative abundance in staves and at depths exposed to higher temperatures. Certain other ellagitannins, such as roburins a and d, also increase in relative abundance at low levels of heating, such as in the more lightly toasted staves. In those samples where nearly all the ellagitannins have degraded, such as the outermost samples of the two heavily toasted staves, correct identification of the remaining peaks was difficult, particularly when their retention times are similar, such as for roburins d, e and vescalagin.

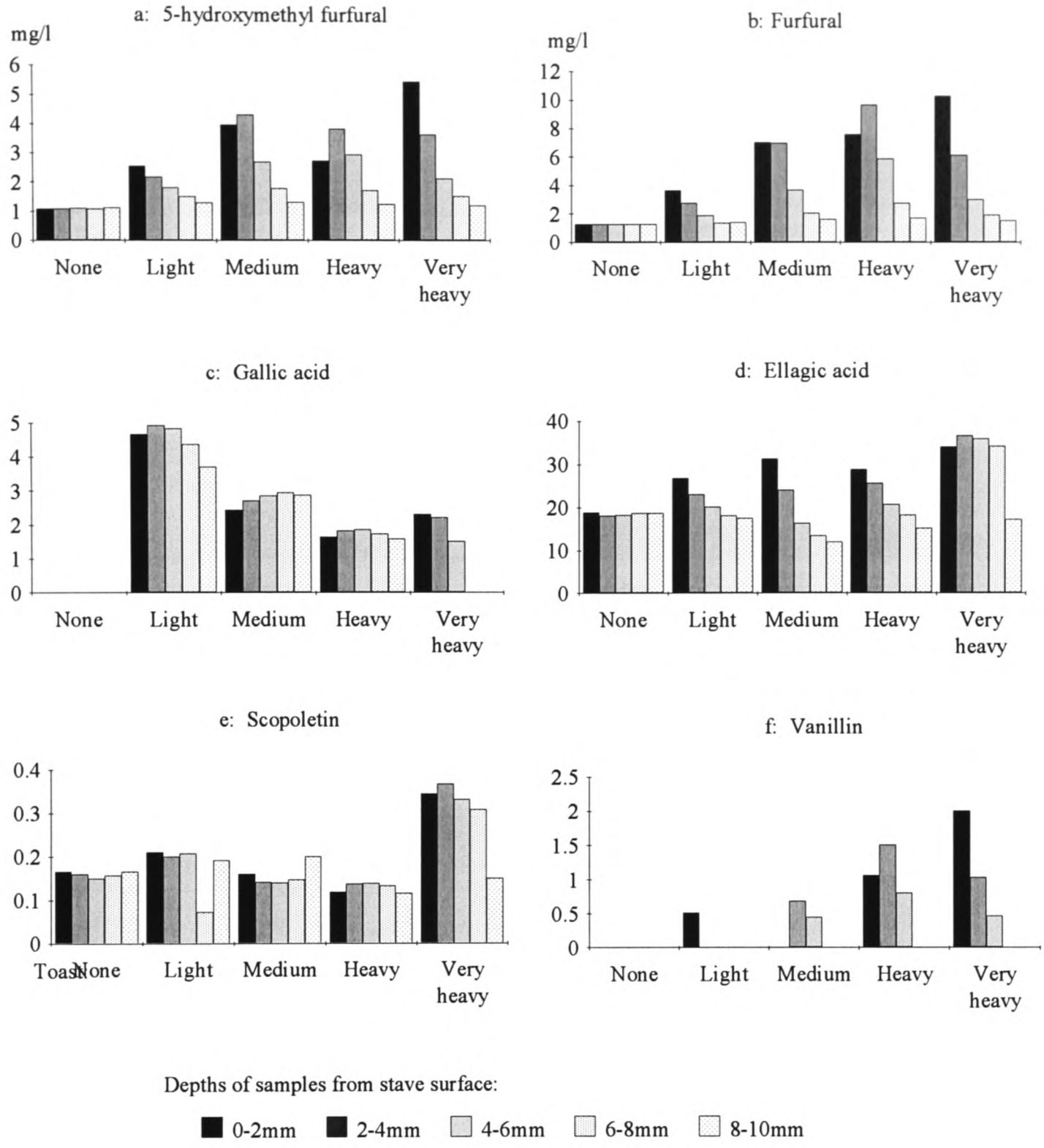
Oak lactones

The levels of oak lactone showed much greater variation between staves than within them, making interpretation of the effects of heating difficult. The single stave from which measurable amounts were extracted does not allow any conclusions on the effect of heating to be made. The very low levels of lactone extracted from these Limousin staves corresponds with the low amounts found in Limousin staves which were examined in the previous study.

Other extractives

Figures 3.6.a-f describe the variation of the compounds within and between staves showing clearly the effects of heating on the concentrations of extractives. Both 5-hydroxymethyl furfural and furfural were predictably very strongly correlated with the intensity of toasting, found at their highest levels in the surface layers of the most highly toasted staves. These compounds result from the thermal degradation of cellulose and hemicelluloses and are characteristic of burnt wood. The results also indicate that the effect of toasting is restricted to the outer 6 to 8 mm of the stave, with levels of furfurals in the inner-most samples of even the very heavily toasted stave being similar to those of the untoasted stave.

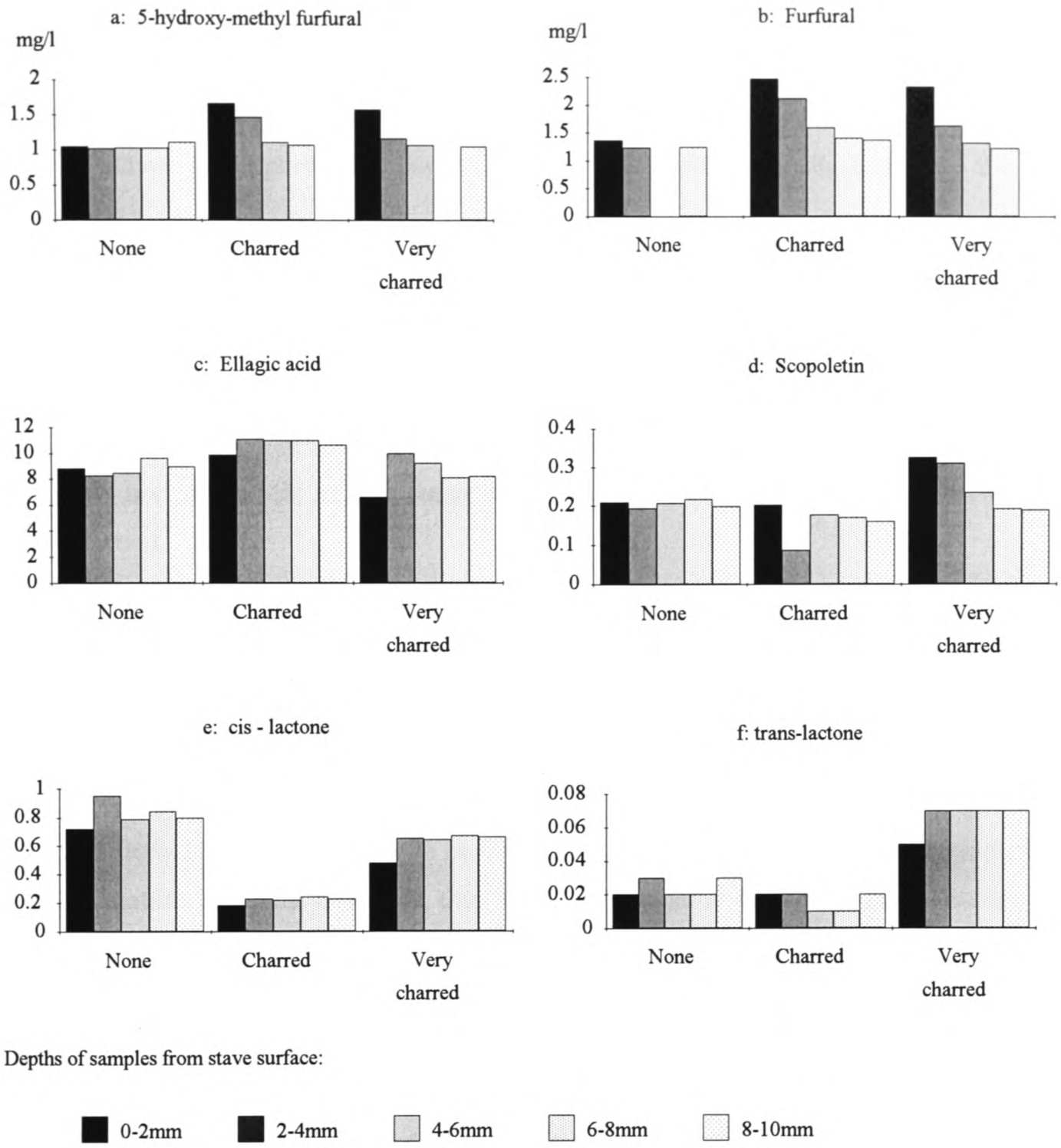
Ellagic acid showed similar variation between samples to that of the furan compounds. It was at its highest levels in the surface samples and more heavily toasted staves. However, gallic acid showed a different, less interpretable, pattern of variation. No peak was detected in any samples from the untoasted stave nor in some from the very heavily toasted stave. It was found at its highest concentrations in samples exposed to low levels of heating, being most abundant near the surface of the lightly toasted stave. Scopoletin appears to be affected only by very high levels of heating, while vanillin and syringaldehyde were only found in measurable quantities within the samples most exposed to high temperatures.



Figures 3.6.a-f: Concentration of extractives in samples taken at different depths from five staves of French Limousin oak each toasted to different intensities.

3.9.2. Charred staves

Figures 3.7.a-f describe the variation found in extractive concentrations between samples. Predictably the variation in the furans was similar to that in the toasted staves, although the levels varied little between the two charred staves, suggesting that there was little difference between the charred and heavily charred treatments. Furthermore the concentrations were much lower than those found in the toasted staves. Scopoletin and the aromatic aldehydes, vanillin and syringaldehyde, also showed a similar pattern of variation.



Figures 3.7.a-f: Concentrations of extractives in samples taken at different depths from the surface of three American oak staves charred at three different intensities.

As has been previously reported for American oak (*eg* Puech 1984; Ford and Done 1991), the amounts of ellagitannins were very low even in the unheated stave, with total ellagitannin concentrations of no more than 6 mg/l. Although the two largest peaks were tentatively identified as vescalagin and castalagin, identification of individual tannins was very uncertain due to their low concentrations and therefore samples were not compared. The low levels of ellagitannins is the most likely cause of the relatively low concentration and variation in ellagic acid. Levels of oak lactones, while much higher than those found in the European oak samples, showed little variation within staves. This indicates that they are relatively unaffected by heating.

3.10. Discussion

Because variation between staves cannot be assumed to be caused solely by the different heat treatments, conclusions on the effects of heating can only be reached where a pattern of variation between staves is supported by the variation within a stave, as illustrated by the two furan compounds.

The results show the effects of heating on the formation of lignin derived extractives and degradation of ellagitannins, as has been observed by previous studies (Sarni *et al.* 1990; Marsal and Sarni 1987; Charrier 1992). Similarly the relative composition of both tannins and other extractives also varied between samples. However, they do not allow one to assume that other factors do not play a role as it is feasible that the effect of heating may vary between different types of wood.

The levels of lactones varied much more between than within staves, suggesting little effect of heating despite the claims of Maga (1989a) and other reports that levels are increased by heating. Given the results of this and the earlier study (3.3), lactone levels seem to be more affected by wood origin than by treatment of the wood during cask manufacture.

Ford and Done (1991) suggested the charring of casks was the reason why ellagitannins are not found in mature whiskies. However, they have been detected during the early months of cognac and brandy maturation (Viriot *et al.* 1993). Although nearly all ellagitannins are degraded in the samples most exposed to heating, they are still present in wood within a few millimetres of the stave surface of even heavily toasted staves. It is likely therefore that they are still available for extraction. More likely explanations for the absence of ellagitannins in whisky are that they rapidly transform into oxidised and decomposition products or undergo complexation with other compounds.

Individual ellagitannins appeared to respond differently to the toasting treatments. Both Roburin a and d increased in relative amounts, compared to other ellagitannins, and absolute amounts at low levels of exposure. Both these are dimeric tannins that may arise by oxidative phenolic coupling at low temperatures. Of the two dominant monomeric tannins, castalagin and vescalagin, the latter was found to be more susceptible to degradation at low temperatures.

3.11. Chapter conclusions

These two studies show that there are significant differences between staves in the levels of extractives. Despite the lack of replication the results support claims that the levels of extractives may vary significantly between forest origins of the staves, with the amounts of extracted lactones and tannins corresponding to descriptions of flavour that had been assigned to the different types of wood. However, these studies also emphasise the importance of cooperage

practices on the levels of available extractives. The heating of wood, by toasting or charring, had a major effect on extractives, while the results also suggested an effect due to air-drying. Any attempt to examine the effect of wood origin in greater detail must ensure that other influencing factors are kept constant or are excluded. Because of the difficulty in ensuring constant cooperage effects (such as seasoning conditions and the degree of heating) it is better that future studies compare untreated wood so as to determine whether real forest or provenance differences exist.

Chapter 4: Preliminary studies on the variation of extractives within and between mature oak trees

Wet ground is acidic, and then the oak will be too bitter. One senses that immediately in a cognac. A tree that was wounded when it was young, will also not give a good cognac. In a wounded tree the juices do not circulate properly, and the wood no longer has that taste.

R. Kapuscinski - *Imperium*

4.1. Chapter summary

Two studies examined the variation of soluble ellagitannins within and between oak trees. While negligible quantities were found in the sapwood, the highest levels were extracted from the outer heartwood. Concentrations declined as heartwood age increased towards the central pith and a simple model is proposed for equating the concentration of soluble tannins in heartwood of different ages. However, the pattern of variation differed among individual ellagitannins, with the dimers Roburins a, b and c, increasing in the first ten to thirty years of heartwood. Despite the large within tree variation, a comparison of the concentrations of ellagitannins extracted from the outer heartwood found significant differences between the nine trees studied. The variation both between and within trees was very large, with the highest concentration of ellagitannins, found in the outer heartwood of trees, being ten times the lowest. A comparison of the early and latewood of 10 annual rings confirmed the findings of Singleton (1974) in that the earlywood had a mean of 121% of the total ellagitannins extracted from the latewood. Levels of oak lactone were found to vary highly between two trees and increased in concentrations from the outer towards the inner heartwood.

4.2. Introduction

If any comparison is to be made between different trees and forests then an understanding of the nature of variation within these categories is necessary to enable the adoption of correct sampling regimes. The use by the cooperage industry of a restricted range of wood (generally the outer heartwood of trees between 90-200 years old) may reduce much of the variation that might otherwise be present within trees. Nonetheless, variation within trees may be considerable, it having being reported in many studies that the level of soluble tannins varies with heartwood age (*eg* Peng *et al.* 1991). It is important at an early stage to estimate the degree of this variation. This will influence how rigidly such factors need be controlled when making comparisons between provenances or trees.

4.3. Variation of extractives between and within the wood of trees from local woods around Oxford

4.4. Material and methods

Sections from two oaks felled at Wytham wood in 1989 and more recently felled trees, between 80 and 110 years old, from three other woods near Oxford were studied. The measurements were made during the autumn and winter of 1992. Table 4.1 describes the trees and number of samples taken.

Table 4.1: Summary of tree origins and the number of different heights and areas of heartwood from which samples were taken.

Site	Species	Date of felling	No. Trees	No. Heights	Heartwood zones	Samples per height x zone
Wytham	*Q. robur & Q. petraea*	Nov. 1989	2	6	3	2
Bagley	*Q. petraea*	Oct. 1992	2	3	2	1
Blenheim	*Q. robur*	Oct 1992	2	3	4	1
Eynsham	Unknown	Nov. 1992	5	2	1	1

Cross sections were cut at different heights from each tree while they were awaiting processing at local saw-mills. Narrow sections across each disk were then cut and stored for 1-2 months before sampling at different locations along their diameter with an electric router (see figure 4.1). Samples were generally taken from the sapwood and from different zones of heartwood, each of which coincided with an approximate 20 year period of growth. Only for the two trees felled in 1989 were the exact heights up the bole known. For the other trees the different heights can simply be taken as different locations along the main bole of the tree, corresponding to the region of wood which, if of adequate quality, would be used for cask manufacture. Generally only a single sample at the same height and wood zone was measured, except from the two trees at Wytham where two samples from opposite sides of the bole were taken.

Samples were also collected of freshly felled healthy, frost damaged and fungal infected oak heartwood during a visit to forests in the Limousin region of France.

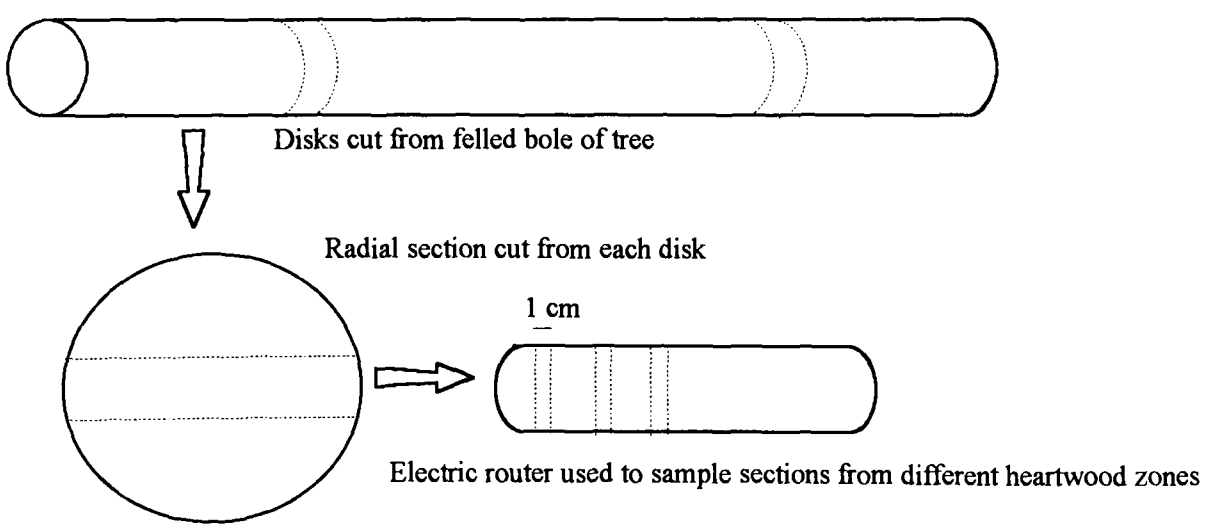


Figure 4.1: Outline of tree sampling methodology.

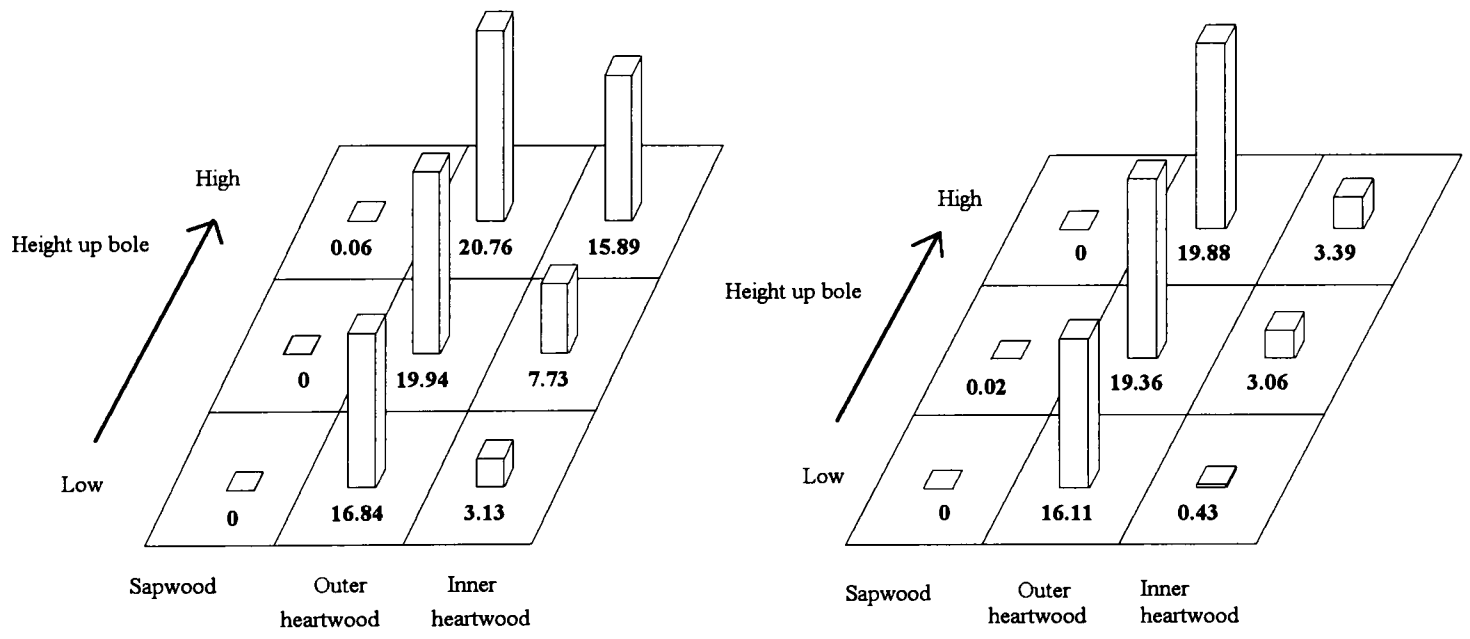
Wood samples were extracted and water soluble ellagitannins measured by HPLC, as described in section 2.2. The concentration of oak lactones in 63% ethanol extractions were also measured (see section 2.5) for a subset of samples from the trees felled at Wytham.

4.5. Results

4.5.1. Variation of ellagitannins

Variation within trees

The pattern of variation found within the two trees felled at Bagley wood is shown in figures 4.2.a-b. Although only a single sample was measured for each zone, both trees showed similar variation. The results indicate a decline in the levels of soluble tannins from the outer towards the inner heartwood. Negligible or no measurable amounts of any ellagitannins were found in sapwood samples and this was found to be true for all the other trees sampled.



Figures 4.2.a-b: Concentrations of total ellagitannins (mg/g) extracted from sections of wood sampled at different heights and depths from two trees felled at Bagley wood.

As well as a sharp decline from the outer to inner heartwood, levels of tannins also drop from the top towards the bottom of each tree. This reflects the different age of heartwood that comprises the two zones at each height. The heartwood age of the inner zone decreased from the lower to upper heights and it was observed that the concentration of tannins in this zone varied most between the three heights.

The mean total ellagitannin concentrations of the two samples taken at each height and heartwood zone, from the two trees felled at Wytham, are shown in figures 4.3.a and b. Quite large variation was found between some of these samples, as indicated by the large error bars, while variation at different heights showed no clear pattern. The trees displayed irregular growth both between years and at different points around the circumference. Therefore samples at the same height and

wood zone do not necessarily correspond to exactly the same wood area, with respect to cambial or heartwood age.

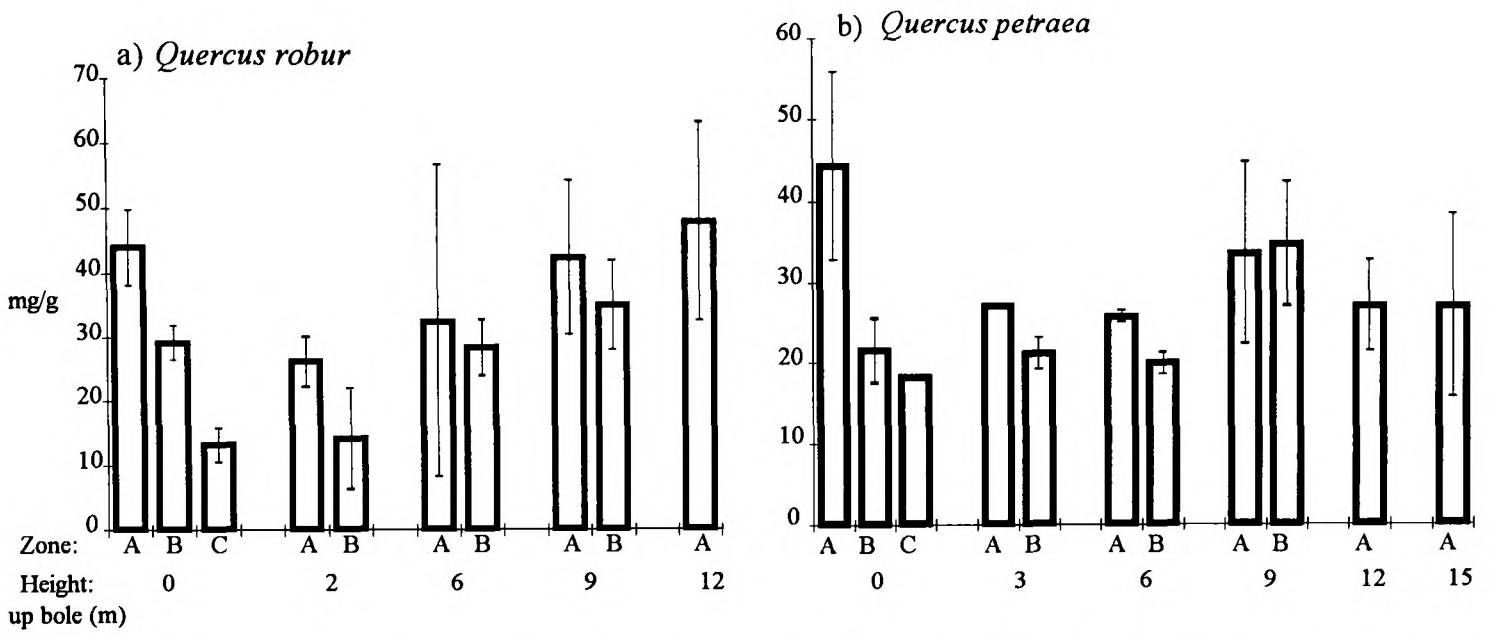


Figure 4.3.a-b: Concentrations of ellagitannins extracted from different heartwood zones, from A (outer heartwood) to C (inner), at different heights up two trees: *Q. robur* (left) and *Q. petraea* (right) from Whytham wood. The mean concentration from two extractions made from different wood samples from each zone and 2 x standard error bars are shown.

Total concentrations of ellagitannins in the two trees from Blenheim were much higher than those found in the four trees from Bagley and Wytham, and better peak separation was achieved. These two trees were used to examine variation in individual ellagitannins as shown in figures 4.4.a-b.

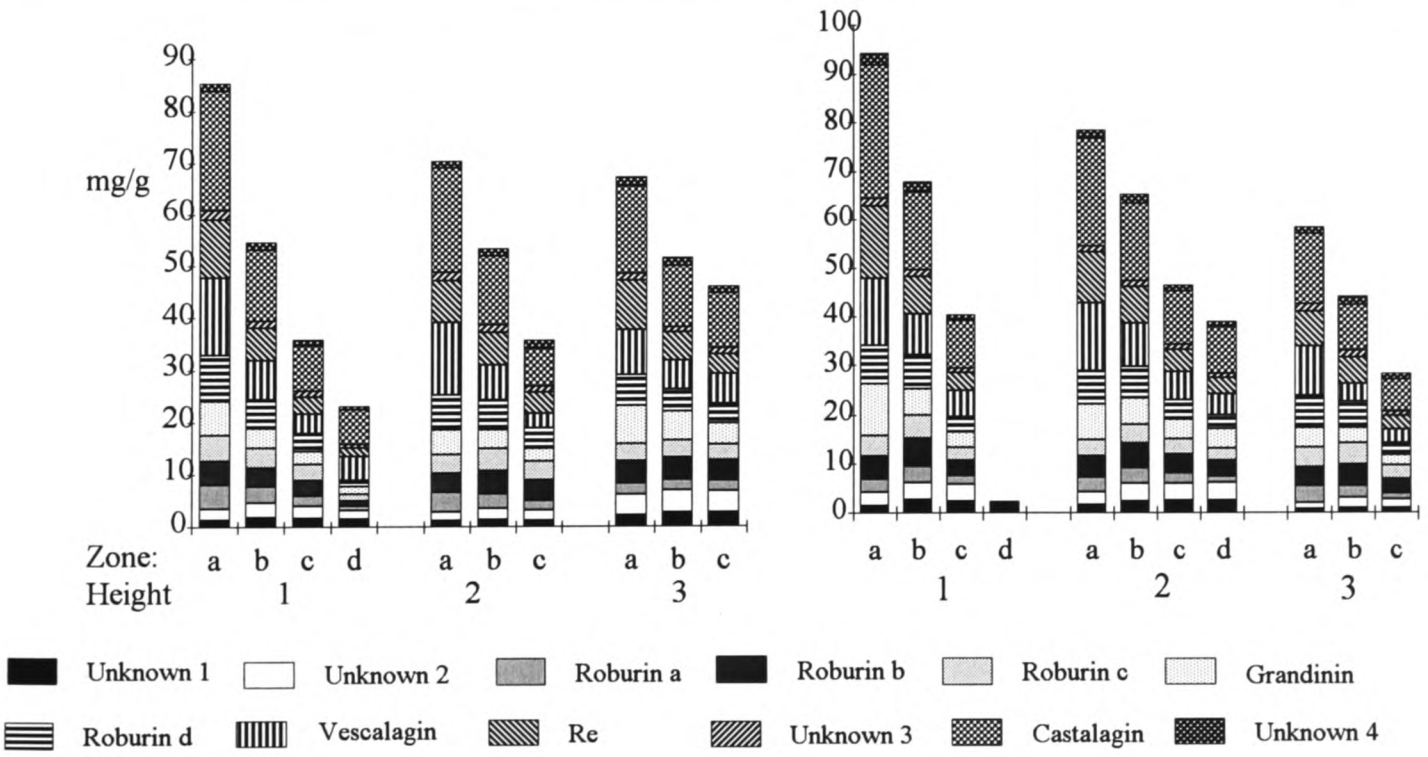


Figure 4.4.a-b: Concentrations of ellagitannins (mg/g) among samples of two trees felled at Blenheim estate. Samples taken from outer (a) to inner (d) heartwood zones from three heights, at approximate one metre intervals (1=low, 3=high) along the bole.

The total concentrations of ellagitannins show the opposite trend, in regards to variation with height up the bole, to that observed for the two Bagley trees (figure 4.2). This is despite the

average age of some heartwood zones declining with increasing height. These differences are, however, relatively small in both cases.

Most ellagitannins, notably vescalagin and castalagin, follow a similar pattern of variation as that observed for total ellagitannins, although the rates of decline with heartwood age appear to vary. However, the amounts of the two unknowns 1 and 2 can be seen from figure 4.4 to be generally at their highest in the middle zones of heartwood. The variation in the identified ellagitannins can be best seen in figure 4.5. This illustrates that the levels of a number of the less abundant ellagitannins, such as roburins *a-c* all frequently increase from the outermost to the next inner zone of heartwood. After this increase the levels generally decline with heartwood age.

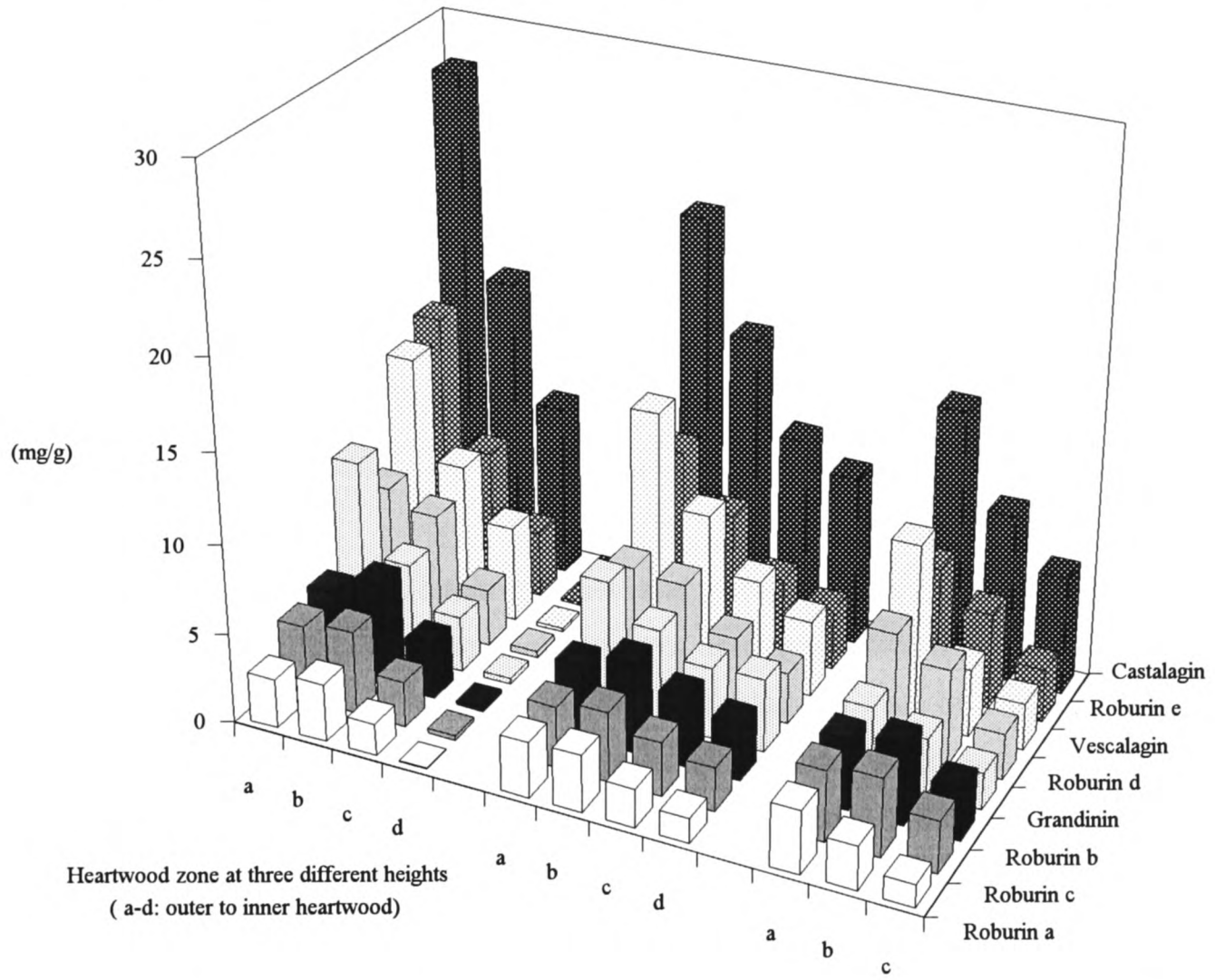


Figure 4.5: Variation of individual tannins between different heartwood zones from three different heights (left lowest; right highest) along the bole of a recently felled *Q. robur* tree from Blenheim estate.

Variation between trees

Samples were taken only from the outer heartwood at two different locations along the boles of the five trees felled at Eynsham. The concentrations of ellagitannins were measured in these samples and their sums determined. The results were pooled with those for the outermost heartwood zones from the highest and lowest sections of the trees felled at Blenheim and Bagley. This gave a total of nine trees, each sampled at two different heights up their boles. A one-way analysis of variance was carried out to determine if there was significant variation between the

trees. Table 4.2 shows the results and estimated variance components expressed as percentages of the total variation. Despite the sampling method being not particularly rigorous, a substantial part of the variation is attributed as occurring between trees. The magnitude of variation is also very large with the highest mean tree concentration being ten times that of the lowest tree mean.

Table 4.2: Comparison of the ellagitannins extracted from the outer heartwood at two different heights up the bole of nine trees deriving from three woods near Oxford (degrees of freedom: 8 between trees and 9 as error). Probability (P) of significant differences between trees and the percentage (%V) of total variation attributed to occurring between trees. Means, maxima, minima and coefficients of variation (CV%) among samples in mg/g of wood.

	Variation between trees		Concentrations in outer heartwood samples			
	P	%V	Mean (mg/g)	CV%	Min	Max
Unknown 1	0.36	14	2.0	65	0.18	3.5
Unknown 2	0.53	0	2.6	64	0.36	4.8
Roburin a	0.12	45	3.6	73	0.34	6.8
Roburin b	0.06	56	3.5	61	0.23	6.8
Roburin c	0.07	55	3.2	61	0.33	5.2
Grandinin	0.04	63	5.2	65	0.31	10.6
Roburin d	0.15	39	6.3	54	0.38	12.7
Vescalagin	0.17	37	15.5	89	1.49	42.9
Roburin e	0.02	68	8.0	70	0.37	14.9
Unknown 3	0.42	7	1.6	33	0.81	3.0
Castalagin	0.14	41	19.5	71	1.60	36.5
Unknown 4	0.09	49	1.3	37	0	2.2
Total	0.01	67	54.4	69	5.00	119.0

Individual ellagitannins show different degrees and patterns of variation. One of the most abundant tannins, vescalagin, while varying the most among samples from the outer heartwood (as indicated by its coefficient of variation in table 4.2) relatively little of this variation occurred between trees. From figures 4.4 and 4.5 vescalagin appears also to be one of the tannins most affected by heartwood zone, decreasing rapidly towards the interior of the heartwood.

Table 4.2 shows that concentrations of total ellagitannins vary enormously, even within the outer heartwood, making up between 0.5 and nearly 120 mg/g of wood. The values obtained from trees at Eynsham mill included the highest of all those recorded during the three years of research. Water content of the wood was estimated as approximately 16%, and so 119 mg/g equates to 14% of wood dry weight, which is greater than the highest values reported by Scalbert *et al.* (1988a) who observed that ellagitannins could make up to 10% of heartwood dry weight.

Frost damage and fungal infection

The samples of both frost damaged and fungal infected oak heartwood obtained from France both gave negligible quantities of ellagitannins. In discussions with coopers it was claimed that even if such wood were structurally sound, the flavour resulting from its use in casks would be unwelcome. From samples collected from a tree felled at Bagley wood in 1990, which had

suffered from heartwood rot, it was found that substantially lowered levels of ellagitannins characterised an area of wood that extended beyond that with observed structural damage.

4.5.2. Variation of oak lactones

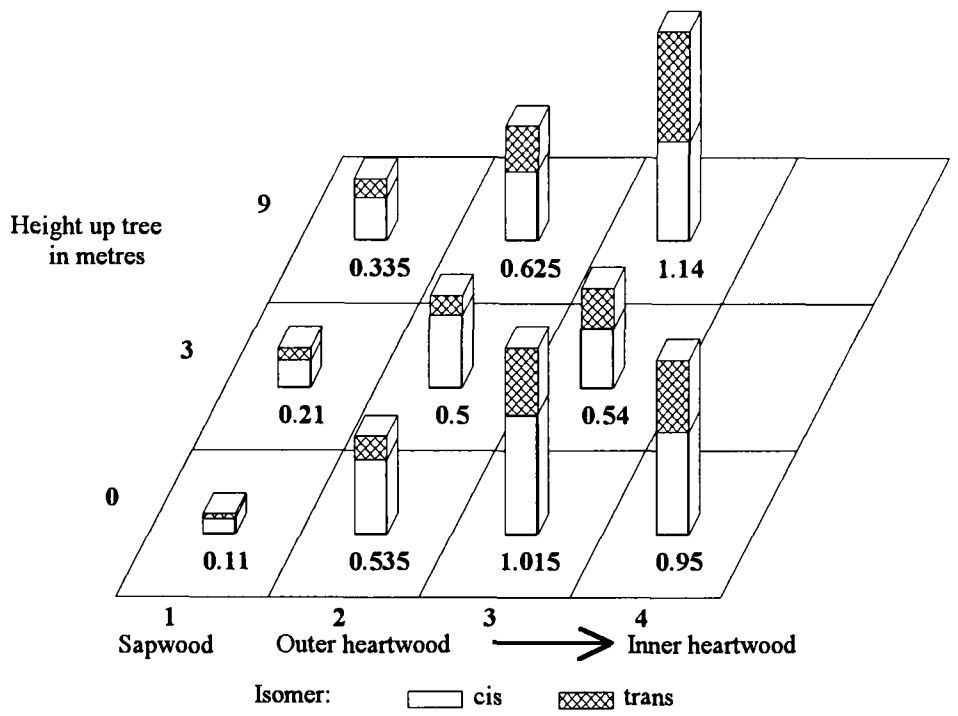


Figure 4.6: Mean concentrations (ppm) of the sum of both *cis* and *trans* isomers of oak lactone from different wood areas of a *Q. petraea* tree felled at Wytham wood. Means are shown from two extractions of samples of each wood zone, taken from opposite sides of the bole.

The concentrations of oak lactones extracted with 63% ethanol from a subset of samples from the two trees felled at Wytham were measured. Only very low concentrations of between 0.01 and 0.02 ppm were found in extractions from the *Quercus robur* specimen and these results are not shown. However, levels in the *Q. petraea* tree were much higher and showed clear variation between samples as illustrated in figure 4.6 and table 4.3. This indicates that the concentration of both isomers display a different trend to that of ellagitannins, increasing from the outer towards the inner heartwood. The *trans* isomer can be seen to increase in relative abundance with the increase in absolute concentrations of both isomers.

Table 4.3: Concentrations of oak lactone in extractions made from a *Quercus petraea* tree felled at Wytham in 1989. Means based on two measurements from different extractions from sapwood and (a) the outer to (b) the innermost heartwood zones.

Height (m) up bole	Wood zone	*Cis* isomer		*Trans* isomer	
		Mean	*S.E.*	Mean	*S.E.*
0	Sapwood	0.085	*0.007*	0.025	*0.035*
0	Heartwood a	0.410	*0.127*	0.125	*0.049*
0	Heartwood b	0.650	*0.014*	0.365	*0.007*
0	Heartwood c	0.560		0.390	
3	Sapwood	0.145	*0.106*	0.065	*0.064*
3	Heartwood a	0.390	*0.156*	0.110	*0.057*
3	Heartwood b	0.320	*0.140*	0.220	*0.014*
9	Sapwood	0.235	*0.007*	0.100	*0.014*
9	Heartwood a	0.380	*0.085*	0.245	*0.148*
9	Heartwood c	0.545	*0.049*	0.595	*0.078*

4.6. Discussion

No ellagitannins were found in the sapwood, while levels in the different regions of the heartwood varied significantly. The highest levels were found in the outer heartwood, while the lowest occurred in the central, older heartwood. Differences between heights may have been due to the different heartwood ages defining each wood zone. The results also indicated that individual tannins varied differently within tree heartwood, with not all being most abundant in the outermost heartwood.

Despite the large within tree variation, a comparison of ellagitannins extracted from the outer heartwood found significant differences between trees. The wood zones sampled were of only approximately the same heartwood age and each tree was sampled at different heights along its bole. Therefore differences between trees could be attributed to differences between the age of these zones, caused for example by different rates of growth. However, the size of the differences in ellagitannin concentrations between trees is unlikely to be explained solely by such relatively small differences in the age of the heartwood samples.

Fungal infestation has frequently been found to correlate with a decrease in the levels of ellagitannins (*eg* Bauch *et al.* 1991), although the exact mechanism of this decrease is still disputed. The results obtained here emphasise the need for careful felling and sample storage, to avoid fungal infection, if valid comparisons of extractive concentrations are to be made.

Levels of oak lactone also varied highly between different heartwood regions. The degree of variation was of a similar magnitude to that found for heartwood tannins. Although the number of samples compared was very small, the presence of only trace amounts of oak lactone in the *Quercus robur* tree suggests that large variation occurs between trees and possibly between species. This supports the findings of the previous chapter which found very large variation between staves.

The results indicate that a large proportion of variation between staves could be explained simply by the area of heartwood from which they are cut. As generally two rows of staves are cut from each tree, the results from this study indicate that the second, inner row will have generally much lower levels of ellagitannins and possibly higher lactone concentrations. Variation in extractive concentrations between the trees could also have been partly due to differences in the age of the wood, caused for example by the width of annual rings. However it is unlikely that this could explain all variation between trees observed in this study and particularly not the large variation found in concentrations of oak lactone between the two trees in which levels were assessed.

4.7. Tannin variation with heartwood age and between early and latewood

The primary aim of this study was to examine more carefully the variation of oak ellagitannins with heartwood age. This required better sampling techniques, which allowed the exact age of each heartwood sample to be known. The most convenient method of obtaining suitable samples is by wood cores. If individual tannins showed different patterns of variation then this points to the possibility of determining the age of heartwood from the composition of water soluble ellagitannins. The ability to do so would allow variation due to heartwood age to be accounted for *post-hoc*, if necessary.

Singleton (1974) reported that the earlywood had an average of 132% the total phenolics extracted from latewood of adjacent annual rings, leading him to predict that slow growing oak would have higher levels of total phenolics and tannins. As previous results have found no obvious link with ring size, a small study was thought prudent to compare ellagitannin concentrations in early and latewood of the same annual rings.

4.8. Methods

4.8.1. Variation in tannin composition with heartwood age

Four Pressler cores, each taken at breast height from a different tree of between 100-120 years old were used in the study. Three of the trees were taken during May 1993 from an oak stand near Voronezh, Russia, while the other was from an isolated field boundary oak near Oxford, taken in 1990. All the trees were *Quercus robur* and displayed fairly regular and rapid growth throughout the core lengths.

The cores were cut into different sections according to the age of the heartwood from the heartwood-sapwood boundary: 0-5 years, 6-10, 11-20 and so on in steps of ten years up to 40 to 70 years according to the tree. After being ground, wood from each zone was extracted and ellagitannins measured by HPLC according to the method described in 2.2.

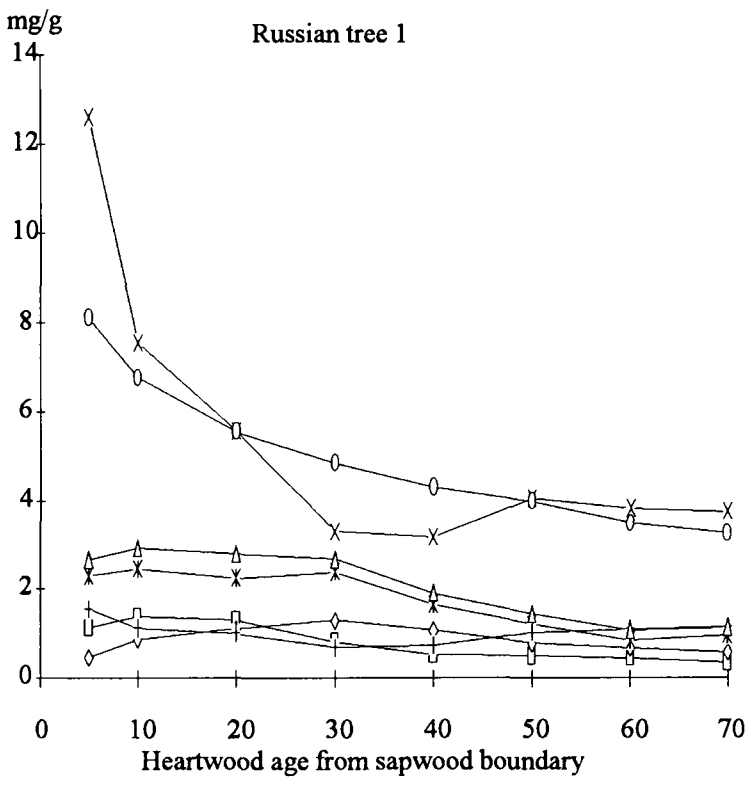
4.8.2. Comparison of early and latewood

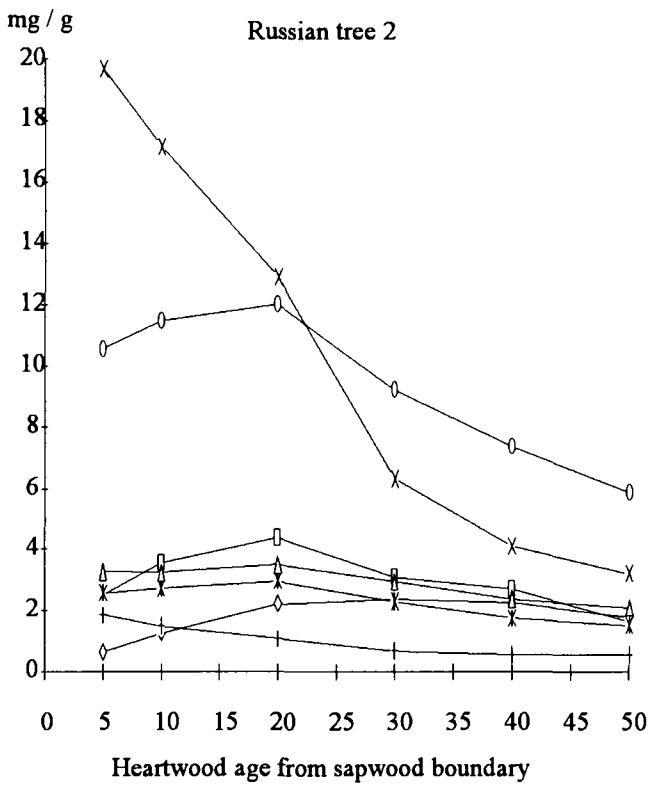
The early and latewood was separated from 10 adjacent annual rings from a section of the outer heartwood of a *Quercus petraea* tree from north-east France. A single extraction was made from each wood zone of each ring.

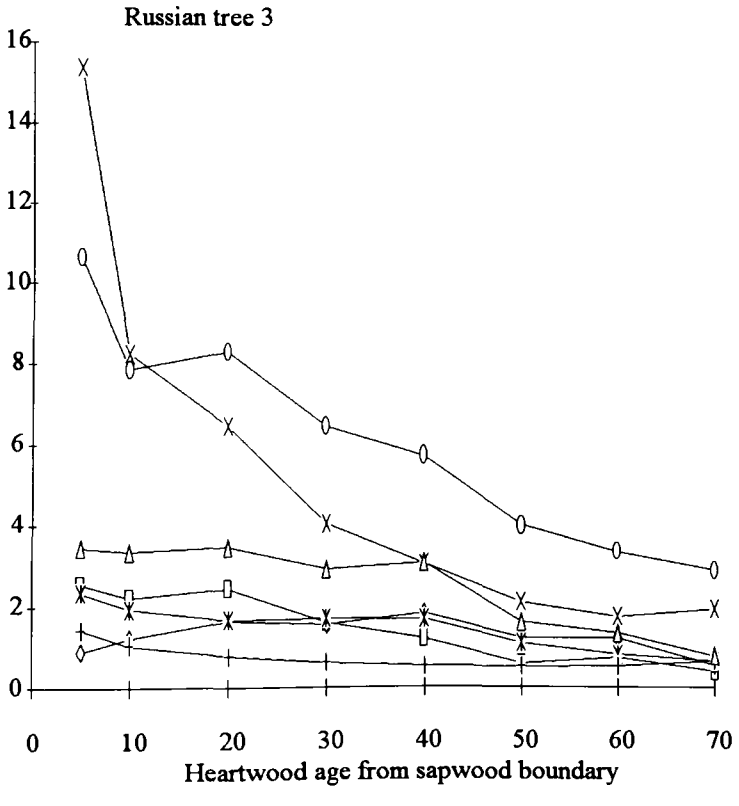
4.9. Results

4.9.1. Variation of ellagitannins with heartwood age

Due to the overlap of the peaks for gallic acid and roburin b in some samples, both these were excluded from the analyses. A 2-way analysis of variance found significant differences in total ellagitannin concentrations between both wood zones and trees. The variations in tannin concentrations by tree are illustrated in figures 4.7.a-d and by ellagitannin in figures 4.8.a-h. Although the same general pattern of variation with wood age is apparent in all four cores, there are still some marked differences, suggesting that the rates of change vary between individual trees.







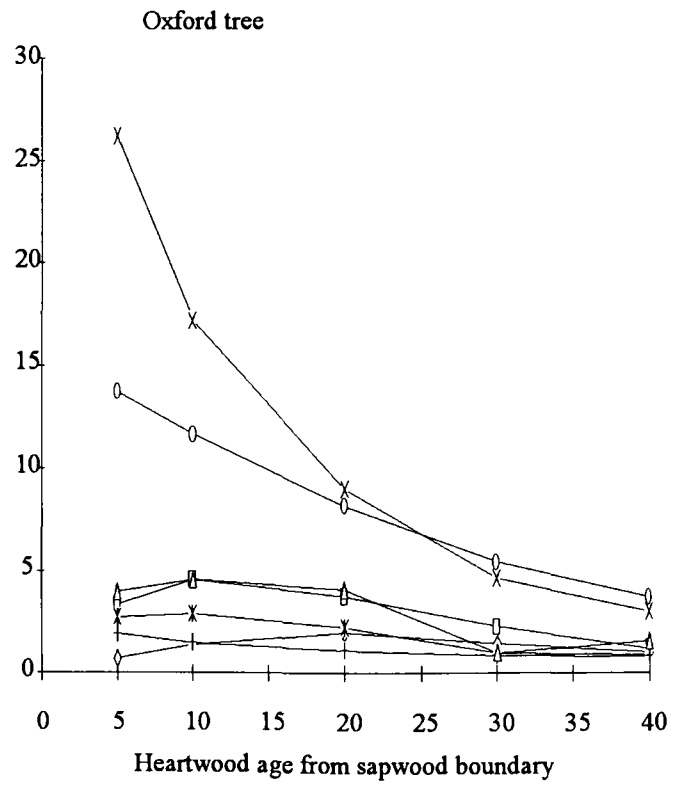
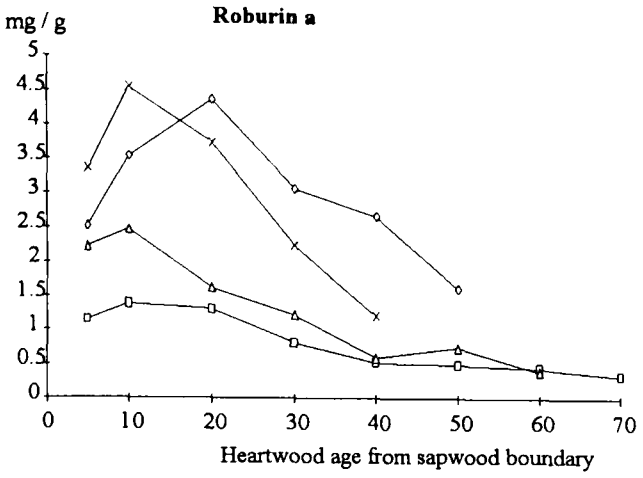
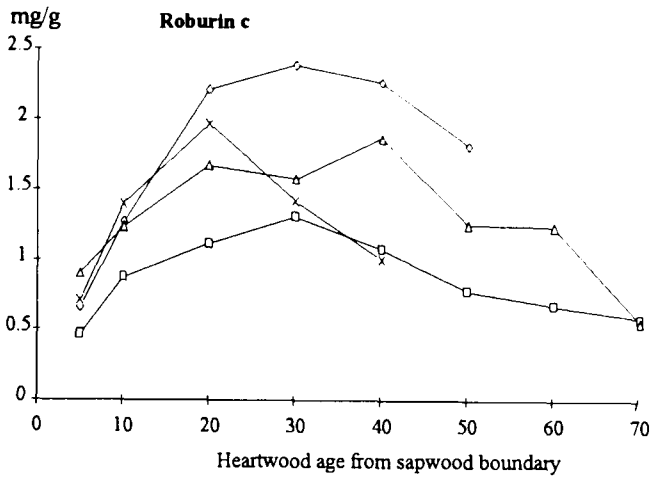
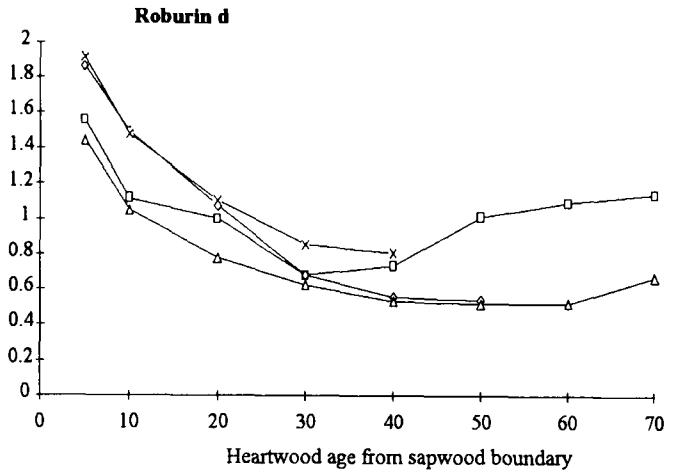
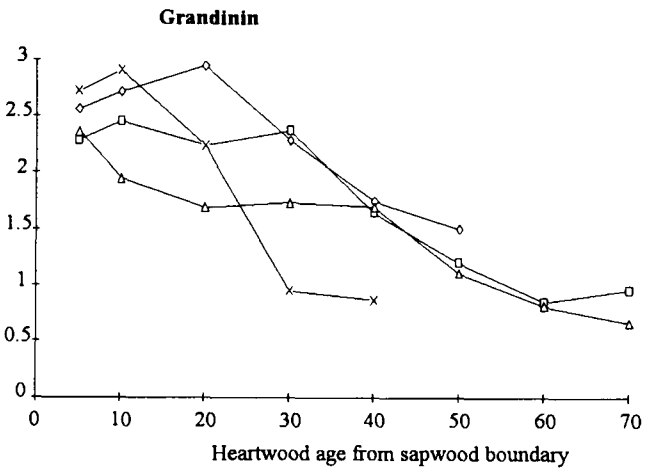


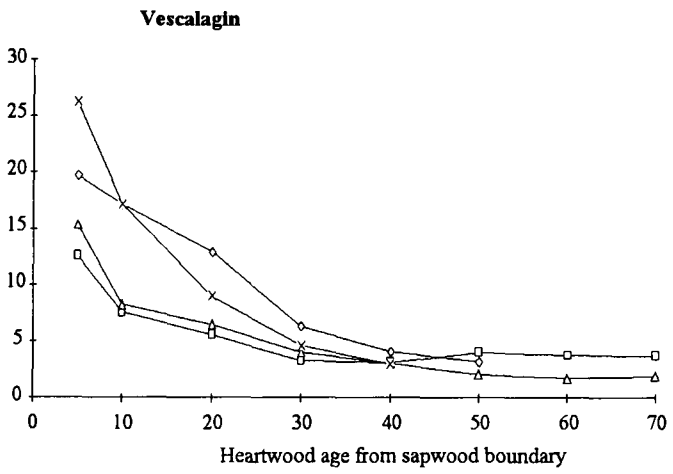
Figure 4.7.a-d: Variation in the concentration of ellagitannins in four tree cores.
Where: □ Roburin a; ◊ Roburin c; * Grandinin; + Roburin d; X Vescalagin; Δ Roburin e; o Castalagin

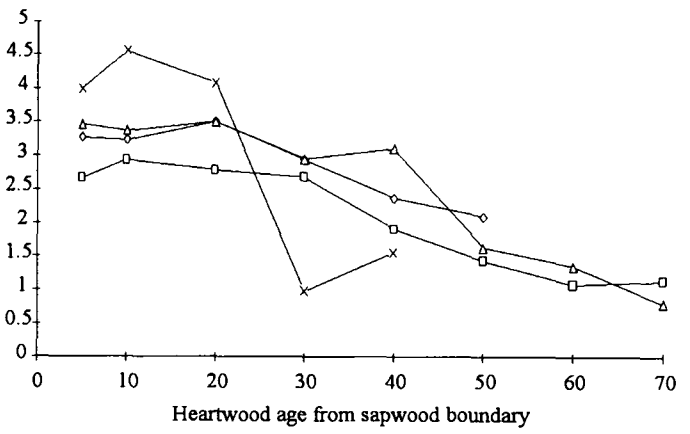


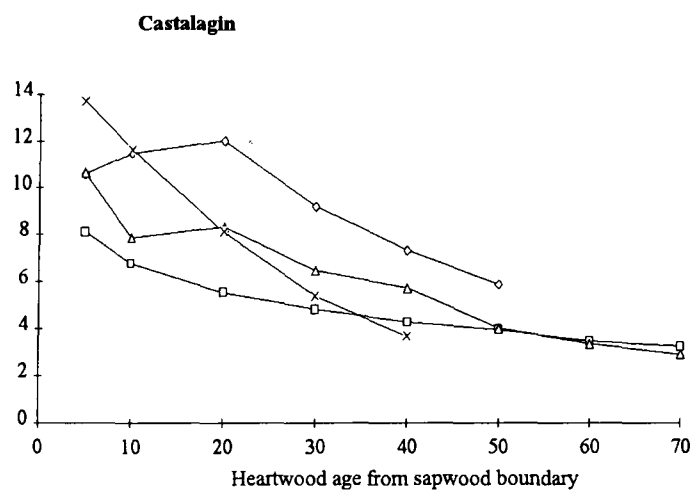


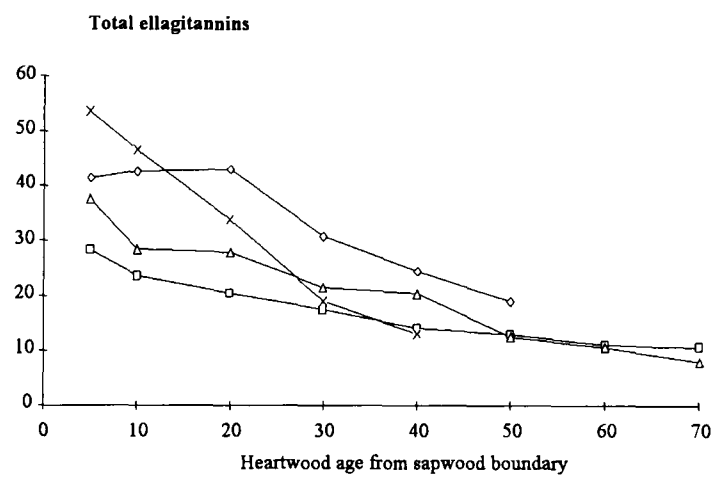












Figures 4.8 a-h: Concentration of ellagitannins between heartwood zones of different ages in four trees.
□ Russia tree 1; ◊ Russia tree 2; Δ Russia tree 3; X Oxford tree

Vescalagin, the most abundant ellagitannin in outer heartwood, is seen to decrease rapidly during the first twenty or thirty years of ageing, after which the decline lessens or even ceases. Castalagin, less abundant than vescalagin in outer heartwood, declines at a slower and more constant rate, becoming the most abundant tannin in older heartwood (see figure 4.7).

The other ellagitannins show more diverse patterns of variation. The dimer roburin d shows a similar pattern to vescalagin, which contrasts with the variation of roburins a and c. Roburin a increases in concentration during the first ten years of ageing and roburin c during the first thirty years, before each declines again in older wood. Grandinin and roburin e show less clear patterns, but in general concentrations remain approximately constant during the first thirty years before declining.

Percentage decline of ellagitannins with wood age

The concentration of total ellagitannins was expressed as a percentage of that found in the outermost heartwood (years 0-5) for each tree. The means and standard errors for all four trees are shown in figure 4.9. This displays a logarithmic decline with heartwood age. The following simple linear model was fitted:

$$\ln(T_a / T_{oh}) = ba$$

Where a is the heartwood age; T_a is the concentration of ellagitannins at age a and T_{oh} where $a=0$.

This gives an estimate for b of -0.0219 with a standard error of 0.0007 and an R^2 of 0.988. Therefore if one knows the level of tannins in the outermost heartwood, that in the heartwood of age a may be estimated by $T_a = T_{oh} \ / \ e^{0.0219a}$.

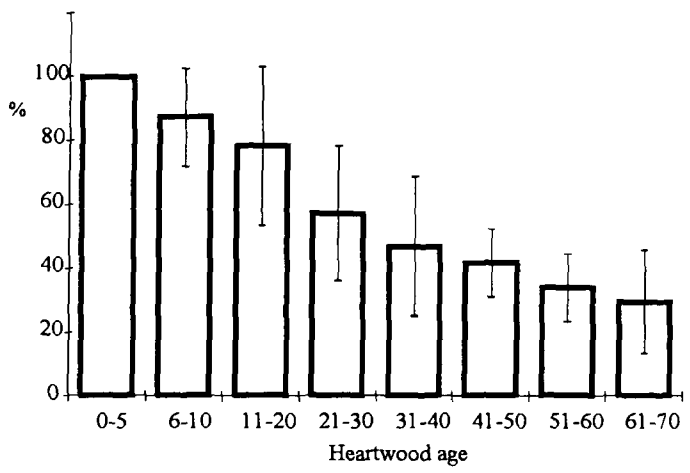


Figure 4.9: Mean total ellagitannin concentrations (and 2 x standard error bars), extracted from heartwood regions of different age, from four trees, expressed as percentages of the concentrations found in the youngest heartwood zone (years 0-5)..

Variation of the ratio of vescalagin to castalagin

The ratio of vescalagin : castalagin was calculated to determine its suitability as an indicator of heartwood age. A similar pattern of variation in the ratio was observed for all four trees, declining during the first 30-40 years before levelling off (see figures 4.10.a-b). This reflects the changes in vescalagin decline against the relatively constant decline of castalagin. Concentrations of vescalagin, and the ratio vescalagin : castalagin, increased in some samples of wood older than fifty years. This result could do with further sampling to confirm its validity. Such an increase obviously restricts the use of this ratio as an indicator of heartwood age.

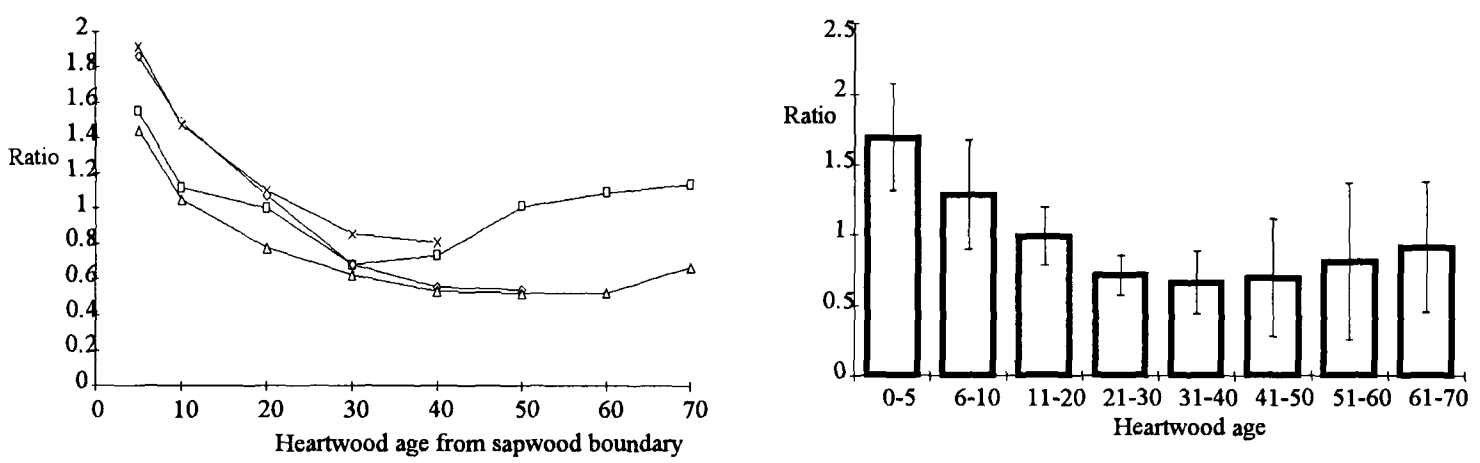


Figure 4.10.a-b: Variation in the ratio of vescalagin / castalagin by tree and means with 2 x standard error bars for heartwood age categories.

4.9.2. Comparison of early and latewood

Higher levels of ellagitannins were extracted from the earlywood than from latewood in all 10 rings measured. An analysis of the variance of total ellagitannins confirmed that there was significant variation between the two wood zones and between rings as shown in table 4.4. These results confirm those reported by Singleton (1974).

Table 4.4: Analysis of total ellagitannins extracted from early and latewood of 10 annual rings.

Source of variation	df	Sums of Squares	Mean squares	F	P
Annual ring	9	2467	274	11.78	0.0006
Wood zone	1	399	399	17.16	0.0025
Error	9	209			

A similar analysis of variance of the vescalagin:castalagin ratio also gave significant variation between rings and wood zones, at probabilities between 0.05 and 0.07 respectively. However as can be seen from table 4.5 the degree of variation was relatively small.

Table 4.5: Variation in the total ellagitannins and the ratio of vescalagin to castalagin in extracts from early and latewood of 10 annual rings. The difference between early and latewood (% difference) is expressed as the percentage of the concentration in the latewood that is found in the earlywood of the same ring. CV%= coefficient of variation as a percentage; SE=standard error of the mean.

		Minimum	Maximum	Mean	S.E.	CV%
Total ellagitannins	Earlywood	43.4	76.3	57.7	*3.68*	20
	Latewood	33.5	66.0	48.8	*4.02*	26
	% difference	102	164	121	*5.95*	16
Vescalagin / Castalagin	Earlywood	1.42	1.61	1.49	*0.02*	4.5
	Latewood	1.42	1.76	1.57	*0.04*	7.2

4.10. Discussion

The results indicate that as well as a general decline in ellagitannins, due most likely to hydrolysis or insolubilization reactions (Peng *et al.* 1991), more complex reactions also occur during heartwood ageing. The increase in the concentrations of Roburins a, c and possibly the other dimers during ageing are only likely to be due to their synthesis from other tannins. This implies that the monomers vescalagin and castalagin may transform directly or indirectly to dimers. The rapid decline in vescalagin corresponds with an increase in concentrations of these dimers. Roburin d (vescalagin-castalagin) increases later than roburins a and c possibly due to the slower decline in castalagin. Such an explanation for similar observations to those reported here was made by Viriot *et al.* (1994).

Castalagin and vescalagin are the two most easily separated and measured of the ellagitannins. Therefore the ratio of vescalagin:castalagin would be a convenient indicator of heartwood age. While this ratio appears to be a reasonable estimator for the first 40 years of ageing, its accuracy for determining the age of older heartwood appears less likely. The increase in the ratio in older heartwood suggests that it could be used as an accurate predictor only in combination with other characteristics, such as absolute concentrations of soluble ellagitannins. The rapid decline of vescalagin with heartwood age and its greater susceptibility to heating and large variation between samples reported in chapter 3 all imply that it is much less stable than castalagin, despite the similarity of their structures.

4.11. Estimating sample size for future studies

The results from this and the previous chapter were used to estimate the replication that would be necessary to detect real differences between populations. Mean tree or stave values were divided into subjective groups on the basis of similar origins and tannin levels, as shown in table 4.6. Overall group means and percentage coefficients of variation (CV%) were calculated.

Table 4.6: Mean concentrations of ellagitannins and percentage coefficients of variation (CV%) calculated for subjectively chosen groups of staves and trees.

Group origins	Description of within group values	No. staves or trees	Mean (mg/g)	CV%
Allier & Nevers	Green and seasoned stave means	4	12	45
Limousin and Chateauroux	Green and seasoned stave means	4	24	21
Wytham and Bagley	Tree outer heartwood means	4	27	37
Blenheim and Eynsham	Tree outer heartwood means	6	74	34
Russian cores	Tree means of outer 20 years of heartwood	3	33	28
			Mean CV%:	**33**

The method described in Sokal and Rohlf (1981) was followed using iterative solutions of the formula: $n \geq 2\left(\frac{\sigma}{\delta}\right)^2 \{t_{\alpha[\upsilon]} + t_{2(1-P)[\upsilon]}\}^2$

Where n is the number of replicates; σ is the true standard deviation of the two populations; δ is the true difference between populations; v is the degrees of freedom of sample n; P is the probability of detecting the true difference at a significance level of α. t values are from tables with the appropriate degrees of freedom defined by the terms P; v and α.

The mean CV% of 33% (from table 4.6) was used as an estimate of the variation, σ, between trees in a population. The mean tannin concentrations varied by 50% between the two stave groups and by 64% between the two groups of English oaks. Therefore the difference between the two populations, δ, was taken as 57% which gives a σ/δ ratio of 0.58. With a 95% chance (P=0.95) of detecting this difference at a 0.05 (α) significance level, the number of required replicates was calculated as 13.

This result emphasises the need for greater replication than has occurred in previous comparisons of oak extractives between forests or different sources of wood (*eg* Miller *et al.* 1993; Puech 1984). Although the amount of within population variation may be reduced by more careful control of wood sampling, the difference between sites assumed here is very large and greater than that suggested in the literature as occurring between different origins of European cooperage oak. High between tree, within provenance variation is not surprising as it is typical of a wide range of wood properties.

4.12. Chapter conclusions

The results indicate the importance of careful sample collection, particularly in regards to heartwood age and also in wood storage to ensure no fungal infection occurs before tannin measurement. The high variation of extractive concentrations supports that found among the staves studied in the previous chapter. Absolute amounts, however, were greater than those

observed in the stave wood. Although the study shows that the concentration of tannins is highly dependent upon heartwood age, there is a large degree of variation between trees, when wood of a similar age is compared. The change in ellagitannin composition with heartwood age appeared predictable and suggests that heartwood age could be estimated from the relative abundance of the different tannins. Although there was very limited sampling, oak lactone showed a similar degree of variation with both wood age and between trees.

Although heartwood age is the most likely cause of within tree variation no consideration was given to tree age at the time of wood formation. Later studies (in chapter 7) suggested that very young trees may lay down lower amounts of tannins in newly formed heartwood. Therefore, in retrospect, a study comparing the levels of tannin in heartwood of the same age but in trees of different age classes would have been a useful extension to these studies on within tree variation.

The results from this and the previous chapter generally support the notion that one may be able to use extractive properties as criteria for the selection of trees and cooperage wood. However any comparison of different sources of oak wood will require careful sampling of trees, to ensure the wood is of suitable quality and of similar age. In addition the results emphasise the need for adequate replication, even if one is interested in detecting only large differences in the levels of extractives. Both these factors must be incorporated into future studies aiming to examine the degree of variation on a broader scale, such as between provenances or forests.

Chapter 5: Variation within and between two forests of the soluble tannins in oak wood.

The entire secret of cognac is hidden in the rings of the oak tree. The oak grows and gathers sun into itself. The sun settles into the rings of the oak as amber settles at the bottom of the sea. It is a long process, lasting decades.

R. Kapuscinski - *Imperium*

5.1. Chapter summary

Two heartwood samples from opposite sides of the bole were taken from each of twenty trees from each of two forests of French oak (*Quercus robur* and *Q. petraea*). The levels of soluble ellagitannins in water extracts were measured by HPLC and total phenolics by the Folin-Denis method. Analysis of variance found significant variation in all ellagitannins between forests and between trees within a forest. Levels of soluble tannins were lower in trees from a forest near Tronçais than in wood from the Limousin region. The results contradict the hypothesis that tannin content will be higher in more slowly grown trees and forests. The composition of ellagitannins suggested that the average age of the wood from the two sites was different. However, any difference in heartwood age was unlikely to explain the difference in total soluble tannin concentrations.

5.2. Introduction

Chapter 3 and 4 suggested that there may be some basis for claims that wood deriving from different origins may vary in extractive properties. However, the results from both chapters 3 and 4 have shown how a wide range of factors may influence these properties both within standing trees and after harvesting. Previous studies have either failed to control these confounding variables or not considered the large degree of variation found for most wood properties between trees within populations. The primary aims of this and the following chapter are to determine whether cooperage wood varies significantly in properties liable to influence flavour and whether the variation occurs on a scale that allows wood to be selected for such effects. The investigation described in this chapter examines the variation, between and within two forest coupes, of the soluble ellagitannins in oak heartwood.

5.3. Methods

5.3.1. Forest and tree sampling

Trees were compared from two forests that typified opposing types of oak forest (see table 5.1) which have been claimed to influence flavour differently. These forest coupes were chosen and purchased by Tonnellerie Taransaud and therefore correspond to trees and coupes from which

coopers are able to select. By the choice of two such contrasting sites it was intended to test the hypothesis that it is not possible to select for cooperage wood with significant differences in wood extractives .

One site was located in the forests near Tronçais, the other in the Limousin region of France. From each of these clear felled sites, twenty randomly selected trees, of suitable quality for cooperage, were chosen. During the splitting of logs and cutting of bolts, two staves from opposite north and south facing sides near the base of the bole were removed and used for this study. Therefore for each site, a total of forty samples from twenty trees were examined.

Table 5.1: Forest characteristics. The dominant species were identified from casual observation of adjacent plots.

Forest location	Limousin	Tronçais
Forest ownership	Private	State owned and managed
Silviculture	Intense thinning and rapid growth	Managed for slow, regular growth
Soil type	Heavy, clay soil.	Sandy more acidic soil.
Dominant species	*Quercus robur*	*Quercus petraea*

5.3.2. Sampling of staves

The 80 staves were stored for approximately four months before a hand held plane was used to remove shavings from their surfaces that would make up the inner face of a barrel. Wood colour was measured, using the CIELAB system described in section 2.10, with ten measurements taken across transverse sections of each sample. Mean ring widths were also determined.

After removal of the frequently discoloured outer surface of the stave, shavings from the top 1-2 mm were taken and ground, before storing in a desiccator for one week. Total phenolics and ellagitannins were measured from water extractions of 50 mg wood samples (see sections 2.4 and 2.2).

One stave of Limousin and one of Troncais wood were used to estimate the amount of insoluble tannins remaining after extractions. Three replicate water extractions of wood samples from each of these staves were carried out. The insoluble ellagitannins left in the wood residues were estimated by degradation in alcohol-hydrochloric acid solutions (section 2.3).

5.4. Results

5.4.1. Comparison between forests

It was suspected that the two samples from each tree may not have been taken rigorously from north and south facing sides of the bole and that for further analyses they might best be treated as random within tree replicates. The assumption that there was no 'orientation' effect was tested before further analysis by calculating the differences in tannin concentrations for each tree

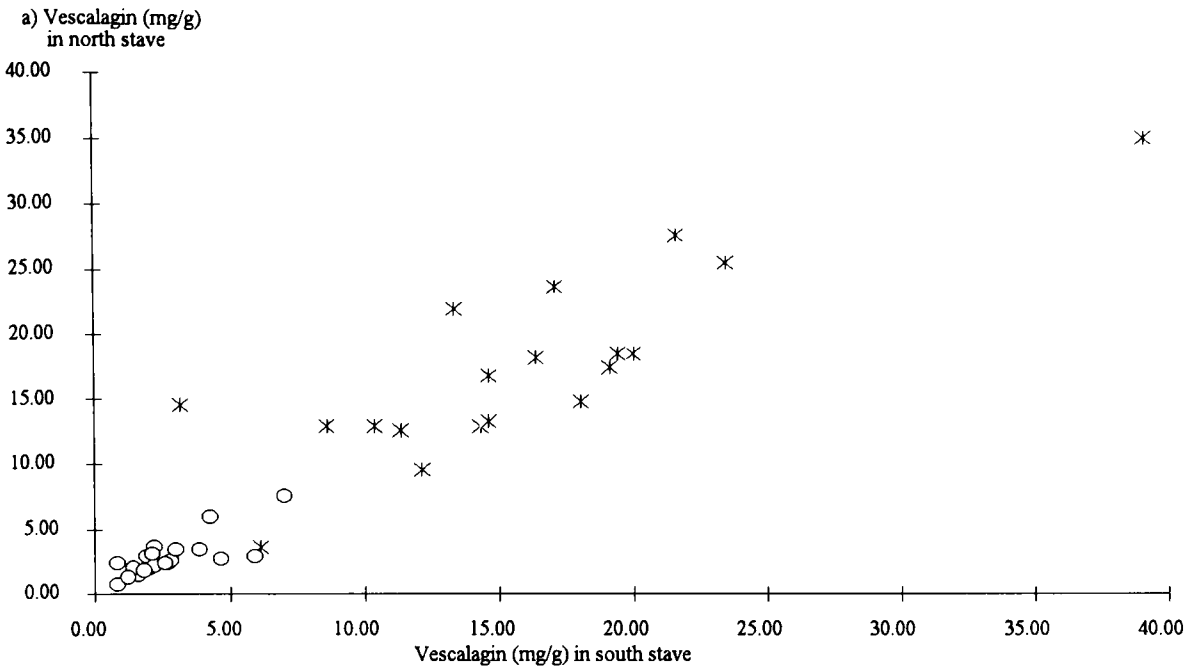
([South]-[North]). As the difference values were not normally distributed, a non-parametric test was used with the null hypothesis that the mean difference equalled zero. A Wilcoxon signed-rank test for matched pairs (Neave and Worthington 1988) was used, where the differences were ranked according to absolute value and then the rank sums for differences above and below zero were calculated. The lowest of these summed ranks was used to test the null hypothesis against critical values. Data from both forests were grouped, resulting in the rejection of the null hypothesis at 5% significance for seven ellagitannins (see table 5.2). For all the significant results the mean concentrations were found to be higher in the south facing samples. However as only some of the less abundant ellagitannins gave significant differences, the within tree samples were treated as random replicates for future analyses.

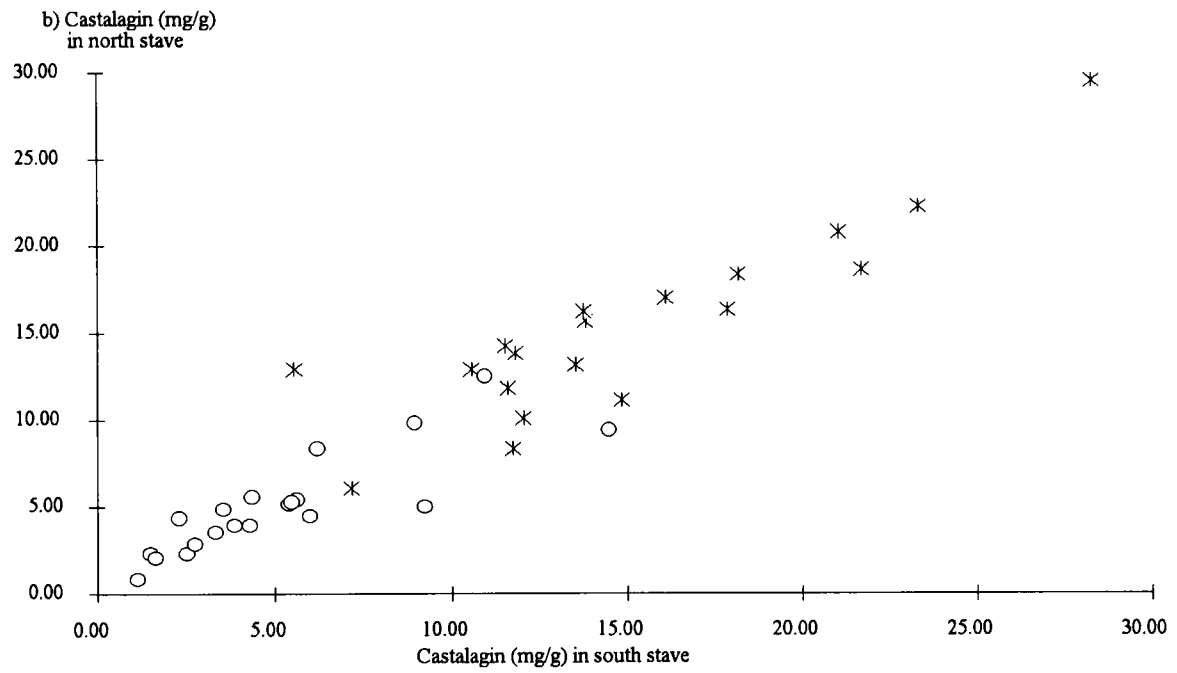
Table 5.2: Comparison of mean ellagitannin concentrations (mg/g wood) of north and south facing sample using grouped data from both forests.

Ellagitannin	South stave Mean n=39	North stave Mean n=40	Wilcoxon test P
U1	1.08	1.17	n.s.
U2	1.31	1.28	n.s.
Roburin a	2.58	2.38	0.05
Roburin b	2.38	1.97	0.01
Roburin c	1.72	1.29	0.01
Grandinin	2.91	2.86	n.s.
Roburin d	3.47	2.99	0.05
Vescalagin	9.28	10.13	n.s.
Roburin e	4.44	4.18	0.05
U3	0.92	0.82	0.01
Castalagin	9.97	10.16	n.s.
U4	1.24	1.01	0.01

Figures 5.1.a-c depict the variation of the two most abundant ellagitannins and total tannins. These emphasise the difference between the two forests and also show the relatively low variation between samples from the same tree, with most of the points lying approximately along a gradient of one. The lower variation among samples from the Tronçais forest than those from the Limousin is also apparent. Tests of the homogeneity of variances using Cochran's test confirmed a similar pattern of heterogeneity for the majority of ellagitannins. Before further parametric statistical analyses were carried out the data were log-transformed, resulting in more homogeneous variances. Tests of the normality of the transformed data indicated acceptable conformity, analyses of variance being fairly robust to slight deviations.

A balanced, nested analysis of variance was used on the transformed data, to compare variation between and within trees and forests. Due to one tree having only a single replicate both this replicate and the data of a random tree from the other site were removed from the analysis, reducing the total degrees of freedom to 37 within each site. These results and the large proportion of variance explained by between tree and between forest variation are shown in Table 5.3..





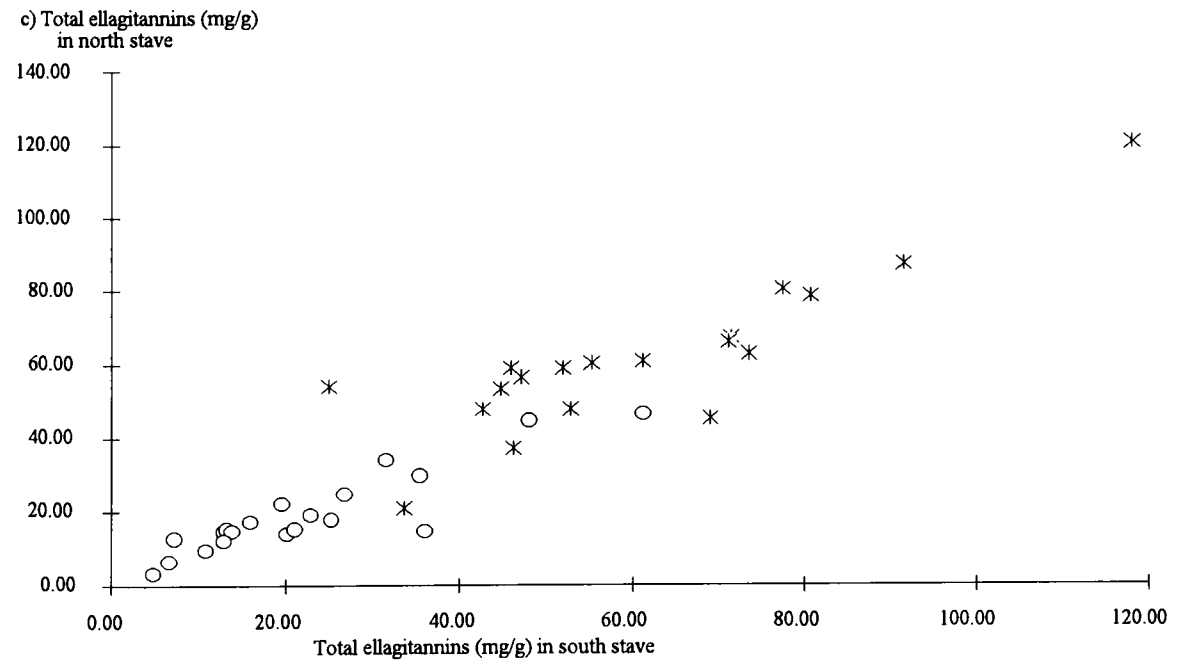


Figure 5.1.a-c: Concentrations of ellagitannins in north and south staves of trees from Limousin (*) and Tronçais (o) forest sites.

Table 5.3: Mean forest values of wood properties; percentage variance components and probabilities from analyses of variance. Units: all tannins concentrations in mg/g wood; ring widths in mm.

Character	Limousin		Tronçais		Between forests		Between trees within forests	
	Mean	S.E.	Mean	S.E.	%var	P	%var	P
U1	1.42	0.10	0.84	0.09	28	**	48	***
U2	1.49	0.09	1.12	0.11	13	*	65	***
Roburin a	4.24	0.36	0.76	0.10	68	***	23	***
Roburin b	2.92	0.18	1.44	0.18	44	***	36	***
Roburin c	1.94	0.24	1.08	0.20	15	*	51	***
Grandinin	3.82	0.19	1.90	0.18	54	***	37	***
Roburin d	4.92	0.37	1.58	0.23	58	***	27	***
Vescalagin	16.68	1.16	2.79	0.25	77	***	19	***
Roburin e	5.80	0.30	2.82	0.30	53	***	35	***
U3	1.36	0.13	0.37	0.04	56	***	29	***
Castalagin	15.13	0.86	4.74	0.49	72	***	24	***
U4	1.50	0.10	0.79	0.11	33	***	36	***
Total tannins	61.21	3.35	20.24	2.00	72	***	24	***
Total phenols	54.53	1.80	27.78	2.02	70	***	25	***
Ring width	3.06	0.15	1.24	0.06	76	***	9	**

Wood colour and ring widths

Of the three variables lightness (L*), a* and b* used to define wood colour, Lightness varied most. However, analyses of variance found that only b* and the derived variables hue and saturation varied significantly between the two forests, while significant variation between trees and samples was found for all three variables. Variance components (see table 5.4) show that the between forest variation of most parameters accounted for a relatively small amount of the total. Greater between forest variation was found for ring width, with the Limousin samples having much wider rings than those from Tronçais (see table 5.3).

Table 5.4: Forest means, variance components (%var) and probabilities of significant variation (P) from analyses of variance of CIELAB parameters of wood colour. df = degrees of freedom.

Colour parameter	Limousin n=117		Tronçais n=120		Between forests df=1		Between trees in forest df=36		Between samples within trees df=38	
	Mean	S.E.	Mean	S.E.	% var	P	%var	P	%var	P
L*	61.07	0.18	60.44	0.28	0.4	0.298	31.6	*	50.6	***
a*	11.25	0.05	11.40	0.05	0.0	0.367	42.6	**	46.7	***
b*	23.46	0.09	22.68	0.09	18.5	*	43.4	***	23.0	***
Hue h	1.123	0.001	1.104	0.001	42.6	***	32.0	***	19.3	***
Saturation C	264.4	2.13	259.1	2.24	10.3	*	44.9	***	29.0	***

Composition of ellagitannins

In order to test whether the composition of ellagitannins varied between sites, the percentage of the total soluble ellagitannins for each ellagitannin was calculated. A nested analysis of variance

was carried out on arcsine-transformed percentage data. Non-parametric comparison of the two sites was also carried out by a Wilcoxon 2-sample test of rank sums (SAS Institute Inc. 1985; Neave and Worthington 1988). Both the parametric and non-parametric tests found significant differences between the two sites for most of the ellagitannins (see Table 5.5).

Table 5.5: Parametric and non-parametric tests for differences between forest and tree means of the percentage composition of ellagitannins in relation to total soluble ellagitannins.

Ellagitannin	Mean percentage		Wilcoxon 2-sample test	Anova of arcsine transformed data	
	Limousin	Tronçais	P of between site	P of between site	Between trees within site
U1	2.4	4.3	***	***	***
U2	2.6	5.7	***	***	***
Roburin a	6.6	3.3	***	***	n.s.
Roburin b	4.9	6.4	***	**	n.s.
Roburin c	3.3	4.5	n.s.	n.s.	**
Grandinin	6.5	10.0	***	***	n.s.
Roburin d	7.8	6.9	**	n.s.	n.s.
Vescalagin	26.7	14.9	***	***	***
Roburin e	9.8	14.0	***	***	n.s.
U3	2.1	1.9	n.s.	n.s.	**
Castalagin	24.7	24.8	n.s.	n.s.	n.s.
U4	2.5	3.3	***	**	n.s.

To present the variation in tannin composition more clearly, star diagrams, of the forest mean ellagitannin compositions, were plotted (see Figures 5.2.a-b). The most prominent feature of these diagrams is the lower proportion of vescalagin in the Troncais samples.

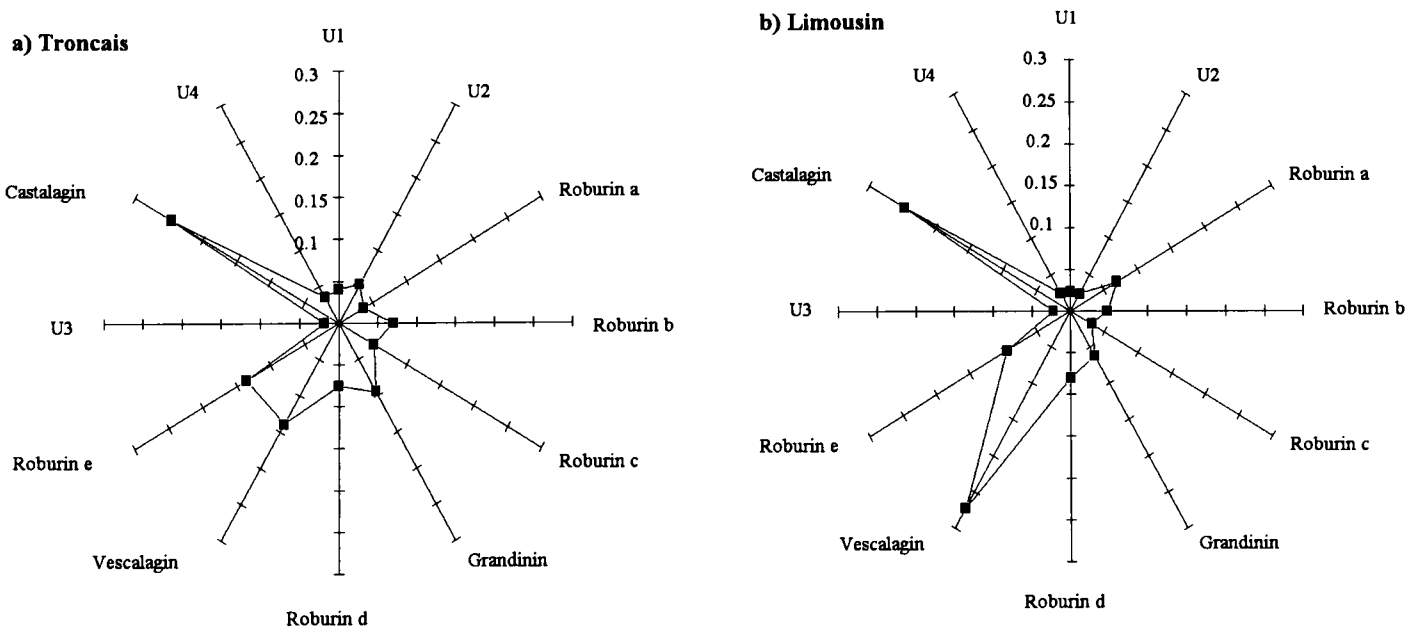


Figure 5.2.a-b: Mean ellagitannin composition of Tronçais and Limousin oak. Values are proportions of the total ellagitannin concentration.

It has been observed in the previous chapter that a lower ratio of vescalagin to castalagin (VC ratio) could be taken as a crude indicator of greater heartwood age. These ratios were calculated for each of the samples, and as the values within each forest were approximately normally

distributed, the forest means were compared by an F-test. Although there was overlap between the two forests in the values of the ratio (see Table 5.6.), the difference between the means was highly significant, with the ratio being generally lower among the Tronçais samples. The result suggests that the Tronçais samples are, on average, cut from older heartwood than the Limousin samples. This corresponds with the likelihood that the Tronçais trees were older when felled, due to the slower growth indicated by the narrower ring widths observed in these samples.

Table 5.6: Statistics of the Vescalagin / Castalagin ratio for samples from Limousin and Tronçais sites.

Statistic	Limousin n=39	Troncais n=40
Mean	1.087	0.606
Standard Error	0.041	0.024
Minimum	0.580	0.279
Maximum	1.671	0.942

5.4.2. Influence of wood age on soluble ellagitannins

One could propose that the difference in tannin concentrations between the two forests is due to a difference in heartwood age of the samples. Greater insolubilization or hydrolysis of soluble ellagitannins may have occurred in the older samples from Tronçais. Three different methods were used to address this possibility.

Use of vescalagin/castalagin ratios as an indicator of wood age

As an initial test of this hypothesis, those samples from each forest where the VC ratio fell between the minimum Limousin value and the maximum Tronçais value were selected. The forest means were compared, using only these samples, for vescalagin, castalagin and total ellagitannin concentrations. Table 5.7 shows that while there was no significant difference in the ratio there were very large differences in the amount of ellagitannins between the forests.

Table 5.7: t-tests for variables from samples with similar vescalagin/castalagin ratios. Tests of significance assume unequal variance between populations.

Variable	Limousin (n=9)		Tronçais (n=23)		P>|T|
	Mean	Std. Error	Mean	Std. Error	
Vescalagin	9.36	1.41	3.14	0.43	0.0026
Castalagin	12.11	1.85	4.64	0.69	0.0047
Total ET	47.44	6.08	19.49	2.74	0.0025
V / C ratio	0.77	0.043	0.70	0.02	0.2330

A nested analysis of variance gave similar variance components to those that had been found using all the data. Figure 5.3 shows variation in total ellagitannins *vs* the ratio for all samples.

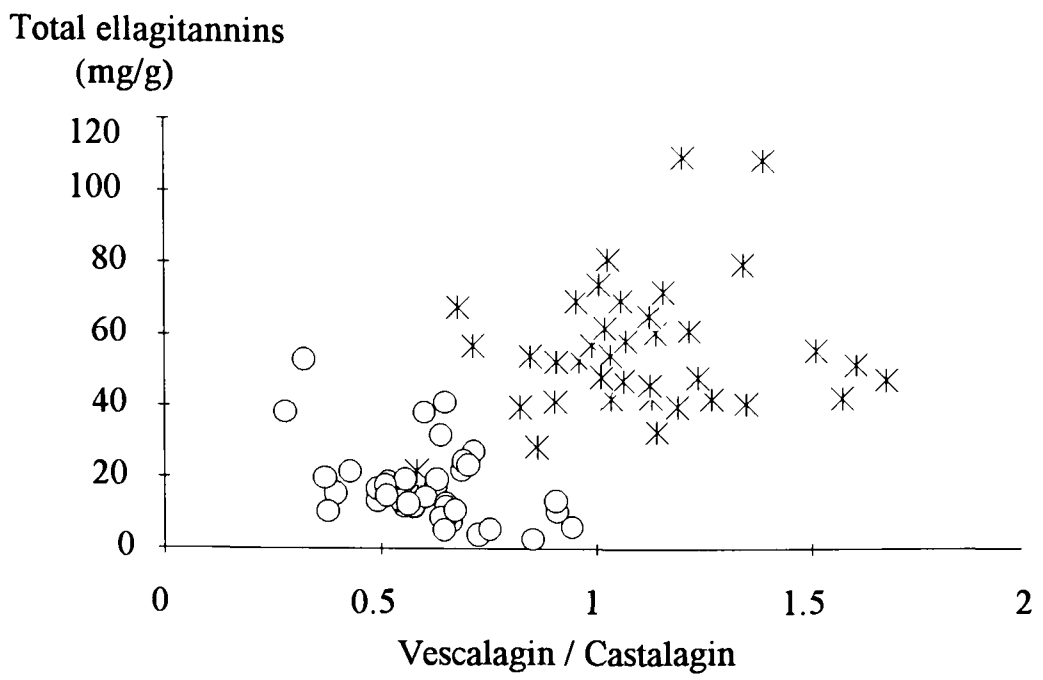


Figure 5.3: Total ellagitannins vs the ratio of vescalagin to castalagin in samples from Limousin (*) and Tronçais (o) forests.

Estimating soluble tannin decline due to heartwood age

The heartwood age of samples from each forest can be estimated from ring widths. If one assumes that all staves are 7 cm across in transverse section with the nearest edge cut 2.5 cm from the sapwood boundary, one can calculate average heartwood age, assuming constant annual growth equal to the mean ring widths for each forest. Therefore mean heartwood age of a stave, a = (25 + 70/2) / mean ring width (mm). Table 5.8 shows the resulting estimates.

Using this estimate of heartwood age as a and the mean total ellagitannins found in samples from each forest (see table 5.3) as T_a, the concentrations of tannins in the youngest heartwood T_{oh} can be estimated using the model developed in section 4.9.1, where $T_{oh} = T_a e^{(0.0219a)}$

Table 5.8 shows that there remains a large difference between the two forests in the estimated soluble ellagitannins of new heartwood.

Table 5.8: Estimated soluble tannins in the youngest heartwood (mg/g wood) and percentage remaining in samples (%r). Calculated from estimated heartwood age (years growth from sapwood boundary) and mean total ellagitannins extracted from samples from each forest.

Forest	Mean ring width (mm)	Mean stave heartwood age	Ellagitannins in young heartwood	%r
Limousin	3.0	20	90.2	67.8
Tronçais	1.25	48	52.0	38.9

Measurement of insoluble tannins in two samples

Table 5.9 gives the levels of insoluble ellagitannins remaining in a single sample of each of Limousin and Tronçais wood after water extractions. The concentration of insoluble ellagitannins

is probably slightly overestimated as the free ellagic acid present in the wood, formed from the natural hydrolysis of ellagitannins, was not measured. Although slightly soluble in water the majority of free acid will have remained in wood residues. Viriot *et al.* (1994) calculated the concentration of ellagic acid at the sapwood : heartwood boundary as representing 10% of the total ellagitannins, with 1% of ellagitannins hydrolysed into ellagic acid every following ten years. The difference in total ellagitannins between the two samples is greater than that indicated from the calculations of the previous section, suggesting that the earlier assumptions are more likely to have over rather than under estimated the effects of heartwood ageing.

Table 5.9: Mean amounts of soluble and insoluble ellagitannins (mg/g) from three extractions, followed by degradation in alcohol-acid solutions from a single stave of each of Limousin and Tronçais wood.

Forest	Total soluble	Insoluble ellagitannins		Total ellagitannins
Sample	ellagitannin	Mean (n=3)	Std Error	
Limousin	87.1	11.5	0.78	98.5
Tronçais	12.8	6.6	1.20	19.3

Overall these results suggest that the differences in tannin concentrations between the forests cannot be explained solely by the difference in the heartwood age of the samples. It is however the likely explanation of the different tannin compositions observed.

5.4.3. Correlations

Correlation matrices for all variables were calculated using grouped data and separately for each forest. Highly significant correlations were found between all the individual ellagitannins. However the strongest correlation was that between total phenolics and total ellagitannins, with an *R* value of 0.99 (see figure 5.4). This suggests that the Folin Denis method is an effective means of comparing the tannin contents of oak wood, supporting results described by Puech *et al.*(1990). However, Klumpers *et al.* (1994) observed that the ratio of ellagitannins to total phenolics (as measured by the Folin Denis test) declines with heartwood age. Calculating this ratio and using the ratio of vescalagin to castalagin as an indicator of wood age, one finds that these two ratios are significantly correlated (R=0.50), as they both are with ring width (R of VC ratio = 0.58; R tannins/phenolics = 0.49).

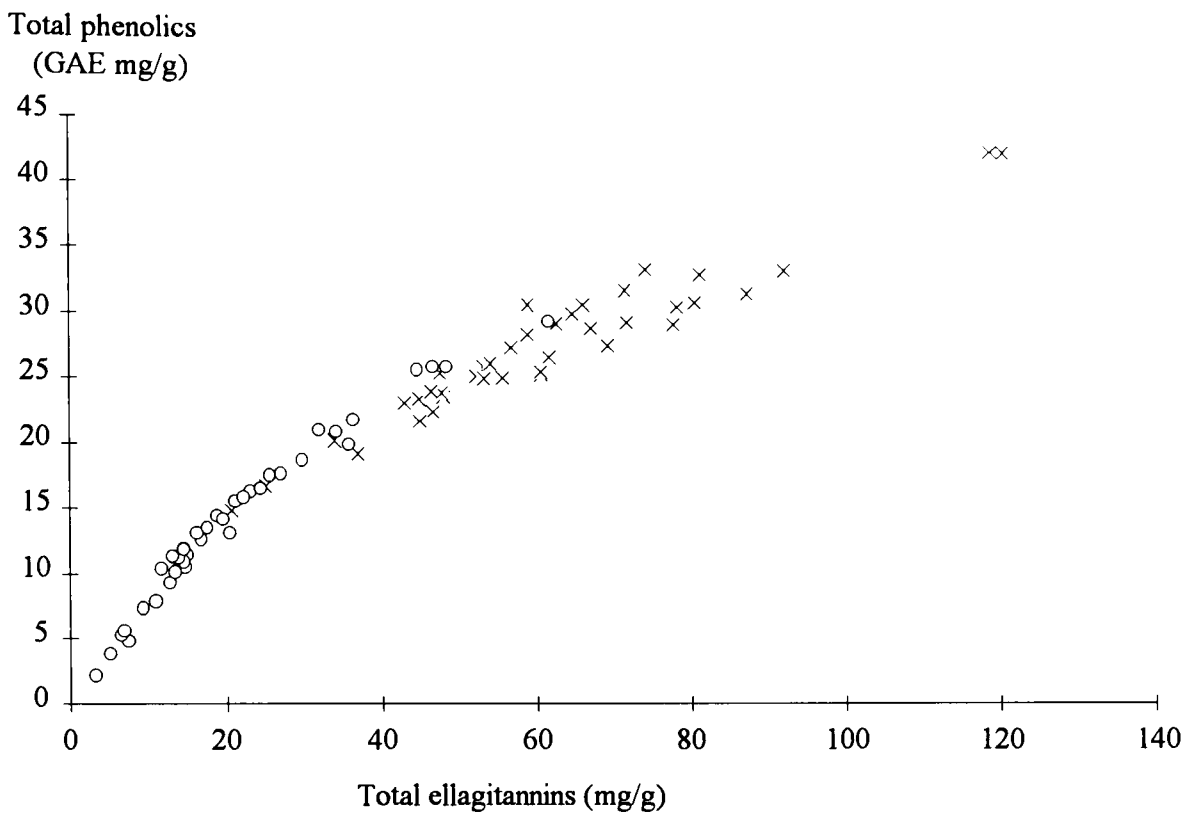


Figure 5.4: Total phenolics measured by Folin Denis method vs total ellagitannins measured by HPLC in samples from Limousin (*) and Tronçais (o) forests.

Among the wood colour parameters, the variable b* correlated most strongly with tannin content both separately for each forest and when the data for the two are grouped (R grouped = 0.640). Despite scatter this trend is perceptible in figure 5.5. Similar correlations were found between total tannins and both hue and colour saturation. There have been previous suggestions that the tannins play a role in the formation of heartwood colour (Klumpers *et al.* 1993 and 1994). It has been observed that inner heartwood is darker and colour saturation more pronounced, affecting colour parameters such as a*, L* and hue (Klumpers *et al.* 1994; Klumpers and Janin 1992), while b* was the parameter least affected by wood age.

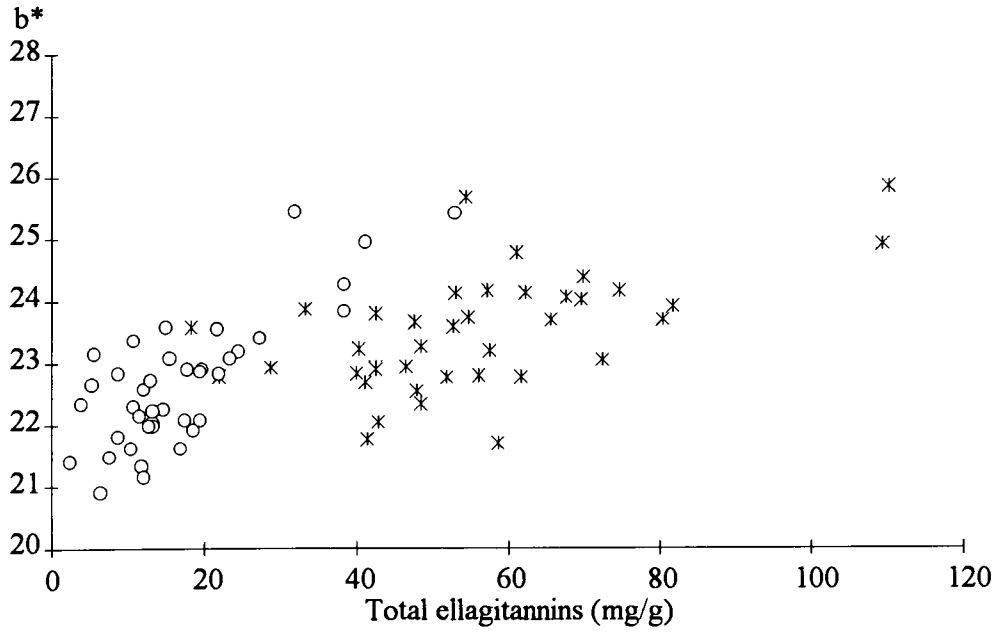


Figure 5.5: Wood colour variable b* vs total ellagitannins in samples from the Limousin (*) and Tronçais (o) forests.

Correlations between characters are difficult to interpret because of the much greater variation between rather than within forests observed for most characteristics. Therefore although a

correlation (R=0.66) between ring width and total tannins is found when using the grouped data, correlations are much lower within each forest, where the range of observations was narrower. It is hazardous to generalise from correlations that are significant when using only grouped data due to the many differences between the two forest sites.

5.5. Discussion and conclusions

The results show that there is a high degree of variation in the soluble tannins between the two forests. Furthermore the within tree variation was much lower than studies in the previous chapter had indicated. This is most likely due to the restrictions placed on the sampling, with samples taken of only cask-quality wood from the outer heartwood near the base of each tree. The forests sampled were selected to correspond to opposing criteria used within the cooperage industry to select wood. Therefore the relative proportion of variation explained by between-forest differences should be treated as an upper limit of the degree of variation that could feasibly be selected for by coopers.

Cask wood is often identified by referring to the 'grain': a term corresponding to ring widths. Singleton (1974) proposed that the higher level of tannins in European oak, compared to American, was due to their slower growth leading to a greater proportion of earlywood, which as shown in chapter 4 contains greater amounts of tannins than the latewood. However the results presented in this chapter refute this as a likely explanation. Although there was a large difference in ring widths between the forests, the opposite trend with regards to tannins was found, with the high-tannin containing Limousin oaks also displaying faster growth.

A more intense thinning regime and consequent more rapid growth are often a characteristic of the mostly privately owned forests in the Limousin region. More slowly grown oaks will normally be felled at a later age and the stave wood from such trees will, on average, be older than that from more quickly grown oaks. Due to the rapid decline in soluble tannins observed in the first twenty years of heartwood ageing this might significantly influence the average tannin content. Samples used in this study comprised between 12-50 years of heartwood growth, depending on ring widths. However, the results and calculations in this study suggest that differences in extracted ellagitannins cannot be explained solely by differences of wood age.

The correlation between total ellagitannins and the wood colour parameter b* was one of the few correlations that held both within each forest and using combined forest data. This finding may provide a visual indicator of tannin levels, although the high degree of scatter suggests any predictive ability will be limited.

The two forests differed in many ways, in addition to the silvicultural regimes, and this makes it impossible to identify the reasons for the large differences found in tannin concentrations.

However, the careful sampling and treatment of wood samples after felling, and the large proportion of variation found between the coupes, suggests that factors beyond the immediate forest environment can influence wood tannin concentrations. Whether these factors be environmental, genetic or differences between species remains unclear. How closely the variation in tannins is matched by variation in other extractive properties, possibly influencing flavour, will be the subject of the next chapter.

Chapter 6: The flavour effects and extractives of charred oak wood from two different forests

One does not need to do anything more; one must wait. The right time will come for everything. The alcohol now enters the oak, and then the wood yields everything it has. It yields sun; it yields fragrance; it yields colour.

R. Kapuscinski - *Imperium*

6.1. Chapter summary

Wood from twenty trees from each of two different French forests was heat treated and then stored in a 63% ethanol solution. After six months the concentrations of lignin degradation products, oak lactones, ellagic and gallic acids in the solutions were measured. The solutions were then assessed by nosing for flavour differences. Significant differences in both the chemical composition of the solutions and in the nosing assessments were found between the two forest origins. Additional trials confirmed that the duration of heating influenced both chemical and nosing characters, but indicated that the effects may vary between wood origins.

6.2. Introduction

Comparison of the ellagitannin content of oak wood from two French forests found significant differences in their concentrations, as described in the previous chapter. Wood collected from a site near Tronçais had lower amounts of ellagitannins than wood from the Limousin region. This study was initiated to compare further the extractive content of the wood and to determine whether any differences in chemical composition are reflected in different flavours imparted by the wood.

During the manufacture of casks the wood may undergo a variety of heat treatments. This may vary from intense charring of casks used for bourbon and subsequently Scotch whisky maturation, to various intensities of toasting used in the manufacture of casks for wine, brandy and other drinks. The heating and charring of wood is known to affect the flavour released from the wood and some of the effects on the composition of extractives have already been examined in chapter 3. As the majority of flavour and nosing assessments (see section 2.13) are carried out on mature whiskies or other cask-matured beverages it was decided that a more applicable comparison of flavour and chemical composition would be made if the wood were heat treated. Among the extractives thought to influence flavour, the oak lactones and lignin degradation products such as vanillin and aromatic aldehydes are among the most often quoted (*eg* Maga 1989b; Nishimura and Matsuyama 1989). Lignin degradation products are known to derive mostly from degradation of lignin during the heating of wood, as shown in chapter 3. No clear effects of heating on the oak lactones were observed. By examining the extractives released by

heated wood one can obtain a better understanding of the relative importance, or otherwise, of oak origins to the maturation of whisky.

6.3. Methods and materials

6.3.1. Methodology

Studies by United Distillers (Ford pers. comm.) had suggested that the flavour released by wood differed according to whether extractions were made from powder or from solid wood and might also depend upon the wood surface exposed to extraction. Therefore four oak sticks, of the proportion and orientation shown in figure 6.1, were cut for each of the flavour samples to be described shortly and heated at 200°C in a convection oven for the appropriate length of time. Studies by United Distillers had suggested that lengthy heating at this temperature should ensure maximum production of lignin degradation products. After being allowed to cool to room temperature the sticks were then placed in 500 ml bottles to which was added 450 ml of a 63% ethanol solution. The bottles were then closed with air-tight tops and stored in darkness for six months.

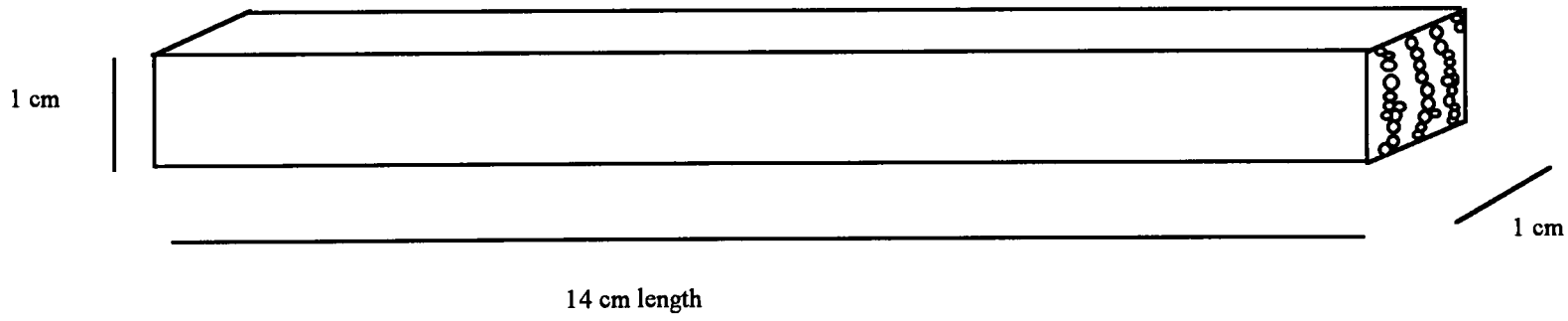


Figure 6.1: Wood sample size and aspect cut for flavour trials.

The geometric surface area of immersed wood was approximately 190 cm^2 giving a surface area to volume ratio of 380 cm^2/litre. This is approximately four times the ratio found in whisky casks which range from 67.5 to 100 cm^2/litre (Philp 1989a). Such a high ratio was chosen to compensate for the shorter extraction time, with the intention being to have a similar intensity of sample flavour to that found in whisky.

6.3.2. Sampling

Wood from the same samples as chapter 5 was used. As the variation in tannins was found to be very low between wood samples taken from the same tree, it was decided that only limited within

tree replication would be carried out. This was done to obtain an estimate of the within-sample variation. Trials were carried out to determine the following:

(i) **Forest comparison:** whether there are significant differences in extract composition and nosing assessments between the two forest origins. Also to typify the flavour characteristics of each forest and examine correlations between physical, chemical and flavour properties. A flavour trial was set up using wood from each of the forty trees sampled, with two sticks cut from each of the two within tree samples and heated for 120 minutes.

(ii) **Replication test:** to test for differences using wood from the same tree and to obtain an estimate of the within sample variation for the other tests. Two trees from each of the forests were chosen and four replicate flavour trials carried out for each. The trees were chosen to represent the upper and lower extremes of tannin content found within each forest. Each trial comprised of two sticks cut from each of the two within-tree samples and heated for 120 minutes.

(iii) **Heat treatment test**: to compare the effect of different heating times on the flavour and chemical properties released by the wood. This may indicate the best treatment at which to assess flavour and whether wood samples respond differently to heating. Two sample sets used wood taken from a single tree from the Limousin and Tronçais sites, sampled as above in (i) and (ii). Another two sets were cut from a single Limousin and American stave which had been previously used for the studies described in chapter 2. For each of the four sets, four different flavour trials were set up, using samples heated for one of four different times (0, 30, 60 and 120 minutes) at 200°C.

The results table 6.2 summarises sample origins and treatments.

6.3.3. Analytical measurements

The following measurements were then made after the solutions had been stored for six months:

i. Absorption of the solution at 520 nm (see section 2.8).

ii. pH of the solution.

iii. Concentrations of lignin degradation compounds, ellagic and gallic acid by HPLC, as described in section 2.62.

iv. Concentrations of *cis* and *trans* isomers of oak lactone (see section 2.5).

6.3.4. Sensory assessment

Samples were diluted 1:2 with water before all nosing tests, thereby reducing the ethanol concentration to 21%.

Difference tests - see 2.13.1

For each of the three oak origins (Limousin, Tronçais and American) one sample (heated for 120 mins) was selected and three triangle tests carried out to compare each pair. Eight triangle tests were also carried out to determine whether differences could be detected between replicate samples. To test for differences between heating times, three tests were carried out on both Limousin and Tronçais samples.

Descriptive profiling - see 2.13.2

Sensory assessment by a panel of judges was carried out on each of the 72 samples. To prevent fatigue the samples were randomly divided into 8 arrays, each assessed at different times.

6.3.5. Multivariate analysis

The multivariate analysis program Canoco (ter Braak 1987-1992) was used to analyse the data. It was particularly suited to the ordination of flavour descriptors, where many terms were used for only a small proportion of samples, as it is designed specifically for such sparse datasets. Initial analyses using both linear and non-linear methods suggested that principal component analysis (PCA) was most suited to the data, explaining more variation than equivalent non-linear methods (such as correspondence analysis). The analyses shown in Table 6.1 were carried out. The unconstrained PCA's, carried out in these studies, assumed a linear model to describe relationships between response variables and principle components with *post-hoc* correlations on any independent variables. Replicate samples 57-72 were not used in any of the analyses to calculate the principal components. However their values along components were calculated afterwards to allow them to be plotted and to compare within-sample with between sample separation.

Table 6.1: Principal component analyses of samples. Analysis is applied to the response variables of the sample set, to calculate derived principal components. These components may be interpreted by calculated correlation coefficients with the response variables and with independent variables, after the unwanted effect of any covariables is partialled out in the analysis.

Study	Sample set	Response variables	Independent variables	Covariables
1.a.	Forest trial samples 1-40	Concentrations of extractives, pH and absorbance.	Sample descriptors (*eg* forest origin) and properties such as wood colour.	None
1.b.	Heating trial samples 41-56	Concentrations of extractives, pH and absorbance.	Sample descriptors and properties such as wood colour.	None
2	1-56	Flavour descriptors: scored by sample.	Concentrations of extractives, pH and absorbance.	Array number of assessment

Ordination diagrams of response and independent variables against principal components give an indication of covariance and correlations between variables (ter Braak 1987-1992). The values of response variables were adjusted for their variances to counteract the effect of response variables having variances proportional to their abundance or frequency of occurrence (ter Braak 1990). Response variable scores are correlations between the variables and eigenvectors, allowing better representation in ordination plots of the mutual correlations between variables. When covariables are used the correlations are more precisely termed partial covariables, but these are interpreted similarly. The only disadvantage of this adjustment of the response variables, is that distances between samples in scatter plots are not Euclidean distances in sample space.

6.4. Results

One of the most striking differences between samples was the colour of the extract solution. Figure 6.2 illustrates the colour range that had developed after only two weeks of extraction. It was also observed that after heating, the Limousin wood was generally darker than Tronçais wood heated for the same length of time. Concentrations of extractives, pH and absorbance measurements, by sample, are shown in table 6.2.

6.4.1. Chemical analysis

Within tree variation

From the results of the replication test, the within tree variances of each extractive for each of the four trees were calculated. These four estimates were then tested for homogeneity at a probability of 0.01. Only furfural gave significant heterogeneity. The results were therefore analysed by one-way analyses of variance to obtain estimates of a pooled within-tree variation for each extractive. As only one of the four replicate trees gave detectable levels of the oak lactones, only these four samples were used as an estimate of within tree variation of these extractives.

Figure 6.2: The colour range of samples, two weeks after the start of trials.

Table 6.2: Concentrations (ppm) of extractives, pH and absorbance (A) at 520 nm (arbitrary scale) for flavour samples.

Sample	Forest origin	Tree	Heat (mins)	Gallic Acid	HMF	Furf	Van Acid	Syring. Acid	Vanil	Syring. aldhyd	Conif. aldhyd	Sinap aldhyd	Ellagic Acid	Scop	Oak Cis	lactone Trans	pH	A
1	Limousin	1	120	22	10	195	49	26	7	35	22	74.5	350	0.25	<0.01	<0.01	4.02	0.22
2	Limousin	2	120	20	10	161	48	28	7	36	32	76.0	352	0.30	<0.01	<0.01	3.89	0.231
3	Limousin	3	120	13	13	131	54	24	5	36	26	79.0	155	0.20	<0.01	<0.01	4.18	0.074
4	Limousin	4	120	10	10	62	27	25	7	41	24	89.5	185	0.35	4.19	1.08	4.11	0.113
5	Limousin	5	120	17	13	140	42	26	8	41	29	82.0	258	0.20	0.04	0.02	3.91	0.194
6	Limousin	6	120	21	11	190	60	26	11	37	30	89.5	233	0.30	0.02	0.01	3.96	0.183
7	Limousin	7	120	10	13	112	45	25	8	40	39	103.5	268	1.05	<0.01	<0.01	4.03	0.163
8	Limousin	8	120	9	11	106	45	31	10	55	32	88.0	250	0.70	<0.01	<0.01	3.90	0.169
9	Limousin	9	120	10	16	173	61	24	9	37	29	77.0	188	0.20	<0.01	<0.01	4.16	0.139
10	Limousin	10	120	9	14	105	40	23	6	35	31	90.0	161	0.10	<0.01	<0.01	4.05	0.122
11	Limousin	11	120	12	15	136	46	21	5	26	29	77.0	110	0.45	1.89	2.68	4.17	0.102
12	Limousin	12	120	17	10	139	44	27	8	39	34	85.0	343	1.55	<0.01	<0.01	4.09	0.214
13	Limousin	13	120	12	14	157	62	26	8	37	23	77.0	172	0.25	0.03	0.02	3.99	0.134
14	Limousin	14	120	10	9	84	36	39	10	54	29	89.5	230	0.60	0.84	0.73	3.93	0.172
15	Limousin	15	120	15	14	159	54	39	10	57	38	100.0	296	1.15	1.14	0.96	4.06	0.189
16	Limousin	16	120	42	14	173	79	28	4	32	22	70.5	324	0.25	0.3	3.4	4.03	0.187
17	Limousin	17	120	22	12	218	56	24	9	31	33	92.0	253	0.20	0.02	0.02	4.05	0.166
18	Limousin	18	120	8	10	138	36	39	13	58	29	92.0	153	0.45	<0.01	<0.01	3.99	0.119
19	Limousin	19	120	12	8	176	40	41	9	50	23	72.0	336	1.60	<0.01	<0.01	3.79	0.316
20	Limousin	20	120	17	10	141	59	37	9	58	23	75.0	285	0.80	<0.01	<0.01	3.75	0.182
21	Troncais	1	120	3	16	126	40	30	12	48	29	90.5	88	0.15	2.82	7.76	4.26	0.045
22	Troncais	2	120	5	18	119	49	22	9	34	32	95.5	79	1.30	0.65	5.94	4.43	0.044
23	Troncais	3	120	3	17	84	34	28	15	59	36	94.5	38	0.20	4.27	2.75	4.46	0.03
24	Troncais	4	120	12	11	102	36	21	8	30	23	61.5	246	1.10	0.57	6.05	4.40	0.095
25	Troncais	5	120	5	17	91	34	29	14	61	36	89.5	29	0.10	4.96	7.32	4.44	0.026
26	Troncais	6	120	7	15	105	54	17	10	26	22	54.5	128	0.70	0.9	6.13	4.51	0.041
27	Troncais	7	120	10	13	81	47	27	9	39	33	84.5	162	0.35	2.34	4.08	4.34	0.052
28	Troncais	8	120	6	15	111	37	22	7	36	29	85.0	89	0.20	0.44	3.3	4.28	0.059
29	Troncais	9	120	10	13	162	41	22	11	34	31	82.5	123	0.65	0.01	0.03	4.17	0.09
30	Troncais	10	120	6	5	14	15	20	4	28	12	49.5	132	0.30	0.01	0.03	4.16	0.084
31	Troncais	11	120	6	19	114	53	20	8	30	27	79.0	89	1.40	0.58	5.32	4.45	0.045
32	Troncais	12	120	10	15	58	38	22	5	31	24	75.0	132	0.40	1.84	7.72	4.24	0.072
33	Troncais	13	120	4	7	22	16	26	7	37	26	86.5	89	0.75	1.37	3.47	4.26	0.059
34	Troncais	14	120	7	14	98	47	25	9	39	23	75.0	86	1.10	0.99	3.42	4.28	0.071
35	Troncais	15	120	7	9	38	16	24	6	32	21	79.0	126	0.30	3.39	5.57	4.30	0.062
36	Troncais	16	120	9	16	135	56	20	7	30	19	56.0	126	0.55	5.99	0.73	4.08	0.078
37	Troncais	17	120	9	8	104	39	17	9	28	22	59.5	119	0.20	1.38	4.02	4.73	0.064
38	Troncais	18	120	4	11	96	44	19	7	26	26	57.0	61	0.30	4.75	0.39	4.43	0.038
39	Troncais	19	120	6	20	102	46	29	13	54	30	84.0	39	0.20	2.83	9.94	4.26	0.045
40	Troncais	20	120	13	8	55	32	23	4	36	20	58.0	190	0.40	0.02	0.04	4.03	0.134
41	Limousin	1	0	15.5	4.5	2	8	6	1	3.5	1.5	2.5	193	0.45	0.01	0.01	4.00	0.032
42	Limousin	1	30	53.5	7	1	11.5	9.5	4.5	15.5	8.5	72.5	329	0.40	<0.01	<0.01	3.91	0.279
43	Limousin	1	60	44.5	10	0.5	14	17.5	4.5	25.5	14.5	93.5	426	0.30	<0.01	<0.01	3.95	0.35
44	Limousin	1	120	26.5	9	2.5	14	32	2	33.5	5	53.0	325	0.20	<0.01	<0.01	4.00	0.314
45	Troncais	19	0	5	1	0.5	6.5	5.5	1.5	1.5	1.5	1.5	123	0.15	2.26	8.11	4.26	0.017
46	Troncais	19	30	10.5	4	0.5	5	10.5	1	13	3.5	23.5	95	0.15	2.45	7.07	4.29	0.023
47	Troncais	19	60	10.5	2	0.5	9	33	2.5	13	2.5	39.0	45	0.15	1.95	6.85	4.27	0.031
48	Troncais	19	120	6.5	2	1	11	35	1	19.5	7	58.5	65	0.10	1.83	7.35	4.33	0.035
49	Limousin	Stave	0	4	0.5	0.5	6	4	2	2.5	2	1.5	67	0.25	0.03	0.2	4.24	0.026
50	Limousin	Stave	30	20	1	0.5	11	12.5	1	10.5	3	66.0	243	0.20	0.02	0.1	4.08	0.149
51	Limousin	Stave	60	12	1	0.5	13.5	21	1	23.5	5.5	75.0	248	0.20	0.01	0.06	4.19	0.161
52	Limousin	Stave	120	8	2	0.5	17.5	28.5	2	33	6.5	69.5	184	0.20	0.01	0.05	4.16	0.152
53	American	Stave	0	6.5	1.5	0.5	5	5	1	1.5	2	1.0	66	1.70	0.09	6.3	4.50	0.009
54	American	Stave	30	12	1	0.5	7.5	10.5	1	10	4.5	58.0	72	1.50	0.2	6.38	4.48	0.031
55	American	Stave	60	4	2.5	0.5	9	14	1.5	20	8.5	90.0	43	1.10	0.23	6.26	4.46	0.039
56	American	Stave	120	4	7	0.5	13	27.5	2	35.5	11.5	103.5	30	0.90	0.26	6.08	4.42	0.052
57	Troncais	20	120	11.5	2.5	1	16.5	26.5	2	28	5	56.0	227	0.25	0.01	0.03	3.98	0.138
58	Troncais	20	120	11.5	8.5	15.5	23.5	34.5	4.5	38.5	10	52.5	172	0.25	0.01	0.03	3.87	0.132
59	Troncais	20	120	12.5	5.5	38	32	24.5	2.5	31	18.5	59.5	179	0.25	<0.01	<0.01	3.94	0.166
60	Troncais	20	120	7	5	7.5	16.5	31	5	37	13	65.5	210	0.25	<0.01	<0.01	3.98	0.151
61	Troncais	19	120	5.5	8	1.5	18	32	2	26.5	12.5	65.5	34	0.25	1.6	6.3	4.34	0.034
62	Troncais	19	120	4	7	1.5	15.5	24	1	29.5	12	66.5	27	0.15	1.77	6.8	4.35	0.053
63	Troncais	19	120	6	9.5	1	19	21.5	2	33.5	14	64.0	33	0.10	1.62	7.18	4.30	0.044
64	Troncais	19	120	6.5	8	1.5	16.5	26.5	1.5	30	13	65.5	55	0.25	1.74	6.36	4.26	0.044
65	Limousin	18	120	5	4	1.5	12.5	27	1.5	33	6	55.5	109	0.10	<0.01	<0.01	3.91	0.172
66	Limousin	18	120	5.5	4.5	2.5	12.5	26	2	36.5	6	57.0	117	0.05	<0.01	<0.01	3.88	0.159
67	Limousin	18	120	8	2	0.5	16	28.5	1.5	31.5	4	55.5	144	0.25	<0.01	<0.01	3.92	0.178
68	Limousin	18	120	5.5	3	1.5	14	29.5	1.5	38.5	4	63.0	168	0.05	<0.01	<0.01	3.93	0.158
69	Limousin	1	120	16	13	262.5	71.5	30.5	5.5	38.5	28.5	89.0	326	0.25	<0.01	<0.01	3.95	0.343
70	Limousin	1	120	20	11	229.5	62	25	5	32	25.5	84.0	334	0.25	<0.01	<0.01	3.99	0.352
71	Limousin	1	120	20.5	11.5	246.5	69	29	5	35	27	85.5	334	0.30	<0.01	<0.01	3.99	0.373
72	Limousin	1	120	24	12	248.5	68	29	6.5	36.5	26.5	86.5	378	0.25	<0.01	<0.01	4.05	0.357

Variance between and within forests

After calculation of forest means and variances (using samples 1-40), no significant heterogeneity between the two within-forest variances was found using Cochran's test at a probability of 0.01. Therefore the forest means of the various properties were compared by analyses of variance. The error mean square, comprising of within and between-tree variation, was subsequently compared against the estimates of within-tree variation obtained as described above. This allows one to test for an effect between trees, within forests. Table 6.3 summarises the results. Most characters, including many lignin derived products, were found to vary significantly between forests.

Table 6.3: Anovas of forest means from flavour trials. All measurements made after six months maturation. Replicate mean squares from anovas of replicate tree samples 57-72.

Character	Limousin		Tronçais		Mean Squares			P of F test	
	Mean	Std. Error	Mean	Std. Error	Forest df=1	Error df=38	Replicate df=12	forest	trees
Gallic acid	15.15	*1.75*	6.85	*0.63*	688.9	34.6	4.95	**	**
5 - HMF	11.45	*0.49*	13.03	*0.96*	24.806	11.7	2.27	0.15	**
Furfural	144.53	*8.61*	90.55	*8.38*	29133	1444	110.95	**	**
Vanillic acid	48.93	*2.62*	38.43	*2.70*	1102.5	141	18.91	**	**
Syringic acid	28.65	*1.45*	22.95	*0.87*	324.9	28.5	12.09	**	ns
Vanillin	7.88	*0.47*	8.43	*0.67*	3.025	6.73	0.74	ns	**
Syringaldehyde	41.43	*2.20*	36.65	*2.35*	228.01	104	12.63	0.14	**
Coniferaldehyde	28.60	*1.13*	25.85	*1.34*	75.625	30.9	8.88	0.12	*
Sinapaldehyde	83.95	*2.08*	74.83	*3.33*	832.66	154	12.24	*	**
Ellagic acid	244.78	*16.72*	108.30	*11.72*	186254	4171	43.66	**	**
Scopoletin	0.55	*0.10*	0.53	*0.09*	0.0023	0.18	0.004	ns	**
Oak lactone-cis	0.45	*0.21*	4.20	*0.66*	140.96	4.77	0.010	**	**
Oak lactone-trans	0.42	*0.23*	2.01	*0.41*	25.027	2.2	0.170	**	**
Cis / Trans ratio	0.87	*0.56*	3.82	*0.70*	86.789	8.03	NA	**	NA
Absorbance	0.17	*0.01*	0.06	*0.01*	0.1161	0.0018	0.00014	**	**
pH	4.00	*0.03*	4.33	*0.04*	1.0401	0.02	0.00014	**	**

The test for between tree variation should be treated with caution, given the low number of replicates used to estimate the error term. However, the results suggest that there is significant variation between trees within forests for many extractives.

It has been reported (Ford pers. comm.) that the ratio of the *cis* to *trans* isomers of oak lactone differ between casks made from American and European wood. To determine whether there was a difference between the forests sampled this ratio was calculated and compared by T-test and the non-parametric Wilcoxon sum rank test. Both found a significant difference between forests at $P<0.01$, with higher relative

amounts of the cis-isomer being found in Tronçais than in Limousin wood. However given the trace levels found in many Limousin samples this result has limited value.

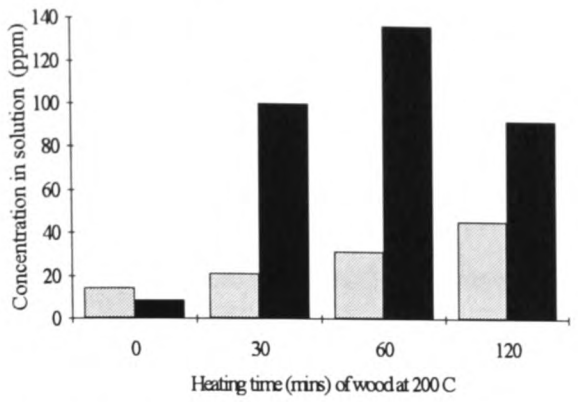
Heating trials

A two way analysis of variance, with trees and heating times being treated as random and fixed factors respectively, was used to analyse samples 41-56. Significant differences were found for both between trees and heat treatments (see Table 6.4). Figures 6.3.a-d show how levels of lignin derived acids (vanillic and syringic acids) and aldehydes (vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde) vary with increasing heating times for the four wood sources studied. Concentrations generally increased with heating time with the exception, in the Limousin samples, of total aldehydes, which either decline or level off at the longest times. Concentrations of both furfural and 5-HMF were very anomalous (see table 6.2), with levels being much lower than expected and relating little to heating time. No explanation could be found for these anomalies.

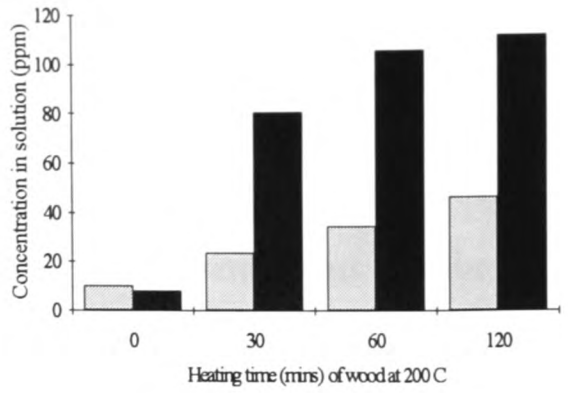
Table 6.4: Anova results of heating trial and probabilities (P) of significant differences between different heating times and between trees. Error mean squares used as denominator for F tests of variation between heating times and trees.

Character	Mean Squares			P of F tests	
	Heating df=3	Trees df=3	Error df=9	Between heat times	Between trees
Gallic acid	206.7	711.0	52.4	*	**
5 - HMF	6.8	32.6	2.9	0.138	**
Furfural	0.307	0.932	0.196	n.s.	**
Vanillic acid	42.1	18.5	2.47	***	**
Syringic acid	517.7	32.5	16.6	***	0.191
Vanillin	0.68	2.39	1.18	n.s.	0.181
Syringaldehyde	572.7	43.1	16.1	***	0.110
Coniferaldehyde	31.3	13.1	9.09	0.065	n.s.
Sinapaldehyde	4539	779	277	***	0.099
Ellagic acid	5203	57660	4202	***	n.s.
Scopoletin	0.066	1.17	0.027	0.133	***
Oak lactone-cis	0.099	61.5	0.074	n.s.	***
Oak lactone-trans	0.016	4.24	0.023	n.s.	***
Absorbance	0.013	0.041	0.004	0.069	**
pH	0.002	0.176	0.002	n.s.	***

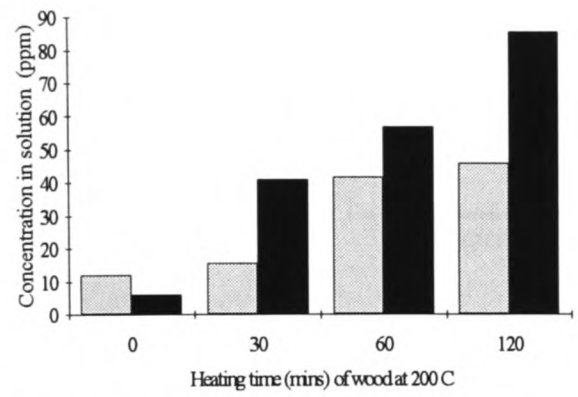
a) Limousin tree 1



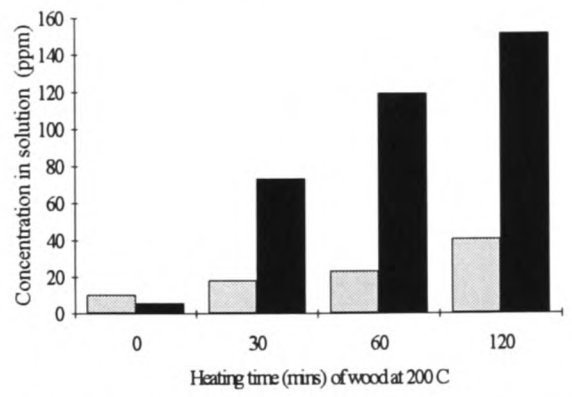
b) Limousin stave.



c) Tronçais stave 19



d) American stave



Figures 6.3.a-d: Total aldehydes and acids extracted from wood after varying heating times at 200° C.
■ Aldehydes ▯ Acids

6.4.2. Sensory assessment

Difference tests

All three triangle tests comparing the different wood origins gave significant results, while tests using replicate samples from the same tree found no significant differences. Five out of the six tests on heating time also found significant differences between the sample pairs.

Table 6.5: Results of triangle tests showing the number of judges, the number who correctly identified different samples and the resulting probabilities (P) of significant differences between sample A and B. Sample numbers refer to those used in table 6.2. Wood origins were American oak (Am.), Tronçais (T or Tron.) and Limousin oak (L or Lim.). Numbers refer to heating time.

Test	Sample A	Sample B	No. Judges	No. correct	Prob.
Lim. vs Tron.	1	21	11	10	***
Lim. vs Am.	1	56	14	10	**
Tron. vs Am.	21	56	14	9	*
Tron. replicates	57	58	10	4	n.s.
Tron. replicates	58	59	10	5	n.s.
Tron. replicates	59	60	11	4	n.s.
Tron. replicates	57	60	11	4	n.s.
Lim. replicates	65	68	11	3	n.s.
Lim. replicates	67	68	11	3	n.s.
Lim. replicates	65	67	11	6	n.s.
Lim. replicates	65	66	11	3	n.s.
L 0 vs 30 mins	41	42	11	11	***
L 30 vs 60 mins	42	43	11	9	***
L 60 vs 120 mins	43	44	11	8	**
T 0 vs 30 mins	45	46	11	7	*
T 30 vs 60 mins	46	47	11	7	*
T 60 vs 120 mins	47	48	11	4	n.s.

Descriptive profiles

Appendix 1 lists the terms used and basic statistics associated with each term. For the 40 terms most frequently used to describe samples 1-40, the number of times they were used to describe samples of each forest was compared by calculating the X^2 statistic (Sokal and Rohlf 1981) and testing vs a χ^2 distribution with one degree of freedom. Seven of these terms were found to have been significantly more frequently applied to one of the two forest origins and these are listed in Table 6.6. This is more than the two terms one may expect to differ by chance at a probability of 0.05.

Table 6.6: Sensory terms varying significantly in frequency of use between Limousin and Tronçais samples from forest trial. *n* = number of assessments made

Descriptive term	Limousin n=202 No. comments	Tronçais n=212 No. comments	Chi Square df=1 Probability
Burnt	66	48	*
Sweet	31	83	*
Pencil shavings	4	18	*
Coconut	1	7	*
Soapy	0	7	**
Sour	6	1	*
Phenolic	4	0	*

Samples were also compared by a single derived score relating to the uniqueness of its descriptive profile. The intention was that samples described by terms used infrequently should receive higher scores than those described by more common terms. Firstly the number of times that each assessor used each descriptive term was determined. Each term was then expressed as the percentage of the total number of comments made by a particular assessor. These percentages were then used to assign a score to each sample where:

$$\text{Sample score } S = \sum_{1}^{tj} \frac{\%\text{use of most frequently used term by judge } j}{\%\text{use of term } t \text{ by judge } j}$$

A frequency chart of the sample scores suggested a positively skewed normal distribution, with values ranging from 45 to 369. Table 6.7 summarises those samples with high scores and therefore the most unusual flavour descriptions.

Table 6.7 : The percentage of samples and the number, according to different heating times and origins, with high flavour scores. Only one American oak sample (unheated) had a score greater than 200 and this is included in the heating time <120 mins.

Flavour Score	% of samples	Number of samples			
		All	Heating < 120 mins	Heating time = 120 mins	
				Limousin	Tronçais
> 300	6.9	5	5	0	0
> 250	12.5	9	5	1	3
> 200	34.7	25	7	6	11
Total No. of samples in category:		72	12	30	29

6.4.3. Multivariate analyses

Table 6.8 summarises the results of the three analyses which were outlined in table 6.1. The mean ellagitannin concentration and mean ratio of vescalagin to castalagin of the samples, before heating, were calculated using the results from chapter 5. These were used as additional independent variables in all three analyses. Sample and variable scores for each analysis are given in Appendix 2.

Table 6.8: Eigenvalues and percentage of variation (% $s^{2)}$ explained by principal components for three principal component analyses described in table 6.1. In PCA analysis 2. percentage variation is that of the residual variation explained after fitting of the covariable: array number.

PCA analysis	No. samples		Principal component axes			
			1	2	3	4
1.a. Forest samples extractives	40	Eigenvalues	0.832	0.124	0.021	0.011
		Cumulative % s^2	83.2	95.6	97.7	98.8
1.b. Heating trial extractives	16	Eigenvalues	0.899	0.07	0.02	0.07
		Cumulative % s^2	89.9	96.9	98.9	99.6
2. Flavour assessments	57	Eigenvalues	0.232	0.112	0.057	0.052
		Cumulative % s^2	25.4	37.7	43.9	49.7

Interpretation of ordination diagrams

Referring to figures 6.4-6.7, variables (response and independent) may be inferred to be positively correlated if their two arrows make a sharp angle and negatively if an obtuse angle is formed. The longer the arrow the higher the correlation. The relationship of variables with the principal components may be similarly inferred. The sample values of variables may be determined by projecting samples along a variable line. However the sample plots do not represent Euclidean distances between samples.

Analysis 1.a of extractives

Figures 6.4a and b show how the two forests are well differentiated along the first component, which correlated most strongly with gallic and ellagic acid concentrations and absorbance. However there is broad overlap of forest samples along the second component, which explains much less of the overall variation and correlates more closely with lignin derived products. Plots of the replicate samples show little within sample variation, with greater variation along the second than the first component. Of the independent variables, the first component correlated most closely with ring width and b* measure of wood colour

(figure 6.4.a). The two nominal variables (not shown in figures) defining forest origin were located at either end of this axis. Figure 6.4.b illustrates the correlations between ellagic and gallic acids with ellagitannins and absorbance, as well as the negative correlations with pH and oak lactones.

Analysis 1.b of extractives

Within tree replicates showed less variation than in analysis 1.a, as would be expected if heating influenced extractives. Figure 6.5.b shows how the two components correlated in similar ways to the forest study: the first explaining most variation due to tannins and lactones while the second related more strongly to lignin derived acids and aldehydes. This second component also correlated strongly with the heating time, with samples distributed along it accordingly. These results emphasise the dependence of lignin degradation products on heating time. The greater variation of Limousin samples along the first component of figure 6.5.a is probably due to the higher levels of ellagic acid produced during heating, than in American or Tronçais samples. It is interesting to note the similarity between the distribution of American and Tronçais samples at different heating times.

Analysis 2 of flavour descriptors

The analysis was carried out using scores of the number of times each flavour term was used to describe a sample. The most common term 'woody' was excluded from the analysis as discussion with judges suggested it had been used primarily to emphasise the difference between the oak extract solutions to the mature whiskies they were more accustomed to assessing. The first two principal components, at 38%, explain much less of the variation than in the previous analyses, even after fitting the covariable array number, to account for variation between different assessment times. However, this is not surprising given the nature of the assessment and that 115 different terms were used to describe samples. Figure 6.6.a shows how the principal components differentiate samples according to heating times and forest origins, strongly suggesting that both these factors influence flavour. Figure 6.7.a displays the flavour terms and extractives that relate most closely to the two components. Modifications to the data analysed were carried out to try to increase the proportion of residual variation explained by components and reduce the within tree variation shown in figure 6.6.b. These included using only scores from judges who had assessed all 72 samples and weighting scores to account for the differing number of judges who assessed samples, but no improvements to the analyses were achieved.

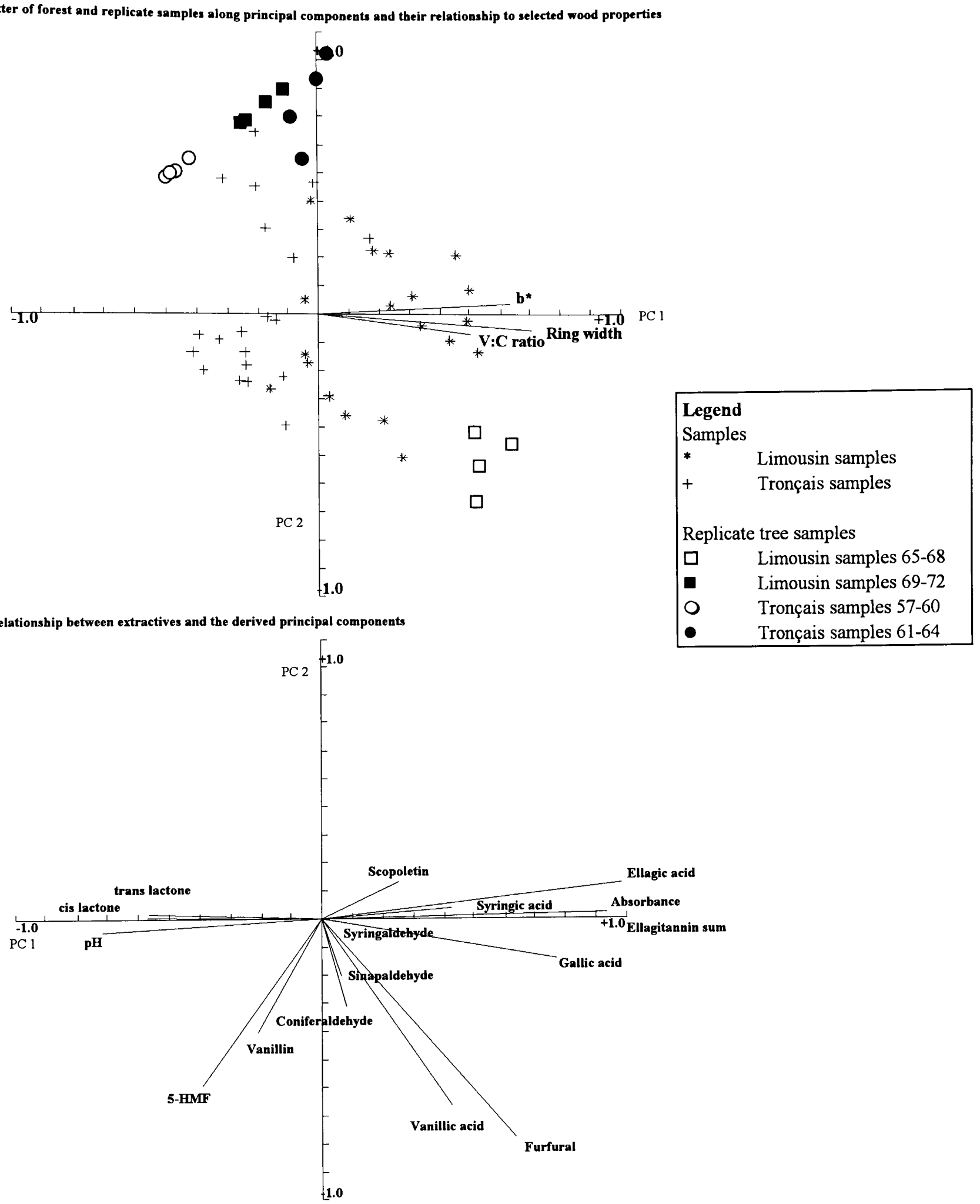
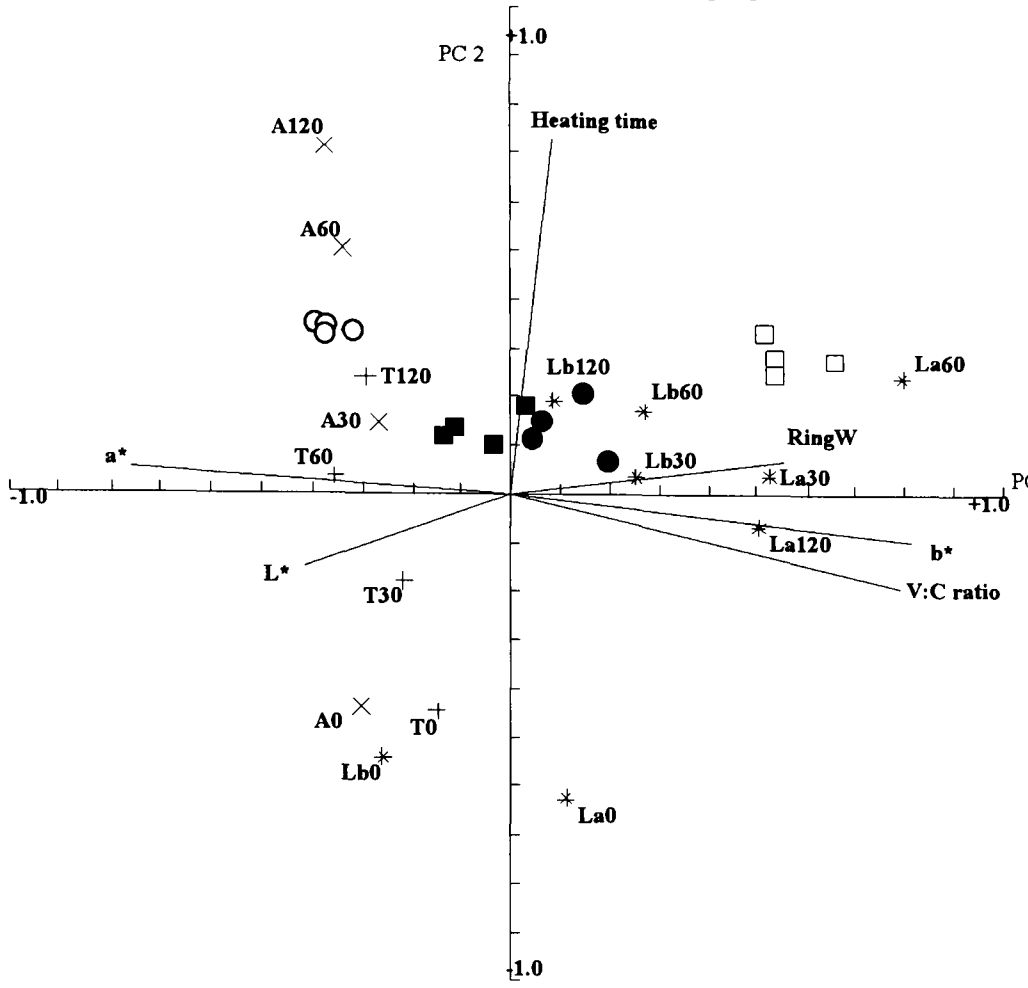


Figure 6.4.a-b: Principal component analysis applied to extractive concentrations, pH and absorbance at 520 nm of samples 1-40 of Limousin and Tronçais wood. *Post hoc* correlations with wood properties and the values of passive within tree replicate samples are shown.

a) Scatter of heating trial and replicate samples along principal components calculated from extractive concentrations. The relationship with selected wood properties and treatments are also shown.



b) Relationship between extractives and derived principal components

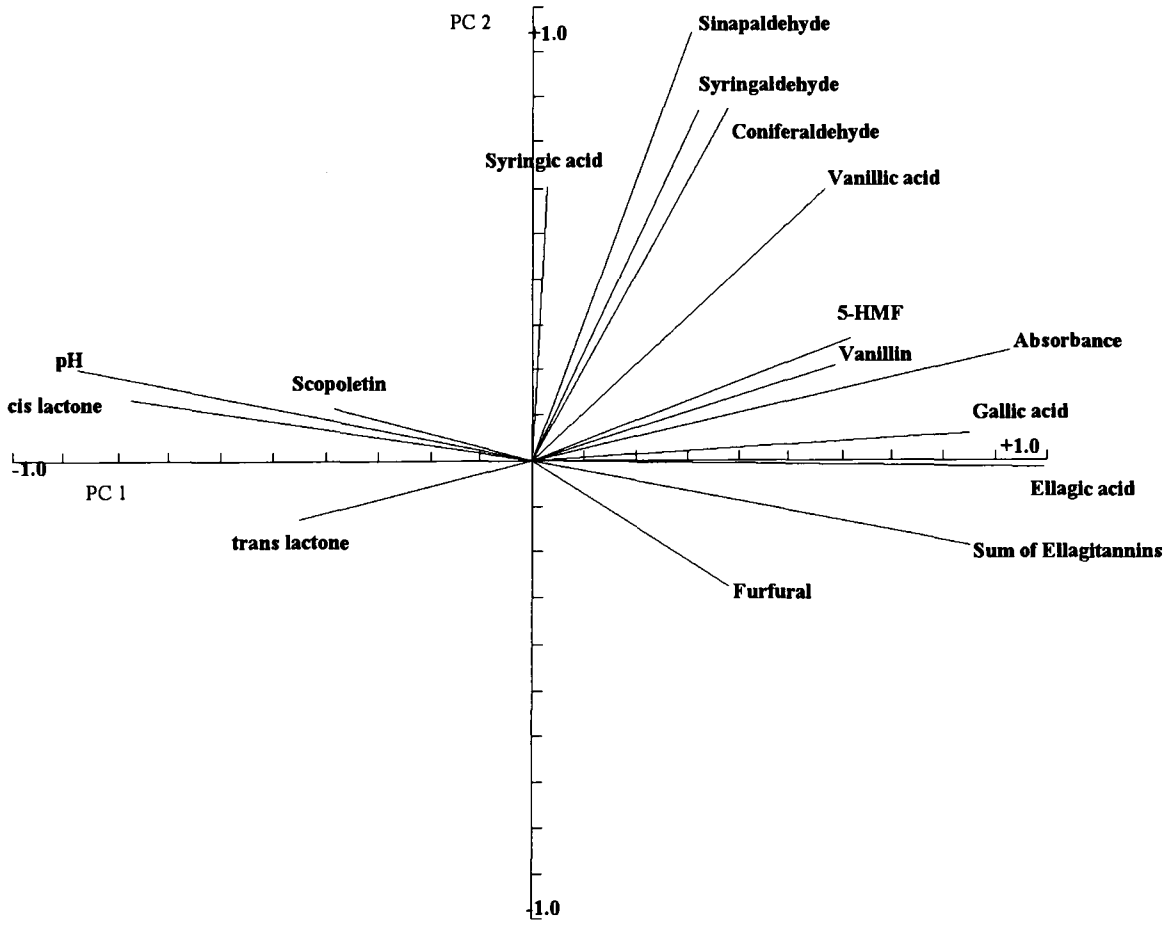
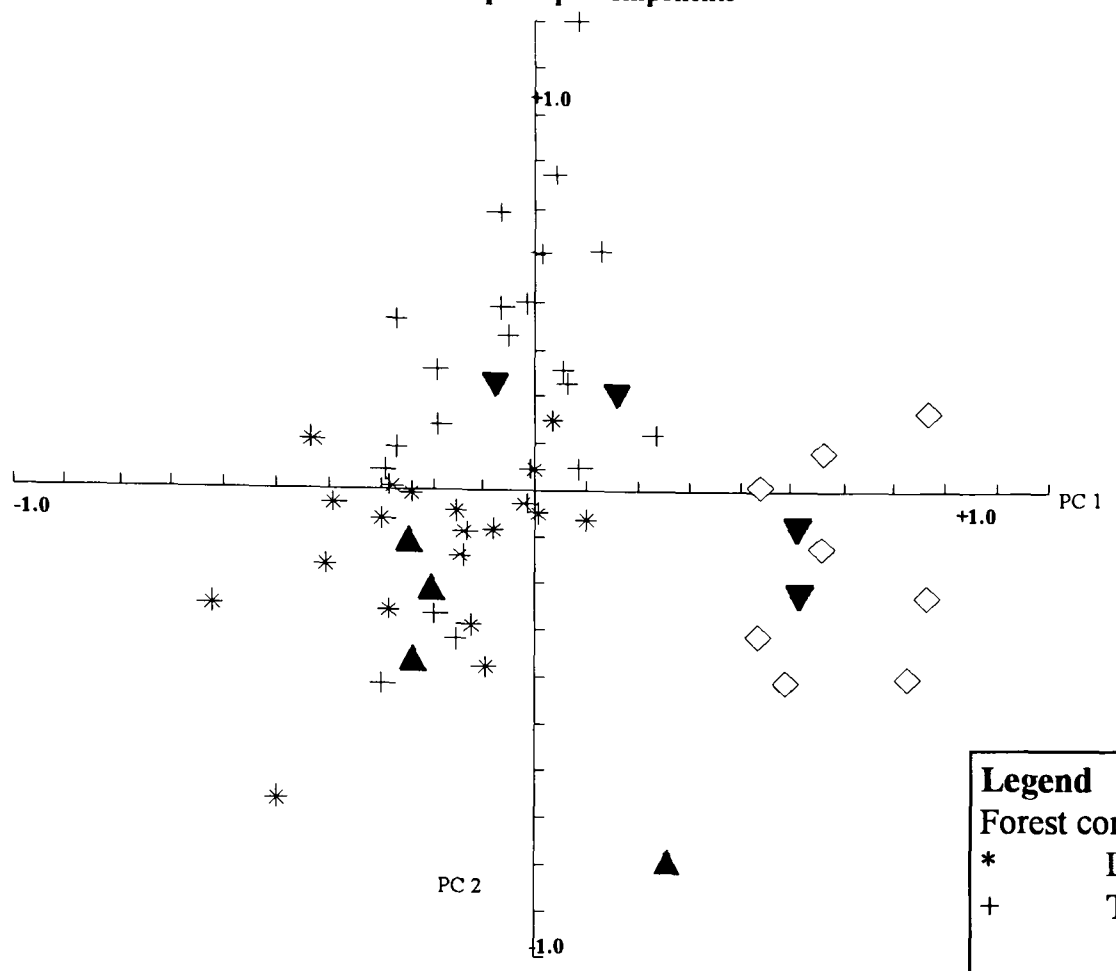
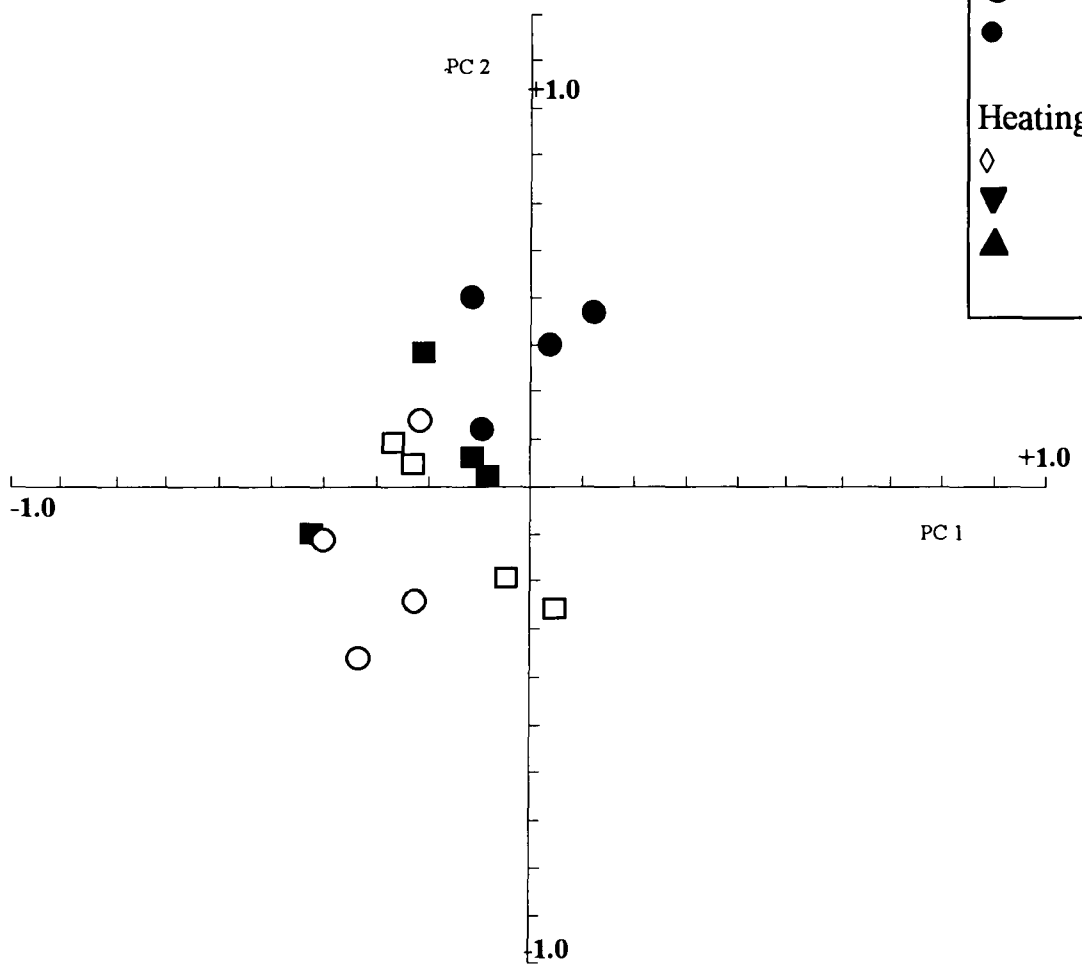


Figure 6.5.a-b: Principal component analysis applied to extract concentrations, pH and absorbance at 520 nm of the samples 41-56 (see table 6.2) of different types of oakwood subjected to different heating times. *Post-hoc* correlations with wood properties and the values of passive within-tree replicates.

a) Plot of all samples used in the calculationof principal components



b) Plot of passive within tree replicate samples along principal components



Legend

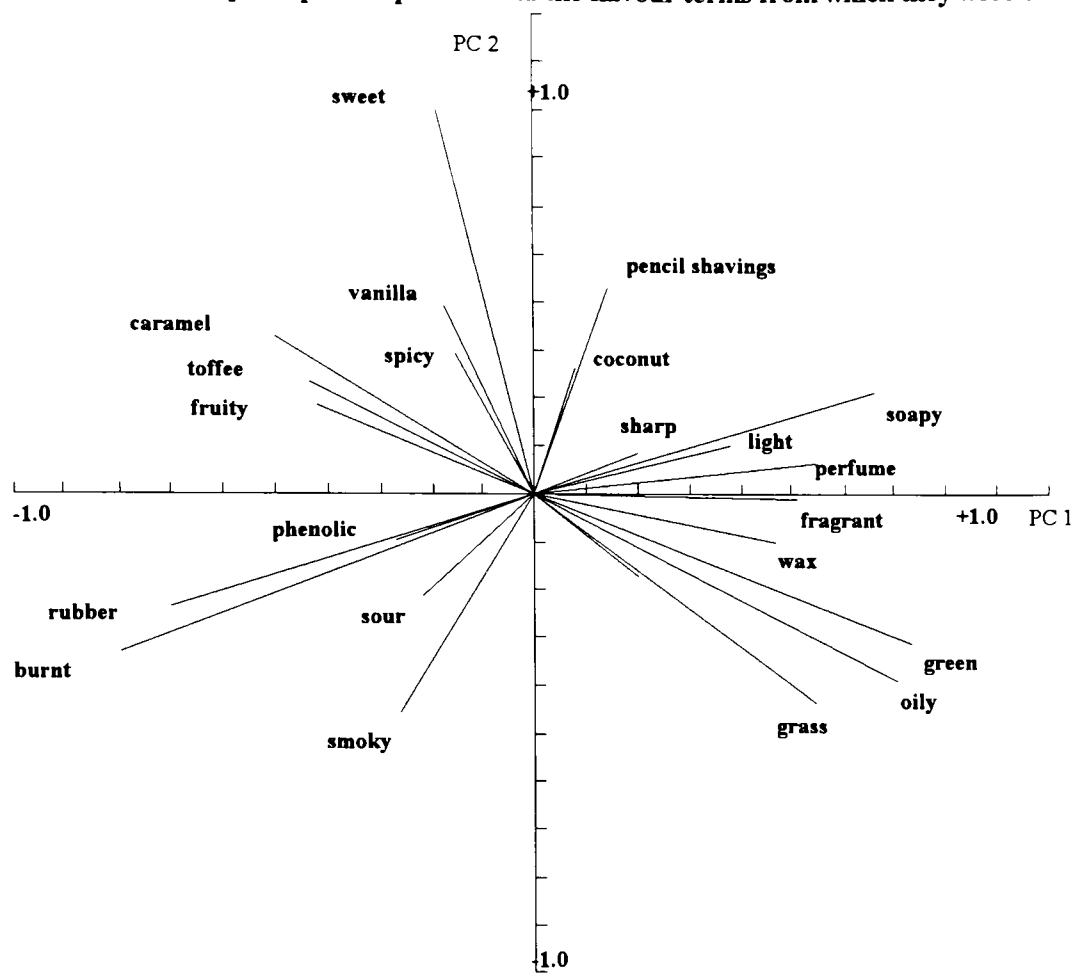
Forest comparison samples
- * Limousin samples 1-20
- \+ Tronçais samples 21-40

Replicate tree samples
- □ Limousin samples 65-68
- ■ Limousin samples 69-72
- ○ Tronçais samples 57-60
- ● Tronçais samples 61-64

Heating Trial samples
- ◊ All samples (0-30 mins)
- ▼ Tronçais and American (60+ mins)
- ▲ Limousin (60+ mins)

Figure 6.6.a-b: Partial component analysis applied to flavour descriptors of all samples, after fitting the assessment array number as a covariable. Scatter of all samples along components are shown.

a) Relationship between principal components and the flavour terms from which they were calculated.



b) Relationship between principal components and extractive concentrations of samples

Figure 6.7.a-b: Partial component analysis applied to flavour descriptors of all samples, after fitting the assessment array number as a covariable. Figures show relationship of components with flavour terms from which they derive and with extractive properties of samples

6.5. Discussion

6.5.1. Variation in extractives

The results show that one may discriminate between the forest origin of samples by a single principal component correlating strongly and positively with gallic and ellagic acids and negatively with the oak lactone isomers. This confirms the findings of the previous study, the acids arising through tannin degradation during the heating of the wood. The first principal component was also found to be very strongly correlated with the absorbance of the extract solution, indicating that extract colour is a reliable indicator of the tannin content before heating (and their products gallic and ellagic acid after heating). This suggests that coloured compounds soluble in ethanol/water solutions may also form from tannin degradation, agreeing with Klumpers *et al.* (1994) who suggested that the reddening of heartwood with ageing may be due to the transformation of polyphenols or dimeric derivatives into more coloured compounds. Others (*eg* Charrier 1992; Haluk *et al.* 1991) have suggested the involvement of ellagitannin degradation products in the discolouration of oak wood during kiln drying. Alternatively the colour may be derived from other compounds, such as proanthocyanidins, which are strongly correlated with the concentration of soluble hydrolysable tannins.

Oak lactones as well as varying highly significantly between the two forests, also varied markedly between trees. Only trace amounts were found in most Limousin samples but a few contained levels similar to those found in most Tronçais samples. This supports the findings of Feuillat (1991) and of chapters 3 and 4. Concentrations of lignin degradation products varied widely, but only some discriminated between the forest origins, with higher levels being found in Limousin wood. There was also greater variation of lignin products between within-tree replicates than oak lactones or tannin products.

The composition of lignin derived products is dependent upon temperature, with the initial formation of aldehydes followed by an increase in acids and decline in aldehydes at higher temperatures (Sarni *et al.* 1990). The colour of the wood and figures 6.3.a-d suggested that the Limousin samples responded as if heated to a higher temperature than the other wood types, aldehydes beginning to decline at longer heating times. This apparent difference in the response to heating found between the different woods should be treated with caution given the lack of replication and fairly crude methodology. However there are many reasons that could explain such different responses. The highly complex structure of oak wood and the interlinking between cellulose, hemicelluloses and lignins may create zones which vary in solvent accessibility. Heating of wood at temperatures less than 180°C results in shrinkage and cracks in cell walls. At higher temperatures the cell walls may break, causing fissures between the secondary walls (Puech and Sarni 1990). The proportion of late to earlywood and the abundance of fibres may also influence these physical reactions to heating and heat conduction. Higher levels of extractives, such as tannins, in the wood may also increase heat conductivity (Tsoumis 1991). Puech and Sarni (1990) suggested that the presence of high levels of soluble tannins or more specifically their products, gallic and ellagic acids, may be

responsible for the breaking of bonds (due to their acidity) and accelerating the delignification process giving rise to lignin degradation products. However the lack of strong correlations between tannin concentrations and any lignin derived extractives does not support the notion that tannins greatly influence the amounts of other extractives released. The effects of heating are further complicated by the possible loss of many volatiles, such as oak lactones, due to the long heating times.

6.5.2. Variation in flavour

The triangle tests clearly found differences between the trees tested while no differences were detected for within-tree replicates. Ideally any classification or distribution of samples according to flavour descriptors should separate those samples between which differences in flavour had been detected in triangle tests, while grouping the replicate samples where no differences were found.

The two principal components, derived from the flavour descriptions, allow one to discriminate not only between different heating times, but also between the forest origins. It is interesting to observe that the high tannin Limousin wood was characterised by appropriate terms such as 'phenolic' and 'sour'. Terms suggestive of a more pleasant flavour characterise the Tronçais samples. Figures 6.7.a-b also show how the terms 'coconut' and 'pencil shavings', both of which had been found to correspond with high lactone levels in other studies (Marco *et al.* 1994; Chatonnet 1991; Ford pers. comm.), relate to the principal components in the same way as the two oak lactone isomers. The results from the PCA are reflected by the significant differences in the frequency with which a number of flavour terms were used to describe samples from each forest.

The majority of judges used to assess samples were experienced in nosing whiskies and many had been trained to identify particular flavours. However there was no means of knowing whether terms were used to describe flavour in the same way by different judges. The same term used by different judges may refer to different flavour characters, as may apparently analogous terms used by the same or different judges (*eg* burnt sugar and caramel). The frequency of use by each judge was calculated for each term, with large variations being found. The term 'burnt' made up 35% of the comments used by one judge while under 2% for several others. Despite this the use of identical terms by different judges were grouped together so as to restrict the number of terms used in analyses. These shortcomings are more likely to hide any genuine flavour differences between samples than produce false ones. The calculation and analysis of flavour scores avoids many of these shortcomings as the samples receive values according to how frequently the individual judges awarded the same descriptors to other samples. The highest values were all given to samples that had been heated for 0 or 30 minutes. This emphasises the importance of heating on flavour released. A larger proportion of unusual flavour descriptions were also found for Tronçais samples rather than Limousin, suggesting greater variation or subtlety. Surprisingly American oak samples, despite making up a very small proportion of the total number of samples, scored relatively low values. This indicates that American oak may not possess any unique flavours compared to European oak wood.

However as all the American oak samples derived from a single stave any such conclusions a very tentative.

The results show that the difference in mean ellagitannin content found between the two forests is reflected by differences in both the extractives and flavour released by the wood after heating. Although the apparent differences in flavour cannot be shown to be caused by any of the extractives measured, known flavour congeners, such as oak lactones, are among those that vary significantly between the two sites. The high tannin containing Limousin wood is characterised by appropriate flavour terms that suggest that tannins may influence flavour despite degradation by heating. The results also show the dominant influence of heating on lignin derived extractives and flavour released while suggesting that wood may respond differently to identical treatments. However, this difference in response, which may be due purely to structural properties of the wood, might not explain the difference in flavour, as many of the extractives that discriminate most between the two origins appear the least affected by heating (*eg* oak lactones). It appears that the chemical composition and concentrations of wood extractives will influence flavour independently from any effects due to the physical properties of the wood.

6.6. Conclusions

The large differences between the two forests, found in both extractives and flavour released, cannot be considered typical of variation between forests as they were selected to typify opposing types. Although the sites had been selected by coopers on the basis of traditional properties used to identify oak types, such as wood grain, one cannot draw any conclusions about their effectiveness in predicting flavour or extractive properties. The large number of factors varying between the two locations prevents speculation on likely causes of the observed differences. However, the study has shown that differences in wood extractives and flavour characteristics do exist between different cooperage oak, on a scale that may be realistically selected for and that could influence the maturation of whisky or other beverages in oak casks.

Chapter 7: Analysis of material from genetic trials

A barrel made from a young oak would not produce good cognac. The oak grows; its trunk begins to turn silver. The oak swells; its wood gathers strength, colour, and fragrance. Not every oak will give good cognac.

R. Kapuscinski - *Imperium*

7.1. Chapter summary

Pressler cores of young clones of *Quercus petraea* and *Q. robur* were analysed from two sites in Germany. Variation of wood colour, ellagitannins and density between clones, species and sites was examined. Similar studies were made of cores from another trial of 20 half-sib families of parent trees deriving from 5 different forests. Despite shortcomings in the experimental design, the results indicated a high degree of genetic control for many wood properties, particularly heartwood ellagitannin content and wood density. Amounts were also found to be much lower and of more limited composition in the younger trees of the progeny trial than in clonal samples. A large proportion of the *inter*-clone variation of wood density and ellagitannin levels occurred between the two species. Less variation was found between the two sites, despite a difference in growth rates reflected by annual ring widths. Anomalous narrow-sense heritabilities of over one were estimated for the majority of characters and there was frequently significant variation between forest origins. This was sometimes due to outlying families but may also have reflected either the experimental design or that the selected parent trees were related. No clear relationship was found between ellagitannin concentrations and either heartwood colour or density.

7.2. Introduction

It has been seen from previous chapters that there is significant between and within-forest variation of oak wood extractives, which are likely to influence the flavour of whisky. However, previous studies, whose aims were primarily to test for any such variation, were unable to indicate likely causes of variation or how particular properties might be selected for. The difficulty of adequately controlling confounding variables made it unlikely that worthwhile results could be obtained from comparisons of natural populations within the time available. Furthermore, the unknown seed origins of most planted oak forests makes such comparisons even more uncertain.

There are not many indications as to which factors are likely to influence the properties of interest, with few published studies of relevance. The results in chapter 5 rejected the suggestion by Singleton (1974) that slowly grown trees will have higher amounts of extractable tannins. In contrast, Henry (1887) claimed that more quickly grown European oaks contain higher levels of extracts. In other species either no correlation has been observed, such as for *Quercus rubra* and *Juglans nigra* (Nelson 1975), or higher levels have been found in more slowly grown trees. Any

relationship is likely to be complicated by the extractives being laid down several years after the annual ring formed. Therefore the detection of any relationship between rate of growth and extractives might only be observed between trees of regular but different growth rates. A variety of other environmental influences may affect extractives but no study has been able to separate the different factors. However, many properties of wood, including extractives, have been reported as being under strong genetic control (*eg* Zobel and Van Buijtenen 1989). The levels of extractives are easily selected for in *Pinus elliotti* (Franklin *et al* 1970 in Klumpers 1994) but less so in *Populus tremuloides* and *Cryptomeria japonica.* Characteristics of oak wood that are thought to be highly heritable include the width of early wood, wood density (Nepveu 1984), vessel size (Mather 1991; Kanowski *et al.* 1991) and sapwood width (Savill *et al.* 1993).

It was decided that interpretable results could be only acquired from a comparison of plantations of known seed origins. The only material of sufficient age to allow the examination of heartwood are clonal, progeny and provenance trials established in Lower Saxony, Germany. Although the experimental designs of these trials have shortcomings, they allow not only an estimate of genetic parameters, but also comparisons of provenances and sites with greater control of confounding variables than would normally be possible.

7.3. Material and Methods

7.3.1. Sample Collection

Pressler cores were taken from progeny and ramets at the three sites in Germany (see figure 7.1), according to the scheme shown in figure 7.2.

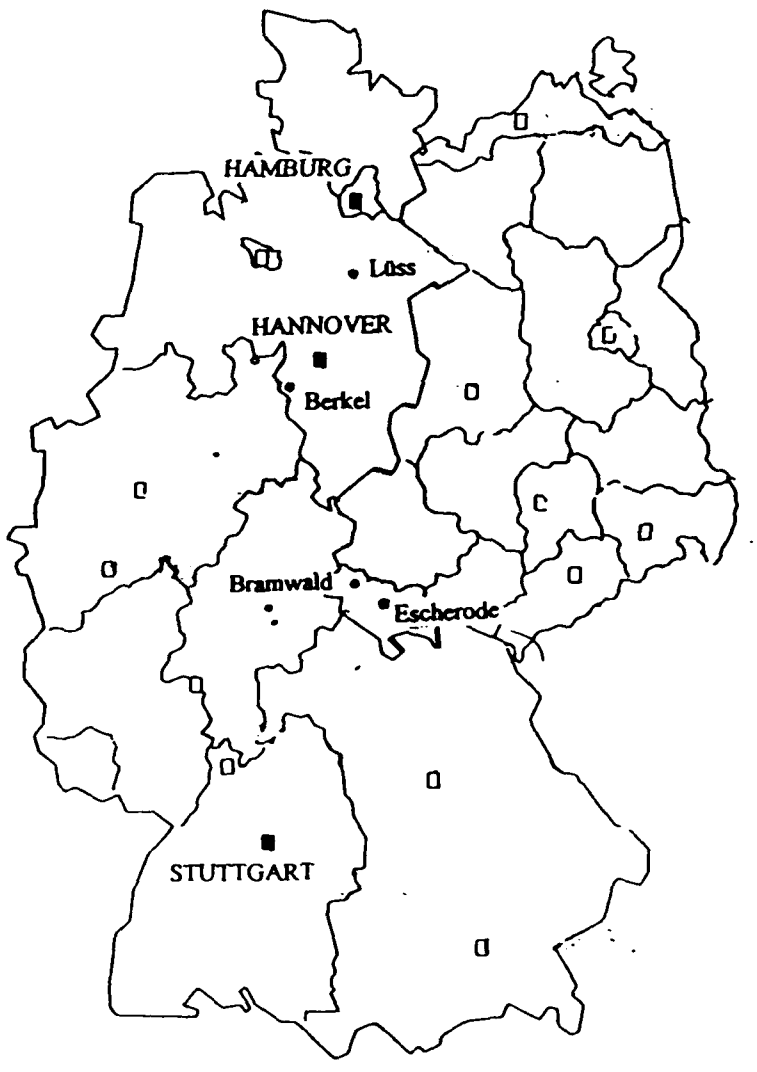
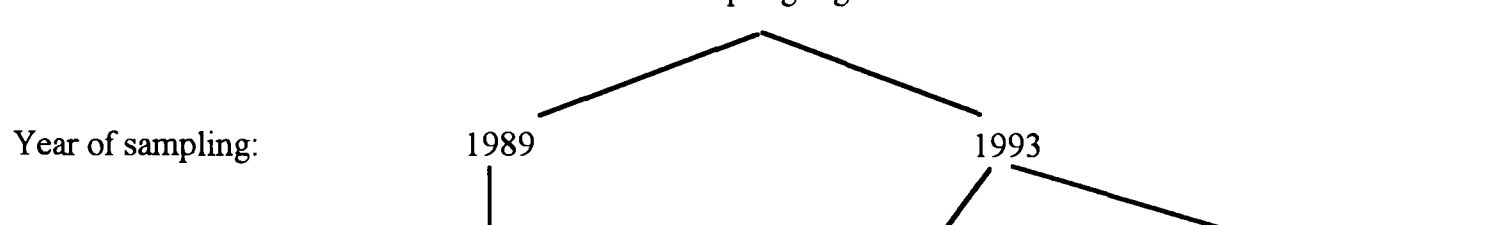


Figure 7.1: Map showing location of clonal and progeny trials in Germany from which samples were taken:
Bramwald; Berkel; Lüss.

Clonal seed orchards were established with ramets of vigorous trees of good form, selected from forests throughout the former West Germany and grafted in the forest districts of Berkel and Lüss. Orchards were established between 1955 and 1957, with ramets randomly located within each site at 6 x 6 metre spacing. Samples of both *Quercus petraea* and *Quercus robur* had been taken at Berkel by Dr Mather in 1989. Additional samples, collected from both Berkel and Lüss, were made in March 1993 by Dr Charrier (see figure 7.2). A number of the clones and trees sampled in 1993 were the same as those sampled in 1989. The two sites differed markedly in their soil characteristics, with that of Berkel being much richer than the poor, sandy soil of the site in Lüss.

Progeny trials were established on a 0.62 ha site in the Bramwald forest, near Kassel in Lower Saxony. Thirty four half-sib families, deriving from five forests throughout West Germany, were planted in an unreplicated design. Progeny of one family had been planted in randomly allocated rows between family plots. However, high mortality among these trees prevented reliable assessment of microsite variation. Two bark to bark cores were taken from eight trees of 21 year old *Quercus petraea*, from each of twenty families (see figure 7.2).



No. of clones	5	5	11	15	11	No. of families	20
Total No. of ramets	19	18	51	39	32	Total No. progeny	160
Cores per tree	1	1	1	1	2		2
Total No. of cores	95	90	51	39	62		320

Figure 7.2: Sampling of *Quercus petraea* and *Q. robur* undertaken in 1989 and March 1993. Cores per tree refers to the number of bark to bark cores taken from each tree. Two hybrid clones sampled in 1993 and one in 1989 are accounted for under *Q. petraea.*

7.3.2. Measurement of wood properties

Cores were allowed to dry until reduced to 11% moisture content before being cut into approximately 2 mm thick strips. Three different regions were defined for the purposes of subsequent measurements, as illustrated in figure 7.3. Sapwood was primarily identified through

the change in wood colour, with the presence of tyloses in early wood vessels used to determine the point of transition for any ambiguous samples. This latter property is not highly suitable as it has been reported that tyloses may be present in sapwood vessels 10-20 rings away from the heartwood boundary (Ziegler 1968 in Klumpers 1994). The outer heartwood was defined as the seven most recently formed heartwood rings, while the remainder comprised the inner heartwood.

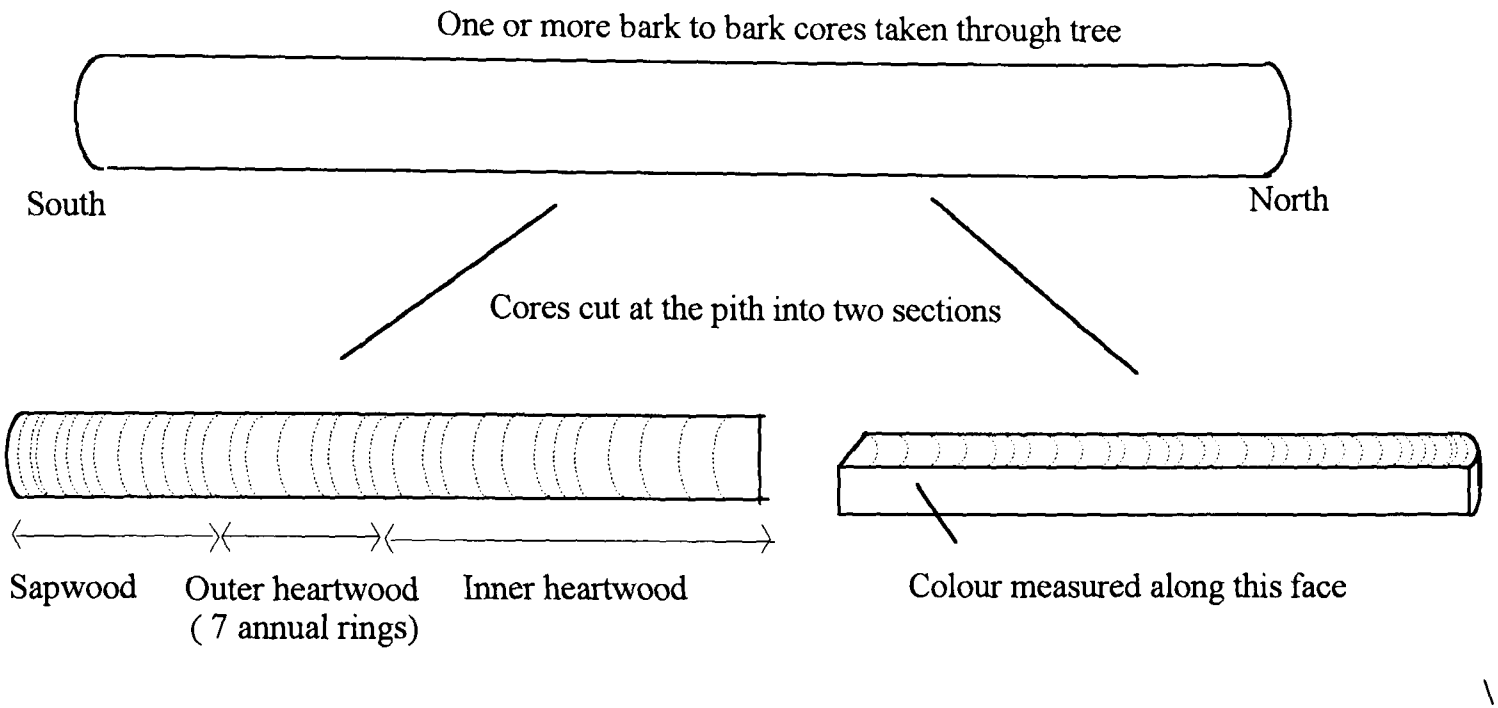


Figure 7.3: Trees in Lüss and in Bramwald were sampled in both directions: north-south and east-west. Trees from Berkel were sampled only in the north-south direction.

The following measurements were made of clonal and progeny cores.

- Colour analysis: all cores were analysed using the CIELAB system of colour measurement (described in section 2.10). Wood colour was measured along the surface indicated in figure 7.3. Ten measurements were made of both the inner and outer heartwood zones of clone cores and either 5 or 10 measurements of the sapwood, depending upon its width. For the progeny samples, 5 measurements of the sapwood and outer heartwood were made.

- Microdensitometry: densities along each core were measured using the method described in section 2.12. For the progeny samples the sapwood width was measured and this allowed each annual ring to be identified as either heartwood or sapwood. Due to the young age of the progeny and damage to some cores, an average of only 4 years of heartwood was measured.

- Ellagitannin analysis: levels of water soluble ellagitannins were measured using the method described in section 2.2. Because the results of the two heartwood zones in the 1989 samples were very similar, only concentrations in the outer heartwood were analysed for those samples collected in 1993. There was irregular replication of clonal samples, with from 1 to 4 extractions made from cores deriving from the same tree. Measurements were carried out over a period of a year using two different HPLC systems and this is likely to

have increased experimental error. Only vescalagin and castalagin were measured and concentrations were generally greater in the cores sampled in 1993 than in those from 1989. Therefore each set of samples was analysed separately. For progeny samples, the outer heartwood from grouped east and west facing cores of each tree was used to determine ellagitannin concentrations, with additional replication for a few trees to give an estimate of within tree variation.

7.3.3. Estimation of heritabilities

Following the example set in Kanowski *et al.* (1991) and Savill *et al.* (1993) samples of the two species from different sites were pooled to estimate heritabilities. The model fitted was:

$$Y_{xs} = \mu + g_s + e_{xs}$$

where Y_{xs} is the value of individual x in genotype s; μ is the overall mean; g_s is the effect of genotype s and e_{xs} is the normally and randomly distributed deviation of the individual value. Individuals were either ramets of a clone or progeny in a family.

Variance components were estimated from analyses of variance by equating the appropriate mean squares to expected values shown in table 7.1. The variation within clones is σ^2_e while σ^2_g is the genotypic variance component between clones or families, while k is the appropriate coefficient dependent on the harmonic mean of observations per family or clone. The SAS GLM and VARCOMP (SAS 1985) procedures were used to estimate variance components. The latter uses a restricted maximum likelihood method (Searle and Yu 1989) which allows more accurate estimates for unbalanced designs.

Table 7.1: Expected mean squares in analyses of variance to estimate variance components in clonal or progeny trials. For a balanced design: k = the number of trees n within a clone or family; s represents the number of clones or families.

Source of variation	Degrees of freedom	Expected mean squares
Clone or Family	s-1	$\sigma^2_e + k\sigma^2_g$
Within group	sn-s	σ^2_e

The clonal data were used to estimate broad-sense heritability from the equation: $h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_e / n)$ where n is the number of ramets per clone. Approximate standard errors were calculated by adapting formulae described in Wright (1976) such that $\sigma_{h^2} = \dfrac{(1-h^2)(1+kh^2)}{\sqrt{k(s-1)}}$ with the terms as previously defined and as in table 7.1.

Progeny data were used to estimate narrow-sense heritability on an individual tree basis, from the equation: $h^2 = 4\sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$ and standard errors were estimated using the formula:

$$\sigma_{h^2} = \frac{(1-(h^2/4))(1+k(h^2/4))^2}{\sqrt{k((s-1)/2)}}$$

7.4. Results

7.4.1. Ellagitannin concentrations

Clonal samples

Tree means and coefficients of variation (CV%) were calculated from those trees for which there were replicate measurements. Coefficients of variation were pooled across site, species, sampling time and wood zone to determine the mean within tree coefficient and related statistics that are shown in table 7.2. The large within-tree variation is probably mostly due to various problems associated with the transfer of the work from Dr Charrier and the long period of time over which samples were analysed.

Table 7.2: Within tree variations, expressed as percentage coefficients of variation from 67 ramets collected in 1989 and 1993.

	Minimum	Maximum	Mean	S.E. of mean CV%
Vescalagin CV%	3.9	97.9	37.7	2.7
Castalagin CV%	1.4	82.0	30.6	2.3
No. of within tree samples	2	6	2.6	

Broad-sense heritabilities are shown in table 7.3 and are all very high, indicating strong genetic control. Figures 7.4.a-b illustrate how the mean levels of tannins in those trees sampled in 1989, of *Quercus robur* clones are generally higher than those of *Q. petraea.* A similar pattern is apparent in figure 7.5 which shows the mean values of extracted ellagitannins for each clone sampled in 1993, grouping samples from both sites.

Table 7.3: Broad-sense heritabilities and variance components of tree mean ellagitannin concentrations in outer and inner heartwood of Pressler cores from clonal ramets. Clones include *Quercus robur* and *Q. petraea* planted at two different sites in Lower Saxony. Referring to table 7.1: $k = 4.37$ for outer and 3.7 for the inner heartwood of the 1989 samples and 4.45 for 1993 samples.

Year and wood zone	Tannin	No. samples Clones	Trees	Variance components Clones	Trees	Heritability h^2	S.E.
1989	Vescalagin	9	32	6.07	1.67	0.93	0.06
Inner	Castalagin	9	32	2.24	1.09	0.90	0.09
1989	Vescalagin	8	35	8.71	2.54	0.94	0.06
Outer	Castalagin	8	35	2.62	1.21	0.91	0.09
1993	Vescalagin	26	116	26.3	6.67	0.95	0.02
Outer	Castalagin	26	116	6.29	4.09	0.87	0.06

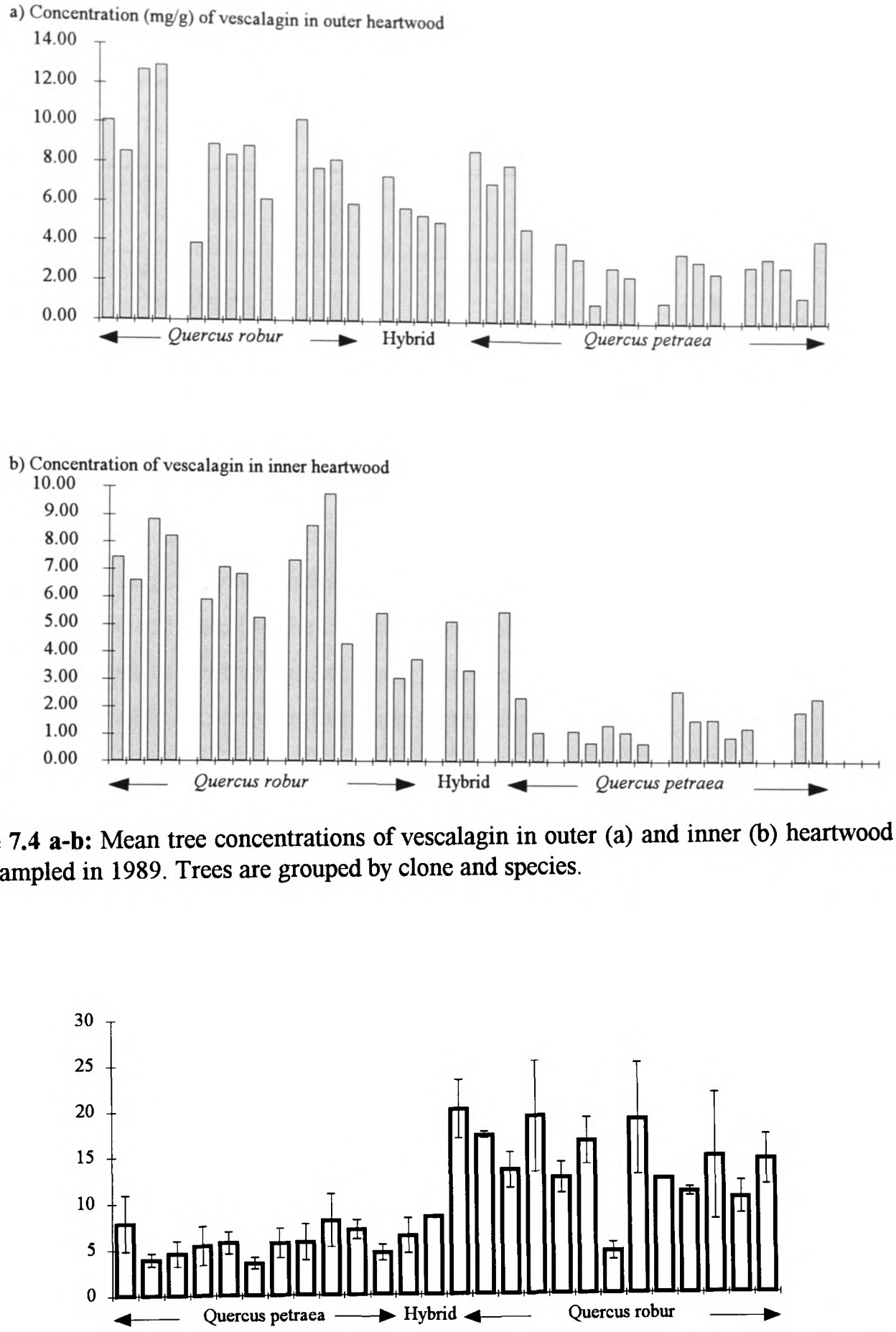


Figure 7.4 a-b: Mean tree concentrations of vescalagin in outer (a) and inner (b) heartwood of Pressler cores sampled in 1989. Trees are grouped by clone and species.

Figure 7.5: Mean concentrations of vescalagin and 2 x standard error bars in outer heartwood of clones from two sites and three orchards.

A nested analysis of variance, with clones nested within each species, confirmed that the majority of the variance in vescalagin concentrations between clones can be attributed to variation between the two species. These results are shown in table 7.4.

Table 7.4: Nested analysis of variance of ellagitannin concentrations in inner and outer heartwood of clones of *Quercus robur* and *Q. petraea*. df = degrees of freedom.

Samples and Heartwood	Tannin	Mean Squares			Probability		% of variance		
		Species	Clones	Within clone	Species effect	Clone effect	Species	Clone	Within Clone
1989 Inner	df	1	6	23					
	Vescalagin	156	7.9	1.75	0.0001	0.0002	74	13	13
	Castalagin	56	2.7	1.14	0.0001	>0.05	69	8	23
1989 Outer	df	1	6	27					
	Vescalagin	163	20	2.5	0.0001	0.0001	55	27	17
	Castalagin	38	8.3	1.2	0.0001	0.0001	37	36	27
1993 Outer	df	1	24	90					
	Vescalagin	1691	45	6.7	0.001	0.001	66	19	15
	Castalagin	230	23	4.11	0.01	0.001	31	35	34

At the Berkel site the clones of each species occupied separate, but adjacent, seed orchards, while the 1993 samples of *Q. petraea* include samples from Lüss. Therefore the apparent species difference could be attributed to the experimental design. Alternatively the difference may be genuine, with clones of the two species being genetically more dissimilar than clones within a species, with regard to those loci influencing tannin content.

To investigate the effect of the Berkel and Lüss sites those clones of *Quercus petraea* that had been planted at both sites were analysed by a two way anova. The results are shown in table 7.5. While there is no significant effect of site there is significant clone and site interaction.

Table 7.5: Analysis of variance of the mean concentration of two ellagitannins in the outer heartwood of 56 ramets of 9 clones planted at two sites. The variation between clones is considered a random effect while the effect of site is fixed. Mean squares and F tests are approximate due to the uneven replication.

Source of variation	df	Vescalagin			Castalagin		
		Mean Squares	F	P	Mean Squares	F	P
Site	1	8.9	0.64	ns	5.14	0.35	ns
Clone	8	15.3	5.96	0.0001	23.6	12.3	0.0001
Site x Clone	8	14.0	5.47	0.0001	14.7	7.7	0.0001
Within clone	38	2.56			1.92		

Figure 7.6. illustrates how the mean clone values vary between the two sites, showing clearly how the majority of interaction between site and clone is due to a single clone. With this exception the clones at the Lüss site have similar values to those at Berkel. This suggests that environmental influences on ellagitannin content are relatively low compared with the overall variation between genetic types.

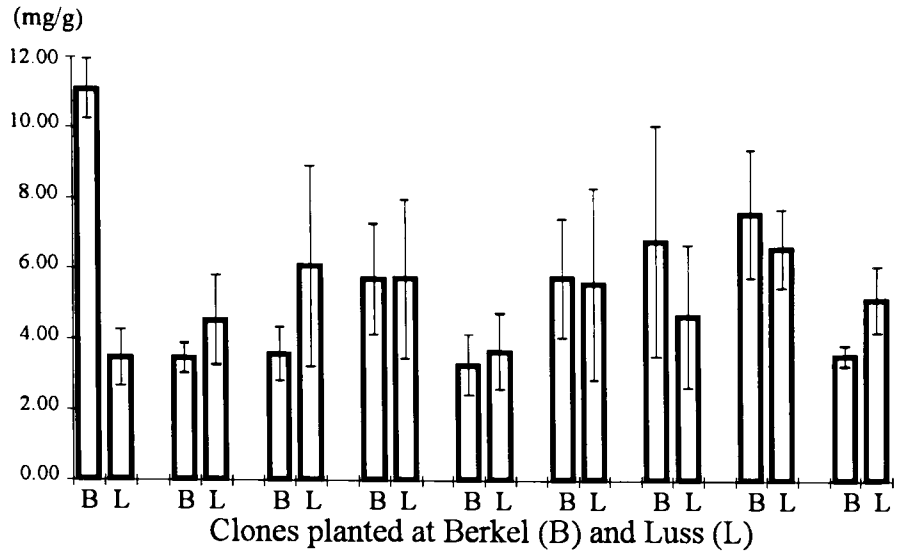
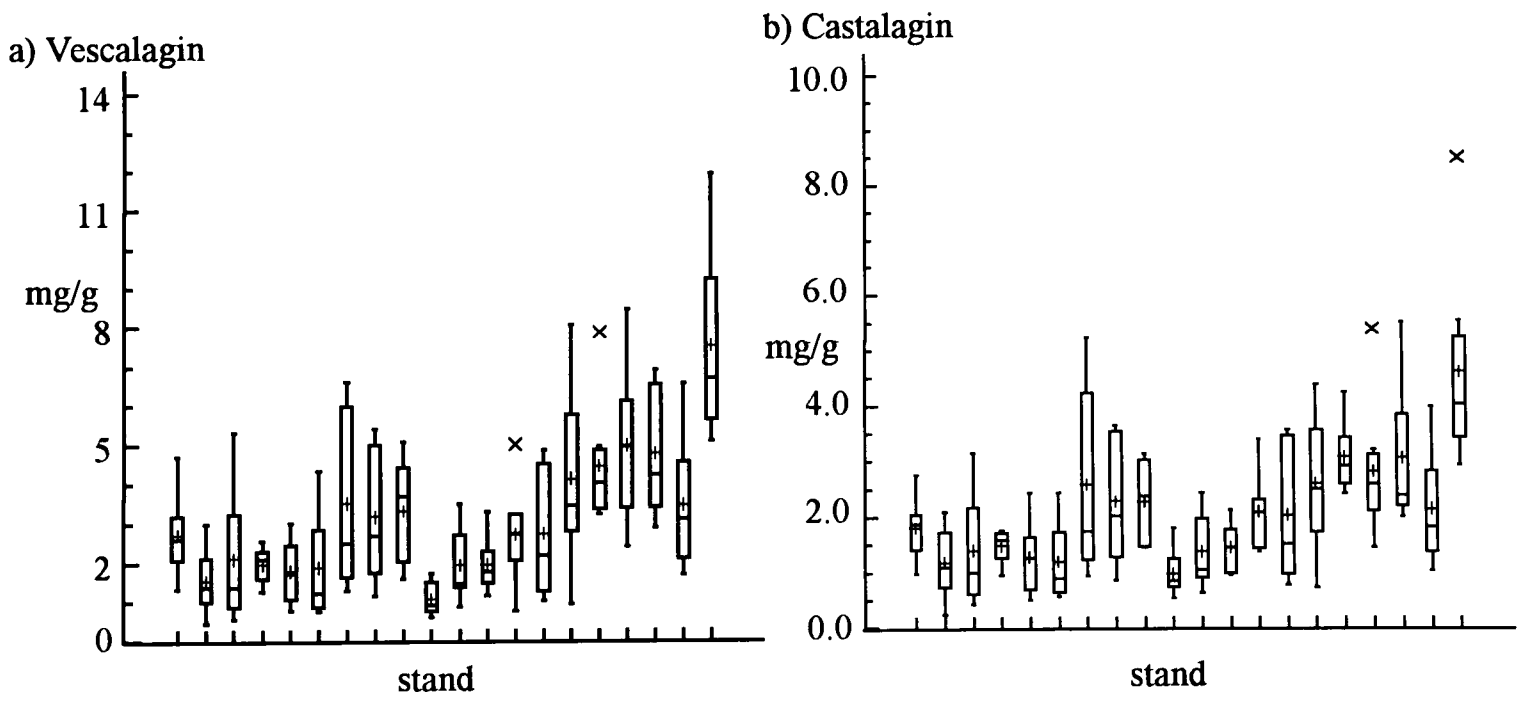


Figure 7.6: Mean vescalagin concentrations and 2 x standard error bars of 9 clones, each grown at two sites (Berkel and Lüss).

Progeny data

To determine within tree variation four individual cores were analysed from each of 3 trees from different families. The mean coefficients of variation were 15.6% for vescalagin and 11.3% for castalagin, each mean having a standard error of 4.4. This is significantly lower than that found for the clonal samples, probably because of the better methodology used for the analysis of progeny samples. Figure 7.7 shows variation between and within families in the concentration of vescalagin and castalagin.



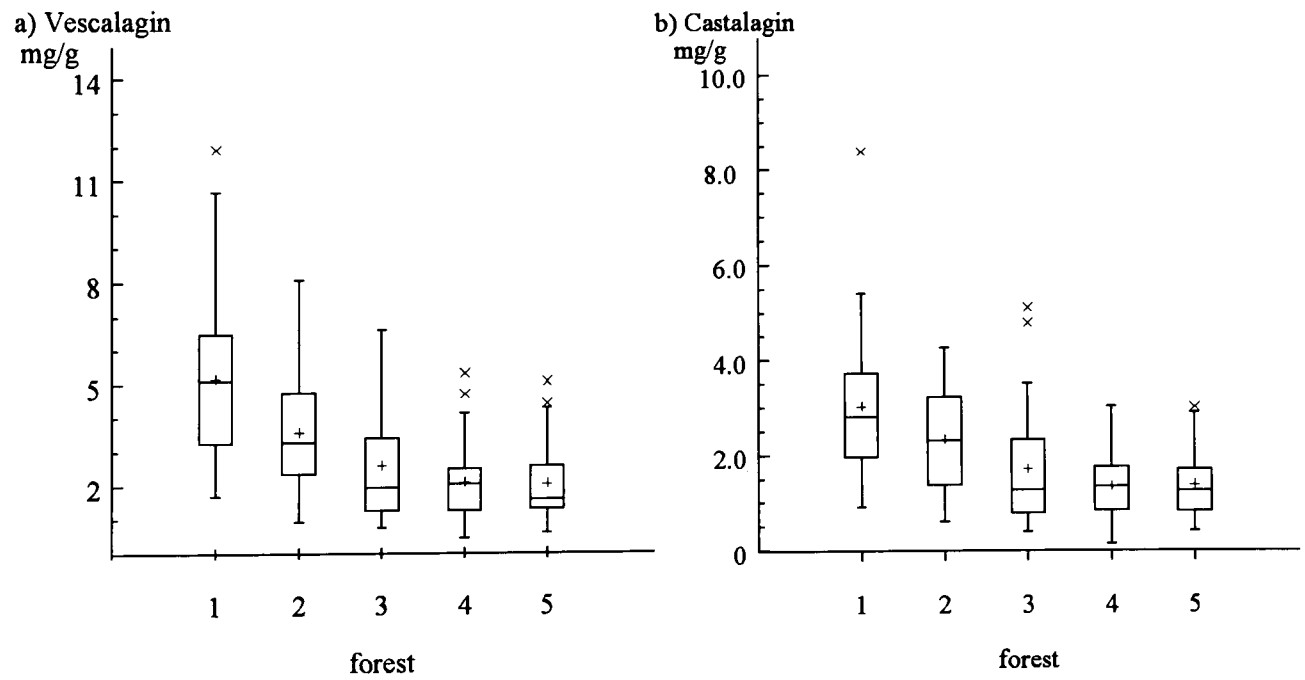
Figures 7.7.a-b: Box and whisker plots of mean tree concentrations of castalagin and vescalagin by family stand. The plot divides the data into four areas of equal frequency. The box encloses the middle 50%, with the horizontal line positioned at the median. The vertical lines connects the respective quartile to the smallest or greatest data point within 1.5 inter quartile ranges. Observations beyond the whiskers are plotted individually as an 'X' with far outliers beyond three inter quartile ranges of the box plotted as '*'. Where given the mean is marked as a '+'.

Log transformations of 10 x ellagitannin concentrations were applied to improve the normality of residual variation. However, as can be seen from figure 7.7, the last family plotted has extreme values, which were not altered by the log transformation. Calculation of narrow-sense heritabilities gave abnormally high values for both vescalagin (h^2=1.73) and castalagin (h^2=1.55). There are no grounds for excluding the outlying family and even without this the resulting heritabilities are still greater than one. As the families derived from five different forests the data were further examined by a nested analysis of variance, as shown in table 7.6.

Table 7.6: Nested analysis of variance of log transformed ellagitannin concentrations in the outer heartwood of eight progeny in each of four families from each of five forests..

		Vescalagin				Castalagin			
	df	Mean squares	% variance	F	P	Mean Squares	% variance	F	P
Forest	4	5.46	30	5.80	**	3.094	23.8	4.83	*
Family	15	0.90	17	3.47	***	0.640	17.4	3.32	***
Within family	137	0.26	53			0.193	58.8		

Figure 7.8 illustrates the significant variation between forest origins. The progeny of each family were planted in single, unreplicated plots, but in addition families from the same forest origin tended to be growing close to each other. However, it was found that the within forest variation was no greater for those forests whose family plots were more scattered across the planting area than for those where the family plots were adjacent to one another. This indicates that the significant variation between forests may not be due entirely to the experimental design.



Figures 7.8.a-b: Box and whisker plots of mean tree concentrations of castalagin and vescalagin by forest origins. See figure 7.7 for an explanation of plots.

Ellagitannin composition

The ratios of vescalagin to castalagin were determined for both clonal and family data and analysed in a similar way to the absolute amounts of each tannin. From the results discussed in chapters 4 and 5 one would expect values of over 1.0 and low variation, as all extractions were made from heartwood of the same age. However, figures 7.7.a-b showed that castalagin varies less than vescalagin, and the results in table 7.7 show how there is wide variation in the ratio. The pattern of variation was similar to that of the absolute amounts of ellagitannins, with high percentages explained by variation between forest origins and species. The variation between and within families is shown in figure 7.9.

Table 7.7: Variation in the vescalagin:castalagin ratio found in trees from different clones of *Q. robur* and *Q. petraea* and 20 half sib families of *Q. petraea*.

Type of samples	Heartwood zone	Min.	Max.	Mean	Standard Error	% variation between species or forests	% variation within clones or families
Clones 1989	Outer	0.69	1.72	1.29	0.28	66.5	16.6
	Inner	0.41	1.54	1.08	0.33	60.3	31.9
Clones 1993	Outer	0.50	1.97	1.18	0.34	77.7	10.5
Progeny 1993	Outer	0.87	1.90	1.32	0.02	34.7	11.8

Lower levels of ellagitannins were observed in the progeny samples than in the clones. While peaks corresponding to all the identified ellagitannins were detected in clonal samples, only peaks corresponding to grandinin and roburin *e*, in addition to those of vescalagin and castalagin, were detected for most progeny samples. The results indicate that the composition of ellagitannins is not determined solely by a *constant* rate of hydrolysis or insolubulization occurring during heartwood ageing.

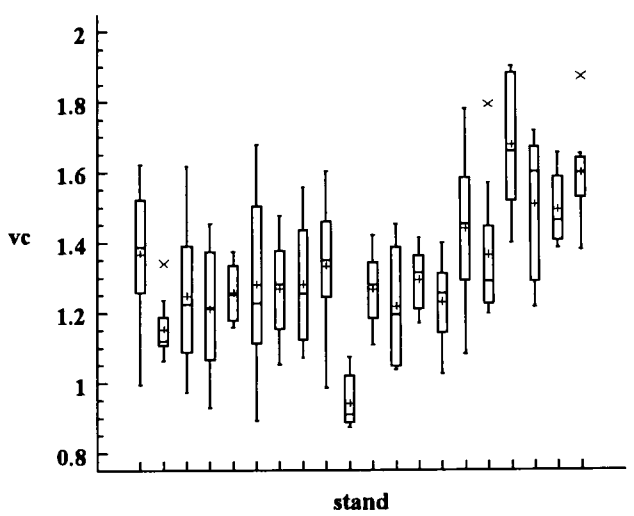


Figure 7.9: Box and whisker plots of tree mean ratios of vescalagin / castalagin by family.

7.4.2. Colour parameters

The progeny data were used initially to explore variation of the different parameters. The difference between the two zones was less than that which is normally observed in oak wood, possibly because of the young age of the trees. Data from two trees were not used in future analyses as no visible heartwood was observed in any of their cores. These tree cores had given correspondingly very low levels of ellagitannins.

Both hue and saturation derive from the parameters b* and a* as described in section 2.10 and this is emphasized by the calculation of correlation coefficients shown in table 7.8. The L, a*, b* variables have been found to relate best to polyphenolic content (Klumpers 1994). However, figures 7.10 a-e show how lightness (L) and hue (h) and a* best discriminate between sapwood and heartwood. The results are generally in accord with other studies on oak wood colour variation which have used the CIELAB system (*eg* Klumpers 1994; Janin 1987; Janin *et al.* 1990). Klumpers (1994) concluded that a level of perceptible difference could be taken as 1.0 for lightness and as 0.5 and 0.6 respectively for hue and saturation. Perceptible differences were not calculated for the parameters a* and b*.

Table 7.8: Spearman coefficients for correlation among parameters of colour from measurements of oak sapwood (upper right of table) and outer heartwood (lower left half). Nearly identical results were obtained from using Pearson correlation coefficients.

	Lightness	a*	b*	Saturation	Hue
Lightness		-0.49	-0.02	-0.11	0.56
a*	0.06		0.54	0.66	-0.7
b*	0.59	0.66		0.99	0.17
Saturation	0.51	0.76	0.99		0.02
Hue	0.73	-0.3	0.45	0.33	

The results were initially analysed to test for any effect due to core aspect. Although significant differences were found, the magnitude of the differences was very small and below perceptible differences. Therefore, for later analyses the cores were treated as random nested within-tree replicates and not as fixed effects according to orientation.

To determine the degree of variation at different levels a nested analyses of variance was carried out (see table 7.9). Although there were high within-core and within-tree variations, as indicated by the percentage of core and error variance in table 7.9, there is also significant between tree and family variation for most parameters. The amount of variation attributed to occurring between forest origins, compared to between families, is much lower than was found for ellagitannin concentrations. However, narrow sense heritabilities were all greater than 1.0, with the exception of sapwood lightness (0.87) and heartwood lightness and b* (0.90 and 0.71 respectively).

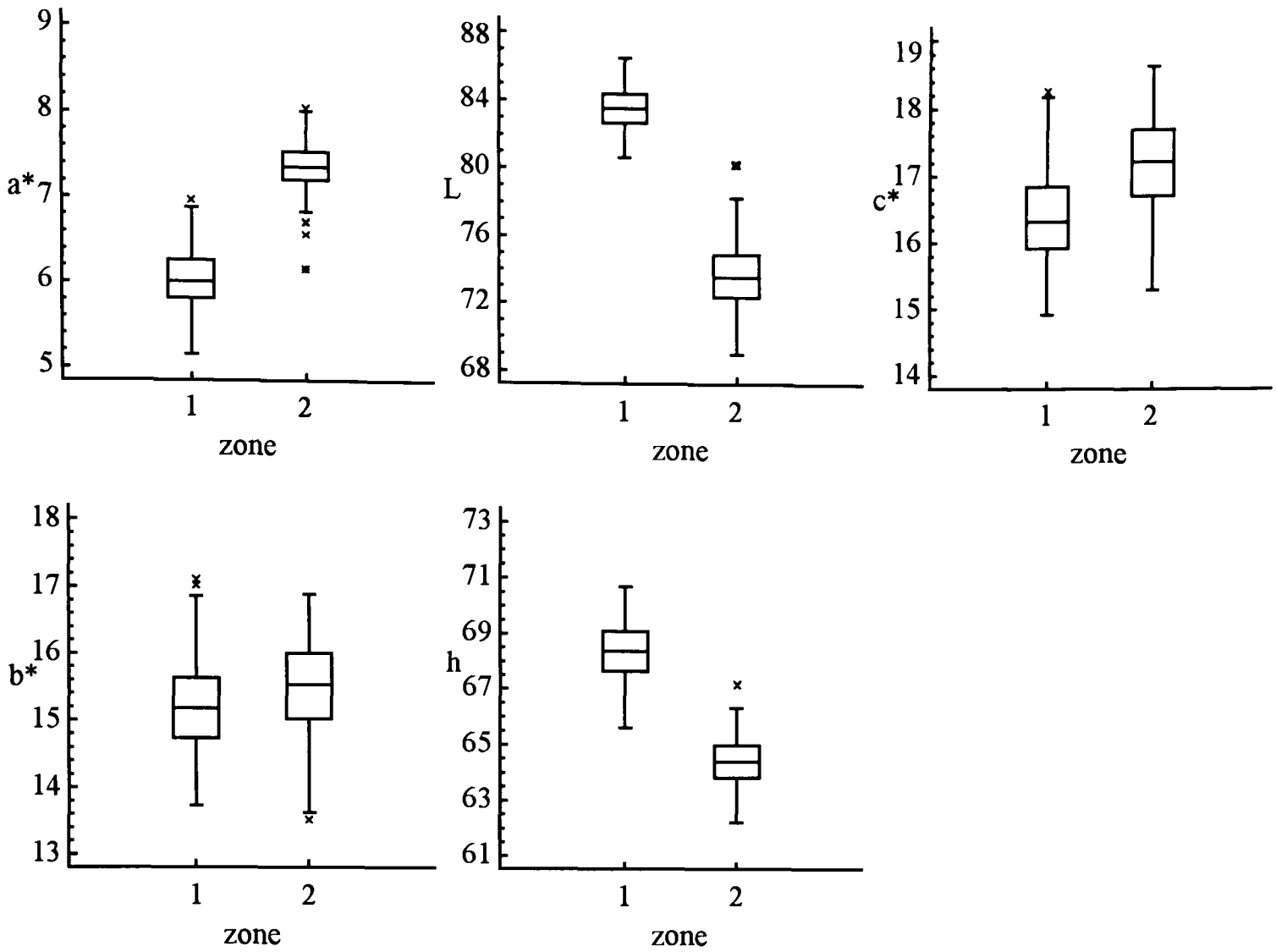


Figure 7.10.a-e: Box and whisker plots of tree means of sapwood (zone 1) and the outer heartwood (zone 2) for the colour parameters lightness (L), a*, b*, saturation c* and hue h. Each of the 160 tree means derives from 4 core means of 5 measurements of colour in each zone.

Table 7.9: Results of a nested analyses of variance of colour parameters of twenty families from five forest origins. Percentages of variance (%), F values (F) and probabilities of significant variation (P), are given for each level of the analyses.

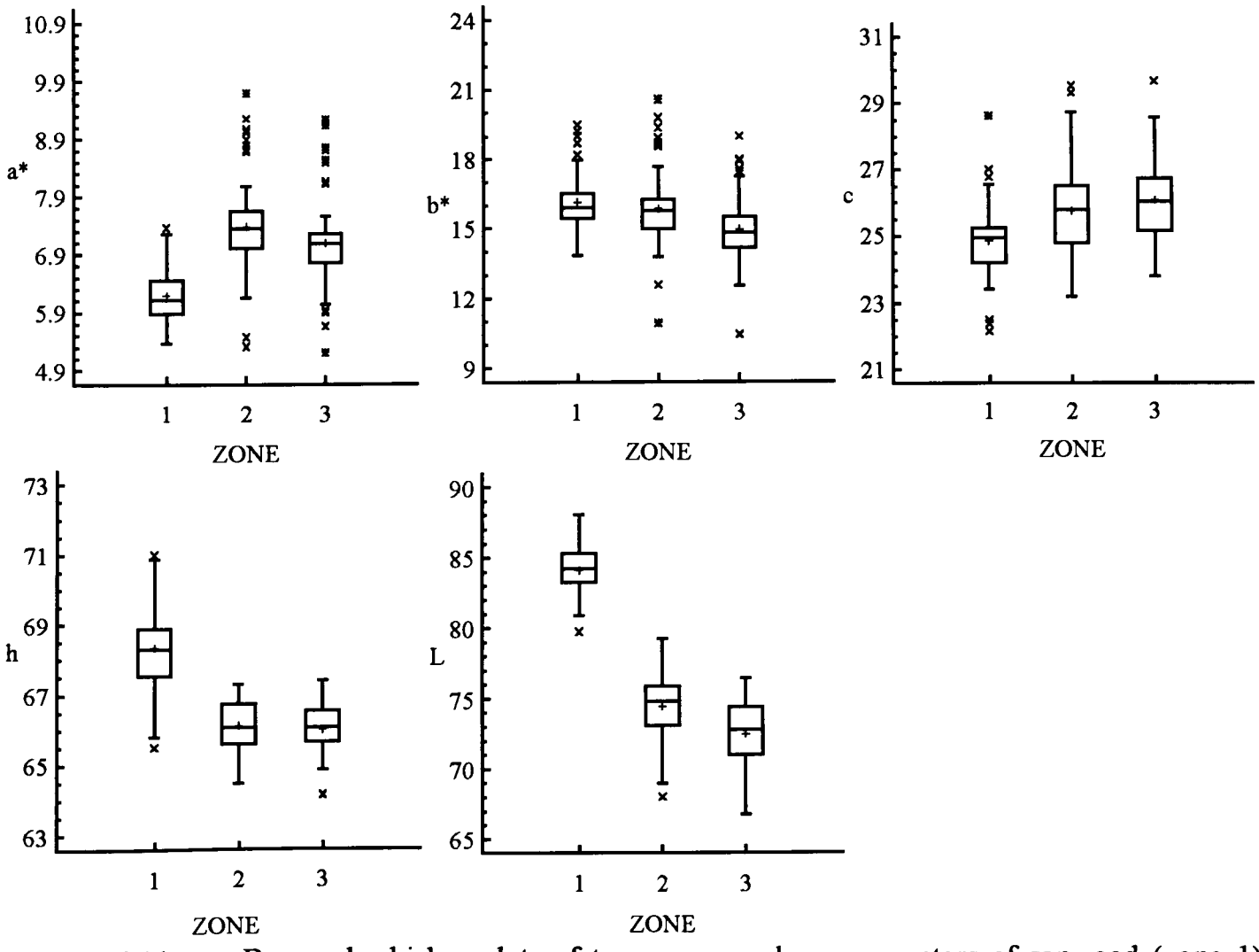
		Lightness			**a***			**b***			**Saturation**			**Hue**		
Sapwood	df	%	F	P	%	F	P	%	F	P	%	F	P	%	F	P
Forest	4	0	0.58	n.s.	7.4	2.60	n.s.	2.4	1.41	n.s.	1.6	1.29	n.s.	15.4	4.03	*
Family	15	12.8	3.57	***	14.6	4.65	***	18.9	6.43	***	18.3	6.36	***	15.6	4.33	***
Tree	140	30.0	3.98	***	21.9	3.16	***	15.4	2.24	***	14.3	2.10	***	32.2	6.97	***
Core	472	35.4	9.10	***	36.0	10.0	***	45.7	14.0	***	47.9	14.3	***	17.4	5.49	***
Within core	2533	21.8			20.1			17.8			18.0			19.4		
Heartwood	df	%	F	P	%	F	P	%	F	P	%	F	P	%	F	P
Forest	4	8.7	4.96	**	0.5	1.18	n.s.	4.6	1.87	n.s.	2.8	1.58	n.s.	12.9	4.00	*
Family	15	1.4	1.19	n.s.	5.2	1.87	*	16.2	4.37	***	14.5	3.96	***	10.9	2.76	**
Tree	140	52.2	8.52	***	36.7	4.20	***	28.8	3.99	***	28.7	3.74	***	44.3	8.94	***
Face	465	24.5	10.3	***	41.5	14.0	***	34.4	11.8	***	37.7	12.5	***	19.3	8.75	***
Within core	2504	13.2			16.1			16.0			16.4			12.6		

Unlike the ellagitannin results, there was no single family which could account for a large proportion of the variation between families. The differences between families of lightness, hue and saturation, were all above perceptible thresholds. However, the level of variation within families was very heterogeneous. Kolmogorov-Smirnov tests rejected the null hypothesis of the

normality of residual variance, after fitting analysis of variance models, for all heartwood parameters and for sapwood lightness. The two most common deviations from normality were either slightly skewed distributions or, more often, the presence of extreme outliers. The effect of outliers was examined by recalculating heritabilities after the elimination of those points lying outside the 99% and 1% extremes in the original data. It was frequently found that this led to lower estimates of heritabilities, although they were still generally greater than one. However, extreme tree mean values were due to numerous colour measurements of several cores, rather than a few anomalous readings. Therefore outliers are unlikely to represent experimental error and does not justify removing them from the data.

Clonal data

Samples taken in 1989 were darker and had higher a* and b* values than those of 1993. Analyses of variance found no significant differences between the inner and outer heartwood of the samples collected in 1989. However, significant differences for the majority of colour parameters were found using the 1993 samples. Therefore the two heartwood zones were examined separately in future analyses.



Figures 7.11.a-e: Box and whisker plots of tree means colour parameters of sapwood (zone 1) outer (zone 2) and inner heartwood (zone 3) of 27 clones from cores collected in 1993.

The variation of colour parameters between the three wood zones in the 1993 samples is shown in figure 7.11. The variation differs from that found in other studies (*eg* Klumpers 1994), notably by the decline of b* and a* from the outer to inner zones of heartwood.

The variances of mean tree values were analysed to determine broad-sense heritabilities, grouping clones across sites and species. Table 7.10 gives the results of these analyses.

Table 7.10: Broad-sense heritabilities of wood colour parameters in sapwood, outer and inner heartwood of clonal ramets. Clones include *Quercus robur* on one site and *Q. petraea* at two sites and sampled in two different years.

	37 ramets of 8 clones collected in 1989						125 ramets of 8 clones collected in 1993					
	Sapwood		Outer heartwood		Inner heartwood		Sapwood		Outer heartwood		Inner heartwood	
	h^2	*SE*	h^2	*SE*	h^2	*SE*	h^2	*SE*	h^2	*SE*	h^2	*SE*
Lightness	0.60	*0.27*	0.60	*0.27*	0.71	*0.22*	0.77	*0.13*	0.61	*0.19*	0.71	*0.16*
a*	0.56	*0.28*	0.38	*0.30*	0.66	*0.24*	0.03	*0.16*	0.64	*0.18*	0.67	*0.17*
b*	0.46	*0.30*	0.02	*0.19*	0.37	*0.30*	0.55	*0.20*	0.75	*0.14*	0.70	*0.16*
Saturation	0.41	*0.30*	0.07	*0.21*	0.44	*0.30*	0.50	*0.21*	0.73	*0.15*	0.69	*0.16*
Hue	0.76	*0.19*	0.66	*0.24*	0.52	*0.29*	0.69	*0.17*	0.19	*0.20*	0.60	*0.19*

The results fail to corroborate the variation found between families. While lightness was one of the parameters that varied the least between families and forests, here it is one of the most consistently and highly heritable of the parameters. The results show wide variation both between the two sampling times and between different wood zones. Samples collected in 1989 have generally lower and more erratic heritabilities. The difference in colour between the two data sets has suggested that the colour of the 1989 samples has probably changed during the intervening years of storage and so the 1993 estimates are considered more reliable. The mean core values of the 1993 samples were further analysed using a fully nested analyses of variance. The percentage variance components, for the sapwood and outer heartwood, are shown in table 7.11. Inner heartwood showed very similar variation to that of the outer heartwood. F-tests were not calculated due to the unbalanced design of the analyses.

Table 7.11: Percentage of the total variation of colour parameters explained by variation between species, clones, trees and within trees. Variance components estimated by a maximum restricted likelihood method using a fully nested anova design. 642-665 measurements of 60-64 ramets of 14 clones of *Quercus petraea* and *Quercus robur* collected from two sites in 1993.

	Sapwood				Outer heartwood			
	Species	Clones	Trees	Error	Species	Clones	Trees	Error
Lightness	7	14	18	61	0	18	42	40
a*	7	0	50	43	38	0	38	19
b*	17	9	46	28	38	9	32	21
Saturation	17	7	48	28	39	8	33	20
Hue	0	14	25	61	0	8	40	52

There are large differences between the parameters in the proportion of variance occurring between species. The highest between species variation is found for a*, b* and colour saturation values of heartwood, although surprisingly hue shows a very different pattern of variation. Figure 7.12 illustrates the difference between clonal ramets of *Q. petraea* and *Q .robur* and the correlation between a* and b* at tree level.

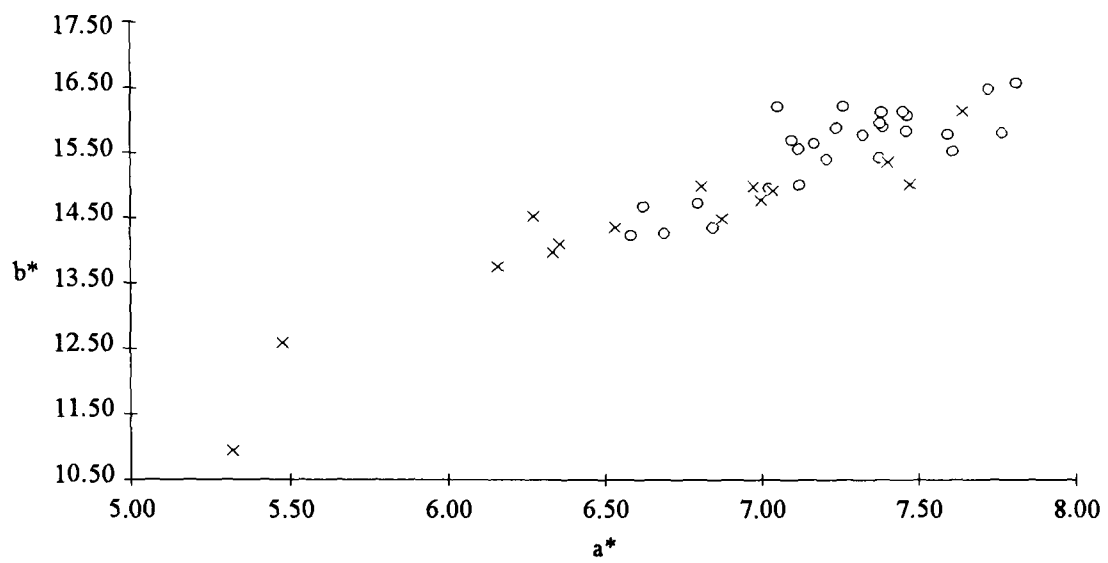


Figure 7.12: Tree means of colour parameters a* and b* of *Quercus petraea* (o) and *Quercus robur* (X) clones planted on adjacent seed orchards at Berkel.

A final comparison was made of the outer heartwood and sapwood of those clones that had been planted on the two different sites, by means of a two-way analysis of variance. Significant variation was found between sites and between clones within sites, for all the parameters except lightness and hue. Figure 7.13 illustrates the difference in tree means between the sites for parameters a* and b*.

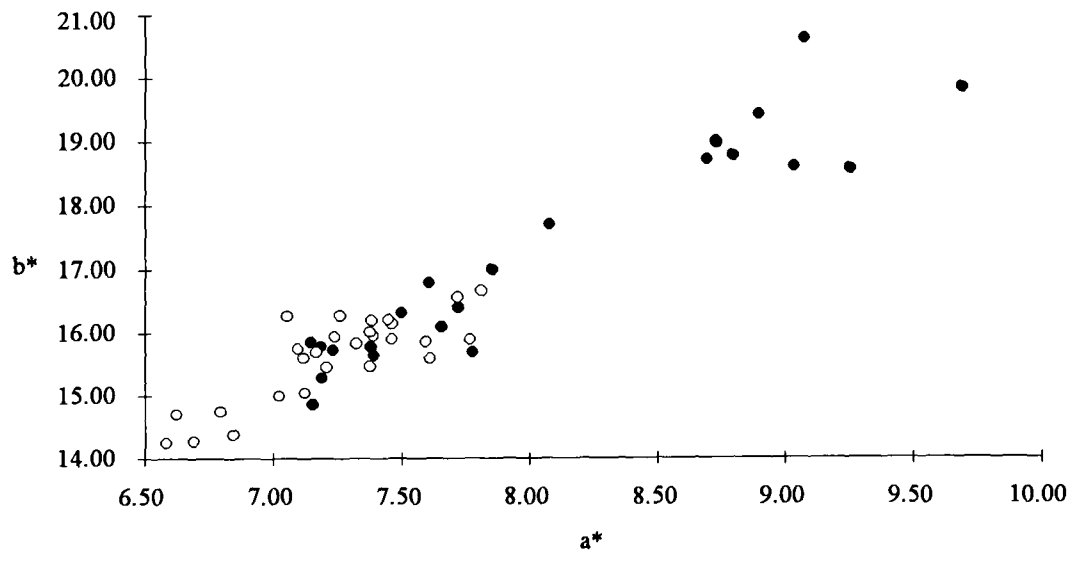


Figure 7.13: Mean tree colour parameters a* and b* of *Quercus petraea* clones planted at two sites: Berkel (o) and Lüss (•).

7.4.3. Microdensitometry

Progeny data

The number of rings comprising the sapwood varied between 4 and 15, with a mean of 9.2. Klumpers (1994) compared the last ten rings of sapwood with the first ten years of heartwood, of mature trees, and found that the latter was on average 40 kg/m^3 more dense. Any comparison between the zones is complicated by the comparison also representing different periods of growth, ring width and cambial age irrespective of heartwood formation. The tree mean values of sapwood and heartwood were initially compared by means of a Wilcoxon matched pairs analyses (see section 5.4.1) and the results for ring width, ring and latewood density and earlywood width are shown in table 7.12. Of those characteristics examined, latewood density differs the most between the two zones, as demonstrated by both the mean values and the non-parametric comparisons. However, in contrast to the findings of Klumpers (1994) the sapwood has a higher latewood and total ring density. This difference between the zones is probably due to the young age of the trees. Although wood density has been reported as declining with increasing cambial age (Zhang *et al.* 1993), the first 20-30 years of growth often show the reverse pattern.

Table 7.12: Comparison of sapwood and heartwood means from 158 trees from 20 half-sib families. Sapwood - heartwood differences are ranked by absolute value. The null hypothesis assumes an equal rank sum for positive and negative differences, the values given being the sums and number of trees comprising the lowest of these two categories.

	Sapwood		Heartwood		Wilcoxon non-parametric comparison		
	Mean	*SE*	Mean	*SE*	Rank sums	No. trees	P
Ring width (mm)	2.27	*0.05*	2.61	*0.05*	3327	53	0.001
Ring density (g/cm^3)	903	*3.64*	869	*3.51*	1302	27	0.001
Latewood density (g/cm^3)	970	*3.51*	918	*3.77*	419	17	0.001
Early wood width (mm)	0.46	*0.01*	0.46	*0.01*	6276	82	n.s.

Despite the differences between the sapwood and heartwood, the pattern of variation of wood properties was generally identical for both zones. Therefore narrow-sense heritabilities were calculated from the mean values for the period of growth 1981-1991, with no discrimination made between sapwood and heartwood. The estimates were all over one and of an even greater magnitude than had been observed previously. The results of a nested analysis of variance of tree mean values is shown in table 7.13. Variation between families and forests of some of the properties are shown in figures 7.14 and 7.15.

Table 7.13: Probabilities of significant variation, and variance components expressed as percentages, between forest origins and families within forests. Calculated from tree means of wood zones corresponding to growth between the years 1981-1991.

	Probability		% of total variation		
	Forests df=4	Families df=15	Forests df=4	Families df=15	Error df=140
Ring width	<0.001	0.003	45	9	47
Ring density	0.06	<0.001	16	29	55
Early wood width	<0.001	0.133	37	3	59
Early wood density	0.08	0.002	11	19	70
Late wood density	0.01	<0.001	29	25	46

From figure 7.14 it can be seen that a large part of the variation of wood density between families is accounted for by a single outlying family. However, this family is not the same as the outlying families for other properties such as ellagitannin concentrations and there are no grounds for excluding it. The proportion of variation between forest origins and families within forests varied between the different characters (see table 7.13). While the majority of variation in ring widths occurred between forests, a similar proportion of the total variation of density properties occurred between forests and between families within forests.

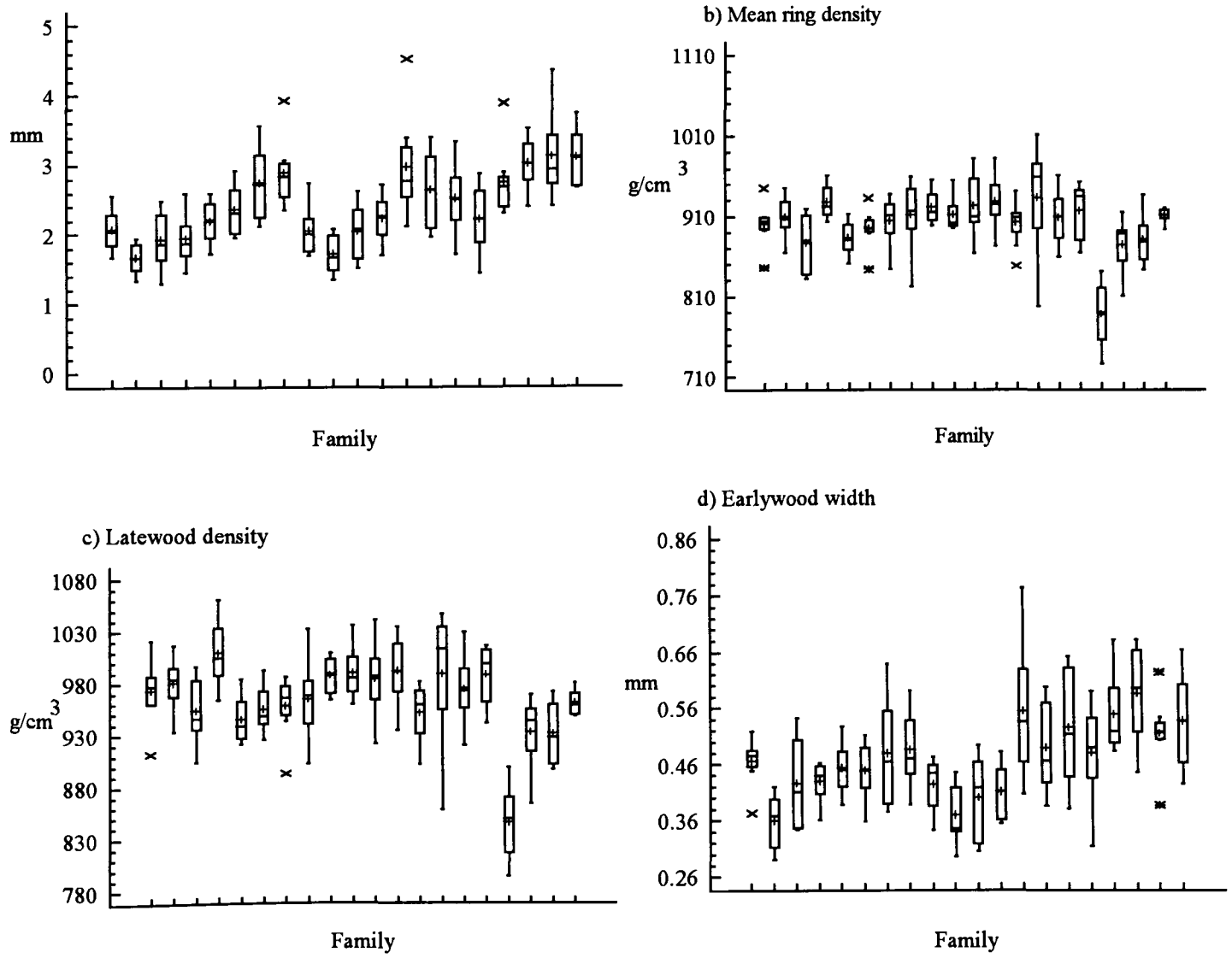


Figure 7.14: Variation of the mean tree values of wood properties between 20 half-sib families. Tree values calculated from annual ring means of wood zones corresponding to growth between the years 1981-1991. See figure 7.7 for an explanation of the plots.

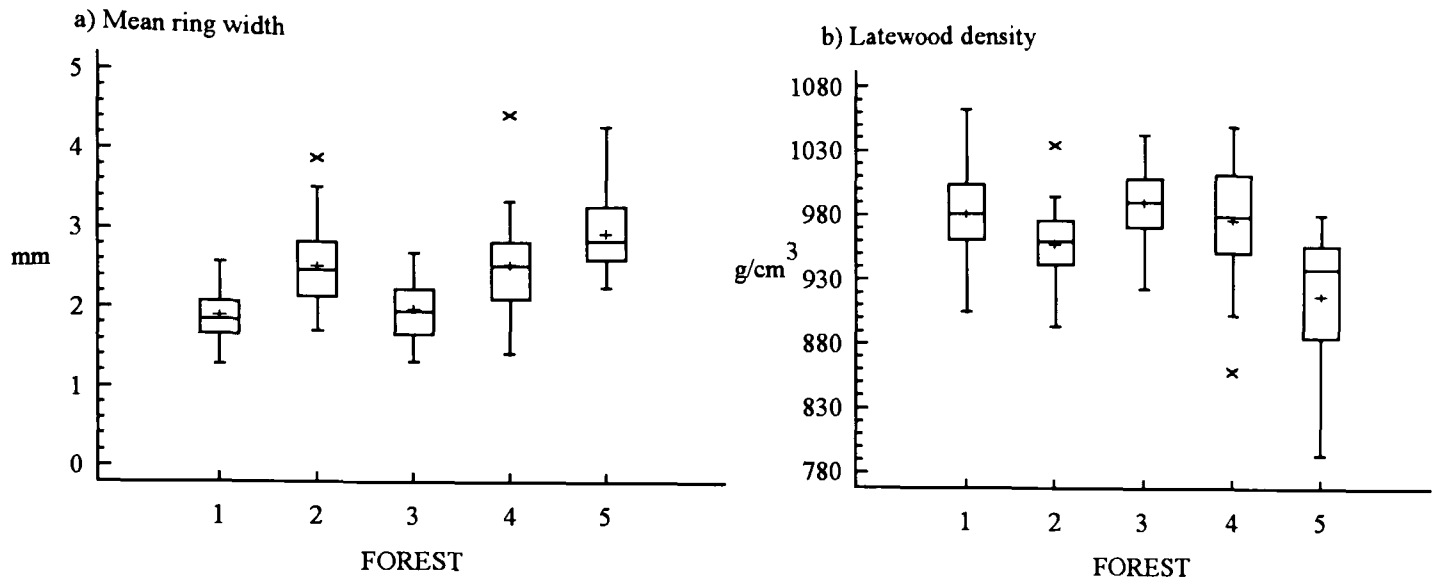


Figure 7.15: Variation of mean tree values of annual ring width and latewood density between five forest origins. Tree values calculated from tree means of wood zones corresponding to growth between the years 1981-1991. Figure 7.7 explains the plots.

Clonal data

Adequate replication was available for growth between the years 1972 and 1992 and these years of growth were therefore used for the calculation of core and tree means. Means of ring width and latewood density were also calculated for two ten year periods of growth (1972-1981 and 1982-1991) and compared by Spearman rank correlation coefficients. Each characteristic correlated strongly between the two periods of growth (0.70 for ring width and 0.78 for density) and neither character varied significantly between the two zones. These results suggest that there is little effect of sapwood, which one would expect to influence predominantly the later 10 years of growth. This conflicting result to that obtained from the progeny samples may be due to the approximately 20 year age difference between clonal ramets and family trees.

Broad-sense heritabilities for the years of growth 1972-1992 were calculated using ramet means and grouping by clones across species and sites. Table 7.14 gives the results.

Table 7.14: Broad-sense heritabilities from 118 ramets of 27 clones.

	Variance components		Heritability	
	Clones	Within clones	h^2	S.E
Ring width	0.862	1.182	0.76	*0.10*
Ring density	4036	1648	0.91	*0.04*
Early wood width	0.026	0.033	0.77	*0.09*
Early wood density	2905	2171	0.85	*0.07*
Late wood density	4797	1791	0.92	*0.04*

Ten clones of *Quercus petraea* for which there were results from each of the two sites were analysed by a two-way anova, the results being shown in table 7.15. Significant differences are found between the sites for most properties, particularly ring width which reflects the poorer growing conditions of the site at Lüss.

Table 7.15: Results of analyses of variance of clones of *Quercus petraea* growing at two different sites. Mean squares (MS), F values (F) and probabilities (P) are all approximate due to uneven replication. *** where P<0.001.

	Site effect (df=1)			Clone effect (df=9)			Clone x Site effect (df=9)			Error (df=42)
	MS	F	P	MS	F	P	MS	F	P	MS
Ring width	38.6	72.5	***	2.66	5.0	***	0.32	0.6	0.79	0.53
Ring density	2975	6.76	0.19	6900	4.09	***	4583	2.71	0.014	1689
Early wood width	0.703	97.7	***	0.053	3.6	0.002	0.028	1.9	0.08	0.015
Early wood density	16.78	10.7	0.002	4070	2.59	0.018	6759	4.30	***	1570
Late wood density	11636	5.84	0.02	7849	3.94	0.001	3979	2.0	0.06	1993

Clones of the two species growing at Berkel were then compared by a simple nested anova of tree mean values. The results (see table 7.16) show significant variation between the two species for all characteristics, particularly wood density.

Table 7.16: Analyses of variance comparing tree means of clones of *Quercus petraea* and *Quercus robur* planted in adjacent seed orchards on the same site. *** where P<0.001. All tests are approximate due to uneven replication.

	Species (df=1)			Clone effect (df=25)			Error (df=60)
	MS	F	P	MS	F	P	MS
Ring width	5.07	6.96	0.01	2.73	3.74	***	0.728
Ring density	127611	96.0	***	10024	7.54	***	1329
Early wood width	0.231	11.51	0.001	0.089	4.48	***	0.02
Early wood density	83899	51.2	***	8076	4.93	***	1637
Late wood density	141088	96.5	***	10855	7.42	***	1462

Mean ring width and latewood density were further compared by calculating variance components using mean core values. The percentage of total variation, explained by each level, is given in table 7.17. This clearly shows that the pattern of variation is very different for ring width and latewood density, the latter showing less within-tree variation but much higher variation between species.

Table 7.17: Percentage of total variation between 148 core means explained by variation between 2 species, 26 clones and 81 trees, estimated by a restricted maximum likelihood method.

	Species	Clone	Tree	Cores
Ring width	1.7	41.0	20.8	36.5
Latewood density	43.9	33.5	13.2	9.4

From these analyses it appears that ring width is most affected by site with less variation between clones and species than for ring and latewood densities, which in turn vary less between the two sites. This supports the higher heritabilities estimated for density properties than ring width. Figure 7.16 illustrates how ring width and latewood density discriminate between clones, species and site.

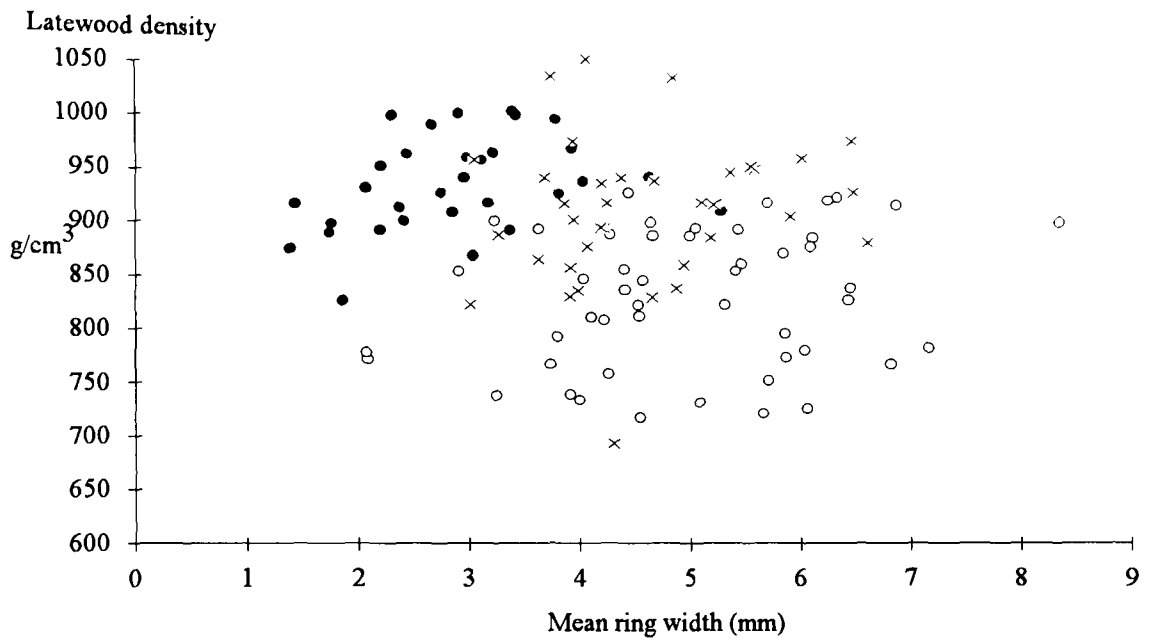


Figure 7.16: Latewood density vs ring width of clonal ramets of two species planted at two sites: *Q. robur* at Berkel (x); *Q. petraea* at Berkel (o) and at Lüss (•).

Sapwood width

Analyses of both the mean tree sapwood width and the number of sapwood rings were carried out using the progeny data. The genotypic component of sapwood width variation was estimated as 0.4, but no significant variation between families was detected for the number of sapwood rings. The variation in sapwood width can be attributed to the variation in mean ring width which is of a similar magnitude. However, the lack of any significant variation between families in the number of sapwood rings conflicts with the findings of Savill *et al.* (1993), which estimated narrow-sense heritability as 0.57. This study examined variation between 192 trees from 26 families of *Quercus robur* growing at an adjacent family plot to that examined in this study. The results of Savill *et al.* (1993) also estimated broad-sense heritability as being 0.83 from examining the same clonal samples studied here, that were collected by Mather in 1989. It is possible that the lack of any family effect may be due to the indirect method of measuring the number of sapwood rings. Alternatively it could be due to the different families and species examined in this study.

Correlations among properties

Tree mean values for the different properties were correlated for the progeny samples and the clonal samples collected in 1993. Only colour parameters of the outer heartwood and sapwood were compared. Spearman rank correlation coefficients are given in table 7.18. Concentrations of vescalagin and castalagin were negatively correlated with late wood density in both the clonal and progeny samples. However, significant positive correlations were found between the ellagitannins and ring width for both clonal and progeny data. Figure 7.17 shows this correlation among progeny trees, illustrating the wide degree of scatter. This pattern is carried over into the mean family values, as demonstrated in figure 7.18. The way the two characters discriminate between

species and sites is illustrated in figure 7.19, but this also indicates that there is little correlation between the two characters within species or sites.

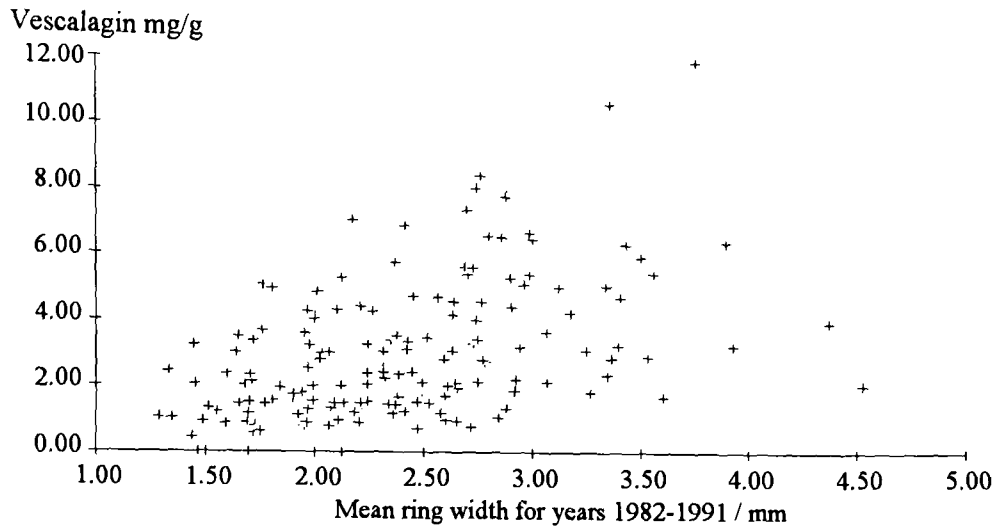


Figure 7.17: Concentrations of vescalagin vs ring width of 157 trees from 20 half sib families.

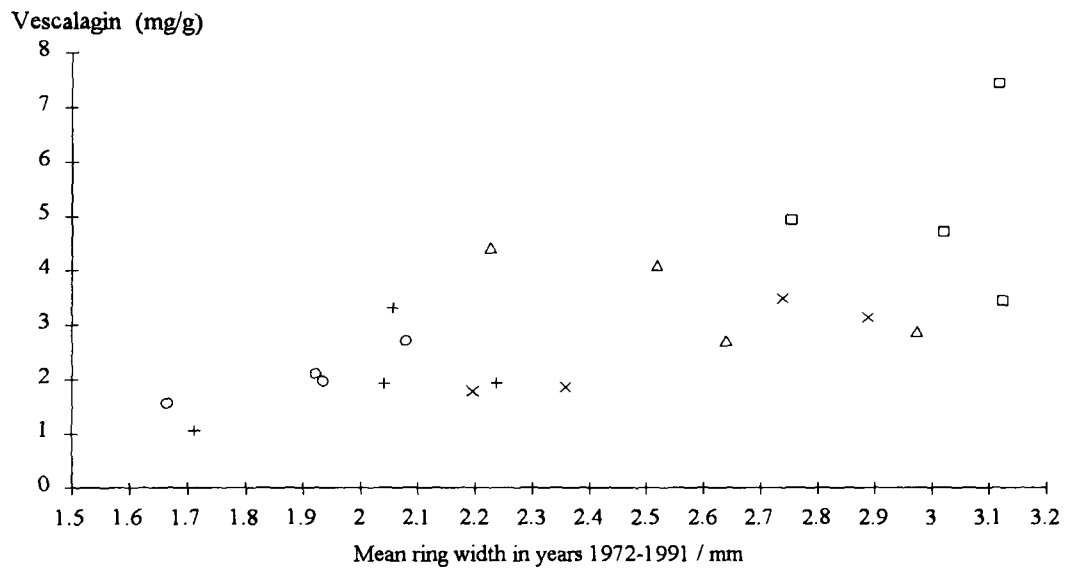


Figure 7.18: Mean levels of vescalagin vs ring width in 20 half sib families. Different symbols represent families from different forest origins. o: Rohrbrunn; x Johanniskreuz; Δ: Waldleiningen; +: Göhrde; □ Escherode.

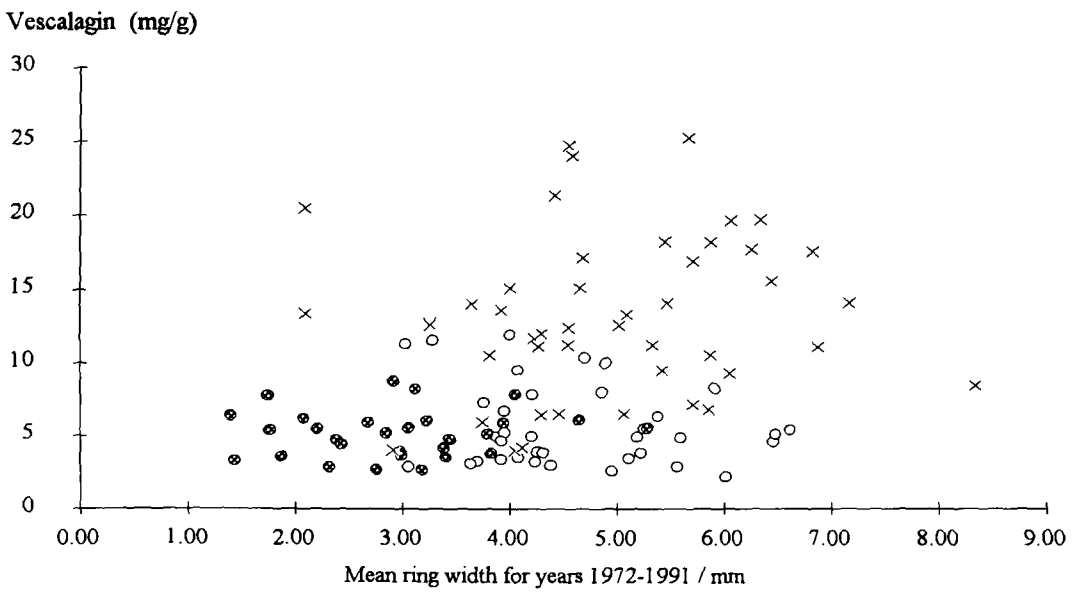


Figure 7.19: Vescalagin vs ring width of clonal ramets of two species planted at two sites: *Q. robur* at Berkel (x); *Q. petraea* at Berkel (o) and at Lüss (•).

Correlations with colour variables show contradictory results between the clonal and progeny samples. Positive correlations between tannin concentrations and many heartwood and sapwood colour parameters were found among progeny trees. Yet negative correlations among many of the same properties and of similar magnitude were found for the clonal data. Additional correlations of interest were those between many colour parameters and ring width, suggesting that the latter may influence wood colour.

It is often assumed that wide ring widths correlate with high ring density, as the proportion of low density earlywood generally falls with increasing ring width. Therefore latewood density has been considered a better estimate of wood density, being independent of ring width. However, contrary to expectations, either no or negative correlations were found between ring width and both ring and latweood density.

Table 7.18: Spearman rank correlation coefficients between properties in progeny (upper right) and 1993 clonal (lower left) samples.

name	V	C	Ring width	Ring density	EW width	EW density	LW density	SW L	SW a*	SW b*	SW c*	SW hue	HW L	HW a*	HW b*	HW c*	HW hue
Vescalagin		0.98	0.46	-0.05	0.38	0.09	-0.19	-0.21	0.02	0.10	0.09	0.06	0.30	0.04	0.28	0.25	0.30
Castalagin	0.90		0.43	0.01	0.35	0.14	-0.12	-0.24	0.07	0.11	0.11	0.01	0.21	0.05	0.23	0.21	0.23
Ring width	0.33	0.25		-0.05	0.82	0.09	-0.29	-0.23	0.04	0.31	0.28	0.19	0.15	0.03	0.26	0.23	0.22
Ring density	-0.50	-0.33	-0.12		-0.18	0.78	0.91	-0.33	0.28	0.06	0.11	-0.28	-0.13	0.07	0.01	0.02	-0.09
Early wood width	0.34	0.27	0.85	-0.11		-0.04	-0.27	-0.13	0.07	0.18	0.17	0.08	0.11	0.02	0.15	0.13	0.10
Early wood density	-0.45	-0.27	-0.23	0.89	-0.15		0.59	-0.28	0.24	0.13	0.17	-0.17	-0.16	0.05	0.00	0.01	-0.07
Late wood density	-0.52	-0.34	-0.25	0.98	-0.19	0.85		-0.26	0.34	-0.02	0.05	-0.39	-0.17	0.08	-0.08	-0.05	-0.18
Sapwood Lightness	-0.14	-0.05	-0.28	0.14	-0.33	-0.01	0.20		-0.47	-0.01	-0.11	0.49	-0.02	-0.04	-0.06	-0.05	0.01
Sapwood a*	-0.10	-0.03	-0.49	0.13	-0.43	0.29	0.23	-0.08		0.39	0.54	-0.74	-0.24	0.30	-0.06	0.00	-0.40
Sapwood b*	-0.34	-0.27	-0.40	0.31	-0.42	0.37	0.36	0.30	0.77		0.98	0.27	-0.03	0.24	0.32	0.32	0.10
Sapwood saturation	-0.31	-0.24	-0.42	0.29	-0.43	0.38	0.36	0.24	0.82	0.99		0.10	-0.09	0.28	0.27	0.29	0.01
Sapwood hue	-0.32	-0.30	0.13	0.32	0.00	0.15	0.27	0.62	-0.30	0.29	0.20		0.22	-0.15	0.27	0.21	0.48
Heartwood Lightness	-0.02	0.00	0.21	-0.31	0.16	-0.41	-0.33	0.12	-0.43	-0.44	-0.46	-0.13		0.05	0.60	0.52	0.72
Heartwood a*	-0.26	-0.12	-0.43	0.19	-0.40	0.28	0.28	0.43	0.46	0.56	0.54	0.28	-0.04		0.60	0.72	-0.27
Heartwood b*	-0.36	-0.23	-0.42	0.25	-0.42	0.26	0.34	0.52	0.36	0.56	0.53	0.39	0.11	0.90		0.99	0.54
Heartwood saturation	-0.34	-0.21	-0.43	0.24	-0.43	0.25	0.33	0.52	0.37	0.56	0.53	0.38	0.10	0.93	0.99		0.41
Heartwood hue	-0.11	-0.21	0.10	-0.03	0.04	-0.20	-0.04	0.01	-0.32	-0.24	-0.25	0.06	0.40	-0.37	-0.02	-0.09	

Future analyses

The analyses of microdensitometry results, although suitable for comparison with tannin and colour properties, do not take full advantage of the data being available on a ring by ring scale. Future analyses, to be described in Mosedale *et al.* (*in preparation*), will incorporate the year of growth as an additional parameter of analysis of variance models. Additionally, it is intended to determine whether there is a significant genotypic component in the residual variation of models that have been developed to estimate wood density, such as those described in Zhang *et al* (1994).

7.5. Discussion

Table 7.19 summarises the variation found between different clones and families and estimates of the broad-sense heritabilities and the proportion of variation between progeny which was attributed to variation between families. Estimates of the heritability of oak wood properties, from previous studies, are shown in table 7.20.

Table 7.19: Mean, minimum and maximum family and clone averages of tree means for different properties. The proportion of variation (G) between tree means explained by variation between families and the broad-sense heritabilities (h^2) are also given. Minimum and maximum clone means were calculated only from those samples collected in 1993 at Berkel. Genetic components were estimated by grouping all samples from both sites. Only values for sapwood and outer heartwood are given.

Wood zone and property	Half-sib families						Clones					
	No. of Families	No. of Trees	Min	Max	Mean	G	No. of Clones	No. of Trees	Min	Max	Mean	h^2
Vescalagin (mg/g)	20	157	1.06	7.52	3.09	**0.43**	26	98	3.58	25.45	10.82	**0.95**
Castalagin (mg/g)	20	157	1.12	4.76	2.23	**0.39**	26	98	3.41	15.08	8.03	**0.87**
Sapwood Lightness	20	160	82.19	85.04	83.47	**0.21**	23	62	81.39	86.07	84.12	**0.77**
Sapwood a*	20	160	5.58	6.46	6.04	**0.39**	23	62	5.50	7.09	6.18	**0.03**
Sapwood b*	20	160	14.61	16.18	15.22	**0.43**	23	62	13.90	18.74	16.11	**0.55**
Sapwood Saturation	20	160	15.81	17.42	16.38	**0.41**	23	62	14.95	20.03	17.26	**0.50**
Sapwood Hue	20	160	66.70	69.77	68.35	**0.43**	23	62	67.66	70.15	68.98	**0.69**
Heartwood Lightness	20	158	71.20	76.12	73.61	**0.23**	23	64	70.22	78.12	74.46	**0.61**
Heartwood a*	20	158	7.14	7.75	7.40	**0.18**	23	64	6.30	9.25	7.42	**0.64**
Heartwood b*	20	158	14.59	16.38	15.47	**0.34**	23	64	13.89	19.53	15.94	**0.75**
Heartwood Saturation	20	158	16.27	18.05	17.15	**0.29**	23	64	15.38	21.51	17.58	**0.73**
Heartwood Hue	20	158	63.45	65.89	64.42	**0.44**	23	64	64.14	66.19	65.05	**0.19**
Ring width (mm)	20	160	1.66	3.12	2.41	**0.50**	26	87	2.92	6.46	4.91	**0.76**
Ring density (g/cm^3)	20	160	787	934	900	**0.44**	26	87	677	924	793	**0.91**
Early wood width (mm)	20	160	0.35	0.50	0.47	**0.37**	26	87	0.65	1.39	1.01	**0.77**
Early wood density (g/cm^3)	20	160	590	723	677	**0.28**	26	87	480	680	573	**0.85**
Late wood density (g/cm^3)	20	160	846	1011	964	**0.51**	26	87	735	1009	859	**0.92**

Abnormally high narrow-sense heritabilities were estimated for nearly all the properties, including ring width which is normally one of the least heritable traits (see table 7.20). These results may be explained partly by a large degree of environmental variation being included in the estimate of genotypic variation, due to the unreplicated experimental design. Alternatively, the parent trees may be more closely related than half-sibs due to the open pollination method of propagation or to the initial selection of parent trees. As family plots from the same forest origins were generally adjacent to one another, the frequently significant variation of many properties between forests may also be due to either of these explanations. The low and, particularly for the clonal studies, uneven replication must also be acknowledged and may explain the lack of harmony between results from clonal and progeny trials. The possibility of root stock effects on the clonal trial also remains unconsidered. Despite these drawbacks, a number of inferences, including the relative importance of genetic variation, can be made from the results.

The results emphasise the high degree of variation in wood properties found between individual trees, even when grown under very controlled and constant conditions. Many of these properties appeared to be under strong genetic control. Wood density properties and ellagitannin concentrations gave particularly high estimates of heritability and discriminated strongly between clones of the two species (see figures 7.16 and 7.19). However, it is difficult to assess the reliability of this difference from the few genetic types represented by the clonal study. The variation between species largely explains the negative correlations between wood density and tannin concentrations. The lack of any positive correlations between density and ring width agrees with recent studies such as that of Ackermann (1994) who found that oak wood of the lowest density often had the widest annual rings. This was explained by the latewood, in more vigorously growing trees, often developing many small vessels and paratracheal parenchyma which lowers the density.

Table 7.20: Heritabilities of wood characteristics of *Quercus robur* and *Quercus petraea* reported in the literature.

Wood characteristic	Type of estimate	Heritability	Species studied	Reference
No. sapwood rings	Broad sense	0.83	*Q. petraea* and *Q. robur*	Savill *et al.* (1993)
	Narrow sense	0.57	*Q. petraea* and *Q. robur.*	
Early wood vessel area	Broad sense	0.87-0.93	*Q. petraea* and *Q. robur*	Kanowski *et al.* (1991)
	Narrow sense	0.60	*Q. petraea* and *Q.robur*	and Mather *et al.* (1993)
Ring width	Broad sense	0.247; 0.212	*Q.petraea; Q.robur*	Nepveu (1984b)
Infradensity	Broad sense	0.56; 0.59	*Q.petraea; Q.robur*	Nepveu (1984b)
Axial shrinkage	Broad sense	0.00; 0.24	*Q.petraea; Q.robur*	Nepveu (1984b)
Tangential shrinkage	Broad sense	0.32; 0.30	*Q.petraea; Q.robur*	Nepveu (1984b)
Radial shrinkage	Broad sense	0.20; 0.12	*Q.petraea; Q.robur*	Nepveu (1984b)
Volumetric shinkage	Broad sense	0.29; 0.28	*Q.petraea; Q.robur*	Nepveu (1984b)
Early wood width	Broad sense	0.62	*Quercus robur*	Nepveu (1984a)
Late wood width	Broad sense	0.27	*Quercus robur*	Nepveu (1984a)
% vessels in early wood	Broad sense	0.43	*Quercus robur*	Nepveu (1984a)
% fibres in late wood	Broad sense	0.27	*Quercus robur*	Nepveu (1984a)

Interpretation of the variation in wood colour is difficult, with many parameters varying as much, or more, between families as between clones. Correlations with other properties are also difficult to interpret as they rarely correspond between the clonal and family sample sets. The heartwood colour parameter b* correlated weakly with clone tannin concentrations, but negatively rather than the significant positive correlation found in chapter 5. Variation is further complicated by factors, such as wood storage after sampling, that appear to influence colour. However, unlike Rink (1987), who, from a study of black walnut, concluded that there was no indication of genetic control over any heartwood colour variable, there are such indicators for oak wood colour even if the method by which such control expresses itself is uncertain. Colour parameters may be influenced by a variety of wood properties, such as ring width, although such relationships are generally weak and complicated, as noted by Klumpers (1994). A more constructive approach to that used here may be to obtain a better understanding of the influence of anatomical and

structural properties on the measurement of wood colour. The degree of genetic control could then be estimated indirectly rather than through the direct measurement of colour.

The equal or higher sapwood than heartwood densities, in contrast to the findings of other studies such as Klumpers (1994), are probably explained by the young age of the trees studied. The low level of ellagitannins and their limited composition in many progeny samples, may also be due to their age. This is an alternative explanation of the low tannin concentrations found in the central heartwood of mature trees. Previous studies, such as Viriot *et al.* (1994), have explained such observations solely by the hydrolysis or insolubilization of tannins that occurs during heartwood ageing. It has been assumed that tannin content is independent of tree age but no studies have examined variation between different tree age classes. Generally only monomeric ellagitannins were found in most progeny samples, although dimeric tannins might form during the later ageing of heartwood.

The composition of heartwood ellagitannins (represented by the vescalagin to castalagin ratio) varied significantly between families and clones. Although this variation may relate to genetic differences, an alternative explanation is that the rate of growth is the main influence upon the composition and concentration of tannins in the wood of such young trees. Correlations between ring width and ellagitannin concentrations, among the progeny, were strongest at the lower range of values for both characters. There is little indication of a similar correlation among clonal ramets, which generally displayed wider annual rings. This suggests that ring width may be an indicator of tannin levels at only very slow rates of growth or in only very young trees.

It is difficult to compare in detail the present results with those in table 7.20. Although the genetic material of all these studies derive from the same or similar genetic trials, the sampling strategy and replication varied widely. Many of the previous studies simply estimated between clone variation, irrespective of sites or species, without further comparisons. The limited origins of the material and the experimental design limitations made apparent in this study emphasise the need for alternative samples of known seed origins. Present estimations of the heritability of properties can only be considered as crude relative indicators of genetic control. Lacking any alternative material for the near future, the interpretation of present results would be most facilitated by estimates of the genetic similarity, by molecular and biochemical markers, of the parent trees, clones and species. These techniques have already been developed and used (*eg* Bacileri *et al.* 1993; Ducousso *et al.* 1993; Petit *et al.* 1993) in estimating the genetic diversity of populations of *Q. robur* and *Q. petraea*.

7.6. Conclusions

The results give valuable information on the feasibility of selecting oak for extractive and other properties. The high level of variation in the concentration of heartwood tannins, as illustrated in

the maximum and minimum clone means in table 7.19, confirms earlier findings, while better controlling variation due to heartwood age. The strong genetic control of tannin concentrations suggests that the identification of high or low tannin trees may best be achieved by examining covarying characteristics rather than by comparing different forest and environmental conditions. The apparent species difference gives support to this conjecture and one of the aims of work reported in chapter 8 was to determine if this is a reliable indicator of tannin levels in more wide ranging and variable material than that studied here. Correlations between ring width and tannin concentrations also suggests the need for further examination to determine if this is due to the young age of the trees studied. The contrary correlations with wood colour, to those observed in chapter 5, suggest that colour is not a good indicator of tannin content. Colour also appears to be influenced by other factors such as the duration of wood storage. It is possible that wood density may influence the permeability of casks and, although not considered in this thesis, the high degree of genetic control indicated in this study may prove of future interest.

7.7. Acknowledgements

The research described in this chapter was carried out with funding from the EEC AIR Program on the 'Production of quality wood from broadleaves'. Samples were collected with the permission and assistance of Jochim Kleinschmit and his colleagues at the Niedersächsische Forstliche Versuchsanstalt, Escherode, Germany. Bertrand Charrier was responsible for initiating the work, the collection of samples in 1993 and a significant proportion of the measurements with the assistance of Gérard Janin and Claude Houssement. Material collected in 1989 by Dr Richard Mather of the Oxford Forestry Institute was also used. The majority of the work was carried out at the INRA Wood Quality Research Team at the Center of Research at Nancy and at the Ecole Supérieure du Bois, Nantes.

Chapter 8: Variation of heartwood tannins and oak lactones between the species and phenological types of *Quercus petraea* and *Q. robur*

The best cognac is given by solitary oaks, which grow in quiet places, on dry ground. Such oaks have basked in the sun. There is as much sun in them as there is honey in a honeycomb.

R. Kapuscinski - *Imperium*

8.1. Chapter summary

The levels of total phenolics in the outer heartwood was measured for trees of the two species *Quercus petraea* and *Q. robur*. Significant differences were found between the two species in material deriving from German provenance trials and from mixed plantations sampled in the UK. Concentrations of oak lactone also varied significantly between the species. However the difference in total phenolics between species was much lower at the mixed plantations than was found for the provenance trial. A weak but nonetheless significant positive correlation between ring width and total phenolics was also detected for both trials. A comparison between late and early flushing types of *Quercus petraea* found no significant difference between these phenological types.

8.2. Introduction

Chapters 5 and 6 described significant variation between two forests in the concentrations of ellagitannins and other extractives. The dominant species differed between these two forests. In chapter 7 it was found that the majority of the significant variation between clones of *Q. robur* and *Q. petraea* could be attributed to variation between the two species. However this variation also coincided with variation between seed orchards and furthermore the range of genetic variation was limited by the number of clones sampled. In both studies the concentration of soluble tannins extracted from *Q. robur* trees was significantly greater than that from *Q. petraea*. The examination of more diverse material was thought necessary to indicate the reliability of this apparent difference between species.

Certain wood properties have also been found to coincide with phenological types of the two species. Savill and Mather (1990) reported a correlation between flushing time and the size of early wood vessels. The identification of any easily observable phenological characteristics which correlate with extractive properties could be of value in selecting and predicting the effects of oak on whisky maturation.

Therefore, the principle aim of this study was to examine the variability in heartwood ellagitannin concentrations of *Quercus petraea* and *Q. robur*, of varying seed origins grown on the same and at different sites. It was also desired to investigate the variation of other oak extractives and for

this purpose oak lactone was chosen as it had been found to vary widely between trees and forests, as reported in earlier chapters.

8.3. Materials and methods

The experimental material used in this study consisted of 5 mm diameter cores, extracted with Pressler borers from oak trees. Four main aspects of variation in tannin levels were investigated:

- Variation between provenances, planted in unreplicated experimental designs on two sites in Germany. These trials were established by Krahl Urban in 1950 in the Bramwald Forest adjacent to the progeny trial described in chapter 7. All sampled provenances derived from stand collections of seed to represent overall provenance traits rather than single tree collections. In 1951 provenance seedlings were also transplanted to a site at Syke, near Bremen. Cores from trees of these provenances had been collected in 1989 for other investigations (described by Mather 1991). For the purposes of this investigation, the same three provenances of each species growing on each site, as shown in Table 8.1, were analysed.

- Variation in mixed plantations of the two oak species which were not specifically established for experimental purposes. For this, a single core was taken in August 1994 at about 30 cm from the ground from each of 12 trees of *Quercus petraea*, and 12 of *Quercus robur* which were growing in close proximity to each other in three woodlands: Bagley Wood, near Oxford; the Forest of Dean, Gloucestershire, and the Eling Estate, near Newbury in Berkshire. In each of the first two woodlands, the 12 trees of each species were taken from two different sites, six from each. All the trees sampled were 90-100 years or more old, and their provenances were unknown, though they were unlikely to be native to the sites where they were growing.

- Variation between trees within a population of *Quercus petraea* which were distinguishable because of their early or late flushing habits. These all came from a stand of locally native provenance in Bagley Wood, near Oxford, which was of coppice origin, and last coppiced about 1900. Samples were taken from paired neighbouring late and early flushing trees of similar form. The cores were collected in 1989 for work described by Savill and Mather (1990).

- Since earlier results suggested that the tannin content might be influenced by the width of annual rings, the widths of the ten outermost rings of heartwood (the part where tannins were assessed) were measured to the nearest micrometre (μm) and averaged for each sample for all the material used.

Table 8.1: Summary of the number of cores and details of experimental material and sampling regimes.

Site and planting year	Species	Seed origin	Number of sample trees	Mean ring width (mm)
Collections from mixed plantations				
Bagley Wood Cpt 16a (1907)	*Q. petraea*	Unknown	6	1.7
	Q. robur	Unknown	6	2.4
Bagley Wood Cpt 16b (1908)	*Q. petraea*	Unknown	6	1.5
	Q. robur	Unknown	6	2.7
Forest of Dean Cpt 446c (1909)	*Q. petraea*	Unknown	6	3.6
	Q. robur	Unknown	6	4.3
Forest of Dean Cpt 402c (1905)	*Q. petraea*	Unknown	6	3.4
	Q. robur	Unknown	6	3.0
Eling Estate Cpt 22 (c.1870)	*Q. petraea*	Unknown	12	1.3
	Q. robur	Unknown	12	1.5
Provenance experiments				
Bramwald (1950)	*Q. petraea*	68 Selzerthurm	12	1.9
		85 Zell/Mosel	12	2.0
		92 Johanniskreuz X/5D	12	2.6
	Q. robur	29 Gifhorn 26A	12	1.9
		39 Ütze 54C	10	1.7
		76 Nordkirchen	12	2.5
Syke (1951)	*Q. petraea*	68 Selzerthurm	12	1.9
		85 Zell/Mosel	11	1.9
		92 Johanniskreuz X/5D	12	2.1
	Q. robur	29 Gifhorn 26A	9	2.3
		39 Ütze 54C	6	2.0
		76 Nordkirchen	6	2.4
Early and late flushing trees				
Bagley Wood Cpt 37a (1900)	*Q. petraea*	Bagley Wood early flushing trees	12	2.8
		Bagley Wood late flushing trees	12	2.9

For the chemical analyses of tannins, only the ten most recently formed heartwood rings were used. Previous studies have shown how the level of soluble tannins declines with heartwood age. These ten rings were separated from the core and ground into particles. 50 mg were then extracted with 5 ml of a 63% ethanol-water solution over 24 hrs and filtered. The Folin Denis method (see section 2.4) was used to measure the concentration of total phenolics in the extract solution. Each sample was measured in triplicate and the mean results expressed as gallic acid equivalents. Earlier studies (described in chapter 5) had supported the conclusions of Puech *et al.* (1990) that the Folin Denis method was an accurate measurement of total tannins in oak wood. The concentration of oak lactones was measured (using the method in section 2.5) in the extractions of a subset of the provenance samples, comprising trees of two provenances of each species growing on each site.

8.4. Results

8.4.1. Variation in soluble tannins

Provenance material

Means for each provenance, site and species were calculated and are shown in Table 8.2.

Table 8.2: Mean total phenolics (GAE mg/g) and standard errors (*S.E.*) in provenance experiments.

Species	Bramwald		Syke		Both sites	
	Mean	*S.E.*	Mean	*S.E.*	Mean	*S.E*
Quercus petraea						
68 Selzerthurm	8.24	*0.12*	7.52	*0.18*	7.88	*0.37*
85 Zell/Mosel	6.38	*0.13*	7.14	*0.15*	6.74	*0.34*
92 Johanniskreuz X/5D	5.92	*0.18*	5.31	*0.12*	5.61	*0.37*
Mean	**6.85**	***0.33***	**6.64**	***0.34***	**6.75**	***0.23***
Quercus robur						
29 Gifhorn 26A	8.71	*0.18*	9.77	*0.23*	9.19	*0.45*
39 Ütze 54C	8.58	*0.20*	7.52	*0.28*	8.15	*0.44*
76 Nordkirchen	8.35	*0.16*	10.81	*0.15*	9.17	*0.47*
Mean	**8.54**	***0.32***	**9.42**	***0.46***	**8.89**	***0.30***
Site mean	7.64	*0.25*	7.68	*0.32*	7.66	*0.20*

Analysis of variance was used to determine the levels at which variation was significant using the following model:

$$T_{ijkl} = \mu + \alpha_i^{Spp} + \alpha_j^{Site} + a_{k(i)}^{Prov(Spp)} + \alpha_{ij}^{Spp.Site} + a_{jk(i)}^{Site.Prov(Spp)} + \beta_{RW}(RW_{ijkl} - \overline{RW}_{ijkl}) + \varepsilon_{lijk}$$

Where α represents fixed and a represents random effects, while effects with sub and superscripts in brackets are nested within these effects. T_{ijkl} is the total phenolics extracted from tree l, of provenance k of species i at site j; while μ is the overall mean. $\beta_{RW}(RW_{ijkl} - \overline{RW}_{ijkl})$ represents covariation with ring width, where β_{RW} is the regression coefficient. The term ε_{lijk} represents residual variation for the i th species, j th site, k th provenance and l th tree.

The results are given in table 8.3 and clearly show significant variation between the two species, as well as between provenances within species. From table 8.2 it can be seen how the two species are clearly differentiated, with all three means of the *Quercus robur* provenances being higher than those of *Q. petraea*.

Table 8.3: Analysis of variance (see text for model) of the total phenolics extracted from provenance samples.

Source	DF	Sums of Squares	Mean Square	F value	P > F
Species	1	139.08	139.08	47.52	0.0001
Site	1	1.89	1.89	0.64	n.s.
Site x Species	1	8.81	8.81	3.01	n.s.
Prov in Species	4	73.03	18.26	6.24	0.0001
Prov in Species x Site	4	31.70	7.92	2.71	0.05
Ring Width	1	33.65	33.6	11.5	0.001
Error	111	324.88	2.93		

No clear pattern in tannin levels between the two sites is indicated in table 8.2 and this is reflected by there being no significant site effect, although a significant interaction between sites and individual provenances was found. The effect of ring width was found to be highly significant, although a Pearson correlation coefficient of 0.18 indicates that this effect is weak, with a very high degree of scatter as shown in figure 8.1.

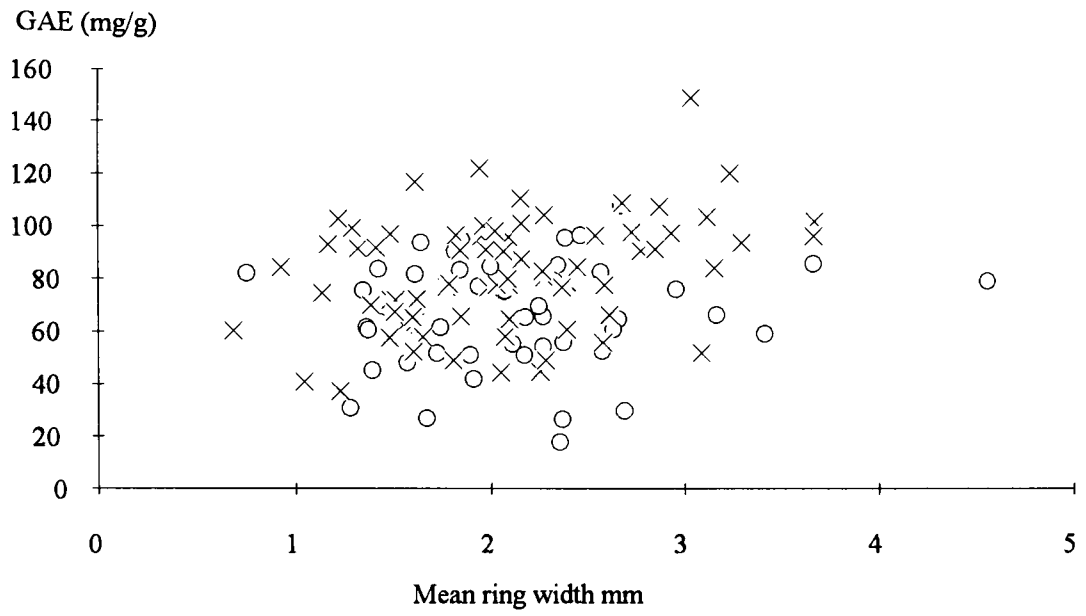


Figure 8.1: Total phenolics vs mean ring width of trees from three provenances, planted at two sites, of *Quercus robur* (x) and *Q. petraea* (o).

Mixed plantations

Table 8.4 shows the mean values for species and sites showing much greater variation in total phenolics than was found in the provenance material. The higher concentrations than those extracted from the provenance samples was probably at least partly due to the more recent sampling. Hydrolysis and insolubilization reactions had probably decreased the level of soluble tannins in the provenance samples, during the three years of storage. The young age of the provenance trees may also explain the low levels.

Table 8.4: Mean tannin levels and standard errors (*S.E.*) in mixed plantations (GAE mg/g)

Site	Q. petraea		Q. robur		Mean	
	Mean	*S.E.*	Mean	*S.E.*	Mean	*S.E.*
Bagley Wood						
Cpt 16a	12.87	*1.78*	18.78	*1.75*	15.82	*1.49*
Cpt 16b	14.48	*1.60*	17.55	*1.49*	16.01	*1.14*
Mean	**13.67**	*1.17*	**18.16**	*1.11*	15.92	*0.92*
Forest of Dean						
Cpt 446c	22.03	*1.18*	22.37	*1.56*	22.20	*0.94*
Cpt 402c	22.47	*0.73*	20.94	*1.26*	21.70	*0.73*
Mean	**22.25**	*0.67*	**21.66**	*0.98*	21.95	*0.58*
Eling Estate						
Cpt ?? (mean)	**18.20**	*1.77*	**21.39**	*1.77*	19.65	*1.28*
Overall mean	**18.04**	***0.93***	**20.34**	***0.77***	18.96	*0.62*

The following model was used for an analysis of variance:

$$T_{ijl} = \mu + \alpha_i^{Spp} + a_j^{Site} + a_{ij}^{Spp.Site} + \beta_{RW}(RW - \overline{RW}) + \varepsilon_{l_{ij}}$$

Table 8.5 describes the results. Although a significant difference was found between the species, the variation between sites was the dominant effect. From table 8.5 it can be seen that although levels of *Quercus robur* were greater for four of the five sites, this pattern was reversed for one of the Forest of Dean sites.

Table 8.5: Analysis of variance (see text for model) of the total phenolics extracted from samples from mixed oak plantations.

Source	DF	Sums of Squares	Mean Square	F value	P > F
Species	1	92.90	92.90	4.54	0.05
Site	4	448.89	112.22	5.48	0.001
Site x Species	4	100.45	25.11	1.23	n.s.
Ring width	1	0.15	0.15	0.01	n.s.
Error	59	1207.78	20.47		

Unlike the provenance material there was also no effect attributed to ring width, although the correlation coefficient was weak but significant. The scatter is again very large, as shown by figure 8.2.

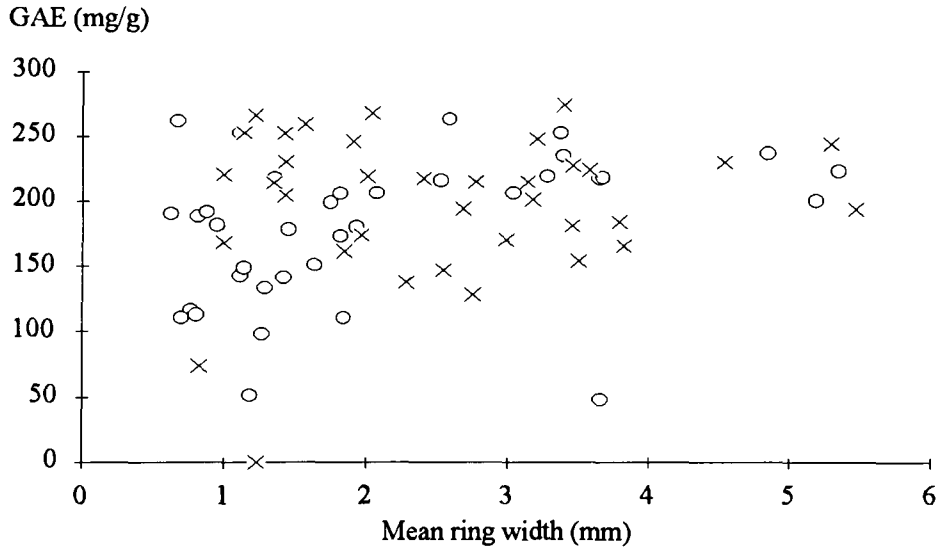


Figure 8.2: Total phenolics vs mean ring width of trees from mixed plantations of *Quercus robur* (x) and *Q. petraea* (o).

Early and late flushing trees

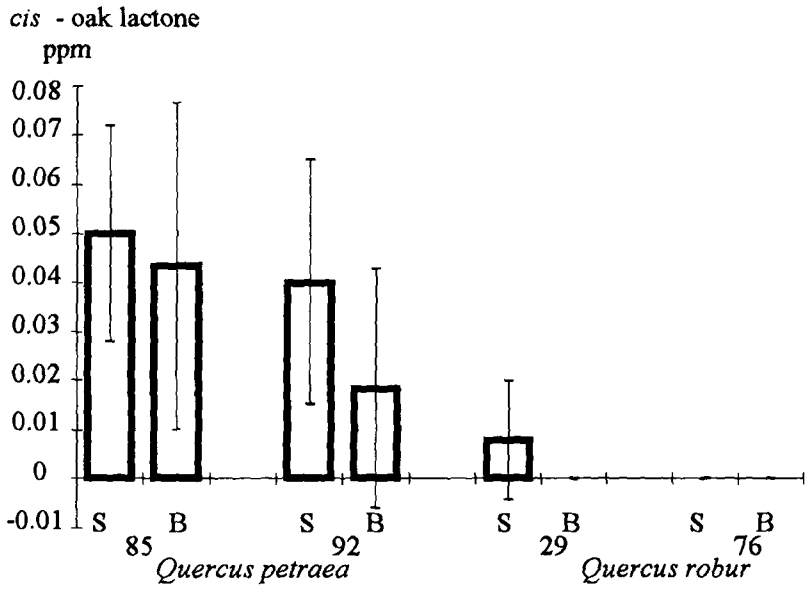
The difference between early and late flushing trees was calculated for each pair and these data used to test the null hypothesis that the mean difference was zero. A paired T-test did not reject the null hypothesis, with a T value of 0.69. There was relatively little variation between trees, compared to that found in the other two studies.

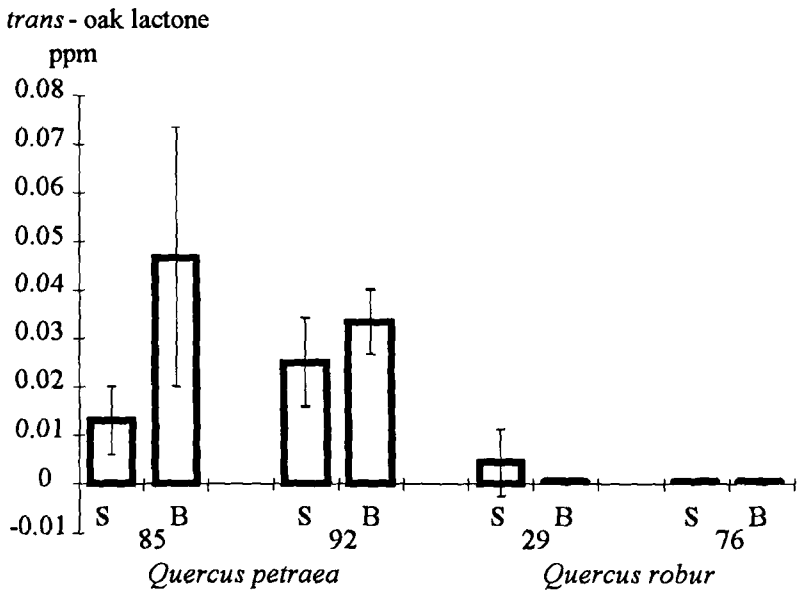
Table 8.6: Overall means and mean difference (earlywood-latewood values) between flushing pairs of *Quercus petraea*.

Time of flushing	Total phenolics (GAE mg/g) Mean	SE
Early	8.01	0.06
Late	7.59	0.11
Difference between pairs	0.42	0.60

8.4.2. Variation in oak lactones

Despite the small number of extractions for which oak lactones were measured, it can be seen from table 8.7 that there was a clear difference between the two species. Statistical analysis did not seem worthwhile given the small number of samples, with figures 8.3 a-b adequately displaying the pattern of variation. The very low levels measured are not surprising as they were assessed from extractions of the outermost heartwood. The results given in section 4.5.2 describe how levels increased with heartwood age. The young age of the trees might also have influenced heartwood concentrations.





Figures 8.3 a-b: Mean concentrations of *cis* and *trans* oak lactones extracted from 6 trees of each of 4 provenances (85, 92, 29, 76), 2 of each species, planted at Bramwald (B) and Syke (S).

Table 8.7: Provenance mean values and standard errors of *cis* and *trans* oak lactone (ppm).

	Syke					Bramwald				
		trans		*cis*			*trans*		*cis*	
Provenance and Species	N	Mean	*S.E.*	Mean	*S.E.*	N	Mean	*S.E.*	Mean	*S.E.*
Quercus petraea										
85 Zell/Mosel	10	0.013	*0.007*	0.05	*0.022*	6	0.047	*0.027*	0.043	*0.033*
92 Johanniskreuz X/5D	12	0.025	*0.009*	0.04	*0.025*	6	0.033	*0.007*	0.018	*0.024*
Mean	22	0.0255		0.045		12	0.040		0.031	
Quercus robur										
29 Gifhorn 26A	9	0.004	*0.007*	0.008	*0.012*	6	0	*0*	0	*0*
76 Nordkirchen	6	0	*0*	0	*0*	6	0	*0*	0	*0*
Mean	15	0.002		0.004		12	0		0	

8.5. Discussion

The results from the provenance trial clearly show a species difference in heartwood tannin, as measured by total phenolics, and lactone concentrations, but this is not found so convincingly in the mixed plantation results. No indication of tannin differences between flushing types was observed.

As provenances are derived from acorns collected from whole stands, the genetic variation of the provenances is likely to have a much broader range than that of the clonal trial studied previously. The method of determining species is not known but the material would have allowed a variety of characteristics to have been used. Trees from the mixed plantations were identified by leaf morphology, with acorns only occasionally being present. *Quercus robur* trees were easily identified as having auricles that ended at the base of a very short petiole. *Quercus petraea* was characterised by a longer leaf stem of generally more than 2 cm and lacking auricles. However, to a certain extent the identification of *Q. petraea* was somewhat subjective, being largely relative to the opposing characters of *Q. robur* trees present at the same site.

The environment of the mixed plantations was also more variable than at the provenance sites. The mean ring widths in table 8.1 indicate the much faster growth obtained at many of the plantations, than was found for the provenance trees. Tree spacing was generally irregular on the mixed plantations and for some sites it was likely that there were wide *intra*-site differences in light and moisture conditions. As the two species generally had uneven, clumped distributions within each site, it is possible that the two species will have been exposed to different growing conditions. The use of ring width as a covariate will have largely removed any effect due to different growth rates, but it can be seen by comparing tables 8.1 and 8.4 that the only site where *Q. petraea* had higher tannin levels than *Q. robur* was where it had also the faster growth.

Given the high degree of genetic control indicated from previous results, it seems unlikely that greater environmental variation is the most important explanation, despite the significant effect of ring width found in this study. However, it should be emphasised that the results of the earlier heritability studies cannot be reliably extrapolated to different growing conditions than those used for the trials.

The two species are known to hybridise but the extent of such diversity is still a matter of controversy, as emphasised by recent papers and reviews of the subject (*eg* Rushton 1993; Dupouey and Badeau 1993). Ducouso *et al.* (1993), Bacilieri *et al.*(1993 and 1992) and Kremer and Petit (1993) have all highlighted the very high levels of gene flow and gene diversity within oak species. Although this may partly be due to hybridisation, the life history characteristics of oaks and human planting are the main responsible factors. Therefore, it is probable that the

greater species overlap of tannin levels in the mixed plantations is at least partly due to the different, and perhaps more diverse, seed origins than were present in the German trials.

8.6. Conclusions

One can propose the following reasons to explain the greater species overlap of tannin concentrations in the mixed plantations: (i) mis-identification of species or presence of hybrids; (ii) confounding variation of growing conditions within the sites; (iii) greater *intra* specific genotypic variation within the mixed plantations than at either the provenance trial or the clonal trial described in chapter 7.

It should be noted that despite the low magnitude of the difference between species in the mixed plantations, significant differences were nonetheless found. Overall the results indicate that species is a good, if perhaps not perfect indicator, of the concentration of heartwood tannins in that *Quercus robur* has generally higher levels than *Q. petraea*. This difference appears to be reflected in the concentrations of extracted oak lactones. Once again this independently supports the findings in chapter 5, where the predominantly *Quercus petraea* Tronçais wood samples were characterised by lower tannin and higher oak lactone concentrations than those in the mostly *Q. robur* Limousin samples.

It is the author's personal view that the two species should be viewed as genetic and phenotypic extremes of a continuum. The species differences reported here are perhaps better understood as genetic linkages between the phenotypic characters used for the classification of the species and the levels of extractives. Although not always reliable they are useful indicators of extract properties. Future studies could examine more closely the degree of correlation with precise phenotypic characters, such as petiole length, to determine which are the most closely correlated.

8.7. Acknowledgements

Peter Savill and Michelle Taylor undertook a large proportion of the fieldwork and preparation of the samples for analysis. Richard Mather was responsible for the collection of provenance samples in 1989, with the assistance of Jochim Kleinschmit and his colleagues at the Niedersächsische Forstliche Versuchsanstalt, Escherode, Germany.

Chapter 9: Conclusions

The advance of knowledge is an infinite progression towards a goal that forever recedes.

J.G.Frazer - *The Golden Bough*

The main conclusions of the studies described in this thesis are outlined below.

- Variation of both ellagitannins and oak lactones within trees relates mostly to heartwood age, with negligible concentrations in the sapwood of trees (chapter 4). Levels of soluble ellagitannins decline while oak lactones increase with increasing heartwood age. The composition of these extractives also varies with heartwood age. Concentrations of dimeric ellagitannins increase during the first 10-30 years of heartwood ageing, while the relative amount of *trans*-oak lactone increases compared to that of the *cis* isomer. Results in chapter 7 suggest that young trees may lay down lower amounts of ellagitannins which are of more limited composition than older trees.

- There is significant and large variation in the heartwood concentrations of ellagitannins and oak lactones within and between European oak trees, provenances, forests and the species *Q. robur* and *Q. petraea*. Concentrations of soluble tannins in the outermost heartwood of different trees may comprise from less than 1% to 14% of the dry weight of heartwood. Even after controlling for differences due to heartwood age, chapter 5 found that over 70% of the total variation in levels of soluble tannins was attributed as occurring between, rather than within, two contrasting forests. Chapter 8 indicated that the concentrations of ellagitannins and many physical properties of oak wood, such as density, are under strong genetic control. A large proportion of variation occurs between the two species *Q. robur* and *Q. petraea,* the latter being typified by lower tannin but higher oak lactone concentrations. It is this difference between species that is likely to explain a large proportion of variation between forest stands.

- The changes in the concentrations and composition of heartwood extractives that occur with heartwood age are also likely to take place during the seasoning and storage of oak wood. Long seasoning of wood, such as occurs during air-drying of timber, is likely to cause a decline in soluble tannins and increased concentrations of oak lactones.

- The heating of the wood during cask manufacture reduces the levels of ellagitannins, while increasing ellagic acid and many lignin-derived products. However, the changes incurred by toasting or charring of casks are generally limited to a shallow surface region of the staves and some extractives, such as oak lactones, appear unaffected by heating. Changes incurred by heating are not so great as to render differences between oak wood origins unimportant. Indeed the concentrations of many extractives produced by heating and the flavour imparted by the wood varied significantly between oaks from the two forests studied in chapter 6.

- The apparent difference between the two species of European oak allows the identification and selection of wood for different extractive properties and effects on flavour. However, there will always be large *inter*-tree variation within species, provenances and forest stands.

While many of these conclusions have been inferred or claimed by other studies and authors, there has been little evidence from studies with adequate replication and control of confounding variables. Furthermore, for every claim that the studies described in this thesis have supported there have been numerous conflicting or alternative explanations and suppositions.

Singleton (1974) and Hillis (1975) suggested that slow rates of growth will produce oak wood with higher levels of heartwood tannins. Although higher levels of tannins were found in the earlywood compared to latewood, any relationship with growth rates was the opposite to that proposed by these two authors. Low levels of ellagitannins, even when confounding factors such as heartwood age were controlled, were associated more with narrow annual rings. However, the influence of growth rate appears to be relatively weak, possibly being only of consequence for extreme values.

There are numerous claims that certain growing conditions will influence the flavour produced by oak wood. While some of these (such as the amount of light a tree receives and references to free standing trees) can be interpreted as referring to the rate of growth, others have been more specific in claiming that factors such as soil conditions may be of importance. The studies described here did not address directly any of these environmental factors and therefore their possible affects cannot be ruled out. However the high levels of genetic control and variation between species suggests that the explanation for differences between oak growing in different regions may lie predominantly in the different genotypes of the oaks.

The two species *Q. robur* and *Q. petraea,* while having overlapping ranges, do characterise different environmental conditions. *Q. petraea* is generally associated with poorer, more acid and freely draining soils than *Q. robur* (Kleinschmit 1993; Jones 1959). Within Britain, *Q. robur* is dominant mostly in the lowlands in the south-east and central parts of England on heavy and especially basic soils. *Q. petraea* is characteristic of silicaceous soils in the north and west of Britain, although planting over many centuries has obscured the differences in their natural ranges (Savill 1991; Morris and Perring 1974). The predominance of *Q. robur* on silviculturally more optimum environments compared to *Q. petraea* may explain the tendency, among for example French coopers, to associate high tannin levels with more quickly grown timber.

The whole issue of characterising the two species of oak is still a matter of controversy. Numerous authors (Dupouey *et al.* 1993; Rushton 1993) have described introgression and hybridization of the species and the problems of defining biological species have frequently been discussed (*eg* Van Valen 1976; Cousens 1965*)*. Other authors (Kremer *et al.* 1993) have

emphasised the high degree of genetic variation that is found within the two species and within forest populations. This variation explains the high *inter*-tree variation of many properties, such as heartwood ellagitannins, that from this study have been found to be under strong genetic control.

Work carried out for this thesis has shown that the selection of oaks for different extractive properties is feasible and furthermore that variations of the extractives correlates with variations in the flavour imparted after heating. Although there were large variations in the properties examined, two opposing sets of characteristics typified the majority of samples. High ellagitannin concentrations and low levels of oak lactones were associated with phenotypic characteristics describing *Q. robur*. These trees also imparted higher levels of lignin-derived products and an intense colour to solutions after heating. In contrast, low levels of tannins and high lactone concentrations, associated with the release of a more complex and subtle flavour and less intense colour after heating were found to be typical of *Q. petraea*. These contrasting sets of properties will be further magnified in stave wood if they derive from trees with different growth rates, or if wood is taken from the outermost or innermost circle of staves cut from a tree. Slower growth will result in the use of a greater proportion of older heartwood, as will the use of the innermost staves, and therefore these staves will contain lower levels of tannins but greater oak lactone concentrations. Air drying over several years is likely to augment these properties further. It is perhaps not surprising to find that the two types of French oak most commonly described as producing different flavours are typical of these opposing sets of characters. Limousin being rapidly grown *Q. robur*, while slowly grown, predominantly *Q. petraea* typify Tronçais oaks.

9.1. Future research on the influence of oak wood on the maturation of whisky

On commencing this research there was little understanding of the variation of extractives and flavour between different origins of European oaks, due to the lack of adequate replication in the few published studies. Even the variation of ellagitannins within trees had been the subject of only a few thorough studies and these were based on single tree samples. During the course of this current research numerous studies of much higher quality than these have been published by other authors (notably by researchers at INRA, Paris, Grignon), which have confirmed many of the findings described here. However, the few studies that have recently appeared which examined variation between oak origins (*eg* Marco *et al.* 1994) are still characterised by very low replication and a lack of details about such factors as wood treatment, seasoning, approximate tree age and species. If studies examining variation of extractive or flavour properties of oak wood are to be worthwhile, they must control or at least describe such factors as the species of oak and heartwood age, quality, storage and treatments. With the better observations and understanding of variation obtained in this thesis, a more wide ranging examination of populations could now be profitable.

Recent papers have compared different methods of extracting and measuring compounds (Scalbert 1992a; Maga 1989b; Puech *et al.* 1990) and have shown how they may give widely differing results. It would be useful to develop more standard methods and determine how different methods of measuring wood extractives and flavour relate to the actual performance of a wood type in whisky maturation. Such methods should ideally take account of any physical properties of the wood that might influence extraction or the conditions of maturation. A major subject of research requiring attention and which has not been focused on by these studies, is how the wood extractives relate to the flavour produced and effects of maturation. As stated by numerous authors (*eg* Paterson and Piggott 1989; Swan and Howie 1985; Maarse and van der Berg 1989) the problem remains of relating chemical concentrations with sensory data on flavour and aroma. Only when swifter, and more predictive methods of estimating flavour effects have been developed will it be possible to examine more fully the variation in the effects of cask and wood types.

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We must remember that at the bottom of the generalisations of science, or in common parlance, the laws of nature are merely hypotheses devised to explain that ever-shifting phantasmagoria of thought which we dignify with the high sounding names of the world and the universe.

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Appendix 1

Flavour terms used to assess samples and their frequency of use in describing Limousin and Troncais samples. Sample numbers refer to those in table 6.2 of chapter 6. G statistic after Williams' correction - see Sokal and Rohlf (1981)

Flavour Term	Number of comments			*Cf* of Forests	
	Limousin No.1-20	Troncais No. 21-40	All No.1-72	G statistic	Chi Square
woody	110	119	423	0.12	0.12
burnt	66	48	189	5.20	5.22
rubber	53	43	163	2.05	2.06
sweet	31	52	134	5.46	5.44
caramel	22	21	63	0.11	0.11
fruity	25	17	71	2.14	2.15
spicy	13	19	52	0.92	0.93
vanilla	10	17	43	1.59	1.60
toffee	12	14	38	0.08	0.08
salami	9	10	25	0.02	0.02
pencil shavings	4	14	29	5.49	5.32
sherry	6	10	27	0.83	0.85
meaty	9	7	25	0.36	0.37
dry	7	8	27	0.03	0.03
pencil rubber	6	9	27	0.47	0.48
coffee	6	7	29	0.04	0.04
smoky	9	4	26	2.21	2.24
musty	8	4	35	1.54	1.58
plastic	5	7	31	0.24	0.25
bland	3	9	16	2.82	2.80
flavour sl	8	4	15	1.54	1.58
heavy	5	6	21	0.05	0.05
sulphur	3	7	11	1.42	1.45
perfume	2	7	24	2.62	2.60
sawdust	3	5	16	0.40	0.42
tobacco	5	3	15	0.58	0.61
coconut	1	7	9	4.57	4.30
green	3	4	34	0.09	0.10
soap	0	7	21		6.78
fragrant	3	4	19	0.09	0.10
sour	6	1	15	3.99	3.88
charred	5	2	9	1.40	1.46
M6	4	3	8	0.19	0.20
cardboard	2	4	14	0.55	0.58
paper	3	2	12	0.23	0.25
light	1	4	11	1.64	1.68
burnt sugar	3	2	10	0.23	0.25
rich	2	3	9	0.14	0.16
dusty	2	2	5	0.00	0.00
phenolic	4	0	5		4.24
treacle	2	1	8		
toasted	2	1	7		
used matches	1	2	7		
tar	2	1	5		
chocolate	1	2	4		
leather	2	1	3		
oily	2	0	25		
esters	1	1	6		
plasticene	2	0	5		
cinnamon	0	2	4		
clean	1	1	4		
H2S	0	2	3		
bourbon	1	1	3		
butter	0	2	3		
curry	0	2	3		
sharp	2	0	3		
acetic acid	1	1	2		

Flavour	No. comments			*Cf* of Forests	
	Limousin No.1-20	Troncais No. 21-40	All No.1-72	G statistic	Chi Square
acrid	2	0	2		
bovril	1	1	2		
onions	0	2	2		
rotten	2	0	2		
cream	1	0	6		
bitter	1	0	4		
wine	1	0	4		
butyric	1	0	2		
fatty	0	1	2		
mud	0	1	2		
pleasant	0	1	2		
rough	1	0	2		
American oak	0	1	1		
acidic	1	0	1		
antiseptic	0	1	1		
astringent	0	1	1		
charcoal	1	0	1		
cigarette	1	0	1		
cocoa	0	1	1		
dirty	0	1	1		
marzipan	0	1	1		
mature	0	1	1		
mellow	0	1	1		
neoprene	1	0	1		
new wood	0	1	1		
off	0	1	1		
p_soup	1	0	1		
raisins	0	1	1		
soft	0	1	1		
thin	0	1	1		
tomatoes	1	0	1		
grass	0	0	13		
bread	0	0	6		
cotton	0	0	6		
wax	0	0	6		
silage	0	0	3		
M3	0	0	2		
aldehyde	0	0	2		
books	0	0	2		
carbolic	0	0	2		
fresh	0	0	2		
grainy	0	0	2		
juniper	0	0	2		
lemon	0	0	2		
malt	0	0	2		
pine	0		2		
tea leaves	0	0	2		
flavour 2	0	0	1		
apples	0	0	1		
carrots	0	0	1		
disinfectant	0	0	1		
eucalyptus	0	0	1		
linseed	0	0	1		
mint	0	0	1		
resin	0	0	1		
stale	0	0	1		
varnish	0	0	1		
vegetable	0	0	1		
No. terms used	515	555	1969		
No. Assessments	202	212			

Appendix 2

Results from principal component analyses described in chapter 6. All sample numbers refer to those in table 6.2.

PCA 1 a: Analysis of extractives of samples from two forests all heated for the same length of time.

Response variable scores (adjusted for variance)

N	NAME	AX1	AX2	AX3	AX4	WEIGHT
	Eigenvalues	0.83	0.12	0.02	0.01	
1	Gallic acid	0.77	-0.14	-0.19	-0.28	1.00
2	5 -HMF	-0.39	-0.59	0.18	-0.27	1.00
3	Furfural	0.63	-0.78	-0.01	0.01	1.00
4	Vanillic acid	0.43	-0.66	-0.18	-0.31	1.00
5	Syringic acid	0.42	0.04	0.66	0.30	1.00
6	Vanillin	-0.21	-0.40	0.60	0.16	1.00
7	Syringealdehyde	0.07	-0.01	0.80	0.31	1.00
8	Coniferaldehyde	0.08	-0.31	0.75	-0.29	1.00
9	Sinapaldehyde	0.07	-0.20	0.91	-0.15	1.00
10	Ellagic acid	0.99	0.13	0.00	-0.02	1.00
11	Scopoletin	0.26	0.13	0.09	0.07	1.00
12	Oak lactone cis	-0.57	0.00	0.05	-0.30	1.00
13	Oak lactone trans	-0.57	0.02	0.05	-0.05	1.00
14	Total ellagitannins	0.92	0.00	-0.06	0.36	1.00
15	pH	-0.72	-0.04	-0.20	-0.28	1.00
16	Absorbance	0.94	0.02	0.08	0.24	1.00

CFit: Cumulative fit per response variable as fraction of variance

N	NAME	AX1	AX2	AX3	AX4	VAR(y)	% EXPL
	Eigenvalues	0.83	0.12	0.02	0.01		
1	Gallic acid	0.59	0.61	0.65	0.73	0.07	59.13
2	5 -HMF	0.15	0.50	0.54	0.61	0.02	25.71
3	Furfural	0.40	1.00	1.00	1.00	2.81	43.86
4	Vanillic acid	0.18	0.62	0.65	0.75	0.22	30.59
5	Syringic acid	0.18	0.18	0.61	0.70	0.05	37.97
6	Vanillin	0.05	0.21	0.57	0.60	0.01	24.29
7	Syringealdehyde	0.01	0.01	0.64	0.74	0.14	33.77
8	Coniferaldehyde	0.01	0.10	0.67	0.75	0.04	33.65
9	Sinapaldehyde	0.00	0.05	0.88	0.90	0.22	50.55
10	Ellagic acid	0.98	1.00	1.00	1.00	11.51	69.53
11	Scopoletin	0.07	0.08	0.09	0.10	0.00	18.31
12	Oak lactone cis	0.33	0.33	0.33	0.42	0.01	66.09
13	Oak lactone trans	0.32	0.32	0.33	0.33	0.00	42.05
14	Total ellagitannins	0.86	0.86	0.86	0.98	0.91	81.04
15	pH	0.51	0.52	0.56	0.63	0.00	73.05
16	Absorbance	0.88	0.88	0.88	0.94	0.00	83.63

PCA 1 a: Analysis of extractives of samples from two forests all heated for the same length of time.

Sample scores

N	NAME	AX1	AX2	AX3	AX4	WEIGHT
	Eigenvalues	0.83	0.12	0.02	0.01	
1	L1	1.95	-0.50	-0.69	0.41	1.00
2	L2	1.85	0.32	-0.22	-0.52	1.00
3	L3	-0.16	-0.53	-0.38	0.07	1.00
4	L4	-0.09	1.49	0.68	0.40	1.00
5	L5	0.88	0.11	0.14	0.61	1.00
6	L6	0.79	-1.40	0.11	-0.15	1.00
7	L7	0.86	0.80	1.40	-0.99	1.00
8	L8	0.67	0.83	1.16	-0.26	1.00
9	L9	0.33	-1.34	-0.56	1.08	1.00
10	L10	-0.17	0.18	0.34	0.68	1.00
11	L11	-0.60	-0.98	-0.70	-0.20	1.00
12	L12	1.70	0.78	0.36	-0.35	1.00
13	L13	0.12	-1.08	-0.61	0.91	1.00
14	L14	0.39	1.25	1.41	-0.52	1.00
15	L15	1.27	-0.16	1.87	-1.11	1.00
16	L16	1.62	-0.35	-1.20	-1.96	1.00
17	L17	1.03	-1.89	0.12	-1.38	1.00
18	L18	-0.13	-0.65	1.40	2.07	1.00
19	L19	1.83	-0.09	-0.10	3.71	1.00
20	L20	1.15	0.23	0.32	0.34	1.00
21	T21	-0.87	-0.90	1.01	-0.33	1.00
22	T22	-0.96	-0.87	0.57	-0.55	1.00
23	T23	-1.46	-0.27	1.68	0.70	1.00
24	T24	0.62	1.00	-1.15	-0.14	1.00
25	T25	-1.54	-0.50	1.54	0.54	1.00
26	T26	-0.54	-0.08	-1.83	-0.91	1.00
27	T27	-0.31	0.74	0.52	-2.03	1.00
28	T28	-0.90	-0.50	0.22	-0.36	1.00
29	T29	-0.41	-1.46	-0.07	-0.12	1.00
30	T30	-0.76	2.38	-1.73	1.10	1.00
31	T31	-0.88	-0.66	-0.47	-0.76	1.00
32	T32	-0.65	1.13	-0.39	-1.09	1.00
33	T33	-1.17	1.77	0.66	0.13	1.00
34	T34	-0.94	-0.24	-0.33	0.49	1.00
35	T35	-0.77	1.68	0.02	-0.08	1.00
36	T36	-0.44	-0.82	-1.79	0.16	1.00
37	T37	-0.63	-0.04	-1.43	-0.23	1.00
38	T38	-1.21	-0.34	-1.58	-0.02	1.00
39	T39	-1.41	-0.74	0.81	0.14	1.00
40	T40	-0.06	1.73	-1.12	0.54	1.00
57	TR257	0.13	3.42	-1.38	0.26	0.00
58	TR258	-0.34	2.59	-1.15	1.38	0.00
59	TR259	-0.20	2.04	-1.21	0.57	0.00
60	TR260	-0.02	3.10	-0.43	0.70	0.00
61	TR161	-1.76	1.85	-0.85	0.64	0.00
62	TR162	-1.82	1.82	-0.83	0.81	0.00
63	TR163	-1.77	1.85	-0.84	0.62	0.00
64	TR164	-1.57	2.02	-0.77	0.31	0.00
65	LR265	-0.95	2.51	-1.40	3.09	0.00
66	LR266	-0.88	2.54	-1.21	3.01	0.00
67	LR267	-0.63	2.79	-1.44	2.35	0.00
68	LR268	-0.41	2.95	-0.77	2.11	0.00
69	LR169	1.94	-2.47	-0.03	0.33	0.00
70	LR170	1.91	-1.55	-0.49	0.19	0.00
71	LR171	1.96	-2.00	-0.32	0.12	0.00
72	LR172	2.38	-1.70	-0.16	-0.65	0.00
73	ORIGIN	-2.12	1.87	-5.36	1.49	0.00

PCA 1b: Analysis of extractives from heating trial samples.

Response variable scores (adjusted for variance)

N	NAME	AX1	AX2	AX3	AX4	WEIGHT
	Eigenvalues	0.90	0.07	0.02	0.01	
1	Gallic acid	0.85	0.06	-0.14	-0.28	1.00
2	5 -HMF	0.62	0.27	-0.06	0.08	1.00
3	Furfural	0.38	-0.28	0.24	0.38	1.00
4	Vanillic acid	0.57	0.60	0.29	0.39	1.00
5	Syringic acid	0.03	0.61	0.06	0.73	1.00
6	Vanillin	0.59	0.21	-0.05	-0.22	1.00
7	Syringealdehyde	0.32	0.77	0.18	0.44	1.00
8	Coniferaldehyde	0.38	0.77	-0.03	-0.13	1.00
9	Sinapaldehyde	0.31	0.94	0.08	-0.09	1.00
10	Ellagic acid	1.00	-0.01	-0.04	0.01	1.00
11	Scopoletin	-0.38	0.12	-0.06	-0.46	1.00
12	Oak lactone cis	-0.78	0.13	-0.59	0.02	1.00
13	Oak lactone trans	-0.45	-0.13	-0.62	0.29	1.00
14	Total ellagitannins	0.85	-0.19	0.49	-0.02	1.00
15	pH	-0.88	0.20	-0.17	-0.07	1.00
16	Absorbance	0.93	0.24	0.00	0.08	1.00

Cumulative fit per response variable as fraction of variance.

N	NAME	AX1	AX2	AX3	AX4	VAR(y)	% EXPL
	Eigenvalues	0.90	0.07	0.02	0.01		
1	Gallic acid	0.73	0.73	0.75	0.83	0.19	66.20
2	5 -HMF	0.39	0.46	0.46	0.47	0.01	81.38
3	Furfural	0.15	0.22	0.28	0.42	0.00	54.59
4	Vanillic acid	0.32	0.68	0.77	0.92	0.01	87.04
5	Syringic acid	0.00	0.37	0.37	0.90	0.11	89.38
6	Vanillin	0.35	0.39	0.40	0.44	0.00	37.30
7	Syringealdehyde	0.10	0.70	0.73	0.93	0.12	90.06
8	Coniferaldehyde	0.15	0.74	0.75	0.76	0.01	49.38
9	Sinapaldehyde	0.10	0.99	0.99	1.00	1.08	57.52
10	Ellagic acid	1.00	1.00	1.00	1.00	13.28	76.96
11	Scopoletin	0.15	0.16	0.16	0.37	0.00	93.61
12	Oak lactone cis	0.61	0.62	0.97	0.97	0.01	99.55
13	Oak lactone trans	0.20	0.22	0.61	0.69	0.00	98.19
14	Total ellagitannins	0.73	0.76	1.00	1.00	1.18	100.00
15	pH	0.78	0.82	0.85	0.85	0.00	95.41
16	Absorbance	0.87	0.93	0.93	0.94	0.00	73.10

PCA 1b: Analysis of extractives from heating trial samples.

Sample scores

N	NAME	AX1	AX2	AX3	AX4	WEIGHT
	Eigenvalues	0.90	0.07	0.02	0.01	
41	SAMP 41	0.31	-1.74	1.38	-0.17	1.00
42	SAMP 42	1.45	0.10	-0.01	-1.79	1.00
43	SAMP 43	2.21	0.66	-1.01	-0.43	1.00
44	SAMP 44	1.39	-0.19	0.19	2.08	1.00
45	SAMP 45	-0.41	-1.24	-1.89	0.21	1.00
46	SAMP 46	-0.60	-0.50	-1.29	0.09	1.00
47	SAMP 47	-0.98	0.10	-0.44	0.98	1.00
48	SAMP 48	-0.81	0.66	-0.49	1.17	1.00
49	SAMP 49	-0.72	-1.51	2.03	-0.57	1.00
50	SAMP 50	0.71	0.10	0.18	-0.79	1.00
51	SAMP 51	0.75	0.48	0.32	0.48	1.00
52	SAMP 52	0.25	0.53	1.25	1.36	1.00
53	SAMP 53	-0.83	-1.21	-0.85	-0.27	1.00
54	SAMP 54	-0.74	0.40	-0.43	-1.16	1.00
55	SAMP 55	-0.95	1.39	0.32	-1.20	1.00
56	SAMP 56	-1.03	1.97	0.73	0.03	1.00
57	SAMP 57	0.53	0.19	-0.61	1.63	0.00
58	SAMP 58	0.11	0.31	0.20	2.50	0.00
59	SAMP 59	0.17	0.41	0.13	1.42	0.00
60	SAMP 60	0.41	0.57	-0.21	2.12	0.00
61	SAMP 61	-1.04	0.95	0.05	1.01	0.00
62	SAMP 62	-1.09	0.96	0.15	0.55	0.00
63	SAMP 63	-1.04	0.92	0.06	0.62	0.00
64	SAMP 64	-0.88	0.92	-0.23	0.85	0.00
65	SAMP 65	-0.37	0.32	1.34	1.33	0.00
66	SAMP 66	-0.31	0.37	1.26	1.41	0.00
67	SAMP 67	-0.10	0.28	0.86	1.52	0.00
68	SAMP 68	0.09	0.50	0.65	1.91	0.00
69	SAMP 69	1.43	0.92	0.82	2.77	0.00
70	SAMP 70	1.48	0.68	0.57	1.96	0.00
71	SAMP 71	1.49	0.78	0.63	2.43	0.00
72	SAMP 72	1.84	0.76	0.03	2.60	0.00
73	ORIGIN	-1.37	-1.15	-0.55	-0.79	0.00

PCA 2: Analysis of flavour descriptions of all samples.

Response variable scores (adjusted for variance)

N	NAME	AX1	AX2	AX3	AX4	WEIGHT
	Eigenvalues	0.23	0.11	0.06	0.05	
2	?	-0.10	-0.15	-0.10	0.05	1.00
3	Am_oak	0.00	0.21	-0.01	-0.10	1.00
4	H2S	-0.03	0.22	0.02	-0.10	1.00
5	M3	0.38	-0.16	0.25	-0.08	1.00
6	M6	-0.24	0.13	0.19	0.03	1.00
7	acet ic_acid	-0.17	-0.09	0.07	0.05	1.00
8	acidic	-0.01	-0.01	-0.05	-0.01	1.00
9	acrid	-0.21	-0.13	0.01	0.04	1.00
10	aldehyde	0.40	-0.04	0.16	0.04	1.00
11	antiseptic	-0.11	0.04	0.00	0.02	1.00
12	apples	0.32	0.07	0.04	0.32	1.00
13	astringent	-0.03	0.16	-0.03	-0.13	1.00
14	bitter	0.28	-0.15	-0.13	-0.03	1.00
15	bland	-0.14	0.09	0.18	0.00	1.00
16	books	0.31	-0.09	-0.26	0.24	1.00
17	bourbon	0.01	0.19	-0.15	-0.19	1.00
18	bovril	-0.17	0.09	0.03	0.12	1.00
19	bread	0.54	-0.25	0.51	-0.09	1.00
20	burnt	-0.80	-0.33	0.14	0.41	1.00
21	burnt_sugar	-0.21	-0.03	-0.02	0.09	1.00
22	butter	0.04	0.37	0.04	-0.17	1.00
23	butyric	-0.12	0.00	0.01	0.25	1.00
24	caramel	-0.50	0.33	-0.14	-0.11	1.00
25	carbolic	0.31	-0.16	0.10	0.03	1.00
26	cardboard	0.45	0.13	0.25	0.19	1.00
27	carrots	0.11	-0.33	0.35	-0.13	1.00
28	charcoal	-0.18	0.04	0.16	0.16	1.00
29	charred	-0.25	-0.06	-0.07	-0.05	1.00
30	chocolate	-0.05	-0.02	-0.10	0.01	1.00
31	cigarettes	-0.04	-0.16	-0.17	-0.05	1.00
32	cinnamon	-0.06	0.32	0.04	-0.05	1.00
33	clean	0.04	0.06	-0.08	0.25	1.00
34	cocoa	-0.11	0.15	0.18	0.06	1.00
35	coconut	0.07	0.26	-0.08	-0.29	1.00
36	coffee	-0.29	-0.08	-0.13	0.02	1.00
37	cotton	0.54	-0.25	0.51	-0.09	1.00
38	cream	0.18	-0.13	0.03	-0.03	1.00
39	curry	0.20	0.24	0.14	-0.10	1.00
40	dirty	0.03	0.42	0.06	-0.08	1.00
41	disinfectant	0.07	0.08	-0.26	0.27	1.00
42	dry	-0.13	0.14	-0.03	0.07	1.00
43	dust y	-0.06	-0.01	0.12	0.09	1.00
44	esters	0.16	-0.09	0.19	0.07	1.00
45	eucalyptus	0.20	-0.17	-0.11	0.05	1.00
46	fatty	0.14	-0.06	0.17	0.00	1.00
47	fragrant	0.51	-0.01	0.04	0.20	1.00
48	fresh	0.30	-0.31	0.35	-0.16	1.00
49	fruity	-0.42	0.18	0.13	0.01	1.00
50	grainy	0.30	-0.31	0.35	-0.16	1.00
51	grass	0.55	-0.44	0.24	-0.08	1.00
52	green	0.73	-0.32	0.38	0.05	1.00
53	heavy	-0.10	0.00	0.08	0.11	1.00
54	juniper	0.40	-0.04	0.16	0.04	1.00
55	leather	-0.02	0.09	-0.11	-0.17	1.00
56	lemon	0.40	-0.04	0.16	0.04	1.00
57	light	0.38	0.10	0.25	0.09	1.00
58	linseed	0.18	-0.13	0.08	-0.09	1.00
59	malt	0.20	-0.17	-0.11	0.05	1.00
60	marzipan	-0.03	0.25	0.11	0.12	1.00
61	mature	0.02	0.28	0.02	-0.16	1.00
62	meaty	-0.20	-0.27	-0.37	-0.25	1.00
63	mellow	0.02	0.11	-0.01	-0.16	1.00
64	mint	0.32	0.07	0.04	0.32	1.00
65	mud	0.03	0.42	0.06	-0.08	1.00

66	musty	0.30	-0.05	-0.48	0.54	1.00
67	neoprene	-0.21	-0.28	0.09	0.28	1.00
68	new_ wood	0.10	0.05	-0.29	0.24	1.00
69	off	-0.02	0.14	0.20	-0.14	1.00
70	oily	0.71	-0.39	0.46	-0.02	1.00
71	onions	-0.17	0.12	0.20	0.10	1.00
72	p_rubber	-0.23	0.09	0.02	0.11	1.00
73	pencil_shavings	0.14	0.43	-0.40	-0.02	1.00
74	p_soup	-0.05	-0.12	-0.19	-0.21	1.00
75	paper	-0.09	-0.07	0.00	0.24	1.00
76	perfume	0.54	0.06	-0.22	0.30	1.00
77	phenolic	-0.27	-0.10	0.07	0.07	1.00
78	pine	0.32	-0.06	0.06	0.17	1.00
79	plastic	0.01	0.01	0.12	0.14	1.00
80	plasticene	0.16	-0.19	0.20	0.06	1.00
81	pleasant	0.04	0.18	-0.14	0.21	1.00
82	raisins	-0.08	-0.11	-0.03	-0.06	1.00
83	resin	0.32	0.07	0.04	0.32	1.00
84	rich	-0.15	0.12	0.03	0.16	1.00
85	rotten	-0.14	-0.15	-0.05	0.14	1.00
86	rough	-0.15	-0.19	-0.08	0.09	1.00
87	rubber	-0.70	-0.24	0.08	-0.32	1.00
88	salami	-0.33	0.08	-0.17	-0.13	1.00
89	sawdust	0.17	0.07	-0.04	-0.18	1.00
90	sharp	0.20	0.09	-0.12	0.04	1.00
91	sherry	-0.03	0.16	-0.08	-0.18	1.00
93	sly	-0.27	0.06	0.17	-0.07	1.00
94	smoke	-0.26	-0.46	0.04	0.06	1.00
95	soap	0.66	0.21	-0.07	0.33	1.00
96	soft	0.02	0.28	0.02	-0.16	1.00
97	sour	-0.21	-0.21	-0.09	0.18	1.00
98	spicy	-0.16	0.29	-0.23	0.04	1.00
99	stale	0.24	0.03	0.08	0.13	1.00
100	sulphur	-0.21	0.18	0.21	0.12	1.00
101	sweet	-0.19	0.80	0.38	0.14	1.00
102	tar	-0.07	-0.03	-0.24	-0.07	1.00
103	tea_ leaves	0.20	-0.17	-0.11	0.05	1.00
104	thin	0.00	0.21	-0.01	-0.10	1.00
105	toasted	-0.09	-0.11	0.20	-0.13	1.00
106	tobacco	-0.19	-0.14	-0.12	-0.12	1.00
107	toffee	-0.44	0.23	0.17	0.00	1.00
108	tomatoes	-0.06	-0.06	-0.04	-0.09	1.00
109	treacle	-0.16	-0.01	-0.01	-0.20	1.00
110	used _matches	-0.17	-0.10	-0.07	0.06	1.00
111	vanilla	-0.17	0.39	0.04	-0.11	1.00
112	varnish	0.22	-0.09	-0.20	0.09	1.00
113	vegetables	0.11	-0.33	0.35	-0.13	1.00
114	wax	0.47	-0.10	0.14	0.14	1.00
115	wine	-0.19	-0.04	0.08	-0.03	1.00

PCA 2: Analysis of flavour descriptions of all samples.

Sample scores

N	NAME	AX1	AX2	AX3	AX4	WEIGHT
	Eigenvalues	0.23	0.11	0.06	0.05	
1	L1	-1.55	-2.05	0.67	2.10	1.00
2	L2	-1.26	-0.50	0.20	-0.08	1.00
3	L3	-0.30	-1.17	-1.27	-0.37	1.00
4	L4	-0.06	-0.09	-0.34	-0.10	1.00
5	L5	-0.24	-0.26	-0.67	-1.49	1.00
6	L6	-1.93	-0.77	0.56	0.50	1.00
7	L7	0.11	0.45	-1.23	-0.65	1.00
8	L8	0.31	-0.20	-1.01	-0.47	1.00
9	L9	-0.45	-0.44	-0.30	-0.65	1.00
10	L10	-0.88	-0.80	-0.10	0.53	1.00
11	L11	0.01	-0.15	-0.98	-1.10	1.00
12	L12	-0.92	-0.20	-1.01	0.81	1.00
13	L13	-1.21	-0.09	0.96	0.98	1.00
14	L14	-0.02	0.14	-0.57	-1.27	1.00
15	L15	-0.39	-0.90	-1.44	-1.53	1.00
16	L16	-1.34	0.33	1.16	1.15	1.00
17	L17	-0.87	0.01	0.07	1.84	1.00
18	L18	-0.74	-0.02	1.31	-0.39	1.00
19	L19	-0.42	-0.28	0.23	0.10	1.00
20	L20	-0.47	-0.15	-0.11	-0.36	1.00
21	T21	0.12	2.08	0.12	-1.18	1.00
22	T22	-0.22	1.83	0.80	0.86	1.00
23	T23	-0.04	1.23	0.48	0.16	1.00
24	T24	-0.47	-0.99	-0.37	-0.89	1.00
25	T25	0.26	3.09	0.42	-0.61	1.00
26	T26	0.17	0.79	-0.04	-1.17	1.00
27	T27	0.03	1.56	-0.05	-0.75	1.00
28	T28	-0.57	0.43	0.71	1.79	1.00
29	T29	-0.92	-1.28	-0.72	-0.94	1.00
30	T30	-0.60	-0.82	-0.19	-0.47	1.00
31	T31	-0.83	1.12	1.36	0.45	1.00
32	T32	0.28	0.14	-1.30	-0.26	1.00
33	T33	0.40	1.57	-1.07	-0.83	1.00
34	T34	-0.20	1.20	-0.24	-0.98	1.00
35	T35	0.72	0.36	-2.13	1.79	1.00
36	T36	-0.59	0.79	1.31	0.77	1.00
37	T37	-0.83	0.28	0.01	0.12	1.00
38	T38	-0.91	0.13	0.68	0.59	1.00
39	T39	0.20	0.71	-0.78	-0.22	1.00
40	T40	-0.16	1.02	1.47	-1.04	1.00
41	H0L41	1.51	-1.29	-0.82	0.36	1.00
42	H1L42	2.25	-1.25	0.81	-0.83	1.00
43	H2L43	-0.63	-0.66	0.14	-0.36	1.00
44	H3L44	-0.75	-0.35	-0.16	-1.33	1.00
45	H0T45	2.37	0.50	0.29	2.38	1.00
46	H1T46	1.35	-0.97	0.63	-0.68	1.00
47	H2T47	1.58	-0.25	-1.19	1.80	1.00
48	H3T48	0.50	0.62	-1.91	1.98	1.00
49	H0L49	2.37	-0.71	1.05	-0.62	1.00
50	H1L50	1.35	0.02	2.04	-0.22	1.00
51	H2L51	0.80	-2.47	2.59	-0.99	1.00
52	H3L52	-0.73	-1.13	-0.73	0.40	1.00
53	H0A53	1.74	0.26	0.63	0.99	1.00
54	H1A54	1.73	-0.39	1.82	-0.02	1.00
55	H2A55	1.60	-0.70	-1.50	0.70	1.00
56	H3A56	-0.24	0.69	-0.29	-0.32	1.00
57	TR257	-0.69	-0.75	-0.45	0.77	0.00
58	TR258	-0.66	0.43	1.09	-0.22	0.00
59	TR259	-1.03	-1.12	0.19	-0.40	0.00
60	TR260	-1.24	-0.35	0.87	-0.31	0.00
61	TR161	-0.31	0.37	-0.51	-0.81	0.00
62	TR162	0.36	1.13	-1.08	-0.79	0.00
63	TR163	0.10	0.93	-0.51	0.17	0.00
64	TR164	-0.35	1.23	0.33	0.47	0.00
65	LR265	0.14	-0.80	-1.65	-0.62	0.00
66	LR266	-0.83	0.29	0.88	0.58	0.00
67	LR267	-0.70	0.14	0.46	-0.19	0.00
68	LR268	-0.14	-0.60	-1.03	-2.52	0.00
69	LR169	-1.31	-0.31	0.89	1.59	0.00
70	LR170	-0.64	0.87	0.75	2.63	0.00
71	LR171	-0.26	0.07	0.01	0.33	0.00
72	LR172	-0.36	0.19	-0.40	0.57	0.00
73	ORIGIN	1.16	-0.19	-1.50	-1.15	0.00

PCA 2: Analysis of flavour descriptions of all samples.

Cumulative fit per response variable as fraction of variance.

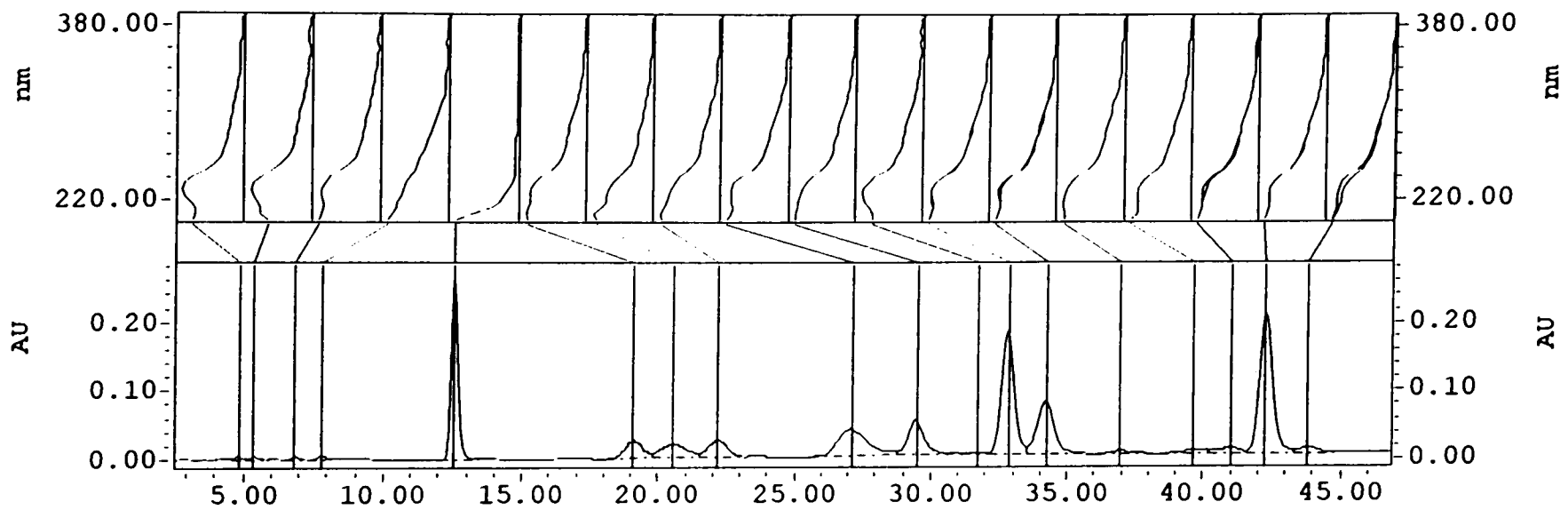
N	NAME	AX1	AX2	AX3	AX4	VAR(y)	% EXPL
	Eigenvalues	0.23	0.11	0.06	0.05		
2	?	0.01	0.03	0.04	0.05	0.10	19.9
3	Am_oak	0.00	0.04	0.04	0.05	0.10	18.54
4	H2S	0.00	0.05	0.05	0.06	0.19	19.77
5	M3	0.15	0.17	0.24	0.24	0.19	50.7
6	M6	0.06	0.08	0.11	0.11	0.68	20.56
7	acet ic_acid	0.03	0.04	0.04	0.05	0.19	16.06
8	acidic	0.00	0.00	0.00	0.00	0.10	18.49
9	acrid	0.04	0.06	0.06	0.06	0.19	30.15
10	aldehyde	0.16	0.16	0.18	0.19	0.19	49.53
11	antiseptic	0.01	0.01	0.01	0.01	0.10	42.72
12	apples	0.10	0.11	0.11	0.21	0.10	43.9
13	astringent	0.00	0.03	0.03	0.05	0.10	14.96
14	bitter	0.08	0.10	0.12	0.12	0.28	27.26
15	bland	0.02	0.03	0.06	0.06	1.58	24.31
16	books	0.09	0.10	0.17	0.23	0.19	30.65
17	bourbon	0.00	0.04	0.06	0.10	0.28	24.68
18	bovril	0.03	0.04	0.04	0.05	0.19	24.22
19	bread	0.29	0.35	0.61	0.61	0.53	49.46
20	burnt	0.64	0.75	0.77	0.93	12.88	59.15
21	burnt_sugar	0.04	0.05	0.05	0.06	0.61	23.19
22	butter	0.00	0.14	0.14	0.17	0.19	26.1
23	butyric	0.01	0.01	0.01	0.08	0.10	26.65
24	caramel	0.25	0.36	0.38	0.39	3.06	43.36
25	carbolic	0.10	0.12	0.13	0.13	0.19	37.11
26	cardboard	0.21	0.22	0.29	0.32	1.19	44.05
27	carrots	0.01	0.12	0.24	0.26	0.10	24.06
28	charcoal	0.03	0.03	0.06	0.08	0.10	57.01
29	charred	0.06	0.07	0.07	0.07	0.61	47.59
30	chocolate	0.00	0.00	0.01	0.01	0.28	28.64
31	cigarettes	0.00	0.03	0.06	0.06	0.10	21.04
32	cinnamon	0.00	0.11	0.11	0.11	0.19	47.74
33	clean	0.00	0.01	0.01	0.08	0.37	35.95
34	cocoa	0.01	0.04	0.07	0.07	0.10	24.28
35	coconut	0.01	0.07	0.08	0.16	0.75	23.66
36	coffee	0.08	0.09	0.11	0.11	1.33	36.31
37	cotton	0.29	0.35	0.61	0.61	0.53	49.46
38	cream	0.03	0.05	0.05	0.05	0.28	32.06
39	curry	0.04	0.10	0.12	0.13	0.28	65.08
40	dirty	0.00	0.17	0.18	0.18	0.10	37.01
41	disinfectant	0.00	0.01	0.08	0.15	0.10	34.73
42	dry	0.02	0.04	0.04	0.04	1.72	10.25
43	dust y	0.00	0.00	0.02	0.03	0.45	16.44
44	esters	0.03	0.04	0.07	0.08	0.45	24.36
45	eucalyptus	0.04	0.07	0.08	0.09	0.10	57.79
46	fatty	0.02	0.02	0.05	0.05	0.19	48.59
47	fragrant	0.26	0.26	0.26	0.30	1.81	39.04
48	fresh	0.09	0.19	0.31	0.33	0.19	31.21
49	fruity	0.18	0.21	0.23	0.23	3.03	26.73
50	grainy	0.09	0.19	0.31	0.33	0.19	31.21
51	grass	0.30	0.49	0.55	0.56	1.78	59.05
52	green	0.54	0.64	0.78	0.78	5.11	66.33
53	heavy	0.01	0.01	0.02	0.03	1.37	26.02
54	juniper	0.16	0.16	0.18	0.19	0.19	49.53
55	leather	0.00	0.01	0.02	0.05	0.28	25.73
56	lemon	0.16	0.16	0.18	0.19	0.19	49.53
57	light	0.15	0.16	0.22	0.23	0.81	38.5
58	linseed	0.03	0.05	0.06	0.07	0.10	27.7
59	malt	0.04	0.07	0.08	0.09	0.10	57.79
60	marzipan	0.00	0.06	0.07	0.09	0.10	19.63
61	mature	0.00	0.08	0.08	0.10	0.10	21.62
62	meaty	0.04	0.11	0.25	0.31	1.41	31.82
63	mellow	0.00	0.01	0.01	0.04	0.10	30.41
64	mint	0.10	0.11	0.11	0.21	0.10	43.9
65	mud	0.00	0.17	0.18	0.18	0.10	37.01
66	musty	0.09	0.09	0.32	0.61	3.96	61.94
67	neoprene	0.04	0.12	0.13	0.21	0.10	31
68	new_ wood	0.01	0.01	0.09	0.15	0.10	14.87

69	off	0.00	0.02	0.06	0.08	0.10	14.93
70	oily	0.50	0.65	0.87	0.87	4.53	66.42
71	onions	0.03	0.04	0.08	0.09	0.19	27.93
72	p_rubber	0.05	0.06	0.06	0.07	1.21	10.09
73	pencil_shavings	0.02	0.20	0.36	0.36	1.97	40.25
74	p_soup	0.00	0.02	0.06	0.10	0.10	25.36
75	paper	0.01	0.01	0.01	0.07	0.68	29.21
76	perfume	0.30	0.30	0.35	0.44	2.69	57.01
77	phenolic	0.07	0.08	0.09	0.09	0.37	39.81
78	pine	0.10	0.11	0.11	0.14	0.19	35.43
79	plastic	0.00	0.00	0.02	0.04	1.70	17.09
80	plasticene	0.03	0.06	0.10	0.10	0.37	31.87
81	pleasant	0.00	0.03	0.05	0.09	0.19	25.04
82	raisins	0.01	0.02	0.02	0.02	0.10	14.99
83	resin	0.10	0.11	0.11	0.21	0.10	43.9
84	rich	0.02	0.04	0.04	0.06	0.53	21.65
85	rotten	0.02	0.04	0.05	0.07	0.19	20.41
86	rough	0.02	0.06	0.06	0.07	0.19	23.16
87	rubber	0.49	0.55	0.56	0.65	11.43	45.78
88	salami	0.11	0.11	0.14	0.16	1.30	24.08
89	sawdust	0.03	0.03	0.03	0.07	1.19	22.84
90	sharp	0.04	0.05	0.06	0.06	0.28	32.8
91	sherry	0.00	0.03	0.03	0.07	1.44	46.16
93	sly	0.07	0.08	0.11	0.11	1.04	17.73
94	smoke	0.07	0.28	0.28	0.28	1.37	44.29
95	soap	0.44	0.48	0.49	0.60	1.44	79.83
96	soft	0.00	0.08	0.08	0.10	0.10	21.62
97	sour	0.05	0.09	0.10	0.13	0.95	26.11
98	spicy	0.02	0.11	0.16	0.17	3.91	38.69
99	stale	0.06	0.06	0.06	0.08	0.10	38.67
100	sulphur	0.04	0.08	0.12	0.13	1.01	30.75
101	sweet	0.03	0.67	0.82	0.83	9.54	38.51
102	tar	0.00	0.01	0.06	0.07	0.28	17.26
103	tea_ leaves	0.04	0.07	0.08	0.09	0.10	57.79
104	thin	0.00	0.04	0.04	0.05	0.10	18.54
105	toasted	0.01	0.02	0.06	0.08	0.53	33.35
106	tobacco	0.04	0.06	0.07	0.08	0.81	30.94
107	toffee	0.19	0.24	0.27	0.27	2.97	42.97
108	tomatoes	0.00	0.01	0.01	0.02	0.10	13
109	treacle	0.03	0.03	0.03	0.07	0.37	27.31
110	used _matches	0.03	0.04	0.04	0.05	0.37	24.87
111	vanilla	0.03	0.19	0.19	0.20	3.16	35.58
112	varnish	0.05	0.06	0.10	0.11	0.10	32.17
113	vegetables	0.01	0.12	0.24	0.26	0.10	24.06
114	wax	0.22	0.23	0.25	0.27	0.53	47.49
115	wine	0.04	0.04	0.04	0.04	0.19	34.93

Appendix 3

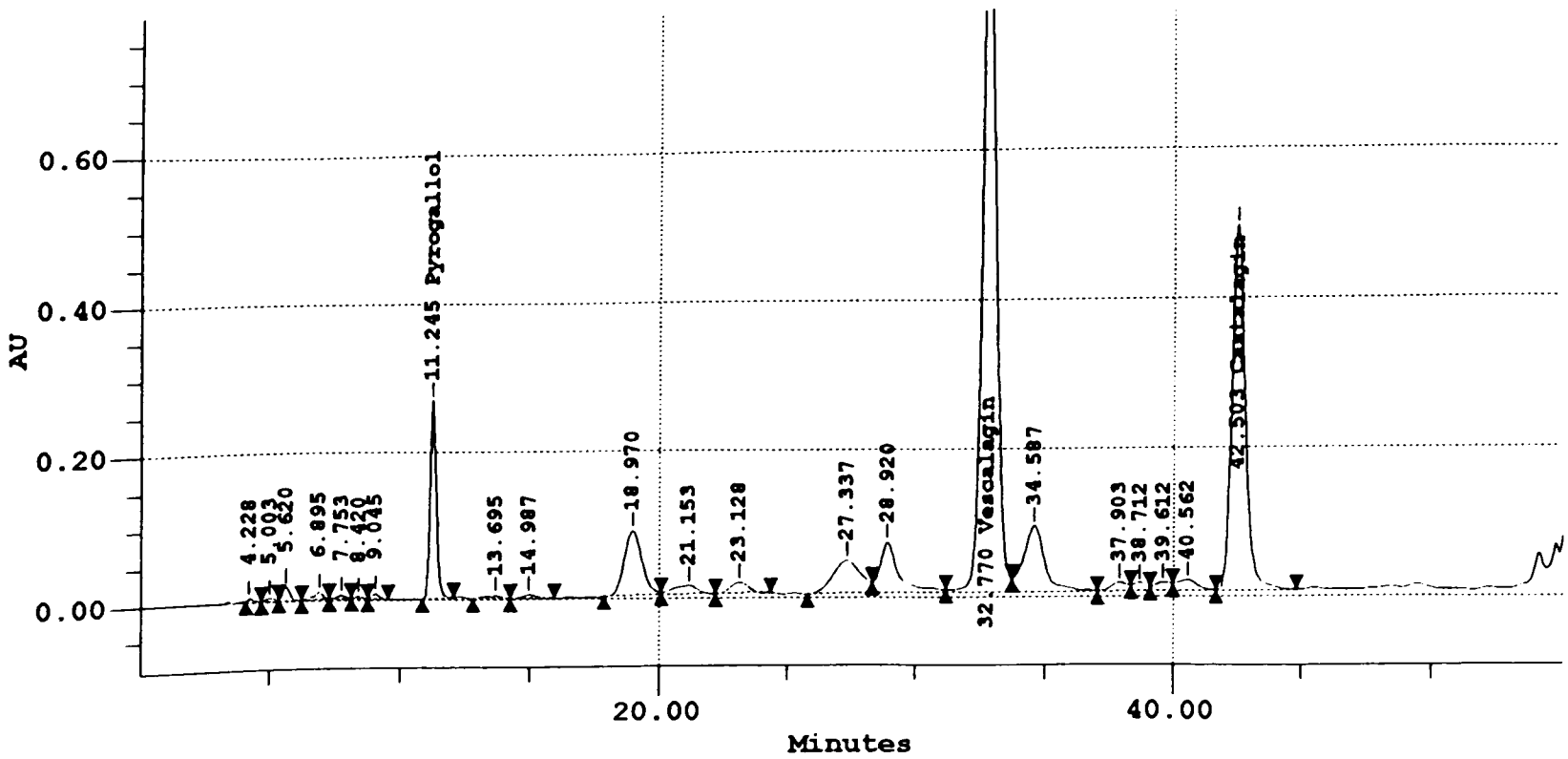
Additional examples of HPLC chromatograms and peak spectra. Chromatograms illustrate the better separation that was eventually obtained than that illustrated on page 40. The chromatograms illustrated here were all obtained using a newly installed HPLC system at the Ecole Supérieure du Bois, Nantes. The gradient was the same as that described on page 39.

Chromatogram and peak spectra illustrating the main identified ellagitannin peaks.

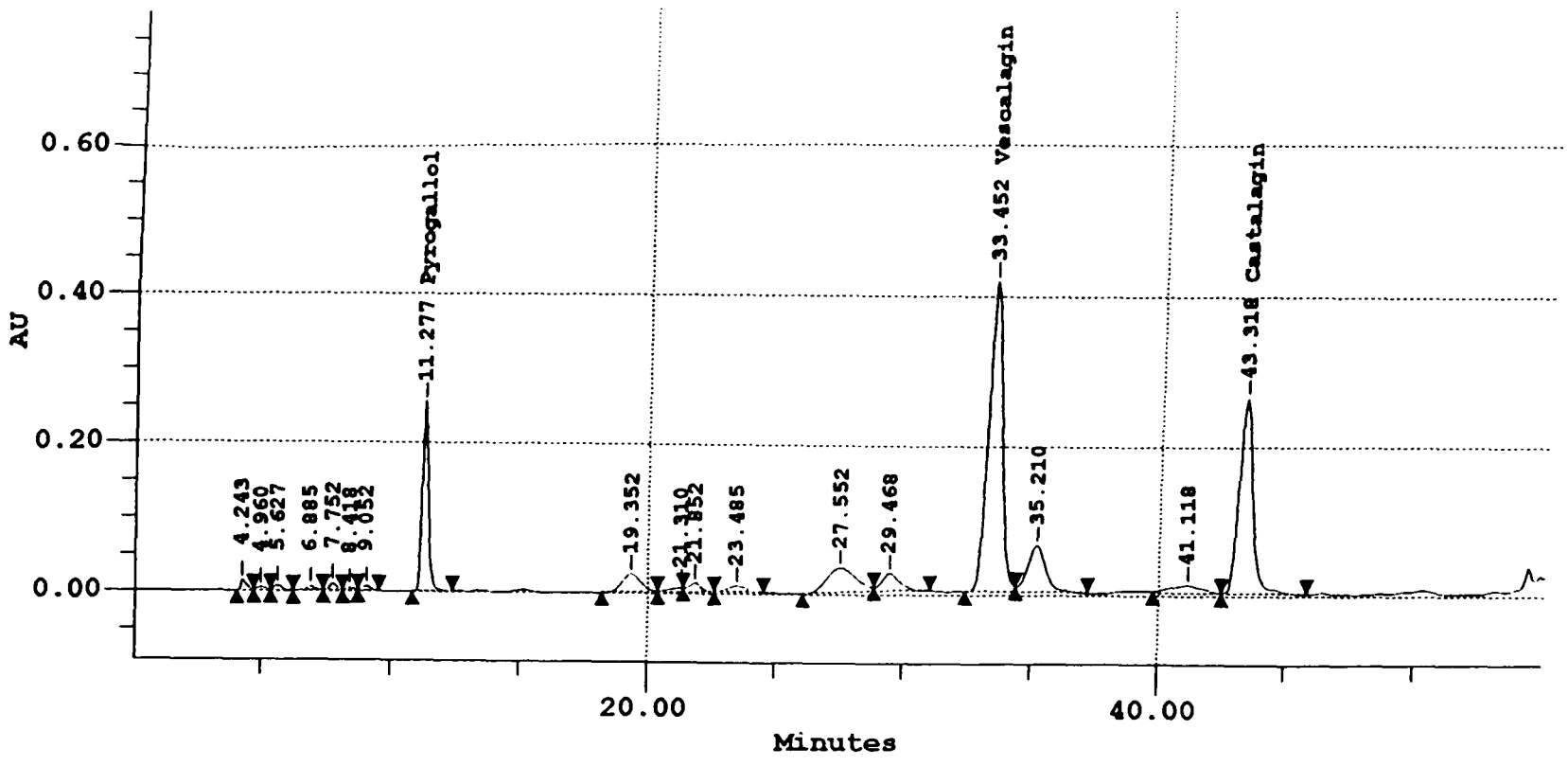


Chromatograms from Russian and English oak samples described in section 4.7.

Oxford tree - heartwood age 0-5 years.



Russian tree 3 - heartwood age 0-5 years.



Russian tree 3 - heartwood age 40-50 years. Interference of roburin B by gallic acid is visible.