Accelerated Seasoning of Manuka and Oak Wood Chips Destined for Wine and Spirit Flavour

Zhongyi Sun

A thesis submitted in partial fulfilment of the requirements

for the degree of Master of Applied Science

AUT University

Auckland

March 2013

Table of Contents

List of Tables

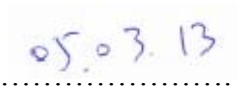
List of Figures

Appendices

Attestation of Authorship

I declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material formerly published or written by another person, which to a considerable level has been accepted for the qualification of any degree or diploma of a university or any other institute of higher learning. All the reference material used in this thesis has been fully referenced.

Signed 

Date 

Acknowledgement

I would like to thank my supervisor, Dr Owen Young for his great effort and extensive knowledge for this research project. With his superior insight and vision in the science, Dr Young always had brilliant ideas and suggestions to guide my works on the right track at all time. His ability to find the value and essence from seemingly irrelevant events is really impressive, so it is truly a great learning experience to work with him. I would also thank my co-supervisor, Dr. John Robertson for his support in the use of GC instrument, and also Dr. Michelle Yoo for her assistance in the submission of my thesis.

A lot of appreciation also goes to Mr Andrew Vincent from South Pacific Timber Limited in Eden Terrace for the supply of the oak wood used in this project, and Bark 'N' Firewood Bin Ltd., Albany, for the supply of the manuka wood.

Last, but not least, the greatest appreciation goes to my family: my wife Grace, and my two sons Kevin and Victor for their unconditional support, and love.

Abstract

Based on the concept of geographic exclusivity, a number of research projects have been conducted at AUT as an effort to explore the applicability of winemaking by using a number of unique New Zealand wood species other than traditional oak to age wines or whiskies. One of the researches has shown that manuka (*Leptospermum scoparium*) previously dry toasted to about 200°C, can substitute for oak in wine. However, two New Zealand wine experts have remarked that the desirable flavours of toasted manuka are overlaid with a flavour note reminiscent of when oak wood is unseasoned prior to barrel construction and toasting. Desirable as some flavours may be, if manuka is to be used as an alternative to oak, this species may also have to be subject to a seasoning procedure.

Designed to simulate natural seasoning but accelerated by extreme conditions, leaching due to water (but not fungal colonisation) was used to treat the wood while parallel treatments were also applied to American oak (*Quercus alba*) as a control. The objectives of the study are to compare the different seasoning methods for manuka which may affect the flavour of either wine or spirit, and to build knowledge about manuka seasoning.

Both manuka and American oak woods were cut into chips 20 x 10 x 4 mm and received various leaching treatments at four different conditions: none; boiling under reflux for 1 h; reflux for 8 h; soaking at ambient temperature for 3 wk. A toasting with 200 °C for 2 h (light toasting) or 210 °C for 3 h (dark toasting) was also applied. With a range of combination from these leaching and toasting methods, a total of 16 treatments were thus established. Weight losses and changes in CIE colour space were monitored at both stages of leaching and toasting, and ultraviolet absorbances due to leaching were also recorded. Subsequent infusions for the toasted chips after the 16 treatments were done using 60% v/v of ethanol. Ultraviolet absorbances and gas chromatographic analyses were both conducted for the 16 infusions using authentic whisky as reference. Meanwhile, semi-formal sensory assessment for these infusions was conducted, and weight changes for the infused chips were also recorded.

Leaching always resulted in weight loss, except in the situations where toasting

preceded reflux. Fewer changes in CIE colour were observed in manuka chips, and ultraviolet absorbances due to leaching were found much lower on manuka curves, both indicating manuka was more stable to leaching. Manuka had greater weight losses than oak on toasting. The absorbance due to whisky in the UV range is much lower than for the experimental infusions, suggesting that the quantity of woody matter in authentic whisky is lower than in the current infusions, in turn suggesting less exposure of commercial whisky spirit to wood. GC results showed that oak infused more strongly, that parallels the results from UV absorbance analyses. The semi-formal assessment confirmed only treatment for oak with dark toasting to be more mellow and lower in a woody note.

The aim of the research is not to emulate existing whisky styles, but rather to produce a whisky-coloured spirit that is closely identified with New Zealand. Current research has discovered the limitation of manuka due to its strong resistance to the treatments. It is recommended that more wood species are to be explored for this purpose where totara has already shown a lot of potential (Young *et al.* 2010). Therefore, The key words for future research might include “totara”, “dark toasting”, limited exposure”, “esters”, and “caramelised sugar” etc.

Chapter 1

Introduction

1.1 Alcoholic beverages

Alcoholic beverages are consumed by people all over the world as part of the diet each day partly as a thirst quencher for drinks, but also for its relaxant and euphoric effects. There are many types of such beverages with different alcoholic levels, and may be broadly classed into beers and similar low alcoholic drinks, wines and spirits. Beers typically range from 3 to 6% alcohol by volume (ABV), wines range from 9 to 16%, while, spirits have higher ethanol concentrations ranging from a minimum of 38% by law in New Zealand and Australia (FSANZ) with no specified upper limit, up to 95%, a point where alcohol is azeotropic with water, which means no change of alcohol concentration by boiling. Anyway, it seems obvious that the concentration must be lower than concentrations that would yield heat on water addition. The most well-known spirit perhaps is whisky with its ethanol level typically around between 40 and 43%.

1.2 The current New Zealand alcohol industry

The beer brewing industry in New Zealand has enjoyed high returns thanks to the deregulation of the liquor industry in 1999. As a relatively seasonal drinks, beer is very popular in New Zealand– it was the 16th highest beer consuming nation per capita in 2008 (Stuff.co.nz 2009). While the market competition remains intense, it seems a trend has been emerging that beer consumption per capita is decreasing in New Zealand. However, it still maintains the most popular alcoholic beverage in New Zealand, representing 66.3 per cent of total alcoholic beverages consumed (Stuff.co.nz 2009).

From a negligible production base of fortified wines in the 1950s, the New Zealand wine industry is worth $1.5 billion to New Zealand's gross domestic product in 2009. Its exports now represent 2.2% of total goods exported. Wine is now the 11th largest export (up from

19th in 2003), behind only dairy, meat, fruit and fish amongst other foods. The production increase was particularly strong in the last 10 to 15 years (Figure 1). It has grown at an annual rate of 9% over the last 10 years due to the strong and growing demand in all major export markets (Price Water House 2007).

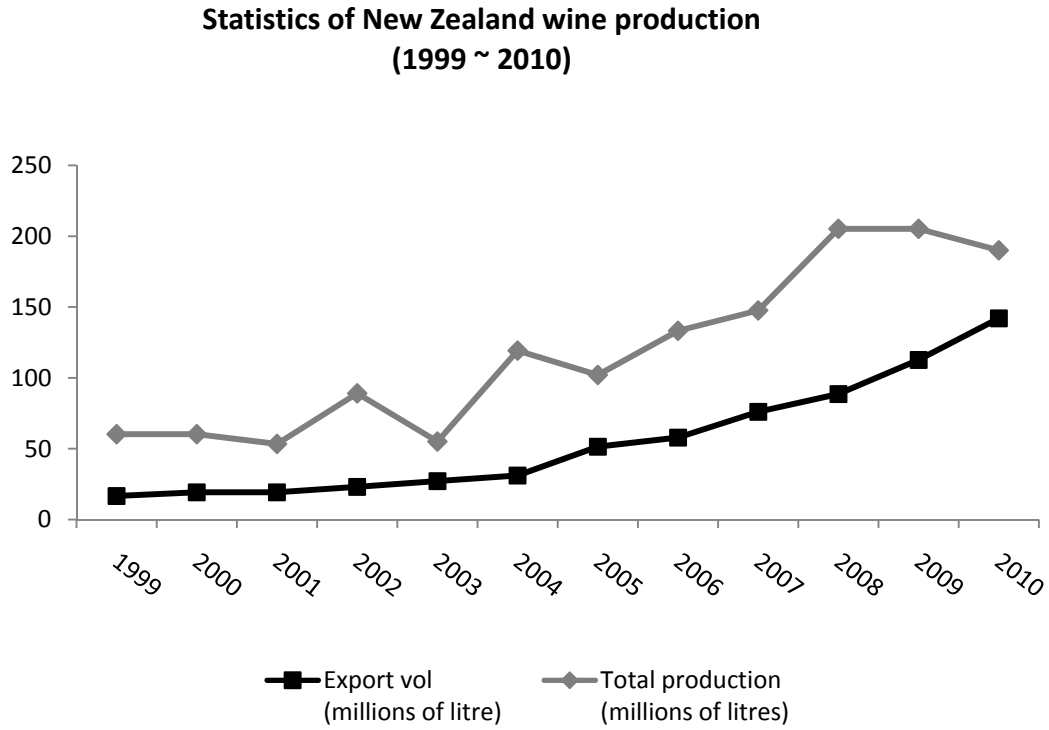


Figure 1 Development of the New Zealand wine industry 1999-2010, data from (New Zealand Wine 2010)

Nonetheless, New Zealand is not a key player in this market both at the aspects of production and consumption (Figure 2). New Zealand is a minor player.

France, Italy and Spain, the three European nations in the Mediterranean region, account for half of the total wine production in the world while New Zealand only took about 0.5% of the total in 2006.

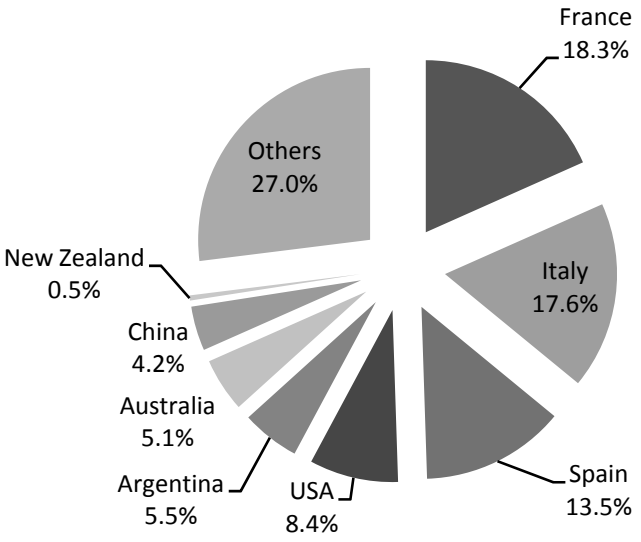


Figure 2 World wine production by country in 2006, data from (The Wine Institute 2009)

The success of the New Zealand wine industry in recent years has been attributed to its ability to occupy the premium quality market rather than competing with some large-scale producers for the oversupplied lower-priced market. As a country with a beautiful landscape and a purported 'clean, green' image, New Zealand has a reputation as a producer of high-quality foods. Premium prices for New Zealand wines have been achieved in the UK market, and second only to French wine in the US and Australia markets among other major wine-exporting countries (Price Water House 2007).

In common with other New World wine producers, New Zealand vintners use European grape varieties – merlot, zinfandel, syrah, chardonnay etc. – to produce often excellent examples and variations of the original European styles. However, they are fundamentally copies that lack the appellations of their origins, and can struggle to establish a point of difference in a commercially crowded international market (Young *et al.* 2010). Young and others 2010 described a novel approach to this problem. They conducted a number of pilot experiments with New Zealand woods, presented to wine as toasted chips, and the results have

shown that some species, notably manuka, yields flavours as attractive as oak as models of how novel wines could be developed. In the present study this approach has been extended to spirits as will become clear later in this chapter.

Spirits are produced by fermentation followed by distillation. The distillation is a refining process to increase the alcohol concentration because the fermentation stops when the alcohol level reaches about 18% (v/v).

New Zealand spirits are almost always distributed with international brands with *Johnnie Walker* (Scotch whisky), *Smirnoff* (vodka) and Diageo (gin) among the most popular and best selling brands. While some brands are made in New Zealand such as *42 Below* for vodka, many are imported and whisky, with the dominant producer being Scotland.

Apart from the NZ made *42 Below*, gin, whisky and other coloured spirit are also locally produced. These include Christies (gin), a Lion Nathan owned brand, Milford (Whisky), a famous brand from Willow bank Distillery of Dunedin.

1.3 Whisky

As one of the most popular distilled beverage worldwide, whiskies are highly variable in terms of base product, alcoholic content, and perceived quality. Typical whisky is produced by fermentation of various malts, grains or their combinations, followed by distillation and ageing in oak barrels, which in turn are highly variable. However, the requirement for ageing (or maturation) in wood is not entirely universal–for example, American corn whisky does not necessarily need to be aged to deemed ready for sale.

It has long been known that oak barrels have an effect on both wines and spirits in terms of flavour, clarity and colour. This is particularly true for spirits, in particular whiskies, which acquire all of their colour and much of their flavour from contact with oak. It is not commonly realised that when whisky is distilled, the distillate is clear and colourless. All whiskies extract colour from oak, but it is not commonly known that in some whiskies, caramel colouring is often used prior to bottling to give a desired colour richness and well-aged appearance (Whisky Megazine 2006).

However, as whisky is a strictly regulated spirit, additional flavourings and colouring compounds are restricted or even prohibited in whisky of some types. With the exception of blended whisky, the addition of colouring and flavouring is not allowed in all other types of American whisky (Justia US Law 2011). Therefore for these types of whisky, oak barrels are the main or only source of flavour and colour acquired during the ageing process.

The type of whisky is strongly related to the type of oak barrel and the duration of ageing, which can vary extensively and are legally defined by the product name. Barrels can be categorised either as new, used or partially used, or as charred or uncharred ones. Under the U.S. Federal Regulations, if American corn whisky is to be matured in oak barrels, it must be matured in either new or used uncharred oak barrels in a short ageing period, usually less than 6 months. In contrast other American whiskies must be aged in charred new oak containers. Scotch whisky is usually matured in barrels which have been previously used for storage of other alcohol such as sherry or bourbon, and the maturation takes at least three years and one day before it can be termed as Scotch whisky (The Scotch Whisky Regulations 2009). However, most of the Scotch whisky is matured far beyond three years, often over 10 years for premium brands.

The relation between the duration of ageing and the quality of whisky is not necessarily as commonly understood as "the older the better". Usually, an age of a decade or two is long enough for a whisky to achieve an optimal level of flavour and colour through the aging process. Additional aging in a barrel may only add a rarity value to the whisky.

1.4 The evolution of alcoholic beverage containers

Historically, an amphora, a type of ceramic vase, was the choice for transporting and storing wine and other alcohols before wooden barrels were later invented by ancient Romans as a better replacement (Sanderson 2010). Amphora first appeared around 3500 years before present and became common in the ancient world as a multi-purpose container for food items. However, being made of clay, amphorae were brittle and prone to break. In contrast to wood, which is a tougher material, barrels made of palm wood were probably first used for wine transportation by ancient Mesopotamians (Wikipedia 2010). However, palm wood is a not

an ideal material to bend and fashion into barrels, and according to this Internet source a wide range of wood species including oak, chestnut, pine, redwood, and acacia were subsequently used to craft wooden barrels. Local availability determined which wood was used. None were as suitable as oak for wine storage. The major reason was its compatibility with wine.

(a) (b) (c)

Figure 3 The historic evolution of wine container from (a) Roman amphorae (Ancient Touch 2009) to (b) Oak barrels (Salut Wine Co. 2009) and (c) Stainless steel tanks (Sierra Stainless 2009)

It has long been known that wine quality improves or certainly does no deteriorate when oak barrel are used in fermentation or maturation. Oak was also favoured by some other reasons: it was abundant in the northern hemisphere across temperate, and subtropical to tropical forests (Nixon 2006); it had superior physical properties in that the wood was more straight-grained with fewer faults and better strength, pliability and lack of porosity than many other species.

Stainless steel tanks are currently very common in the liquor industries. New Zealand has been a pioneer in this respect, deriving the technology from dairy sector where such containers are being used heavily. The main advantages include cost effectiveness because it

is permanently reusable, is non-reactive, is easy to clean and sterilise to eliminates microbial spoilage, and is suited to precise temperature control for example double-jacket containers that provides efficient heat exchange (Mueller 2009). Despite of the multiple benefits of the new technology, not all wine producers use stainless steel for fermentation and maturation. There are several reasons for this - Oak imparts flavours that stainless steel cannot, and premium wine producers avoid connection to stainless steel because they rely on the romance of barrels in cellars to sustain their market position.

1.5 Characteristics of oak barrels

As indicated in the previous section, oak exhibits the desired strength when shaped into a cask (Gunther 1986), the required flexibility and water-tightness, and importantly a lack of overpowering flavour compounds (Margalit 2004). Within the genus *Quercus*, three species are normally used in the liquor industries. These are French oak, *Q. robur* and *Q. sessilis*, and North American oak, *Q. alba*. These oak woods have different structures that dictate the way barrels are made, and the different compositions of the oaks are responsible for distinct sensory characteristics in liquor.

Making oak barrels can be separated into four steps, cutting, seasoning, toasting and making with the traditional method of European cooperage. The wood logs are cut along the grain to the desired length and then split into bolts followed by stave wood with suitable thickness (Jackson 2000). Importantly for this thesis, the stave wood then is stacked outdoors for drying by means of exposure to the weather for up to three years during which time the harshest tannins are leached out (Wikipedia 2010), a so called seasoning process. Seasoning is discussed in more detail later in this chapter.

Figure 4 Oak staves were stacked outdoors to undergo natural seasoning process for three years at the purpose of *wood softening* through incomplete drying of the wood, along with hydrolysis and degradation of the wood components (Nishimura 1983)

Subsequently, it is followed by a toasting process which involves an open fire or steam applies to the seasoned wood to bend the staves (Figure 5). Iron rings with various diameters are held in places around the staves with the pressure exerted by the tensile force of stretching staves (Figure 3). Depending on the oak species and alcohol to be aged, the barrel is then toasted at various levels from light to heavy to generate different effects on the spectrum of compounds and flavours the oak will diffuse into the maturing alcohol (Woodworth 2009). Toasting is also discussed in more detail later in this chapter.

Figure 5 Oak barrels being toasted at a cooperage where barrels are being bent under a brazier of burning oak blocks (Neeley 2009).

Several techniques have been developed to extend the barrel life because oak barrels gradually lose flavour compounds over prolonged use normally in three to five years of time. One technique involves shaving off the inner layers followed by toasting at original degree (Jackson 2000). Another similar method includes shaving same as above then inserting toasted new staves (Miller 2009). One technique is also used to improve the barrel performance to reduce the extraction differences due to barrel-to-barrel variation and maintain a constant oak character in the alcohol by keeping new and used barrels at a constant ratio and blending before bottling (Jackson 2000).

All wood, oak included, is slightly pervious to air and thus the oxygen in it. The concentration of oxygen in wine is much lower than water exposed to air. This is because the loss of CO_2 during fermentation acts as carrier of oxygen such that fermentation is an anaerobic process. When fermentation ceases and wine is stored in an oak barrel, the law of mass action means there is a net flow of oxygen through the wood into the wine. This must go beyond solubility effects because a range of chemicals in wine are capable of being oxidised. However, the rate of diffusion across the wood typically does not reach the point of over-oxidation and spoilage. Mass action also dictates that some water and alcohol is lost to the atmosphere. The net result is that an accumulationofflavour and aroma intensify through

evaporation while an initially astringent taste is softened by the oxidation of tannins that derive from grapes and oak wood. As mentioned above, mass action effects also apply to desirable toasted oak compounds that diffuse out of the wood into the wine.

1.6 Oak barrel alternatives

Oak barrels are expensive to construct and maintain. The cost of a barrel can vary in a wide range depending on the wood source, process of making, market demand and reputation or the barrel maker. A standard 225 L American oak barrel costs about US$ 300 and a French oak barrel at same size can double the cost to US$ 700 plus (World Cooperage 2011). Also there is an accompanying cost for barrel maintenance (Manuel 2002) and storage of wine in barrels rather than large tanks requires a larger and more costly storage building than if wine were stored in huge tanks. It has been estimated that barrel maturation adds about US$2 to every bottle of wine, a significant fraction of the retail price.

However, this cost can be greatly reduced by the application of oak alternatives which are basically oak wood in various forms and sizes including oak staves, chips, cubes and powder. Moreover, barrels do not have to be made, an intrinsically expensive process. Thus, oak chips – to cite a popular form –can add a significant amount of oak character at a small fraction of the cost of new barrels (barrelbuilders 2008). The principle is based on the notion of oak-in-wine rather than wine-in-oak. Oak barrel alternatives also reduce the cost associated with topping up and lost volume via evaporation (Jackson 2000) and work well in long-lasting stainless steel tanks.

Apart from the cost benefits of oak wood in these new forms, these alternatives significantly improve productivity by reducing maturation time, which is achieved by the increased ratio of surface area to volume since the entire surfaces are immersed not just the inner surface of the barrel. Oak chips have the ability to impart intense oak flavouring in a matter of weeks while traditional oak barrels would need a year or more to show a similar intensity (Wikipedia 2010). However, the quick process makes the extraction of flavour compound less manageable. As a result, it is claimed that some of the oak alternatives are likely bring a monochromatic, harsh profile, missing a delicate mixture of aromatic

compounds that a wine barrel usually can deliver. However, it is not known how true this is or if this is merely propaganda by vested wine and barrel interests.

The most common oak alternative is oak chips (Figure 6) which come with a diversity of different factors including geographical origin, chip size and toasting level etc. (Gawel 2009). All these factors impact on wine character differently, and the diversity available gives winemakers many options for experimentation. They can be added during either fermentation or maturation except right before bottling. It is said they are best added during fermentation so that the oak flavours integrate well with the wine (barrelbuilders 2008). The use of oak powder (Figure 6) is less common than the use of chips, but they serve as a very practical alternative when oak character is required at fermentation stage(Gawel 2009).

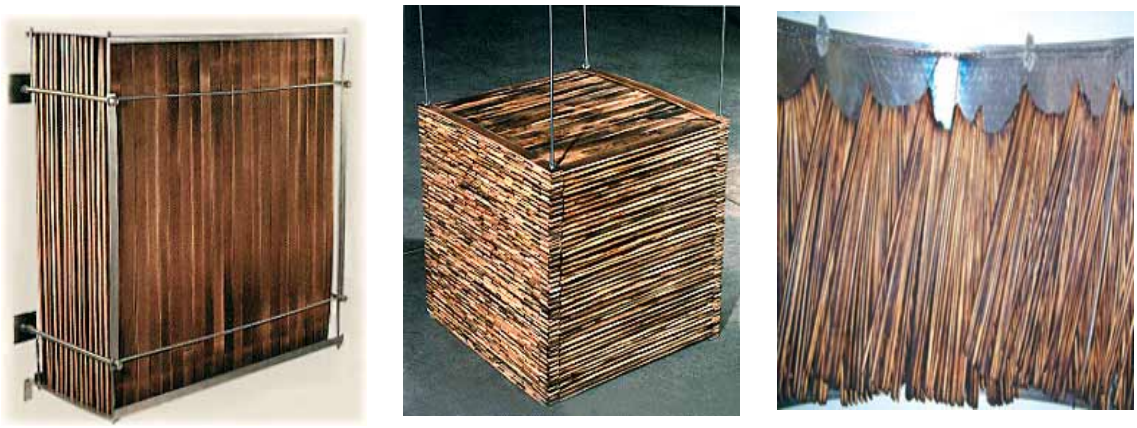
(a) (b) (c)

Figure 6 Oak alternatives in smaller sizes (a) toasted oak chips (ibrew 2009) (b) oak powder (Suber Lefort Int 2010) (c) oak cubes (WeekEnd Brewer 2009)

Oak cubes are another alternative (Figure 6). One study shows that oak cubes are superior than oak chips due to a standardised optimised shape and size and thus toasting level (Alexander 2004), factors that cannot be achieved with chips. Oak cubes are about 6 mm thick and wine can fully penetrate to this depth (2 x 3 mm). Oak cubes replicate the complex flavours of a barrel better than chips because the cubes are able to have multiple toasting levels like a barrel would.

Oak staves or planks are other useful techniques (Figure 7). Oak character is imparted by wine through holding staves or planks vertically or by stacking them at the bottom of tanks

from where they can be easily withdraw at will (StaVin 2009). Although these practices are a little more costly than using oak chips, some tasters believe they do not leave bitterness or harshness that oak chips may cause (Manuel 2002), although it is difficult to see how this claim might be true. Equipped with other contemporary technologies like micro-oxygenation, these oak alternative can yield a consistent good quality as the wine undergoes temperature controlled fermentation and maturation.



(a) (b) (c)

Figure 7 Oak alternatives (a) Oak stave packet held vertically in a tank (b) Oak stave packet stacked at the bottom of a tank (c) Oak stave fans that are staves bundled together and suspended in a tank (StaVin 2009)

The use of alternatives is currently seen in many cheaper and mid-priced wines mainly due to the reduced costs, but is still not favoured by premium brands. The reason for this is probably one of image. Oak barrels in a cellar evoke an image that chips in a stainless steel barrel cannot match. Oak alternatives are even prohibited in the production of premium wines in some countries. The oak chips practice was just approved in recent years in Europe (2006) and the USA (1993) with many restrictions still imposed, probably because of vested commercial interests of industry participants, e.g. barrel makers. However, nowadays the oak alternatives have been proved that it can compete with barrels for equal quality.

Regarding oak's influence on wines and spirits during maturation, it is important to realise that the flavours imparted by oak are the result of seasoning (claimed) and heat

treatments (verified) on oak wood that allow complex modifications of the major components of oak to occur.

1.7 The composition of oak and its flavour chemistry

The major constituents of oak, and indeed all woods, are cell wall ingredients which consist of the three main building blocks: cellulose (45 to 50%), hemicelluloses (20 to 25%), lignins (25 to 35%) plus tannins (5 to10%) and small amounts of lipids and terpenes (0.1 to 0.5%) (Chatonnet 1998).

The three major components are linked to each in such a way as to impart oak's strength, resilience and impermeability to water. Cellulose microfibrils are locally grouped together in bundles which are deposited in different planes, forming a stable interlacing framework, and this structure is meshed into a matrix of hemicellulose and lignin polymers (Figure 8) (Jackson 2000), in a way analogous to steel reinforcing rods in a matrix of concrete. Within the structure, cellulose supplies strength and resilience, hydrophobic lignins fill the spaces in the cell wall and limit water permeability, while hemicelluloses act as binding substance (Jackson 2000).

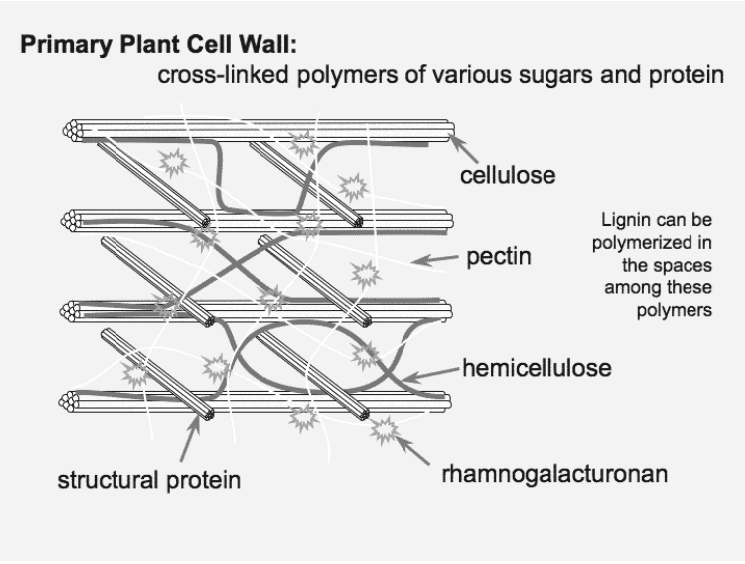
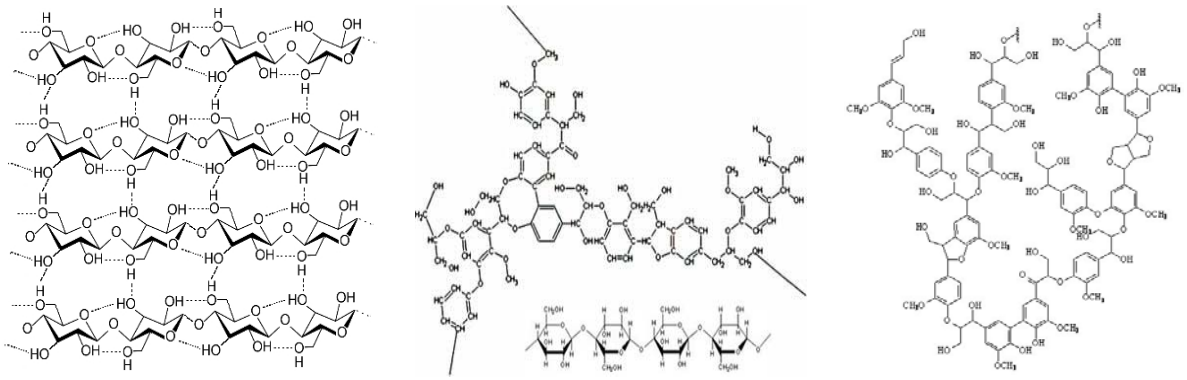


Figure 8 Primary oak cell wall (Koning 1994)where cellulose forms an enduring interlacing framework, and hemicelluloses along with pectins hold the cellulose and lignins together

1.7.1 Cellulose

Cellulose is the most abundant and major structural component in oak. Chemically it consists of linear chains of glucose units linked by straight β (1→4) bonds. Inside the chains, the multiple hydroxy groups are held each other into a spatial network via hydrogen bonding, which holds the chains firmly to form the structural building block microfibrils (Figure 9(a)).

Cellulose may not be directly involved in the development of oak flavour during wine maturation due to its high resistance to both enzymatic and non-enzymatic degradation (Jackson 2000). However, under the action of seasoning and toasting, cellulose can undergo low-level decomposition such that pairs of glucose units are released during partial hydrolysis, resulting in disaccharide compound cellobiose, supplying nutrition for *Brettanomyces* growth and subsequent spoilage in wines (Gunther 1986). In addition, cellulose may have a role in the ageing of whisky where heavy toasting and charring of the barrel may promote sufficient degradation and thereby facilitate extraction of the resultant compounds (Crum 1995).



(a) (b) (c)

Figure 9 Molecular structures of the major components of oak cell wall. (a) Cellulose (Laghi 2007) (b) Hemicellulose (Electregy 2009) (c) Lignins (Gregory 2007)

1.7.2 Hemicellulose

In terms of the structure, hemicellulose (Figure 9 (b)) contains many different sugar monomers mainly pentoses and hexoses while cellulose consists purely of glucose units. The chains of hemicellulose are shorter, two dimensional and heavily branched, having a

random, amorphous structure with weak strength (Electregy 2009).

Unlike cellulose, the hemicellulose class of compounds have a major impact on sensory characteristics in oak aged wines. During the toasting process, hemicellulose is partially decomposed into smaller components which participate in chemical reactions, and ultimately produces compounds such as furfural, maltol, cyclotene and ethoxylactone (Crum 1995). These aromatic compounds are characterised as having toasty and caramel aromas.

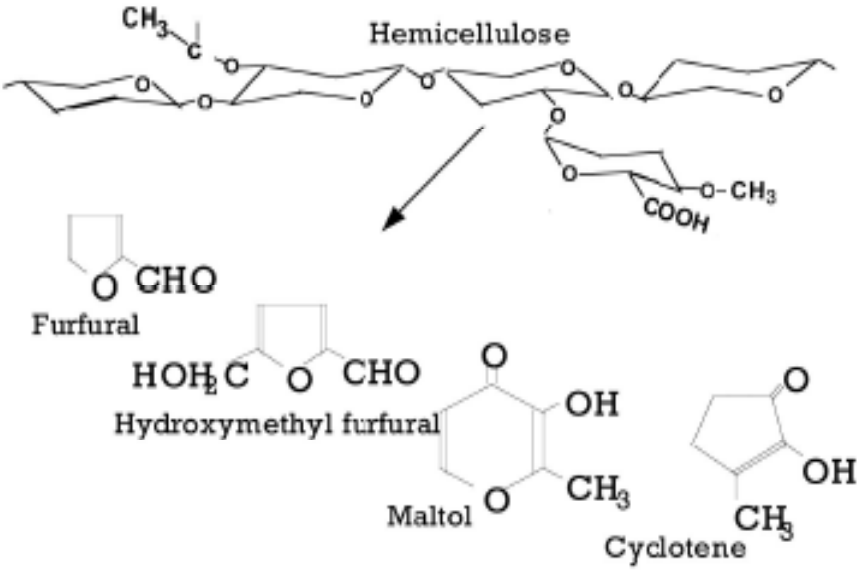


Figure 10 Production of known toasty flavours by breakdown of oak hemicellulose (Nishimura 1983)

Hemicellulose can form additional volatile compounds through Maillard reactions in the presence of amino acids and heat (Chatonnet 1998). Most compounds generated are also characterised as toasty and caramel-like aroma. It is considered that the enolic group was specifically responsible for the toasty aroma of these compounds (Hodge 1967). The hemicellulose degradation process involving toasting and charring is rather complex but is clearly very important in the development of toasty flavours (Gunther 1986).

1.7.3 Lignin

As a hardwood lignin, oak lignin (Figure 9 (c)) is large, complex, three dimensionally

branched polymer mainly coniferyl and sinapyl alcohols that can generate a variety of phenols based on the guaiacyl and syringyl nucleus (Zoecklein 1995; Jackson 2000). The structure of lignin is similar to a crystal lattice that provides skeletal support to other components in the wood (Crum 1995).

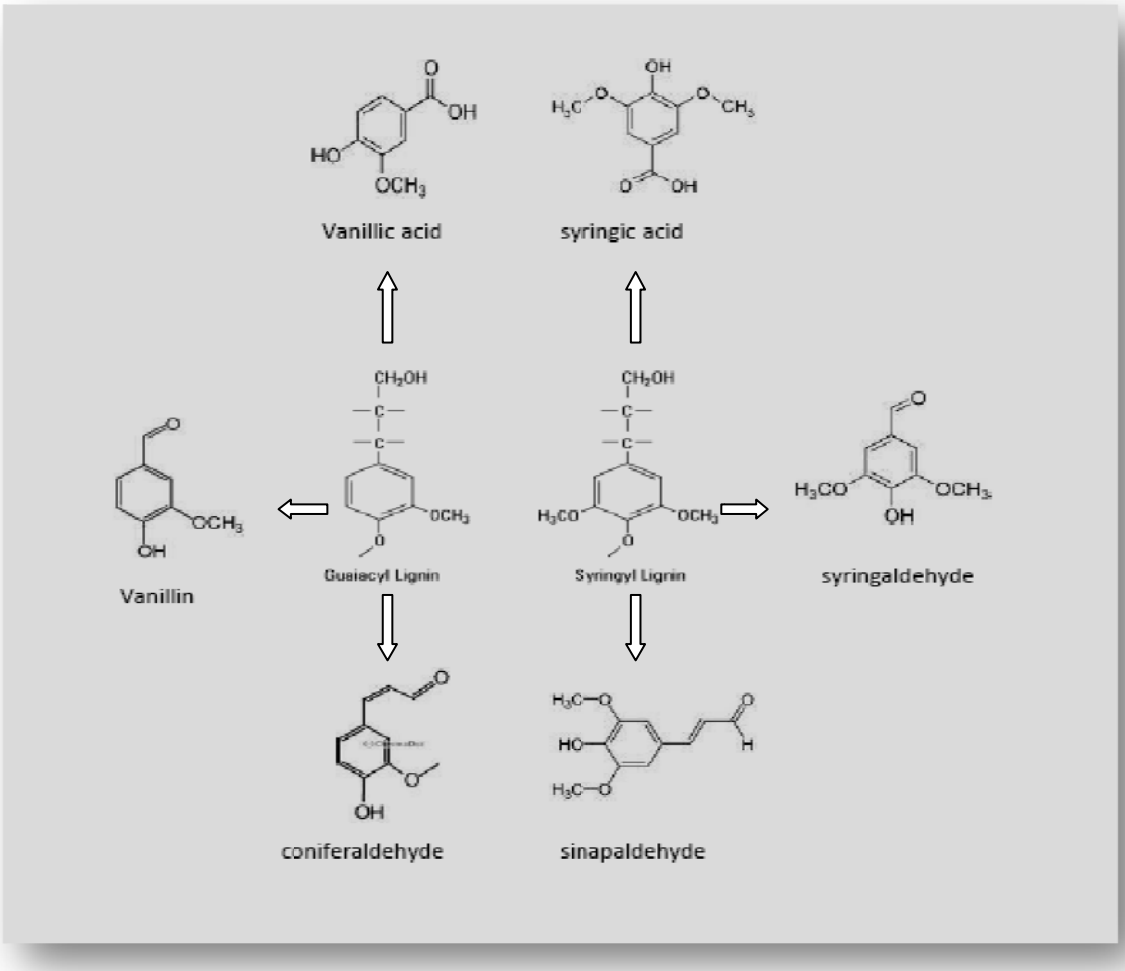
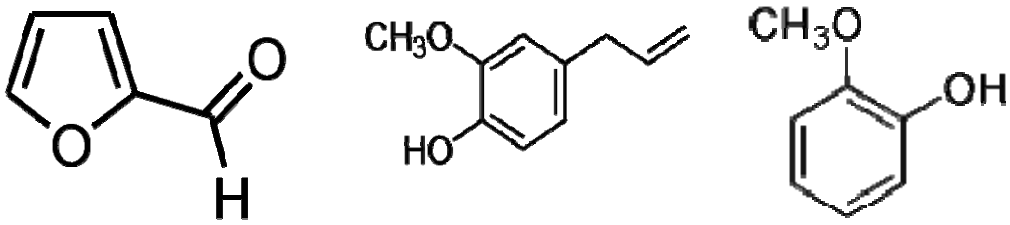


Figure 11 Phenolic aldehydes formed from the degradation of lignin of the guaiacyl and syringyl building blocks, redrawn from (Emerald 2009)

The majority of aromatic compounds in wine resulted from exposure to oak are produced by lignin degradation. Lignin degradation products can be classified into phenolic aldehydes and volatile phenols. With the action of alcohol and oxygen, the degradation involves lignin ethanolysis and subsequent oxidisation (under mild acidic condition) to release aromatic compounds including vanillin, vanillic acid and coniferaldehyde from guaiacyl lignin; syringaldehyde, syringic acid and sinapaldehyde from syringyl lignin (Figure 11). Of

all these compounds, vanillin with its characteristic aroma at very low odour threshold is the most significant and the most recognised. Gentle heating at about 200°C enhances degradation, leading to the production of aromatic phenolic aldehydes, notably vanillin (Jackson 2000). The degree of toasting will determine the degree to which lignin is broken down into aromatic compounds.

At toasting/charring temperatures higher than 200°C,volatile phenols as opposed to phenolic aldehydes (Figure 11), are also produced (Gunther 1986). In this process, lignin is degraded into simple structured phenols typically furfural associated with a caramelised aroma, eugenol with a spicy, clove like aroma, and guaiacol perceived as smoky (Chatonnet 1998).



(a) Furfural (b) Eugenol (c) Guaiacol

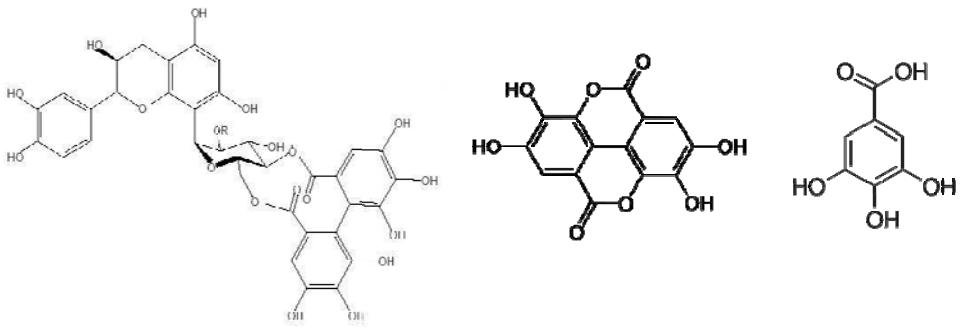
Figure 12 Typical volatile phenols produced by the breakdown of lignin complex through extra heating

There is also a small proportion of lignins, called native lignins, which can directly dissolve into wine as these are ethanol-soluble (Jackson 2000).

1.7.4 Tannins

Barrels used for liquor ageing are constructed from oak heartwood, and heartwood tannins are the most abundant constituents of oak wood which are extracted during ageing. Oak tannins are typified by the ellagitannins (Figure 13a), which are chemically related to lignin.

Ellagitannins are a class of hydrolysable polymers formed when ellagic acid (Figure 13b) and sometimes gallic acid (Figure 13c) esterifies with the hydroxy group of a polyol carbohydrate such as glucose (Belitz *et al.* 2009). The esterification yields molecular complexes typically above 500D, which have the protein-binding ability exploited in leather tanning, and which are responsible for many of their biological activities and properties including bitterness and astringency (Puech *et al.* 1999; Jackson 2009), which are caused by tannins binding with proteins in our saliva. Apart from the role as protein precipitating agents, tannins also have other diverse effects on biological systems working as metal ion chelators and biological antioxidants.



a b c

Figure 13 a) Ellagitannins (Tripod 2010), b) Ellagic acid, c) Gallic acid

To be discussed in more detail later (1.8.1), both the seasoning and toasting processes affect the degradation of the ellagitannins in oak. In the case of seasoning for oak staves destined for barrel production, the seasoning process progressively leaches out as well as hydrolyses the harshest tannins from the wood during the 10 to 36 month period (Vivas 1998). Hydrolysis of the tannins results in a reduction of bitterness due to higher perception thresholds of the resultant subcomponents. During toasting, there is a further breakdown of ellagitannins (Hale 1999).

At the same time oak tannins play an important role in spirit maturation through

oxidation and the creation of a particular fragrance. The tannins react with oxygen in the presence of a transition metal to release hydrogen peroxide. The peroxide oxidises alcohol to produce acetaldehyde which then combines with more alcohol to create the fragrant compound diethyl acetal in the spirit (Gunther 1986).

1.7.5 Lactones

It is believed oak lactones are present in the lipids in oak, but additional quantities can be generated during the seasoning and toasting processes of cooperage (Wilkinson 2009). They increase markedly during toasting or charring through dehydration of the resulting volatile compounds generated from thermal degradation (Garde-Cerdan *et al.* 2006).

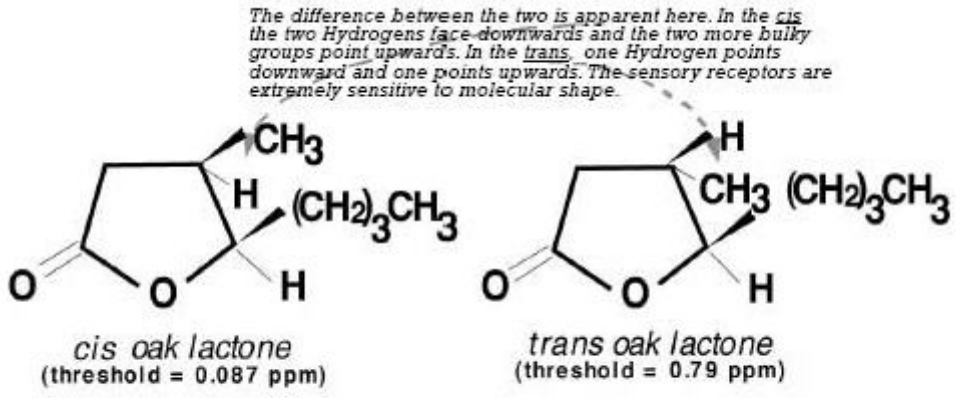


Figure 14 *cis*- and *trans*- isomers of oak lactone. Redrawn from (Gunther 1986)

Of the many oak-derived volatiles extracted during maturation, the *cis*- and *trans*-isomers of oak lactone (Figure 14) are considered to be the most important (Wilkinson 2009). Both are described as woody and coconut-like with the *cis*- version much more intense due to its much lower odour threshold (Gunther 1986). Although they are found in all oak woods used for cooperage, the content and ratio of *cis*- to *trans*- varies greatly among different species. *cis*-Lactones occur in much higher concentrations in American oak than European varieties (Gunther 1986).

1.8 The maturation of oak wood

1.8.1 Seasoning

Traditionally, oak wood undergoes seasoning treatment prior to barrel construction. Typically, sawn timbers are left in open air in large space. It serves a number of practical purposes, involving dehydration followed by a period of maturation, thus improving its aromatic qualities. One is to dry out the wood by reducing the moisture content from around 60% to about 15%. This moisture content is required to make barrels watertight in cooperage (Vivas 1998; Waterhouse 2001). Seasoning requires a simultaneous combination of all the factors (rainfall, temperature variation, UV radiation, fungi etc.), because simple dehydration is not enough to make a quality barrel (Vivas 1998; Cadahia *et al.* 2001).

Drying primarily stabilises the dimension of the wood, making the barrel watertight when in contact with wines or spirits. According to (Vivas 1998), oak planks undergoing seasoning usually take about a year to dry out regardless of weather conditions and the thickness of the wood planks. However, climatic condition with insufficient rainfall is adverse to wood seasoning as rapidly-dried wood will lead to broken wood fibres and delamination of ligneous segments, with most of the extractable elements remaining trapped in the wood mass.

Leaching involves rain water washing away part of the hydrosoluble substances which are mainly water-soluble ellagitannins, and are mostly depleted during the first 3 or 4 months of drying. This contributes to a significant decrease in the bitterness and astringency. Thus it has been found that total rainfall and rainfall distribution are both important in leaching effects (Vivas 1998).

The loss of the ellagitannins in the heart wood of oak during seasoning is primarily due to the action of fungal enzymes. Although ellagitannins are easily oxidised in a solution, Vivas (1998) suggested the chemical oxidation plays a minor role in the loss of ellagitannins. Also, leaching has virtually no effect on lignin degradation because lignin is insoluble in water. Rather, under seasoning condition, fungi colonise in the wood tissues during the whole drying

period. Colonisation requires a condition that drying must not proceed rapidly, and at the same time high moisture contents facilitate leaching process. By maintaining sufficient water content, the growth of this limited flora is encouraged. The fungi digest the cell wall and release enzymes into the tissues, liberating glucose and simple phenols including phenol acids. The ellagitannins are used as a source of carbon for the fungi species to develop. Therefore, it has been a standard practice to periodically spray water over the stave stacks to regulate the effects of the microclimate for the woodpiles (Vivas 1998).

Thus, unlike kiln-drying or other artificial drying method, drying by seasoning allows a fraction of the ellagitannins to be removed. The resulting wine tastes less astringent and bitter compared to artificially dried equivalents.

1.8.2 Toasting

Subsequent toasting of oak barrels (or chips) has its important influence on the resulting wine or spirit. The heating process causes thermo degradation of mainly the three components – cellulose, hemicellulose and lignin in oak, producing numerous volatile compounds as some of these were discussed in Section 1.7. Chemical bonds among the three polymers are disrupted, with hemicellulose and lignin being more affected than cellulose. Meanwhile, the structure and amount of oak tannins undergo major changes during toasting (Hale 1999). Approximately, toasting process accounts for the occurrence of 70% of the phenolic compounds found in wine (Chatonnet 1998).

The degree of toasting also affects the level of degradation of lignin and of ellagitannins remaining after seasoning. (Chatonnet 1998) found that medium to high toasting generated maximum levels of phenolic aldehydes like vanillin while very heavy toasting and charring of oak can convert phenolic compounds into their respective acids, and therefore lower the aromatic contribution of vanillin (Mosedale 1995). It was also found that increased heating led to a reduction in the content of ellagitannins and a corresponding increase in ellagic acid (Sarni *et al.* 1990).

Apart from its main function to increase the concentrations of aroma enhancing

compounds due to toasting, toasting also has the effect of reducing undesirable volatile compounds. The fat oxidation product (E) 2-nonenal is one of the important compounds volatilised during toasting. It has a low odour threshold, and is characterised as a sawdust-type smell and is not desirable in wine flavour (Crum 1995).

1.9 Aims of the study

Recent research at AUT has shown that wood chips from a number of New Zealand unique species, and previously dry toasted to about 200°C, can substitute for oak in wine (Young *et al.* 2010). This technological innovation provides a variety of distinct and previously unavailable flavour possibilities, as well as more flexible options in wine handling.

Although results with New Zealand woods have been encouraging, particularly for toasted manuka (*Leptospermum scoparium*), evidence from the research shows that the desirable flavours of toasted manuka are overlaid with a flavour note reminiscent of when oak wood is unseasoned prior to barrel construction and toasting.

As discussed earlier, it seems clear that leaching and colonisation could be replicated by exposing chips to the New Zealand climate. Considering oak chips made for example from French oak (*Quercus sessilis* or *Quercus robur*), if seasoned for many months, toasted, and used in wine or spirits, it will be highly likely that the flavours of authentic French oak barrels could be closely paralleled. Obvious as this approach seems, there is no known literature on this concept. Seasoning of oak – and of oak chips if this is done at all – remains an arcane activity, not helped by a language barrier and an understandable lack of transparency to maintain the international wine barrel market.

In choosing a project that can be done in a year of full-time research, it was practicable to focus mostly on only one of the two major aspects of seasoning, leaching due to water rather than colonisation due to fungi. Two woods were chosen, the New Zealand native manuka and American oak as the control, and these have been subject to a wide range of leaching treatments. At the outset, it was decided to source single samples of wood, but realising that variations of extractables in wines and spirits depends on species, geographical locality, the tree itself, and position within a tree, as discussed by many authors for example, Marco *et al*.

(1994), Masson *et al*. (1995).

The objectives of the study are to compare the different accelerated seasoning methods for manuka which may affect the flavour of either wine or spirit, and to build knowledge about manuka seasoning. After exposure to seasoning treatments, manuka and oak chips have been compared for differences in their extract profiles in 60% alcohol, the base concentration used in whisky production. The approach here has been largely physiochemical but with some sensory analysis.

Chapter 2

Materials and Methods

2.1 Wood

2.1.1 The choice and sourcing of wood

As discussed earlier, two species of wood, manuka (*Leptospermum scoparium*) and American oak (*Quercus alba*) were selected for this research. These are both dense woods. The manuka was collected from Bark 'N' Firewood Bin Ltd., Albany, Auckland City. The collection came in a form of lower-end stem approximately 400 mm in diameter and 500 mm high. From its cross-section, the heart wood showed a colour range from dark brown to red-brown with a tight-grained fine texture. The exact origin within New Zealand was unknown but was certainly close to Auckland to minimise transport costs. The American oak was supplied by South Pacific Timber Ltd., Eden Terrace, Auckland, and again the exact origin was unknown. The wood had been mechanically processed as tongue-in-groove flooring, which had not been subject to any chemical treatment according to the supplier.

2.1.2 Wood chip preparation

Both woods were cut into chips to a standard shape and size (approximately 20mm x 10mm x 4mm) with electric saws. A consistent size was important because of the small volumes involved, typically 250 mL for each replicate within treatment. Intuitively the surface area to volume ratio will be important in the extraction of compounds from wood, and irregular shapes would result in lack of control of ratios, even though the chip mass to spirit volume ratio might be constant. Also, other research in this laboratory with toasted wood chips used these dimensions, and for which suitable toasting protocols were already established.

The first stage in cutting was an electric circular saw cutting through the manuka stem and oak flooring along the grain to produce a large number of long strips of approximately

equal width (10 mm) and thickness (3mm), followed by inspection. Only strips with no major exterior faults and low size variation went to the next stage. Accepted strips were sawn to a length of 20mm with an electric band saw.

2.1.3 Size, mass and weight change of chips

As discussed in 2.1.2, the surface area to volume of ethanolic solution is important in the extraction of compounds from wood. Thus, a minimal variation of the surface area to mass ratio was required. If all the chips were uniformly cut, the total surface area of the chip samples can be approximately calculated by the surface area of one piece chip multiplied by the quantity of the pieces. Thus, the chip samples were measured to determine the variation of the surface area.

Twenty raw manuka and American oak chips were randomly sampled, and the length, width and thickness were measured with a digital calliper. The chips were also weighed. Provided all the chips were dimensionally very similar, mass would provide a good guide to surface area. Mass was recorded at every stage of the treatments for both species.

Weight change has been considered important to both of the processes of accelerated leaching and toasting. At leaching stage, weight change was not only a direct reflection of the degree of wood dehydration to its ambient humidity (Cadahia *et al.* 2007) but also the result of the extent of loss of hydrosoluble compounds. At toasting stage, weight change was associated with a series of complex degradation of wood, and weight loss occurred through gradual breakdown of the wood mass with a controlled incomplete combustion. So, weight change at both stages in Table 2 was recorded. The data were analysed directly, and also analysed with other data including colour change to explore more information upon wood maturation process.

2.1.4 Soaking, accelerated leaching, and toasting

The experimental designs are shown in both Table 1 and Table 2 below. Whether toasting was done before or after leaching, the toasting procedure was always as follows. Two levels of toasting were applied. For light toasting the chips were exposed to 200 °C for

2 h while dark toasting was at 210 °C for 3 h in a calibrated laboratory oven. Approximately 60 g of chip were accurately weighted, loosely and evenly spread in an aluminium baking tray, and lightly covered with aluminium foil to minimise air contact and thus the chance of burning.

Leaching of hydrosoluble substances such as ellagitannins is reportedly an important process during seasoning of oak staves prior to barrel construction. The main body of the research was conducted in ways to accelerate leaching, but a preliminary experiment was also conducted under three different acidity conditions.

As a complex process comprising hydrolysis, degradation and oxidation, leaching might be affected by the acidity of solution, although there is no precedent for such a possibility. Both oak and manuka were each leached by refluxing under neutral, acidic and alkali conditions, which accounted for six treatments in total.

Table 1 Accelerated leaching in solutions with different pH values

Code	Wood	Liquid for leaching or refluxing	Sequence of action		
			Phase 1	Phase 2	Phase 3*
A	Oak	0.01M HCl	Reflux (1 h)	Light toasting	None
B	Manuka				
C	Oak	Deionised water			
D	Manuka				
E	Oak	0.01M NaOH			
F	Manuka				

* Phase 3 is included to make this table compatible with Table 2

Reflux boiling was conducted by a standard laboratory apparatus (Figure 15). The randomly selected wood chips were accurately weighed to 20 g and placed in a 1 L round-bottom boiling flask with 500 mL of the solutions described in Table 1 and Table 2. After reflux the mixtures were cooled to room temperature with cold tap water. Through a funnel plugged with glass wool, the reflux solution was transferred into a 1 L volumetric flask and chips were recovered in the funnel, rinsed with three quantities of 50 mL of water.

The solution and combined washings were diluted to 1 L. The solutions were then stored under refrigeration prior to spectrophotometric analysis.

Figure 15 The reflux apparatus

Subsequent to the pH experiment, accelerated leaching by reflux boiling was also combined with toasting in two different ways, reflux before toasting – as is the standard procedure in the liquor industry – and, unconventionally, reflux after toasting (Table 2).

Table 2 Wood treatments at neutrality

Code	Wood	Liquid for leaching or refluxing	Sequence of action		
			Phase 1	Phase 2	Phase 3 with treated chips split into 4 replicates
1	Oak	Deionised water	None	Light toasting	Infusion in 60% ethanol
2	Manuka				
3	Oak		Reflux (1 h)		
4	Manuka				
5	Oak		Light toasting	Reflux (1 h)	
6	Manuka				
7	Oak		None	Dark toasting	
8	Manuka				
9	Oak		Reflux (1 h)		
10	Manuka				
11	Oak		Reflux (8 h)		
12	Manuka				
13	Oak		Soaking for 3 wk		
14	Manuka				
15	Oak	0.1% $HgCl_2$			
16	Manuka				

The leaching/reflux methods involved here are combinations of four different conditions: none; boiling under reflux for 1 h; reflux for 8 h; soaking at ambient temperature for 3 wk. The last treatment was also split into two treatments, where $HgCl_2$ at a concentration of 0.1% was added to Treatments 15 and 16 to suppress anticipated microbial growth. This was the one situation where the role of microbes like wood-rotting fungi was considered.

There were six light toasting and 10 dark toasting treatments. Treatments 1 and 2were directly comparable with Treatments 5 and 6, the difference being the order of action, reflux before toasting and the reverse. This reversal was not done for dark toasting. Rather, the effect of reflux to 8 hours was explored (Treatments 7 to 12) along with extended soaking at ambient temperature (Treatments 13 to 16).

Reflux boiling was conducted in a same way as in the pH experiment. The only

difference was the scale, three times larger than in the pH experiments. Thus, 60 g wood chips were accurately weighted and placed into a 2 L round-bottom flask with 1.5 L of deionised water. For soaking over 3 weeks, the 60 g and 3 L of water were held in reagent bottles. The reflux liquids were all recovered and made to 3 L for subsequent analysis, compared with 1 L from 20 g in the pH experiment. An aliquot of each of these 3 L volumes was held refrigerated awaiting spectrophotometric analysis.

2.2 Alcohol

2.2.1 The choice of alcohol and its concentration

As stated in the Chapter 1, the primary objective of the research was to compare the different accelerated seasoning methods, reducing or eliminating undesirable flavour notes. Alcohol here serves as a medium to extract flavour to be examined from the treated wood samples. Solutions of only alcohol and water were used rather than more complex commercial spirits, because a model alcohol solution should yield clearer results.

A 60%-ethanol solution was chosen for this work for two reasons. Firstly, this is a typical concentration used in whisky industry, although at retail the concentration is usually diluted to about 40 and 50%, rather more close to the former. Unpublished experiments at AUT have confirmed that highest extraction of colour and flavour compounds occurring around 60% ethanol regardless of the wood species. Secondly, a high concentration of alcohol (a minimum of 60%) is a requirement for the application of direct injection method in gas chromatography. In this application, lower alcohol levels should be avoided as this damages chromatographic column. Although this problem can be solved by solvent extraction and enrichment, direct injection has a number of advantages over it. Solvent extraction is often selective, involves extra cost in materials and time, and can result in contamination from impure solvents. Moreover, enrichment through evaporation can result in loss of compounds of interest.

A 16 kg drum of pure ethanol was a product of APS Specialty Chemicals (NZ) Ltd. This absolute alcohol has alcohol strength at minimum 99.85%. Because the alcohol was not

denatured, it would be safe for sensory evaluation if required. Twenty litres of 60% v/v of alcohol solution in deionised water was prepared for infusion of the wood chips.

2.2.2 The alcohol infusion of the wood chips

The infusion process includes following steps: Accurately weighed chips around 10 g were placed in a 250 ml Schott bottles previously filled with 200 mL of 60% alcohol. After all the chips settled down to the bottom, the bottles were then completely filled with the alcohol to exclude air and minimise void space, and then tightly screw-capped. The final volume of the liquid in these bottles ranged from 290 to 300 mL due to size variation among these bottles. For 300 mL of the solution the infusion rate was approximately 33.3 g L^{-1}. No vacuum was applied to the bottles so that the infusions retained trace amount of air, as would be the situation in wine and whisky barrels where some oxygenation takes place.

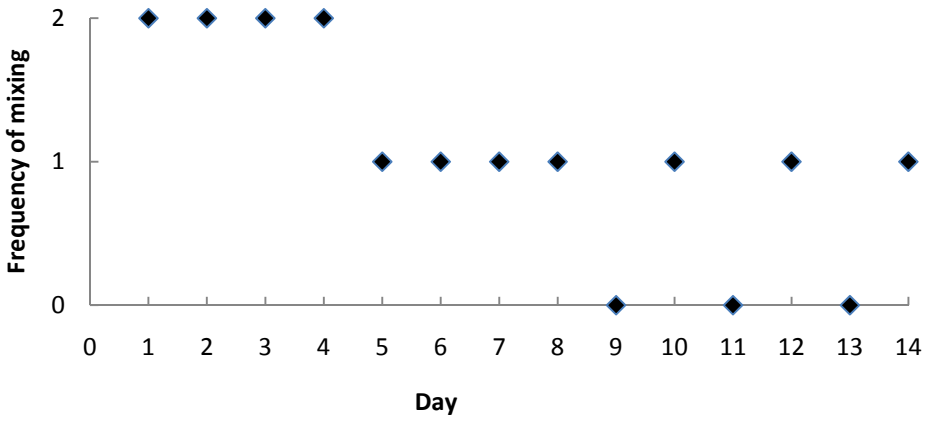


Figure 16 The frequency of infusion mixing

The bottles were kept in a dark place at ambient temperature (about 20°C) for two weeks. During this time the bottles were subject to regular gentle mixing as shown in Figure 16, with the aim of aiding the release of compounds from the chips to the solutions. It was unavoidable that the alcohols were brought into contact with the blue plastic caps (though no notable effects to the alcohols due to its inert nature). Apart from this contact the alcohol solution contacted only glass and wood.

After two weeks, all the chips were separated from the solutions through a glass funnel plugged with glass wool, and the solutions along with the combined washings (60% ethanol) were collected in a 500 mL measuring cylinder, and diluted to 300 mL with 60% ethanol. The clear, amber-coloured infusions were then collected in the same Schott bottles and stored in dark and cool place, waiting for further experiments including spectrophotometric and gas chromatographic analyses.

2.2.3 Colour measurements

The colour of the wood chips underwent changes after various treatments including leaching, toasting and infusion. To some extent, colour changes due to the treatments were probably related to physical and sometimes chemical changes. This could be tested for statistical significance with other data.

Visual inspection is not an ideal approach for colour experiment in research. Human colour perception of individuals varies widely and is also affected by illumination, sample size, background, and angle of observation. However, colorimetric instruments provide a set of standardised conditions that scientifically assure the data consistency and repeatability.

A Hunter Colorflex colorimeter (Hunter Associates, Virginia, USA) was used to record colour in CIE colour space (CIE 1976), where colour is defined by three coordinates L*, a*, and b*values (Figure 17). The vertical coordinate L* is lightness from 0 (complete black) through 50 (mid grey) to 100 (complete light reflectance); the horizontal coordinate a* is greenness/redness, running from – 60 (green) through grey to + 60 (red); an orthogonal horizontal coordinate b* is yellowness from – 60 (blue) to + 60 (yellow).

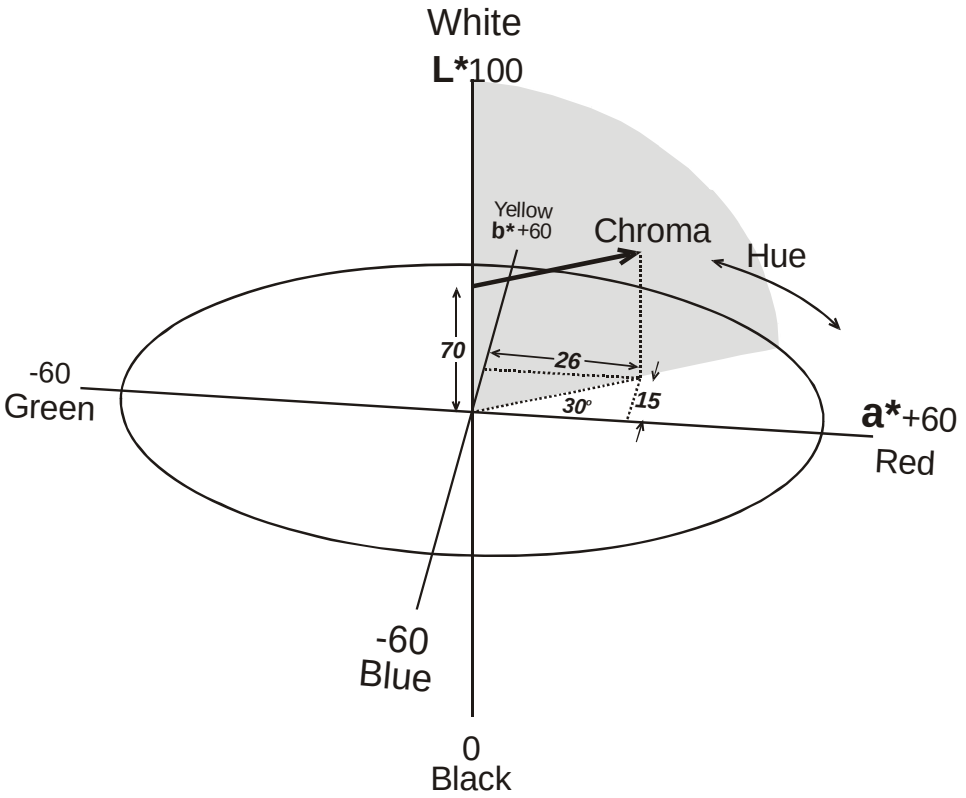


Figure 17 A mathematic model of Hunter colour space. The spatial position of the tip representing a colour can be resolved into coordinate components by its lightness +70, redness +26 and yellowness +15. The hue is arctangent 15/26 (=30 º) and the chroma, is the length of the thick line, a hypotenuse described by √(152 + 262) (= 30, by chance numerically the same as 30 º) (Young *et al.* 2001)

The system also introduces two terms, hue angle and chroma which effectively describe colour and intensity of colour of any object. Hue angle is the gradation of colour within the visible spectrum of light. Hue angle, which equals to arctangent (b*/a*), is determined by the angle formed by the corresponding values of a^* and b^* of the colour on the plane. Chroma, also called saturation, is the intensity of a specific hue. For example, a strongly saturated hue has a distinct, intense colour while a hue at low saturation appears pale and vague. Chroma is defined as $\sqrt{(a^{*2} + b^{*2})}$, the Pythagorean hypotenuse of a* and b*.

Figure 18 A Hunter colorimeter (ColorFlex, Hunter Associates, Virginia USA). The dish completely covers the light source, and the dish is covered with black cylindrical shroud, not shown in this picture.

L*, a* and b* values were recorded for many but not all wood chips from the various treatments described in Table 1 and Table 2. The wood chips were evenly spread in a cylindrical transparent dish 100 mm in diameter. The dish was then placed in the illuminant path, and covered with an opaque metallic black shroud. For each sample measurement, the mean of ten readings was taken to minimise the variation caused by the irregular distribution of the chips, by means of random redistribution after each reading. Prior to sample readings, ten blank readings were taken with the empty dish. Therefore, the actual values of colour were the sample means subtracted by the blank means.

2.2.4 Ultraviolet light absorption

As discussed earlier in Chapter 1, most compounds released by leaching during

seasoning are water soluble substances e.g. ellagitannins (polyphenols), and the substances extracted during infusion are aromatic compounds such as furanic (C5) compounds and phenols (C6). These compounds are derived from seasoning and toasting of the wood, or extractable without those treatments.

Ultraviolet (UV) absorption of light by solution is commonly used in the quantitative determination of highly conjugated organic compounds. Furanic compounds and phenols are conjugated and show strong absorption in UV region. Thus scanning plot in the UV range may provide useful information from the pattern of scanning curve, which is especially useful to analyse treatment effects among different treatments. Therefore, the post-leaching and –refluxing solutions, and the post-infusion alcoholic solutions were measured and compared for their UV/visible absorption from 200 to 800 nm.

Area under curve (AUC) from within any wavelength range can be used as a mathematic tool to give an approximate guide to the concentrations of the compounds in total, but realising that different compounds and even classes of compounds have different molar absorbance coefficients. Thus, it may be useful to compare the AUC data between the leaching and infusion solutions within a treatment and between treatments. The relation between AUC, weight loss and colour change can also be examined. The basis for this idea is that weight loss beyond moisture loss will also represent the loss of these compounds; colour change mainly due to toasting may also be linked to UV absorption and thus the AUC.

The UV measurements were carried out in a UV/VIS spectrometer Ultrospec 2100 pro (Amersham Biosciences), in the wavelength range 200 to 800 nm, using a single 1cm quartz cuvette. The spectrophotometer was linked to Biochrom data collection software version 2.0 which was used for simultaneous gathering of absorbance reading at a 2 nm interval during slow scanning, and export to Excel spreadsheets. AUC were calculated as the sum of the total absorbance from 200 to 450 nm.

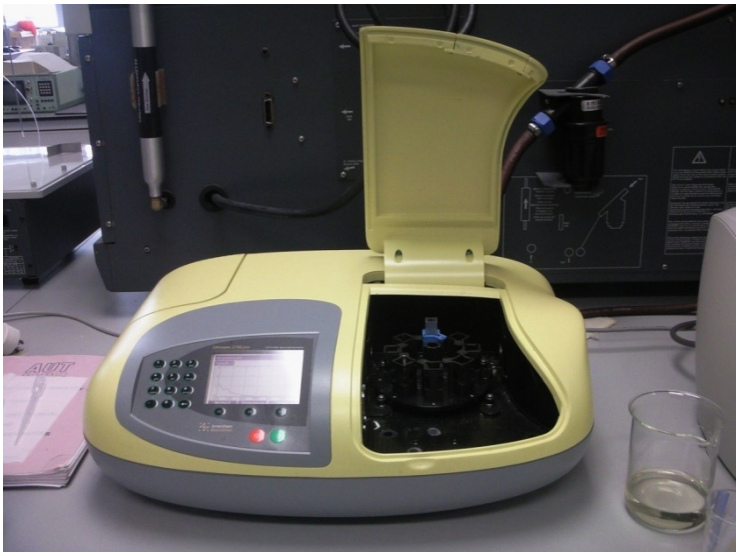


Figure 19 A UV/VIS spectrometer Ultrospec 2100 pro

Prior to the absorbance measurements, all solutions after leaching, reflux and infusion were diluted in water (after the first two) or 60% ethanol (after infusion). This was done because all these solutions exceeded the upper limit of the measuring range of the instrument, from 0 to 3. However, the extent of dilution varied with treatment because leaching, reflux and infusion were variously effective in extracting UV-absorbing matter, and indeed there were differences between oak and manuka. Data presented have been corrected for these dilution factors.

2.2.5 Gas chromatography

Alcohol infusion solutions described in Table 2 were ultimately analysed by gas chromatography (GC) after direct injection. GC is a common type of chromatography used in analytic chemistry for separating and analysing compounds that can be vaporised without decomposition in an inert atmosphere. After the various leaching and toasting treatments, it was expected that some variation in the resultant compounds along with their concentration could be revealed by GC analysis. The retention times of the peaks that often represent a

single compound can provide qualitative information about compounds while the area ratios (internal standard method) give quantitative information about the compounds. Prior to sample analyses, 2-ocatanol (BDH reagent) as the internal standard was accurately added into each test solution at 10 ppm concentration.

While all the infusion treatments in Table 2 had the alcoholic strength at 60%, the reference whisky (Johnnie Walker Red Label) has a declared 40% alcohol concentration. Pure ethanol was added to this reference to increase its concentration to match that of the infusion treatments, 60%.

To reiterate from 2.2.1, direct injection to detect volatiles in a spirit has several analytical advantages over solvent extraction methods. In solvent extraction, the chosen organic phase (e.g. n-pentane, dichloromethane, diethyl ether or their mixtures) selectively extracts different classes of whisky-soluble compounds (Pérez-Coello 1997), such that some classes of compound that may be important remain trapped in the aqueous phase. Direct injection overcomes this problem but necessarily yields huge ethanol peaks.

Taking account of the nature of the solutes, the column capacity and the availability of carrier gas, an optimised GC method was established with a number of trial runs. The use of the relatively higher ramp rate of temperature and low linear velocity (required for nitrogen carrier gas) was to minimise the run time without significant loss of resolution.

The chromatographic analysis of the extracts was carried out in a Shimadzu (Model 2010) gas chromatograph, equipped with a flame ionisation detector and a new ZB-Wax*plus* column (30m x 0.25mm internal diameter x 0.25 µm film thickness of 100% polyethylene glycol able to withstand 250 ºC) (Phenomenex, Auckland). Compared to ZB-Wax columns – widely used for analysing alcohol samples – ZB-Wax*plus* column is much more tolerant to water (Phenomenex 2010).

An AOC-20i auto injector was coupled to execute automated batch analysis of the samples. The injection volume was 0.5µL at split ratio 1:20. The nitrogen carrier gas had the flow rate of 0.71 mL min^{-1} (linear velocity 20.0 cm sec^{-1}). The injector temperature was set at 200 ºC. The column temperature was programmed as follows: initial 50 ºC (held for 1

min) was increased to 100 ºC (held for 2 min) at a rate of 25 ºC min^{-1}, then to 170 ºC at 25 ºC min^{-1}, to 240 ºC at 15 ºC min^{-1}, then held for 50 min at that temperature. Total run time was 62.47 min, necessary to ensure no carry-over peaks to next run. The detector temperature was set at 250 ºC.

2.2.6 Odour of the infused alcohols

As a major interest of the research, the odour characteristics of the resultant alcohols (Table 2) were evaluated by the author. One AUT staff member and two AUT students also participated in the trial. Dr Owen Young was also involved in the trial, monitoring and examining the whole process.

Unlike some sensory methods which involve many elaborate procedures, this method consists of just a few simple steps aiming to describe the odour types and intensities for the alcohol samples. The method included comparisons between species (oak treatments vs. respective manuka), and within species (light toasting vs. dark toasting).

As described in detail later, the ethanolic odour of all the treated alcohols was strong to the point of being overwhelming. The differences of odour among these infusions were very subtle.

2.3 Data analysis

The recording and handling of raw data were performed using Microsoft Excel. This work included the calculation of treatment means and relative standard variation for chip size, weight loss and colour measurement. Data for area under curve of ultraviolet scans were also drawn using the scatter plot function in Excel. Statistical tools in Excel such as correlation and simple linear regression were used to test the strength of the relationship among the data, while some data i.e. colour and AUCs were analysed for statistical significance using SPSS software.

Chapter 3

Change in Weight and Colour

3.1 Dimension and mass of wood chips

The main interest for the dimension measurement here was to explore the relationship between surface area and mass for wood chips as both may be important in respect of extraction into 60% ethanol. Wine can penetrate about 6mm into the oak staves of a barrel (Alexander 2008), although the literature is vague about the kinetics of this for wine, and data for spirits is proprietary information (the whisky industry is arcane for obvious commercial reasons.). Light toasting penetrates oak barrel stave to a depth of about 2 mm and dark toasting to 3 to 4 mm (Alexander 2008). Therefore, the entire chip mass in the present study (nominally 3 mm in thickness) will be within the range of complete toasting and extraction according to the literature. The behaviour of water, acidic or alkaline, is not known, although is more likely to approximate the behaviour of wine rather than spirits.

Oak chips in commercial use are simply irregular wooden fragments from which the surface areas vary greatly at equal mass. It would be difficult to work out the relationship between surface area and mass for irregular wooden fragments. To reiterate from 2.1.3, surface area can only be measured easily for chips with uniform shape and size. Besides, accurately cut chips are mandatory for a systematic study of infusion where volumes are low, as in the present study, and both area and mass should be well controlled.

After cutting, 20 pieces of chips from both woods were randomly selected from the totals and their dimensions and mass were individually measured (Table 3). The greatest variation was in thickness, reflecting the fact that the chips were cut on a manually-controlled band saw. If thickness were the most important factor controlling leaching (water washing) and infusion (60% ethanol), then the greater mean thickness of the oak chips (4.23 vs. 2.89) might be important.

Table 3 Mean chip dimensions and masses of both woods

		Oak		Manuka	
		Mean	[1]RSD (%)	Mean	[1]RSD (%)
Length	(mm)	20.07±0.11	0.55	19.88±0.14	0.70
Width	(mm)	10.20±0.26	2.55	9.47±0.38	4.01
Thickness	(mm)	4.23±0.20	4.73	2.89±0.14	4.84
Surface area	(mm^2)	665.4±17.2	2.58	546.2±19.9	3.64
Mass	(g)	0.68±0.02	2.94	0.47±0.02	4.26

[1] RSD means relative standard deviation.

With regularly cut chips, such as used here, the relationship between surface area and mass should be linear. Shown as in Figure 20, both plots indicate a strong linearity. The respective r^2 values for oak and manuka chips were 0.83 and 0.89, meaning 83% and 89% of the total variation in surface area of the chips was explained by their respective masses. The rest 17% and 11% of the unexplained variation might be due to measurement error, shape and density variations etc.

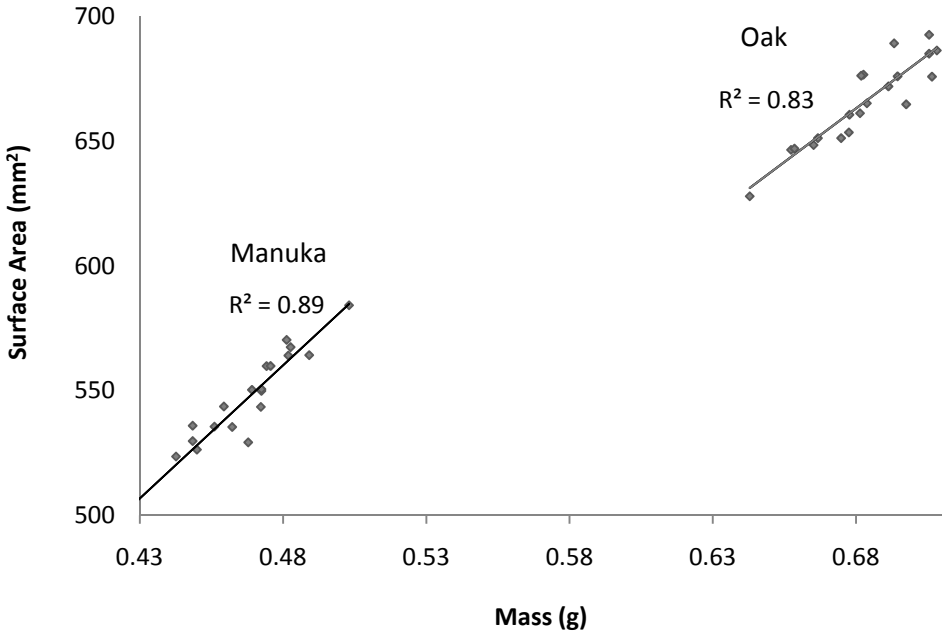


Figure 20 Scatter plots of surface area and mass for unleached, untoasted oak and manuka chips.

3.2 Weight change

Data for weight changes at all 3 phases (Table 2) were recorded. The weight changes

were then compared and analysed within and between the oak and manuka due to toasting, reflux and light toasting, reflux (or leaching) and dark toasting, and infusion.

3.2.1 Weight loss due to toasting

Consider first the 'big picture' of toasting with or without reflux treatment (Figure 21). Both woods had more weight loss through dark toasting than light toasting for both untreated and previously refluxed chips.

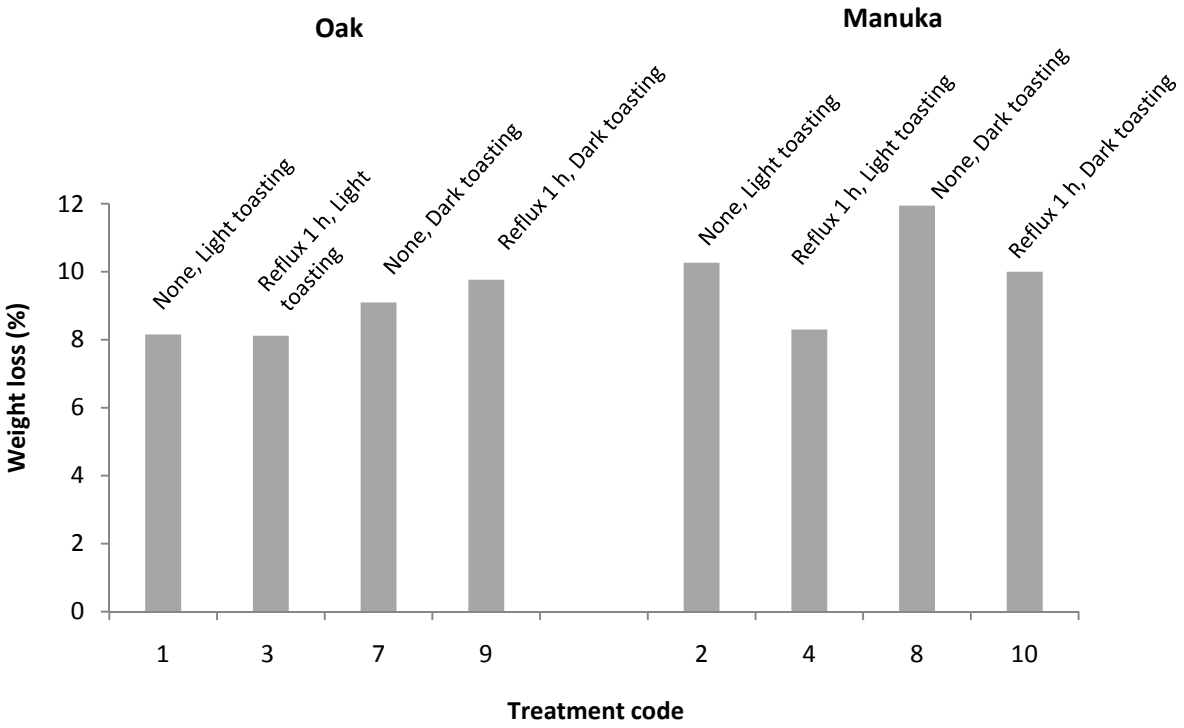


Figure 21 Weight losses due to toasting, irrespective of prior reflux (code numbers according to Table 2).

On light toasting, oak Treatments 1 (no reflux) and 3 (reflux 1h) had similar total weight losses of 8.2 and 8.1% while manuka Treatment 2 (no reflux) and Treatment 4 (reflux 1h) had total weight losses by 10.3 and 8.3% respectively. It is noted that prior reflux of manuka resulted in reduced total weight loss, but no change or an increase in oak.

On dark toasting, oak Treatments 7 (no reflux) and 9 (reflux 1h) lost weight by 9.1 and

9.8% while manuka Treatments 8 (no reflux) and 10 (reflux 1h) lost by 12.0 and 10.0%, respectively, paralleling the pattern seen for light toasting.

It was also clear that untreated manuka lost relatively more weight than untreated oak during both light and dark toasting, although this may not be mainly due to thermal degradation. Young *et al*. (2010) showed that the oak they used was drier than the manuka they used, where both were equilibrated to ambient conditions. If the same were true for the samples used here, then the weight loss in manuka may be more due to water loss than thermal degradation.

On the other hand, a different pattern of weight loss was also found between oak and manuka in Figure 21. In oak, there was more weight loss after dark toasting in Treatment 9 (reflux 1h) than in Treatment 7 (no reflux) while manuka showed an opposite effect. Treatment 10 (reflux 1h) lost more weight after dark toasting than in Treatment 8 (no reflux) possibly due to the difference of water content. However in oak, any effect due to water content was possibly swamped by degradation through reflux where the dynamics of thermal hydrolytic power might facilitate the breakdown or structural change, creating a condition more in favour of degradation.

3.2.2 Weight change due to reflux and light toasting

This section looks at the individual effects of reflux and light toasting.

During Phases 1 and 2 (Figure 22), the total weight loss for oak in Treatment 5 (light toasting, reflux 1h) (2.9%) was much lower compared to Treatments 1 (reflux only) (7.7%), and Treatment 3 (Reflux 1h, light toasting) (9.0%). For manuka, Treatment 6 (light toasting, Reflux 1h) also had a weight loss (5.3%) significantly lower than Treatment 2 (reflux only) (10.1%), and Treatment 4 (reflux 1h, light toasting) (11.5%). For both Treatments 5 and 6, the weight gains in Phase 2 were the main reason for the reduction in net weight loss, in both cases reflux after toasting.

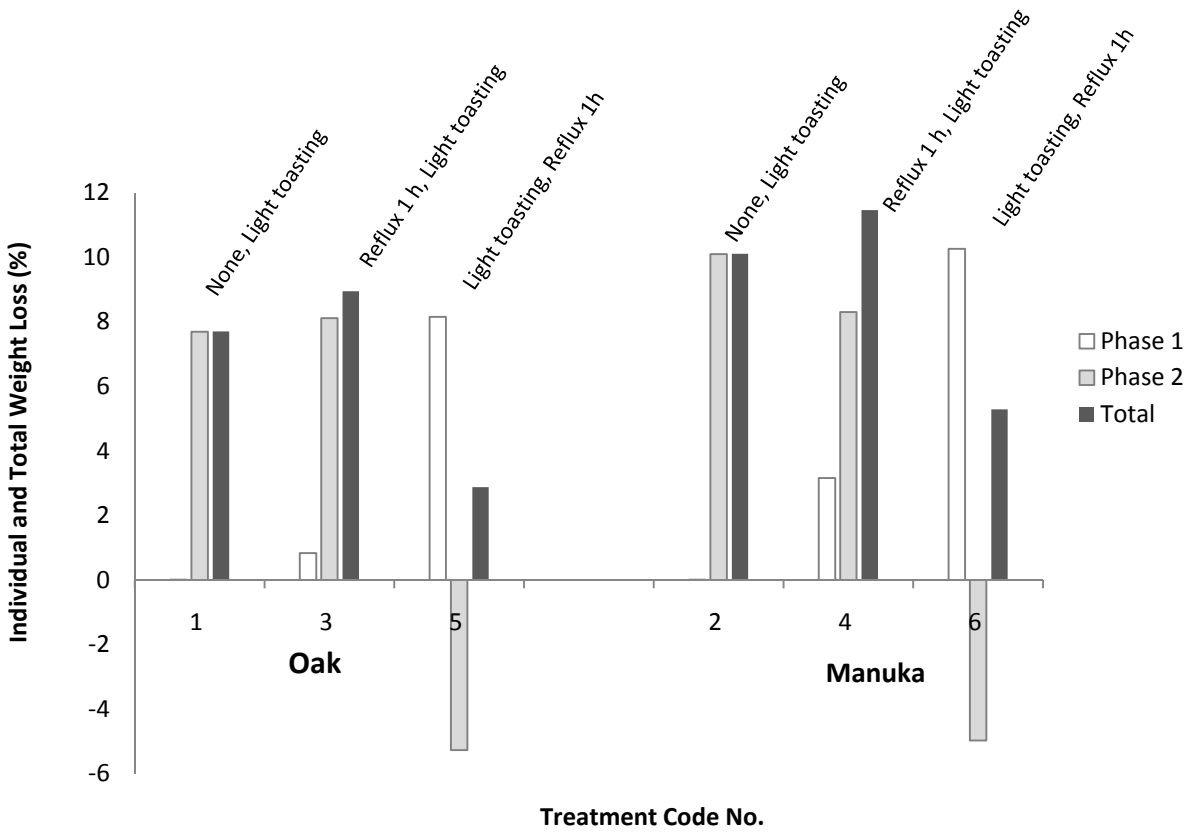


Figure 22 Weight losses for treatments involving light toasting during Phases 1 and 2. Weight gain at Phase 2 in Treatments 5 and 6 is shown as negative.

For oak, after reflux for 1 hour Treatment3 (reflux 1h, light toasting) had a 0.8% weight loss while Treatment 5 (light toasting, reflux 1h) gained weight by 5.7% (Figure 22). While a small amount of water soluble compounds was leached to the reflux water causing weight loss in Treatment 3, the weight gain in Treatment 5 suggested that water was probably absorbed during reflux through the cavities in the layer of charred surface formed by light toasting, which increased the water-binding capacity of the wood and the water was still partially retained in after air drying. A similar situation was also found in manuka with roughly more weight losses than their oak equivalents (Figure 22).

3.2.3 Weight loss due to reflux or leaching and dark toasting

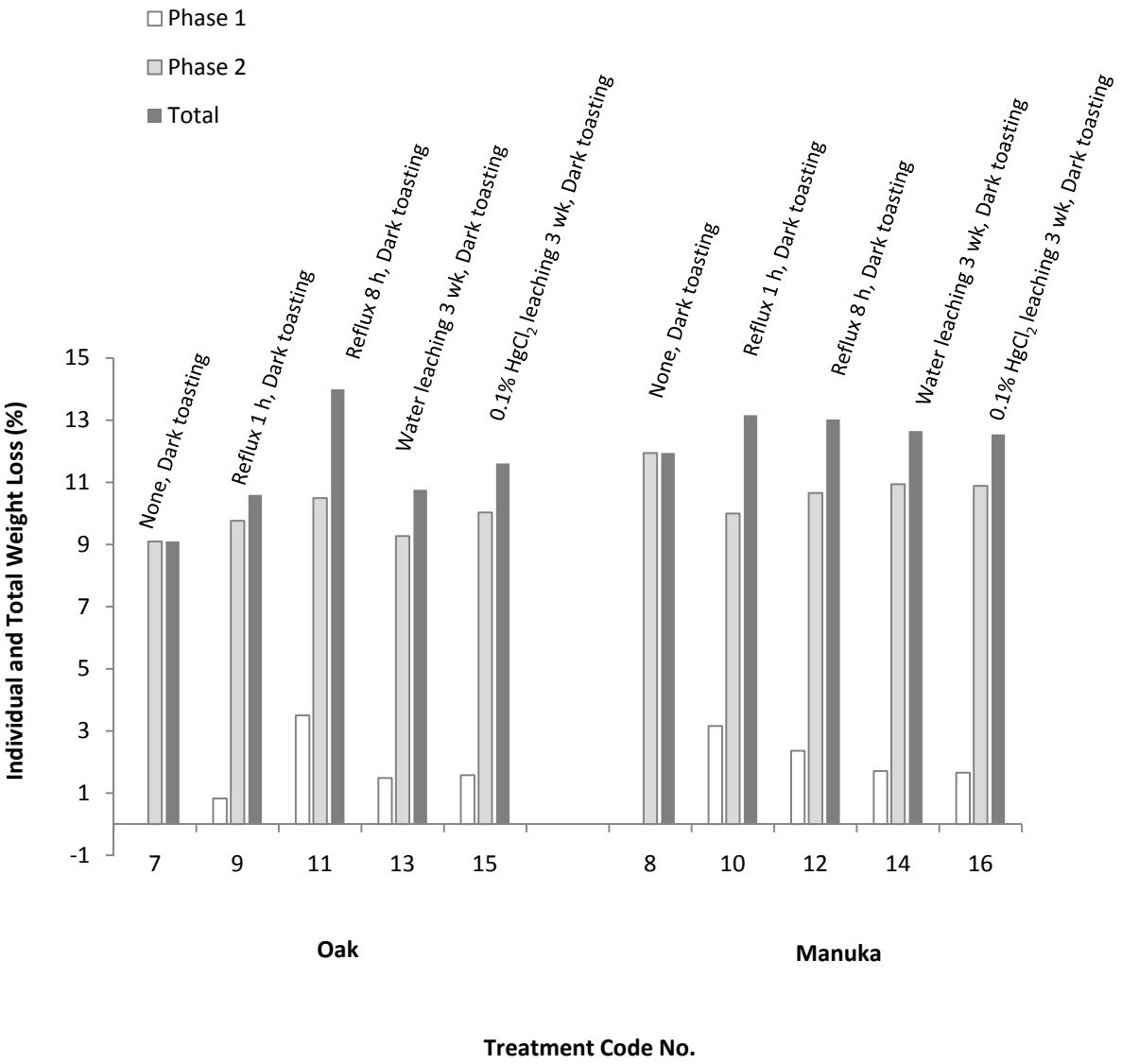


Figure 23 Weight losses for the treatments involving dark toasting during Phases 1 and 2.

It is shown (Figure 23) that Treatment 11 (reflux 8h) had a greatest weight loss both at Phase 1 (3.5%) and Phase 2 (10.5%). Treatment 9 (reflux 1h) had a least weight loss during Phase 1 (0.8%) and Treatment 7 (no reflux) had least weight loss (9.1%) during Phase 2. The data clearly suggest that reflux for 8 hours was the most effective way to remove hydrosoluble substances from oak among the other treatments. This is possibly not surprising.

On the other hand in manuka, Treatment 8 (no reflux) lost most weight (11.9%) on toasting (Phase 2) compared to others in Phase 2. Unlike oak, Treatment 10 (reflux 1h) had the greatest weight loss (3.2%) in Phase 1 of all manuka treatments, and also the total weight loss (13.2%), but necessarily the least weight loss in Phase 2.

3.2.4 Discussion on weight change in Phase 1 and 2

In comparing oak and manuka, there was relatively more weight loss in manuka, particularly in Phase 1. The involved reflux or leaching in these treatments caused weight losses in various degrees while the only exception was toasted then refluxed woods which gained weight from both equivalents. Looking at these parallel treatments between the two woods, the general patterns were rather similar.

3.2.5 Weight change in Phase 3 due to infusion

Weight change in both chips (Figure 24) also occurred in Phase 3 (the alcohol infusion). After infusion, mainly due to water uptake both woods had weight increase except Treatments 5 and 6, the two treatment equivalents for light toasting then reflux 1 h. Manuka regained more weight than oak in all the parallel treatments. Manuka also had less weight loss from Treatment 6 (0.7%) than oak equivalent Treatment 5 (1.5%) while the probable reasons for the weight losses were the losses of water (gained in Phase 2 after reflux) when infusion process took place on the surface, and also more importantly the loss of certain amount of leachable matters. Figure 24 also shows both light toasted woods regained more weight than those of heavy toasted. The reason could be light toasting had less alteration to the structure of the wood mass compared to heavy toasting while the alteration was associated with the reduction of water-binding capacity. It also clearly shows in Figure 24 that both woods with extensive leaching (from Treatments 11 to 16) had least weight gain.

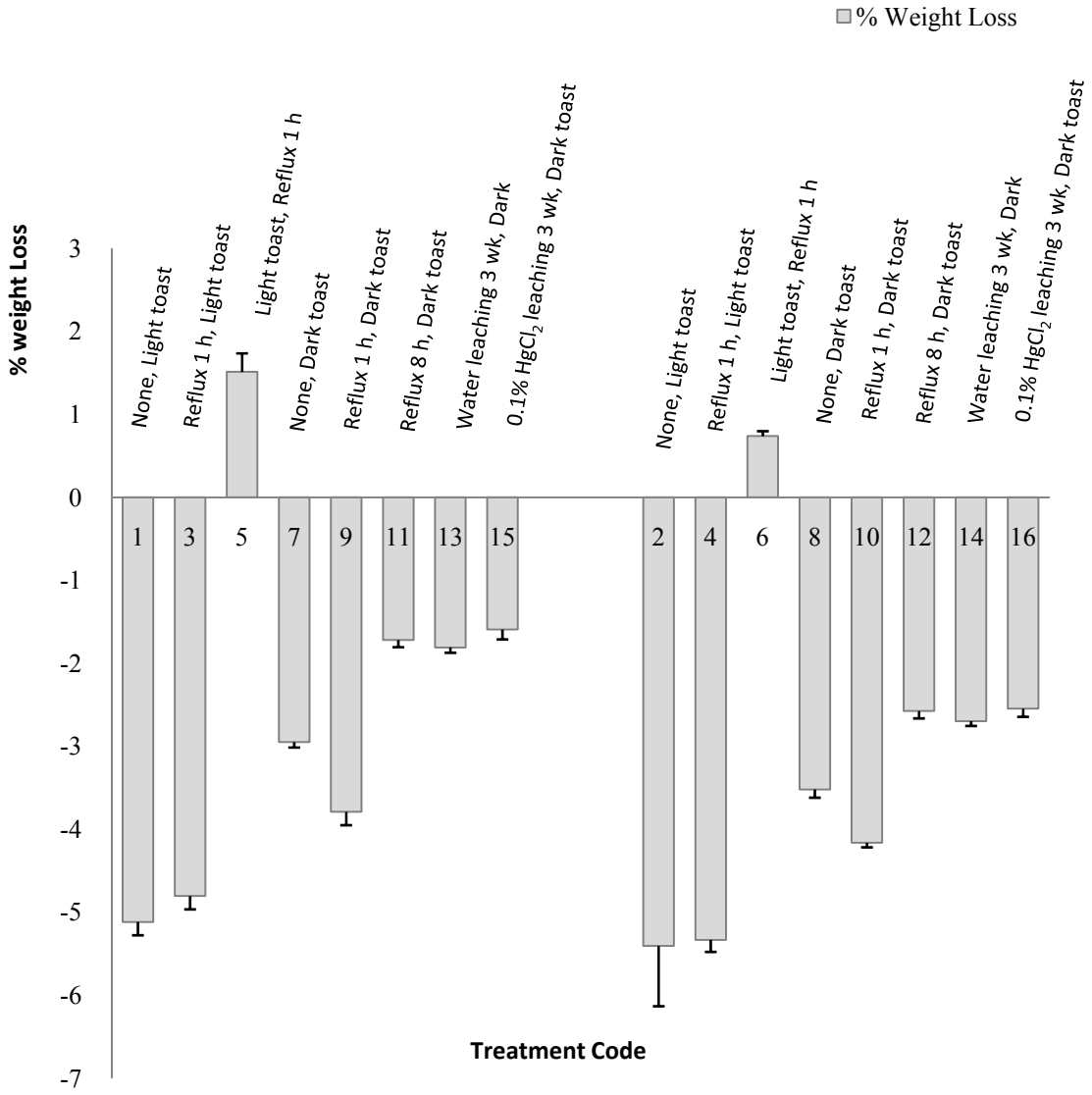


Figure 24 Weight losses during infusion (Phase 3) for all the treatments. The weight losses in Treatments 5 and 6 are shown as positive. Error bar = standard deviation for the four replicates.

Inspecting the parallel treatments in Phase 3 between oak and manuka (Figure 24), the general patterns were even more similar than those occurred in Phase 1 and 2 (Figure 22 and Figure 23).

3.3 Colour change

3.3.1 Colour change during the accelerated leaching

3.3.1.1 *Lightness*

Colour change in all 16 treatments (Table 2) was measured in ten replicates using a Hunter colorimeter. As expressed by lightness (L*), hue angle, and chroma, the data were tabulated and statistically analysed for the post-leaching changes using both untreated chips as controls against the other treatments.

Table 4 Chages in lightness in Phase 1 prior to toasting

Oak			Manuka		
Code	L*	*p* value	Code	L*	*p* value
7 (1)[1]	52.61±3.12[3]		8 (2)[1]	42.79±2.12	
9 (3)[2]	56.89±3.17	0.007	10 (4)[2]	42.73±1.79	0.951
11	48.63±1.38	0.019	12	40.66±2.81	0.122
13	54.99±1.57	0.137	14	43.89±2.42	0.381
15	52.24±2.01	0.815	16	40.82±1.93	0.106

[1]As a control for each wood group, Treatments 7 and 8 were the respective replicates of Treatments 1 and 2 in Phase 1.

[2]Treatments 9 and 10 were the respective replicates of Treatments 3 and 4 in Phase 1.

[3]Data are means of 10 readings ± standard deviations.

Oak chips show more changes in lightness due to reflux (Treatment 9 and 11) compared to water soaking for 3 weeks (Treatments 13 and 15) according to Table 4. The degree of changes was reflected by *p* value ($p < 0.05$) in both reflux treatments while fewer changes ($p > 0.05$) were found in both water soaking treatments. Oak for 1 hour reflux (Treatment 9) gained lightness while lightness declined in 8 hours reflux (Treatment 11). By moderate reflux for 1 hour, oak chips might lose some hydrosoluble substances and turned brighter upon drying. However, the extended reflux for 8 hours possibly disrupted the microfibril structure of the oak, reducing its water binding ability. As a result, the surface became over dry and rough upon drying, and light was more easily to be absorbed, thus lowering the lightness. In respect of manuka, no significant differences in lightness were found in all the four equivalent

treatments (Table 4).

A negative linear relationship ($R^2 = 0.89$) between weight loss and change in lightness was found for the four treatments in the oak group (Figure 25), suggesting that the more loss in weight the more reduction in lightness. The oak plot shows both Treatments 9 and 11 were at the two extremes. On the other hand, no relationship between weight loss and change in lightness was observed from the parallel treatments in the manuka group (Figure 25).

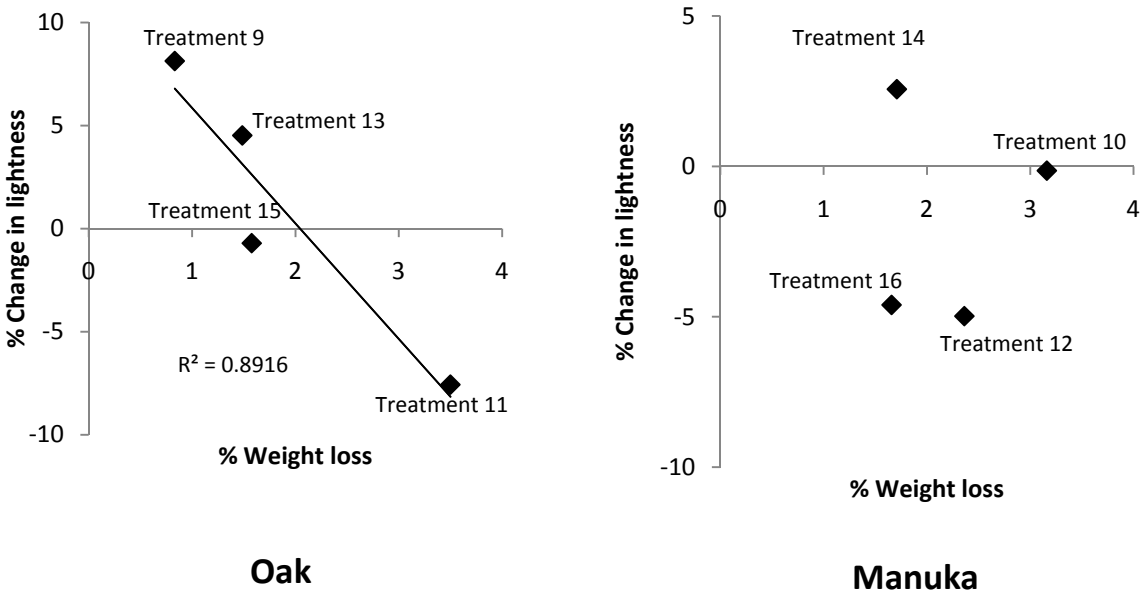


Figure 25 Correlation plots between weight loss and change in lightness.

3.3.1.2 *Hue angle*

As with lightness, both refluxed oak chips (Treatments 9 and 11) had more changes ($p < 0.05$) in hue angle compared to the rest two oak chips treated by water-soaking (Treatments 13 and 15) as shown in the *p* values ($p > 0.05$) (Table 5). The wood colour was brown i.e. dark orange in technical term, which lies between yellow and red. In comparison with untreated oak (Treatment 7), a yellower hue with higher value was found in oak for 1 hour reflux (Treatment 9), and a reddish yellow hue with lower value was seen in 8 hours reflux (Treatment 11) (Table 5). However, all the four manuka equivalents were not significantly

affected after the treatments at this aspect (p> 0.05).

Table 5	Changes in hue angle at Phase 1 prior to toasting				
Oak			Manuka		
Code	Hue angle (°)	*p* value	Code	Hue angle (°)	*p* value
7 (1) [1]	71.71±1.05[3]		8 (2) [1]	58.21±0.69	
9 (3) [2]	73.04±0.46	0.002	10 (4) [2]	57.71±0.72	0.129
11	68.89±0.55	0.000	12	57.57±0.85	0.134
13	71.24±0.10	0.318	14	57.76±0.71	0.259
15	71.26±0.45	0.357	16	57.55±1.32	0.216

[1] As a control for each wood group, Treatments 7 and 8 were the respective replicates of Treatments of 1 and 2 at Phase 1.

[2]Treatments 9 and 10 were the respective replicates of Treatments 3 and 4 at Phase 1.

[3]Data are means of 10 readings ± standard deviations.

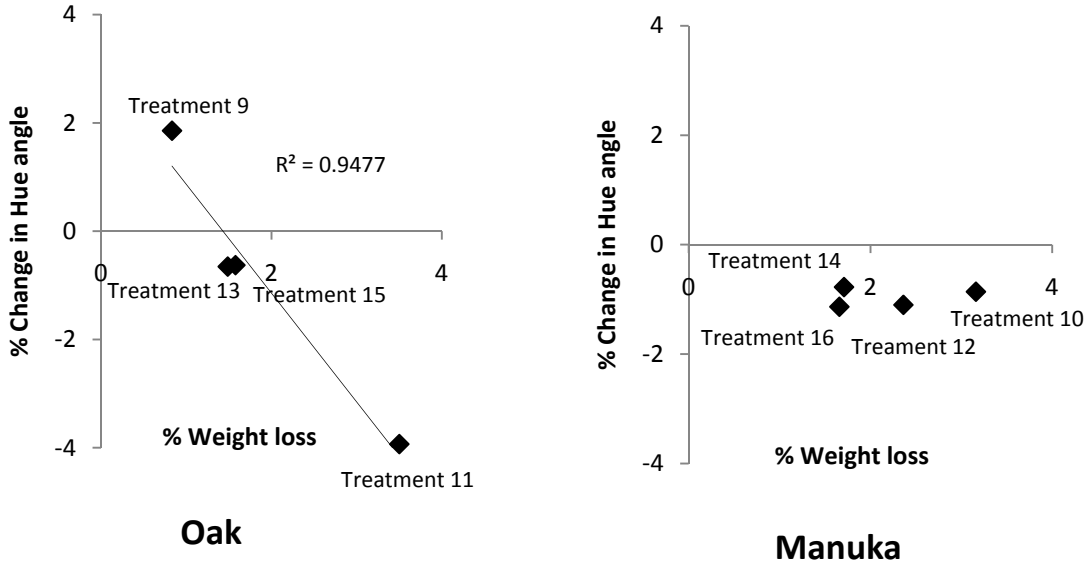


Figure 26 Correlation plots between percentage weight change and percentage change in hue angle.

A negative linear relationship (R^2 = 0.95) was also found between weight loss and change in hue angle in the oak group (Figure 26). As for lightness, hue angle decreased as weight loss increased. The greatest reduction in hue angle was found in Treatment 11 (reflux 8h) while an increase in hue angle was seen in Treatment 9 (reflux 1h). Similar changes both in weight and hue angle were also seen in both water soaking treatments (13 and 15). There

was no relationship observed among the four manuka equivalents.

3.3.1.3 *Chroma*

In oak, significant changes in chroma were found in both Treatments 9 and 11 ($p< 0.05$) while fewer changes ($p> 0.05$) were seen from both Treatments 13 and 15 (Table 6). As mentioned in the previous chapter, chroma is the intensity of a specific hue. With 1 hour and 8 hours reflux (Treatments 9 and 11), there were certain degrees of loss in chroma ($p< 0.05$), so the resultant colour appeared paler than the control (untreated oak). However, the chroma values were similar (17.61 and 17.70) between the two treatments (Table 6), suggesting that the levels of change in chroma were almost same. Meanwhile, fewer significant changes in chroma were found in both water soaking treatments (13 and 15) ($p> 0.05$).

On the other hand, significant changes in chroma (Table 6) were found in all the four treatments in manuka with more changes from the two reflux treatments (10 and 12) than the two water soaking treatments (14 and 16).

Inspection of the data reveals that in the two oak reflux treatments (9 and 11) that no difference was detected in chroma while significant differences were found in lightness and hue angle between the two treatments.

Table 6 Changes in chromaat Phase 1 prior to toasting

Oak			Manuka		
Code	Chroma	*p* value	Code	Chroma	*p* value
7[1]	20.47±0.80[3]	N/A	8[1]	21.28±1.09	N/A
9[2]	17.61±0.81	0.000	10[2]	17.95±0.74	0.000
11	17.70±0.68	0.000	12	17.76±1.02	0.000
13	20.16±0.58	0.426	14	19.66±0.50	0.008
15	19.65±0.69	0.056	16	19.06±1.04	0.002

[1] As a control for each wood group, Treatments 7 and 8 were the respective replicates of Treatments of 1 and 2 at Phase 1.

[2]Treatments 9 and 10 were the respective replicates of Treatments 3 and 4 at Phase 1.

[3]Data are means of 10 readings ± standard deviations.

There was no relationship found between weight loss and change in chroma in both oak

and manuka (Figure 27). From oak plot below, a big gap for the weight losses between the two reflux (0.83% for 1 hour and 3.50% for 8 hours) merely resulted in rather small gap for the chroma loss (-13.97% for 1 hour and -13.53% for 8 hours).

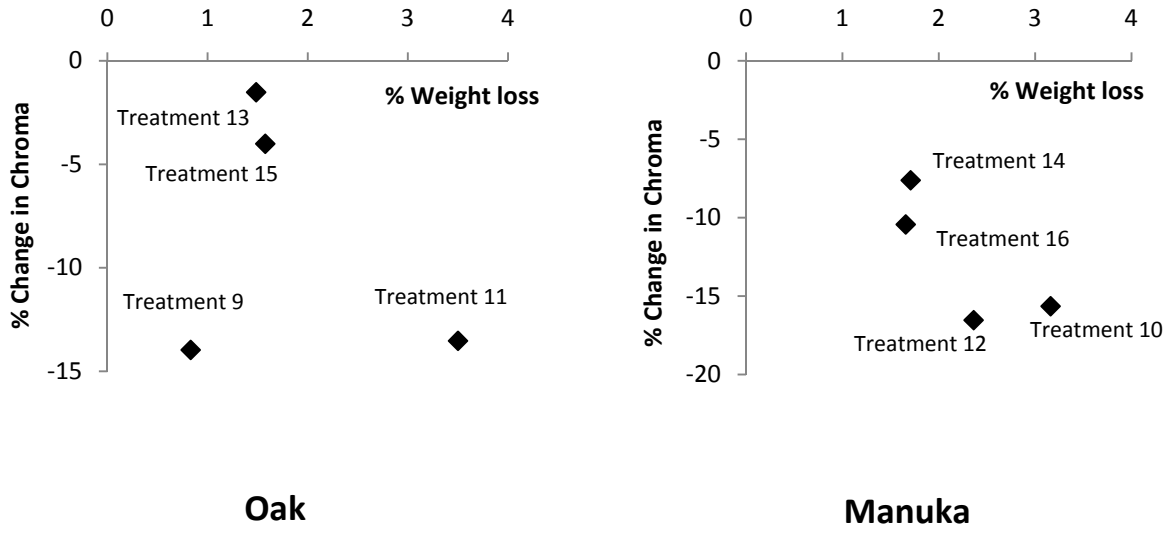


Figure 27 Correlation plots between percentage weight change and percentage change in chroma.

3.3.1.4 *Conclusion*

In terms of colour change, data above suggested that oak was generally more susceptible than manuka to the various water treatments. In oak group, the reflux treatments (Treatments 9 and 11) were more effective to change the colour than water leaching (Treatments 13 and 15). It was also found that there were no significant changes in lightness and hue angle, but noticeable changes in chroma were observed after the water treatments in manuka group.

3.3.2 Colour change due to toasting

3.3.2.1 *Light toasting*

For lightness in oak group (Table 7), a marked increase (22.75%) occurred in reflux before light toasting (Treatment 3) vs. light toasting only (the control, Treatment 1). However, there was few change (-0.50%) for oak with reflux after light toasting (Treatment 5) vs. light toasting only (the control, Treatment 1). This was the situation where the sequence of reflux and toasting was inversed. The probable reason for the difference could be that the

reflux (in Treatment 3) removed the hydrosoluble substances including sugars containing tannins, thus reduced the level of caramelisation i.e. darkening after toasting. The difference in lightness between Treatments1 and 3 could be easily examined as the latter was much brighter (Figure 28). In manuka group, by contrast there were no significant changes in lightness after the inversed treatment – with only -1.31% for reflux before light toasting (Treatment 4) vs. light toasting only (the control, Treatment 2), and -4.15% for reflux after light toasting (Treatment 6) vs. light toasting only (the control, Treatment 2). The reason for the difference between the two species is unknown.

In the case of hue angle (Table 7) in oak, Treatments 3 had minor increase for 2.21% while Treatment 5 had slight decrease for 1.39% against the control (Treatment 1). In manuka, both Treatments4 and 6 also had minor decrease for 2.49% and 3.74% respectively. The data suggest that he light toasting is not effective to alter the hue angle for both the oak and manuka groups.

Figure 28 A comparison of colour between untreated oak light toasting only (the control, left tray) and oak with reflux then light toasting (right tray).

In terms of chroma (Table 7), both treatments in the oak increased strongly with 40.71% for Treatment 3 and 15.87% for Treatment 5. The change was best illustrated from above picture (Figure 28). The difference in chroma (as colour saturation) between the control and the treated oak could be seen as the oak chips from the right tray were more saturated in the

specified hue. On the other hand, relatively minor increase was seen in the parallel treatments with 5.37% for Treatment 4 and 11.27% for Treatment 6.

3.3.2.2 *Dark toasting*

In the case of dark toasting (Table 7), the data indicates that the lightness of oak decreased in Treatments 11 (reflux 8 hours) for 8.55%, Treatment 13 (soak in water 3 weeks) for 9.13% and Treatment 15 (soak in 0.1% $HgCl_2$ 3 weeks) for 14.38% against Treatment 7 (the control, untreated oak dark toasting only). By contrast, Treatment 9 (reflux 1 hour) had marked increase for 10.9% rather than decrease. Table 8 also shows that manuka had similar pattern and level of change in lightness in the three parallel treatments (Treatments 12, 14 and 16, from 9.03% to 11.39%) against Treatment 8 (the control) while rather slight increase (2.29%) occurred in Treatment 10 (reflux 1 hour).

For hue angle, only minor reductions were found in both woods. In oak (Table 7), the reduction for Treatment 11 (reflux 8 hours) against the control (Treatment 7) was small (1.41%) while the greatest reduction in Treatment 15 was 4.91%. In manuka (Table 8), the biggest change was seen in Treatment 12 (8 hours reflux) by 3.26%. Similar to lightness, both woods with reflux 1 hour then dark toasting (Treatments 9 and 10) had similar degrees of increase (rather than decrease instead) for 7.70% and 6.47% respectively.

In terms of chroma, Table 7 shows that the oak had marked increase in the two reflux methods (Treatments 9 and 11) by 49.3% and 23.66%. On the other hand, there were minor changes in the parallel treatments in manuka ranging only from 1.85% to 8.93% (Table 8).

3.3.2.3 *Conclusion*

The results show few colour changes in the manuka no matter what of the treatments used. It suggests that the chemistry of manuka was relatively less altered as oak is more susceptible to the treatments explored so far.

Table 7 Colour change due to light toasting during Phase 1 and 2

Treatment Code	L*	%change	*p* value	Hue angle	%change	*p* value	Chroma	%change	*p* value
1 (control)	33.72±0.84	-	-	67.40 ± 0.44	-	-	15.82 ± 0.50	-	-
3	41.39 ± 1.62	22.75	0.000	68.89 ± 0.68	2.21	0.003	22.26 ± 0.44	40.71	0.000
5	33.55 ± 2.34	-0.50	0.883	66.46 ± 0.76	-1.39	0.046	18.33 ± 0.56	15.87	0.000
2 (control)	27.44 ± 1.80	-	-	63.89 ± 0.91	-	-	11.18 ± 0.23	-	-
4	27.08 ± 1.29	-1.31	0.727	62.30 ± 1.66	-2.49	0.098	11.78 ± 0.31	5.37	0.009
6	26.30 ± 1.81	-4.15	0.350	61.50 ± 0.87	-3.74	0.003	12.44 ± 0.52	11.27	0.001

Table 8 Colour change due to dark toasting during Phase 1 and 2

Treatment Code	L*	%change	*p* value	Hue angle	%change	*p* value	Chroma	%change	*p* value
7 (control)	29.13 ± 1.95	-	-	59.66 ± 0.77	-	-	13.95 ± 0.72	-	-
9	32.30 ± 1.8	10.88	0.028	64.25 ± 0.78	7.69	0.000	20.83 ± 1.28	49.32	0.000
11	26.64 ± 1.81	-8.55	0.069	58.82 ± 0.56	-1.41	0.085	17.25 ± 1.05	23.66	0.000
13	26.47 ± 2.00	-9.13	0.066	57.41 ± 0.79	-3.77	0.002	16.45 ± 0.86	17.92	0.001
15	24.94 ± 1.29	-14.38	0.004	56.73 ± 0.95	-4.91	0.001	15.03 ± 1.03	7.74	0.092
8 (control)	27.03 ± 1.04	-	-	58.91 ± 1.41	-	-	12.43 ± 0.92	-	-
10	27.65 ± 2.10	2.29	0.571	62.72 ± 1.74	6.47	0.005	13.54 ± 1.32	8.93	0.162
12	23.95 ± 0.81	-11.39	0.001	56.99 ± 0.66	-3.26	0.025	13.10 ± 0.59	5.39	0.209
14	24.54 ± 1.15	-9.21	0.007	57.79 ± 1.36	-1.90	0.237	12.66 ± 0.38	1.85	0.622
16	24.59 ± 0.98	-9.03	0.005	57.49 ± 0.87	-2.41	0.092	13.21 ± 0.24	6.28	0.107

Chapter 4

Ultraviolet absorption

4.1 Leaching solution

As described in 2.2.4,UV absorbance was measured for all solutions recovered after leaching or reflux and infusion. The absorbances shown in the charts and tables below have all been corrected for the dilution factors applied to initial solutions that were experimented to bring the absorbances on scale.

4.1.1 UV absorption under different pH conditions

As discussed in 2.1.4 (Table 1), a primarily experiment of UV absorption was conducted by using the leaching solutions at 3 different pHs. The main purpose was to collect data which could be useful for the design of primary experiment.

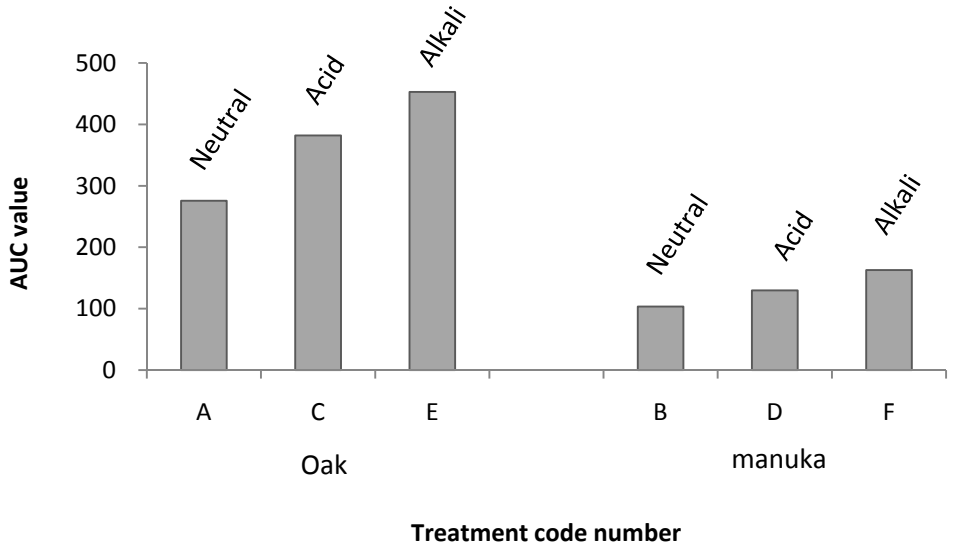


Figure 29 Area under curve (AUC) values for the leaching solutions at pH neutral (DI water), acid (0.01M HCl) and alkali (0.01M NaOH) conditions.

Figure 29clearly shows that the oak leaching solutions had AUC values significantly higher than the values from manuka under all the three pH conditions, a probable indication

that more water soluble UV-absorbing substances - mostly phenolic matters were extracted from oak. It was also found that AUC value increased with the increase of pH value in both species.

At first sight, it appears that more UV-absorbing materials were extracted in alkaline conditions. However, it was later realised that absorbance of phenolic matters are affected by pH (Young *et al.* 2010). Assuming that the pH of the reflux solutions are unchanged by reflux with wood, the pHs of the solutions after dilutions are supposed to be approximate pH = 3 for acid, pH = 7 for neutral and pH = 11 for alkali. At the first of this experiment, this possibility was no thought of and the post-reflux solutions were not recovered. Thus, the possibility of residue pH effects on absorbance remains a major uncertainty in inspecting these data. However, this pH issue did not affect later work because deionised (DI) water was used for all subsequent works.

4.1.2 UV absorption by water treatments

DI water was chosen as the solution to conduct the leaching and reflux for the chips. It was considered as a primary focus in this project mainly because there were so many still unknown in the area for accelerated leaching using water. It should be the starting point for the research.

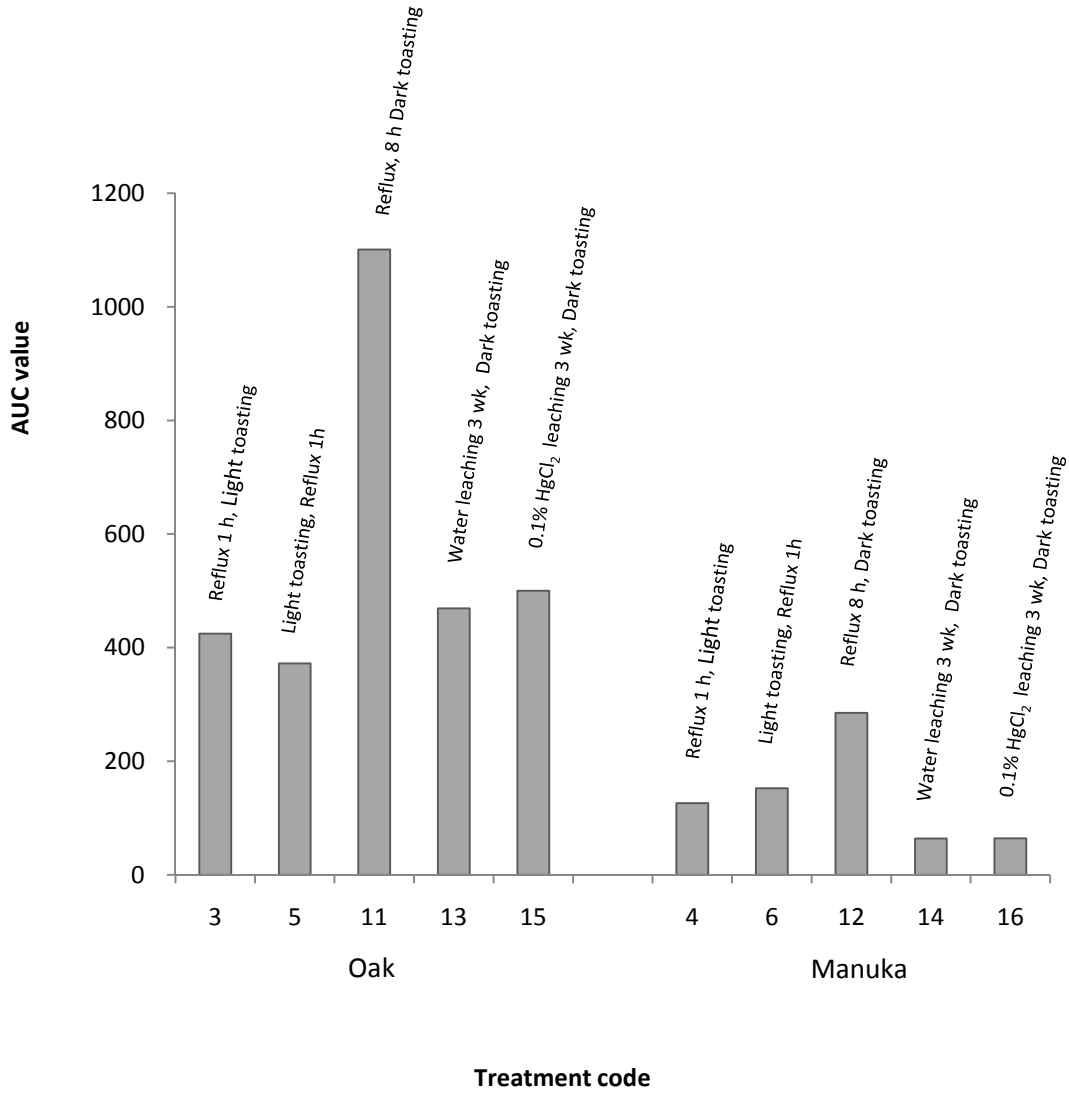


Figure 30 AUC values for the leaching solutions from the different treatments

4.1.2.1 *Oak*

The most obvious point was that the oak had higher AUC values than the manuka (Figure 30), which is an evidence that oak species contained more UV-absorbing compounds and/or structurally more prone to materials loss under the various leaching treatments.

It is also evident that Treatment 11 (reflux 8 hours) had the highest AUC value after leaching, indicating that vigorous reflux strongly increased the release of UV-absorbing matter compared to other relatively milder treatments (Figure 30). There was only marginal difference between Treatments 3 and 5, suggesting less effect due to the sequence of 1h reflux

and light toasting. Similar AUC values were found between Treatments 13 and 15, suggesting no fungal or bacterial effect in the leaching process.

The UV scanning curves for the oak leaching solution (Figure 31) also provided some useful information. One interesting point was the flat curve shape of Treatment 5, showing decreased absorbance between 200 and 300 nm but a stable zone between 300 and 450nm compared to others. It was evident that thermal degradation caused a broad change in compounds composition at the stage of toasting.

Another interesting part of the plots was that similar UV curves both in shape and intensity were found among Treatments 3, 13 and 15. It showed that two different leaching methods i.e. 1 hour refluxing and 3 weeks water soaking could produce quite similar UV spectra, quite likely leaching similar compounds. On the other hand, the near identical curves for Treatments 13 and 15 confirmed the previous finding for AUC values (Figure 30).

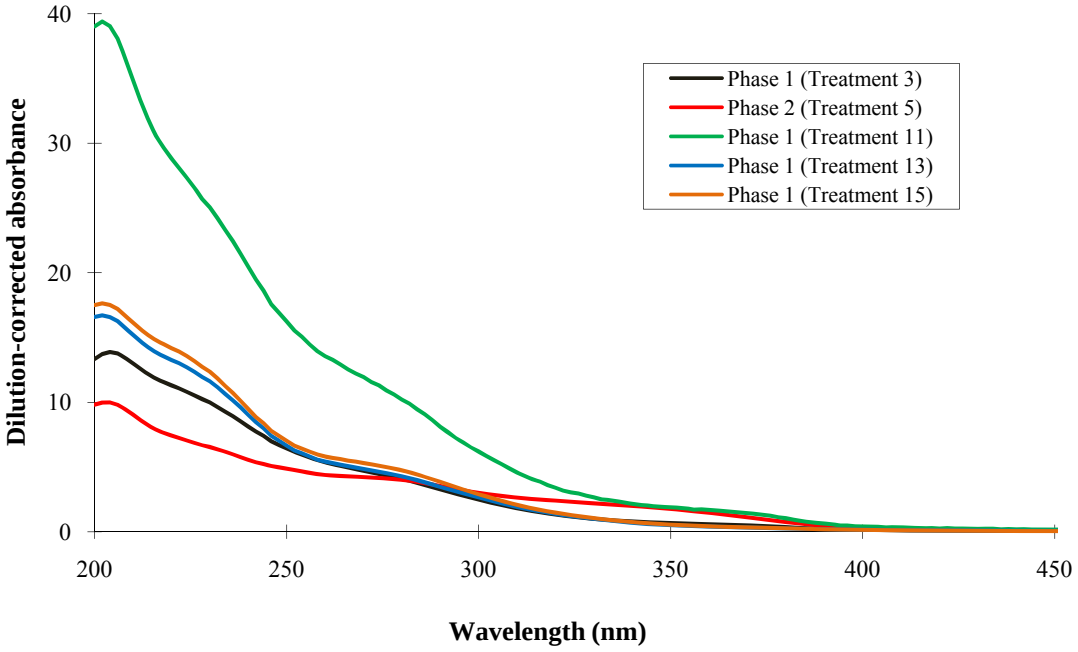


Figure 31 UV scan plots of the oak leaching solutions

4.1.2.2 *Manuka*

To reiterate from Figure 30, oak yielded more water soluble UV-absorbing compounds than manuka. Similar to the finding in oak treatments, the highest AUC value belonged to Treatment 12 – reflux for 8 hours. Another result was that there were no fungal or bacterial effects involved in long term leaching (Treatments 14 and 16), as judged by the closely similar AUC values. However, unlike oak, the reflux methods (Treatments 4, 6, 12) had significantly higher AUC values than the water leaching methods (Treatments 14 and 16).

UV scanning curves for the manuka leaching solutions have been vertically scaled in two ways. The scale 0 to 40 (Figure 32a) enables direct comparison with oak (Figure 31), while the scale 0 to 15 shows more detail (Figure 32b). The manuka curve showed parallel patterns to equivalent oak curves. From 250 nm to 400 nm, Treatment 6 (light toasting then reflux) exhibits a different plot pattern from the others. Similar to Treatment 5, it is suggested a big change in composition after the light toasting process.

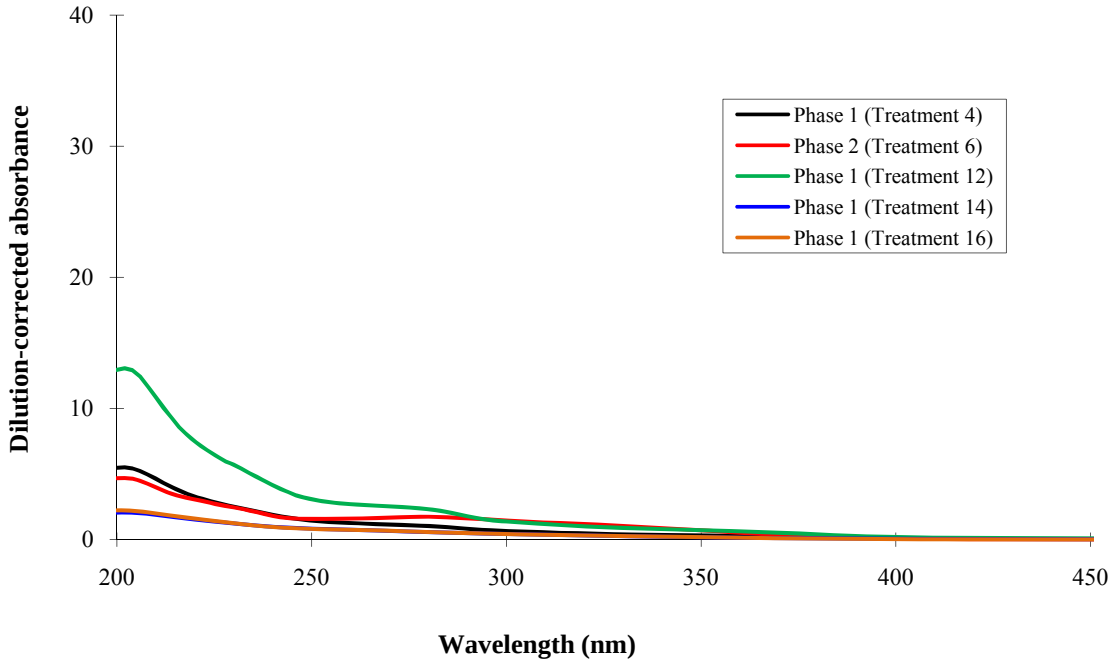


Figure 32a UV scan plots of the manuka leaching solutions

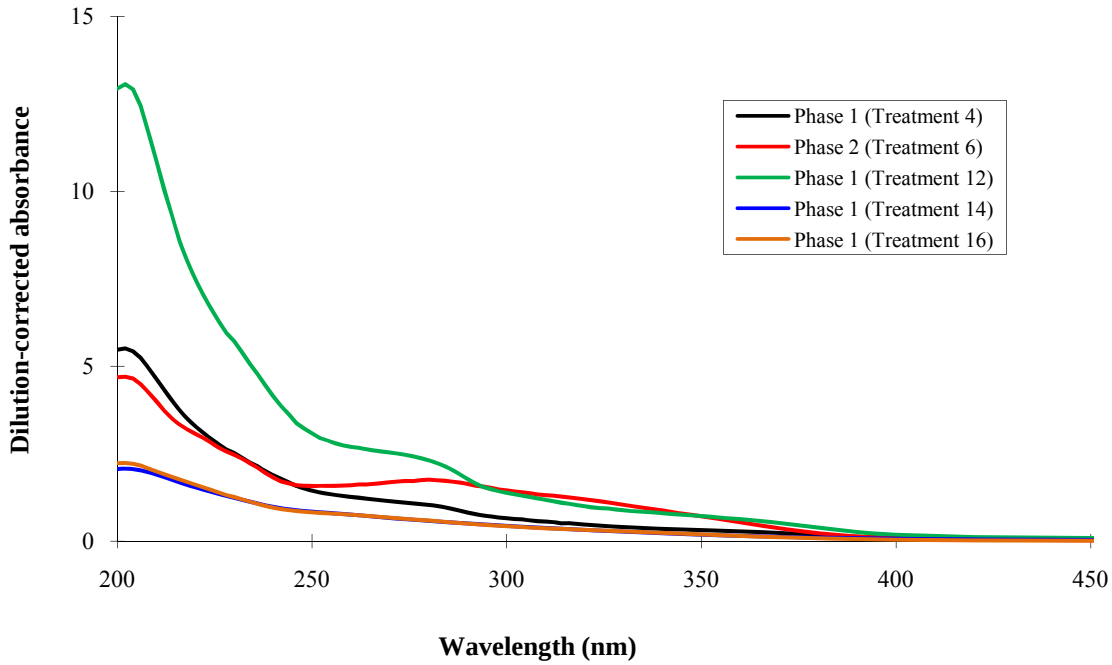


Figure 32b Rescaled UV scan plotsof the manuka leaching solutions

Another point observed was the identical curves for Treatments 14 and 16. This was also seen in the oak group i.e. Treatments 13 and 15, suggesting microorganisms had no effect on the UV absorbance. Anti-fungi treatments probably were not essential during the leaching processes provided the solutions were refrigerated, and measured without delay.

4.2 UV absorption after alcohol infusion

In Phase 3 the treatments were identically infused in 60% ethanol described in 2.2.2. The resultant solutions were measured for UV absorbance from which the distinct UV profiles and also the AUC data were collected.

It was clearly shown (Appendix 2) that the oak group exhibited higher AUC values than the manuka group by approximate 36% on average. This result paralleled AUC values in the leaching solutions (Figure 30), indicating higher leaching and extraction capacity of the oak wood. The phenomenon could suggest that the manuka as a species was more resistant to leaching and infusion, or had lower level of UV-absorbing compounds than the oak.

4.2.1 Oak

From Figure 33, the position of the absorption curve for the authentic whisky was the very lowest, indicating a lower level of presence of total UV-absorbing matter in the whisky than in the other treatments. Meanwhile, the curve showed a shallow valley at about 240 nm and a peak at 270 nm. These suggest the possibility due to the different composition of compounds from the wood-only treatments here.

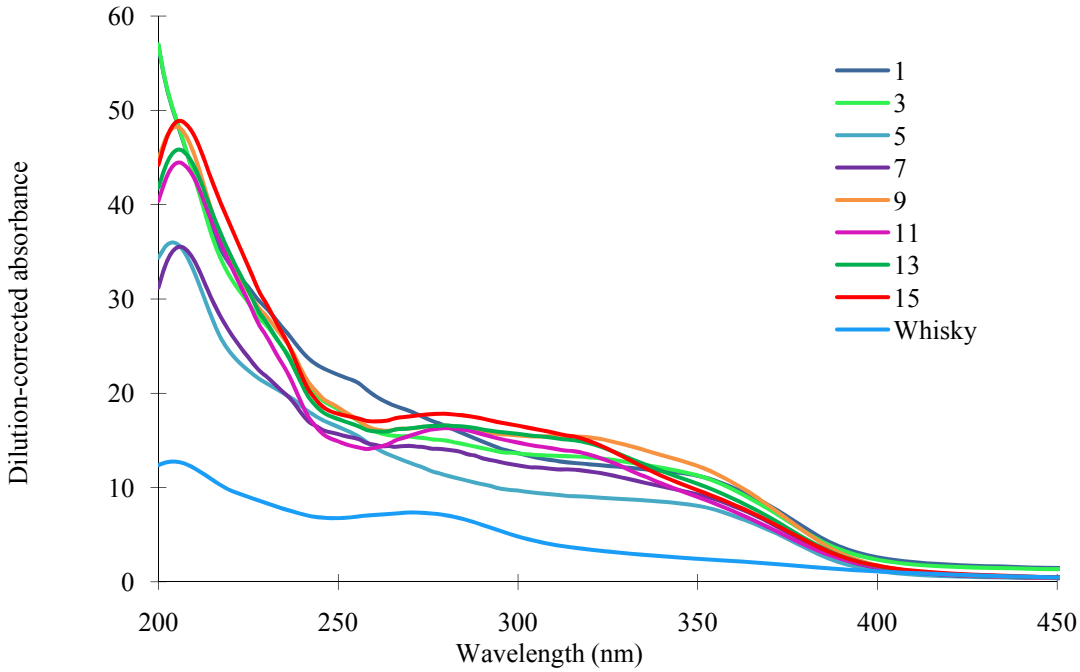


Figure 33 UV scan curves for the alcohols after infusion for the oak group with the authentic whisky (Red Label from Johnnie Walker) as a reference.

The curve position for Treatment 5 (light toasting then reflux) was lowest among the other treatments (Figure 33). The curve was rather similar to that for Treatment 1 as both showed smooth shape without any obvious bump after 220 nm, indicating a probable similar composition of compounds between the two solutions.

The curves for Treatments 3, 9, 11, 13 and 15 also exhibit similarity both in shape and

intensity, showing a shallow valley at about 250 nm, signifying the resemblance both in composition and concentration of the compounds for these treatments.

The AUC value for Treatment 5 (1227) was significantly lower than for Treatment 1 (1831) while only minor difference was found between Treatments 1 and 3 (1737) (Appendix 2). It suggests that reflux after toasting ultimately results in a reduced extraction of UV-absorbing compounds into 60% ethanol.

Appendix 2 also shows the effectiveness of the toasting level on the release of UV-absorbing compounds. It is clear that light toasting (Treatments 1) (1831) resulted in higher AUC value than dark toasting (Treatments 7) (1419) from previously untreated oak, which according to Crum (1995) was because very hot temperature and charring markedly degraded phenolic materials on the surface, thereby reduced the extractable fraction into the alcohol and limited aromatic compounds contribution.

Unlike the treatments with light toasting (Treatments 1 and 3) (1831 vs. 1737), the dark toasting involved treatments i.e. Treatments 7 (no reflux) and 9 (reflux 1h) had a clear difference in AUC value (1419 vs. 1801) after infusion, with a higher AUC value in Treatment 9. The leaching process in Treatment 9probably modified the wood surface through the combined actions of disintegration and hydrolysis etc., leading to a higher level of release of aromatic compounds after infusion in comparison to Treatment 7- the same treatment without leaching. However, no significant difference was found in AUC values between Treatments 3 (light toasting) and 9 (dark toasting) where same leaching process but different toasting levels were both involved.

It was also found AUC values from both water soaking Treatments 13 (1726) and 15 (1802) were markedly higher than that from Treatment 7 (1419). The results confirmed that the extended leaching enhanced the extraction level during infusion.

4.2.2 Manuka

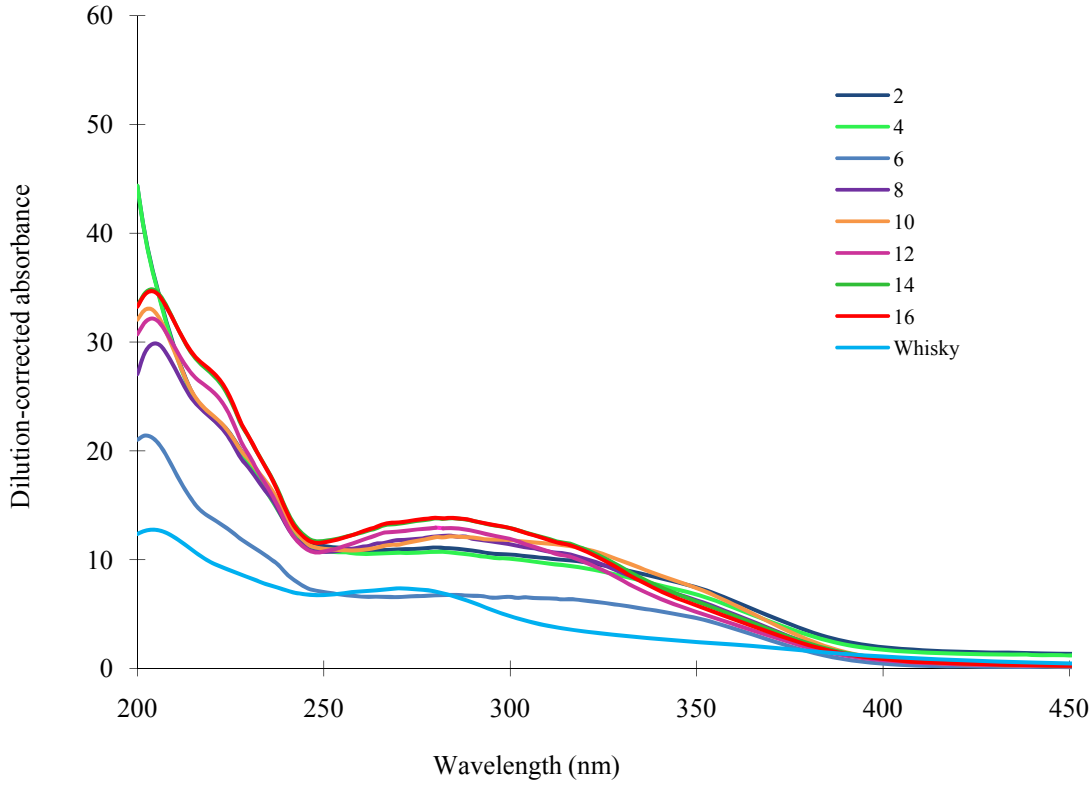


Figure 34 UV scan curves for the alcohols after infusion for the manuka group with the authentic whisky (Red Label from Johnnie Walker) as a reference.

In comparing Figure 34 with Figure 33, it is clear that manuka yielded less UV-absorbing matter. The curve for Treatment 6 (toasting then reflux) paralleled Treatment 5- the equivalent oak treatment. Likewise, there was a strong similarity both in curve shape and intensity for Treatments 2, 4, 8, 10, 12 and 16, as was seen with oak.

Similar to the oak group, as the lowest among other treatments in the manuka group, AUC value for Treatment 6 (toasting then reflux) (718) was significantly lower than Treatments 2 (1232) and 4 (1185) while no difference in AUC value was found between Treatments 2 and 4 (Appendix 2). The results were probably related to the greater loss of compounds (generated by toasting) through leaching.

In the manuka group, the differences on AUC value were far less among the treatments (except for Treatment 2) than those in the oak group, which exhibited a wide range of variations among the equivalent treatments. This was also clearly reflected by the *p* values (Appendix 2). The data showed strong evidence that leaching had less effect on manuka compared to oak.

It was also found (Appendix 2) that the AUC level for water soaking Treatments 14 and 16 were significantly higher than Treatment 8 while no difference was found for Treatment 8 against Treatments10 and 12, indicating no connection between the degree of leaching and AUC result in the manuka, which was different to that in the oak group.

4.3 Assessment of woody odour by smell

The alcohols after infusion were also smelled for their odour differences between and within species.

Table 9Flavour comparison of the alcohols between and within species by four assessors

	Treatment			Result
Within species	1	vs.	7	Treatment 7 smelt more mellow, less woody
	2	vs.	8	Inconsistent opinion
	9	vs.	11	Inconsistent opinion
	10	vs.	12	Inconsistent opinion
Between species	1	vs.	2	Inconsistent opinion
	9	vs.	10	Inconsistent opinion

The result (Table 9) confirms the casual observations made throughout the project that woody notes persisted. However, Treatment 1 and 7 gave different results. Treatment 1 was no preliminary treatment of oak in Phase 1 followed by light toasting. Treatment 7 was the same except for dark toasting. The manuka equivalents (Treatments 2 vs. 8) by contrast did not show this difference. Discussion of these limited sensory data is deferred to Chapter 6.

Chapter 5

Gas Chromatographic Analysis

The treatments in Table 2 were also analysed by gas chromatography using flame ionisation detection. The main purpose was to identify any differences in the patterns between the various treatments rather than identification and quantification of the individual peaks of the extracts. As explained in Chapter 2, three of the four replicates of infused alcohols from each treatment were randomly selected and analysed by direct injection after addition of the internal standard.

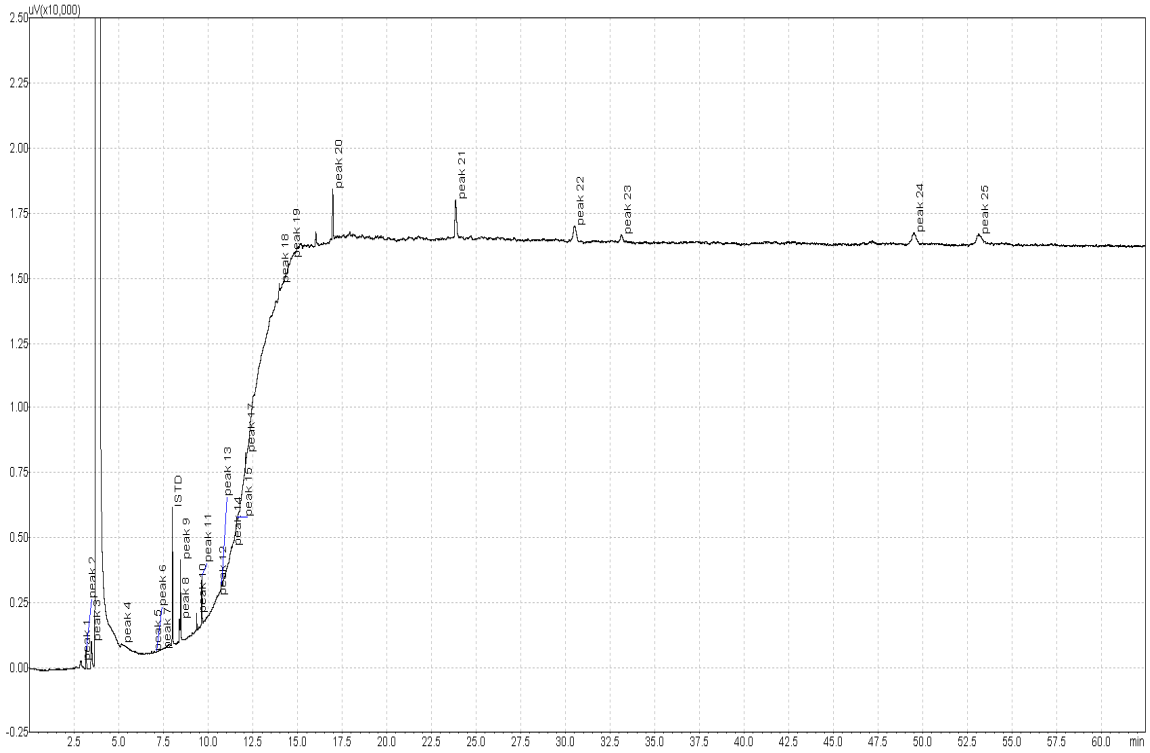


Figure 35 Typical chromatogram for Treatment 1 (oak, non-reflux, light toasting)

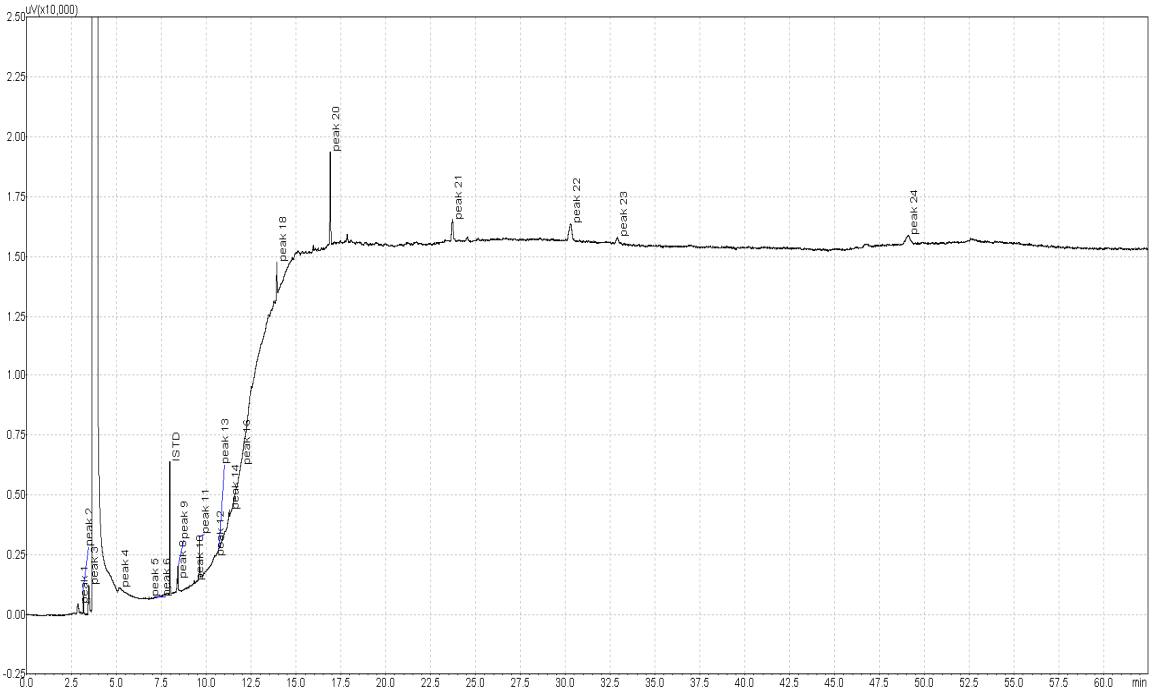


Figure 36 Typical chromatogram for Treatment 2 (manuka, none-reflux, light toasting)

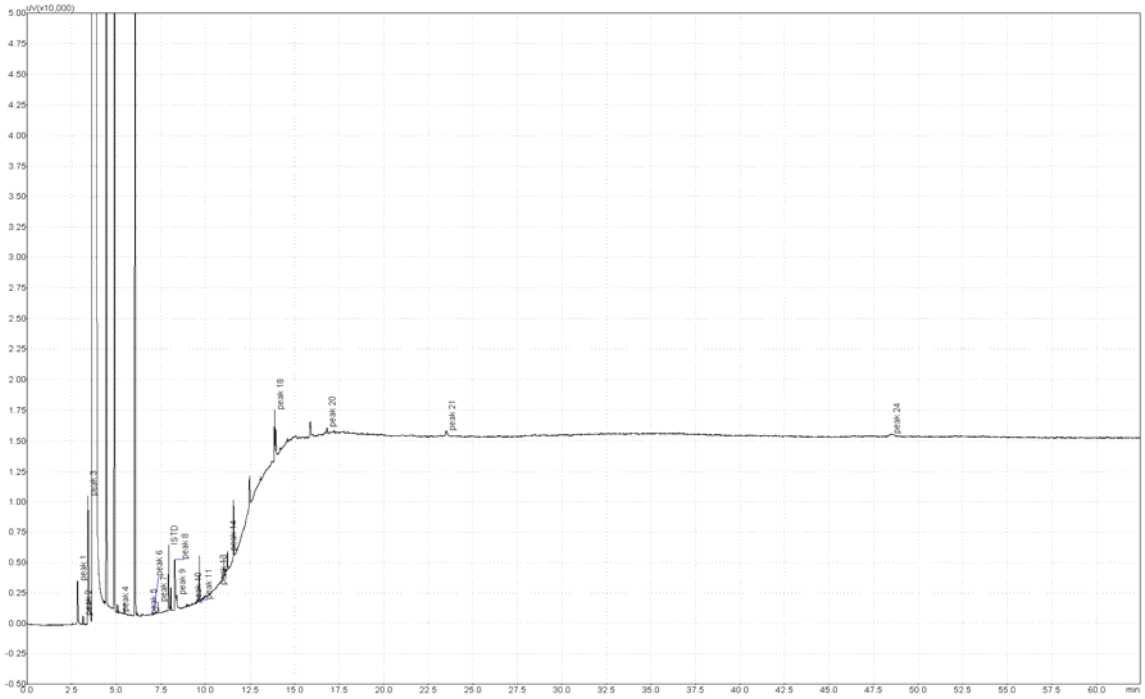


Figure 37 Chromatogram for authentic whisky (ethanol level adjusted to 60%)

5.1 Relative concentrations of peaks from oak, manuka and whisky

Figure 35, Figure 36 and Figure 37 were typical gas chromatographic profiles for oak, manuka and whisky, where the peaks of the internal standard were similar. All treatments had same long run time of 62 min, of which the last 50 min were isothermal at 240 °C. This extended isothermal hold was needed to ensure all compounds had eluted before reinjection. Inspection reveals that many common peaks could be identified in the spirits; the variation of retention time was less than 1% (the confidence limit) for all those peaks. However, there was a preponderance of very early eluting peaks in authentic whisky, which are unnumbered and not included in the analysis. These early eluting peaks in whisky (Figure 37) were probably alcohols and volatile flavouring compounds ultimately derived from fermentation of grain or grapes, realising that the Scotch whisky is aged in oak barrels previously used to store fortified grape wines.

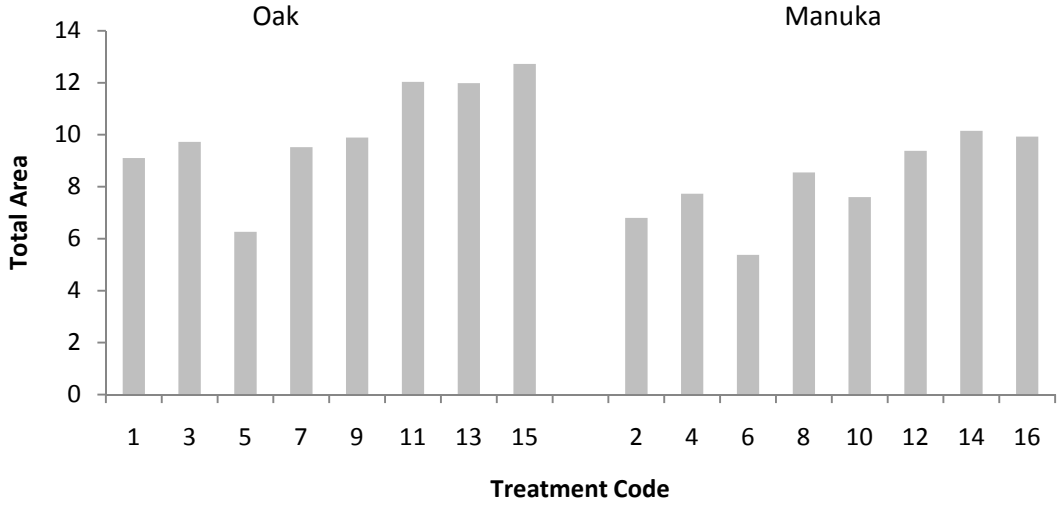


Figure 38 Total peak areas of the detected compounds for all the treatments, normalised with the internal standard as 1.

The total peak areas for all the oak and manuka treatments (Figure 38) provide approximate information on the overall scale of all the infusions. It shows that oak infused more strongly, but the general appearance of the two patterns is similar. The greater infusion from oak parallels the results from UV absorbance analyses (Appendix 2).

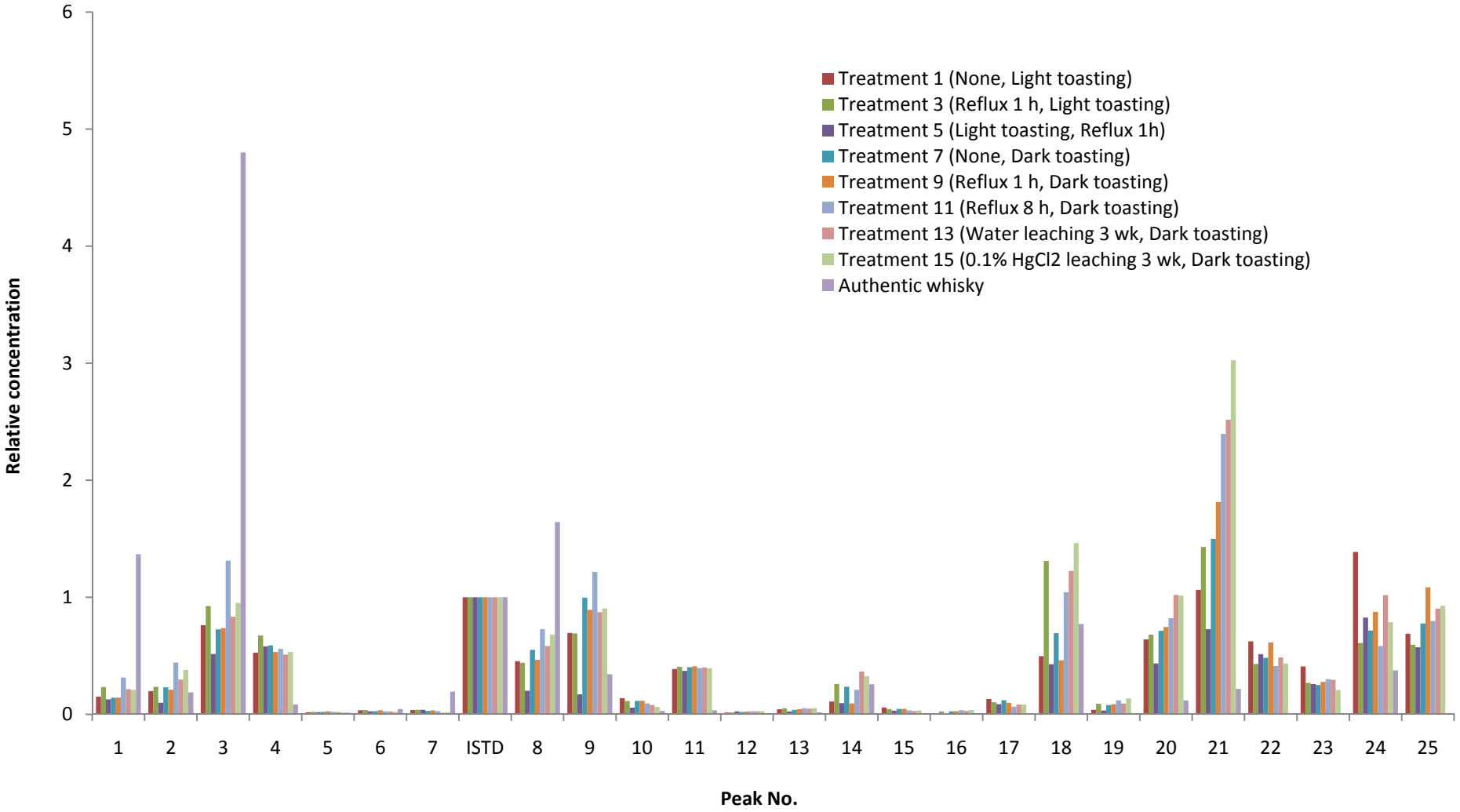


Figure 39 Relative concentration (internal standard as 1) of detected compounds for all the oak treatments and authentic whisky

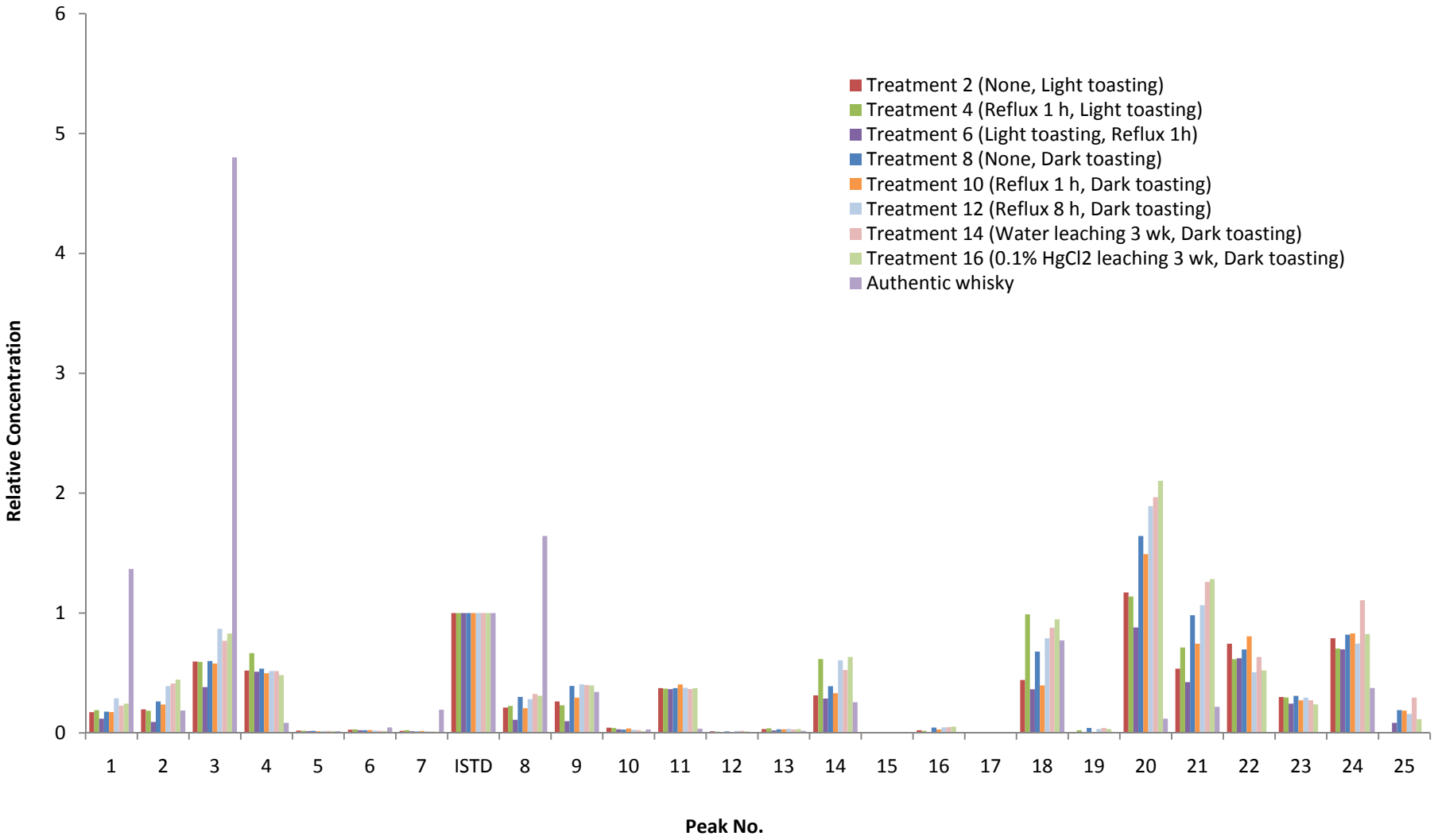


Figure 40 Relative concentration (internal standard as 1) of detected compounds for all the manuka treatments and authentic whisky

The data can also be viewed in another way, essentially 'drilling down' into more detail for oak (Figure 39) and manuka (Figure 40) treatments, and for authentic whisky. As in Figure 38 the data have been normalised to the internal standard. Authentic whisky comprises the extreme right hand bars in both graphs.

First consider Figure 39 for oak. An inspection of the general pattern of relative concentration reveals that treatments involving dark toasting yielded relatively higher concentrations of the several peaks particularly some later eluting peaks, for example, Peak 20 and 21. Other peaks have complex patterns in which Treatment 5 (light toasting, reflux for 1h) was especially low in relative concentrations, as also noted in Appendix 2 for AUC (area under the curve) data for UV absorbance. This was the only treatment where reflux was performed after toasting, and the result suggests there is no benefit in this particular treatment. Peaks 1 and 3, the two most volatile compounds were notably dominant in authentic whisky. Given that both eluted before ethanol – the largest unnumbered peak – suggests Peak 1 and 3 may be acetaldehyde and methanol respectively, as previously observed in whisky (Phenomenex 2012). Peaks 7 and 8 in authentic whisky were also dominant compared to the same peaks in other treatments. However, there were also many peaks (Peaks 4, 9, 20, 21 and 24) in authentic whisky that were markedly lower than in other treatments. Moreover, many peaks – especially very late eluting peaks including Peaks 17, 19, 22, 23, and 25 – were absent in authentic whisky, suggesting a possible connection between these compounds and the woody notes.

In Figure 40, where manuka data are shown, Peak 20 rather than 21 was the dominant peak, and as for oak, the dark toasting treatments generated greater detector response. As for oak, authentic whisky shows that some earlier eluting peaks including Peaks 1, 3, 7 and 8 were particularly higher, and some peaks were particularly lower such as Peaks 2, 4, 20, 21 and 24 than in other manuka treatments. Likewise, the presence of some later eluting peaks in manuka treatments but the absence of these same peaks in authentic whisky confirm the oak results. As for oak Treatment 5, manuka Treatment 6 (light toasting, reflux for 1h) was particularly low in relative concentrations. Finally, comparison of Figure 39 and Figure 40

confirms the result in Figure 38 where overall detector response was greater in oak than in manuka.

Appendix 3 and Appendix 4 give all the data plus standard deviations that in many cases were large, especially where peaks were small. This contrasts with the UV data generated from the same samples. This strongly suggests that there was more variation due to the gas chromatograph behaviour that to the infusion replicates. However, for clarity, the standard deviations bars in the following graphs have not been added. Because of the high standard deviations (in mostly small peaks), only obvious differences in means will be discussed.

In comparing treatments there are many possible comparisons between the 16 treatments. However, for simplicity and clarity those selected to be shown were only the comparisons where differences in treatments might occur in a commercial situation. Thus the pairs reflux for 1 h vs. no reflux and light toasting vs. dark toasting were compared in both species.

5.2 Effects of treatment on oak chromatograms

The GC results show few clear difference in concentrations from the eluted compounds between Treatment 1 (light toasting only) and 3 (reflux 1h, light toasting) (Row 1, Figure 41). Treatment 3 had higher extraction levels in the earlier eluted peaks which were compounds with lower molecular masses and higher volatility. The later eluted peaks shows a mixed extraction results between the two treatments: Treatment 3 had much higher concentrations for Peaks 18 and 21 but a lower concentration for Peak 24. Overall, the earlier involvement of reflux prior to light toasting had no substantial effect on the degree of infusion.

For Treatment 3 (reflux 1h, light toasting) and 5 (light toasting, reflux 1h), it is clear from the graph (Row 2, Figure 41) that the level of infusion was significantly different between the two treatments. The concentrations of almost all the detected compounds from Treatment 3 were higher than those from Treatment 5. The process of light toasting followed by reflux (Treatment 5) probably leached off substantial amount of the compounds

arising from toasting, subsequently lowering the extraction capacity of the treated oak during infusion. Clearly, reversing the sequence of reflux and toasting markedly changes the degree of infusion.

No marked difference was found between Treatment 1 (light toasting only) and 7 (dark toasting only) (Row 3, Figure 41). Close inspection of the graphs shows higher concentrations of mainly middle order peaks in Treatment 7 while mixed concentrations of later eluted peaks were observed in both treatments. Peak 21 was more dominant in the dark toasting treatment while Peak 24 was less dominant.

Likewise, there were only minor differences between Treatment 7 (dark toasting only) and 9 (reflux 1h, dark toasting) (Row 4, Figure 41). The right hand graph shows Treatment 7 had slightly higher concentrations of earlier eluted compounds (from Peak 1 to 20) than Treatment 9. On the other hand, Treatment 9 was higher in later eluted compounds (from 20 to 25).

It is useful to compare Rows 1 and 4, where the only difference is degree of toasting. Greater toasting reduced the effect of prior reflux.

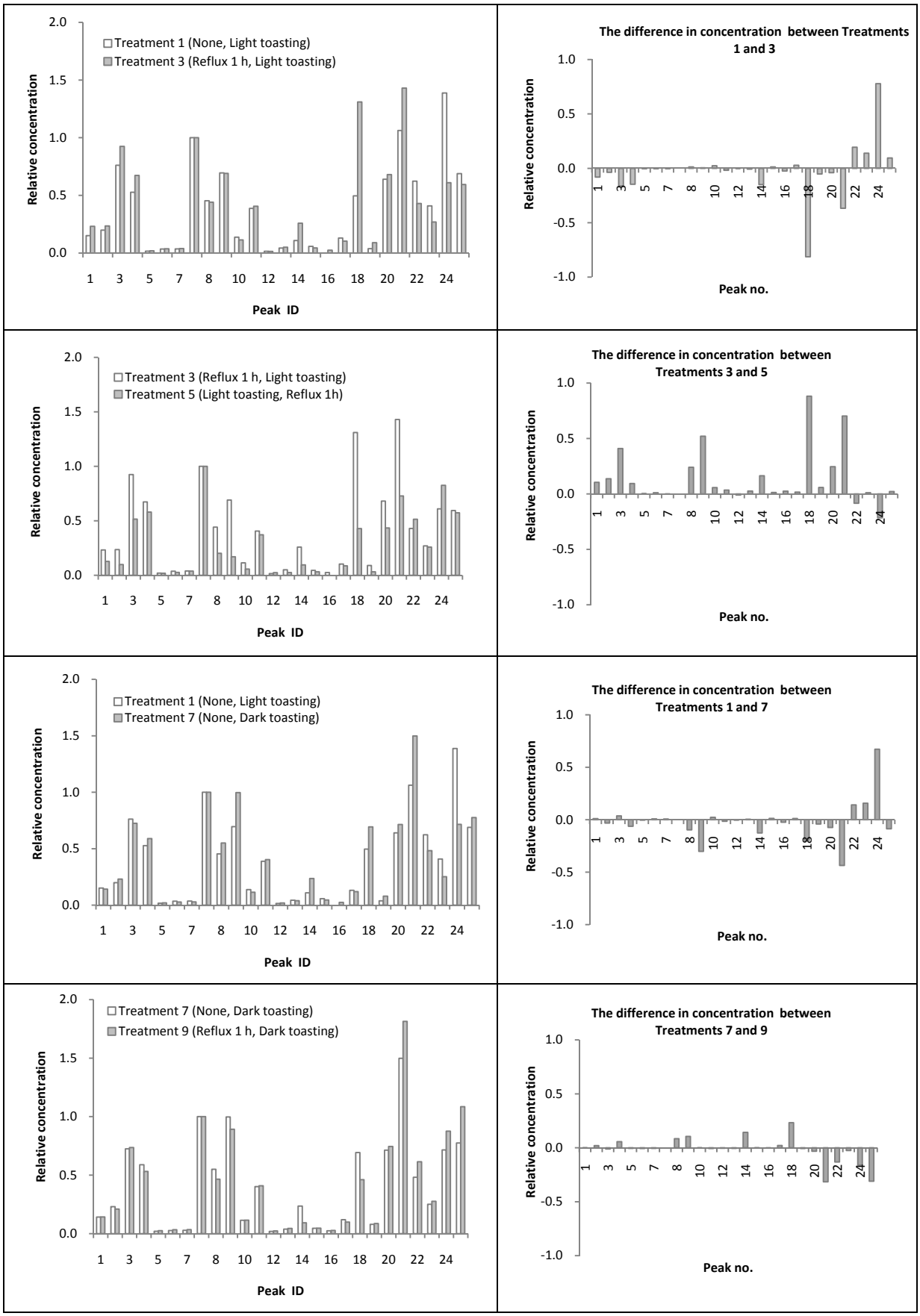


Figure 41 Comparisons of selected some oak treatments by chromatographic difference

5.3 Effects of treatment on manuka chromatograms

The same comparisons made for oak were made for manuka (Figure 42). Row 1 shows that Treatment 2 (light toasting only) had relatively higher concentrations of extracted compounds than Treatment 4 (reflux 1h, light toasting) (Row 1, Figure 42). Compared to their oak equivalents, there were also higher concentrations of earlier eluted compounds in Treatment 2 than in Treatment 4, but for the later eluted compounds Treatment 4 peaks were only slightly higher than in Treatment 2. Overall the effects of reflux were subtle, and less obvious than in oak.

Similar to their oak equivalents, the concentrations of almost all the detected compounds in Treatment 4 were higher than in Treatment 6 (Row 2, Figure 42). As with oak equivalents, reversing the sequence of reflux and toasting changes the degree of infusion.

The graph on Row 3, shows higher concentrations of extracted compounds in Treatment 8 (dark toasting only) than in Treatment 2 (light toasting only). Inspection reveals that the difference in concentrations for the earlier eluted compounds was minor as the major difference only occurred for the later eluted compounds. A striking difference between oak and manuka in this comparison is seen in peak 24. Light toasting favoured this compound in oak (strongly positive) but not in manuka (close to zero). Overall, dark toasting had more effect on oak than manuka.

Figure 42 (Row 4) illustrates that the concentrations of almost all the detected compounds in Treatment 8 (dark toasting only) were generally higher than in Treatment 10 (reflux 1h, dark toasting). That is to say potentially infusible compounds were generally lost on reflux. This contrasts with their oak equivalents, where later eluting compounds were more dominant (negative after reflux).

Further discussion of these differences between oak and manuka GC patterns is deferred to Chapter 6.

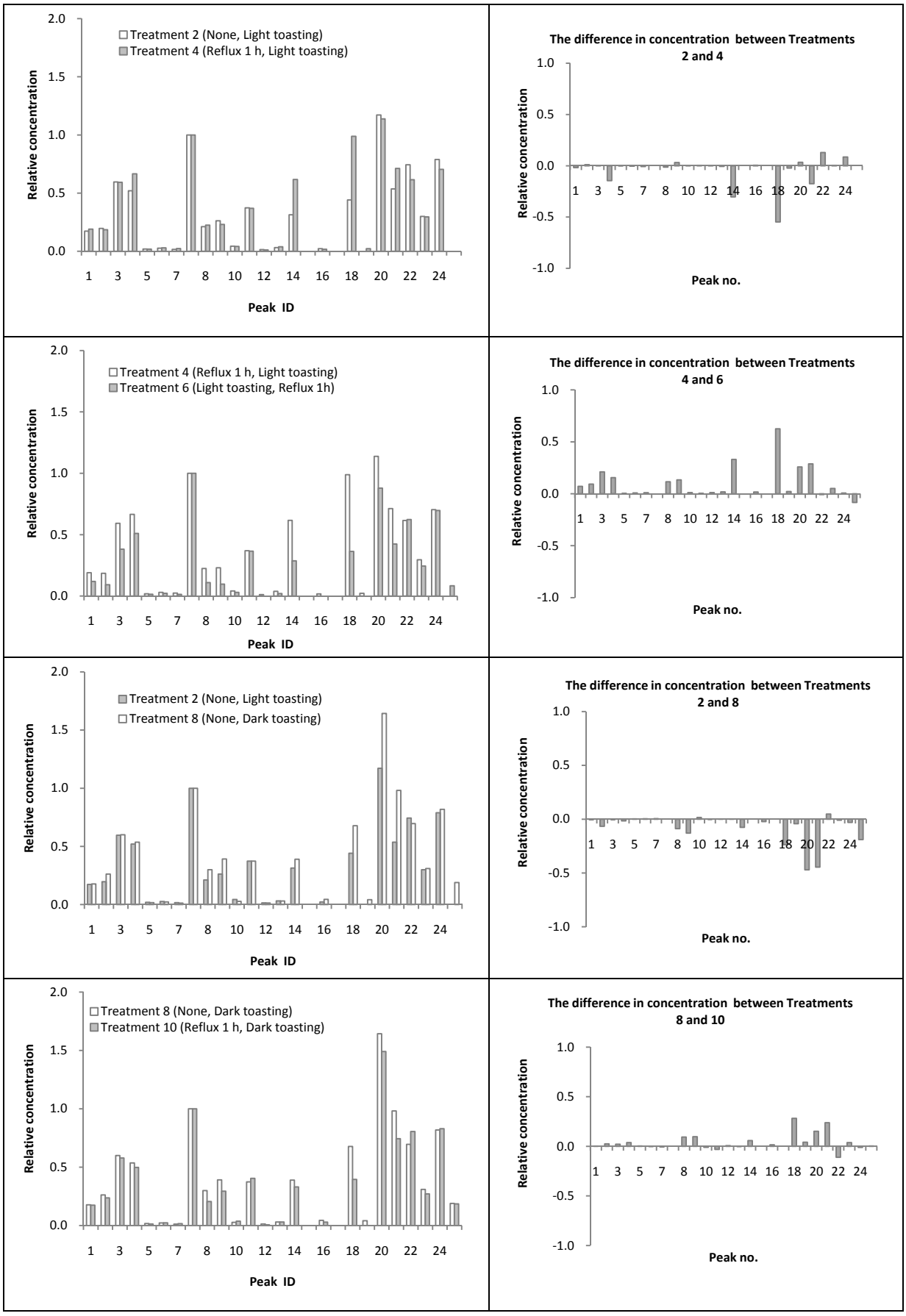


Figure 42 Comparisons of selected some manuka treatments by chromatographic difference

Chapter 6

Effect of the various treatments and overall comparison with whisky

6.1 Seasoning

The Introduction's Section 1.8, emphasised the claimed importance of seasoning oak before barrel construction and toasting. The literature to support the claims of fungal colonisation and loss of ellagitannins by their metabolism and by leaching from rain is scant. What is not emphasised in seasoning is the slow drying, which does appear to be important in final barrel stability and possibly liquid-tightness. Certainly oak has to be dried in order to make barrels. Given that ellagitannins are leached off by rain, it stands to reason that wood near the plank surfaces will be more leached than wood deeper in the planks that are more than 20 mm thick. Importantly, one (widest) surface of the oak planks is the part of the final stave that is in contact with the wine. However, planks are surfaced-planed before barrel construction, so it is unclear how much of the outer plank surface – the most leached part – is lost by planning and there not in contact with wine after toasting.

Now consider oak chips in commercial application. Nothing is known of the treatment – if any – that the oak chips are subject to before toasting. It is possible that oak is chipped before drying, which would then occur in the toasting oven before pyrolysis took place. Thus, toasted oak chips may or may not have had prior seasoning, realising at the same time that prior seasoning would obviously add to cost.

6.2 Surface area, weight loss and colour changes on toasting

Table 3 shows that the mean surface area of the oak was greater than that of manuka by a factor of 1.2. Then one question arises that oak might be expected to result in greater extraction as was indeed found to be the case. However there are some reasons as to why this difference in surface area may not be important. First, despite of the greater surface area per chip unit, the total surface area of oak used in each treatment was rather similar to that of manuka because more pieces of manuka chips required for the same weight of oak. Second, both woods were found to undergo even colour change throughout the chip after

cross-section inspection, indicating a consistent chemical change of all wood. Thus infusion was controlled by mass, and indeed equal masses of both species were used in all infusions.

Reflux always resulted in weight loss, except in the situations where toasting preceded reflux, Treatments 5 and 6. The greatest weight loss was on reflux for 8 h and this was reflected in the AUC values for leaching solutions, for both oak and manuka, particularly the former (Figure 30). Much more UV-absorbing material was lost from oak than from manuka, an indication that the manuka was more stable to leaching.

It is clear that manuka suffered greater weight losses than oak on toasting. At first sight this means that manuka suffered more pyrolytic weight loss. However, what was not known was the moisture content of the chips prior to toasting. This would have to have been determined by drying at 100 °C prior to toasting. In hindsight this should have been measured but was not.

Within species, both woods had more weight loss through dark toasting than light toasting for both untreated and previously refluxed chips. This is not surprising and has been well documented (Young *et al.* 2010).

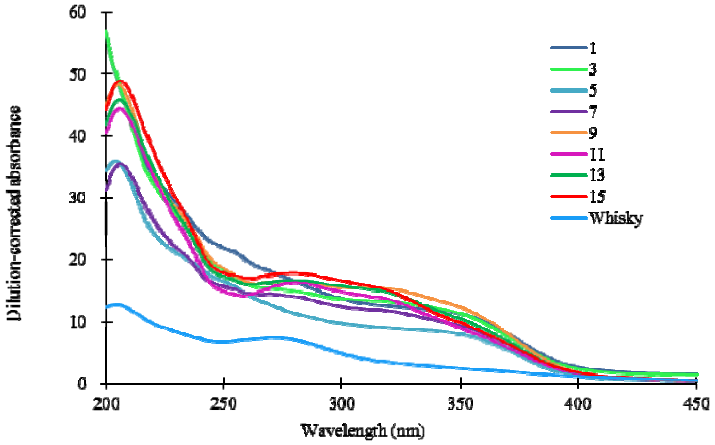
It is also clear that oak is more susceptible to colour change than manuka both on accelerated leaching and toasting. To some degree, colour change after treatments is likely to be a reflection on changes in physical structure and/or chemical modification in the woods. Indeed, colour changes generally confirmed the findings from the other experimental results. In summary that manuka is more enduring than oak towards various leaching treatments and toasting. The correlation analyses in 3.3.1 on weight loss and colour change also confirm this general outcome.

Intuitively, weight losses should occur after toasted chips are infused into60% ethanol because of the extraction of compounds into the spirit. However, expect where toasting was followed by reflux (Treatments 5 and 6) weight changes were reverse of the expected outcome. Weight was gained rather than lost. Because water rather than ethanol is the

naturally occurring liquid in wood, it is highly likely that water is being lost from the spirit and being bound stably when the chips are dried after infusion before reweighing. This result does not provide an insight on the infusion effects of compounds into spirit, but it adds to our understanding of the overall process. As water uptake is the dominant factor here, and it is also a direct reflection and measure of water-binding capacity of the woods. Therefore, the data for weight losses after infusion (Figure 24) are the approximate measure for water-bind capacity, thus in turn the degree of damage to the woods from all the various water treatments i.e. the reflux and water soaking. It is clear from Figure 24 that the less damaged light toasting treatments were able to reabsorb more water than the more severely damaged dark toasting treatments. In the limiting case, wood would turn to charcoal – essentially graphite – there would be no expected uptake of water (hydrophilic) on charcoal (hydrophobic).

6.3 The importance of UV light absorption after infusion for woody odour

The failure of reflux to reduce the woody odour was disappointing, but spectrophotometric data presented in this thesis suggest that the woody odour may be less of a technical problem than first thought. Consider Figures 33 and 34, the first of which is reproduced below to support the following argument. The absorbance due to whisky in the UV range is much lower than for the experimental infusions. This strongly suggests that the quantity of woody matter in authentic whisky is lower than in the current infusions, in turn suggesting less exposure of commercial whisky spirit to wood. This may in part be responsible for lower woody odour in whisky.



This argument can be extended by pointing out that Scotch whisky barrels (at least) have been previously used to age fortified wines like port, sherry and Madeira. Not only will residues of these wines impart a distinctive flavour to whisky that cannot be generated from wood alone, but at the same time some of these grape-derived flavour compounds are likely to absorb UV light. Thus the lowest (whisky) curve in the figure above will represent the sum of absorption from wood and an unknown contribution from grape-derived compounds. In short, the woody odour appears at least in part to derive from too much exposure to wood.

6.4 Other possible causes of woody odour

Throughout the project it was evident that if the woody odour were lost through any of the reflux and similar treatments, the effect was subtle possibly due to the overwhelming odour of the ethanol. The semi-formal assessment confirmed this (Table 9), although the comparison between Treatments 1 (no reflux, light toasting) and 7 (no reflux, dark toasting) showed the dark toasting to be more mellow and quite probably lower in a woody note. Thus, none of the reflux treatments worked, suggesting that ellagitannins leaching prior to toasting is not important. However, it suggests that the woody odour is lost in oak when it is dark toasted, which probably was because of the reduction of some compounds(linked to woody odour) volatilised through increased heating (Crum 1995). Perhaps significantly, descriptions of American whisky production often refer to the use of charred barrels according to Title 27 Code of Federal Regulations Pt. 5.22.

Thus, dark toasting of oak chips appeared to be effective but not so dark toasting of manuka. This may reflect a fundamental advantage of oak which the results show is more changed by toasting than is manuka. However, the probable advantages of dark toasting might also extend to manuka if that species were toasted more heavily. This possibility was not tested.

If dark toasting is linked to a reduction in woody odour, then the chemical causes might

be found by comparing the gas chromatographic profiles of infused spirits and authentic whisky.

6.5 Gas chromatographic profiles and the cause of woody odour

In looking for the chemical cause(s) of woody odour it seems logical to compare peaks present in oak and manuka infusions that are absent in whisky. In this type of comparison, oak is the better wood to compare because that is the wood used to age whisky. Appendix 3 for GC data shows that Peaks 12, 13 to 17, 19, 22, 23 and 25 are candidates for woody odour although smaller peaks could contribute or even be primarily important. This importance, if any, cannot be decided from the present data. Assuming one or more of these peaks is involved does not necessarily mean that they were not present when the authentic whisky had begun its contact with oak staves. It is important to remember that Scotch whisky is aged in oak barrels for 3 years as minimum and is subject to low concentrations of oxygen in this time. The present infusions were done for two weeks although there was a headspace containing oxygen beneath the Schott bottle caps for up to a year after infusion. At the same time the whisky barrels have been previously used to age fortified wines and woody notes may have been lost to those drinks prior to exposure of spirit.

6.6 The way to make a successful spirit from wood of New Zealand species

It is important to state at the outset that the aim is not to emulate existing whisky styles such as Scotch and Bourbon, but rather to produce a whisky-coloured spirit that is closely identified with New Zealand. Thus oak would not be used. In the present study the only New Zealand wood used was manuka, which has iconic status in respect of firewood (it is dense) and food smoking. Its density may be related to the resistance of the wood to toasting and better results might have been obtained with another New Zealand species. Of the woods tested in wine by Young *et al.* (2010), totara was identified as a wood with a lot of potential. It is abundant and is not a protected species on private land, and was the most liked by 180 consumers (Young *et al*., (2010). It is proposed that woody odour should be re-examined in totara and manuka where toasting is light, dark and very dark.

As discussed earlier, woody odour may be present in young whiskies and may take years to be lost, perhaps explaining why whisky is held so long before retail release. However, the evidence that dark toasting of oak shows signs of reducing the odour is encouraging, because the envisaged spirit is intended to be produced and sold for immediate consumption if that were desired. Storage bears costs and should be avoided where possible.

Having chosen the wood and toasting treatment, it is important to limit its exposure to the 60% ethanol, as argued in Section 6.4 above. Assuming that the woody odour problem can be solved by dark toasting and limited exposure, there are other challenges in product design. Whiskies contain esters and other compounds derived from conventional grain-sugar fermentations. Gas chromatographic analyses of Fonterra's whey alcohol at AUT University have shown this alcohol to be very 'clean'. Therefore esters should be added. It is proposed that New Zealand white wine be used for this purpose, either in whole or in part to dilute the 60% 'barrel strength' spirit to the final retail concentration. New Zealand white wines are known for their fruitiness, which is caused by esters. Dilution with water or wine or both would obviously dilute the colour, but it is reiterated that the aims is not to emulate whisky but to create something geographically distinct.

Finally it may be useful to use caramel compounds to intensify colour (if required) and to round out the flavour in another sensory dimension. Lightly caramelised sugar, if added judiciously, could greatly add to the drink's complexity. Whereas any sugar could be used, Fonterra lactose is proposed as the sugar of choice. The reason is that a drinks label could justifiably claim to be made from all New Zealand ingredients.

What would the final retail product be called? This thesis has focused on chemistry and it is beyond the scope of the thesis to seriously propose names and artwork for a retail label, although names come to mind such as 'Totara', 'Manuka' 'Totara Gold' and 'Podocarpus'. However, naming is the province for marketing experts and the more pressing problems are further exploration of woody odour – by the methods discussed above – and the effects of wine and caramel additions on flavour. All this work would have to be accompanied by consumer sensory testing, which is perhaps the most difficult challenge of all

give the highly alcoholic nature of the spirit.

Appendices

Appendix I Weight changes by percentage after infusion (Phase 3)

Oak			Manuka		
Code	% Weight gain[1]	% RSD	Code	% Weight gain[1]	% RSD
1	5.12±0.16	3.10	2	5.41±0.73	13.43
3	4.81±0.16	3.35	4	5.33±0.15	2.72
5	-1.51±0.22[2]	14.78	6	-0.74±0.06[2]	8.05
7	2.95±0.07	2.22	8	3.52±0.10	2.81
9	3.79±0.16	4.24	10	4.16±0.05	1.30
11	1.72±0.09	4.98	12	2.58±0.09	3.43
13	1.81±0.06	3.43	14	2.70±0.06	2.09
15	1.59±0.12	7.45	16	2.55±0.10	3.85

[1]Data are mean values from 4 replicates with the relative standard deviation

[2]Negative values represent weight loss

Appendix II Area under curve (AUC) for the alcohols after infusion

Oak				Manuka			
Code	AUC	*p* value_1	*p* value_2	Code	AUC	*p* value_1	*p* value_2
1	1831±116[1]	N/A	N/A[3]	2	1232±32	N/A	N/A
3	1737±91	0.250 (1)[2]	0.001 (5)	4	1185±47	0.153 (2)	0.000 (6)
5	1227±132	0.000 (1)	N/A	6	718±42	0.000 (2)	N/A
7	1419±92	0.001 (1)	N/A	8	1135±65	0.037 (2)	N/A
9	1801±126	0.003 (7)	0.442 (3)	10	1199±24	0.116 (8)	0.636 (4)
11	1608±86	0.024 (7)	0.044 (9)	12	1164±50	0.513 (8)	0.258 (10)
13	1726±56	0.001 (7)	0.214 (15)	14	1272±33	0.009 (8)	0.682 (16)
15	1802±95	0.001 (7)	N/A	16	1261±35	0.014 (8)	N/A

[1] Data are mean values from 4 replicates ± standard deviations

[2] ( ) The code number in bracketsis the relevant reference treatment for the statistical comparison

[3] N/A = not applicable

Appendix III Detectorresponserelativeto the internal standard of thepeaks in the oak infusion treatments

Code#	1	3	5	7	9	11	13	15	whisky
peak 1	0.15 ± [1]0.01	0.23 ± 0.04	0.13 ± 0.03	0.14 ± 0.01	0.14 ± 0.02	0.31 ± 0.04	0.22 ± 0.06	0.21 ± 0.04	1.37 ± 0.01
peak 2	0.20 ± 0.01	0.24 ± 0.02	0.10 ± 0.01	0.23 ± 0.08	0.21 ± 0.10	0.44 ± 0.07	0.30 ± 0.12	0.38 ± 0.16	0.19 ± 0.00
peak 3	0.76 ± 0.32	0.92 ± 0.26	0.52 ± 0.28	0.72 ± 0.15	0.74 ± 0.24	1.31 ± 0.11	0.83 ± 0.19	0.95 ± 0.21	4.80 ± 0.03
peak 4	0.53 ± 0.28	0.67 ± 0.04	0.58 ± 0.30	0.59 ± 0.32	0.53 ± 0.28	0.56 ± 0.28	0.51 ± 0.21	0.53 ± 0.15	0.08 ± 0.01
peak 5	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
peak 6	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.00
peak 7	0.04 ± 0.05	0.04 ± 0.04	0.04 ± 0.05	0.03 ± 0.04	0.03 ± 0.04	0.03 ± 0.04	0.01 ± 0.01	0.02 ± 0.01	0.19 ± 0.03
ISTD	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
peak 8	0.45 ± 0.18	0.44 ± 0.15	0.20 ± 0.12	0.55 ± 0.18	0.47 ± 0.16	0.73 ± 0.24	0.58 ± 0.10	0.68 ± 0.05	1.64 ± 0.02
peak 9	0.70 ± 0.02	0.69 ± 0.01	0.17 ± 0.02	1.00 ± 0.06	0.89 ± 0.15	1.22 ± 0.05	0.87 ± 0.09	0.90 ± 0.05	0.34 ± 0.04
peak 10	0.14 ± 0.00	0.11 ± 0.01	0.06 ± 0.01	0.11 ± 0.03	0.12 ± 0.03	0.09 ± 0.00	0.08 ± 0.03	0.06 ± 0.01	0.03 ± 0.01
peak 11	0.39 ± 0.02	0.41 ± 0.07	0.37 ± 0.01	0.40 ± 0.06	0.41 ± 0.02	0.40 ± 0.02	0.40 ± 0.04	0.39 ± 0.02	0.03 ± 0.01
peak 12	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.00 ± 0.00
peak 13	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.02 ± 0.01
peak 14	0.11 ± 0.07	0.26 ± 0.07	0.10 ± 0.07	0.24 ± 0.22	0.09 ± 0.07	0.21 ± 0.16	0.37 ± 0.32	0.33 ± 0.05	0.26 ± 0.09
peak 15	0.06 ± 0.02	0.04 ± 0.01	0.03 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.00 ± 0.00
peak 16	0.00 - 0.00	0.02 ± 0.00	0.00 - 0.00	0.02 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.00 ± 0.00
peak 17	0.13 ± 0.02	0.10 ± 0.01	0.09 ± 0.02	0.12 ± 0.02	0.10 ± 0.02	0.07 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.00 ± 0.00
peak 18	0.50 ± 0.31	1.31 ± 0.30	0.43 ± 0.27	0.69 ± 0.40	0.46 ± 0.28	1.04 ± 0.59	1.23 ± 0.72	1.46 ± 0.50	0.77 ± 0.33
peak 19	0.04 ± 0.02	0.09 ± 0.01	0.03 ± 0.03	0.08 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.09 ± 0.03	0.14 ± 0.01	0.00 ± 0.00
peak 20	0.64 ± 0.04	0.68 ± 0.04	0.43 ± 0.03	0.71 ± 0.11	0.75 ± 0.11	0.82 ± 0.05	1.02 ± 0.11	1.01 ± 0.10	0.12 ± 0.09
peak 21	1.06 ± 0.01	1.43 ± 0.27	0.73 ± 0.01	1.50 ± 0.29	1.81 ± 0.44	2.40 ± 0.38	2.52 ± 0.56	3.03 ± 0.39	0.22 ± 0.10
peak 22	0.62 ± 0.13	0.43 ± 0.07	0.51 ± 0.20	0.48 ± 0.04	0.61 ± 0.14	0.41 ± 0.08	0.49 ± 0.05	0.43 ± 0.03	0.00 ± 0.00
peak 23	0.41 ± 0.17	0.27 ± 0.07	0.26 ± 0.05	0.25 ± 0.05	0.28 ± 0.10	0.30 ± 0.06	0.29 ± 0.02	0.21 ± 0.07	0.00 ± 0.00
peak 24	1.39 ± 0.58	0.61 ± 0.09	0.83 ± 0.18	0.72 ± 0.19	0.88 ± 0.35	0.58 ± 0.43	1.02 ± 0.40	0.79 ± 0.27	0.38 ± 0.00
peak 25	0.69 ± 0.20	0.59 ± 0.10	0.57 ± 0.04	0.78 ± 0.29	1.08 ± 0.40	0.80 ± 0.39	0.90 ± 0.18	0.93 ± 0.12	0.00 ± 0.00

[1] = standard variation of 3 replicates

Appendix IV Detectorresponse relativeto the internal standard of thepeaks in the manukainfusion treatments

Code#	2	4	6	8	10	12	14	16	whisky
peak 1	0.17 ± [1]0.02	0.19 ± 0.04	0.12 ± 0.02	0.18 ± 0.02	0.17 ± 0.01	0.29 ± 0.04	0.23 ± 0.01	0.24 ± 0.02	1.37 ± 0.01
peak 2	0.20 ± 0.04	0.19 ± 0.02	0.09 ± 0.02	0.26 ± 0.04	0.24 ± 0.03	0.39 ± 0.03	0.41 ± 0.06	0.44 ± 0.07	0.19 ± 0.00
peak 3	0.60 ± 0.10	0.59 ± 0.13	0.38 ± 0.09	0.60 ± 0.05	0.58 ± 0.08	0.87 ± 0.06	0.77 ± 0.08	0.83 ± 0.05	4.80 ± 0.03
peak 4	0.52 ± 0.27	0.67 ± 0.16	0.51 ± 0.20	0.54 ± 0.18	0.50 ± 0.18	0.52 ± 0.20	0.52 ± 0.14	0.48 ± 0.17	0.08 ± 0.01
peak 5	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
peak 6	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.00
peak 7	0.02 ± 0.02	0.02 ± 0.02	0.01 ± 0.02	0.01 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.19 ± 0.03
ISTD	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
peak 8	0.21 ± 0.06	0.22 ± 0.11	0.11 ± 0.06	0.30 ± 0.05	0.21 ± 0.07	0.28 ± 0.04	0.32 ± 0.03	0.31 ± 0.02	1.64 ± 0.02
peak 9	0.26 ± 0.02	0.23 ± 0.02	0.10 ± 0.02	0.39 ± 0.02	0.29 ± 0.02	0.40 ± 0.05	0.40 ± 0.02	0.40 ± 0.05	0.34 ± 0.04
peak 10	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.00	0.03 ± 0.01
peak 11	0.37 ± 0.02	0.37 ± 0.06	0.36 ± 0.02	0.37 ± 0.03	0.40 ± 0.06	0.38 ± 0.01	0.37 ± 0.01	0.37 ± 0.02	0.03 ± 0.01
peak 12	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
peak 13	0.03 ± 0.02	0.04 ± 0.01	0.02 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
peak 14	0.31 ± 0.24	0.62 ± 0.27	0.29 ± 0.18	0.39 ± 0.19	0.33 ± 0.17	0.61 ± 0.28	0.52 ± 0.31	0.63 ± 0.36	0.26 ± 0.09
peak 15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
peak 16	0.02 - 0.01	0.02 ± 0.00	0.00 - 0.00	0.04 ± 0.01	0.03 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.00 ± 0.00
peak 17	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
peak 18	0.44 ± 0.11	0.99 ± 0.32	0.36 ± 0.08	0.68 ± 0.32	0.40 ± 0.07	0.79 ± 0.22	0.88 ± 0.28	0.95 ± 0.18	0.77 ± 0.33
peak 19	0.00 ± 0.00	0.02 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.00 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.00 ± 0.00
peak 20	1.17 ± 0.15	1.14 ± 0.08	0.88 ± 0.11	1.64 ± 0.30	1.49 ± 0.23	1.89 ± 0.34	1.97 ± 0.31	2.10 ± 0.11	0.12 ± 0.09
peak 21	0.54 ± 0.03	0.71 ± 0.13	0.42 ± 0.04	0.98 ± 0.22	0.74 ± 0.16	1.07 ± 0.25	1.26 ± 0.08	1.28 ± 0.17	0.22 ± 0.10
peak 22	0.74 ± 0.21	0.62 ± 0.03	0.62 ± 0.13	0.70 ± 0.11	0.81 ± 0.15	0.51 ± 0.11	0.63 ± 0.15	0.52 ± 0.05	0.00 ± 0.00
peak 23	0.30 ± 0.04	0.30 ± 0.06	0.24 ± 0.03	0.31 ± 0.13	0.27 ± 0.02	0.29 ± 0.11	0.27 ± 0.09	0.24 ± 0.11	0.00 ± 0.00
peak 24	0.79 ± 0.27	0.70 ± 0.24	0.70 ± 0.47	0.82 ± 0.25	0.83 ± 0.33	0.74 ± 0.18	1.11 ± 0.23	0.82 ± 0.36	0.38 ± 0.00
peak 25	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.14	0.19 ± 0.16	0.19 ± 0.21	0.16 ± 0.27	0.30 ± 0.10	0.11 ± 0.12	0.00 ± 0.00

[1] = standard variation of 3 replicates

Reference

Alexander, J. (2004). "Oak Cubes: A Good cost effective alternative to Barrels." Retrieved 19 December 2009, from http://www.mainbrew.com/media/oak/oak_cubes.pdf.

Alexander, J. (2008) Oak Alternatives. Brew your own**Volume**, DOI:

Ancient Touch. (2009). "Ancient Roman Pottery." Retrieved 24 October 2009, from http://www.ancienttouch.com/roman-pottery-closed-shapes.htm.

Barrelbuilders. (2008). "What you should know about Oak Chips." Retrieved 02 January 2010, from http://www.barrelbuilders.com/admin/content/oak_chips_info.pdf.

Belitz, H. D., W. Grosch, et al. (2009). Food Chemistry, Springer.

Cadahia, E., B. F. de Simon, et al. (2007). "Volatile Compound Evolution in Spanish Oak Wood (Quercus petraea and Quercus pyrenaica) during Natural Seasoning." Am. J. Enol. Vitic.**58**(2): 163-172.

Cadahia, E., S. Varea, et al. (2001). "Evolution of Ellagitannins in Spanish, French, and American Oak Woods during Natural Seasoning and Toasting." Journal of Agricultural and Food Chemistry**49**(8): 3677-3684.

Chatonnet, P. (1998). Volatile and Odoriferous Compounds in Barrel-Aged Wines: Impact of Cooperage Techniques and Aging Conditions. Chemistry of Wine Flavor. Washington, DC, American Chemical Society**:** 180-207.

Crum, J. D. (1995). The Influence of Toast Level of the Barrel on the Flavor of Wine. Proceedings of the International Oak and Cork Symposium, California State University, San Francisco, International Wine Academy.

Electregy. (2009). "What is Cellulosic Ethanol?" Retrieved 15 January 2010, from http://www.electregy.com/what-is-cellulosic-ethanol.php.

Emerald. (2009). "Lignin from waste black liquors - II: different lignins in phenol formaldehyde resin." Retrieved 13 January 2010, from www.emeraldinsight.com/fig/1290280301001.png.

Garde-Cerdan, T. and C. Ancin-Azpilicueta (2006). "Review of quality factors on wine ageing in oak barrels." Trends in Food Science & Technology**17**(8): 438-447.

Gawel, R. (2009). "Oak Barrel Alternatives in Winemaking." Retrieved 12 November 2009, from http://www.aromadictionary.com/articles/oakalternatives_article.html.

Gregory, A. (2007). "Green Energy." Retrieved 19 January 2010, from http://www.research.uky.edu/odyssey/winter07/green_energy.html.

Gunther, C. M., A. (1986). The Composition of Oak and an Overview of its Influence on Maturation. Liebigs Ann. Chem.**:** 2112-2122.

Hale, M. D. M., Katherine. Larmie, Ed. Newton, Jennifer. Swan, James. S (1999). "The

Influence of Oak Seasoning and Toasting Parameters on the Composition and Quality of Wine." Am. J. Enol. Vitic.**50**(4): 495-502.

Hodge, J. (1967). Origin of Flavors in Food: Nonenzymatic Browning Reactions. The Chemistry and Physiology of Flavors. Westport, CT, AVI Publishing.

ibrew. (2009). "MAGRENNAN AMERICAN Oak Chips." Retrieved 28 December 2009, from http://www.ibrew.com.au/collections/oak-barrels/products/magrennan-american-oak-chips.

Jackson, R. S. (2000). Wine science :principles, practice, perception. San Diego, Academic Press.

Jackson, R. S. (2009). Wine Tasting: A Professional Handbook, Academic Press.

Justia US Law (2011). § 5.22 The standards of identity. Title 27 - Alcohol, Tobacco Products and Firearms

Koning, R. E. (1994). "Basic Plant Cytology 1." Retrieved 20 December 2009, from http://plantphys.info/plant_physiology/basiccytology1.shtml.

Laghi, l. (2007). "Cellulose strands." Retrieved 12 January 2010, from http://commons.wikimedia.org/wiki/File:Cellulose_strand.jpg.

Manuel, D. (2002). "Oak." Retrieved 13 April 2010, from http://archive.supermarketguru.com/206?CurrentPage=3&archive=1&currentdate=2010-04-13.

Margalit, Y. (2004). Concepts in wine chemistry. San Francisco, Wine Appreciation Guild.

Miller, D. (2009). "Wine Barrels." Retrieved 19 November 2009, from http://www.cellarnotes.net/wine_barrels.htm.

Mosedale, J. R. (1995). "Effects of oak wood on the maturation of alcoholic beverages with particular reference to whisky." Forestry **68**(3): 203-230.

Mueller, P. (2009). "Wine Storage Tanks." Retrieved 25 August 2009, from http://www.muel.com/ProductDivisions/ProcessingSystems_Equipment/Beverage/Wine/WineStorageTanks.cfm.

Neeley, Z. (2009). "Oak Barrels: French or American?" Retrieved 5 Apr 2011, from http://trefethenfamilyvineyards.wordpress.com/2009/07/22/oak-barrels-french-or-american/.

New Zealand Wine. (2010). "Annual Report 2010." Retrieved 02 May 2011, from http://www.nzwine.com/assets/sm/upload/hp/ds/41/cq/NZW_Annual_Report_2010_media.pdf.

Nishimura, K., Ohnishi, M., Masuda, M., Koga, K., Matsuyama, R. (1983). Flavour of Distilled Beverages. Chichester, UK, Ellis Horwood.

Nixon, K. (2006). "Global and Neotropical Distribution and Diversity of Oak (genus Quercus) and Oak Forests." Ecology and Conservation of Neotropical Montane Oak Forests: 3-13.

Pérez-Coello, M. S., Sanz, J. & Cabezudo, M. D. (1997). "Gas chromatographic-mass spectrometric analysis of volatile compounds in oak wood used for ageing of wines and spirits " Chromatographia**47**(7): 427-432.

Phenomenex. (2010). "Zebron ZB-WAXPLUS ™." Retrieved 15 June 2010, from http://www.phenomenex.com/cms400min/zebronzbwaxplus.aspx.

Phenomenex. (2012). "Scotch Whiskey Main Compounds on ZB-624." Retrieved 02 February 2012, from http://www.phenomenex.com/Application/Detail/16045?returnURL=/Compound?id=07692_Fluka&alias=07692_Fluka.

Price Water House, C. (2007). "New Zealand Wine Industry." Retrieved 18 September 2009, from http://www.investinnz.co.nz/industry/wine/wine-industry.

Puech, J. L., F. Feuillat, et al. (1999). "The Tannins of Oak Heartwood: Structure, Properties, and Their Influence on Wine Flavor." Am. J. Enol. Vitic.**50**(4): 469-478.

Salut Wine Co. (2009). "A primer on cooperage & barrel making." Retrieved 24 October 2009, from http://salutwineco.wordpress.com/2009/05/14/cooperage/.

Sanderson, L.-A. (2010). "A short history of Italian wine." Retrieved 23 October 2010, from http://www.lifeinitaly.com/wines/roman-history.asp.

Sarni, F, et al. (1990). Effect of heat treatment of oak wood extractable compounds. Berlin, ALLEMAGNE, de Gruyter.

Sierra Stainless, I. (2009). "Sierra Stainless, Inc. - The West's Premier Tank Builder." Retrieved 24 October 2010, from http://www.sierrastainless.com/format.html.

StaVin. (2009). "StaVin Tank Products." Retrieved 28 December 2009, from http://www.micro-ox.com/tank_oak.htm.

Stuff.co.nz. (2009). "The business of beer." Retrieved 20 April 2011, from http://www.stuff.co.nz/business/industries/2729409/The-business-of-beer.

Suber Lefort Int. (2010). "Product application." Retrieved 30 December 2009, from http://www.suber-lefort.com.au/.

The Scotch Whisky Regulations (2009). http://www.legislation.gov.uk/uksi/2009/2890/contents/made.

The Wine Institute. (2009). "World Wine Production By Country." Retrieved 25 November 2009, from http://www.wineinstitute.org/resources/worldstatistics/article87.

Tripod. (2010). "MALDI-TOF Mass Spectrometric Analysis of Hydrolysable Tannins." Retrieved 12 March 2010, from http://taninos.tripod.com/hidrolisables.htm.

Vivas, N. (1998). Physical and chemical aspects of oak wood air drying. Bordeaux France, Demptos cooperage posted to Faculty of Enology University Victor Segalen Bordeaux II.

Waterhouse, A. (2001). "Natural products of wine." Retrieved 10 January 2010, from http://waterhouse.ucdavis.edu/ven219/composition_of_oak.htm.

WeekEnd Brewer. (2009). "Wine Barrels - Spigots - Oak Cubes & Staves." Retrieved 06 January 2010, from http://www.weekendbrewer.com/oakbarrels.htm.

Whisky Megazine. (2006). "Is there caramel coloring in Japanese Whisky?" Retrieved 30 May 2011, from http://www.whiskymag.com/forum/viewtopic.php?t=3557.

Wikipedia. (2010). "Oak (wine)." Retrieved 18 September 2009, from http://en.wikipedia.org/wiki/Oak_(wine).

Wilkinson, K. (2009). Oak Lactone Formation In Wine And Spirits, LAP Lambert Academic Publishing.

Woodworth, S. (2009). "Whiskey barrels - Oak gives real character to the Whiskey." Retrieved 12 December 2009, from http://www.whiskeywise.com/whiskey-barrels.html.

World Cooperage. (2011). "Price List." Retrieved 02 Apr 2011, from http://www.worldcooperage.com/library/documents/price12-Napa-WCweb.pdf.

Young, O. A., M. Kaushal, et al. (2010). "Use of Species Other than Oak to Flavor Wine: An Exploratory Survey." Journal of Food Science**75**(9): S490-S498.

Young, O. A. and J. West (2001). Meat Colour. Meat science and applications. New York, Marcel Dekker.

Zoecklein, B. W. (1995). Wine analysis and production. New York, Chapman & Hall.