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Rapid Maturation of Whiskey

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Abstract

Whiskey is among the oldest and one of the most consumed spirit based drinks in the world. Traditionally whiskey is aged for three or more years in wooden barrels to mature the spirit. During maturation, a variety of compounds are extracted from the wood to give a complex mixture of flavours and aromas. It is desirable to speed up the reactions which take place during maturation including, extraction of compounds from wood and reactions between the distillate and wood compounds. The benefits of ageing the spirit faster are the reduction in storage space, time and costs associated with the ageing process.

This project was carried out for a commercial company with a focus on maturing fresh distillate using different ageing techniques; heat, light, heat-freeze and sonication with wood chips to obtain the flavours and aromas associated with mature whiskies in a short timeframe. Quantitative (concentration, ppm) and qualitative (peak areas, ISTD corrected) analysis was carried out on samples stored over time using gas chromatography mass spectrometry (GC-MS).

Initially, different sized wood pieces (toasted to varying degrees) were soaked in 65% ethanol, to replicate a whiskey matrix, and heated over four weeks to confirm what compounds were extracted from the wood and their relative quantities. The ethanol experiment also allowed determination of the optimal temperature (70 °C), time (≤ 14 days), wood size (small chips) and toasting level (medium toast) to extract compounds in a short timeframe with similar levels to the analysed commercial samples.

Heat, light, heat-freeze and sonication treatments were separately applied to fresh distillate (corn and malt) with different wood sizes and toasting levels. Experiments were sub-sampled over time. The determination of the compounds present and the effects of different treatments on the concentrations/peak areas were compared. Experiments were also compared to commercial samples (n = 51) to investigate whether a mature whiskey profile had been achieved.

Samples heated (70 °C) for six days had some desirable compounds (furfural, 1,1-diethoxyethane, several esters and several phenolic aldehydes) at concentration/peak areas similar to commercial samples. Sonication (≤ 8 hours) with medium toast small chips also produced good results for several reasons; the colour and aroma were similar to commercial

samples and it had a similar compound profile to commercial samples for the analysed compounds. Furthermore, untargeted analysis using principal component analysis indicated that the corn distillate sonication samples (non-heated and 50 °C) and heat freeze (day 9) samples were clustered with the 10-12 year-old whiskey sample group whereas the heat-only samples were clustered further away. In comparison, the malt distillate samples did not cluster near any of the commercial samples. Linear discriminant analysis was used to categorise the heat, heat-freeze and sonication samples to an age group based on the model created from the analysed commercial samples with 90% predicted to have a chemical profile ≥ 10 years old (≥ 72% probability). Overall, several age treatments show promise in rapidly maturing whiskey.

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Abbreviations List

5-HMF	5-Hydroxymethylfurfural
Ave	Average
DCM	Dichloromethane
DMTS	Dimethyl trisulphide
DVB-CAR-PDMS	Divinylbenzene–Carboxen on polydimethylsiloxane
ESI-MS	Electrospray Ionisation Mass Spectrometry
FID	Flame Ionisation Detector
GC	Gas Chromatography
HS-SPME	Headspace Solid-Phase MicroExtraction
HPLC	High Performance Liquid Chromatography
Lge	Large Oak Block
LVI	Large Volume Injection
LoD	Limits of Detection
LoQ	Limits of Quantification
LDA	Linear Discriminant Analysis
LC	Liquid Chromatography
MS	Mass Spectrometry
%ABV	Percent Alcohol By Volume
PA	Polyacrylate
PDMS	Polydimethylsiloxane
PC	Principal Component
PCA	Principal Component Analysis
PTV	Programmable Temperature Vapourisation
PTFE	Polytetrafluoroethylene
Q-ToF MS	Quadrupole-Time of Flight Mass Spectrometer
RSD	Relative Standard Deviation
RT	Retention Time
Sml	Small Oak Chips
NaCl	Sodium Chloride
SPE	Solid Phase Extraction
SPME	Solid-Phase MicroExtraction
SBSE	Stir Bar Sorptive Extraction
8TMS	Tetramethylsilane
UHPLC	Ultra High Pressure Liquid Chromatography
UV	Ultra Violet
v/v	Volume to Volume

Chapter 1 - Introduction and Literature Review

Whiskey is amongst the oldest and most consumed spirit based drinks in the world, with Scotch being one of the most popular whiskies [1,2]. Whiskey (or whisky) is an alcoholic beverage distilled from fermented grain and aged in wooden barrels, also known as casks [3]. In the United Kingdom (UK) the term cask is preferred over barrel because it encompasses all wooden container sizes, whereas the term barrel generally refers to 180-200 L containers. In this thesis the term barrel will be used to define the container of whiskey storage with no specified volume [4,5].

The production of whiskey dates back to as early as the 7th century, allegedly originating in Ireland [1]. During the early 1900's, the production of whiskey was taxed by the British Parliament resulting in the appearance of many small illegal camouflaged distilleries, each of which used recipes passed down within their Scottish clans. This naturally created diversification of whiskey. This diverse range of whiskies includes blended, grain and malt which are now commercially available [1,3]. Grain whiskey is produced from corn, rye, wheat or raw barley with a little malted barley (for enzymes); malt whiskey is made from 100% malted barley which is barley that has been germinated briefly to release the enzymes required for alcohol production [6–8]. Blended whiskey is a mixture of two or more whiskies. No longer is whiskey production only limited to Scotland, Ireland and the United States of America (USA); it is now produced around the world including: Japan, Pakistan and New Zealand [3,9].

Most distillers are bound by law to mature their spirits for a minimum of three years to be classified as whiskey, however, in New Zealand and Australia a minimum of two years is required [9–11]. Most whiskey sold has a strength of 40% alcohol by volume (%ABV), however, some are sold at cask strength, ~60% ABV [6,9,10,12].

1.1. Stages of Whiskey Production

There are six main stages to whiskey production (Figure 1.1). The production process, ingredients used (type and source of water, type and quality of yeast and the type of grain), and maturation storage conditions (temperature, humidity and ventilation) are all important factors that will influence the aroma, taste and strength of the final product [3,13–15]. The fermenting, distilling and ageing stages are discussed in more detail in the following subsections because these contribute the most to the flavour and aroma of whiskey.

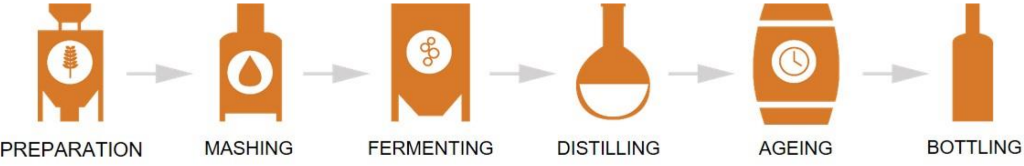

Figure 1.1: Stages of whiskey production [16].

1.1.1. Fermentation

Fermentation is generally carried out in wooden tanks because the natural flavours and aromas of the wood can infuse into the wort (liquid containing the sugars from malt) [5,9,17]. As the pH of the fermentation liquid decreases (from pH 5 to ~4.2), organic acids (e.g. acetic and succinic acid), higher alcohols (characterised by two or more carbons), carboxylic acids, esters, aldehydes and ketones are produced. These compounds carry over into the fresh distillate (prior to maturation) and contribute to the flavour of the whiskey [14,18].

1.1.2. Distillation

During distillation, the liquid is heated until boiling and the compounds within the liquid which have boiling points lower than this temperature will vapourise. These vapours are collected, cooled and condensed back into a liquid, referred to as the distillate. [3,19]. This process is often carried out in copper pot stills, which have a lantern shape where only the small molecular weight compounds reach the top. Some important reactions are catalysed by the copper, including removal of sulphurous compounds [6,17]. In some distilleries the stills are in pairs; the 'wash still' is heated to evaporate alcohol, which then condenses and forms 'low wines' (20-35% alcohol by volume (%ABV)). This is transferred into the 'spirit still' for distillation and is 'cut' into three fractions; the head (foreshots), heart (spirit) and tail (feints) [9,17]. The head has highly volatile compounds (boiling point lower than ethanol (78.6 °C)) and contains undesirable flavours and aromas (e.g. ethyl acetate, methanol and acetaldehyde) at high concentrations, however, a low concentration of some of these compounds may end up in the heart cut such as ethyl acetate. The heart cut (spirit fraction) is the desired fraction, it is also referred to as the fresh distillate, which goes on to be matured. The tail cut contains low volatility compounds (boiling point higher than ethanol) such as propanol, isobutanol, and isoamyl alcohol. The tail cut also has nitrogen containing compounds and too much water for maturation. The head and tail fractions are often recycled into the next batch for re-distillation to recover more ethanol [6,17,20].

1.1.3. Maturation

The focus of the thesis is on the ageing process of whiskey which is described by the change in colour, odour and flavour as well as the decrease in volume and increase/decrease in alcoholic strength (depending on the relative rates of water and ethanol loss). For example, due to the cool, but humid warehouses in Scotland, the alcoholic strength decreases. In contrast, the strength increases in the USA due to their warehouse conditions [13,21]. The fresh distillate has undesirable flavours and aromas so it needs to be modified to make it more appealing. Similar to wine, there are four objectives of maturation; to remove the harsh taste while extracting wood compounds (e.g. phenols) which helps develop desirable aromas/flavours, and ultimately to achieve body and complexity by developing a broad mixture of compounds. Only water and pure caramel (colouring agent produced from heat treatment of carbohydrates with alkali or acid) can be added to whiskey, any other additions are forbidden by law [12,22,23]. Traditionally maturation of the fresh distillate is carried out in barrels constructed from hardwoods, no larger than 700 L. The barrel not only acts as a container, but also acts as a semi-permeable membrane. Alcohol, water and oxygen vapours diffuse through the barrel, and are involved in maturation reactions. The slow evaporation of the distillate is known as the 'angel's share' [1,17,24,25].

Whiskey is generally matured in barrels made of American oak (*Quercus alba*), however, barrels are also made of European oak (*Quercus robur*) and its two subspecies (*Quercus sessilis* and *Quercus pendunculata*), with very few being made from other oak species [4,25,26]. Bourbon is aged in new oak barrels; in comparison, whiskey is generally matured in old charred bourbon or sherry barrels (200 L), but can also be matured in new charred/uncharred barrels [22,27].

Prior to maturation, the inside of the barrel is exposed to a flame at high temperatures (200-1950 °C) to cause thermal degradation which influences the physical (e.g. wood surface area) and chemical (e.g. degradation/generation of compounds) characteristics of the barrel and ultimately the whiskey. Different levels of toasting (heating wood without combusting) or charring (charcoal layer development) is created depending on the flame temperature and flame exposure time [9,28]. The wood cell wall components and extractives present (Figure 1.2) vary between tree species. Many flavouring compounds are extracted from the wood during maturation; the size and type of barrel, origins of the barrel wood, barrel toasting/charring level, characteristics of the fresh distillate and storage environment are all factors that contribute to

the colour, flavour and aroma of the final product. These factors will influence the maturation time. Changes in the composition and concentration of compounds in turn change the flavour of the maturing spirits [4,26,29,30].

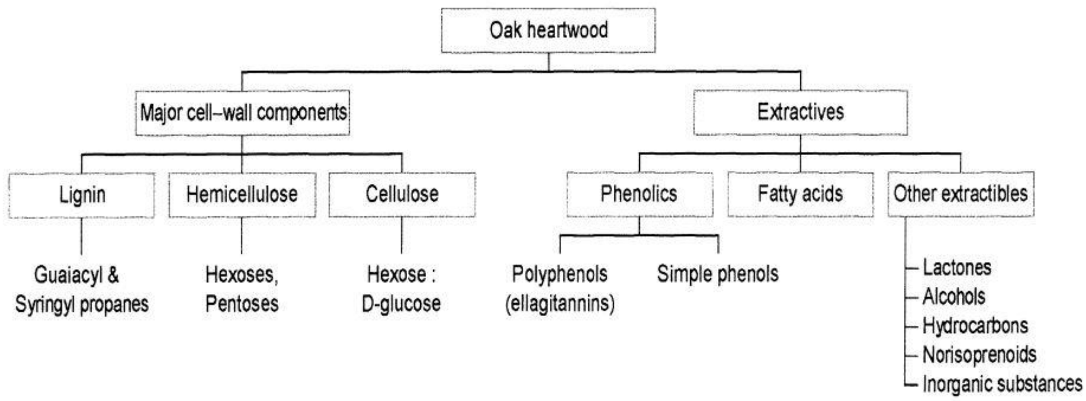

Figure 1.2: Components of oak heartwood [13].

Causes for the change in composition of whiskey can include [13,31]:

1. Evaporation of volatile compounds
2. Reactions with wood compounds, distillate compounds or between the wood compounds and distillate compounds
3. Decomposition of macromolecules into the distillate

It has been shown that different storage temperatures influence the rates of physical and chemical reactions, with higher temperatures promoting faster reactions, however, the change in temperature can alter the compounds present and their concentrations. The increase in temperature can also increase the loss of product by evaporation, hence care needs to be taken when changing the storage temperature [3,18,31–33].

1.2. Compounds and Flavours Associated with Whiskey

There are a large variety of flavours and aromas associated with whiskey. Therefore whiskey assessors use flavour wheels (Figure 1.3) to describe the complex mixture of compounds which make up a particular whiskey [6]. The compounds in whiskey mostly consist of a range of aldehydes, esters, phenolic compounds, organic acids and wood extractives, with their concentrations increasing with maturation time, although most compounds rarely reach 50 ppm [11,32]. This section discusses the common compounds that are present in whiskey

and also provides a starting point for what compounds are desirable when rapidly maturing a whiskey in the experimental section of this thesis.

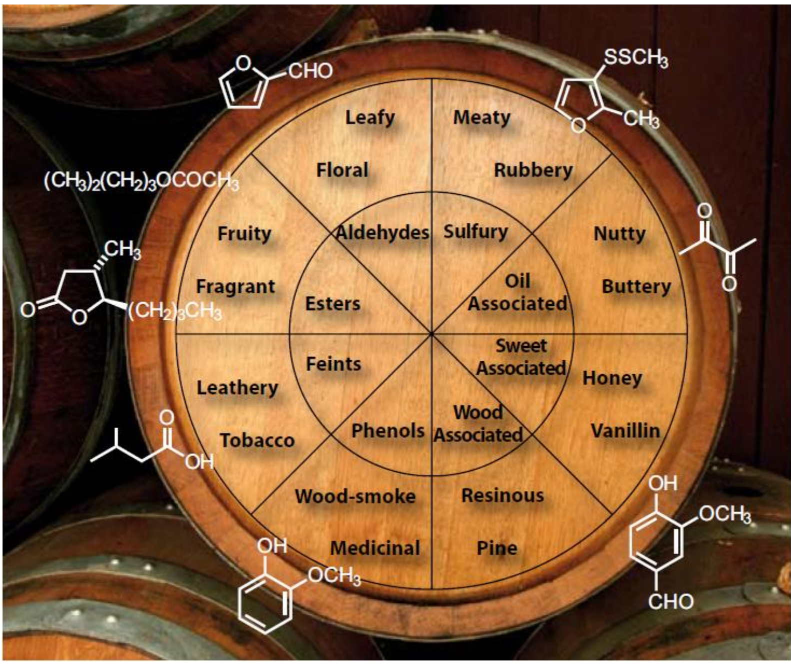

Figure 1.3: General whiskey flavour wheel [6].

Most compounds are derived from the barrel wood which is composed of the macromolecules: cellulose, hemicellulose and lignin together with lower molecular weight extractives. The structure is chemically heterogeneous and the composition of the macromolecules vary depending on the age of the wood and the region in which they were grown [34,35]. The breakdown of cellulose to melanoidins (high molecular weight polymers formed through Maillard reactions) contributes to the colouration of the whiskey [6,31]. Caramelisation and Maillard reactions are discussed in section 1.4.4.

Lignin and hemicellulose provide rigidity to the cell walls making the walls hydrophobic and water impermeable. The bending of wood during barrel manufacture results in delignification (depolymerisation of lignin) which helps to release extractives [34–36]. The extractives from wood consist of a diverse range of compounds which impart colour, odour and taste, some common compounds are described in Appendix A, Table A.1 (page 174) [34,35].

Lignin related compounds are associated with the development of the mature whiskey flavour, therefore, degradation of lignin polymers is required [13,26]. Lignin consists of different phenylpropane subunits which are linked together by both ether and carbon-carbon bonds.

Three hydroxycinnamyl alcohols (coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol) are precursors to the lignin structure with coniferyl alcohol and sinapyl alcohol in various proportions making up the lignin of hardwoods such as oak [34,35]. High temperatures are needed to break the aryl-ether bonds in the polymer to break down the lignin-cellulose matrix to produce the different lignin degradation products (Figure 1.4) [13,23,31,37,38]. The degradation of macromolecules is achieved by exposing the interior of the barrel to a thermal gradient for a specified time, referred to as toasting the barrel. This disrupts the surface of the wood, increasing the surface area which releases flavour congeners (contributing to taste and aroma, Figure 1.5) by oxidation and also increases the polyphenolic content. For the purpose of this thesis, the term congener will refer to all taste and aroma compounds. The purpose of barrel charring is to remove as many undesirable congeners as possible by forming a layer of active adsorbent while catalysing other reactions such as formation of glucose, mannose, galactose, xylose and arabinose (hexose and pentose monosaccharides) and short polysaccharides from the degradation of heterogeneous hemicellulose (structural polymers). The sugars then form furan products (e.g. furfural and 5-hydroxymethylfurfural) due to their susceptibility to thermal degradation (the high heat used during toasting/charring) [13,39]. It has been reported that at the high temperatures (up to 200 °C) of barrel toasting, the release of more aromatic aldehydes are obtained [9,28]. Aromatic aldehydes can be lost by carbonisation due to higher temperatures (> 200 °C) and charring (~1950 °C). Therefore the degree of toasting/charring applied to a barrel will affect the final flavour and aroma of the whiskey. Table 1.1 summarises the concentrations of various aromatics in wood at different toasting temperatures. Some of the common compounds are discussed further in the below sub-sections according to their functional group.

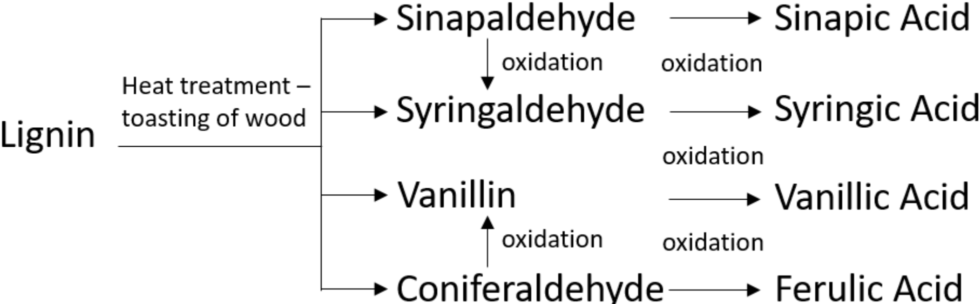

Figure 1.4: Schematic of lignin breakdown into phenolic aldehydes and subsequent conversion to acids during heat treatment of wood. Modified from [40,41].

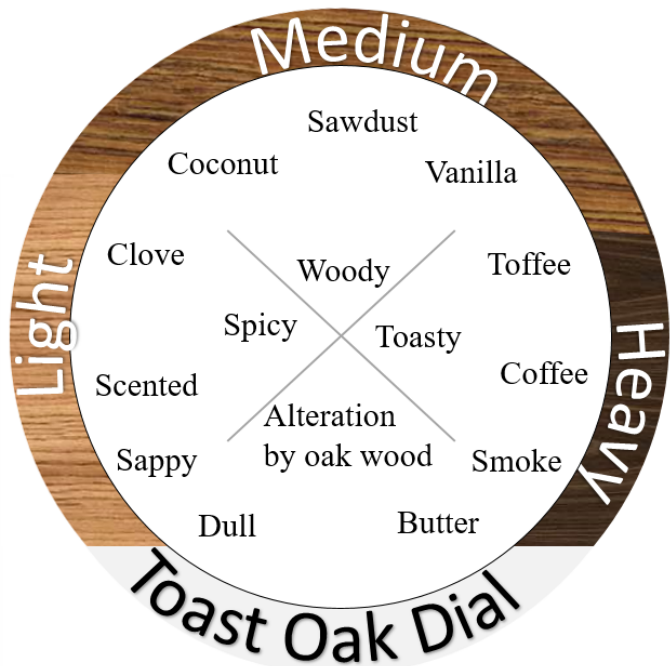

Figure 1.5: Toast Oak Dial to show transition of flavours as the barrel is heated and toasted to different levels. Modified from [26].

Table 1.1: Several toasting temperatures and the associated concentrations (ppm) for several aromatic compounds in wood. Reproduced from [28,42].

	Toasting temperature (°C) and compound concentrations (ppm)			
Compound	**100 °C**	**150 °C**	**200 °C**	**charred**
Vanillin	1.1	3.8	13.5	2.8
Propiovanillone	0.6	1.1	1.4	0.9
Syringaldehyde	0.1	3.8	32.0	9.2
Acetosyringone	-	0.03	1.5	0.6
Coniferaldehyde	trace	4.3	24.0	4.8
Vanillic Acid	-	1.8	6.1	1.1
Sinapaldehyde	trace	6.5	60.0	9.0

1.2.1. Acids

The presence of organic acids influences the clustering of ethanol molecules in the distillate. During maturation the wood components are broken down and acetic acid (sour/vinegar flavour) concentrations increase due to both oxidation of ethanol as well as extraction from the barrel. Several other organic acids (gallic, vanillic, syringic and ellagic acid) are extracted from the lignin and tannin components of wood. Breakdown of wood lipids produce octanoic and decanoic acids. Dicarboxylic acids (succinic, adipic and methylsuccinic acids) are also added to the mixture from the breakdown of wood lipids [21,43].

1.2.2. Aldehydes

Aldehydes, which are mostly part of the head fraction, are congeners and are formed as by-products during fermentation by yeast. Acetaldehyde is prominent and gives the strong and sharp aroma to the fresh distillate. Some aldehydes are oxidised into acids during maturation, which in turn results in esterification (formation of esters by reaction of acids and alcohols). The charring of barrels results in lignin and polysaccharide degradation which introduces aromatic aldehydes (Figure 1.6) such as cinnamaldehyde (cinnamon), syringaldehyde (fruity) and furfural (nutty, caramel) by condensation reactions. High charring has shown to produce greater levels of syringaldehyde, but lower levels of coniferaldehyde and sinapaldehyde compared to light and medium toasting. Aldehydes, such as vanillin, come from storage in barrels and the oxidation of lignin monomers in wood; aldehydes also help colour the distillate [13,15,31,44–46].

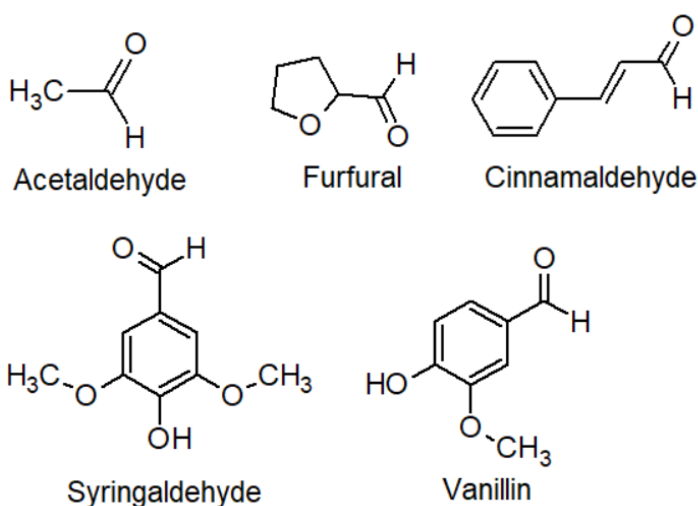

Figure 1.6: Common aldehyde compounds present in whiskey.

1.2.3. Esters

Esters are mostly responsible for the aromas present in whiskey, but most are outside the heart cut so do not make it into the barrel for maturation. Short, medium and long chain esters, which are cut in the heart fraction, produce different aromas (Table 1.2). Figure 1.7 shows some esters that make it into the whiskey. Isoamyl acetate (banana) and ethyl laurate (cream) are short chain esters and are responsible for delicate aromas. Richer and fruiter aromas (apples, grapes, cherries) come from medium chain esters, while herbal and earthy aromas coming from long

chain esters. Most short chain esters are lost during maturation due to their high volatility, however, the barrel introduces new acids (e.g. acetic acid) which in turn react with the alcohols present to form new esters (e.g. ethyl acetate, Figure 1.8) [28,47]. To reduce the effects of ethyl esters, which in high concentrations are associated with undesirable flavour characteristics, their solubility needs to be increased. Increasing the solubility decreases the amount that is in the headspace and hence the effects on the flavour and aroma are decreased; this is done by addition of wood derived compounds (compounds extracted from wood) [13]. The most common esters found in whiskey, besides those mentioned above, include ethyl acetate (~50% of total ester content), ethyl butyrate and ethyl hexanoate [20,45].

Isoamyl Acetate

Ethyl Laurate

Ethyl Acetate

Ethyl Butyrate

Ethyl Hexanoate

Figure 1.7: Structures of common esters present in whiskey: isoamyl acetate, ethyl laurate, ethyl acetate, ethyl butyrate and ethyl hexanoate.

Table 1.2: Common esters present in whiskey with their associated aromas [48–50].

Compound	**Flavour**
Ethyl Acetate	Fruity, ethereal, glue*
Ethyl Butyrate	Pineapple, Sweet, fruity
Ethyl Hexanoate	Pineapple, apricot, waxy*
Ethyl Octanoate	Apple, fruity, wine, pear
Ethyl Decanoate	Fruity, apple, grape
Ethyl Laurate	Sweet, soapy*, floral
Phenethyl Acetate	Honey, floral, fruity
Isoamyl Acetate	Sweet, banana, fruity

* At high concentrations

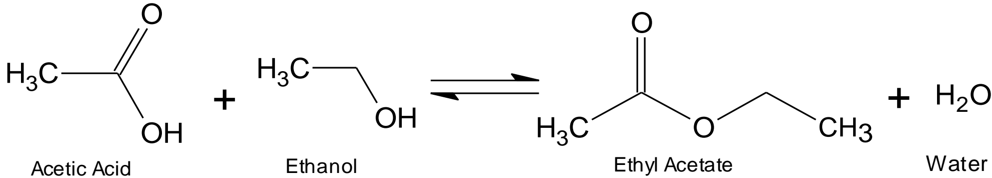

Figure 1.8: Reaction of acetic acid and ethanol to form ethyl acetate [28,51].

1.2.4. Phenols

Phenols contribute to the smokey characters in whiskey and mostly come from barley that has been smoked using peat or from peat smoke introduced after fermentation. The peat produces phenolic compounds that adsorb onto the barley and are released during the mashing and distillation stages. Most phenolic compounds are lost in the head and tail fraction, but some are present in the heart fraction [52]. For example, cresol and guaiacol (volatile phenolic compounds) form during the burning of malt and are responsible for the typical smokey taste in whiskey [1,12]. Volatile phenols can also originate from charred oak wood which results in degraded wood carbohydrates (cellulose and hemicellulose) and lignin), with 4-vinyl guaiacol often being reported as a wood derived compound [13,52,53]. Flavours from common phenol compounds are presented in Table 1.3. As previously mentioned (section 1.1.3), whiskey is aged in used barrels. Each time a barrel is filled, the easily extracted phenol concentrations decline, therefore, over time only hydrolysable phenols remain. This is due to other phenols being released once diffusible phenols are extracted from the surface of the wood [23].

Table 1.3: Phenol compounds present in whiskey and their associated flavours [52].

Compound	Flavour
Phenol	Carbolic
o-Cresol (2-methylphenol)	Musty, medicinal
m-Cresol (3-methylphenol)	Woody, ethereal
p-Cresol (4-methylphenol)	Medicinal
Xylenols (dimethylphenols)	Medicinal, sweet
Guaiacol or creosol (2-Methoxiphenol)	Medicinal, woody, smokey
4-Ethylguaiacol	Smokey, meaty
4-Vinylguaiacol	Spicy, clove
Eugenol (4-(2-propenyl) guaiacol)	Cinnamon, cloves, spicy

1.2.5. Fatty Acids/Fusel Oils

Sterols (e.g. glycerol) and fatty acids (as well as their derivatives) can produce precipitation/cloudiness in the whiskey when present at high concentrations. The literature reports that these compounds have been associated with poor quality whiskey [46,53]. The

production of glycerol is from thermal breakdown of wood and can be released (along with fatty acid ethyl esters) by trans-esterification of ethanol and glycerides. Aroma descriptors for wood characters such as sawdust and resinous substances are often associated with defective barrel character resulting in poor quality whiskey [13,15,46,54]. Common fatty acids present include isobutyric, propionic, butyric, hexanoic, octanoic, decanoic and dodecanoic. Fatty acids are produced during the fermentation stage. Despite the boiling points being higher than the distillation temperatures, they are soluble in water/ethanol mixtures so are carried by the vapour during the distillation process. Fatty acids directly provide reaction substrate for ester production. In good quality whiskies, the degree of esterification of fatty acids with ethanol is higher, although both the acids and ethyl esters are also present [20,46].

Fusel oils also referred to as higher alcohols, are by-products of fermentation and are characterised by having two or more carbons (e.g. 1-propanol, isoamyl alcohol and isobutanol). Fusel oils which have up to five carbons (e.g. amyl alcohols) contribute to the aroma, however, at high concentrations can negatively affect the quality [30].

1.3. Development of Undesirable Compounds

The complex mixture of compounds in whiskey are present at different concentrations and it is the concentration of an individual component that determines whether its presence has a positive or negative effect on the flavour and aroma of the final product. If the concentrations are too high they can cause bitterness and astringency (e.g. tannins) and/or cloudy solutions (e.g. high fatty acids) [15,46,53].

Acetic acid forms in the traditional barrel maturation process (by oxidation of ethanol and degradation of wood) and is a precursor for the formation of many esters present in whiskey, however, if there is a large amount of unreacted acetic acid present in the final product, the sour flavour will be distinguishable [31,54].

Ethyl carbamate is a carcinogen and trace contaminant in distilled spirits. The presence of epiheterodendrin (glycosidic nitrile) in some varieties of barley is the precursor to ethyl carbamate formation. This is regulated particularly in the malting process where non-producing epiheterodendrin barley varieties are selected [9].

Heterocyclic nitrogen compounds such as pyridines produce unpleasant aromas, however, pyrazines and thiazoles give the nutty, roasted aromas which are desirable. During traditional

maturation, the pH of the distillate drops sufficiently (due to the extraction of acids), to minimise the pyridine concentration, but pyrazines and thiazoles are mostly unaffected [55].

Tannins are major wood components which are also extracted from wood during maturation and are related to a bitter flavour. The structure and biological activity of tannins vary. They are complex polyphenolic polymers which are broadly split into two groups; hydrolysable tannins and condensed (non-hydrolysable) tannins. Ellagitannins (hydrolysable tannin) are extracted from wood and contain hydroxyl groups (–OH). These hydroxyl groups promote oxidation and polymerisation within the whiskey. Ellagitannins have a large effect on both taste and quality. Hydrolysis of fatty acid esters and oxidation of ketones have also been related to contributing to bitterness [13,15,30,54,56]. In a study carried out by Liebmann and Scherl (1949) [15], the addition of toasted chips to the distillate showed a quicker increase in tannins, but after 12 months there was no significant difference between the amount of tannins in the traditional barrel matured whiskey and the distillate with chips.

1.4. Key Reactions During Whiskey Maturation

The maturation process of whiskey involves complex reactions including natural reactions from the compounds present in the distillate and those influenced by the barrel environment (type of wood, toast level, storage etc.) [37,57]. There is an increase in volatile and non-volatile compounds with four major types of reactions occurring in the barrel during the maturation stage: extraction of wood compounds by the distillate (ethanolysis), reactions between compounds present in the distillate (esterification), oxidation of compounds in the distillate (oxidation) and breakdown of wood compounds (Maillard and caramelisation) [15,43]. The establishment of equilibria among compounds (e.g. acetaldehyde, ethanol and the corresponding acetal) also occurs [13,21]. These reactions change the flavour profile of the distillate by formation of different congeners, however, the physical and chemical changes are slow during traditional maturation because only low energy reactions are possible [37,58].

Maturation can be separated into additive, subtractive and interactive reactions. With additive reactions, new compounds are introduced by extraction from the wood. For example, the extraction of phenolic aldehydes (sinapaldehyde, syringaldehyde, coniferaldehyde and vanillin). With subtractive reactions, compounds in the distillate are removed or altered by evaporation, adsorption onto the charred barrel layer or degradation of wood compounds. For example, coniferyl alcohol and sinapic alcohol are released from lignin by ethanol. Interactive

reactions occur when compounds present in the distillate react with each other or with wood extractive compounds to form new compounds (A+B→AB). For example, the formation of esters by reaction of alcohols and acids. [6,9,21,31].

During maturation, the proportion of non-volatile compounds increase while the less desirable characters decrease. Most of the compounds associated with flavour concentrate over time due to the evaporation of ethanol and water [31,43]. The effect the barrel has on the chemical reactions can vary depending on the type of wood used, treatment of the wood (level of toasting) and how many times the barrel has been used [22,37].

1.4.1. Ethanolysis

Ethanol reacts with lignin from the barrel; the wood polymers are broken down to release taste and aroma congeners. The distillate aids release of the congeners by diffusing through the wood bringing colour, flavour and aroma compounds into the distillate. The diffusion of water, ethanol and oxygen through the barrel is controlled by the thermal gradient through the barrel and affects the rate of breakdown [31,37].

1.4.2. Esterification

Esterification is an equilibrium reaction between an alcohol and acid to form an ester. When an acid catalyst is used it is referred to as Fischer esterification. In order to achieve complete reaction (shift the equilibrium right) a large excess of alcohol is required. This is easily achieved in whiskey because ethanol is in excess and the distillate (containing many acids) is acidic which acts as a catalyst. Fischer esterification can go to completion using weak acids, however, it takes significantly longer compared to strong acids. Ethanol reacts with acids such as acetic, octanoic (Figure 1.9) and decanoic acid to form ethyl acetate, ethyl octanoate and ethyl decanoate respectively, which are commonly found in commercial samples. Esters have pleasant odours and a number of them are fruity. [20,32,59,60].

Figure 1.9: Example of Fischer esterification. Reaction between octanoic acid and ethanol in an acidic environment to form ethyl octanoate, one of several commonly found esters in whiskey [59].

1.4.3. Oxidation Reactions

Oxidation reactions are very important in the maturation of whiskey and occur as oxygen slowly diffuses through the barrel into the headspace of the spirit [41]. As evaporation occurs, the part of the barrel which does not have contact with the distillate dries causing minute cracks making the barrel more porous allowing more oxygen to diffuse through. Oxidation reactions are also involved in lignin reactions to form phenolic aldehydes, an example reaction scheme is shown in Figure 1.10 [20,31].

Lignin + Ethanol → Coniferyl alcohol + sinapic alcohol

Sinapic alcohol —oxidation→ Sinapaldehyde —oxidation→ Syringaldehyde

Coniferyl alcohol —oxidation→ Coniferaldehyde —oxidation→ Vanillin

Figure 1.10: The proposed reaction between lignin and ethanol to form alcohols which oxidise to form the phenolic aldehydes found in whiskey. Modified from [31].

1.4.4. Maillard and Caramelisation Reactions

Maillard reactions consist of three stages (initial, intermediate and final) which induce browning and have an effect on the flavour of food and beverages. The initial stage is a condensation reaction (reversible) involving an amino acid and reducing sugar (free C=O group) producing *N*-glycosylamine or *N*-fructosylamine. In Maillard reactions the amino acid acts as a catalyst resulting in faster reactions and higher levels of intermediate products. However, in their absence the degradation of sugars requires higher temperatures (~150 °C) and is termed caramelisation. The second stage starts after the products from the first stage have rearranged via Amadori or Heyns rearrangement (depending on whether it is an aldose or ketose sugar, respectively). In the second stage, at pH 4-7 the Amadori or Heyns products undergo degradation releasing amino acids resulting in deoxyosones (di-carbonyl compounds). For example, 3-deoxy-2-hexosulose and 1-deoxy-2,3-hexodiulose (deoxyosones) give rise to secondary products from subsequent dehydrations. Furfural originates from pentose sugars and 5-hydroxymethylfurfural (5-HMF) originates from hexose sugars. In the final stage, the compound can react via a number of different pathways: dehydration, fragmentation and polymerisation reactions involving amino acids. The Strecker reaction leads to deamination and decarboxylation of amino acids; the amino acids are degraded by dicarbonyls produced in the second stage. This produces aldehydes and aminoketones to ultimately produce pyridines,

pyrroles and pyrazines amongst others [61,62]. Pyrazines, pyrroles and pyridines (heterocyclic nitrogen compounds) are also produced (from Maillard reactions) during wood charring. The concentration of pyridine should be minimised because it contributes to bitter and astringent characteristics [13,55,61].

1.5. Chemical Profile of Traditionally Aged Whiskey

Taste, aroma and body profile are all characteristics associated with matured spirits. A general concentration range (if available in the literature) of common compounds found in whiskey is listed in Table 1.4. The %ABV (known as proof in America), total and fixed acidity, aldehydes, furfural, fusel oils (higher alcohols), solids, colour, tannins and pH are often measured during whiskey maturation. A study conducted by Liebmann and Scherl (1949) [15] measured all of these parameters during traditional whiskey maturation across 469 American whiskey barrels (bourbon and rye) from 0-8 years. The results showed that %ABV, esters and fusel oils increased almost linearly. The increases observed are due to the storage conditions being optimised to increase the relative rate of water evaporation, therefore, yielding a higher alcohol content and concentrating the fusel oils; esters increased because they are formed by reactions of alcohols and acids. Acetic acid is the main acid present in whiskey and can account for 50-95% of volatile acids. Consequently, the large amount of acetic acid led to a rapid initial decrease in pH but levelled off around 30 months [11,15].

Liebmann and Scherl (1949) [15] also reported an initial rapid increase in total acidity, fixed acidity, aldehydes, furfural, colour and tannins but the rate of increase slowed down after six months. These increases are related to the extraction of compounds from wood and the reduction in the rate of increase after six months would most likely be due to the establishment of chemical equilibria.

The quality of whiskey is correlated to several compounds. Good quality whiskey has been correlated with whiskey lactone (3-methyl-4-octanolide or β-methyl-γ-octalactone), a volatile compound in unheated oak wood. During maturation, whiskey lactone gives a sweet and evocative coconut flavour (provided it is not present at excessive concentrations), therefore, the compound is desirable in most distilled beverages. Charring the barrels produces higher levels of whiskey lactones with the *trans*-lactone being spicier and the *cis*-lactone having a stronger coconut aroma [6,13,47]. The quality of whiskey has also been shown to have a direct relationship with 5-HMF, phenolic compounds (e.g. vanillin), and possibly even

hydroxymethyl-pyranone compounds [13]. In a study by Reid (1993) [46], it was shown that higher levels of syringaldehyde, 5-HMF and vanillin were found in good quality whiskies, therefore, they can be used as a quality markers. The difference between good and poor quality whiskies are the levels of phenolic compounds, with higher levels being associated with better quality whiskey. The phenolic compounds include the phenolic aldehydes such as vanillin, syringaldehyde and coniferaldehyde as well as the phenolic acids such as vanillic acid and syringic acid [25,46].

Table 1.4: List of common compounds found in whiskey and their reported concentration ranges [11,28,37,48,63–65].

Compound	Concentration (ppm)
Esters	
Ethyl Acetate	100-700
Ethyl Hexadecenoate	0.51*
Ethyl Hexanoate	0.1-3
Ethyl Tertradecanoate	0.04-0.08*
Ethyl Butyrate	0.5-2
Ethyl Decanoate (Ethyl Caprate)	1.2 - 44
Ethyl Octanoate (Ethyl Caprylate)	3.6-14.8
Ethyl Laurate (Ethyl Dodecanoate)	10-20
Isoamyl Acetate (3-Methyl-1-butanol Acetate)	1.21-8.7
2-Methylbutyl Acetate (2-Methyl-1-butanol Acetate)	0.24-3
Phenethyl Acetate	0.84-5.6
Acids	
Acetic Acid	500-800
Butyric Acid	1.6-13.2
Decanoic Acid	20-33
Dodecanoic Acid	0.6-15
Hexadecanoic Acid	1-3.2
Hexanoic acid	3-20
Octanoic acid	5-28
Tetradecanoic acid	1-2.3*
Alcohols	
Isoamyl Alcohol (3-methyl-butanol)	140-950
2-methyl-1-butanol	32-194
Isobutanol	350-800*
1-octanol	0.42*
2-phenylethanol	0.84-30
1-decanol	0.10*
1-dodecanol	0.08*
1-hexanol	0.28*
Hexadecanol	0.54*

Compound	Concentration (ppm)
Phenolic Aldehydes	
Syringaldehyde	0.67-10.2
Coniferaldehyde	0.6-1.2
Vanillin	0.04-5.96
Sinapaldehyde	0.2-1.6
Benzaldehyde	0.07*
Miscellaneous	
1,1-Diethoxyethane (acetal)	3.6-60
3-Methyl-4-octanolide (*cis*- and *trans*- whiskey lactone)	0.22-2.4
5-Methylfurfural	0.02-2*
Furfural	Up to 20-30
5-Hydroxymethylfurfural	4-20*

* Concentration reported from one source

1.6. Current Methods for Rapid Ageing of Whiskey

The process of maturing the fresh distillate in wooden barrels has been used for many years, and to this day is still used because it is required by law in some countries (e.g. Scotland) and it also helps improve the distillate characteristics [21,37].

During the early 1900's quick ageing of spirits was important because aged whiskey was almost completely exhausted due to the British parliament taxing whiskey production and the prohibition period (1919-1933) in the USA (a period where there was legal prevention of manufacture, sale and transport of alcoholic beverages) [1,5,15,21]. After the prohibition period ended, attempts to speed up the whiskey maturation process were abandoned by most distillers; it is unknown whether this was due to producing an inferior product or to uphold tradition [5,15]. A century later, a new age of distillers are now introducing new processes to speed up the maturation stage with some no longer ageing the spirit in wooden barrels [37,57]. The success of these new rapid maturation processes varies; they are often commercially sensitive, therefore, any details published are deliberately vague. Three main types of rapid ageing (heating-cooling, light and ultrasound) are discussed below.

1.6.1. Heating and Cooling

Thermodynamics (energy of a system) and kinetics (rate of reaction) are fundamental in determining whether a chemical reaction will occur. A reaction may be thermodynamically possible, however, it may be kinetically unfavourable (occur very slowly) [51,66]. It is known

that the rate of chemical reactions increase with increasing temperature (heating) because the temperature increases the kinetic energy of molecules consequently increasing the number of molecular collisions in which energy is transferred [51,66]. Therefore increasing the number of collisions results in more molecules having sufficient activation energy for a chemical reaction to occur [66].

Refrigeration (cooling) has been used to increase the speed of precipitation of less soluble wood related compounds (e.g. lipids) which are removed by filtration without too much impact on colour and flavour [66]. In traditional maturation, the daily and seasonal temperature changes affect the movement of the distillate. Hot temperatures heat the wood which makes it more porous resulting in more diffusion of ethanol, water and oxygen through the wood. When the wood is cooled it contracts (becoming less porous) resulting in release of the distillate from the wood into the barrel. The expansion and contraction of the wood results in transference of sugars and flavour compounds (e.g. furfural) into the solution within the barrel. Maximising the temperature change also maximises the sugar and flavour compounds introduction [67]. The upper section (higher storage racks) in storage warehouses is considered to be the best for production of premium products because of the heat generated from solar energy (conducted through the roof) and heated air circulation. However, the upper section in storage warehouses are small in comparison to the rest of the storage warehouse space. Consequently new methods are being developed to increase the expansion and contraction of the wood while utilising the full storage space. For example Brown Water Spirits LLC [67] store barrels for a 4-72 month period on a floatation structure/barge creating natural temperature variations which aid maturation. During the day, the humidity and heat generated from solar radiation expands the pores of the wood and at night the water draws heat away from the barrels (on the floatation structure) contracting the wood pores. The interaction between the distillate and barrel is increased by movement of the barge in the surrounding waters.

In Cleveland, bourbon has been aged in six days using a pulsing pressure tank with charred strips of oak submerged in the tank. The process involves heating and cooling cycles which causes reactions between the wood and distillate to impart flavours. A smoother taste is produced by use of the charred strips, that act like a charcoal filter to extract unwanted compounds [57].

1.6.2. Light

Exposure to light at wavelengths ranging from 400-1000 nm has been used to activate photochemical reactions such as degradation of wood by breaking down polymers within the wood [32,68]. When light meets matter, it will either be reflected or absorbed. If the light is absorbed by the molecules within a material, it provides energy which activates/excites the molecules within. The energy is lost via fluorescence/phosphorescence, thermal energy or chemical energy because the molecule is unstable in the excited state. Therefore the application of UV or visible light to spirits promotes chemical reaction by molecular excitation or addition of heat (thermal energy). The electronic configurations have to be specific for each compound due to molecules having discrete energy levels. Hence a wide wavelength range is required to ensure energy is absorbed by many compounds present in whiskey; making light less effective compared to other treatments [51,66,69]. Autooxidation (generation of free radicals) using light (or oxygen) is associated with spoilage of beer, however, with whiskey maturation it can help speed up photochemical reactions [28].

Lost Spirits Distillery [70] invented a method to age rum in one week which has the same characteristics as a 32-year-old rum; they also use this process for whiskey. The process involves applying heat at temperatures between 60-76 °C and light in the 400-1000 nm range for 1-14 days. Lost Spirits have won several awards using this method, in particular, 'Jim Murray's Whisky Bible' liquid gold award in 2018 and the Wizards of Whisky "Best World Whisky" in 2019 for their "*Abomination - The Crying of the Puma"* [71,72].

1.6.3. Ultrasound

The demand for quality wine has increased, therefore, production in a shorter time frame has led to alternative methods, which can also be applied to rapidly maturing spirits (e.g. whiskey) [73]. Sonication is believed to accelerate diffusion of the liquid through the wood and increase the surface contact of the liquid and wood by cavitation, which is the formation, growth and implosion of mini bubbles. Upon collapse of the bubbles, a large amount of energy is released enabling chemical reactions (production of radicals and breaking of polymer chains) to occur and there is localised pressure supporting the release of compounds from the wood. The increase in temperature and pressure may accelerate ageing [58,74–76]. Sonication has been shown to be an efficient extraction method particularly for polyphenols [58]. Hydroxyl and hydrogen peroxide are formed by sonication of pure water; other oxidising species can be

formed based on oxidation reactions, for example, through the ageing of spirits in wooden barrels [76,77]. Delgado-González *et al.* [76] showed continuous movement (50 L/hr flow rate) of '*Holanda*' (wine distillate, 55% ABV) over wood chips with 6 minute ultrasound pulses (with 24 minute rest periods) aerated at room temperature in the dark for three days gave the best results for polyphenolic extraction (e.g. vanillin and syringaldehyde) whereas continuous flow with no ultrasound pulses or movement gave the worst extraction.

In Tuthilltown, New York, a distillery ages bourbon (Hudson Baby bourbon) for four months in small barrels to increase the surface area to volume ratio which results in more contact between the distillate and wood. They also use sonic maturation to agitate the distillate within barrels. This is achieved by using large speakers to play music with heavy bass which forms, enlarges and collapses bubbles in the distillate to accelerate certain reactions such as extraction of vanillin and syringaldehyde [57].

1.7. Gas Chromatography Mass Spectrometry Methods for Whiskey Analysis

Spirit based drinks, such as whiskey, have a complex matrix (hundreds of compounds), therefore, the ability to identify and quantify compounds relies on the employment of separation techniques such as gas chromatography (GC) and liquid chromatography (LC), which have been reported to have useful applications in the analysis of whiskey [1,20,22,78]. The literature is divided between using GC by itself for analysis, or using both GC and LC to cover volatile and non-volatile compounds [20,78,79]. When analysing complex matrices, many LC methods have poor resolution and problems with co-eluting peaks, whereas GC has better resolution and is a robust analytical technique with high accuracy and high sensitivity [80]. It is mainly used for composition studies as it enables detection of characteristic compounds allowing determination of chemical profiles which are used to define the quality and authenticity of the whiskey [1,12,22,81]. There are a number of detectors that can be coupled to GC, although the majority of methods present in the literature for whiskey analysis use a mass spectrometer (MS) or flame ionisation detector (FID). Most of the compounds contributing to the aromas and flavours in whiskey are volatile, therefore, the detection and quantification of volatile and semi-volatile compounds present in whiskey are important in determining the unique profiles of different whiskies. [12,22,81,82]. The focus of this literature review is on GC-MS due to its robust nature and because it is the chosen method of analysis

for this thesis. A brief outline of GC-MS is given, including different sample introduction systems and how the MS works and is followed by a small discussion on a few methods used in the analysis of whiskey which includes the use of other detectors which are not discussed (e.g. FID).

Prior to analysis, compounds in whiskey samples are often extracted with a solvent (e.g. diethyl ether or dichloromethane or ethyl acetate) using techniques such as liquid-liquid extraction, solid phase extraction (SPE) or derivatisation in order to separate the analytes of interest from the matrix and/or modify analytes to compounds that are more easily detected using GC-MS (e.g. methylated analytes) [1,23,28,37,43,79]. However, these techniques require a large amount of sample so solid-phase microextraction (SPME) is becoming more frequently used because it is automated, relatively quick and does not require solvent extraction (see 1.7.1.2 for further information on SPME) [1,83]. Once the samples have been prepared, introduction into the GC (Figure 1.11) differs for direct injection and SPME. For direct injection (liquid or headspace), the sample (liquid or headspace) is drawn up in a syringe and inserted it into the inlet via the injection port. For SPME, the fibre is inserted into the sample (headspace or liquid) for a specified time to adsorb/absorb compounds before it is inserted into the inlet (via the injection port) for desorption of compounds. The syringe pierces through the septum (Figure 1.12) to reach the vapourisation chamber where the sample is vapourised and condenses onto the front of the analytical column. The sample flows through the analytical column eluted by carrier gas (generally helium or nitrogen) where separation occurs by partition between the mobile (carrier gas) and stationary (analytical column) phases. More volatile (lower boiling point) compounds elute faster if a non-polar column is used, whereas polar columns separate compounds based on functionality (e.g. alcohols and esters) [23,82]. A temperature program (or oven ramp) is used to help separate compounds and ensure elution times are kept short. After eluting from the column, the molecules flow to the detector (see section 1.7.2 for further information) which is required to rapidly respond to compounds even at minute concentrations. A chromatogram (retention time versus peak area) for the sample is obtained and displayed on the computer ready for interpretation. For quantitative analysis, standards at various known concentrations are analysed and used to form calibration curves to allow calculation of unknown sample concentrations [23,82]. An internal standard (ISTD) is included (at the same concentration) in each standard and the ratio of the compound peak area to the ISTD peak area is used in the concentration calculation to minimise variation in reported concentrations due to fluctuations within in the system (injection volume, flow rate etc.) [82].

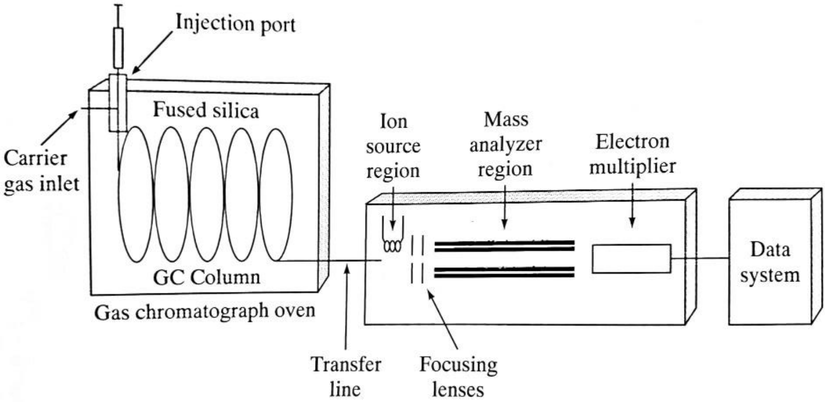

Figure 1.11: Block diagram of a gas chromatography instrument showing the main components: sample injection into injection chamber, oven with column, mass spectrum detector and data system [82].

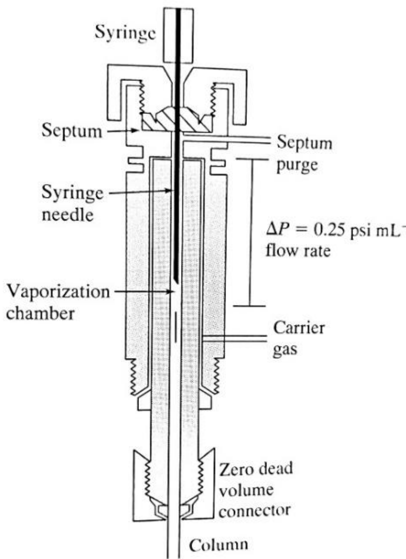

Figure 1.12: Cross sectional view of GC injector showing the septum, syringe needle, vapourisation chamber and column connection [82].

Characterisation is limited by the selection of the analytical column which is generally a fused-silica capillary column with a diameter of 0.1-0.5 mm and a stationary phase (immobilised liquid) coated on the interior. There are several different stationary phases available (Table 1.5) and selection is based on the required application such as what functional groups are of most interest to the researcher [12,22,81,82]. The focus of this thesis was on phenolic aldehydes, esters phenols and alcohols (based on information from sections 1.2.2-1.2.5). Polyethylene glycol columns are most suited for spirit analysis because they are commonly used for detection of free acids, alcohols and essential oils which consist of compounds like aldehydes, ketones, esters and phenols [82,84]. Despite the common applications of the 5% phenyl-polydimethyl

siloxane column not including alcohols and phenols, several articles have successfully used this column (e.g. HP-5MS) for the detection of esters and phenolic aldehydes [32,85].

Table 1.5: Stationary phases in some available GC columns with their common applications listed and maximum temperatures [82].

Stationary Phase	Common Applications	Maximum Temperature (°C)
Polydimethyl siloxane	Hydrocarbons, polynuclear aromatics, drugs, steroids, polychlorinated biphenyls	350
5% Phenyl-polydimethyl siloxane	Fatty acid methyl esters, alkaloids, drugs, halogenated compounds	350
50% Phenyl-polydimethyl siloxane	Drugs, steroids, pesticides, glycols	250
Polyethylene glycol	Free acids, alcohols, ethers, essential oils, glycols	250
50% Cyanopropyl-polydimethyl siloxane	Polyunsaturated fatty acids, rosin acids, free acids, alcohols	240

1.7.1. GC Sample Introduction/Compound Extraction

There are a number of techniques for injecting samples into the GC including direct injection, headspace and SPME which all have their own advantages and disadvantages. The focus within this literature review is on direct injection (traditional injection) and SPME because these are the techniques that were used within this thesis.

1.7.1.1. Direct Sample Injection

The most common GC sample introduction method is direct injection; a few microlitres (typically 1-3 μL) of a given sample is directly injected into the GC system (via the injector). The advantage of direct injection is the ability to use split and splitless injectors which have been used for alcohol analysis due to the ability to split the carrier gas stream (to the column and to waste). When analyte concentrations are high, split injectors are used because it dilutes the sample by only injecting a portion of the sample. When low to trace level analysis is required splitless injectors are used because all of the injected sample is loaded onto the column, increasing the sensitivity [12]. There are two disadvantages of directly injecting the sample into the system. Firstly, adding the whole matrix onto the column could produce poorer resolution and noisy baselines. Secondly, the solvent might deteriorate the column stationary phase, for example many columns are not compatible with water [86].

1.7.1.2. SPME - Headspace and Immersion

Solid Phase Micro-Extraction (SPME) is becoming more popular due to the simplicity and reduction in manual sample preparation. It has been widely used for flavour analysis in malt beverages, vodka and particularly in wine [85]. The SPME technique concentrates volatile compounds onto a fibre in the headspace or immersion of the fibre into the sample. The concentration technique is based on fibre sorption (absorption/adsorption) and the volatiles are thermally desorbed into the injection port of a GC [83,87]. The partition coefficient of the analyte can be used to determine the amount of analyte extracted by the fibre coating. Enhancement of the partition coefficient can be achieved by adjusting the pH or ionic strength of the sample which is generally done by addition of an inorganic salt (normally sodium chloride, NaCl). This technique is advantageous over techniques using liquid extraction because it is fast, simple and solventless [81,88,89].

There are several SPME fibres available (Table 1.6) all of which are a thin silica whisker (fibre) coated with a polymer material to reduce the chance of breakage. The polymer material can consist of one (absorption or adsorption) polymer or several polymers (absorption and adsorption). The fibre choice is based on the polarity of the target analytes with coatings of different polarities being used for quantitative and qualitative analysis due to the high extraction selectivity and reduction of extracting interferences. The conditioning of the fibre, the extraction temperature and extraction time are important parameters in SPME analysis especially because analytes in the atmosphere can also adsorb onto the fibre [81,83,87].

Headspace SPME (HS-SPME) is cleaner than immersion SPME because there are no adverse effects on the fibre due to it not coming into direct contact with non-volatile (potentially high molecular weight) compounds present in the sample. In immersion SPME, the fibre is submerged into the liquid sample where the analytes are transported (by diffusion) directly from the matrix to the fibre. Agitation or sonication of the sample is generally required to help speed up the diffusional analyte transport when using immersion SPME [83]. In HS-SPME, the analytes from the gas phase adsorb/adsorb onto the fibre after equilibration. The equilibration time, defined as the time after which the amount of analyte extracted remains constant, is generally shorter for HS-SPME than immersion SPME for three reasons: a large portion of analytes are already present in the gas phase (prior to equilibration), a large interface (boundary between two phases) exists between the gas and liquid and the diffusion coefficients (rate at which an analyte diffuses across a concentration gradient) are larger. One limitation of

HS-SPME is the enhancement of concentrations for some compounds (due to increased adsorption/adsorption) and the suppression of others (decreased adsorption/adsorption) making it hard to interpret chromatograms. Therefore, selection of the correct fibre is essential to ensure detection of the desired compounds regardless of whether headspace or immersion is used [83,87].

Table 1.6: Properties of SPME fibres including the fibre coating, type, polarity, coating stability and extraction mechanism [87,90,91].

Fibre coating	**Type**	**Polarity**	**Coating stability**	**Extraction mechanism**	**Recommended use (Molecular Weight)**
Polydimethylsiloxane (PDMS)	Homogenous polymer	Non-polar	Non-bonded	Absorption	
100µm					Volatiles (60-275)
7 µm					Non-polar high molecular weight compounds (125-600)
30 µm					Non-polar semi-volatiles (80-500)
Polyacrylate (PA)	Homogenous polymer	Polar	Bonded crosslinked	Absorption	
85 µm					Polar semi-volatiles (80-300)
Carboxen Polydimethylsiloxane (CAR/PDMS)	Porous particle/polymer	Bipolar	Partially crosslinked	Adsorption	Gas and low molecular weight compounds (30-225)
75 or 85 µm					Gas and low molecular weight compounds (30-225)
Carbowax Divinylbenzene (CW/DVB)	Porous particle/polymer	Polar	Partially crosslinked	Adsorption	
65 µm					Alcohols and polar compounds (40-275)
Divinylbenzene Carboxen Polydimethylsiloxane (DVB/CAR/PDMS)	Porous particle/polymer	Polar	Highly crosslinked	Adsorption	
50 and 30 µm					Flavour compounds – volatile and semi-volatiles (40-275)
50 and 30 µm (2 cm stableflex fibre)					Trace compound analysis (40-275)
Polydimethylsiloxane Divinylbenzene (PDMS/DVB)	Porous particle/polymer	Bi-polar	Bonded partially crosslinked	Adsorption	
65 µm					Volatiles, amines and nitro-aromatic compounds (50-300)

1.7.2. Mass Spectrometer Detection

A mass spectrometer detector has high accuracy and sensitivity [82,92]. After the sample molecules elute from the GC column they enter the ion source where they are bombarded with high energy electrons (typically at 70 eV). The energy transferred on collision between the electrons and compounds is high enough to cause ionisation and fragmentation. Neutral molecules often form a molecular ion by loss of one electron which provides useful information by defining the molecular weight, however, if the transferred energy is too large the molecule may fragment and a molecular ion may not be seen. The molecular ion can undergo fragmentation and rearrangement producing positively charged ions that are separated based on their mass/charge (*m/z*) with the number of ions for each *m/z* unit recorded as a spectrum of signals. The mass spectrum is reproducible and the fragmentation patterns of compounds within a sample are used to identify the compounds within complex mixtures by searching a library database [23,51,93,94].

1.7.2.1. Fragmentation Patterns

The fragmentation pattern is characterised by the signals (ions present and their relative intensities) within a spectrum which differs for different functional groups [93]. Examples of compounds found in whiskey are given below.

Aromatic wood extractive compounds (e.g. syringaldehyde) have fairly strong molecular ions (resonance stability) and/or strong fragmentations with a loss of a proton (M^+-1) for aldehydes, a loss of a methyl group (M^+-15) for methyl ketones and a loss of a methoxy group (M^+-31) for methyl esters [23,93]. For example, syringaldehyde has a strong molecular ion at *m/z* 182, and a high intensity at *m/z* 181 because it loses a hydrogen radical to form an acylium ion (Figure 1.13) [95].

For primary and secondary alcohols, the molecular ion is generally small and fragments with a loss of a hydroxymethyl group (M^+-31) [93]. For example, there is a prominent ion at *m/z* 57 for 2-methyl-1-butanol (Figure 1.14) [95].

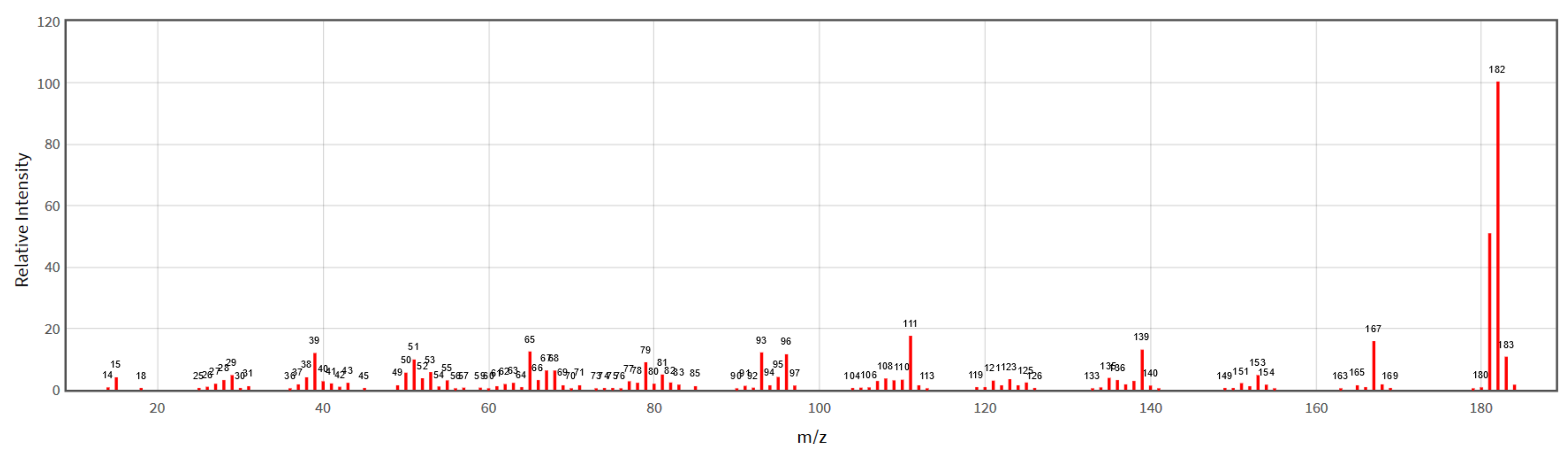

Figure 1.13: Mass spectrum for syringaldehyde showing a prominent molecular ion (*m/z* 182) and loss of a proton (*m/z* 181) [95].

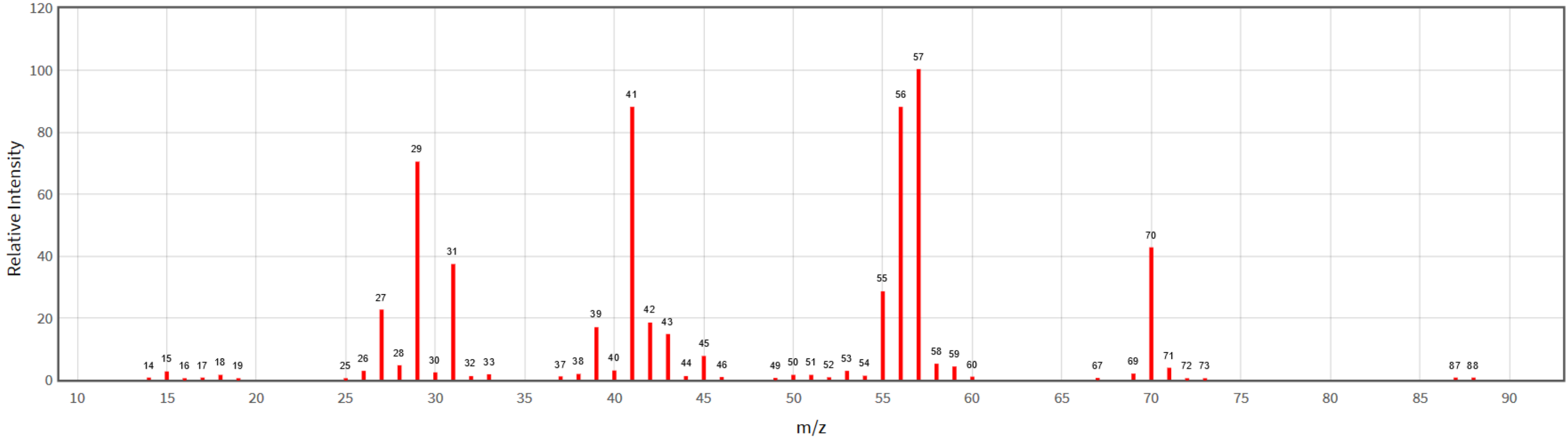

Figure 1.14: Mass spectrum for 2-methyl-1-butanol showing the small molecular ion (*m/z* 88), loss of H_2O (*m/z* 70), loss of a hydroxymethyl group (*m/z* 57) and loss of CH_3-CH-CH_2OH (*m/z* 29) [95].

Ester fragments include elimination of alkene units and the McLafferty rearrangement. For example ethyl hexanoate has a prominent ion at *m/z* 88 (McLafferty rearrangement), *m/z* 43 (propyl cation) and an ion signal at *m/z* 99 represents the loss of the ethoxy group from the molecular ion (*m/z* 144) (Figure 1.15) [93–95].

Aliphatic acids have weak molecular ions whereas aromatic acids have strong molecular ions due to resonance stability. Aliphatic acid have a strong ion at *m/z* 60 which represents the McLafferty rearrangement and there is also the loss of the hydroxyl group ($M^{+}-17$) and carboxyl group ($M^{+}-45$). Additionally for long chain acids, there are two fragmentation series from the cleavage of C-C bonds; one series is for the charge on the oxygen containing fragment and the other is for the charge on the alkyl fragment group. For example, *n*-decanoic acid has signals at *m/z* 45, 60, 73 etc. for the oxygen containing fragments and signals at *m/z* 29, 43, 57 etc. for alkyl fragments (Figure 1.16) [93–95].

GC-MS can be operated in full scan (scans over a wide *m/z* to produce an overview of the sample composition) or selected ion monitoring (SIM) (selectively target certain compounds by selecting specific *m/z*). For this research full scan was used to gain an understanding of the compounds present in whiskey and in rapid maturation experiment samples.

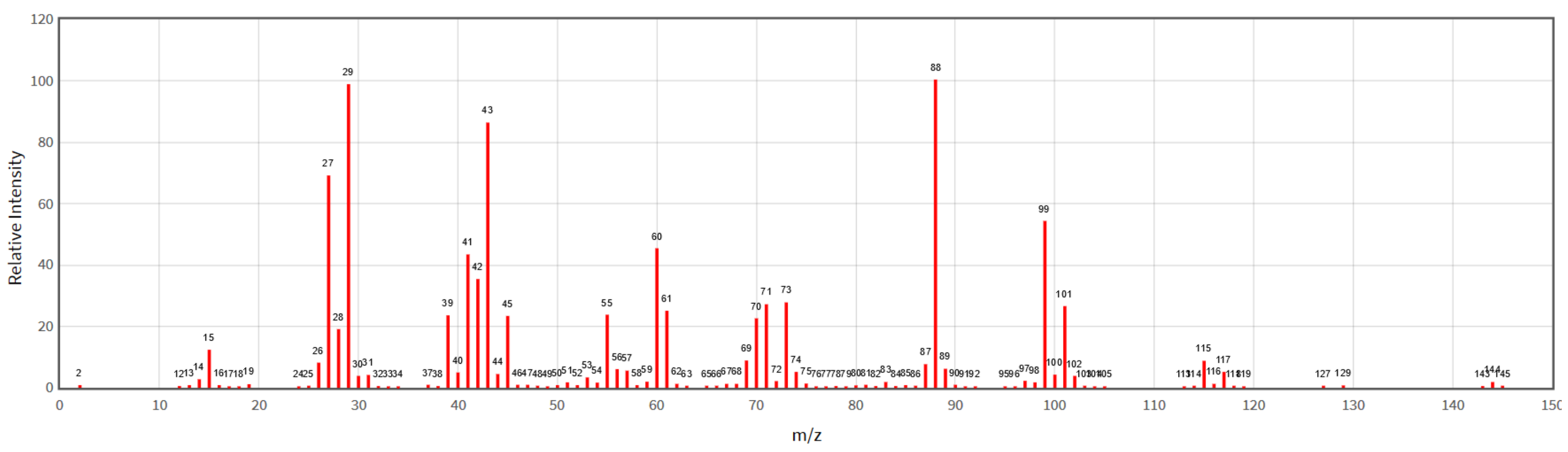

Figure 1.15: Mass spectrum for ethyl hexanoate with several prominent ion signals including McLafferty rearrangement (*m/z* 88), propyl cation (*m/z* 43) and loss of ethoxy group (*m/z* 99) [95].

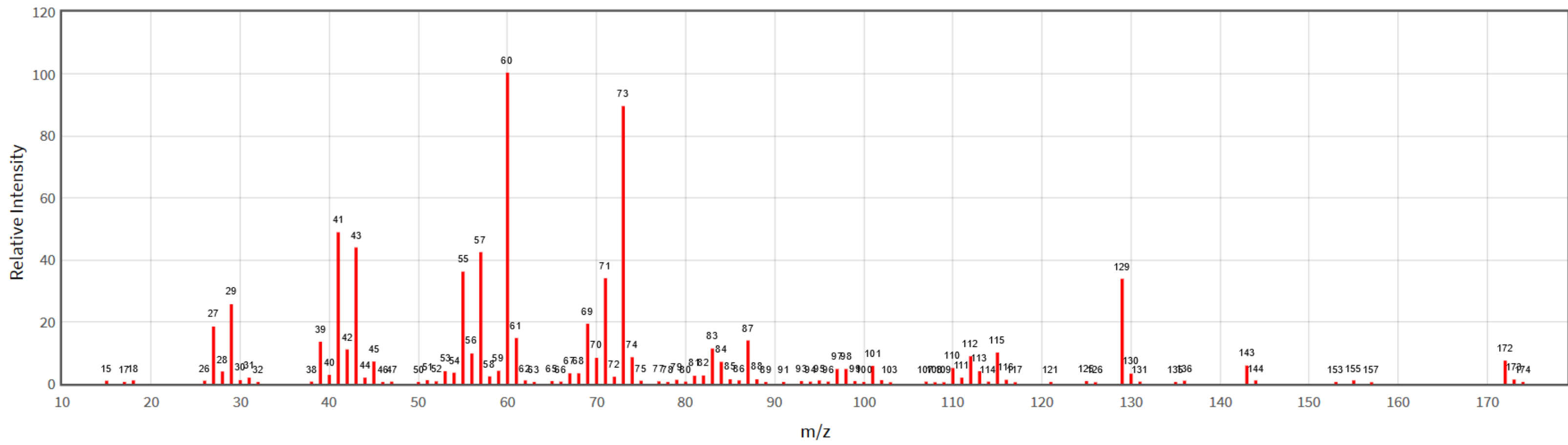

Figure 1.16: Mass spectrum for *n*-decanoic acid showing the strong ion at *m/z* 60 (McLafferty rearrangement), oxygen containing fragments (*m/z* 45, 60, 73 etc.) and alkyl fragments (*m/z* 29, 43, 57 etc.) [95].

1.7.3. Gas Chromatography Methods for Whiskey Analysis

The methods chosen from the literature for comparison used gas chromatography for analysis of spirits with ethanol percentages > 30% and different sample introduction methods (SPME and direct injection). The focus of this section is on methods which used columns that had a polyethylene glycol phase or (5%-phenyl)-methylpolysiloxane stationary phase because these were the two columns best suited for spirit analysis (polyethylene glycol) or have been reported in spirit analysis (5% phenyl-polydimethyl siloxane) [32,82,84,85].

Demyttenaere *et al.* [85] used GC-MS to study the composition of volatiles and semi-volatiles (esters) present in blended Scotch whiskey using two different sample preparations. The two extraction methods were SPME and stir bar sorptive extraction (SBSE). It was found that SPME was better due to SBSE requiring a thermal desorption device which desorbs volatiles into a cold trap and transfers the compounds to the GC-MS. When comparing immersion SPME and HS-SPME, Demyttenaere *et al.* reported more enrichment of the fibre (more volatile and semi-volatile compounds present) using immersion SPME (compared to HS-SPME) with no salt addition. The salt (NaCl) reduced enrichment of the fibre due to the low solubility of the salt and high alcohol content of the whiskey samples, therefore, the authors chose to carry out sample injection without the addition of NaCl. To determine which fibre was best suited for volatile analysis of esters, three different fibres (PDMS, PA and DVB-CAR-PDMS) were tested. The conclusions drawn were to use immersion SPME (PDMS fibre) stirred at 25 °C during 30 min extraction without addition of salt to analyse whiskey samples and achieve high fibre enrichment and good reproducibility [85,87].

Fitzgerald *et al.* [96] measured the congeners present in Scotch and Irish whiskey by GC-MS using a high polarity wax phase column. It was noted that SPME (headspace and immersion) performed better than direct injection for congener detection, therefore, Fitzgerald *et al.* compared immersion and HS-SPME using different fibres. Fitzgerald *et al.* found that HS-SPME with the polyacrylate (PA) fibre (polar fibre) was the best for detecting the analytes of interest (several alcohols and esters) at the selected concentrations and the reproducibility was also better than immersion SPME. This is in contrast to the conclusion by Demyttenaere *et al.* who used immersion SPME with a PDMS fibre [85]. Just like Demyttenaere *et al.*, Fitzgerald *et al.* showed that there was no advantage to salt addition [85,87,96]. The final SPME parameters determined by Fitzgerald *et al.* for the PA fibre was a 35 minute extraction time at room temperature (22 ± 1 °C) and a desorption time of 5 minutes [96]. The extraction

time and temperature were similar to those used by Demyttenaere *et al.* (30 minutes and 25 °C) [85].

Stupak *et al.* [79] developed a GC-MS analysis method using a polyethylene glycol column for quality and authenticity analysis of whiskey. Two different sample extraction methods (SPME and acetate extraction) were compared. For SPME extraction, the incubation and extraction times (5, 10, 20 and 30 min), as well as the incubation and extraction temperatures (30, 40, 50, 60 and 70 °C) were varied for comparison of three different SPME fibres (PDMS, PA and DVB/CAR/PDMS). Optimal results were obtained using the DVB/CAR/PDMS SPME fibre with 10 minute incubation and extraction times and 60 °C incubation and extraction temperatures. These parameters (fibre, incubation/extraction times and temperatures) were different to those determined by Demyttenaere *et al.* [85] (PDMS fibre, 25 °C for 30 minutes) and Fitzgerald *et al.* [96] (PA fibre, 22 °C for 35 minutes). Stupak *et al.* [79] also tested different dilutions of the whiskey samples (1:1, 1:2, 1:3) and addition of NaCl. The optimal dilution ratio was found to be 1:3 whiskey to water (with addition of NaCl) resulting in approximately 10% EtOH. The results for the addition of salt showed improvement of detection for the compounds of interest (*N*-(3-methylbutyl)acetamide, ethyl 5-oxoprolinate, 5-oxooxolane-2-carboxylic acid and 4(2-hydroxyethyl)phenol). This showed that the addition of salt could improve detection depending on the target compounds as Demyttenaere *et al.* and Fitzgerald *et al.* found no advantage to adding salt [79,85,96].

In the method used by Clyne *et al.* (1993) [37], samples were extracted with dichloromethane (DCM) then analysed by direct injection GC-MS. Thirty compounds were identified using retention times and authentic compounds (reference materials) with an additional 18 compounds tentatively identified. Many volatile compounds were identified including several which are known to have a large impact on aroma (e.g. furfural and ethyl esters), flavour (e.g. vanillin and several ethyl esters) and the quality of whiskey (e.g. 5-methyl furfural and vanillin), however, this preparation should be avoided for routine procedures due to the toxicity and difficulty of disposal of DCM.

Jeffery (2012) [20] used a polyethylene glycol column to analyse oak extractives using a large volume injection (no sample preparation). It was found that a long solvent delay was required because ethanol and other early eluting volatiles caused sensitivity issues, particularly because large volume injection was used. Peak separation was achieved using a slow oven temperature ramp. This method was different from Demyttenaere *et al.* [85], Fitzgerald *et al.* [96] and

Stupak *et al.* [79] because none of them reported sensitivity issues or peak separation problems which required a slow oven temperature ramp which was most likely due to injecting 5 μL whereas the others used SPME.

McNamara *et al.* [78] and Nascimento *et al.* [97] did not carry out any sample preparation prior to direct injection of samples into the GC-MS. McNamara *et al.* [78] analysed volatile compounds in distillates at different intervals during the maturation process (extending 20 years) in American and European oak barrels using a dimethylpolysiloxane phase column [78]. Whereas Nascimento *et al.* [97] used GC-MS to quantify esters (specifically ethyl esters) in various spirits including whiskey using a polyethylene glycol phase column. Neither author reported any issues arising from the lack of sample preparation or their chosen columns indicating sample preparation was not necessary for direct injection.

In summary, most methods concluded that SPME headspace and no salt addition gave better recoveries (except Stupak *et al.*). There was no consensus on which fibre was best suited for whiskey analysis. The DVB/CAR/PDMS is used for the analysis of flavours and includes volatile and semi-volatile compounds [87,90]. McNamara *et al.*, Jeffery and Nascimento *et al.* were successful in using direct injection without sample preparation. Both direct injection (with no sample preparation) and SPME were investigated in this research during method development (Chapter 3) to determine which was most suitable for the analysis of esters, phenolic aldehydes, phenols and alcohols. Table 1.7 summarises the GC detectors, sample introductions, columns, and column phases from the literature discussed and the compounds targeted.

Table 1.7: Summary of GC analysis methods for spirits with the author, instrument, sample introduction, column, column phase, type of compounds identified and ISTD used.

Author	Detector	Sample introduction	Column	Column phase	Type of compounds identified	ISTD
Jeffery (2012) [20]	FID	Direct injection	Agilent Stabilwax column	Polar phase (polyethylene glycol phase)	volatile congeners	N/A
Balcerek *et al.* [45]	FID	Direct injection	Agilent HP-Innowax	Polyethylene glycol phase, high polarity	major volatiles (alcohols and some aldehydes)	
Clyne *et al.* [37]	MS	Direct injection	SGE Carbowax BP20	Polar phase (polyethylene glycol phase)	acids, alcohols, esters, aldehydes	2,3-dimethylphenol
Mosedale *et al.* [98]	MS	Direct injection	J&W DB-Wax	Polyethylene glycol phase, high polarity	whiskey lactone (*cis* and *trans*)	
Lost Spirits [32]	MS	Direct injection	Restek Rxi-5Sil MS	Non-polar – 1,4-bis(dimethylsiloxy)-phenylene dimethyl polysiloxane	acetyl, ethyl decanoate, ethyl laurate, sinapaldehyde, furfural and syringaldehyde	N/A
Masuda and Nishimura (1981) [99]	MS	Direct injection	various columns used (HPLC used to separate fractions)		volatile sulfur compounds	methyl ethyl sulfide
McNamara *et al.* [78]	MS	LVI, PTV inlet	Agilent CP-sil 5 CB	Non-polar - dimethylpolysiloxane phase	volatile	N/A
Demyttenaere *et al.* [85]	MS	PTV inlet - immersion SPME (PDMS fibre)	HP-5MS	Nonpolar - 5%-phenyl-methylpolysiloxane	volatile and semi volatile	N/A
Nascimento *et al.* [97]	MS	Split mode	HP-FFAP		ester analysis	4-methyl-2-pentanol
Fitzgerald *et al.* [96]	MS	Splitless - headspace SPME (PA fibre)	Chrompack/Agilent CP Wax 57 CB	high polarity	congeners present	4-methyl-2-pentanol

Author	Detector	Sample introduction	Column	Column phase	Type of compounds identified	ISTD
Stupak *et al.* [79]	Q-ToF	Splitless - SPME (DVB/CAR/PDMS)	HP-INNOWax	Polyethylene glycol phase, high polarity	metabolomics fingerprinting of semivolatile substances	N/A
Jeffery (2012) [20]	MS	Splitless injection	DB-Wax	Polyethylene glycol phase, high polarity	oak extractives	N/A
Balcerek *et al.* [45]	MS	SPME DVB/CAR/PDMS fibre	Agilent VF-WAX	Polyethylene glycol phase	minor volatiles	4-heptanone

N/A No internal standard used/listed

1.8. Objectives and Aims

The exact science behind the maturation process is slowly being uncovered. There are still many unknowns (the changes in concentrations and compounds) in traditional maturation because production of whiskey is considered an art; methods are passed down through the generations and whiskey is produced through trial and error rather than focusing on the science to manipulate processes in order to achieve desired flavours and aromas. For example, experienced distillers assess whiskey by sensory analysis which includes assessment of: aroma, flavour, astringency, sweetness, appearance and mouthfeel (the way food or drink feels on the tongue). The distillers use these parameters to determine whether the whiskey has aged long enough or if it requires more time [9,28,100].

This work focused on the compounds present and the reactions and concentration changes during maturation. This research was carried out for a small commercial company who want to produce whiskey which has the flavours and aromas of aged whiskey (≥ 10-year-old) within a shorter time frame for commercial advantage and economical viability. The benefits of rapid maturation include; reducing time, storage space and loss of product via evaporation, therefore, more product is available within a shorter time [13]. Development of a rapid ageing process requires investigation of several areas including the compounds present in commercial samples and the effects of different ageing techniques on the distillate.

The aims of this research were to:

1) Carry-out method development and validation for analysis of samples using analytical instrumentation (GC-MS) (Chapter 3)
2) Analyse a range of commercial whiskey samples of various ages and origins to determine the compounds present and their relative compositions (Chapter 4)
3) Conduct exploratory experiments to investigate the effects of ageing techniques on ethanol and distillate (Chapter 5)
4) Determine which ageing treatment is the most suitable to achieve a > 10-year-old whiskey profile (Chapter 5)

Chapter 2 - Experiment Methodology

This chapter outlines the materials, analytical instrumentation and samples used for this project. Experimental details on each of the ageing techniques investigated are also detailed in this chapter.

2.1. Consumables

The following consumables were used: pipette tips (1 mL and 10 mL), amber vials (2 mL), blue screw caps (2 mL) lined with a PTFE/silicone septa, headspace vials (20 mL), magnetic screw caps (20 mL) lined with blue PTFE/white silicone septa, glass pasteur pipettes, sample storage vials (20 mL) with white polypropylene screw lid (PTFE lined).

2.2. Chemicals

Food grade ethanol (96%), malt distillate (75% ABV), Georgia Moon corn distillate (40% ABV) were supplied by the client.

All chemicals were ≥ 98% purity: 1-naphthol, 2-phenylethanol, 3-propanol, glacial acetic acid, acetone, benzaldehyde, cinnamaldehyde, ethyl acetate, ethyl butyrate, ethyl decanoate, ethyl laurate, ethyl octanoate, gallic acid, guaiacol, isoamyl acetate, isoamyl alcohol, *m*-cresol, *m*-methoxyphenol, *n*-decanoic acid, *n*-pentanol, pentane, succinic acid, syringaldehyde, syringic acid, vanillic acid and vanillin. Specific purities and supplier details are given in Appendix B, Table B.1 (page 177).

2.3. Equipment

- Grade A volumetric flasks (10-100 mL)
- DURAN® bottles (100 mL)
- Transonic 700/H Elma sonicator (frequency of 37kHz, 200W power and an energy density of $50W.L^{-1}$)
- BioLab Velp Scientifica Vortex
- Contherm Thermotec 2000 Oven
- 40 Watt light - Growsaber LED tube (120 cm long)
- Mylar foil (stretched polyethylene terephthalate)
- Fisher and Paykel Chest freezer (−18 °C)

- Thermo Fisher adjustable pipette (1-10 mL)
- Eppendorf adjustable pipette (100-1000 µL)
- Graduated SGE syringe (500 µL)
- Beko oven (60cm White Solid Hotplate Upright Cooker)

2.4. Analytical Instruments and Software Details

Instruments

An Agilent 7890B gas chromatograph coupled to a mass spectrometer with triple quadrupole detection (GC-QQQ, Agilent 7000D GC/TQ) was equipped with a HP-5MS (nonpolar, 5%-phenyl-methylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 µm) and used for comparison between SPME headspace and direct injection sample introductions. Analysis was performed in full-scan, electron-impact mode. The system contained a PAL-RSI 120 autosampler equipped with a SPME fibre holder for SPME headspace analysis and a liquid tool (10 µL syringe) for direct injection with an injection volume of 1 µL. The incubation and extraction temperatures were both set to 25 °C and the injector temperature was set to 250 °C. The GC-MS parameters are summarised in Table 2.1. Analysis was carried out with the following oven parameters: initial oven temperature; 35 °C, 35-220 °C at 5 °C/min; 220-240 °C at 10 °C/min with a hold time of 5 minutes. For HS-SPME, each fibre was conditioned following manufacturer instructions (Table 2.2) prior to use. The incubation time, extraction time and desorption time (into inlet) were set to 5, 30 and 3 minutes respectively.

An Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass spectrometer was equipped with a ZB-5 MS (nonpolar, 5%-phenyl-arylene-95% dimethylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 µm) which was equivalent to the HP-5MS column used in the GC-QQQ. Analysis was performed in full-scan, electron-impact mode and an Agilent autosampler (Agilent 7683B) was used for the direct injection of samples at a volume of 1 µL. This instrument was used for method validation and analysis of commercial and experimental samples. Table 2.1 contains the GC-MS parameters used for the analysis of all commercial and experimental samples. The oven parameters used are listed in Table 2.3.

Table 2.1: GC-QQQ (SPME and liquid) and GC-MS parameters used for method development and the analysis of commercial and experimental samples.

Parameter	GC-QQQ SPME*	GC-QQQ Liquid injection*	GC-MS Liquid injection†
GC			
Injector Temperature	250 °C	265 °C	265 °C
Injection Mode	Split	Split	Pulsed Split (25 psi for 1 min)
Split Ratio	10:1	20:1	50:1
Flow Rate	1.12 mL/min	1.12 mL/min	1.2 mL/min
Carrier Gas	Helium	Helium	Helium
Carrier Gas Saver	On	On	On
Carrier Gas Saver Time	2 min	2 min	2 min
	Constant flow	Constant flow	Constant flow
MS			
Ion source	230 °C	230 °C	230 °C
Aux	280 °C	280 °C	280 °C
Quads	150 °C	150 °C	150 °C
Solvent Delay	1.8 min	1.8 min	0.8 min
Start m/z	35	35	35
End m/z	500	500	500

* Used for method development

† Analysis of commercial and experimental samples

Table 2.2: Conditioning temperature (°C) and time (min) for each fibre [101].

Fibre	Conditioning temperature (°C)	Conditioning time (min)
DVB/CAR/PDMS	270	60
PDMS/DVB	250	30
CAR/PDMS	300	60
PA	300	60

Table 2.3: Oven temperature gradient for analysis of commercial and experimental samples (ZB-5MS column).

Oven Temperature (°C)	Rate (°C per min)	Hold (min)
35		0
220	5	0
240	10	5
275	30	0

Software

Sequencing - Masshunter GC/MS Acquisition (version B.07.06.2704) was used for sequencing of standards and samples. Each sequence followed the same format, for example:

condition injection → blank → full calibration → QC → blank → 10 samples → blank → 1 ppm check standards → 10 samples → blank → full calibration + QC → standby

Data Workup

Quantitative analysis: Quantitation using calibration standards was carried out on most exploratory experiments for comparison between ageing treatments and commercial samples. Mass Hunter MS Quantitative Analysis (version B.09.00 build 9.0.647.0) was used to create calibration curves and quantify commercial and experiment samples. If two peaks co-eluted, the drop perpendicular integration method was used, otherwise baseline-to-baseline integration was used [102,103]. Peaks were identified using retention time matches to standards and the NIST17 library (> 75% match).

Qualitative analysis: Mass Hunter Qualitative Analysis Workflows (version B.08.00 service pack 1) was used to automatically integrate all peaks (threshold > 5×10^4) within the chromatograms and identify the compounds using retention time and the NIST17 library (> 75% match). The peak areas (ISTD corrected) for compounds not present in the calibration standards were compared with those found in commercial and experiment samples.

2.4.1. Standard Preparation

Calibration Standards

Ten calibration standards were prepared with a calibration range of 0.13-10 ppm. Note that throughout this thesis, all concentrations were reported in ppm which is equivalent to mg/L. Table 2.4 summarises the concentration of the compounds in each standard. More in-depth information on the preparation can be found in Appendix C (page 179). Blank samples (65% ethanol) were injected during method validation to monitor the background and ensure no contamination/carryover was occurring.

Table 2.4: Concentration (ppm) for compounds in calibration standards 1-10.

	Concentration in vial (ppm)									
	Std 1	**Std 2**	**Std 3**	**Std 4**	**Std 5**	**Std 6**	**Std 7**	**Std 8**	**Std 9**	**Std 10**
Mix 1*	0.13	0.19	0.25	0.50	0.750	1.00	1.25	2.50	5.00	10.00
Mix 2†	0.50	0.75	1.00	0.13	0.19	0.25	5.00	10.00	1.25	2.50

* Benzaldehyde, Ethyl Acetate, Isoamyl Acetate, Ethyl Octanoate, Cinnamaldehyde and Ethyl Butyrate

† *m*-Cresol, Vanillin, Syringaldehyde, Isoamyl Alcohol, Guaiacol, 3-Methoxyphenol (*m*-Guaiacol) and 2-Phenylethanol

Internal Standard

The working internal standard (ISTD) solution, 1-Naphthol (3.5 ppm), was added to samples (250 µL in 0.75 mL) and calibration standards (2.5 mL in 10 mL) to give an overall concentration of 0.875 ppm in the vial. Preparation details for the working ISTD solution can be found in Appendix C (page 179).

System Monitoring Compound

The system monitoring compound (SMC), *n*-pentanol (10 ppm) was added to calibration standards (0.5 mL in 10 mL) and to experiment samples (5 mL in 100 mL) prior to age treatment to give an overall concentration of 0.5 ppm in the vial. Preparation details for the 10 ppm solution can be found in Appendix C (page 179).

2.4.2. Spike Recovery Experiment

Three commercial samples (1, 3 and 28) were used to analyse the recovery of compounds. Low spike (0.3 and 0.5 ppm) and high spike (5 ppm) recoveries were carried out in duplicate and repeated on three separate days. An additional high spike (6 ppm) recovery was carried out in duplicate and repeated three times (for ethyl acetate, ethyl octanoate and vanillin due to their high endogenous levels).

2.5. Whiskey Samples

2.5.1. Samples

All commercial samples were sourced from colleagues and acquaintances. The brand, alcohol percentage and whiskey age statement for each sample is detailed in Appendix D, Table D.1 (page 181). The %ABV ranged from 30% to 64% and the whiskey age statement ranged from no age statement to 21 years old.

2.5.2. Sample Preparation

For direct injection, commercial samples and corn distillate (40%) were inverted three times then aliquots were taken (0.75 mL) without dilution into 2 mL GC vials. The malt distillate (75%) was diluted to 65% (13.4 mL of water added to 86.6 mL of malt distillate), then 0.75 mL aliquot was taken and put into a 2 mL GC vial.

For headspace SPME analysis, commercial whiskey samples (17 mL) were prepared in 20 mL headspace vials (~3 mL headspace).

The ISTD and SMC were added to all samples (overall concentrations of 0.875 ppm and 0.5 ppm. respectively) prior to analysis.

2.6. Maturation Experiments

2.6.1. Oak Chip Toasting

One stave from a used bourbon barrel (*Quercus robur*, European/French oak) was sanded down to remove any residues from the previous alcohol prior to cutting and chiselling into three different sizes. The different sized oak chips (shavings, small chips (~35×25×5 mm) and large blocks (~40×35×10 mm)) were each divided into three fractions. A household oven was pre-heated on the fan forced setting to the required temperature prior to toasting and all fractions were toasted for 30 minutes. One fraction was toasted at 140 °C (oaky/sweet) to achieve a light toasting, the second fraction at 180 °C (sweet/vanilla) to achieve a medium toasting and the last fraction at 220 °C (toasty/vanilla) to achieve a heavy toasting.

2.6.2. Preparation of Samples for Maturation Experiments

All samples in the maturation experiments were prepared the same way before being exposed to different ageing treatments. Appendix E, Table E.1 (page 183) summarises the weight of wood chips for each ageing experiment. The weight of small and large sized wood chips (light, medium and heavy toasting) were 4.2 ± 0.7 g (n = 32) and 6.8 ± 0.6 g (n = 9), respectively. Shavings were weighed to 0.39 ± 0.01 g (n = 2) and 1.02 g ± 0.02 g.

All wood chips were placed into 250 mL DURAN® bottles with either ethanol, corn distillate or malt distillate (100 mL). Food grade ethanol (> 95%) was diluted to 65% ± 2% (680 mL of 96% EtOH + 320 mL distilled water to make 1L) with water to provide a blank matrix, act as a pseudo-alcohol matrix and standardise all experiments. The malt distillate was diluted to 65% ± 2% (866 mL of 75% malt distillate + 133 mL distilled water to make 1 L). The system monitoring compound (*n*-pentanol) was added to all samples (except for the 70 °C ethanol heat experiment) with a final concentration of 0.5 ppm in the vial.

Samples were placed in the required conditions for each ageing experiment (detailed in sections 2.6.4-2.6.7).

2.6.3. Sub-Sampling of Maturation Experiments

For each maturation experiment (section 2.6.4-2.6.7), sub-sampling was initially carried out every 1-2 days, then with reduced frequency. Samples were removed from the ageing treatment and left to equilibrate at room temperature for 30-60 minutes. The samples were inverted three times to ensure homogenisation before a sub-sample was removed (0.75 mL). During sub-sampling, a single sub-sample was taken per sample to prevent excessive decrease in volume which would affect the surface area to solution ratio. Samples were returned to the ageing treatment and the sub-samples were stored in sealed amber vials in a refrigerator (4 °C) until analysis.

Each set of ageing experiments were carried out in single replication because a broad view of ageing techniques were undertaken and any anomalies in the trend could be identified by analysis of the same sample over time as well as analysis of the QC within each sample batch.

Prior to GC-MS analysis, samples were allowed to equilibrate to room temperature for > 30 minutes. ISTD (1-naphthol, 250 μL) was added to give a final concentration of 0.875 ppm.

In terms of reference to time throughout this research, one "day" was equivalent to 24 hours, i.e. 2 days = 48 hours.

2.6.4. Heat Experiments

2.6.4.1. Small and Large Chips in Ethanol

Heat experiments using ethanol as the matrix ran for a period of four weeks. The samples were placed into an oven set to 50 or 70 °C and sub-sampled every few days using the method outlined in section 2.6.3. After 2 weeks, samples stored at 70 °C were aliquoted (20 mL) in duplicate into storage vials. One set of vials were returned to the 70 °C oven and the other set stored in the dark at room temperature for two weeks. For samples held at 50 °C, samples were aliquoted (20 mL) into storage vials then stored in the dark at room temperature and the DURAN® bottles returned to the 50 °C oven for two weeks.

2.6.4.2. Small Chips (Medium and Heavy Toasting) in Distillates

Samples were placed in a 70 °C oven and sub-sampled periodically. After 9 days the wood chips were removed and the samples were returned to the oven for a further 9 days with periodic sub-sampling.

2.6.4.3. Wood Shavings in Corn Distillate

Samples were placed in a 70 °C oven for 17 days with sub-sampling for the first 4 days then at the end (day 17).

2.6.5. Sonication Experiments (Non-Heated and 50 °C)

In the first experiment, the temperature of the sonicator bath was not regulated (temperature turned off), however, a thermocouple was used to record the temperature of the sonicator bath and ethanol throughout the experiment. In the second experiment, heat was controlled by setting the water bath to 50 °C.

The following procedure was carried out for both experiments: The samples were sonicated for one hour and then left idle in the bath for one hour. After completion of one cycle (sonicated then idle) each sample was inverted 3 times and a sub-sample was taken. This was repeated over an 8 hour period (i.e. 4 completed cycles; sonicated 4 times and idle 4 times) with a sub-sample taken after completion of each cycle (after every idle period).

2.6.6. Light Experiment

This experiment was carried out using corn distillate as the matrix. Light experiments were performed over a two week period with continuous exposure to a 40W Growsaber LED light. A stretched polyethylene terephthalate film (Mylar foil) was used to cover samples to maximise light exposure (reflect the light). All samples were placed alongside the LED tube for maximum exposure underneath the cover and exposed to light for 15 days.

A sample of fresh distillate and a 50 °C (medium toast small chip) heat treated sample were taken at 0 hours to analyse for the compounds present before light treatment.

2.6.7. Heat-Freeze Experiment

The flow diagram (Figure 2.1) details the process for heat-freeze experiment where the samples were transferred between a 70 °C oven and a −18 °C freezer.

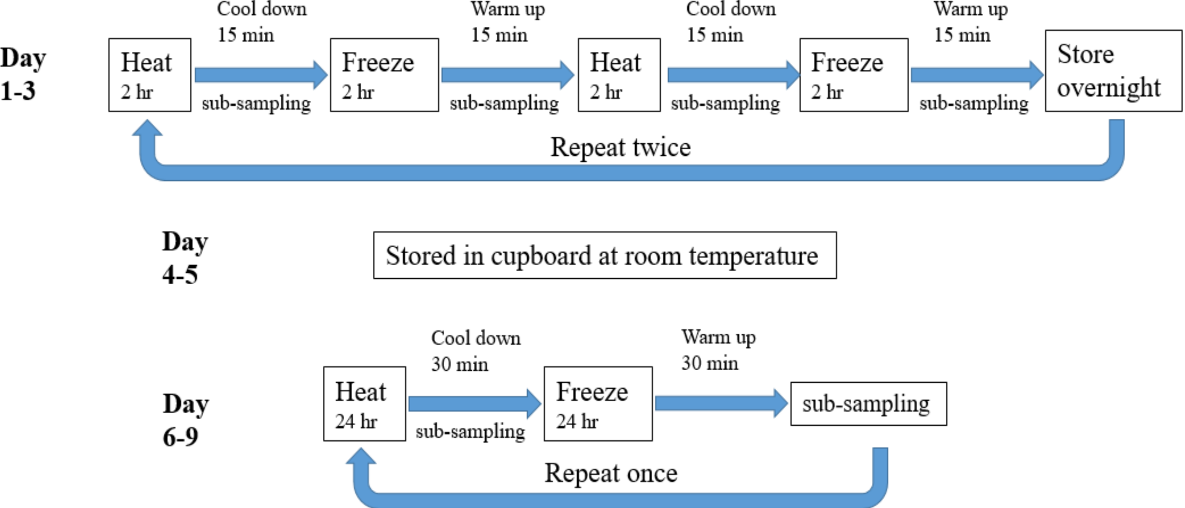

Figure 2.1: Flow diagram of Heat-Freeze experiment procedure for day 1-3, 4-5 and 6-9.

2.6.8. Statistical Analysis

Microsoft Excel 2013 (version 15.0.4433.1506) was used to carry out statistical analysis (SD, LoD, LoQ, average, min and max), peak area comparisons and create graphs.

Minitab 17 (version 17.1.0.0) was used for principle component analysis (PCA) and linear discriminant analysis (LDA) of commercial and experiment samples.

Chapter 3 - GC-MS Method Development and Validation

Gas chromatography (GC) coupled with a mass spectrometer (MS) or flame ionisation detector (FID) are the most widely used instruments for whiskey analysis. For this research GC-MS was chosen because it has high accuracy and sensitivity, and it allows identification of compounds within complex mixtures [104]. Two sample introduction methods (direct injection and HS-SPME) were compared to determine which was more suitable for the analysis of whiskey. To ensure a representative analysis of the samples, various parameters were investigated as part of method development. Method validation (section 3.3) was carried out to determine linearity, limits of detection and quantitation as well as recovery and robustness.

The methods reported in the literature by Lost Spirits LLC [32] and Demyttenaere *et al.* [85] were used as a starting point for this research; these methods used equivalent columns (and are equivalent to the column used in this research). A summary of the parameters used by Lost Spirits LLC, Demyttenaere *et al.* and the chosen analysis parameters are shown in Table 3.1. The MS parameters were taken from Lost Spirits LLC [32] because these were not stated by Demyttenaere *et al.* [85]. The SPME fibre parameters were used from Demyttenaere *et al.* with the extraction time set to 30 minutes and temperature to 25 °C. Lost Spirits had a fast oven ramp (35-300 °C at a rate of 16 °C/min) because the focus was on the detection of six compounds, whereas Demyttenaere *et al.* wanted to detect a range of esters and alcohols. The GC oven temperature program by Demyttenaere *et al.* was used (Table 3.2) because the slower temperature ramp would allow better separation and, therefore, detection of as many compounds as possible.

The GC-MS method was set to fullscan because it was unknown what compounds would be present in commercial and experimental samples. The use of fullscan also allowed more accurate matching with the NIST library which was beneficial due to standards not being available for all compounds detected throughout this research.

Table 3.1: GC-MS parameters used by Lost Spirits, Demyttenaere and the final chosen parameters for method development [32,85].

Parameter	Lost Spirits LLC	Demyttenaere	Chosen parameters
GC Parameters			
Column	Rxi-5Sil MS	HP-5ms	HP-5ms/ZB-5ms
Injector Temp (°C)	265	250	265 (250 for SPME)
Injection Type	Direct injection	SPME immersion	
Injection Mode	Split	Split	Split
Injection Volume	–	N/A	1 µL (for direct injection)
Split Ratio	20:1	10:1	20:1 (10:1 for SPME)
Flow Rate (mL/min)	1.12	1.20	1.12
Carrier Gas	–	Helium	Helium
Carrier Gas Saver	On	–	On
Carrier Gas Saver Time (min)	10	–	2
MS Parameters			
Ion Source (°C)	220	–	230
Auxiliary (°C)	280	–	280
Quadrapoles (°C)	–	–	150
Solvent Delay (min)	1.8	–	2
Start *m/z*	35	40	35
End *m/z*	500	300	500

Parameters not specified in the literature are denoted with a –

Table 3.2: Initial oven temperature program used for method development [85].

Oven Temperature (°C)	Rate (°C/min)	Hold (min)
35		0
220	5	0
240	10	5

3.1. GC-MS Sample Introduction

Exploratory work was carried out to compare two sample introduction methods; direct injection and HS-SPME with three different fibres (Carboxen Polydimethylsiloxane (CAR/PDMS), Divinylbenzene Carboxen Polydimethylsiloxane (DVB/CAR/PDMS) and Polyacrylate (PA)). All parameters (GC and MS) were kept constant during this comparison apart from the sample introduction (and inlet temperature) which avoided any bias that would be caused by changes to instrument parameters (e.g. oven temperature or flow rate) and also allowed for easy comparison between direct injection and HS-SPME as well as comparison between different

SPME fibres. The detection of esters, phenolic aldehydes (e.g. vanillin) and wood extractives (e.g. furfural) was essential because they are important compounds in whiskey due to having a positive or negative effect on aroma and flavour depending on the concentrations at which they are present [1,11,32,45,64]. Therefore, for comparison between sample introductions a set of 8 compounds were analysed: vanillin, vanillic acid, ethyl acetate, succinic acid, *m*-cresol, isoamyl alcohol, 2,4-dimethylphenol and syringaldehyde [1,41,46]. For the sample introduction comparisons, each compound was prepared at a concentration of 2 ppm in 95% ethanol because 2 ppm is within the low to middle concentration range for many of the selected compounds and 95% ethanol was initially used to protect the column by decreasing the amount of water injected into the system during sample introduction investigation [11,37,63]. Three commercial whiskey samples (samples 1-3, Appendix D, page 181) were also analysed to investigate matrix effects. All samples were analysed neat because the %ABV was between 40-65% which is the general percentage of consumed spirits [1,20,78]. The injection of samples with high water content onto the GC column is not recommended because of the detrimental effects to the column phase such as change in selectivity due to absorption of water and column phase bleed. However, many bonded and cross-linked columns (polar and non-polar) can tolerate small volumes of water and several authors have performed analysis of whiskey samples at similar percentages on equivalent columns and no negative effects were noted [20,45,78,85,86].

An additional two compounds that are not present in whiskey but of similar chemical nature (1-naphthol and *n*-pentanol) were also analysed to assess their suitability as an internal standard (ISTD) and system monitoring compound (SMC); assessment included if the compounds were detected at 2 ppm, their resolution and whether they co-eluted with other compounds known to be present in whiskey.

This was a preliminary experiment to determine the best sample introduction method. Single injection analysis was carried out for the 10 individual standards and three commercial samples rather than the preferred triplicate analysis due to a limited supply of samples and time constraints.

3.1.1. Direct Injection

Direct injection is the most common sample introduction method for GC-MS [12]. All 10 individual standards (2 ppm, listed in section 3.1) were visible with a high level of detector

response; examples of chromatograms for 1-naphthol (potential ISTD) and syringaldehyde are shown in Figure 3.1. For the analysed compounds, there were no peak overlaps and resolution was good for most compounds; the acid compounds (vanillic acid and succinic acid) had poor resolution (broad peaks) which suggested incompatibility with the stationary phase (5%-phenyl)-methylpolysiloxane). Therefore, quantitation of acids would not be possible with the given column. The potential ISTD (1-naphthol) and potential SMC (*n*-pentanol) had good peak response, good resolution and did not co-elute with any of the analysed compounds. Therefore if direct injection was used going forward these two compounds would be suitable for use as the ISTD and SMC.

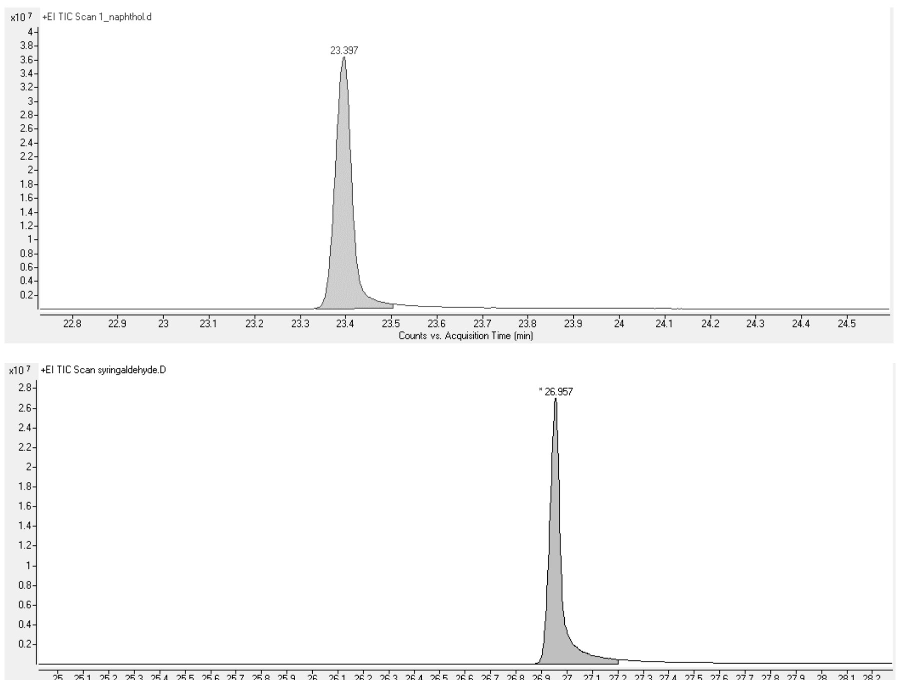

Figure 3.1: Chromatogram for direct injection of 2 ppm standards of 1-naphthol (top, RT = 23.40 min, potential internal standard) and one compounds present in whiskey (syringaldehyde; bottom, RT = 26.96 min). All compounds had good detector response ($\geq 2\times10^7$).

Analysis of three commercial samples showed 13-20 compounds (distinct peaks within the chromatograms) were detected (threshold 2×10^5) with 12-14 compounds tentatively identified (> 75% match) using the NIST library and/or retention time matches to standards (Figure 3.2). There was no baseline noise and no peaks co-eluted showing good separation was achieved for the injected samples using the oven temperature program. Furthermore, there were no peak overlaps between the compounds detected in the commercial samples and individually analysed compounds.

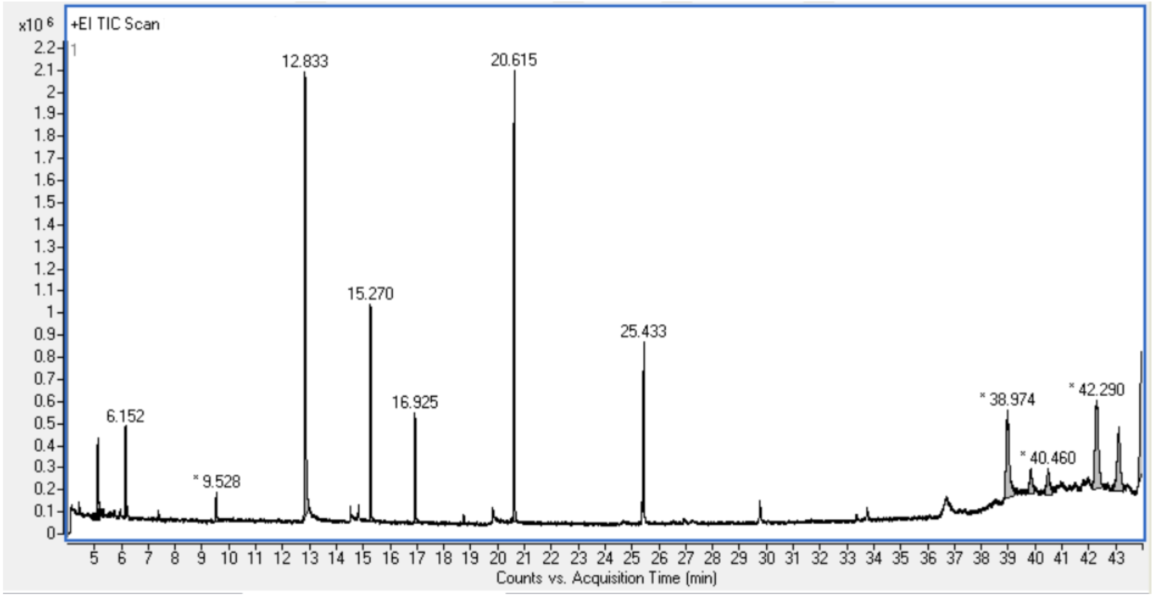

Figure 3.2: Chromatogram obtained for one commercial whiskey (sample 3) using direct injection (peak threshold, 2×10^5). The peaks tentatively identified using standards and the NIST library (retention time in brackets) were: furfural (5.12 min), isoamyl acetate (6.15 min), ethyl hexanoate (9.53 min), 2-phenylethanol (12.83 min), ethyl octanoate (15.27 min), phenethyl acetate (16.93 min), ethyl decanoate (20.61 min), ethyl laurate (25.43 min), unknown compound (38.97 min), maltose tetramethylsilane (TMS) derivative (42.29 min, contamination).

3.1.2. Headspace SPME

All three fibres (CAR/PDMS, DVB/CAR/PDMS and PA) were tested using the same GC and column parameters as direct injection (Table 3.1) with the exception of the injector temperature which was changed to the same temperature as Demyttenaere *et al.* (from 265 to 250 °C) [85]. The current research used HS-SPME instead of immersion SPME to protect the fibre from potential fouling by non-volatiles and high %ABV, however, it was acknowledged that the change did potentially affect the number of compounds that could be detected [85,87]. Injection of the same compounds (2 ppm) and commercial samples (1-3, Appendix D, page 181) analysed by direct injection were also analysed by HS-SPME to determine which fibre was the most suitable with the set parameters and for comparison with the direct injection results.

3.1.2.1. Comparison of Different Fibres

Analysis of blank samples (clean empty vial) and standards showed moderate fibre bleed for CAR/PDMS (Figure 3.3) and significant fibre bleed for DVB/CAR/PDMS (Figure 3.4) with most of the peaks tentatively identified as siloxanes. Column bleed was ruled out because no bleed was observed during analysis with direct injection and because the fibres are coated in

polymer material which is generally siloxane based. The PA fibre was the only fibre that did not have fibre bleed (Figure 3.5). This could have been due to the fibre coating (poly acrylate) and/or because the PA fibre was brand new, whereas the other three fibres had been previously used and there is no record of whether the correct conditioning was carried out prior to first use and during use.

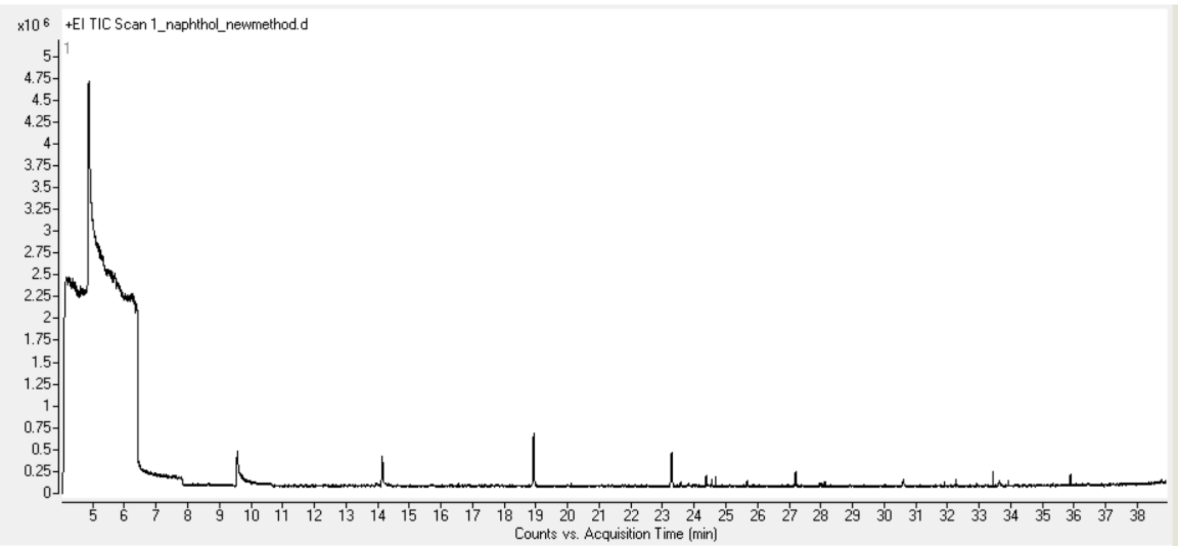

Figure 3.3: Fibre bleed visible in 1-naphthol (potential ISTD) in 95% EtOH using the CAR/PDMS fibre. Siloxane peaks identified at 4.87 min and 9.55-35.89 min and had responses > 10^3. The ISTD retention time was 23.5 minutes, however, the peak at 23.5 minutes was identified it as fibre bleed (tetradecamethyl-cycloheptasiloxane) by the NIST library.

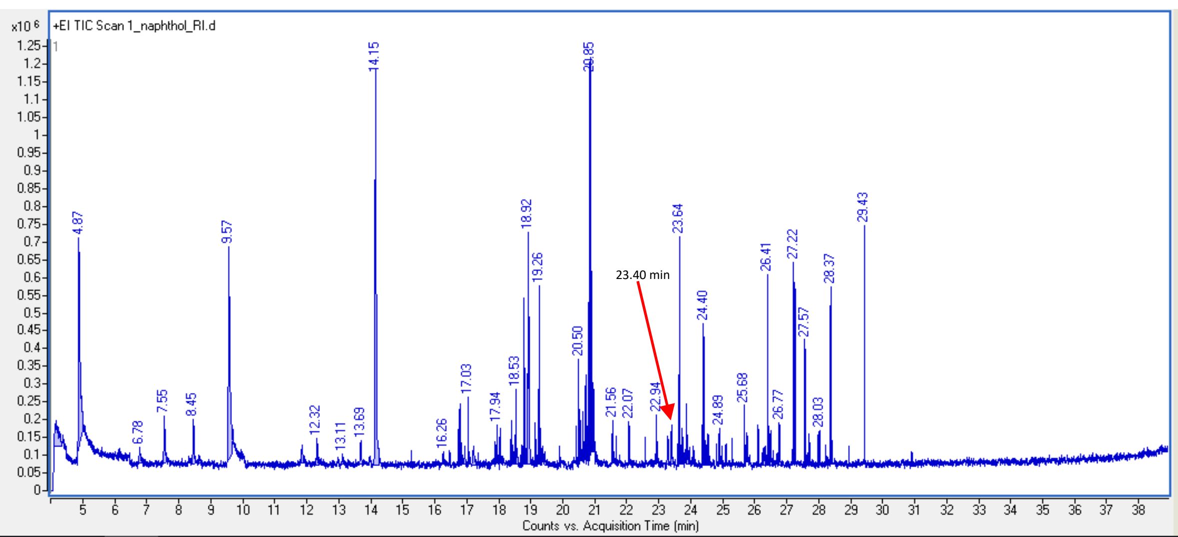

Figure 3.4: 1-naphthol (potential ISTD, 23.40 min) in 95% EtOH chromatogram showing significant bleed using the DVB/CAR/PDMS SPME fibre after conditioning. The majority of peaks were identified as siloxanes (fibre bleed) with more than 13 peaks having a response > 10^3.

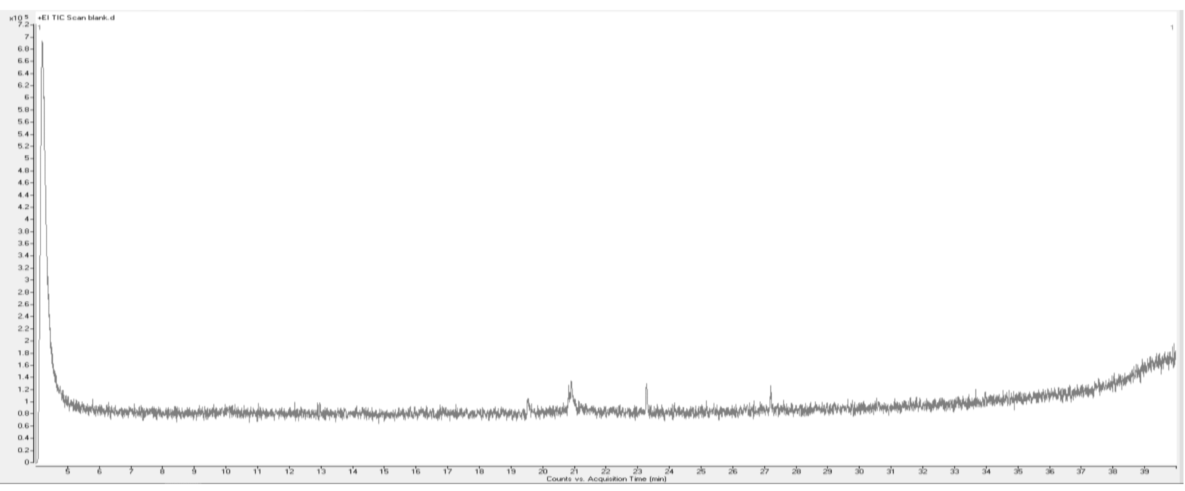

Figure 3.5: Chromatogram of blank injection obtained using PA fibre showing no fibre bleed.

None of the standards were detected with the CAR/PDMS fibre with the set instrument parameters. There was a peak at the ISTD retention time (23.40 min), however, the peak was identified as a siloxane (tetradecamethyl-cycloheptasiloxane). Due to the response of the fibre bleed being similar to the responses of whiskey compounds, method development with this fibre was discontinued. Out of the ten standards (2 ppm) analysed, only one compound, 2,4-dimethyphenol (volatile), was detected with the PA and DVB/CAR/PDMS fibres. In comparison, Jeleń *et al.* [105] proposed that the high responses for alcohols present in distillates (corn, rye and potato, ~10% ABV) was because of the high affinity to the PA fibre coating; this fibre is often used in the food and beverage industry [90]. The analysis of three commercial whiskey samples by HS-SPME detected the same 9-12 compounds (mostly esters) with each fibre. The PA fibre was the best in terms of minimal fibre bleed (cleaner background), however, the response achieved with the PA fibre was lower when compared to the other fibres (Figure 3.6).

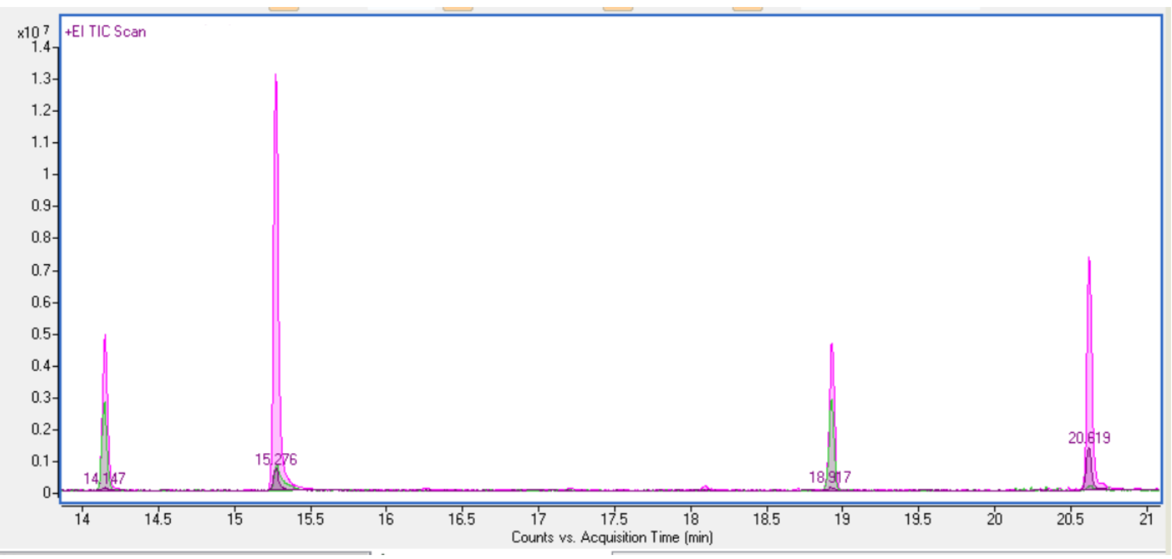

Figure 3.6: Zoomed in chromatograms (14.0-20.9 min) of a commercial whiskey sample obtained using the different SPME fibres (threshold > 2×10^5) to show the difference in response (pink = DVB/CAR/PDMS, green = CAR/PDMS and purple = PA) for decamethyl-cyclopentasiloxane (14.147 min, fibre bleed), ethyl octanoate (15.276 min), dodecamethyl-cyclohexasiloxane (18.917 min, fibre bleed) and ethyl decanoate (20.619 min).

The PA fibre is best suited for detection of polar semi-volatiles, therefore, the lower response (when compared to the other fibres) could be explained by the selectivity of the fibre and the extraction mechanism (absorption or adsorption). The CAR/PDMS fibre is used for the analysis of gas and low molecular weight (MW, 30-225) compounds and the DVB/CAR/PDMS is used for the analysis of flavour compounds (e.g. esters and phenolics) and includes volatile and semi-volatile compounds; in general both of these fibres have shown their suitability for the target compounds [65,79,85,87,88,90,106]. The PA fibre works by absorption whereas the other fibres (CAR/PDMS and DVB/CAR/PDMS) use adsorption (Table 1.6). Furthermore, the absorption/adsorption of compounds onto the fibre can be influenced by the concentration of the solvent (i.e. ethanol) and the distribution equilibrium (between headspace and sample matrix). Jeleń *et al.* [105] and Fitzgerald *et al.* [96] reported that the PA fibre was suitable for alcohol and ester analysis in whiskey. Therefore, due to the difference in matrix (10% ABV used by Jeleń *et al.* [105] versus 95% ABV in this research), syringaldehyde and vanillin were re-analysed at a concentration of 2 ppm using 65% ethanol because their detection is important due to being quality markers in whiskey [1,23,37,46,53]. Despite the reported use of the other fibres in literature [105], the fibre bleed caused too much interference to reliably distinguish between desired compounds and siloxanes, thus, syringaldehyde and vanillin were analysed with the PA fibre. Syringaldehyde and vanillin were not detected in 65% ethanol, therefore,

several method parameters were adjusted to determine whether the 10 original compounds (2 ppm in 95% EtOH) and syringaldehyde and vanillin (2 ppm in 65% EtOH) could be detected with increased extraction time. To account for some variation between instruments and samples as well as the different equilibrium times between immersion and headspace, the time the fibre was in headspace for (extraction time) was increased from 30 to 45 minutes. After the increase in extraction time, no additional compounds were detected with the PA fibre. Fitzgerald *et al.* determined that there was minimal fluctuation in the peak areas after 30 minutes, but chose an extraction time 35 minutes for subsequent studies. Therefore, further changes were made to the method. The incubation temperature (temperature set to aid sample in reaching equilibrium prior to fibre exposure) was increased from 25 to 30 °C and the injection mode was changed from split to splitless. However, no extra compounds were detected with these changes. The lack of compound detection with the PA fibre suggested it was potentially sensitive to the %ABV compared to the other fibres. This suggestion is based on the preparation methods reported in literature; Jeleń *et al.* diluted samples to ~10% ABV prior to analysis and Fitzgerald *et al.* diluted standards containing volatiles found in whiskey to ~20% ABV. Jeleń *et al.* and Fitzgerald *et al.* both used the same type of wax phase column (CP Wax 57) which is cross-linked and bonded making it suitable for the analysis of samples in a water matrix. The analysis of commercial samples in this research (discussed below in section 3.1.3) was carried out successfully at 40.0-50.5% ABV on a 5%-phenyl-methylpolysiloxane column. Therefore, it was postulated that the concentration in the headspace may have been lower than the LoD using the current instrument parameters. The HS-SPME method could have been further developed (e.g. salt addition), but direct injection gave better results; it detected a range of functional groups, peak resolution was good and no siloxane contamination was present. Therefore, further SPME development was not carried out.

3.1.3. Conclusions for Sample Introduction

The analysis of 10 standards and 3 commercial samples did not produce satisfactory results with HS-SPME (using 3 different fibres). In comparison, literature states SPME is a preferable sample introduction method because it is automated and does not require solvent sample preparation (e.g. liquid-liquid extraction) and it is also used to pre-concentrate volatiles which gives better detection limits than direct injection [105]. The number of compounds detected using direct injection and the different SPME fibres were compared (Table 3.3).

Table 3.3: Number of peaks detected (threshold > 1×10^6) using each SPME fibre and direct injection during exploratory analysis (one injection) of three whiskey samples.

Fibre	Number of peaks detected		
	Whiskey 1	**Whiskey 2**	**Whiskey 3**
DVB/CAR/PDMS	10	9	12
CAR/PDMS	7	9	10
PA	N/A	6	N/A
Direct injection	18	24	16

N/A Not analysed

Overall, direct injection was the better sample introduction method for two reasons. Firstly the background was cleaner since there was fibre bleed when using the SPME fibres (excluding the PA fibre). Secondly, target compounds in whiskey are present at low to medium concentrations, therefore, the ability to see < 2 ppm was required. Not all of the compounds detected in the commercial samples via direct injection were detected with SPME, for example 2-phenylethanol (RT = 12.83 minutes) and furfural (RT = 5.12 minutes). The most likely reason less compounds were detected with SPME is because it is a more complex method (multiple parameters to change) and because the equilibrium between the whiskey and headspace may not have been reached for many compounds. Esters are important compounds in whiskey; their detection was achieved with both direct injection and HS-SPME. Higher responses were observed with SPME for several ester compounds (isoamyl acetate, ethyl hexanoate, ethyl octanoate (Figure 3.7), and ethyl decanoate, (Figure 3.8)). One alcohol (1-hexanol) was detected with the CAR/PDMS fibre, however, this research focused on the composition of whiskey, not on any one particular functional group. Therefore, direct injection was more suitable to detect a broad range of compounds and was chosen as the sample introduction method. Since SPME was not used for the remainder of this project, the GC-MS-QQQ (with automated SPME sampler) was not required so the decision to switch to a GC-MS equipped with an equivalent column (ZB-5MS) was made; all analyses from this point forward were carried out using the Agilent GC-MS with direct injection.

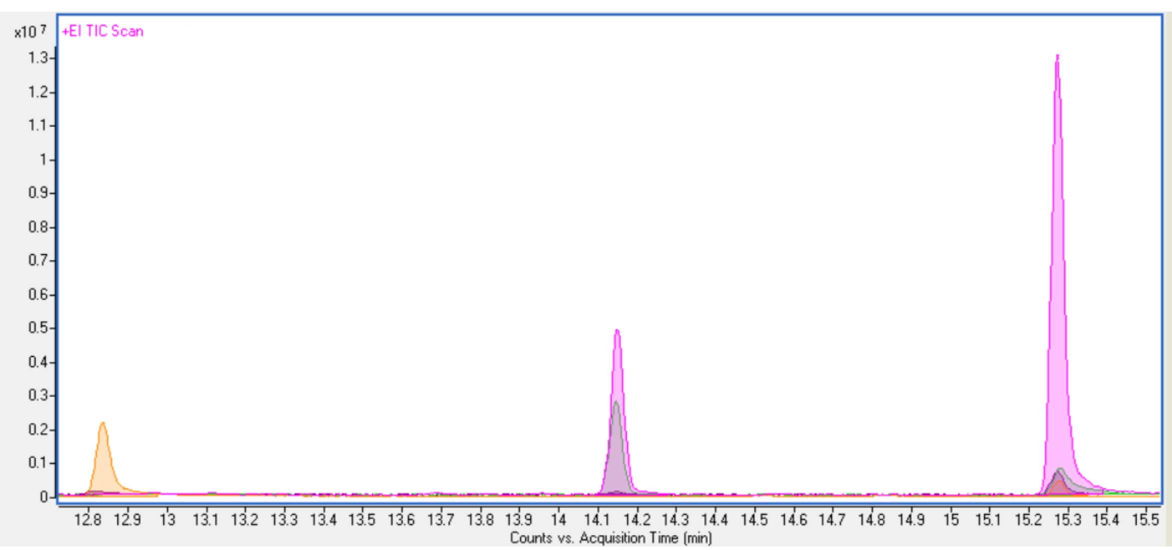

Figure 3.7: Zoomed in chromatograms (12.7-15.5 min) of a commercial whiskey sample showing response difference obtained using the different SPME fibres (pink = DVB/CAR/PDMS, green = CAR/PDMS and purple =PA) and direct injection (orange). Several compounds identified as; phenylethyl alcohol (12.85 min) decamethyl-cyclopentasiloxane (14.147 min, fibre bleed), and ethyl octanoate (15.276 min). The peak area threshold was set to 2×10^5.

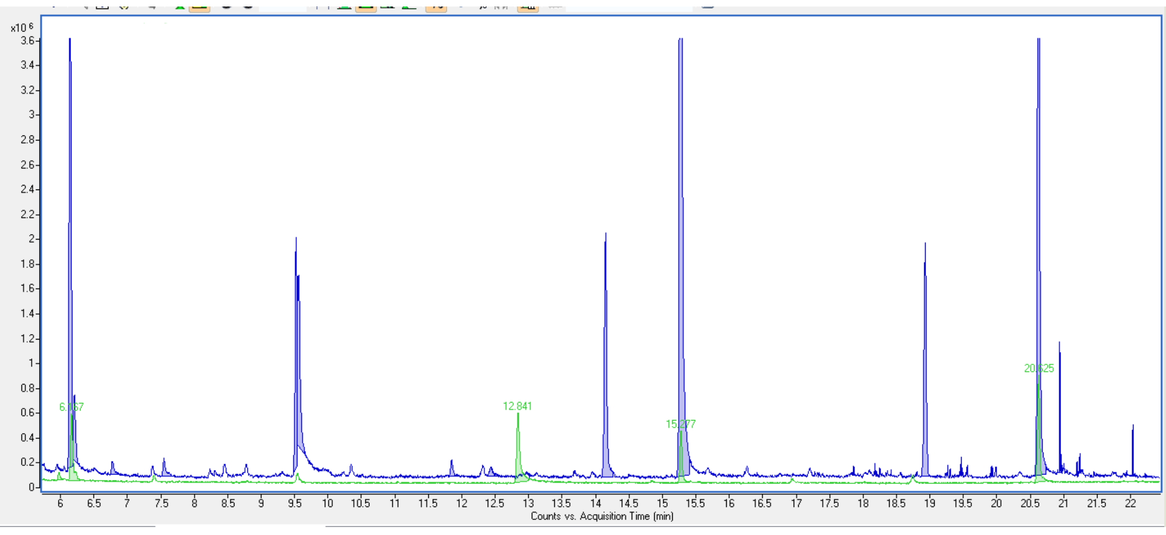

Figure 3.8: Zoomed in chromatogram (5.50-22.5 min) of direct injection (green) overlaid with SPME DVB/CAR/PDMS fibre (blue) chromatogram of a whiskey sample. SPME achieved greater response for several compounds including: isoamyl acetate (6.16 min), ethyl hexanoate (9.54 min), ethyl octanoate (15.27 min) and ethyl decanoate (20.62 min). Fibre bleed was observed at 6.80-8.88 min, 17.90-18.45 min and 19.32-20.02 min.

3.2. Optimisation of Direct Injection GC-MS Parameters

Optimisation of the direct injection method was carried out to improve the number of compounds detected, increase the detector response and decrease analysis time. This required

minor adjustments to analysis time, oven temperature, carrier gas flow rate and injection mode (split ratio). To do this, calibration standards with a concentration range from 0.1-5 ppm in 65% ethanol were prepared. The compounds in the standards were: isoamyl alcohol, *n*-pentanol, ethyl butyrate, isoamyl acetate, benzaldehyde, *m*-cresol, *m*-guaiacol, vanillin, 1-naphthol and syringaldehyde.

The first adjustment was to decrease the solvent delay from 1.8 minutes to 0.2 minutes to determine whether several expected compounds (e.g. ethyl acetate and acetic acid) eluted earlier because they were not detected with a 1.8 minute solvent delay. Three whiskey samples were analysed and the results showed that the delay could be set to 1.0 min to encompass all required compounds and prevent ethanol (RT = 0.863 min) swamping the detector.

To shorten the analysis time, the carrier gas flow rate was changed from 1.12 mL/min to 1.20 mL/min. The faster flow rate decreased the elution times of compounds by 0.2 minutes and did not cause any peak overlaps or loss in resolution.

The initial split flow (20:1) produced several overloaded peaks, particularly ethyl acetate when it was present at concentrations > 30 ppm. The concentration of ethyl acetate in whiskey can be higher than 95 ppm [11]. Therefore, two different split flows (50:1 and 80:1) were tested to reduce peak overloading, whilst still maintaining detection at low concentrations of other compounds [107]. As expected, the frequency of overloaded peaks was reduced when using these higher split ratios. Syringaldehyde was not detectable at concentrations < 0.5 ppm with either split flows, however, as syringaldehyde is generally present at concentrations between 0.67-4.04 ppm this was deemed acceptable [63,64]. The 80:1 split flow had a much lower response for all compounds, and was unable to detect compounds at the two lowest concentrations (0.10 and 0.25 ppm). These concentrations were detectable with the 50:1 split, therefore, the lower split (50:1) was chosen.

A higher inlet flow during injection helps compress the vapourised sample in the liner and provides better transfer of the sample onto the column [108]. Therefore, pulsed injection (high initial flow rate) at 25 psi was tested and showed better detection for the low standards (0.13 and 0.19 ppm) for a number of compounds compared to the non-pulsed injection. Therefore pulsed injection was used and set to 0.2 min.

The oven column temperature program was tested at two different starting temperatures (35 and 40 °C) to determine whether peak elution and shape were affected. The change in the

oven starting temperature did have a minor effect on the baseline; 40 °C gave a slightly higher baseline than 35 °C, therefore, 35 °C was used going forward.

An additional temperature ramp at the end of the column temperature program (240-275 °C at 30 °C/min) was added to ensure all compounds were eluted from the column before the next sample was introduced.

The final method parameters for GC-MS are detailed in Table 2.1.

3.3. GC-MS Method Validation

For a method to be considered suitable, it has to have high accuracy (closeness of a result to the true value) and high precision (agreement between multiple injections of the same sample). As a subset of precision, reproducibility (agreement of results within a method over time) and repeatability (agreement between repeated analysis) are also important [104,109]. Method validation was carried out to demonstrate if the method performance was fit for purpose [110].

To ensure sufficient testing of the method was carried out, validation guidance by International Accreditation New Zealand (IANZ) [111,112] and Eurachem [110] were used as a guideline. Validation which is normally carried out using real sample matrices could not be done because of the wide variation in compound concentrations. Therefore, validation was carried out by analysing standards (in 65% EtOH) to obtain the required concentrations and occurred in triplicate (intra-day) over three days (inter-day). The data was used to determine the linearity (R^2), precision, limits of detection (LoD) and limits of quantification (LoQ). The acceptance criteria was established prior to undertaking the validation. The R^2 for the calibration curves were evaluated with a minimum of 7 points (out of 10); a minimum R^2 of 0.990 was required, although $R^2 \geq 0.995$ was optimal. Precision was assessed by calculating the percentage relative standard deviation (%RSD). A RSD of < 5 % is optimal, while 10-20% was considered good. A RSD < 20% was deemed acceptable for this research [111–114]. The LoD and LoQ would ideally be in the low ppm range (e.g. 0.1 ppm) because some of the target compounds have detectable flavours in this range (e.g. 0.1 and 0.2 ppm for vanillin and sinapaldehyde) and other compounds have detectable aromas at very low ppm (e.g. guaiacol at 0.0069 ppm and vanillin at 0.022 ppm). Some exceptions were made for compounds that are commonly found at higher concentrations. For example, ethyl acetate generally ranges from 4 ppm to greater than 95 ppm and isoamyl alcohol is reported to occur at high concentrations (e.g. 300 ppm) in whiskey [11,20,28,63,64,80].

3.3.1. Linearity, Limits of Detection (LoD) and Limits of Quantification (LoQ)

Quantitative analysis was used for common compounds found in whiskey and for which standards were readily available. For linearity, a set of calibration standards (0.13-10 ppm) were analysed in triplicate and repeated a total of three times (n = 9) to obtain sufficient data to perform statistical analysis [110]. The results were used to plot calibration curves to assess the R^2. Table 3.4 summarises the compounds present in the calibration standards, retention time, average R^2, LoD and LoQ.

The linearity for all compounds met the established criteria, the average R^2 for each compound was ≥ 0.995 with many compounds being ≥ 0.998 (Table 3.4) which proved linearity was acceptable between the measured concentration range of 0.13 and 10 ppm.

The limits of detection (LoD), the lowest concentration detectable above the baseline for a given analyte, was calculated with the lowest standard (0.13 ppm, n = 9) using Equation 1 [115]. Syringaldehyde, vanillin and *m*-methoxyphenol were not detected at the lowest concentration. The lowest concentration that these compounds could be detected at was 0.5 ppm and this was used in the LoD and LoQ calculations. The LoD was between 0.03 and 0.26 ppm for the majority of compounds (Table 3.4), however, ethyl acetate, isoamyl alcohol and ethyl butyrate were higher (1.45 ppm, 0.93 ppm and 0.4 ppm respectively). The standards were in a simple matrix (ethanol) so the calculated LoD would potentially be higher in a more complex sample matrix (distillate). The limits of quantitation (LoQ), the lowest concentration detectable with acceptable accuracy and precision was calculated using Equation 2 [115]. The phenolic aldehydes and phenols were between 0.34 and 0.68 ppm. The LoQ for ethyl acetate and isoamyl alcohol were the highest at 4.82 and 3.09 ppm respectively. Several ethyl esters (octanoate, decanoate and laurate) were between 0.1 and 0.3 ppm, however, isoamyl acetate was in the mid-range at 0.85 ppm and ethyl butyrate had a high LoQ (1.37 ppm). The high LoD and LoQ for ethyl acetate and isoamyl alcohol were acceptable because the common concentrations found in commercial samples are significantly higher (> 95 ppm and ≥ 10 ppm respectively) [11,28]. The high LoD and LoQ for ethyl butyrate and isoamyl acetate could affect accurate quantitation of these compounds because they are both generally reported at 1-2 ppm in literature, however, ethyl butyrate has been detected as low as 0.0095 ppm in American whiskey [28,63,116].

Table 3.4: Retention time (RT, min), R^2, LoD and LoQ for each compound present in the calibration standards (*n* = 9).

Compound	RT (min)*	R^2 †	LoD (ppm)	LoQ (ppm)
Esters				
Ethyl Acetate	1.246	0.996 ± 0.003	1.45	4.82
Ethyl Butyrate	4.136	0.996 ± 0.002	0.41	1.37
Isoamyl Acetate	5.991	0.996 ± 0.002	0.26	0.85
Ethyl Octanoate	15.232	0.997 ± 0.002	0.09	0.29
Ethyl Decanoate	20.591	0.996 ± 0.003	0.06	0.20
Ethyl Laurate	25.416	0.996 ± 0.003	0.03	0.11
Alcohols				
Isoamyl Alcohol	2.861	0.998 ± 0.002	0.93	3.09
2-Phenylethanol	12.775	0.999 ± 0.001	0.10	0.34
Phenolic Aldehydes				
Benzaldehyde	8.246	0.998 ± 0.001	0.20	0.68
Cinnamaldehyde	17.242	0.998 ± 0.002	0.11	0.37
Vanillin‡	20.635	0.999 ± 0.001	0.15	0.49
Syringaldehyde‡	26.879	0.995 ± 0.003	0.10	0.34
Phenols				
m-Cresol	11.704	0.999 ± 0.001	0.14	0.45
Guaiacol	12.068	0.999 ± 0.001	0.16	0.54
m-Methoxyphenol‡	16.055	0.999 ± 0.001	0.18	0.58

* Retention time differs from the sample introduction section (3.1) due to changing from GC-QQQ to GC-MS

† 0.13-10 ppm curve

‡ 0.5 ppm standard was used in the LoD and LoQ calculations

$$LoD = \frac{3 \times SD\ (of\ concentration)}{slope\ of\ calibration\ curve} \quad \text{(Eq. 1)}$$

$$LoQ = \frac{10 \times SD\ (of\ concentration)}{slope\ of\ calibration\ curve} \quad \text{(Eq. 2)}$$

3.3.2. Precision, Reproducibility and Repeatability

The precision, reproducibility and repeatability were assessed using the calibration data from section 3.3.1. The %RSD (Eq. 3) for each compound was calculated and summarised in Table 3.5 [114]. Excellent repeatability and reproducibility results (≤ 5%) were obtained for 8 of the

15 compounds; a further 7 compounds had good %RSD (5-8%). Only isoamyl alcohol had an RSD of 10% but this was deemed acceptable because in many applications 20% is allowed when close to the LoD [114]. These results indicated the method precision was high and suitable for use.

Table 3.5: Summary of precision for compounds in the calibration standards (1 ppm) analysed on three separate days in triplicate (n = 9).

Compound	Average (ppm)	SD	%RSD
Esters			
Ethyl Acetate	1.13	0.08	7
Ethyl Butyrate	1.12	0.07	6
Isoamyl Acetate	1.13	0.06	6
Ethyl Octanoate	1.12	0.06	5
Ethyl Decanoate	0.93	0.04	4
Ethyl Laurate	0.94	0.04	4
Alcohols			
Isoamyl Alcohol	1.00	0.10	10
2-Phenylethanol	0.98	0.02	2
Phenolic Aldehydes			
Benzaldehyde	1.07	0.06	6
Cinnamaldehyde	1.04	0.05	5
Vanillin	0.93	0.06	6
Syringaldehyde	0.96	0.07	8
Phenols			
m-Cresol	0.99	0.02	2
Guaiacol	1.00	0.04	4
m-Methoxyphenol	0.99	0.02	2

$$\%RSD = \frac{standard\ deviation}{mean} \times 100 \qquad \text{(Eq. 3)}$$

A commercial sample (28, Appendix D, page 181) was selected to use as a QC. The average, standard deviation and precision (%RSD) were calculated for isoamyl alcohol, 2-phenylethanol, ethyl decanoate and ethyl laurate (Table 3.6). The %RSD for each compound was between 19 and 33%. The concentrations were close to the calculated LoD and the %RSD limit varies depending on the concentration of the analyte; with a higher tolerance allowed for values closer to the LoD [117,118]. Therefore, for this research a 30% RSD was deemed acceptable. Ethyl decanoate had a large %RSD (33%) due to it being close to the LoQ (0.20 ppm), but it was still used to monitor changes during sample analysis although data would not be dismissed solely based on the QC results for ethyl decanoate. The validation data (n = 9) was used to generate

warning (average ± 2SD) and action (average ± 3SD) limits for QC plots that were used during the analysis of commercial and experimental samples to monitor instrument performance [110,117]. The QC plot for isoamyl alcohol, 2-phenylethanol, ethyl decanoate and ethyl laurate are shown in the Appendix F (page 184).

Table 3.6: Calculated average (ppm), standard deviation and %RSD in the QC samples (*n* = 9).

Compounds	Average (ppm)	SD (ppm)	%RSD
Ethyl Acetate	1.09	0.31	28
Isoamyl Alcohol	5.53	1.60	29
2-phenylethanol	0.26	0.05	19
Ethyl Decanoate	0.12	0.04	33
Ethyl Laurate	0.11	0.03	24

3.3.3. Spike Recovery Experiments

Accuracy could not be calculated because there were no certified reference materials or whiskies with known compound concentrations available, however, recovery of compounds in sample matrices (spike recoveries) was used as a pseudo measure of accuracy. Commercial samples 1, 3 and 28 (Appendix D, page 181) were used. The compounds present in the calibration standards were used to investigate the recovery and reproducibility of the developed method (GC-MS direct injection). Isoamyl alcohol was not included in the spike recovery experiments because the endogenous concentration in the samples (17.0[a], 7.0 and 7.7 ppm, respectively) were higher than the high spike (5 ppm) and the top calibration standard (10 ppm). Ethyl decanoate and ethyl laurate were also excluded from spike recoveries because of their known solubility issues (1 mL in 9 mL of 80% EtOH and 1 mL in 4 mL of 80% EtOH, respectively) [50]. Low (0.3 ppm) and high (5 ppm) spikes were used to measure the recovery of the compounds within whiskey sample matrices; analyses were carried out in triplicate over three days (*n* = 9). An additional low spike with a concentration of 0.5 ppm was analysed for benzaldehyde, 2-phenylethanol, ethyl octanoate, vanillin and syringaldehyde because the LoQ was ≥ 0.3 ppm (low spike concentration). The results are summarised in Table 3.7 with all results presented in Appendix G, Table G.1 (page 187). The method showed good recovery (90-110%) for all compounds in all samples, except for syringaldehyde. Syringaldehyde had a poor recovery for sample 3 (46%); theoretically there should have been a 100% recovery because the unspiked sample (0.51 ppm) was the same concentration as the low spike (0.5 ppm).

[a] Calculated after sample dilution

The results were averaged between six injections and the individual concentrations ranged from 0.85-0.60 ppm, suggesting quantification of syringaldehyde is likely to be underestimated. However, the goal of the rapid maturation experiments was to create a similar profile to commercial samples and the syringaldehyde range in literature (0.20-1.60 ppm) is wide so minor underestimation was not foreseen as a problem.

The spike recoveries showed the method was able to quantify compounds accurately over a wide concentration range (0.13-10 ppm) in Scotch, American and other whiskies [80].

Table 3.7: Average recovery (n = 6) for low (0.3 ppm) and high (5 ppm) spike addition.

Compound	Average Recovery (%) of Low Spike (0.3 ppm)	Average Recovery (%) of High Spike (5 ppm)
Esters		
Ethyl Acetate	N/A*	104 ± 10
Ethyl Butyrate	102 ± 5	95 ± 5
Isoamyl Acetate	96 ± 6	93 ± 4
Ethyl Octanoate†	101 ± 7	93 ± 2
Alcohols		
2-Phenylethanol†	94 ± 2	94 ± 1
Phenolic Aldehydes		
Benzaldehyde†	100 ± 3	92 ± 1
Cinnamaldehyde	100 ± 4	92 ± 1
Vanillin†	92 ± 2	109 ± 1
Syringaldehyde	91‡	96 ± 4
Phenols		
m-Cresol	99 ± 6	97 ± 1
Guaiacol	94 ± 4	96 ± 1
m-Methoxyphenol	110 ± 0.5	101 ± 1

* Endogenous concentration in all samples were over 2-fold higher than the low spike (0.3 ppm)
† 0.5 ppm low spike as LoQ > 0.3 ppm
‡ Sample 3 excluded due to high endogenous level which affected recovery

3.4. Summary of Final Method

The GC-MS method allowed detection of the compounds present in commercial samples using standards and the NIST library. Two different sample introductions were compared and direct injection proved to be superior to SPME with the chosen analysis parameters. The method was

further optimised by changing the solvent delay, flow rate, split ratio and oven program to decrease analysis time and improve the detection of compounds.

Esters, phenolic aldehydes (e.g. vanillin) and wood extractives (furfural) are important compounds to detect and monitor in whiskey because they can have a positive or negative effect on the aroma and flavour depending on the concentrations at which they are present. Method validation was carried out and showed the ability to detect and quantify these functional groups. It was determined that the developed analysis method did not allow for accurate quantification (LoQ) of compounds below 0.1 ppm, however, the LoD (0.03-1.45 ppm) and LoQ (0.11 4.82 ppm) were acceptable for all compounds because the reported concentrations in literature are > 0.1 ppm. Good recoveries (92-110%) were achieved for all compounds. Therefore, the method was deemed fit for the purpose [11,28,37,63,64].

Chapter 4 - Analysis of Fresh Distillates and Commercial Whiskies

Whiskey is a complex mixture of compounds and analysis by several complementary GC methods would be required to determine a wide variety of the compounds present as demonstrated in Table 1.7 (page 34) and the collated table of different instrument parameters (column, injection type and detector) for whiskey analysis by Wiśniewska *et al.* These tables show a minimum of four methods are required to cover volatile compounds, sulphur compounds and compounds responsible for aroma and taste [6,12]. The developed GC-MS method (section 2.4) was used to analyse fresh distillates (prior to maturation) and commercial samples to determine the variety of compounds present in whiskies of various ages and their related concentrations. As previously mentioned (section 3.1) the focus was on esters, phenolic aldehydes and wood extractives because they are important compounds in whiskey [1,11,32,45,64]. Other compounds that were detected during analysis have been mentioned in the discussion (e.g. 1,1-diethoxyethane), however, only qualitative analysis of these compounds was carried out because no standards were available. The concentrations of identified compounds were compared to the rapid maturation experimental samples (Chapter 5) to determine the success of each ageing technique.

4.1. Analysis of Fresh Distillates

There is not a lot of published data on the composition of fresh distillate, and the data that is available varies between brands [105,119]. Therefore, analysis of the corn and malt distillates used during this research were required to determine what compounds were present and their concentrations prior to commencement of experiments.

4.1.1. Corn Distillate

Six compounds were detected in the corn distillate (40% EtOH). The compounds (summarised in Table 4.1) were identified using the NIST library (based on characteristic fragmentation patterns) as well as retention time matches to standards (if available).

The peak at 1.32 minutes had three possible compound identifications in the NIST library; pentane (86.27%), isobutane (86.16%) and isobutanol (84.03%). Isobutanol and pentane standards (2 ppm) were analysed to confirm retention times and identification; the analysis of

these standards was carried out at a later date because they were not available when the distillates and commercial samples were analysed. The retention times for isobutanol and pentane were 1.31 and 9.80 minutes, respectively. The NIST library identified the isobutanol standard as three possible compounds; pentane (81.5%), isobutane (85.6%) and isobutanol (84.2%). Comparison of the obtained mass spectrum for isobutanol to the spectrum in the NIST library (Figure 4.1) showed the peak at 1.31 minutes was highly likely to be isobutanol because the molecular ion (*m/z* 74) and ion fragmentation patterns matched. Isobutanol is also mentioned in literature as being present in whiskey whereas pentane is not [8]. Therefore, going forward the peak at 1.32 minutes was referred to as isobutanol (if > 75% match with NIST library).

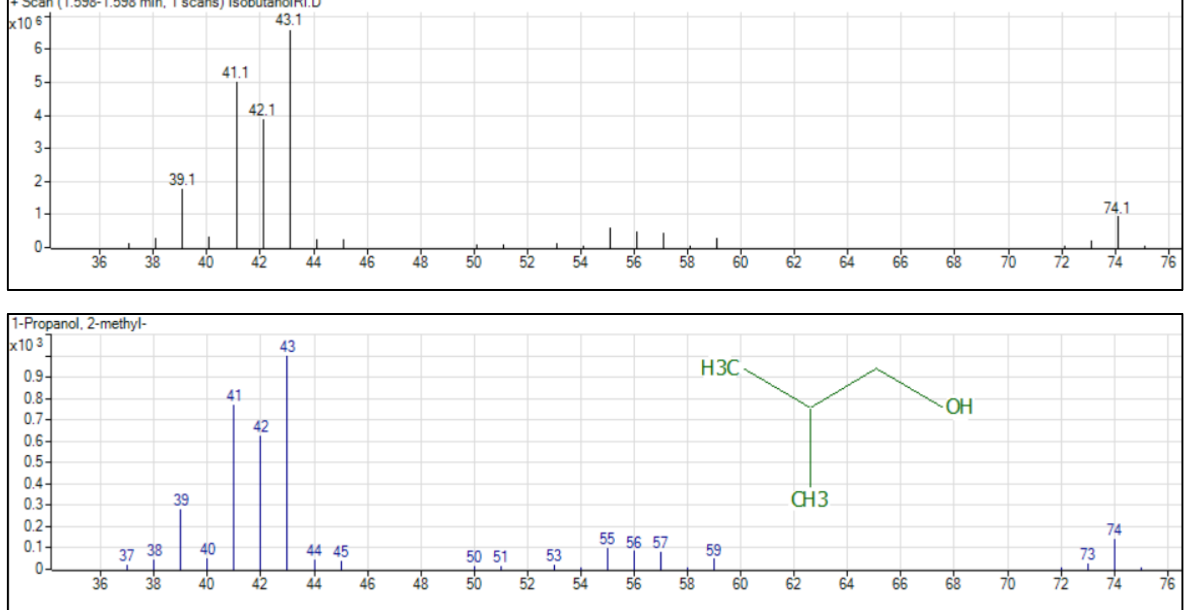

Figure 4.1: Top: Isobutanol standard (2 ppm) mass spectrum (*m/z* 35-76); Bottom: NIST library mass spectrum for isobutanol viewed in the Mass Hunter Quantitative Analysis software (*m/z* 35-76). The only difference between the mass spectra is the number of decimals, these differ between mass spectrometers and most round up to the nearest integer, the mass spectrometer used in this research did not round up [120].

All of the compounds detected in the corn distillate (Table 4.1) are reported in literature as being present in fresh distillate, with isoamyl alcohol generally having the highest concentration amongst the alcohols [8], which aligned well with the results obtained from analysis of the corn distillate. Jeleń *et al.* [105] reported an isoamyl alcohol concentration of ~739-823 ppm (40% ABV), this was considerably higher than the analysed corn distillate isoamyl alcohol concentration (14.30 ppm). The concentration differences for isoamyl alcohol between batches of distillate arise from the fermentation stage in the production of the distillate.

The amino acids that are present and their concentrations determine what higher alcohols are produced. Furthermore, temperature, pH and the amount of yeast used all affect the abundance of higher alcohols like isoamyl alcohol [21,28]. These differences highlighted the importance of analysing a range of commercial samples (section 4.2) to determine what concentration range of isoamyl alcohol (and other compounds) would be required to achieve a > 10-year-old whiskey. Ethyl acetate is reported as having the highest ester concentration, with other esters present at much lower concentrations, often < DL [8]; this could not be confirmed by the corn distillate analysis because ethyl acetate was < LoD (1.45 ppm). Ethyl decanoate was present at a concentration of 0.21 ppm. The concentration of ethyl acetate (< LoD) and ethyl decanoate were significantly lower than that which was reported by Jeleń *et al.* in corn distillates (10% ABV) [105] (2-492 ppm and 2-3 ppm, respectively).

Table 4.1: Compounds detected in corn distillate (40%) and their retention time (min), average concentration (ppm, $n = 3$) and standard deviation (ppm).

Compound	Retention Time (min)	Concentration (ppm)	SD (ppm)
Esters			
Ethyl Acetate	1.19	< LoD	–
Ethyl Decanoate	20.40	0.21	0.02
Alcohols			
Isobutanol*	1.32	–	–
Isoamyl Alcohol	2.75	14.30	1.45
2-Methyl-1-butanol	2.81	–	–
2-Phenylethanol	12.63	0.21	0.01

* Tentatively identified using NIST library as no standard available at time of analysis
No standard for quantification denoted with "–"

The concentration of 2-phenylethanol (0.22 ppm) in the corn distillate could not be compared with literature. Heinz and Elkins [119] detected 2-phenylethanol but they did not report any concentrations and Jeleń *et al.* [105] did not report detection of 2-phenylethanol. 2-methyl-1-butanol was also detected, however, no standard was available for quantification to allow comparison with literature.

4.1.2. Malt Distillate

A malt distillate manufactured by the client was analysed to determine what compounds were present prior to any age treatment. Table 4.2 summarises the compounds and the concentrations at which they were detected. Most of the compounds detected in the malt distillate were the same as the corn distillate, however, 2-phenylethanol was not detected in the malt distillate despite the reported detection in some fresh distillates [8]. None of the compounds present in

the malt distillate could be compared to literature as there is no published data for the concentrations of these compounds in fresh malt distillate. Although, their presence in fresh distillate is well known because they arise from fermentation and are desired in the final product because they impart floral and fruity aromas [20,28]. The factors that affect the obtained distillate are the yeast type, starch hydrolysis conditions (temperature and pH) as well as the fermentation and distillation conditions [8].

Table 4.2: Compounds detected in malt distillate (65%) and their retention time (min), average concentration (ppm, n = 3) and standard deviation (ppm).

Compound	Retention Time (min)	Concentration (ppm)	SD
Esters			
Ethyl Acetate	1.19	10.62	0.39
Isoamyl Acetate	5.87	0.66	0.02
Ethyl Octanoate	15.06	0.12	0.01
Ethyl Decanoate	20.39	0.40	0.02
Ethyl Laurate	25.21	0.20	0.01
Ethyl Hexanoate	9.31	–	–
Isobutyl Acetate	3.64	–	–
2-Methylbutyl Acetate	6.12	–	–
Alcohols			
Isobutanol	1.31	–	–
Isoamyl Alcohol	2.74	15.50	1.18
2-Methyl-1-butanol	2.81	–	–

No standard for quantification denoted with "–"

4.2. Analysis of Commercial Whiskey Samples

A collection of 53 samples comprising of Scotch whiskey (33 samples), American Whiskey (11 samples) and other whiskey (9 samples), including one from New Zealand, were analysed by GC-MS (fullscan) using the parameters outlined in section 2.4.

4.2.1. Qualitative Analysis

The qualitative results obtained were used to determine the presence of common compounds present in whiskey (Appendix H, Table H.1, page 188); any compound identified above the threshold ($> 4\times10^4$) was counted as being present in the sample. These results provided a framework for the compounds which required monitoring (section 4.5) during rapid maturation experiments and would allow comparison to experimental samples. Various sugar compounds (e.g. levoglucosan (1,6-anhydro-β-D-glucopyranose) and melezitose) were detected in only

three samples; the peaks were small ($< 2\times10^4$ threshold) and the match to the NIST library was low (< 75%), therefore, these have been omitted from discussion.

There were eight compounds present in the majority of samples (if not present in all samples) and these are listed here in order of highest frequencies with the percentage of samples ($n = 51$) containing the compound in brackets; isoamyl alcohol (100%), 2-methyl-1-butanol (100%), acetic acid (100%), 2-phenylethanol (84%), ethyl acetate (78%), isobutanol (76%), furfural (75%) and 1,1-diethoxy-ethane (65%). The most noticeable difference between samples with age statements was the number of compounds detected. In general, the older Scotch whiskies (> 12 years old) had 2-10 additional compounds present compared to younger Scotch whiskies (≤ 12 years old). For example, 7 compounds were identified (threshold $> 5\times10^4$) in the chromatogram obtained for a 12-year-old Scotch whiskey (Figure 4.2) and 19 compounds were identified (threshold $> 5\times10^4$) in the chromatogram obtained for a 15-year-old Scotch whiskey (Figure 4.3) using standards (retention time matches) and/or the NIST library.

The > 12-year-old Scotch whiskey samples differed in compound composition to samples with no age statements. For example, Jack Daniels and Jamieson only had some of the most common compounds present (ethyl acetate, acetic acid, isoamyl alcohol, 2-methyl-1-butanol and 1,1-diethoxyethane). In addition to the eight most common compounds present in all samples, the 15-year-old Scotch sample contained phenethyl acetate, $C_6H_{12}O$ (formula suggested by NIST), and decanoic acid amongst others. The two most likely reasons for the difference in the specific compounds and the number of compounds present between the aged Scotch and no age statement samples was because of the starting materials used and/or the ageing time. Corn is used in American whiskey (e.g. Jack Daniels) and the whiskey tends to be aged for 3-4 years (the minimum age time). Whereas in Scotland malt is used and ageing is generally no less than 7 years despite the minimum ageing time being three years [21].

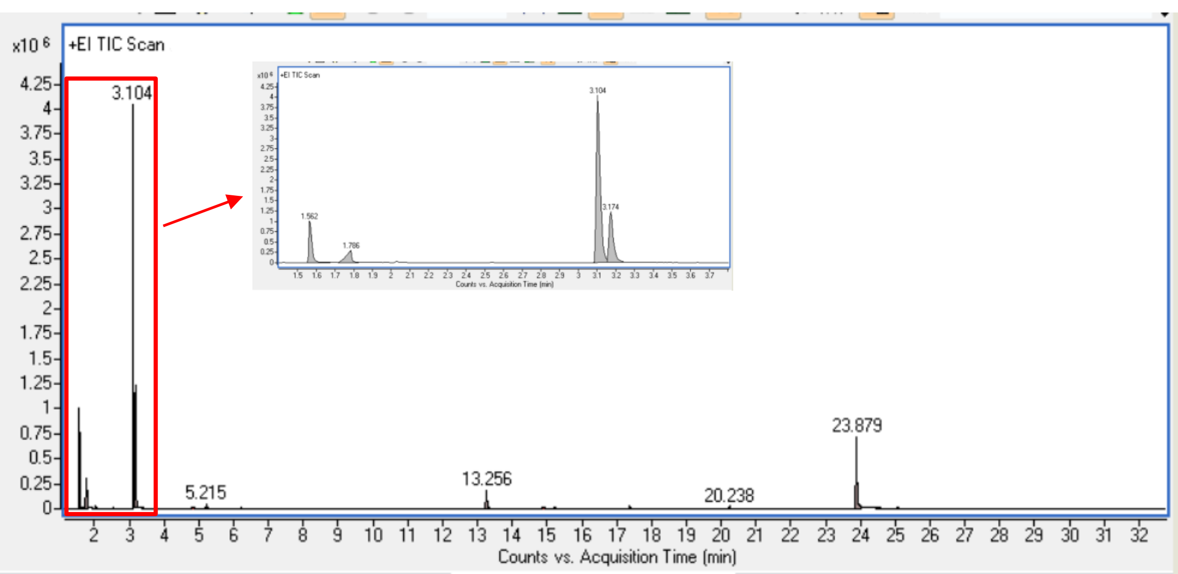

Figure 4.2: Chromatogram obtained for a 12-year-old Scotch whiskey. Compounds identified were: isobutanol (1.56 min), acetic acid (1.78 min), isoamyl alcohol (3.10 min), 2-methyl-1-butanol (3.17 min), furfural (5.21 min), 2-phenylethanol (13.26 min), *n*-decanoic acid (20.24 min) and 1-naphthol (ISTD, 23.88 min), zoomed insert for compounds in red box (0 – 3.8 min).

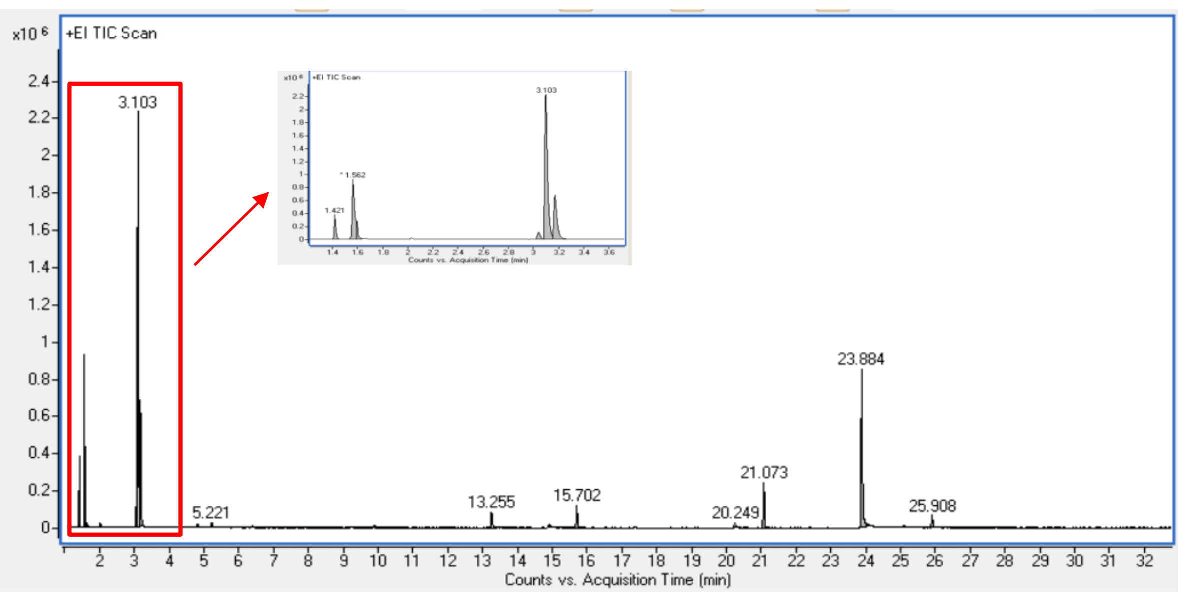

Figure 4.3: Chromatogram obtained for a 15-year-old Single Malt Scotch Whiskey. Compounds identified were; ethyl acetate (1.42 min), isobutanol (1.57 min), acetic acid (1.64 min), $C_6H_{12}O$ (2.04 min), 1,1-diethoxyethane (3.04 min), isoamyl alcohol (3.11 min), 2-methyl-1-butanol (3.18 min), ethyl butyrate (4.48 min), 2-hydroxyethyl propionate (4.79 min), furfural (5.22 min), $C_6H_{14}O$ (6.22 min), isoamyl acetate (6.38 min), ethyl hexanoate (9.89 min), 2-phenylethanol (13.25 min), octanoic acid (14.91 min), diethyl succinate (15.24 min), ethyl octanoate (15.70 min), phenethyl acetate (17.37 min), *n*-decanoic acid (20.24 min), ethyl decanoate (21.07 min), 1-naphthol (ISTD, 23.88 min) and ethyl laurate (25.91 min), zoomed insert for compounds in red box (0 – 3.7 min).

Two of the acquired commercial samples (Jack Daniels Cinnamon Fire and Sortilege) were not true whiskies because they had liqueur/syrup added. Over 20% of the compounds 'identified' in the Jack Daniels Cinnamon Fire sample were sugars which was most likely because of the cinnamon liqueur added to Jack Daniels No.7 whiskey. This produced a distorted baseline and poor resolution within the chromatogram which made compound identifications unreliable. The Sortilege Canadian Whiskey + Maple Syrup sample also produced a distorted baseline and poor resolution within the chromatogram (Figure 4.4), this was thought to be due to the addition of maple syrup. Maltol which gives whiskey a sweet burnt caramel aroma [28] (can be undesirable), was detected along with several other sugars. Based on the poor chromatography and not being true whiskies, the Jack Daniels Cinnamon Fire and Sortilege samples were removed from the summary table (Appendix H, Table H.1, page 188) and were not included in any statistical analysis.

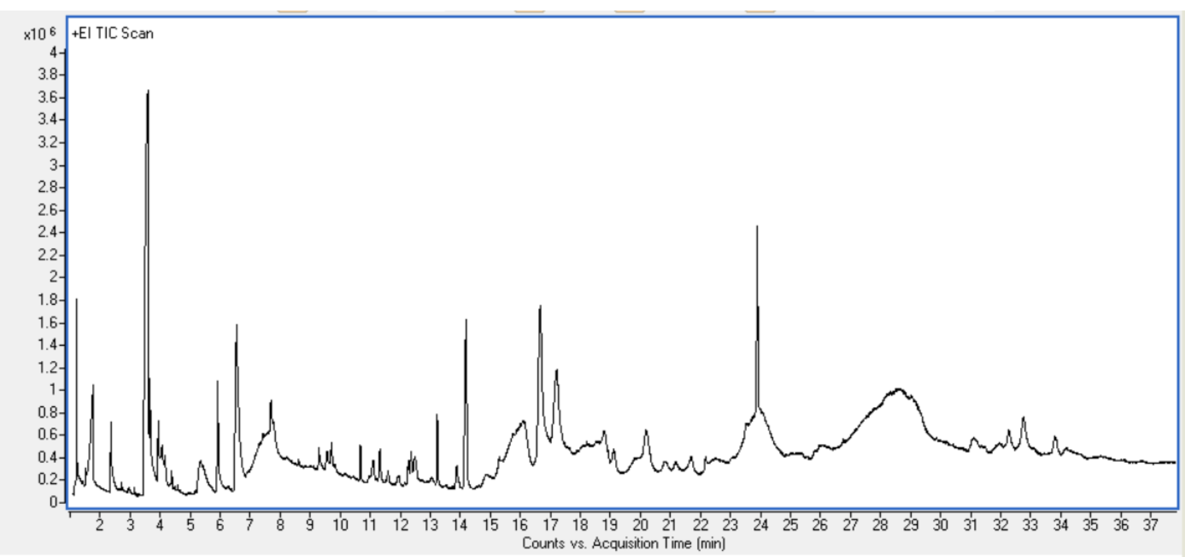

Figure 4.4: Chromatogram obtained for Sortilege Canadian Whiskey + Maple Syrup sample showing the effect of maple syrup addition on the baseline. A distorted baseline and broad peaks within the chromatogram made compound identification unreliable and qualitative and quantitative analysis difficult.

Most samples had at least one other ester present besides ethyl acetate, with many of them having more than two additional esters. The additional esters detected and the number of samples that they were present in (%) were: ethyl decanoate (57%), ethyl octanoate (49%), phenethyl acetate (46%), ethyl laurate (39%), isoamyl acetate (37%), diethyl succinate (31%), 2-hydroxyethyl propionate (29%) and ethyl hexanoate (27%). Octanoic and decanoic acids (precursors to ethyl octanoate and ethyl decanoate, respectively) are generally more abundant

in the distillate compared to butyric acid (precursor to ethyl butyrate). This explained why ethyl butyrate was only present in 8% of samples compared to the higher fatty acid esters (C_6-C_{12}) [63,64]. It was noted that many of the samples that contained ethyl decanoate also contained ethyl octanoate and ethyl laurate which further supports the reason for the greater presence of higher fatty acid esters.

Only a small number of samples (6%) had 2-methylbutyl acetate present. The absence of 2-methylbutyl acetate in commercial samples was unexpected because 2-methyl-1-butanol was present in 100% of the samples and reacts with acetic acid to form 2-methylbutyl acetate [9,50]. The formation of some esters is determined by the availability of the relevant higher alcohol, therefore, because isoamyl alcohol is present at approximately three times that of 2-methyl-1-butanol [63,121], it was expected that the presence of isoamyl acetate (37%) would be higher than 2-methylbutyl acetate (6%). The ratio was lower than expected which was thought to be because of the detection limit; which was postulated to be relatively high [28].

Phenolic aldehydes (e.g. vanillin) and wood extractives (e.g. furfural) are also important because they contribute to the final flavour and aroma of the whiskey [11,32].Vanillin and syringaldehyde were only detected in 35% and 37% of samples respectively, while furfural was detected in 75% of samples. Vanillin and syringaldehyde are desirable because they contribute to the overall flavour and aroma of whiskey and are also used as markers (quality, authenticity and age) [27,32,46,79,122]. Furfural is formed from some grains and also extracted from wood; the concentration increases with age and gives a marzipan flavour and aroma as well as an amber colour (due to polymerisation) which is desired in whiskey. Furfural and 5-hydroxymethylfurfural (5-HMF) are also used as age markers [27,28,122]. The disadvantage of using both of these compounds as age markers is that they can be present from the addition of caramel colouring (E150A), which is the only additive allowed in most whiskies [12,27,28,123].

4.2.2. Quantitative Analysis

The results for quantitative analysis of 15 compounds in 51 commercial whiskey samples are summarised in Table 4.3. The mass spectra for the cresols (*o*-, *p*- and *m*-) and retention times are almost identical; the fragmentation patterns have subtle differences which made it difficult for the NIST library to determine the position of the methyl group with great certainty and the researcher could not distinguish which of the cresols were most likely because they are all

present in whiskey [52]. Therefore all cresol stereoisomers detected in samples were combined as a total frequency present in samples.

Table 4.3: Summary statistics (ppm) for compounds quantified in the commercial samples analysed (n = 51).

Compound	Frequency reported in samples (%)*	Average (ppm) ± SD	Min (ppm)	Max (ppm)
Esters				
Ethyl Acetate	78	2.03 ± 0.44	1.48	2.71
Ethyl Butyrate	8	< LoD	< LoD	< LoD
Isoamyl Acetate	37	< LoD	< LoD	< LoD
Ethyl Octanoate	49	0.14†	0.14	0.14
Ethyl Decanoate	57	0.10 ± 0.03	0.06	0.16
Ethyl Laurate	39	0.08 ± 0.05	0.04	0.21
Alcohols				
Isoamyl Alcohol	100	6.25 ± 4.53	1.09	23.00
2-Phenylethanol	84	0.28 ± 0.13	0.11	0.79
Phenolic Aldehydes				
Benzaldehyde	N/D			
Cinnamaldehyde	N/D			
Syringaldehyde	37	0.46 ± 0.13	0.24	0.70
Vanillin	35	0.32 ± 0.02	0.28	0.34
Phenols				
Cresol (*m*-, *o*-, *p*-)	12‡	0.14†	0.14	0.14
Guaiacol	8	< LoD	< LoD	< LoD
m-Methoxyphenol	8	< LoD	< LoD	< LoD

N/D Not detected

* Calculated frequency based on qualitative results reported in Appendix H, Table H.1, page 188

† Only one sample, no standard deviation

‡ Cresol stereoisomers combined %

The average ethyl acetate concentration in the analysed commercial samples (2.07 ppm) was considerably lower than that which is reported in literature. Ethyl acetate accounts for > 50% of the ester concentration in spirits. It gives a vinegar note at high concentrations, however, at low concentrations, it aids in reducing the 'burn' associated with fresh distillate [1,9,45,64]. There is no clear concentration at which ethyl acetate is considered too high, with various limits reported in the literature. For example, Lee *et al.* [53] stated an ethyl acetate concentration > 175 ppm was considered "solvent-like". The odour threshold for ethyl acetate in air is 3.9 ppm [28], however, concentrations well above the odour threshold have been reported in whiskey. For example, a whiskey has a general ethyl acetate concentration range of

100-460 ppm; the concentration in American and Scotch whiskies has been reported as 144-716 ppm and 44-336 ppm, respectively [20,28,63]. The reason for this variability is that the yeast, temperature and pH used affects how much ethyl acetate is produced and carried over into the fresh distillate. In addition, the maturation conditions contribute to the ethyl acetate variation because it is also formed during the maturation process [28,63].

Ethyl butyrate was < LoD (0.41 ppm), however, ethyl butyrate has been reported at concentration of 1.68-1.86 ppm in rye whiskies (Wild Turkey and Rittenhouse). As previously mentioned (section 4.2.1), ethyl butyrate is not as abundant as higher chain esters such as ethyl octanoate and ethyl decanoate [20,28]. Therefore, the most likely cause for the absence of ethyl butyrate in commercial samples was because of the high limit of detection (0.41 ppm) and quantification (1.37 ppm).

The average isoamyl alcohol concentration for the analysed commercial samples was 6.25 ppm. This was much lower than what is reported in literature [20,28,64,105]. For example, González-Arjona *et al.* [124] analysed 58 samples consisting of Scotch, American and Irish whiskies and reported that the concentration range for isoamyl alcohol was 140-950 ppm. The maximum concentration of isoamyl alcohol detected in the analysed commercial samples was 23 ppm, which was close to the reported concentration (24 ppm in 40% ethanol) by Zhao *et al.* [65].

The average concentration for 2-phenylethanol in commercial samples was 0.28 ppm and the highest concentration was 0.79 ppm (Booker's bourbon, 6-8 year-old). These concentrations were lower than what has been reported in literature. For example, 5.18 ppm in Rittenhouse Rye whiskey, 20.10 ppm Wild Turkey whiskey, 10.61 ppm in Laphroaig single malt Scotch whiskey, 2.60 ppm in a general 40% ABV spirit and 2-80 ppm in 23% grain whiskey [28]. The low concentration of 2-phenylethanol is expected because it decreases with age despite coming from multiple sources (peated malt, produced by yeast and extracted from the barrel) [28].

Benzaldehyde was not detected in any of the analysed samples, however, it has previously been reported in whiskey at 0.07 ppm [63,65] and is found in peated malt, seasoned wood and fresh distillate [9,28,63,125]. It was also reported by Balcerek *et al.* [8] in distillates using different starting materials for fermentation (e.g. unmalted rye and wheat malt). The most likely reason benzaldehyde was not detected in the analysis of commercial samples was because the LoD (0.2 ppm) and LoQ (0.68 ppm) were higher than reported concentrations in the literature.

In a study by Conner *et al.* [126], the influence of new oak barrels versus re-used barrels on the maturation of whiskey was tested for 6 and 48 months of ageing. The results showed that the levels of benzaldehyde were similar for the fresh and re-used barrels at both storage times. The authors showed there was a small change over time and suggested the majority of the benzaldehyde came from the fresh distillate. However, the collection of the heart fraction (desired fraction) is stopped at 94-96 °C depending on the desired %ABV and the boiling point of benzaldehyde (179 °C) is higher hence it is unlikely to be carried over into the fresh distillate [19,50].

Guaicols (2-methoxyphenol and 3-methoxyphenol) and cresols (*m*-, *o*- and *p*-) were only detected in 8% and 12% of samples respectively. Their presence was noted in all Islay single malt Scotch whiskey samples ($n = 7$) which comes from the drying of malt on peat fires [11,28].

4.3. Principal Component Analysis of Commercial Samples

Principal component analysis (PCA) is a non-linear form of untargeted discriminant analysis and is used to simplify large datasets that contain many interrelated variables while maintaining as much variation as possible. This is achieved by creating new uncorrelated variables called principal components (PC) which are listed in order of the highest variance (spread of data) within the original variables [127,128]. PCA was carried out on 51 samples (excluding Jack Daniels Cinnamon Fire and Sortilege – Canadian Whisky + Maple Syrup) to observe groupings and see the differences in compound profiles for whiskies of different ages and country of origin. These plots were used to compare with rapid maturation experiment samples to determine whether the same profile as a > 10-year-old whiskey sample had been achieved (section 5.6). The compounds chosen to include in the PCA plot were based on the detected frequency in commercial samples and/or their importance within literature (e.g. syringaldehyde). The compounds chosen for PCA were made up of quantitative and qualitative data to increase the separation of samples by inclusion of a variety of compounds. If a compound was < LoD or was not detected in a sample a zero value was used. The quantitative compounds used with the frequency found in the commercial samples analysed denoted in brackets were: isoamyl alcohol (100%), 2-phenylethanol (84%), ethyl acetate (78%), ethyl decanoate (57%), ethyl laurate (39%), syringaldehyde (37%) and vanillin (35%). The qualitative compounds used with their frequency within commercial samples in brackets were; acetic acid (100%), 2-methyl-1-butanol (100%), isobutanol (76%), furfural (75%), 1,1-diethoxyethane (65%), octanoic acid (51%) and phenethyl acetate (46%). The PCA matrix

type was set to correlation matrix to standardise the data [127] because both concentrations (ppm) and peak areas were used.

Initially, PCA analysis was carried out on all samples with age statements. Samples were categorised by age to observe which compounds influence the separation and provide insight into which compounds should be focused on in maturation experiments as the aim of the research was to rapidly mature a whiskey with a chemical profile similar to a ≥ 10-year-old whiskey. For the PCA calculations the samples were sorted into three groups based on their age, regardless of whether they were Scotch or American whiskey (Table 4.4).

Table 4.4: Samples grouped by age, the key used in PCA analysis and the number of samples per age group.

Age Statement (Years)	Key	No. of samples
4-8	B	4
10-12	C	18
> 12	D	5

No outliers were detected within the sample set. The first two PCs explain 50% of the variation in the data (PC1 = 28.4% and PC2 = 21.9%), PC3 explained 10.8% of the variation and the remaining 38.9% could be explained by PC4-12. The score plot (Figure 4.5) showed no separation between the group C (10-12 years old) and D (> 12 years old) which suggested only subtle changes occurred in samples ≥ 10 years old. Samples 1 and 2 did not fit within their relevant groupings in the score plot, initially thought to be because of the higher %ABV (50.5%) of both samples compared to the usual 40%. Moreover, sample 19 was the same brand (Wild Turkey 101) as sample 1 but did not identify in the same region of the score plot. Samples were collected through citizen science and the method of sub-sampling was not standardised. Furthermore, it was unknown how long bottles had been open for or the conditions in which they were stored prior to sub-sampling; some of the more volatile compounds may have been lost. This could have contributed to the spread in compound concentrations/peak areas in the samples and hence the spread seen in the PCA.

Group B contained samples aged 4-8 years old which were broadly clustered together in the score plot. The difference in %ABV (37-63.7%) could contribute to the broad clustering because all the samples were of American origin (Figure 4.6). Sample 18, 19 and 36 from group B were separated from sample 1 (group B) on the score plot. Review of the loading plot (Figure 4.7) showed none of the compounds strongly influenced the first or second principal

components; eigenvectors were < 0.30 or > -0.30 for all compounds except acetic acid (PC2 $= -0.47$) and isoamyl alcohol (PC2 $= -0.44$).

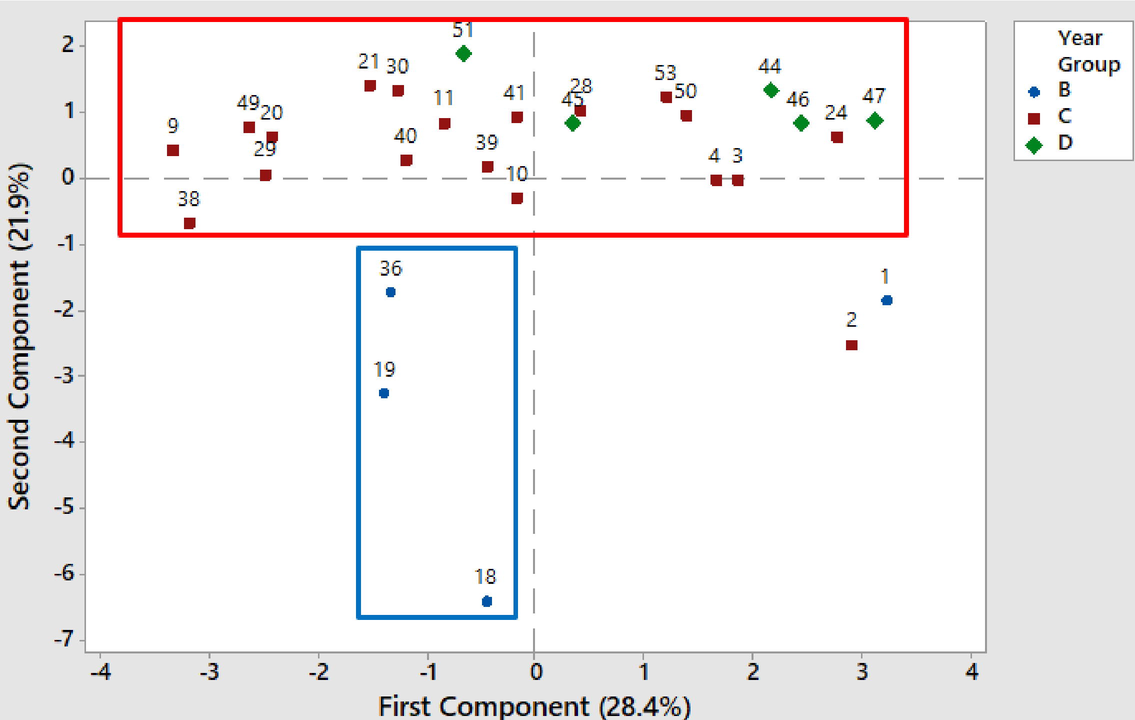

Figure 4.5: Score plot for commercial whiskies categorised by age statement. The samples in group C (10-12 years old) and D ($>$ 12 years old) were clustered together (red square) suggesting subtle changes occur for samples $\geq$ 10 years old. The group B samples were dispersed (blue square) which was thought to be because of the limited sample numbers in group B. Samples 1 and 2 did not fit into either cluster which could have been due to the higher %ABV (50.5%) of both samples or the sample collection procedure employed.

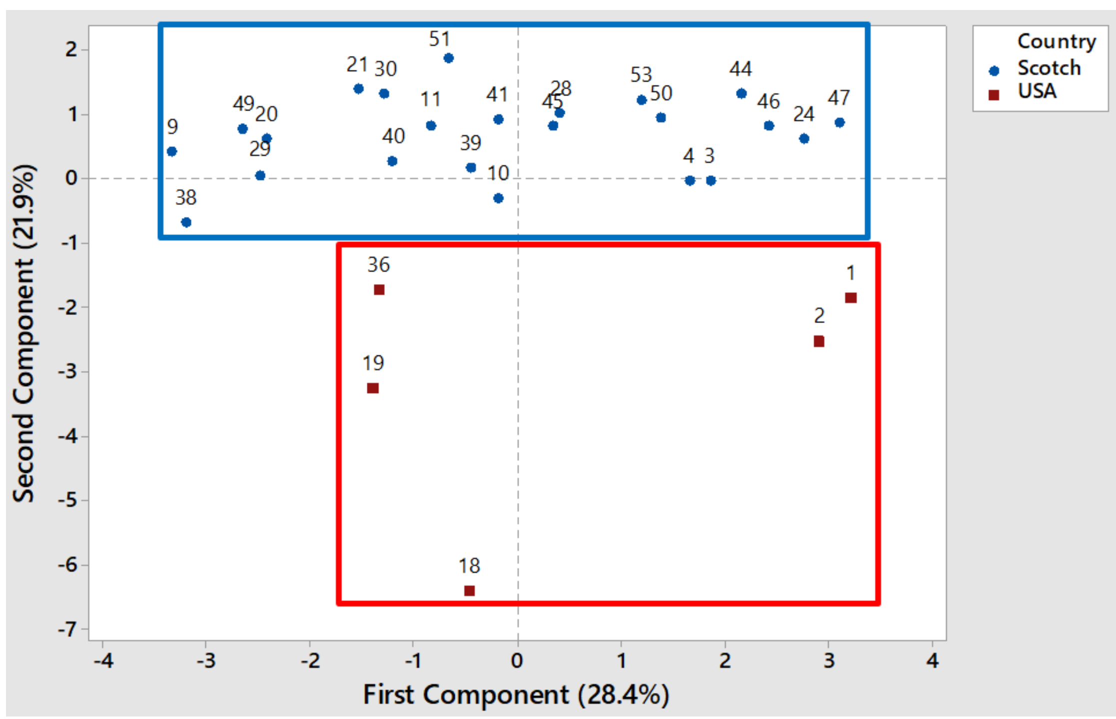

Figure 4.6: Score plot for commercial whiskies categorised by country of origin. The samples from Scotland (blue circles) were all clustered together, whereas the American (maroon squares) samples were more dispersed.

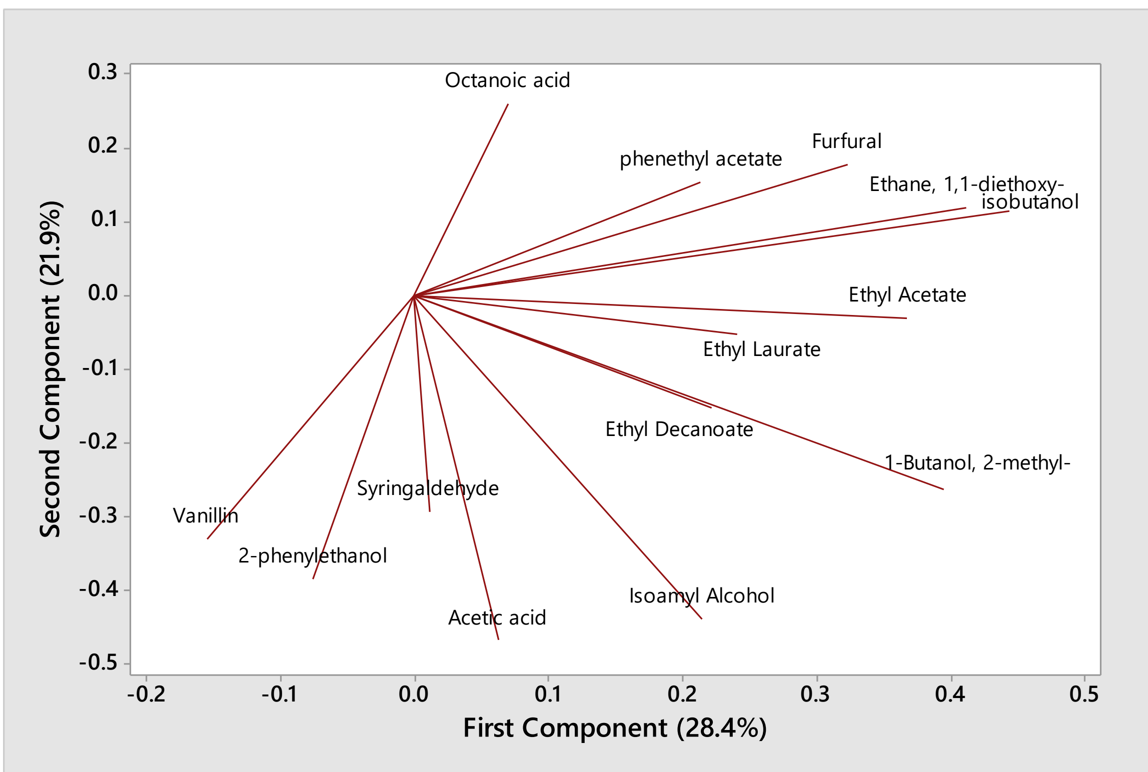

Figure 4.7: Loading plot for commercial whiskey samples with age statements. Most compounds had an influence on PC1 and PC2, however, none of the compounds were close to −1 or 1 so did not have a strong influence on the first or second component.

4.3.1. Scotch Whiskey by Age

PCA was carried out on Scotch samples with age statements to observe the spread of samples based on age. There were no outliers and the samples in group C (10-12 years old) were dispersed, whereas the samples in group D (> 12 years old) were clustered together (Figure 4.8). The loading plot (Figure 4.9) indicated which compounds (and their relevant concentrations/peak areas) were responsible for the group D clustering and these were focused on during the maturation experiments. In the score plot there were a few samples from group C which were in the group D clustering. Sample 10 (Laphroaig), 53 (Ardbeg) and 50 (Jura) are all produced on islands (Islay and Jura) and the remaining three group C samples which were in the group D clustering are produced in the Highlands.

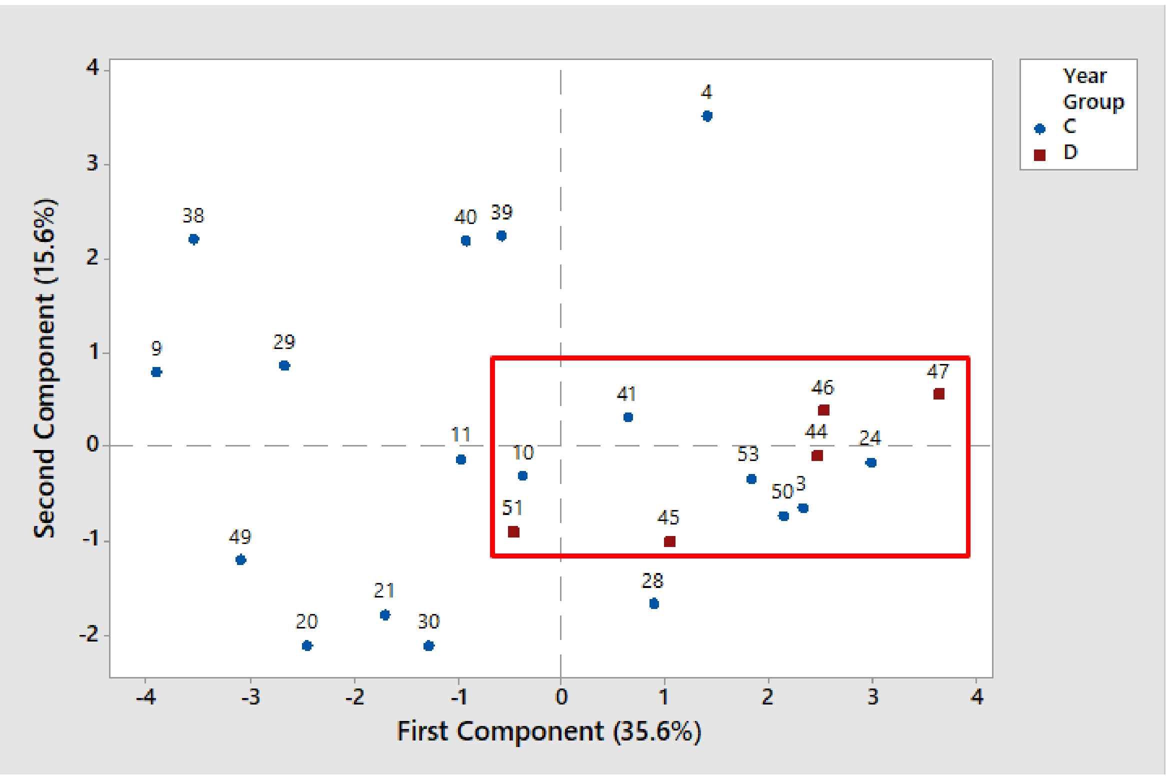

Figure 4.8: Score plot of Scotch whiskies with age statements. Group C (10-12 year-old samples) was more dispersed than the group D (> 12 year-old samples) suggesting samples > 12 years old are more refined in the composition of compounds and their relevant concentrations/peak areas.

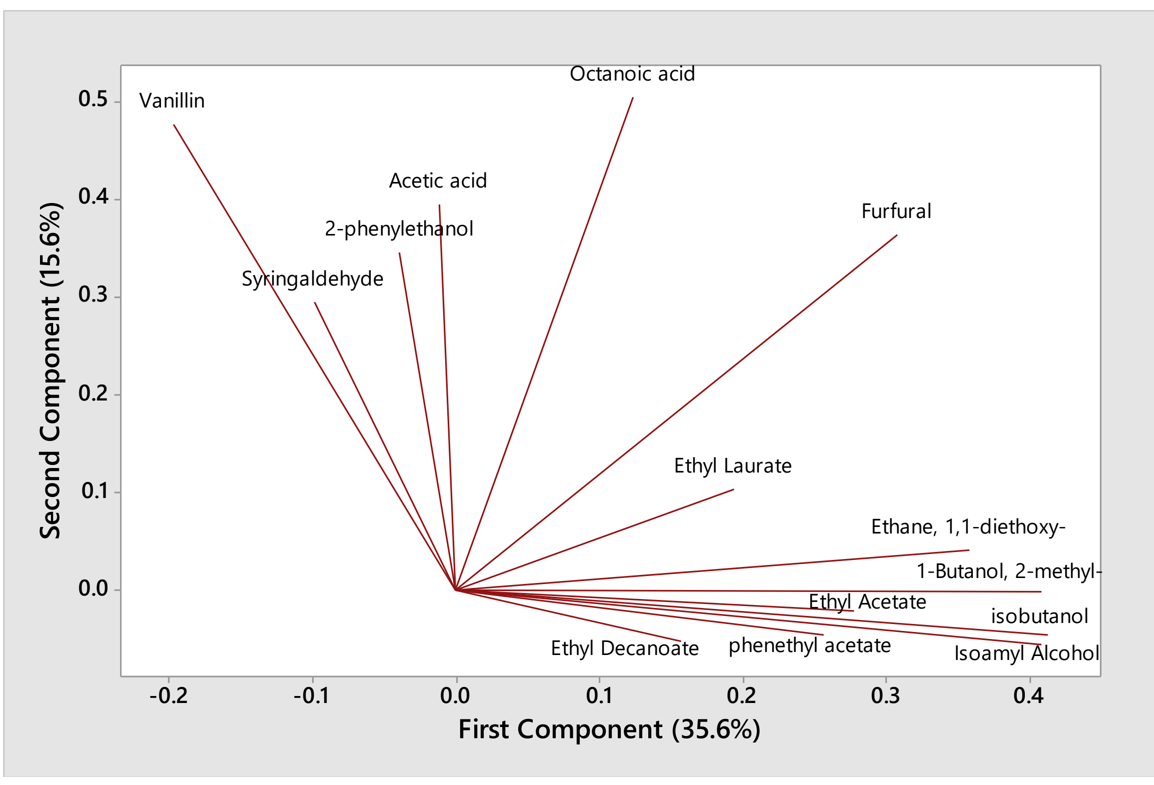

Figure 4.9: Loading plot for all Scotch samples with age statements. The compounds in the lower right corner were responsible for the clustering of the group D samples.

Another PCA was carried out using all the Scotch samples, including the no age statement samples to see which defined age cluster the no age statement samples aligned with. The first two principal components were not able to separate 10-12 year-old samples from > 12-year-old samples (Figure 4.10). As expected, the no age statement samples were scattered, however, many fell within the group C clustering (red square) on the score plot (Figure 4.10) and one sample (K5) was in the group D clustering (green square). Sample 4 and 6 were separated away from the other samples and this was most likely due to their production because there were no outliers in the outlier plot. Sample 4 (Bunnahabhain) was distilled in small batches and sample 6 is a Legacy edition (1815) Laphroaig which was aged in new bourbon barrels and new European oak hogshead barrels whereas Scotch whiskey is generally aged in used barrels [6,11]. The loading plot (Figure 4.11) showed several compounds including 2-methyl-1-butanol and isobutanol influenced PC1, however, there were no compounds that strongly influenced PC1 and PC2 (eigenvectors all < 0.50 and > − 0.50).

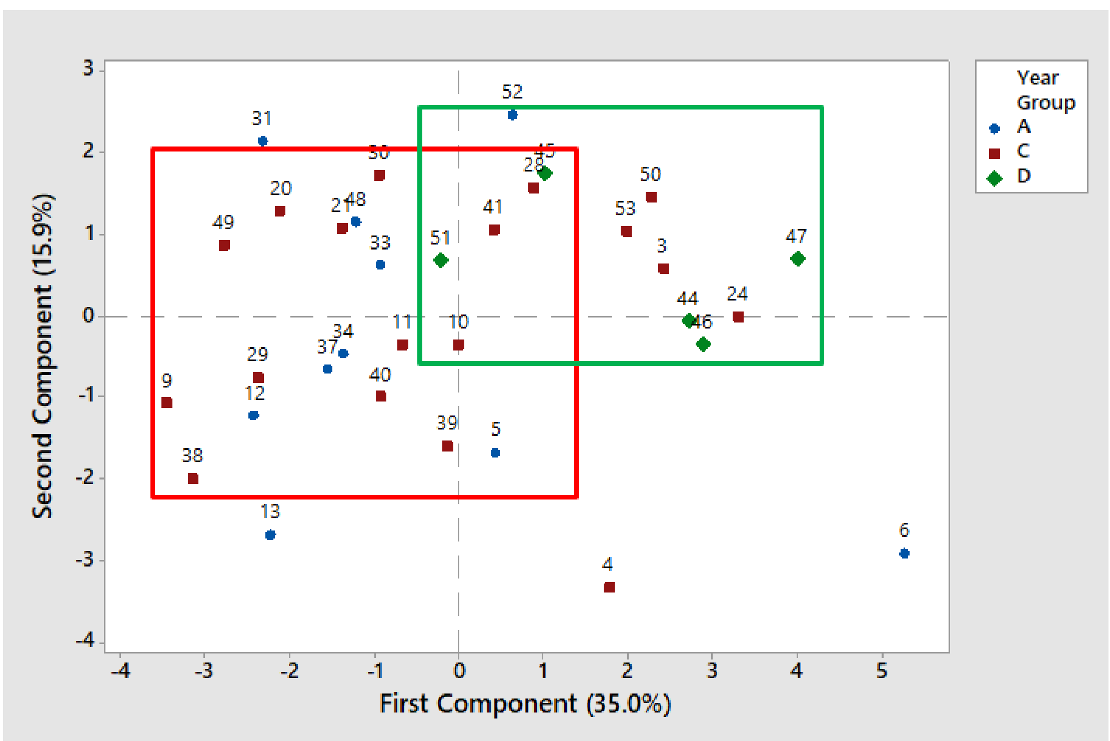

Figure 4.10: Score plot for Scotch samples grouped by age statement. An overlap was seen between group C (10-12-year-old samples, maroon squares) and D (> 12-year-old samples, green diamonds) which showed they have some similarities, however, group C were more dispersed. Sample 4 and 6 did not fit into any clusters most likely because sample 4 was small batch distilled and sample 6 is a Legacy edition (1815) that was aged in first-fill bourbon barrels and new European oak hogshead barrels.

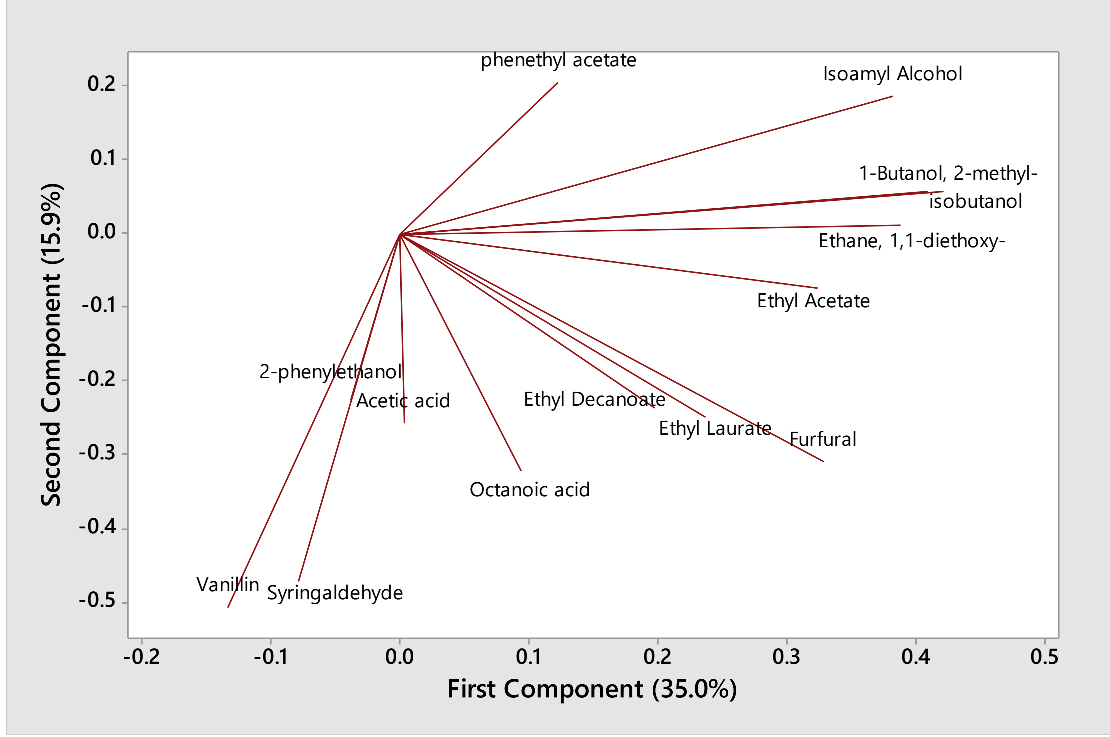

Figure 4.11: Loading plot showed several compounds influenced PC1 and PC2, however, none of them strongly influenced PC1 and PC2.

4.4. Linear Discriminant Analysis of Commercial Samples

Linear discriminant analysis (LDA) is a linear classifier tool which looks for independent variables relative to a dataset that best separate two or more groups. The independent variables which give the largest differences are used to create a 'model' which can be used on new observations to determine to which group they belong [129]. LDA was carried out on 27 samples to see whether there were clear differences between Scotch and American whiskies. The compounds used to perform LDA were the same compounds used in PCA (section 4.3).

The first LDA was carried out using samples with Scottish and American origin to see how successfully the two groups could be separated. There was a clear difference (100%) between the Scotch and American whiskies (Appendix I, page 190) which was expected because of the different base ingredients used to make Scotch (malted barley) and American whiskey (corn, rye, wheat and barley) [21,28,130]. A second LDA was performed on samples to see whether there were any clear discriminants between different age groups using the same key listed in Table 4.4 (section 4.3). Despite the different base ingredients (corn, malt, wheat, rye), the compounds present in the different distillates have the same reactions occurring during maturation (e.g. ethyl acetate increases over time). Hence all age statement samples were used, regardless of the country of origin to investigate if samples could be categorised by age. The proportion of correct age group assignments was 93% ($n = 28$). Two samples did not fit within their assigned group, a 12-year-old Glenfiddich and 14-year-old Balvenie, the predicted groups were > 12 years old (D) and 10-12 years old (C), respectively.

The no age statement samples were added into the age dataset and the 'predict group membership' function in Minitab was used to estimate the age of these whiskies. Of the 23 no age statement samples, 74% were predicted to be part of a particular group with > 75% probability. All but one of the Scotch samples were predicted to be 10-12 years old; this assignment matched information in the literature which states that whiskey produced in Scotland is generally aged for ≥ 7 years [21]. The one exception was Talisker Skye (sample 48) which was assigned with a 50:40 probability between 10-12 years old and > 12 years old suggesting the chemical profile was on the border of a 10-12 year-old profile and > 12 years old profile. The calculated group probabilities and the overall group assignments for the remaining 26% of samples is summarised in Table 4.5, the probability was generally split (50:40) between two groups, B and C or C and D.

Table 4.5: Calculated group probabilities and the overall group assignments for several samples ($n = 6$).

Sample ID	Country	Predicted Group	Group Probability B (4-8 years old)	Group Probability C (10-12 years old)	Group Probability D (> 12 years old)
17	Other	D	0.000	0.466	0.534
23	Other	D	0.000	0.427	0.573
26	Other	D	0.000	0.398	0.602
27	USA	B	0.506	0.009	0.485
32	USA	B	0.655	0.345	0.000
48	Scotch	C	0.000	0.583	0.417

The probabilities were relatively even for sample 17 (Jameson, triple distilled whiskey), 23 (Canadian Club, blended Canadian whiskey) and 26 (Canadian Club). For these samples, a limited number of compounds were detected which may be due to the prior treatment of samples before they were received. This may have affected the ability to discriminate. Samples 27 (Jim Beam) and 32 (Jack Daniels) were likely to be part of group B (4-8 years old) because whiskey is required to be aged for a minimum of three years and bourbon for a minimum of two years in America and is generally only aged for 3-4 years [9,21].

4.5. Desirable Compounds in Rapidly Matured Whiskey

The compounds detected in the corn and malt distillates (section 4.1) as well as in the commercial samples (section 4.2) revealed what common compounds are present in different whiskies and their concentration ranges. The aim of the research (section 1.8) was to develop a method to rapidly mature whiskey so that it has a similar chemical profile to a ≥ 10-year-old whiskey. Therefore the compounds and concentrations detected in samples ≥ 10 years old were used as indicators (Table 4.6) for the compounds required to be present in the rapid maturation experiment samples (Chapter 5). These indicators were also used to gauge how successful each ageing technique was at forming these compounds in the relevant concentration range. The concentration range of the analysed commercial samples was used because of the large variation of chemical profiles between the obtained results and literature. Furthermore a similar profile to a ≥ 10-year-old whiskey was desired, however, as reported, the profile (compounds present and their concentrations) varies between whiskies. Several other compounds were identified as important due to their presence in > 10 years old whiskies, however, standards were not available for 2-methyl-1-butanol, 1,1-diethoxyethane, $C_6H_{12}O$, furfural, ethyl hexanoate, phenethyl acetate and isobutanol so qualitative analysis was carried out. Due to the

broad peaks when analysing acids on the column, qualitative analysis was also carried out for acetic acid. It is noted that acetic acid is the main acid extracted from wood, however, there are many other acids which are also extracted including; octanoic, *n*-decanoic, lauric, *n*-butyric and hexanoic acid [28]. The detection of these compounds using the developed method was possible, however, no quantitative analysis was carried out. There are > 12 acids present in whiskey [28,37] but only three acids (acetic acid, octanoic acid and decanoic acid) were detectable with the column used in this research. The acids had broad peaks and the column used was not compatible with routine acid analysis, hence to protect the column only qualitative analysis was carried out for any detected acids to avoid degradation of the column from routine standard analysis.

Principal component analysis and linear discriminant analysis displayed the similarities and differences between commercial samples of different ages. PCA provided insight into what compounds influence the clustering of a specific age group which would later be used to compare to rapid maturation experiment samples. LDA was used to build a model to determine what year group no age statement samples and rapid maturation experiment samples would approximately fit into. Overall, the results obtained from the analysis of commercial samples showed what compounds are present and their relative concentrations for different aged samples. PCA and LDA were used to observe age clusters and for producing a model which would be used later (section 5.6) for experiment comparisons. The information from this chapter assisted in identifying the desired compounds required to be in rapid maturation samples (Chapter 5).

Table 4.6: Summary of desirable compounds required in rapid maturation experiments (quantitative and qualitative) determined from the analysis of commercial samples with age statements > 10-year-old (n = 23).

Compound	Concentration Range (ppm)	Peak Area (ISTD Corrected)*
Esters		
Ethyl Acetate	< LoD - 2.22	–
Isoamyl Acetate†	< LoD	–
Ethyl Decanoate	< LoD - 0.16	–
Ethyl Octanoate	< LoD - 0.14	–
Ethyl Laurate	< LoD - 0.14	–
Ethyl Buyrate	< LoD	–
Ethyl Hexanoate‡	–	n.d. - 0.17
Phenethyl Acetate‡	–	n.d. - 0.03
Alcohols		
Isoamyl Alcohol	< LoD - 9.32	–
2-Phenylethanol	0.1. - 0.60	–
Isobutanol‡	–	n.d. - 1.19
2-Methyl-1-butanol‡	–	0.04 - 1.57
Phenolic Aldehydes		
Syringaldehyde	< LoD - 0.70	–
Vanillin	< LoD - 0.34	–
Phenols		
Guaiacol†	< LoD - 0.20	–
m-Methoxyphenol†	< LoD - 0.11	–
m-Cresol†	< LoD - 0.15	–
Acids		
Acetic Acid‡	–	0.09 - 0.59
Miscellaneous		
Furfural‡	–	n.d. - 0.06
1,1-Diethoxethane‡	–	n.d. - 0.26
$C_6H_{12}O$‡	–	n.d. - 0.03

* Ratio of the compound peak area to the ISTD peak area
† Optional, but preferable
‡ Qualitative results

Chapter 5 - Exploratory Experiments with Wood Chips in Ethanol (Model System) and Fresh Distillate (Whiskey Matrix)

The literature review (Chapter 1), method development (Chapter 3) and the analysis of commercial whiskies (Chapter 4) provided a focus for the experiments described below by providing information on what compounds are expected to be present from wood extraction and what compounds were present in the fresh distillates. Analysis of maturation experiment samples indicated whether the desired compounds were extracted from the wood and available for further reaction or if additional unpleasant compounds were also being introduced. The results from Chapter 4 also allowed comparison between the commercial samples and maturation experiments to determine whether the compounds were present at similar levels.

To investigate the compounds that are extracted from wood which influence the maturation of whiskey, the focus of this chapter was to conduct experiments using several ageing treatments based on literature. The aim was to determine how each treatment affected the extraction and subsequent conversion of compounds within a model system (ethanol) and in whiskey matrices (corn and malt distillates). The ageing treatments were: heat, light, heat-freeze and sonication. The variables considered were: time, oak chip size and level of wood charring. Oak chips were placed into the liquid (ethanol and distillate) instead of storing samples in oak barrels because it has been shown to speed up the extraction of volatiles and non-volatiles in a shorter timeframe and lower associated production costs due to increased surface area, which are two key reasons for carrying out this research [74].

As the main aim was to speed up the maturation process of whiskey, the factors affecting the rates of reactions that were taken into account include [51]:

1. The physical state of reactants – liquid (ethanol and raw distillate) and solid (wood chips)
2. The concentration of ethanol – to standardise all experiments 65% ethanol was used as this is a common %ABV in barrels [20]; the supplied corn distillate was 40% ABV. The alcohol concentration within a barrel is generally 55-75%ABV and it is known to

affect the extraction of wood compounds producing different profiles depending on the %ABV [20,37,131].

3. Reaction temperature – heat and sonication
4. Presence of catalyst – acids present in distillate act as an acid catalyst. Also light can act as a catalyst.

As this chapter focused on exploratory work, analysis using untargeted GC-MS was used to identify all compounds present (qualitative analysis) and quantitative analysis was carried out on the compounds for which standards were available. The compounds present in the GC-MS chromatogram were identified using the NIST library and pure compounds (if available) for retention time matches.

5.1. Experimental Preparation

5.1.1. Oak Chip Toasting

Studies around the charring of oak have reported that lower levels of extractives are obtained when compared to toasting (due to volatilisation and carbonisation) [24,28]. Based on this information, oak chips were toasted rather than charred. The origin of oak chips used in experiments were from a used barrel made from *Quercus robur* (European/French oak). Toasting for 30 minutes was chosen for two reasons; firstly, research by Farrell *et al.* showed that after 30 minutes the rate of increase for several wood extractive compounds (e.g. vanillin, guaiacol and oak lactone) dropped off, and secondly to ensure the same preparation was applied to all three oak chip sizes without burning the shavings [76,132]. The three temperatures (140, 180 and 220 °C) were chosen to increase the concentrations of oaky, sweet and vanilla compounds (Figure 5.1).

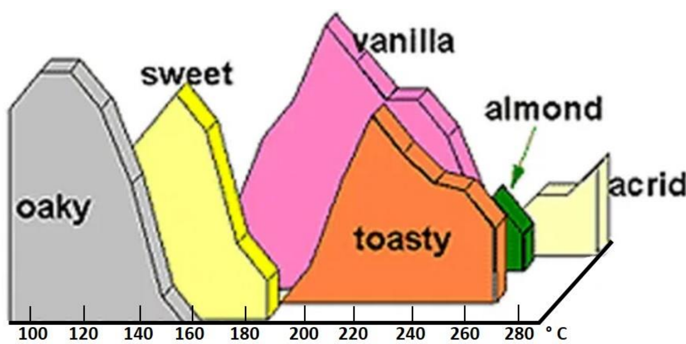

Figure 5.1: Oak toasting temperatures and the associated intensities for specific flavours. Modified from [133].

5.1.2. Surface Area Calculations

Traditionally whiskey is aged in a range of barrel sizes, however, 200-300 L is the most common [26]. An estimation for the surface area to which whiskey is traditionally exposed to was calculated. It should be noted that the actual surface area to volume ratio will be larger than the calculated result because diffusion also occurs through the wood [20]. The surface area of one wood shaving, chip and block were also calculated to determine how many would be required to achieve a similar surface area to a barrel; this was then increased by a minimum of 3-fold to speed up compound extractions and reactions.

Surface area calculation for 300 L barrel

Based on a barrel volume of 300 L with a height of 1000 mm and an average diameter of 700 mm (head (630 mm) and bilge (widest part of the barrel, 770 mm) diameters), the surface area of a barrel was calculated using the surface area equation (Equation 3) [134]; the surface area per 100 mL (experiment volume) calculation is also shown in Equation 3 [135].

$$2\pi \times r^2 + 2\pi rh \qquad \text{(Eq. 3)}$$

$$2\pi \times 350mm^2 + 2\pi \times 350mm \times 1000mm = 2{,}968{,}805\ mm^2$$

Therefore, the surface area per 100 mL:

$$(2{,}968{,}805\ mm^2 \div 300\ L) \div 1000\ mL \times 100$$
$$= 989.6\ mm^2\ per\ 100\ mL$$

Surface area of a small sized wood chip

The surface area of one small sized wood chip, one large sized wood block and one shaving were calculated using the average dimensions (in mm) of the width (w), height (h) and length (l) for each wood size (small chips, large blocks and shavings); An example calculation is shown for the small sized wood chips, Equation 4. The calculated surface area was then used to estimate the number of wood chips/blocks/shavings required for traditional ageing in a 300 L barrel which was then used to indicate the minimum wood required per 100 mL (Equation 5). For all experiments, 100 mL of solution (ethanol or distillate) was used, therefore, to achieve

the same surface area to volume ratio as traditional maturation, approximately ½ a small wood chip, a ¼ large block or 1 shaving would be required.

$$2wl + 2lh + 2hw \equiv 2[wl + lh + hw] \quad \text{(Eq. 4)}$$

$$2 \times [(5\ mm \times 35\ mm) + (35\ mm \times 25\ mm) + (25\ mm \times 5\ mm)]$$

$= 2350\ mm^2$ per small sized wood chip

$$2968805\ mm^2 \div 2350\ mm^2 = 1263\ small\ chips\ per\ 300\ L\ barrel$$

Therefore, the number of chips per 100 mL:

$$300\ L\ \div wood\ chips = per\ 100\ mL \quad \text{(Eq. 5)}$$

$$300\ L \div 1263\ chips = 0.24\ L\ per\ small\ chip$$

$= \sim$ ½ chip per 100 mL

The aim was to speed up the ageing process by increasing the surface area to volume ratio, a minimum of a 3-fold increase was applied to provide excess wood to aid rapid maturation. Overall, an average of 2-3 small chips (4-6 fold increase) were used for all experiments with an approximate total weight of 4.2 ± 0.7 g (n = 24). The large blocks were heavier with a standard weight of 6.7±1.0 g (n = 9) per block, therefore, only one block was used per 100 mL (4-fold increase) to keep the weight similar to the small chips. Three shavings were used per sample (3-fold increase) in the shavings experiment with a weight of 0.38-0.40 g (n = 2). The decision to use weight rather than the surface area for each wood size was to simplify preparation and allow easy comparisons between different ageing treatments as well as replicate the process used in commercial settings.

5.1.3. Rapid Maturation Experiments

The following sections report the results for exploratory maturation experiments. Comparisons between each experiment as well as with commercial samples showed which ageing treatment/s achieved the desired results in a short time frame which would be used in future work. The results for each treatment are presented in separate sections (heat (section 5.2); light (section 5.3); sonication (section 5.4); heat-freeze (section 5.5)). Within each section the results

of important compounds are reported and some comparisons to other treatments were made although overall comparisons are detailed in section 5.6 after discussion of all treatments. The intention of the comparison between the different ageing treatments was to look at the overall chemical profile (compounds present and their relative concentrations/peak areas) produced with each ageing treatment. This would aid in the determination of whether a balance between the compounds present and their relative concentrations/peak areas and if an overall chemical profile similar to ≥ 10-year-old whiskies were achieved. The ageing treatment/s which resulted in the extraction/formation of desired compounds in the shortest time were selected for future experiments to refine the treatment to produce a method which ages distillate in a short time frame and has similar flavours and aromas to a ≥ 10-year-old whiskey.

All samples in the maturation experiments were prepared the same way; the only difference was the ageing treatment applied. Appendix E (page 183) summarises the weight of wood chips used for each ageing experiment. These experiments were all conducted in the laboratory so tasting was not possible. However, nosing the samples was carried out by the researcher to provide a general aroma profile description. Some experiments were only conducted with corn distillate due to the limited quantity and variation in the availability of malt distillate from the client.

Initially, the wood chips were soaked in 65% ethanol to allow qualitative determination of compounds in a matrix that closely represented a fresh whiskey distillate, but did not contain compounds from the distillate production process to aid determination/identification of the compounds of interest. It was acknowledged that peak area does not equal the concentration and the detector response differs for each compound (response factor). Therefore, the peak areas between two different compounds could not be compared, only the peak areas for the same compound could be compared across samples. Furthermore, the detector response changes over time so limited comparisons for a given compound (qualitative analysis) could be made between different age treatments and commercial samples. Examination of the QC sample peak areas showed only small changes in the peak areas of compounds (e.g. isobutanol fluctuated between 0.6 and 0.7 over a 4 month period), hence experimental samples were compared taking minor peak area variation into account to avoid reporting false trends.

Each set of ageing treatments were carried out in single replication and any anomalies in the trend were identified by analysis of the same sample over time as well as analysis of the QC within each sample batch. The calculated standard deviation for compounds detected

quantitatively in the analysis of the fresh corn (n = 3) and malt (n = 3) distillates (section 4.1) were used for error bars on graphs as a proxy to estimate the variability in instrument detection over the duration of the research.

It was noted that there was an initial decrease for most of the compounds from time zero to the first time point in all experiments. The decreases were likely to be from the absorption into wood [28,136] and establishing equilibria between related compounds [28]. The initial decrease will not be discussed going forward and has been ignored when discussing general trends of the data, however, the data has been included on all graphs.

5.2. Heat Experiments (50 °C and 70 °C)

Weak acids, amongst other compounds, are extracted from wood and these weak acids act as a catalyst for ester formation (Fischer esterification). This is a slow process under room temperature conditions, however, in warmer environments acceleration of physical reactions (e.g. extraction of weak acids) and chemical reactions (e.g. formation of esters) have been observed which aligns with the relationship between reaction rate and temperature [24,32,51]. Storage warehouses in some parts of America have large temperature differences between the top and lower storage levels. During summer the temperature differences are most significant where the top layers can reach up to 50-60 °C, whereas the bottom layers are between 18 and 21 °C [24].

Using the method outlined in section 2.6.4, heat experiments were conducted to investigate:

1) The compounds that were extracted from the wood chips and their relative concentrations/peak areas
2) Effect of temperature on the extraction of compounds and any resulting reactions
3) The time taken to extract compounds

The results from this experiment were used to refine the temperature, wood chip size and toasting levels for further heat experiments using fresh distillate (section 5.1.2).

Untargeted qualitative analysis was carried out for oak chips in ethanol (section 5.2.1); all peak areas have been corrected using the internal standard (1-naphthol). Quantitative and qualitative analyses were carried out for heat experiments with distillate (section 5.1.2 and 5.1.3).

5.2.1. Small and Large Oak Chips in Ethanol

The experiment with small wood chips and large blocks in ethanol (65%) was carried out over four weeks and samples were analysed periodically to determine what compounds were extracted from wood, if any of the extracted compounds reacted with each other and which temperature (50 °C or 70 °C) was more efficient at extracting compounds. Initially, the extraction of compounds from wood in the first 14 days while the chips were in the ethanol is discussed. After this time the wood chips were removed from the ethanol and each sample was split into two; one set was returned to the oven and the other set was stored in the dark at room temperature. The purpose of this was to determine whether any compounds extracted from the wood underwent chemical conversion, without the influence of further compound extraction from the wood. This is discussed separately to compare whether or not heat exposure was required for any of the conversions.

All samples were analysed by GC-MS and each chromatogram consisted of 5-25 compounds with 3-13 compounds (threshold $\geq 2\times10^4$) identified by the NIST library (> 75% match). Several compounds (e.g. diethyl succinate and ethyl vanillate) were identified by the NIST library in the medium toast 70 °C samples, but are not discussed because they were only detected once (day 22) during the entire experiment. There were a small number of peaks that were below the pre-determined threshold ($> 2\times10^4$) in the 50 °C samples, hence were not identified by the NIST library and are not discussed further.

5.2.1.1. Day 1-14

A colour change was observed in all samples (50 and 70 °C). The light toast samples (small chips and large blocks) had a light amber colour and the colour was progressively darker for medium and heavy toast samples on day 1 (Figure 5.2). Table 5.1 summarises the compounds which were detected in the samples stored at 50 and 70 °C with different wood sizes and when the compounds were first detected with each toast level (light, medium and heavy). These compounds are discussed in detail below according to their functional group with the exception of acetic acid and ethyl acetate which are discussed together. Appendix J, Table J.1 (page 192) and Table J.2 (page 193) summarise the 50 and 70 °C peak areas respectively on day 1 and 14 for each compound.

Figure 5.2: Colour change of 65% ethanol solutions with large oak chips (left to right: light, medium and heavy toastings) after one day at 70 °C. The light toasting (left) was a light amber whereas the heavy toasting solution was a dark amber (right).

Table 5.1: Summary of the first day of detection of compounds extracted from wood (small chips and large blocks) with various toasting (light, medium and heavy) into ethanol at 50 and 70 °C.

Compound	**Day first present at 50 °C**		**Day first present at 70 °C**	
	Med & Heavy	Light	Med & Heavy	Light
Acetic Acid	8 (med toast) † 9 (heavy toast) †	9	1	1
Ethyl Acetate	1	1	1	1
Syringaldehyde	3	9	1	3
Sinapaldehyde	3 (med toast) 8 (heavy toast)	9	1	8†
Vanillin	9†	n.d.	7* and 10†	22*
1,1-Diethoxyethane	1	3	1	1
Furfural	1	4	1	1
2,6-Dimethoxyphenol	9 (heavy toast only)	n.d.	1 (heavy toast only)	n.d.

Note: The numbers without footnotes indicate the compound was present in both wood sizes (small and large).
n.d. Not detected
* Detected in small chips
† Detected in large blocks

Acetic Acid and Ethyl Acetate

Acetic acid was present in all of the 70 °C samples on day 1 with peak areas of 0.03-0.07 (n = 6). However, it was not detected in any of the 50 °C samples until analysis on day 8. No sampling occurred between day 4 and 8 so it is unknown when it first appeared in the 50 °C samples. Although, it was unlikely to be present before day 8 because the peak area at this time

was ≤ 0.01 (n = 3). The presence of acetic acid is from the oxidation of acetaldehyde (which itself is produced by oxidation of ethanol) and it is also extracted from wood [28]. It is required (amongst other acids) to create an acidic environment (low pH) to allow reactions such as esterification to occur. Acetic acid is also the precursor to ethyl acetate (and other esters) and can account for up to 95% of volatile acids present making it an important acid [11,28,137]. The lower acetic acid levels in the 50 °C samples were most likely due to the reduced extraction of acetic acid from wood as well as slower oxidation of acetaldehyde (not detected) to acetic acid (Figure 5.3).

$$H_3C{-}CH_2OH + 1/2\,O_2 \xrightleftharpoons{H_2O} H_3C{-}C(=O)H + 1/2\,O_2 \rightleftharpoons H_3C{-}C(=O)OH + H_3C{-}CH_2OH \rightleftharpoons H_3C{-}C(=O){-}O{-}CH_2{-}CH_3$$

Ethanol — Acetaldehyde — Acetic Acid — Ethanol — Ethyl Acetate

Figure 5.3: Reaction scheme for the formation of ethyl acetate starting from the oxidation of ethanol. Modified from [28].

Acetic acid was present in 79% of the 70 °C samples (both sizes and all three toast levels) throughout the experiment and decreased over time. High concentrations of acetic acid are undesirable because of the associated vinegar smell and taste [53,137]. After the first three days, the acetic acid started decreasing linearly until day 10 after which it remained constant until day 14; this decrease was thought to be from the formation of ethyl acetate and the levelling off (from day 10) was due to equilibrium between the various species being established. The peak areas at day 14 for both the small chips and large blocks (0.03-0.06) for all toast levels were in the lower acetic acid peak area range found in commercial samples (0.04-1.02) and did not increase to the average acetic acid peak area (0.27 ± 0.18, n = 51) in commercial samples over the 4-week experiment (Figure 5.4).

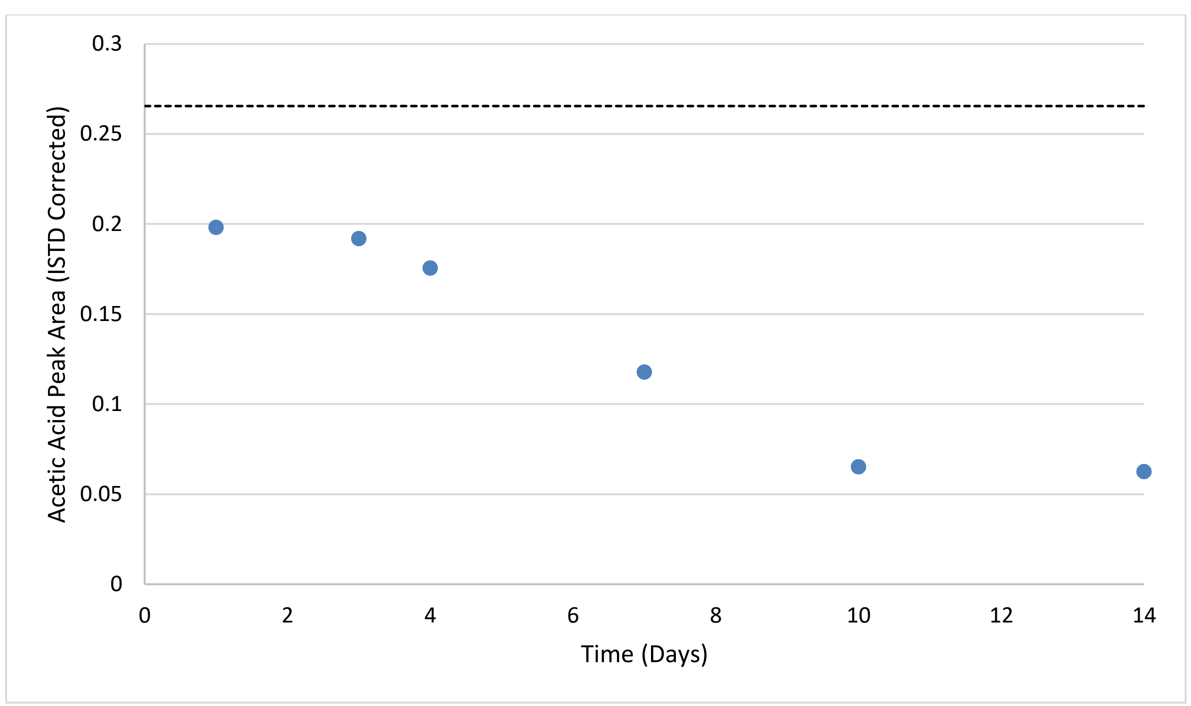

Figure 5.4: Acetic acid peak area from day 1-14 for the 70 °C large block light toast sample. The average acetic acid peak area for commercial samples analysed (0.27 ± 0.18, n = 51, black dashed line) is displayed on the graph and shows the experiment samples never reached the average peak area of commercial samples. After the first three days, the acetic acid started decreasing linearly until day 10 where it remained constant until day 14.

Ethyl acetate was detected from day 1 in all samples, indicating it is easily formed from the reaction between ethanol and acetic acid (Figure 5.3). It was present in 78% of the commercial samples analysed (0.002 to 0.706). The ethyl acetate peak areas observed in the 50 °C samples were lower compared to 70 °C samples (Figure 5.5) especially for the light toast large block because less acetic acid was present in the 50 °C samples to form ethyl acetate. The ethyl acetate peak area (0.04 ± 0.004, n = 3) on day 8 for samples stored at 50 °C was equivalent to the peak area on day 1 of storage at 70 °C which helped demonstrate that higher temperatures increase the extraction and reaction rates [138]. Ethyl acetate did not increase after 7 days at 70 °C. In comparison, during traditional maturation, ethyl acetate increases with age, suggesting possible equilibrium ($ethanol + acetic\ acid \rightleftharpoons ethyl\ acetate$) in the experimental samples and exhaustion of acetic acid extraction from the wood [28]. The peak areas of ethyl acetate in the 70 °C samples from day 3-14 (Figure 5.6) were similar to the peak areas for many commercial samples; it illustrated that ≤ 14 days at 70 °C was required to obtain similar peak areas to those in well-known commercial brands like Glenfiddich, Johnnie Walker and Glenlivet.

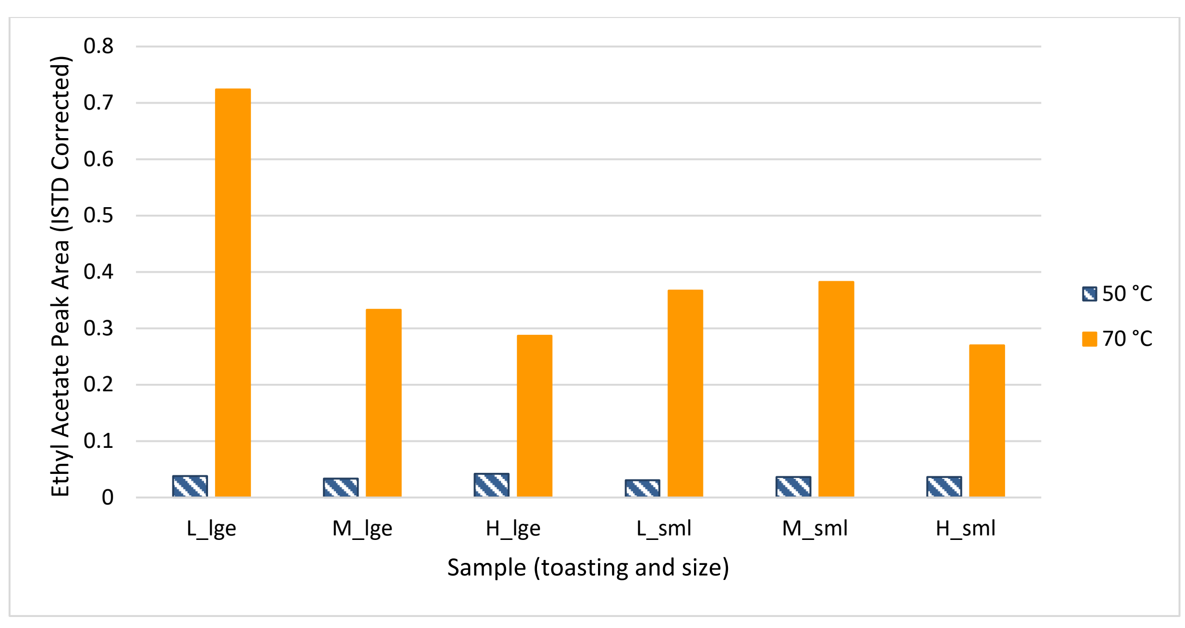

Figure 5.5: Comparison between the peak areas of ethyl acetate at day 8 in 50 °C (blue diagonal) and 70 °C (orange) samples. Sample code: first letter = toasting level (L:light, M:medium, H:heavy) and the three letters = the wood chip size (Sml:small and Lge:large).

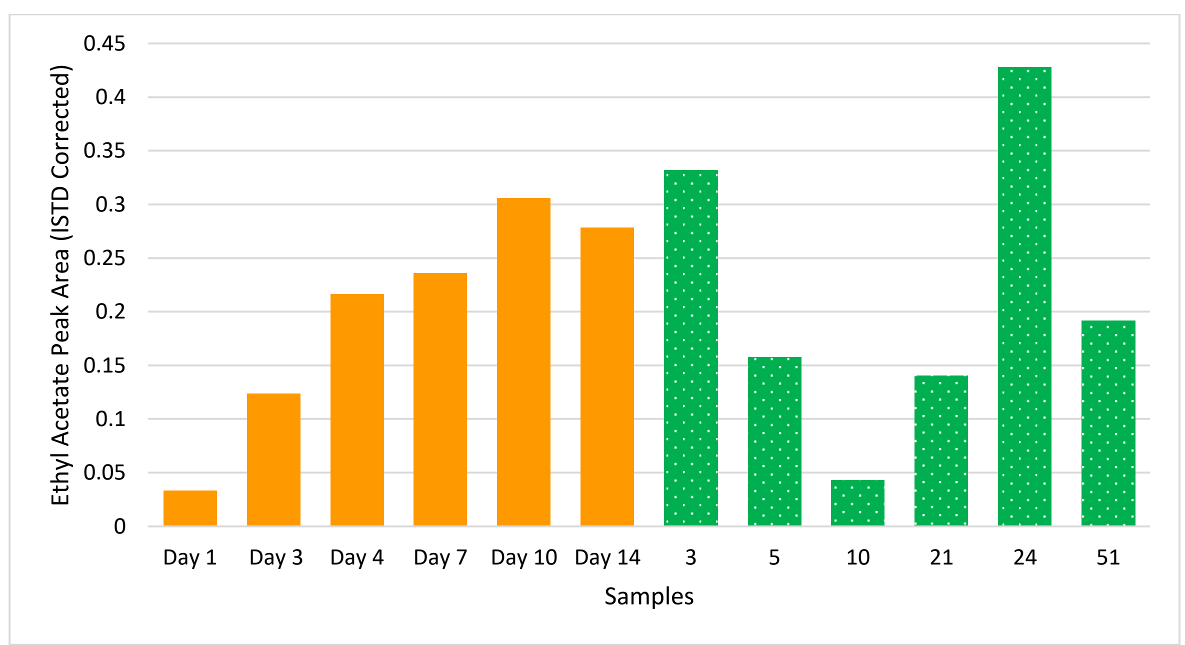

Figure 5.6: Peak areas for ethyl acetate from day 3-14 (orange) in the 70 °C medium toast small chips samples and the peak areas for several commercial samples (dotted green).

Phenolic Aldehydes

Phenolic aldehydes are directly extracted from wood (Figure 1.4, page 6) and are also formed from the reaction of ethanol with precursors extracted from wood (Figure 1.10, page 14)

[31,40]. The extraction of two of these 'wood' compounds, sinapaldehyde (3,5-dimethoxy-4-hydroxycinnamaldehyde) and syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde), were detected at both temperatures but first appeared on different days depending on the toast and wood size (Table 5.1).

In the 70 °C samples, sinapaldehyde increased from day 1-7, then minor fluctuations were observed until day 14, most likely due to continued extraction (increase) and oxidation to syringaldehyde (decrease) or from the variation in peak area over time with GC-MS analysis. A similar trend was seen in the 50 °C samples, however, the peak areas were lower than the 70 °C samples showing temperature was a factor contributing to extracting sinapaldehyde and oxidising it to syringaldehyde (Figure 1.10, page 14). Sinapaldehyde was not detected in any commercial samples [1,28,41,46].

Syringaldehyde was detected in 37% of commercial samples. The peak areas for syringaldehyde in the medium and heavy toast 70 °C samples (large blocks and small chips) on day 3 (0.01-0.03) were similar to 12-year-old Wild Turkey (0.03), Laphroaig (0.02) and Glenrothes (0.01) samples. Syringaldehyde continued to increase the longer it was heated because temperature aided extraction and oxidation. After 8 days, the syringaldehyde peak areas in all 70 °C samples were higher (0.038-0.076) than commercial samples (0.008-0.032). It has been reported that the rapid increase in compound levels is linked to the increased surface area to volume ratio because it aids the diffusion of compounds into solution [139]. Jeffery (2012) [20] demonstrated the rate of release of oak extractives (phenolic compounds) and the associated congener concentrations from different barrel sizes over 200 days. For the first 30-40 days, each of the compounds analysed (vanillin, syringaldehyde, guaiacol, 2-methoxy-4-methylphenol and eugenol) increased roughly the same amount despite different barrel sizes. However, from 40-60 days there was a rapid increase in all compounds (vanillin, syringaldehyde, guaiacol, 2-methoxy-4-methylphenol and eugenol) in the small barrels (7.57 and 11.4 L) compared to the large barrels (18.9 and 37.9 L). Overall the 7.57 L barrel had the highest levels of all compounds [20]. The presence of syringaldehyde (sweet aroma) is desired not only for flavour and aroma but also because it is used as a quality marker, e.g. the higher the syringaldehyde concentration, the better the quality. It is also used to determine age because syringaldehyde is extracted during the ageing process. Thus the higher the concentrations of syringaldehyde, the older the whiskey [1,23,37,46,53].

Vanillin (4-hydroxy-3-methoxybenzaldehyde) was also extracted from wood (Table 5.1) but the day it was first detected differed based on the wood size. Vanillin was first detected in the small chips then the large blocks at 70 °C; the hypothesis for this result is that there is a larger surface area when using small chips [138]. Conversely, vanillin was only detected in the large block samples (medium and heavy toast) at 50 °C. It was hypothesised that the presence of acetic acid in the 50 °C samples with large blocks was the reason because Puech (1987) reported a higher extraction of phenolic aldehydes in solutions (wine 12% ABV) buffered with acid (pH 3.5) [25,140]. The detection of vanillin in the 70 °C samples was earlier than in the 50 °C samples, again showing temperature aided the extraction and formation of vanillin (by oxidation of coniferaldehyde) [41,141]. The peak areas for vanillin in the medium toast (180 °C) were double the heavy toast (220 °C) peak areas which can be explained by vanillin increases with toasting temperature (100-200 °C) [28]. The presence of vanillin (vanilla flavour and aroma) is desired for the same reasons as syringaldehyde [1,23,37,46,53]. Vanillin was detected in 35% of commercial samples and the peak area for the heavy toast small chips 70 °C sample (0.010) was similar to commercial samples (0.008 ± 0.002, $n = 4$). Interestingly, the peak area for the medium toast small chips 70 °C sample on day 7 (0.020) was larger than 80% of the commercial samples (0.006-0.010) but lower than Jack Daniels (0.062).

Miscellaneous Compounds

On day 1, 1,1-diethoxyethane was present in all samples with more extracted at 70 °C (Figure 5.7). 1,1-Diethoxyethane has a fruity aroma and is known to be present in whiskey from the reaction of ethanol and hemiacetal (Figure 5.8) [28,63].

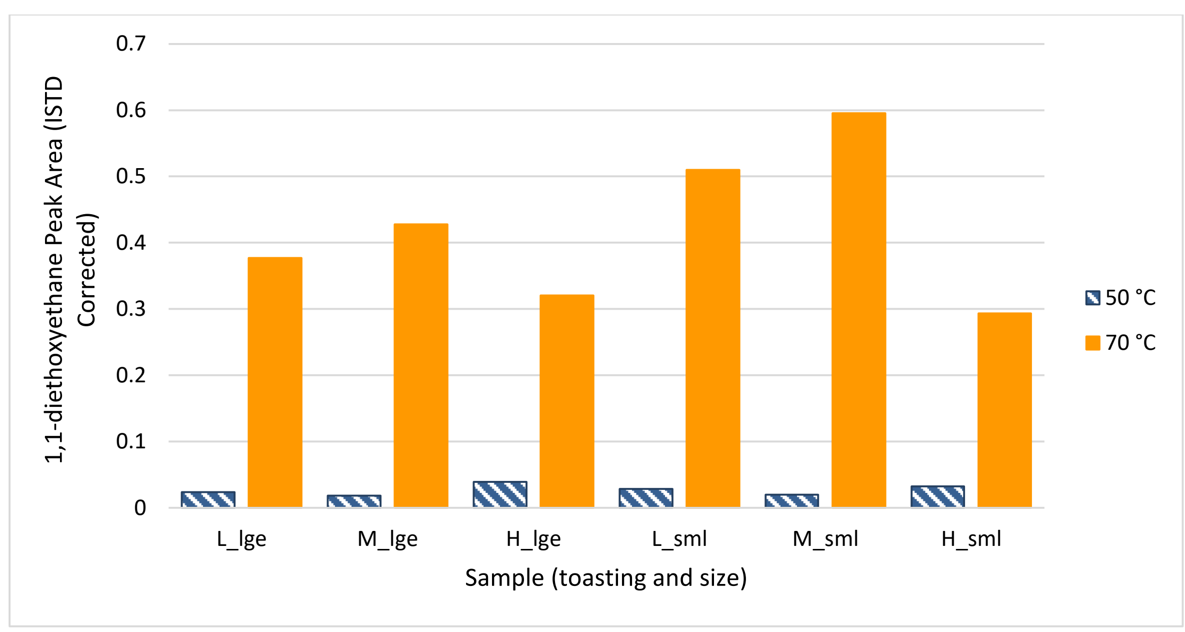

Figure 5.7: Comparison between the peak areas of 1,1-diethoxyethane at 8 days in 50 °C (blue diagonal) and 70 °C (orange) samples. Sample code: first letter = toasting level (L:light, M:medium, H:heavy) and the three letters = the wood chip size (Sml:small and Lge:large).

Acetaldehyde $\underset{}{\overset{EtOH}{\rightleftharpoons}}$ Hemiacetal $\underset{H_2O}{\overset{EtOH}{\rightleftharpoons}}$ 1,1-diethoxyethane

Figure 5.8: Reversible reactions of ethanol with acetaldehyde, hemiacetal and acetal. Modified from [28].

The peak areas for 1,1-diethoxyethane increased over time (Figure 5.9); it was suspected that an analytical error occurred on day 8 (in the 70 °C samples) due a 18-70% increase in all samples. This point was removed and the data showed a logarithmic trend (R^2 = 0.80-0.97). In traditional maturation, 1,1-diethoxyethane increases with age; this was seen in the first 7 days of the heat experiment suggesting equilibrium between acetaldehyde, hemiacetal and 1,1-diethoxyethane. Although this could not be confirmed because acetaldehyde and hemiacetal were not detected (either too low or not detected with the instrument parameters) [28]. On day 3, the samples stored at 70 °C had similar peak areas to several commercial samples including several ≥ 10-year-old whiskies (Figure 5.10). The 50 °C samples did not reached similar peak areas to the 70 °C samples within 14 days, but on day 3 the peak areas (0.01-0.02) in the 50 °C samples were similar to Irish whiskey (Jamieson, 0.015) and other

American whiskies (Jack Daniels, 0.011). These differences highlight the importance of knowing the targeted flavour profile so that the correct experimental parameters can be applied. On day 7 the peak areas (0.18-0.43) in the 70 °C samples were higher than those which were found in commercial samples (highest peak area = 0.26). Depending on what concentration the peak areas would equate to, storing samples at 70 °C for more than 7 days may be undesirable.

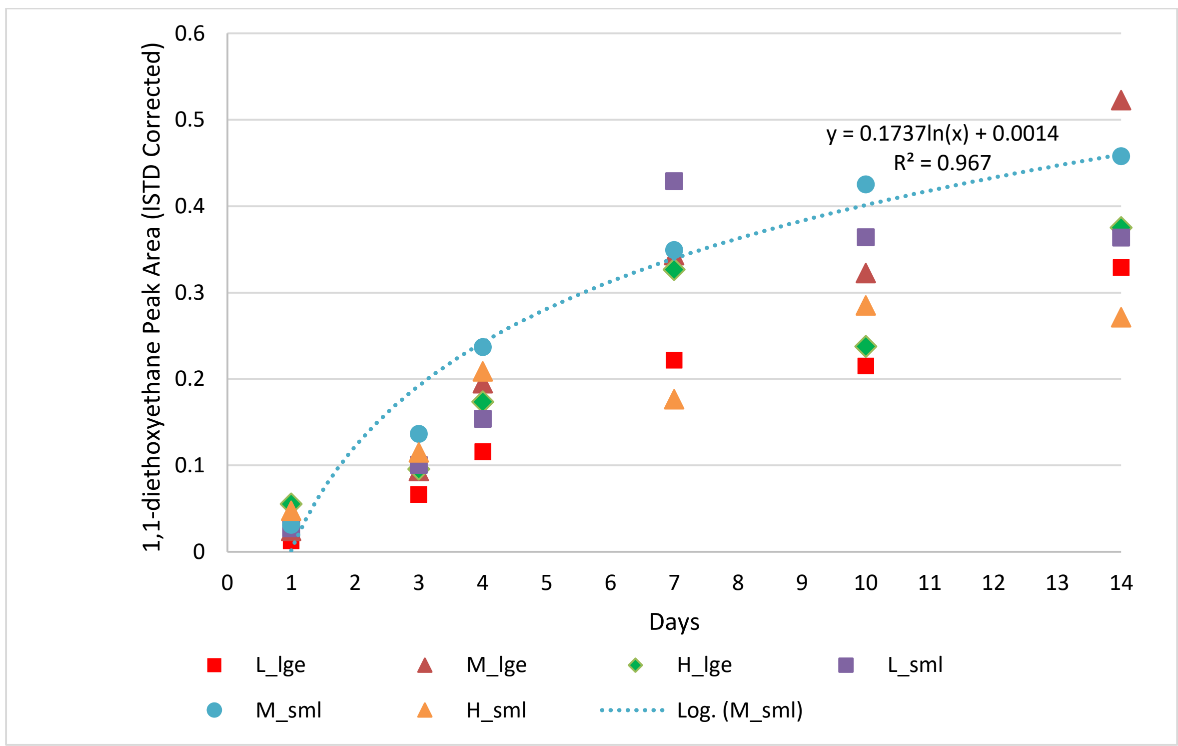

Figure 5.9: Peak area increased over time (0-14 days) for 1,1-diethoxyethane in all 70 °C samples. A logarithmic trend was observed in all samples with an example shown for the medium toast small chips sample (blue dashed line).
Sample code: first letter = toasting level (L:light, M:medium, H:heavy) and the three letters = the wood chip size (Sml:small and Lge:large).

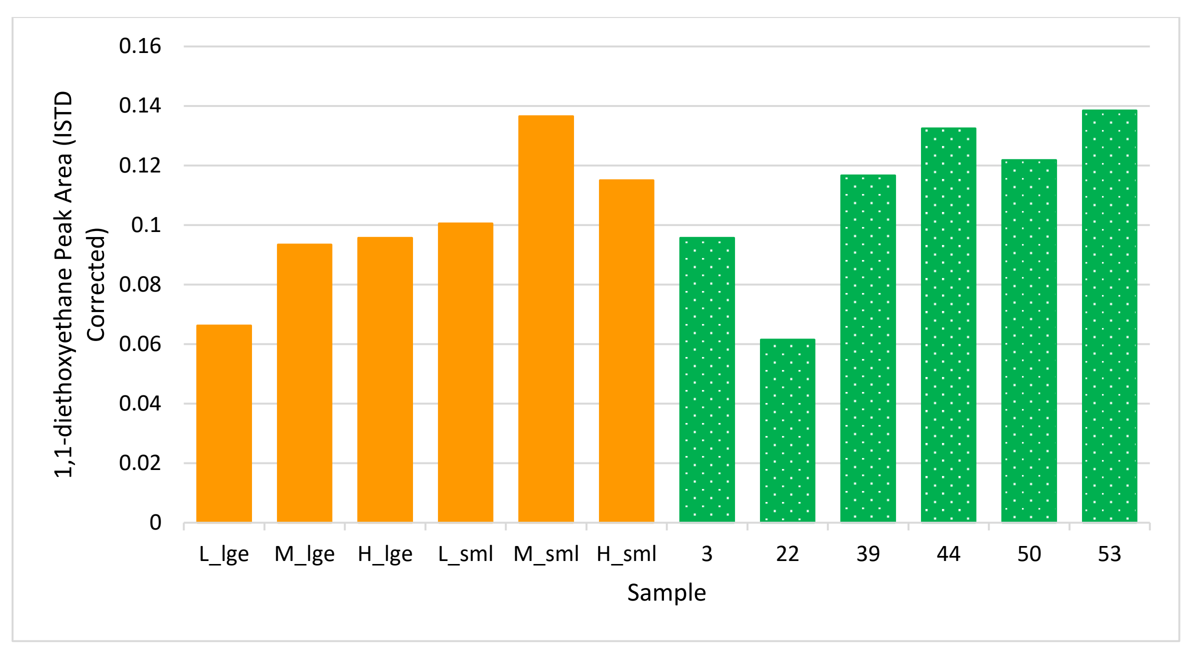

Figure 5.10: The 1,1-diethoxyethane peak areas for the heat experiment samples (orange) after three days at 70 °C and several commercial samples (dotted green). Sample code: first letter = toasting level (L:light, M:medium, H:heavy) and the three letters = the wood chip size (Sml:small and Lge:large).

Furfural was present from day 1 except in the light toast 50 °C sample (Table 5.1). The presence of furfural arises from Maillard reactions between amino acids and sugars and is desired for colouration, flavour and aroma (caramel and nutty) [12,28,123]. Furfural was detected in 75% of the analysed commercial samples (Appendix H, page 188), therefore, was desirable to extract in the rapid maturation process. All samples (70 and 50 °C) showed an initial rapid increase (day 1-4) which is also observed in the first six months of traditional maturation [28]. The difference between the large block and small chips samples was that more furfural was extracted from the large block (Figure 5.11) but exhausted quickly suggesting reaction to form another compound (e.g. maleic acid). Comparison of the furfural peak areas between the commercial samples (0.004-0.13) and ethanol samples (day 1-14) showed furfural was higher in both the medium and heavy toast 70 °C samples (small chips and large block) and 50 °C (large block) samples. While the 70 °C light toast samples (small chips and large blocks) and the 50 °C small chips samples (medium and heavy toast) were similar in peak area to many commercial samples including; Bunnahabhain, Jura, Glenfiddich, Glenfarclas and Wild Turkey (0.03 ± 0.01, n = 8). Based on these results, the time samples are stored for at 50 or 70 °C will influence the caramel/nutty flavour and aroma. The results also suggested the light toast would be the most suitable to avoid high extraction of furfural. Overall, the results highlighted that an

increase in temperature increased the extraction of furfural to the point that 6 months of traditional maturation was condensed into ≤ 14 days at 70 °C.

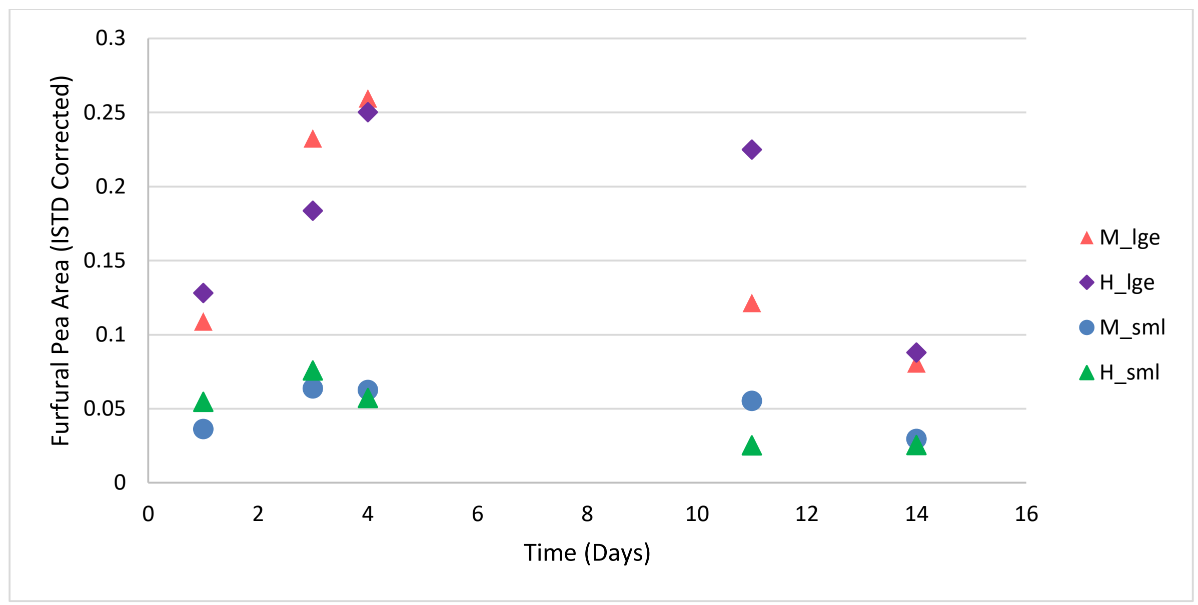

Figure 5.11: Changes in furfural peak area from day 1-14 in the 50 °C samples. The large block showed a rapid increase from day 1-4 followed by a decrease until day 14. Sample code: first letter = toasting level (L:light, M:medium, H:heavy) and the three letters = the wood chip size (Sml:small and Lge:large).

Phenols

2,6-Dimethoxyphenol (syringol) was only detected in the heavy toast samples (Table 5.1). The presence of 2,6-dimethoxyphenol is from the breakdown of lignin which is comprised of syringyl and guaiacyl units [53] and the taste is described as balsamic, bacon and medicinal; it adds smokey characteristics to whiskey [28,50]. The absence of 2,6-dimethoxyphenol in the light and medium toast samples matches literature and suggested heavy toasting was required to achieve smokey characteristics. Despite the absence of 2,6-dimethoxyphenol in the analysed commercial samples, it has been reported in whiskey; Lahne [116] showed it to be a key aroma compound in American rye whiskey.

5.2.1.2. Day 14-22

After 14 days, wood chips were removed from the samples and the ethanol was subdivided for storage at heat and room temperature. The room temperature samples and 70 °C oven samples were stored in sample storage vials and the 50 °C oven samples were kept in the DURAN® bottles. The reason for the difference in oven storage vials was that the 70 °C experiment was conducted first and it was noted that there was inconsistent evaporation over time in the 70 °C

oven samples (Figure 5.12). The evaporation was suspected to be a combination of temperature and the seals on the storage vials. The 50 °C samples had the system monitoring compound (*n*-pentanol) added at the start of the experiment and was stable over the experiment timeframe (4 weeks) with no loss of sample by evaporation, confirming the evaporation in the 70 °C samples was due to the seals on the storage vials. Therefore, the results obtained after 14 days in the 70 °C samples could be a result of the unintentional introduction of oxygen, temperature effects and volatile loss. There were two sets of evidence to support this theory. Firstly, over the 22 days for samples stored at 70 °C, vanillin showed a rapid increase when oxygen was unintentionally introduced (Figure 5.13). However, this also resulted in the loss of other desirable compounds such as acetic acid, sinapaldehyde and 1,1-diethoxyethane. Secondly, after 14 days there were 5 new compounds (diethyl succinate, *trans*-whiskey lactone, ethyl vanillate, ethyl syringate and acetosyringone) detected in the heavy toast 70 °C samples, but there were also many compounds extracted which could not be identified (6-20) due to a combination of low threshold and the inability of the NIST library to match spectra. This indicated too much breakdown of the oak and caused dark colouration of the solutions (Figure 5.12). These results re-iterated the conclusions drawn by Clyne *et al.* [37] and Lee *et al.* [53], who reported that more charring increases the surface area and causes higher degradation of wood carbohydrates which increases the phenolic content resulting in the extraction of more compounds [13,37,53]. Furthermore, these results suggest addition of oxygen in a sealed vessel could help produce a different profile (e.g. more vanillin and esters). Due to the unreliability of the 70 °C samples after 14 days, the 70 °C samples were not discussed further.

Figure 5.12: Aliquots (20 ml measured on day 14) of 70 °C samples at day 22 showing the difference in evaporation over eight days due to the poor seal created by the lids. Furthermore, the difference in colouration between light (vial 1 and 4), medium (vial 2 and 5) and heavy (vial 3 and 6) samples. Samples labelled from left to right; L_lge, M_lge, H_lge, L_sml, M_sml, H_ sml.

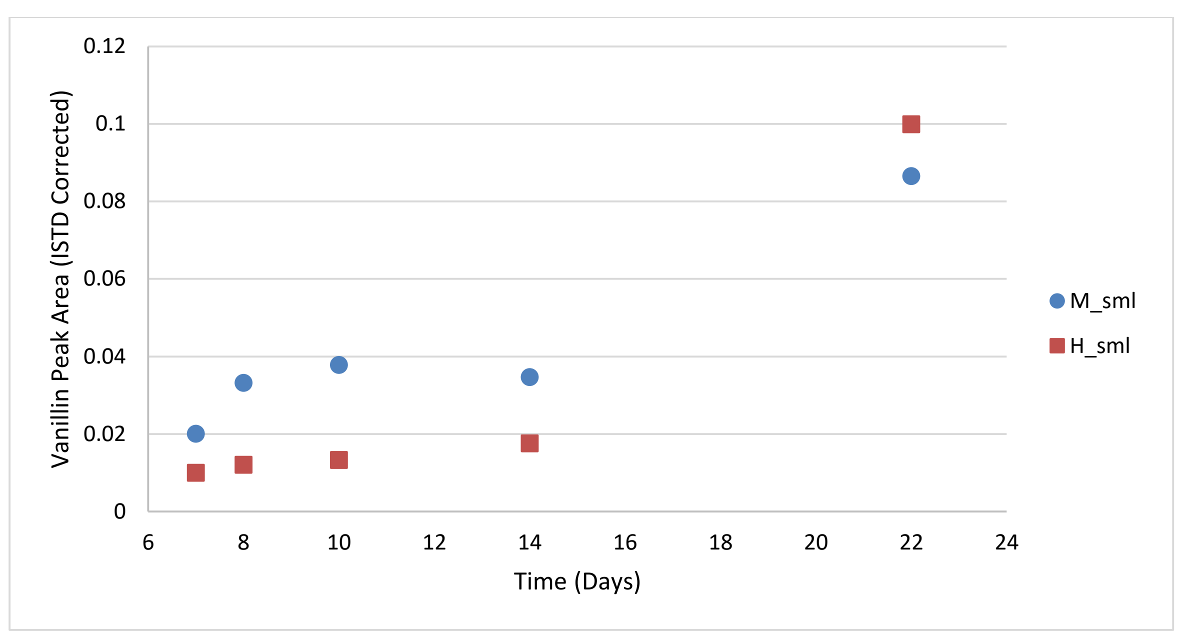

Figure 5.13: The vanillin peak areas for medium (blue circle) and heavy toast (orange square) 70 °C samples from when it was first detected (day 7) to day 22. The rapid increase in vanillin was observed between day 14-22 when oxygen was unintentionally introduced.

Vanillin was not detected in any of the 50 °C light toast samples throughout the experiment [6,46]. Vanillin increased in the medium and heavy toast 50 °C samples from day 14-22, most likely from the oxidation of coniferaldehyde (because the wood chips had been removed) [41]. Two factors can affect the presence of vanillin. Firstly, different toasting temperatures of wood result in different levels of vanillin, with higher levels obtained when toasted at temperatures > 150 °C (medium and heavy toastings). Secondly, the formation of vanillin is a multistep reaction as shown earlier in Figure 1.10 (page 14) [28,42,132]. The absence of vanillin in the 50 °C light toast samples showed 50 °C was not sufficient to accelerate extraction and formation of vanillin.

After 22 days, the peak areas for the 50 °C samples and room temperature samples for the compounds discussed above were compared to each other and the day 14 results. The 50 °C and room temperature sample levels for furfural were similar, however, the peak areas for ethyl acetate, 1,1-diethoxyethane and syringaldehyde were higher in the 50 °C samples. After day 14, no additional compounds were detected in the samples held at 50 °C samples or room temperature. This further illustrated the presence of wood and/or higher heat was required to increase the rate of extraction/formation of compounds.

5.2.1.3. Ethanol Summary

The samples stored at 70 °C extracted more of the desirable compounds in a shorter timeframe compared to 50 °C. The loss of volatiles was noted in the 70 °C samples after day 14 due to poor seals on the storage vials. The comparison between room temperature and 50 °C oven samples showed heating was required to accelerate extraction and reactions (e.g. oxidation). However, most of the desired compounds were extracted at levels similar to commercial samples such as Glenfiddich, Laphroaig and Jim Beam in less than 14 days. Therefore, removal of wood chips and continued heating after 14 days was unnecessary for 50 °C and it is likely that a similar conclusion can be drawn for 70 °C.

The large blocks and small chips (particularly medium and heavy toasts) were shown to extract compounds in a short timeframe. The 70 and 50 °C large block samples (medium and heavy toast) as well as the 70 °C small chips (medium and heavy toast) extracted too much furfural and the peak areas were above those found in commercial samples. However, the small chips were better for the formation of ethyl acetate and vanillin extraction and also minimises the amount of wood used. Therefore large blocks would not be used in further experiments (excluding light experiment which was run concurrently).

There was not much of a difference between the medium and heavy toast samples in terms of compound extraction (excluding 2,6-dimethoxyphenol) but the colour of the medium toast samples were more similar to the colour of traditional whiskey. The light toasting was inadequate because it did not extract as many compounds, and for those it did extract, it took more than double the time compared to the medium and heavy toast samples. Therefore light toasting would not be used in further experiments (excluding light experiment which was run concurrently).

Based on the above conclusions, 70 °C was the chosen temperature to conduct the same heat experiment using distillate coupled with small chips (section 5.2.2) because these gave the best results in terms of extracting desired compounds in a short time. Heavy and medium toasts were chosen for use in experiments from this point forward because the colouration of the medium toast samples was amber and the heavy toast gave a dark amber colour (≥ 14 days produced an undesirable charcoal colour).

A minimal effect on samples was seen when they were returned to the oven after the wood was removed. Despite this, more compounds are present in the fresh distillate (complex matrix)

which may be involved in reactions. Therefore removing the wood chips after a set number of days and returning samples to the oven was carried out to see if any reactions were occurring without wood chips in a more complex matrix.

5.2.2. Medium and Heavy Toast Small Oak Chips in Distillate (Corn and Malt)

The aim of this experiment was to determine how the formation of compounds and the overall composition of the samples differed when the matrix was changed from a simple matrix (ethanol) to a more complex (distillate) matrix. A 70 °C heat experiment was performed using corn and malt distillates. The ethanol results showed ≤ 10 days were required to achieve peak areas similar to commercial samples for several compounds. The number of days samples were stored at 70 °C for was extended to a total of 18 days; wood chips were removed on day 9 and samples were returned to the oven for a further 9 days to monitor the changes in compound levels. Removal of wood chips on day 9 was chosen because the ethanol results showed 8 days of heating at 70 °C produced ethyl acetate and 1,1-diethoxyethane at similar peak areas to commercial samples as well as the presence of acetic acid, sinapaldehyde, syringaldehyde, vanillin, furfural and 2,6-dimethoxyphenol (heavy toast only). Returning the samples to the oven after wood chip removal was to aid any potential reactions (e.g. ester formation) not seen in the ethanol (due to the presence of more precursors in distillate). The wood chips were also removed to reduce the amount of colouration to the solution, especially for the heavy toast samples. Quantitative and qualitative analysis was carried out on all samples. The compounds detected are summarised with their concentrations/peak areas in Appendix K for corn distillate (Table K.1, page 194) and malt distillate (Table K.2, page 196). Day 12 results were removed due to poor calibration and QC results (Appendix F, page 184), injection 30). During the analysis of samples from day 16-18, the heating element blew on the GC-MS so samples were transferred to another GC. A significant change in response was seen (sensitivity was poor), therefore, the data for day 16-18 was also removed.

Acetic Acid and Ethyl Acetate

Acetic acid was detected in all samples and increased over the experiment. The linear (R^2 = 0.88-0.90) increase of acetic acid was similar in the corn distillate medium (from 0.045 to 0.383) and heavy toast (from 0.068 to 0.383) samples. Acetic acid increased faster in the medium toast samples; the malt distillate showed the same linear trend (Figure 5.14). The results for the corn and malt distillate on day 1 (0.11-0.14, n = 4) were higher than the acetic

acid peak areas in the ethanol experiment (day 1, 0.03-0.08, $n = 4$), most likely because of the change in matrix which potentially had acetic acid present in the distillates but below detectable levels [28]. The acetic acid peak area range from day 1-11 for the corn distillate samples (0.05-0.38) and the malt distillate samples (0.13-0.41) fell within the commercial sample peak area range (0.04-1.02). The day 6 results for the medium and heavy toast corn distillate (0.30 and 0.35, respectively) and malt distillate (0.28 and 0.34, respectively) samples were similar to many commercial samples including; Jim Beam, Laphroaig and Glenfiddich (Figure 5.15). At day 11, the efficiency of the column had deteriorated to the point where acetic acid and isobutanol (RT = 1.55 minutes) co-eluted. This resulted in the installation of a new analytical column.

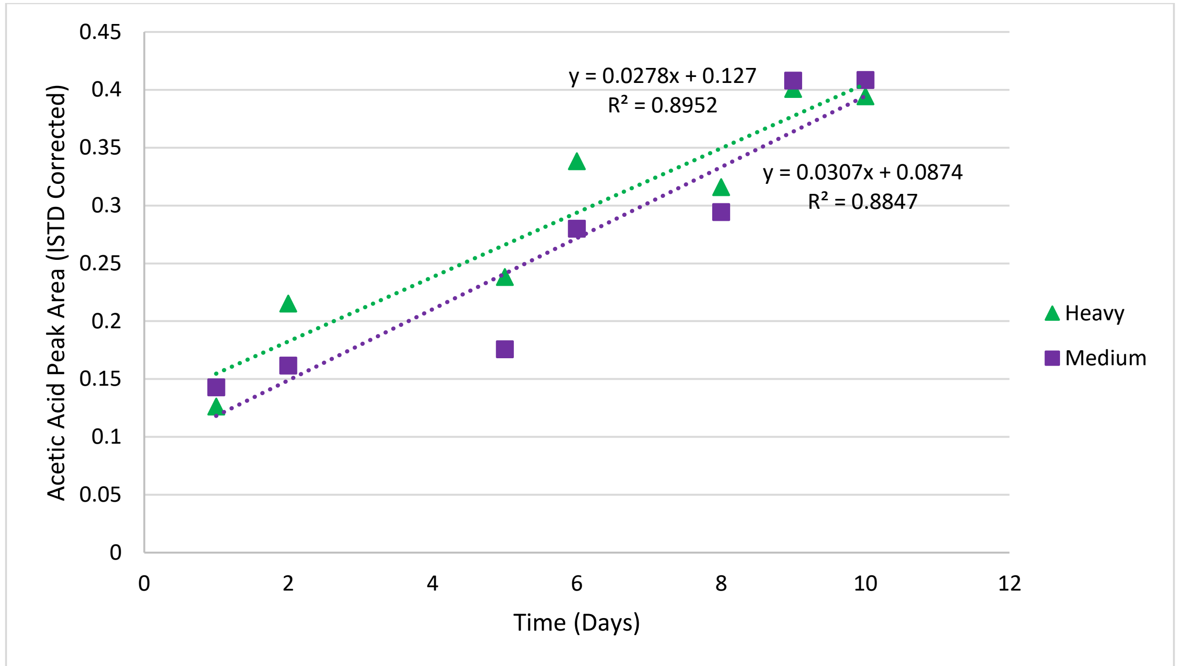

Figure 5.14: Acetic acid peak area increase over 11 days for medium (purple square) and heavy toast (green triangle) malt distillate samples stored at 70 °C. A slightly faster acetic acid increase was seen for the medium toast sample, however, from day 9 the peak areas were similar to the heavy toast sample.

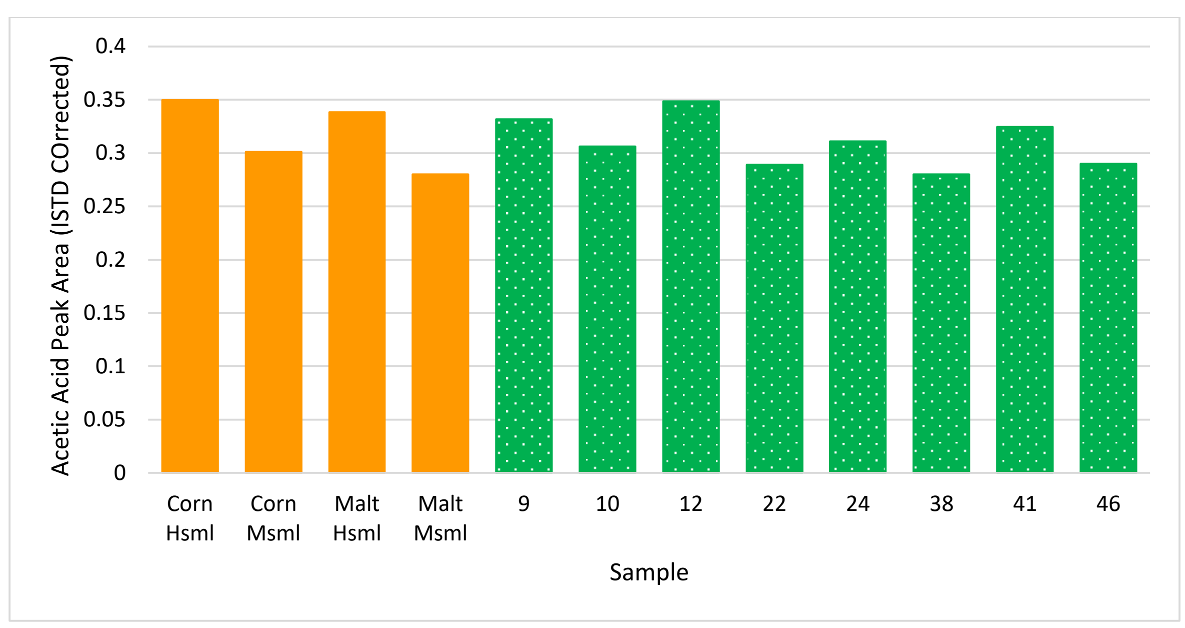

Figure 5.15: Comparison between the acetic acid peak areas obtained in the malt and corn distillate samples (orange) stored at 70 °C for 6 days and 8 commercial samples (dotted green; 9, 10, 12, 22, 24, 38, 41 and 46). Sample code: first letter = toasting level (M:medium, H:heavy) and the three letters = the wood chip size (Sml:small).

Ethyl acetate was present in the corn (0.55 ppm) and malt (10.62 ppm) distillates (section 4.1) prior to heat treatment. Traditionally the ethyl acetate concentration increases during maturation [11,28,31]; the concentration fluctuated in the heavy toast malt distillate sample (Figure 5.16) but never increased above 10.62 ppm. The concentrations for ethyl acetate from day 1-11 in the corn distillate was < LoD (1.45 ppm). The naturally occurring concentration of ethyl acetate in the malt distillate prior to heat treatment was higher than after treatment (3.73-9.17 ppm). It was postulated that the increases seen from day 4-10 (Figure 5.16) were linked to the acetic acid production. This trend (increase then decrease) was reported by Jeffery [20] who conducted research into the relationship between the extraction rates and surface area to volume ratio in smaller barrels (7.5-38 L). Ethyl acetate is a desirable compound and was found in 78% of the analysed commercial samples, however, the concentrations in the malt distillate samples over the course of the experiment (3.73-9.17 ppm) were higher than the maximum concentration found in the analysed commercial samples (2.70 ppm) but considerably lower than those reported in literature [63]. Given the odour threshold for ethyl acetate in air is 3.9 ppm [28], but 17 ppm in a 62% distillate [20] and the broad concentration range of ethyl acetate reported in the literature [20,28,63] as well as those determined by analysis of commercial samples, the concentration of ethyl acetate in the malt distillate (day 1-11) was deemed acceptable.

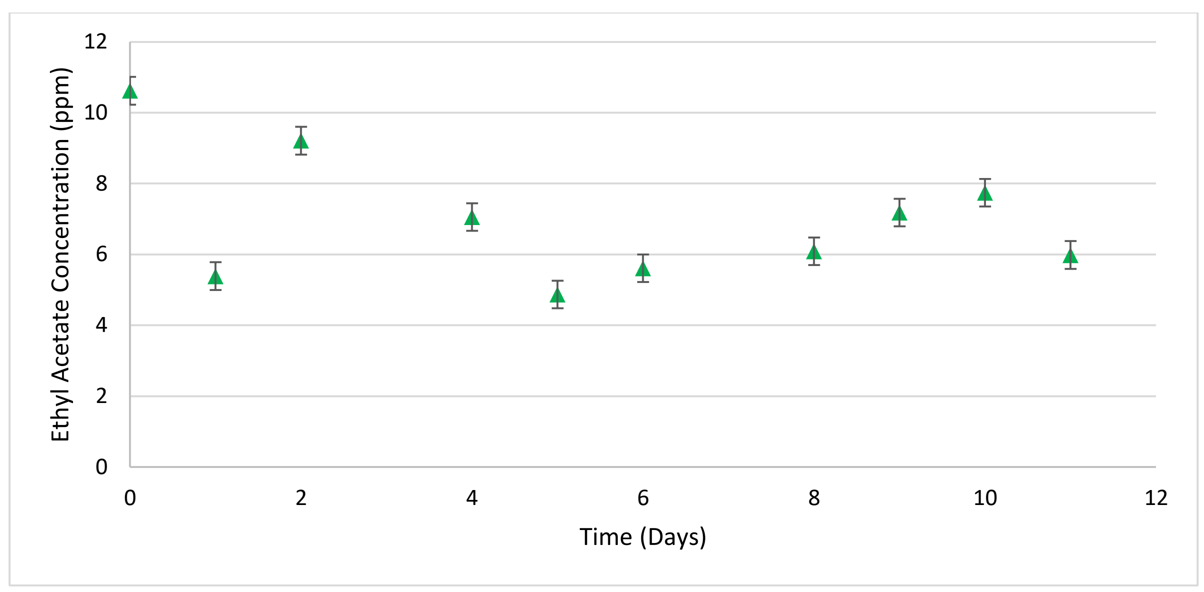

Figure 5.16: Ethyl acetate concentration (ppm) over time in the heavy toast malt distillate samples stored at 70 °C. Error bars have been added using the standard deviation calculated in the malt distillate for ethyl acetate (n = 3, section 4.1.2) as a proxy measurement of instrument variation overtime.

Phenolic Aldehydes

Sinapaldehyde was not detected in the distillates before treatment but was detected from day 1 in all samples except for the heavy toast corn distillate sample (Appendix K, Table K.1 (page 194) and Table K.2 (page 196)). The peak areas fluctuated throughout the experiment. These fluctuations were most likely because of continued extraction from wood and subsequent oxidation to syringaldehyde. There was a large difference between the medium and heavy toast peak areas throughout the experiment with the medium toast samples (corn and malt) being ≥ 10-fold higher than the heavy toast samples. The toasting temperature affects the amount of sinapaldehyde in the wood with higher temperatures (> 200 °C) decreasing the sinapaldehyde available [28,122]. Comparison to the ethanol samples (section 5.2.1) showed sinapaldehyde was higher than in the ethanol samples. For example, on day 1, the peak areas in the medium toast corn and malt distillate samples (0.09-0.10) were 2-5 fold higher than the ethanol samples (0.02-0.04); the reason for this is unknown because the same type of wood chips were used and they were all toasted together.

Syringaldehyde was detected throughout the experiment in both the corn and malt distillate samples. The medium and heavy toast samples had similar concentration ranges in the corn distillate (0.37-0.70 ppm and 0.38-0.73 ppm, respectively) and malt distillate (0.37-0.71 ppm and 0.39-0.73 ppm, respectively). These results suggested there was limited syringaldehyde

present in the wood for extraction because the concentration fluctuated (Figure 5.17) rather than continuing to increase as it does in traditional maturation [1,23,37,46,53]. It was hypothesised that the decreases were from syringaldehyde oxidation to syringic acid, however, this could not be confirmed because syringic acid was not detected with the instrument method [28,31,41]. The concentration range for syringaldehyde (0.37-0.73 ppm) in the corn and malt distillate samples overlapped the commercial samples concentration range (0.23-0.70 ppm) and the general concentration range reported in literature (0.67-4.04 ppm) [21,64]. Comparison of the syringaldehyde concentrations for commercial samples which were ≥ 10 years old (0.4-0.6 ppm) showed the day 6 results (0.51-0.57 ppm) were similar.

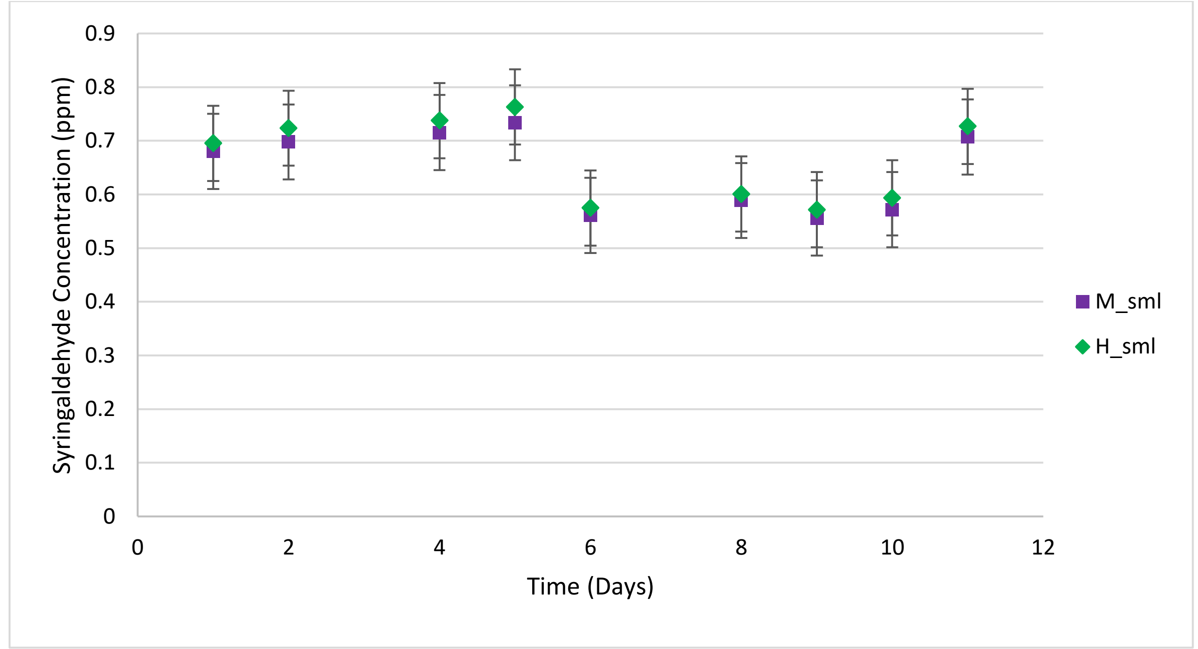

Figure 5.17: Syringaldehyde concentration in the medium and heavy toast malt distillate samples over 11 days of storage at 70 °C; a subtle change in concentration was observed. The calculated standard deviation in the method development (section 3.3.2) was used as a proxy for the variation in instrument detection.

Coniferaldehyde was detected throughout the experiment except for day 1 in the heavy toast corn distillate sample and showed a very similar trend to sinapaldehyde. The medium toast samples had higher peak areas than the heavy toast samples which as previously mentioned is because the toasting temperature affects the amount of coniferaldehyde in the wood, with higher temperatures (> 200 °C) decreasing the coniferaldehyde available to oxidise to vanillin [28,31,122]. Coniferaldehyde was not detected in any commercial samples.

Vanillin was detected in all samples with a small concentration range of 0.23-0.43 ppm. Vanillin was detected in 35% of commercial samples and had a concentration range of 0.32-0.34 ppm. As previously mentioned (section 5.1.1), vanillin is desired not only for flavour and aroma but also because it is used as an age and quality marker, with higher concentrations associated with better quality and older whiskey [1,23,37,46,53]. Therefore, the concentrations detected in the corn and malt distillates were deemed acceptable because they were close to those found in the analysed commercial samples (0.32 ± 0.02 ppm, n = 18) and they fell within the concentrations reported in literature (0.04-5.96 ppm) [21,37,64].

The decomposition of lignin occurs when the release of syringyl and guaicyl units are proportional to each other. Syringaldehyde and vanillin are measured as indicators of each of these units respectively and the syringaldehyde/vanillin ratio will be approximately 1.4:2.5 when decomposition is balanced [53]. The ratio was calculated for day 1-11 in all samples and is presented in Table L.1 (Appendix L, page 198) along with the equations used for the calculation. The results showed the heavy toast corn distillate samples were the only samples for which the ratio was too low suggesting the wood was too charred. Excluding the heavy toast corn distillate samples, vanillin started high in the day 1-5 samples but decreased over the experiment with day 11 having the closest syringaldehyde/vanillin ratio (1.4:2.3) to the reported ratio (1.4:2.5).

Syringylacetone (4-(4-Hydroxy-3,5-dimethoxyphenyl)butan-2-one) was detected (> 75% NIST match) in both the medium and heavy toast samples. The medium toast samples had an undulating trend while the heavy toast samples increased then decreased before levelling off. Syringylacetone is a syringyl derivative from wood like syringaldehyde and sinapaldehyde [142]. It is assumed that the presence of syringylacetone in the corn and malt distillate samples was due to the heating process (of the wood and distillate) because it was not detected in any commercial samples and is not mentioned in literature as being present in whiskey. It was detected in the ethanol samples (section 5.2.1), however, was not discussed due to the NIST library match being < 75%.

Miscellaneous Compounds

1,1-Diethoxyethane was detected on day 1 in the malt distillate and day 2 in the corn distillate. Both distillates had similar trends, however, the increase was more subtle in the corn distillate samples and followed a more expected trend of increasing over time then levelling off. The drop on day 5 was suspected to be because of instrument response as the QC plot indicated the

compounds were close to the warning or action limits (Appendix F, page 184, injection 23), the data was kept in but treated with caution (Figure 5.18). All the medium toast malt distillate samples had peak areas above those in the commercial samples (0.002-0.46), however, the heavy toast malt distillate samples up to day 6 (0.21-0.45, excludes day 2) were within the commercial sample range. The corn distillate samples (medium and heavy toast) were similar to commercial samples with the day 6 peak areas (0.09-0.16) being similar to several Single Scotch malt whiskies; Glenlivet (0.10), Laphroaig (0.16), Glenfiddich (0.15) and Jura (0.12). These results showed the %ABV was important in extracting a similar level of 1,1-diethoxyethane to commercial samples because the corn distillate (40%) was in the same range as the commercial samples but the malt distillate (65%) extracted too much.

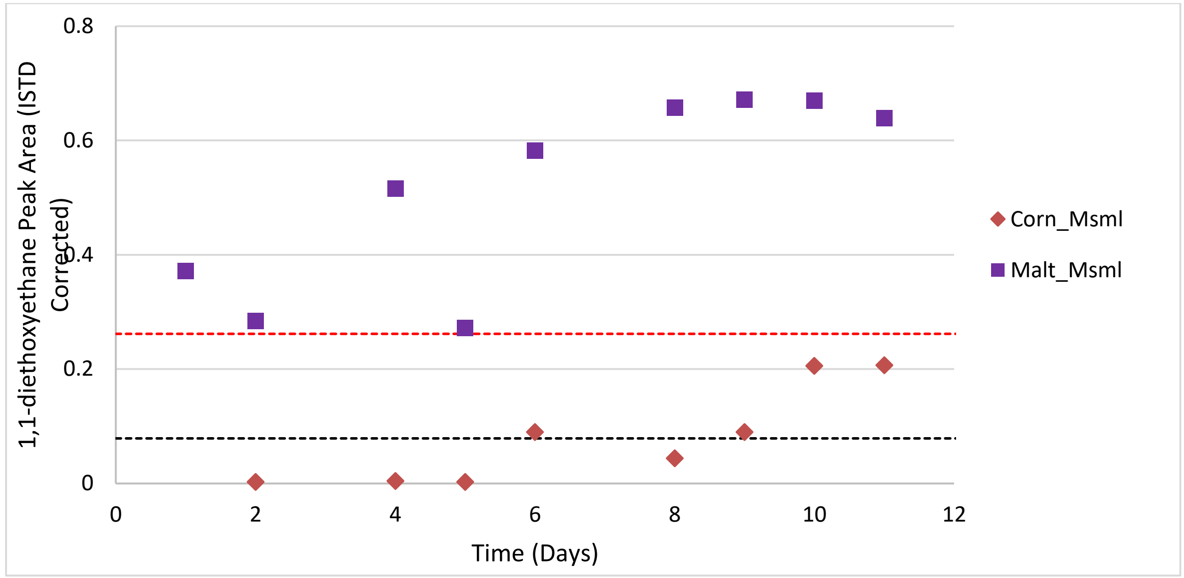

Figure 5.18: Changes in peak area for 1,1-diethoxyethane in the medium toast corn distillate (orange diamond) and malt distillate (purple square) over 11 days stored at 70 °C. The drop in response on day 5 was suspected to be because of instrument response, compounds in the QC plots were close to the warning or action limits. The average (0.08, black dashed line) and maximum (0.26, red dashed line) 1,1-diethoxyethane peak areas for commercial samples are indicated on the graph.

Furfural was relatively consistent in the malt distillate samples, whereas the corn distillate samples had large increases and decreases throughout the experiment (Figure 5.19). As previously mentioned (section 5.2.1.1), the presence of furfural in whiskey arises from Maillard reactions and also from the addition of caramel colouring (E150A) [12,28,123,143]. The furfural present in the samples was due to Maillard reactions because no colouring was added. It was noted again that the %ABV seemed to influence the extraction of furfural because the corn distillate samples (day 1-6) were within the commercial samples peak area range

(0.004-0.13). Whereas the malt distillate samples were mostly outside the range observed for commercial samples; except for day 2 and 5 in the medium toast (Figure 5.19). The furfural peak areas in the medium and heavy toast malt distillate samples (0.15 ± 0.02, n = 18) were similar to the medium and heavy toast ethanol samples (0.25 ± 0.09, n = 24, section 5.2.1). Whereas the furfural peak areas in the medium and heavy toast corn distillate samples (0.09 ± 0.06, n = 18) were similar to the light toast ethanol samples (0.07 ± 0.03, n = 12, section 5.2.1).

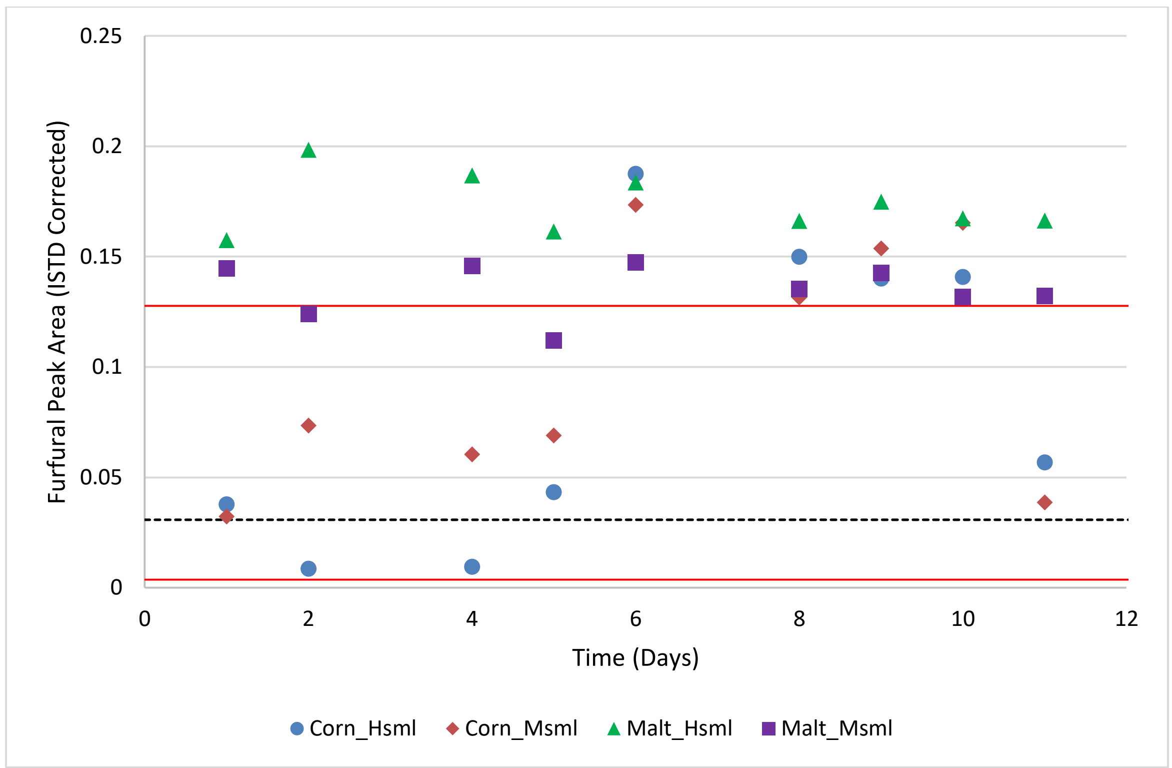

Figure 5.19: Furfural peak areas for the corn distillate medium (orange diamond) and heavy toast (blue circle) samples and malt medium (purple square) and heavy toast (green triangle) samples stored at 70 °C for 11 days. The malt samples stayed relatively constant from day 1-11. The corn samples had large increases and decreases over the 11 days with some levelling out occurring at day 8-10. It was noted the commercial sample average (0.04, black dashed line) was fairly low, however, this was due to the number of samples which had low peak areas. The minimum and maximum furfural peak areas in commercial samples are displayed on the graph (red lines, 0.004 and 0.128, respectively).

Phenols

There was a slight increase in the peak area for 2,6-dimethoxyphenol from day 1-11 in the medium toast corn and malt samples (0.01-0.03), whereas it remained constant in the heavy toast corn and malt samples (0.01 ± 0.01, n = 18). Lahne [116] reported that 2,6-dimethoxyphenol is a key aroma compound in American rye whiskey, however, it was not detected in any commercial samples analysed within the current research. These results showed

the medium toast was better at extracting 2,6-dimethoxyphenol, which is the opposite trend reported in the ethanol heat results (section 5.2.1).

Guaiacol was detected in medium and heavy toast malt distillate samples on day 9-11. Guaiacol was only detected in the heavy toast corn distillate samples on day 9-11 and was not detected in the medium toast. The toasting level as well as the distillate type played a role in the extraction of guaiacol. Higher toasting levels produce more guaiacol [28] and Lahne [116] reported guaiacol was one of the key aromas in American rye whiskey, with rye being closely related to barely [144]. Guaiacol is described as sweet, phenolic and smokey, it is a component of peat smoke and a product of lignin pyrolysis [28,50,116,145]. Quantitative analysis of commercial samples showed all concentrations were < LoD (0.16 ppm). However, the calculated LoD may have been conservative because qualitative analysis showed guaiacol was detected in 8% of commercial samples. Guaiacol has been reported in literature at low concentrations: Jim Beam (0.06 ppm) and 18-year-old Macallan single malt Scotch (0.18 ppm) and higher concentrations in 10-year-old Laphroaig (1.57 ppm), Wild Turkey (3.76 ppm) and Rittenhouse (3.15 ppm) [28]. Therefore the concentrations obtained throughout the experiment (< LoD-0.3 ppm) were acceptable; it would come down to the client's personal preference on how dominant they required the smokey aroma to be.

Esters

Esters are formed during fermentation and also during maturation from the reaction between ethanol and acids [28,59,139]. The temperature has been shown to affect ester production, if the temperature is too high it causes volatilisation and ester hydrolysis [139]. Ester production is also affected by the pH; the lower the pH, the more acid available to react to form esters [28].

Ethyl butyrate and ethyl hexanoate were not detected in the corn distillate samples. Ethyl hexanoate was detected in the malt distillate throughout the experiment but the peak area did not change (0.04 ± 0.01, $n = 18$); it was detected in 27% of commercial samples and the malt distillate peak areas were similar to Glenfiddich (0.02) and Clynelish (0.03), which were both ≥ 15-year-old single malt Scotch whiskies.

Ethyl octanoate (0.12 ppm), ethyl laurate (0.20 ppm) and ethyl decanoate (0.40 ppm) were present in the malt distillate before treatment, while only ethyl decanoate was present in the corn distillate (0.21 ppm) before treatment. The malt distillate was a better starting matrix due to the presence of several esters prior to age treatment.

In the malt distillate samples, minor changes in the ethyl octanoate and ethyl laurate concentrations were observed while ethyl decanoate had larger changes over time (Figure 5.20). These results also showed that time and temperature had an effect on ester formation/concentrations, especially for ethyl decanoate. The concentrations for ethyl octanoate and ethyl decanoate in the day 6 and 8 (0.12-0.16 ppm) malt distillate samples were similar to commercial samples, all other time points had higher concentrations. Conversely, the ethyl laurate concentration (0.10-0.20 ppm) throughout the experiment (excluding day 4-5) were within the commercial sample range (0.01-0.21 ppm) and were similar to several ≥ 12-year-old samples (0.10-0.19 ppm).

In the corn distillate samples, the absence of ethyl octanoate and ethyl laurate was most likely due to the requirement to react with wood compounds (e.g. octanoic acid) which were not extracted [137]. Ethyl decanoate started with a concentration of 0.21 ppm in the corn distillate and increased to a maximum concentration of 0.29 ± 0.003 ppm (n = 4) on day 4-5. After this time the concentration decreased from day 6-11 to an average of 0.14 ± 0.005 ppm (n = 10) which was similar to several commercial samples including; Wild Turkey (0.10 ppm), Glenlivet (0.12 ppm), Jim Beam (0.08 ppm) and Laphroaig (0.12 ppm). The decrease may have been due to hydrolysis [139] suggesting samples should not be stored at 70 °C for more than 5 days.

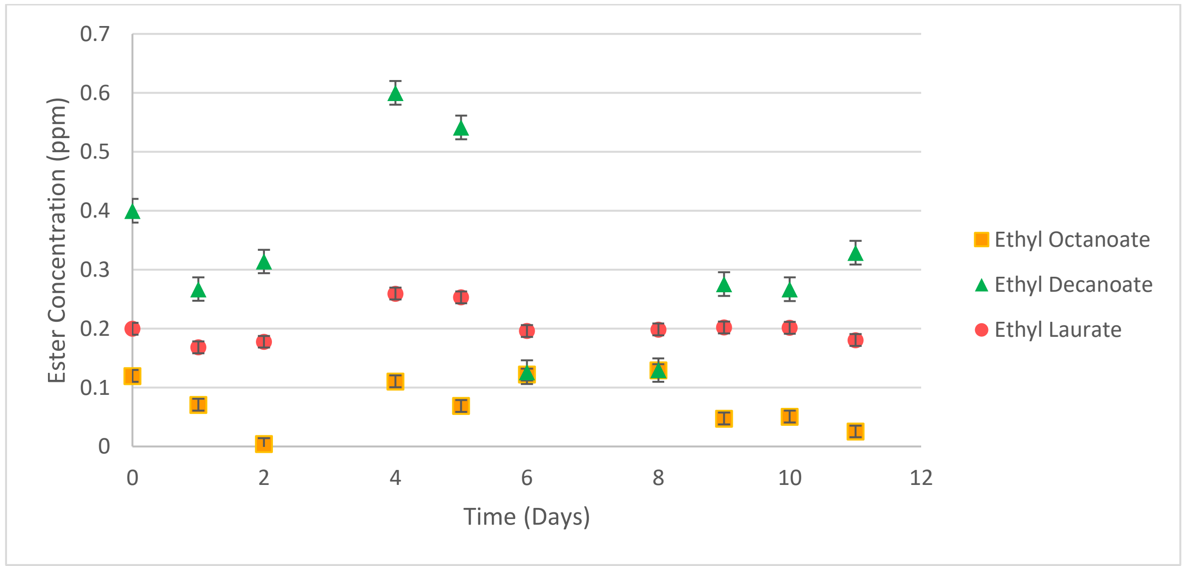

Figure 5.20: Change in concentration for ethyl octanoate, ethyl decanoate and ethyl laurate in the heavy toast malt distillate samples stored at 70 °C for 11 days. Error bars have been added using the standard deviation calculated in the malt distillate for ethyl octanoate, ethyl decanoate and ethyl laurate (n = 3, section 4.1.2) as a proxy measurement of instrument variation overtime.

Isoamyl acetate was present in the malt distillate prior to treatment (0.66 ppm) and had an overall downward trend during the 11 day experiment (Figure 5.21). The formation of isoamyl acetate is from the reaction of isoamyl alcohol and acetic acid and is desirable because of its fruity (banana) aroma [9,28]. Isoamyl acetate was not detected in the corn distillate and as previously mentioned (section 4.2.1) the availability of the relevant higher alcohol determines the formation of some esters [63]; there was a high abundance of isoamyl alcohol in both distillates. Therefore, the absence/decrease of isoamyl acetate in the corn and malt distillates, respectively, was proposed to be because other compounds (e.g. ethanol) were preferentially reacting with acetic acid over isoamyl alcohol in both the corn and malt distillates.

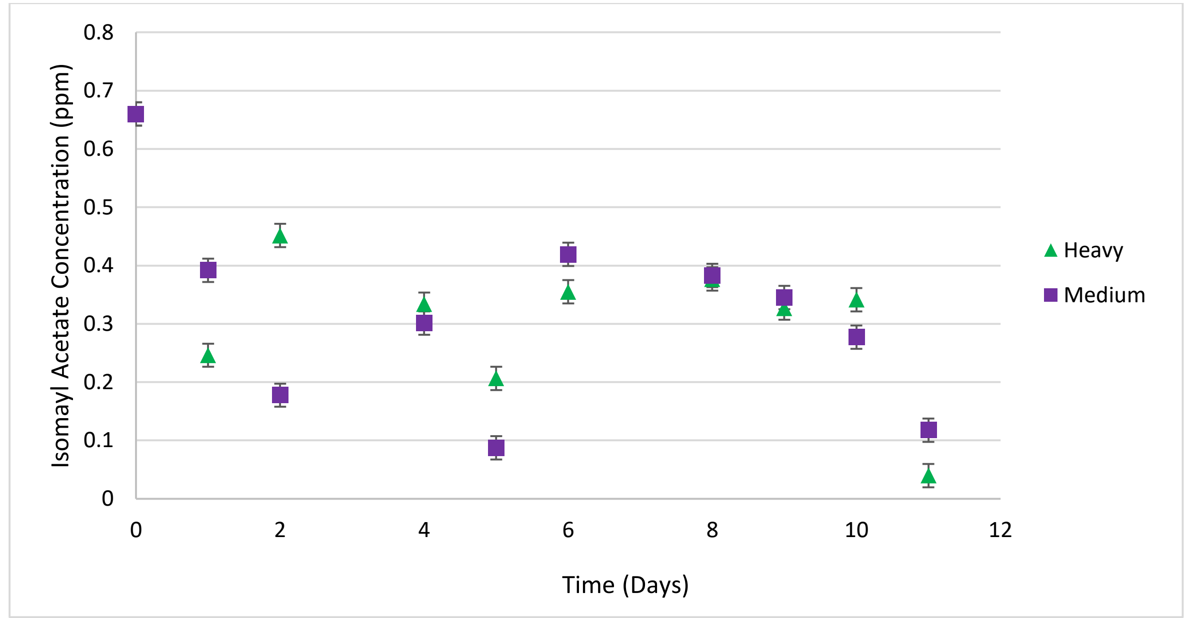

Figure 5.21: Change in isoamyl acetate concentration in the medium (purple square) and heavy (green triangle) toast malt distillate samples over the 11 day experiment. Error bars have been added using the standard deviation calculated in the malt distillate for isoamyl acetate (n = 3, section 4.1.2) as a proxy measurement of instrument variation overtime.

Isobutyl acetate and 2-methylbutyl acetate were present in the malt distillate prior to treatment (Appendix K, Table K.2, page 196). Their aroma and flavour are described as sweet and fruity [50]. The trend for the heavy toast samples differed to the medium toast, however, after 11 days both compounds had the same peak area as they started with (Figure 5.22). Isobutyl acetate was not detected in commercial samples, but 2-methylbutyl acetate was detected in 6% of samples. The peak areas for 2-methylbutyl acetate (0.02-0.06) were much higher than commercial samples (0.002-0.007).

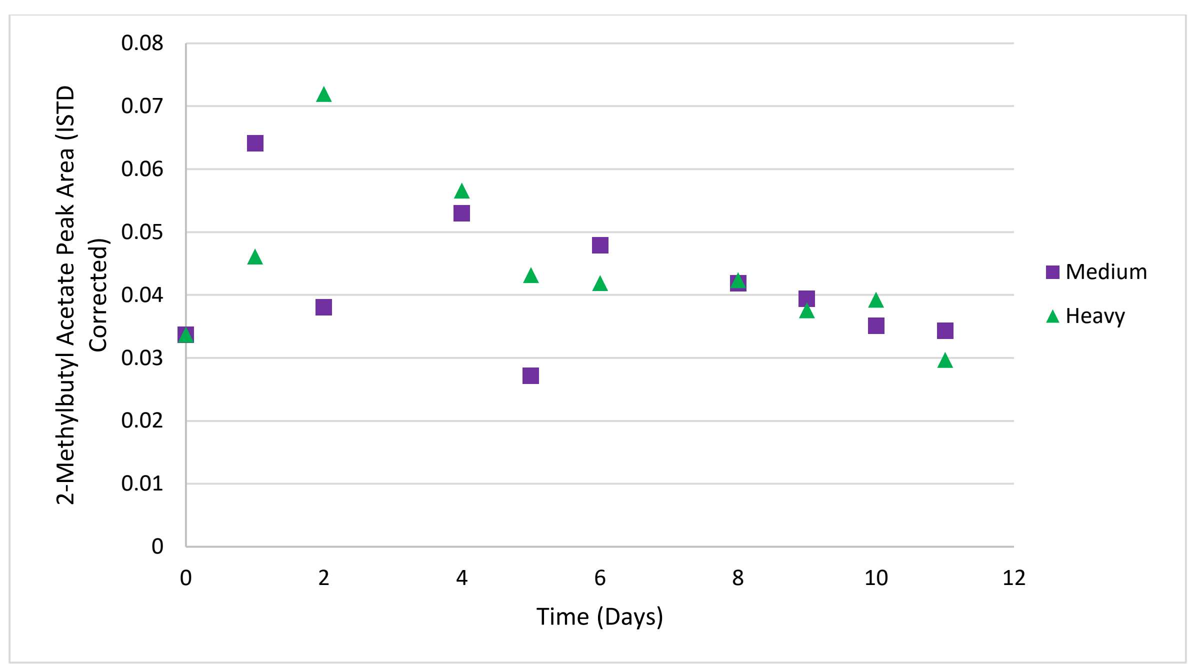

Figure 5.22: Minor peak area changes in the medium and heavy toast malt distillate samples for 2-methylbutyl acetate over 11 days of storage at 70 °C.

Alcohols

Isoamyl alcohol was detected at high concentration in the corn (14.3 ppm) and malt (15.5 ppm) distillates before heat treatment. There was variation in the concentration of isoamyl alcohol in the corn and malt distillates throughout the experiment (Figure 5.23). From day 6, the isoamyl alcohol concentration in the malt distillate (medium and heavy toast) remained fairly constant (8.89 ± 0.52 ppm, $n = 10$) which is expected because it is a product of fermentation [21,28,116]. The initial decrease in both distillates was most likely due to the absorption into the wood, this was demonstrated by Conner *et al.* [126], Coelho *et al.* [136] and by Allen, 1897, as cited in Miller [28]. Despite the difference between the traditional trend and the heat trend, a decrease in concentration in both the corn and malt distillates was desirable because not only does it have a pungent odour, but also isoamyl alcohol along with other higher alcohols (propanol, butanol and 2-methyl-1-butanol) are known to cause headaches and hangovers [21]. The concentrations in all samples fell within the wide concentration range in commercial samples (0.21-23 ppm) with the day 6 results being similar to 27% of samples including; Glenlivet (8.56 ppm), Jim Beam (9.04), Glenfiddich (6.77) and four ≥ 15-year-old single malt Scotch whiskies (6.6-8.8 ppm).

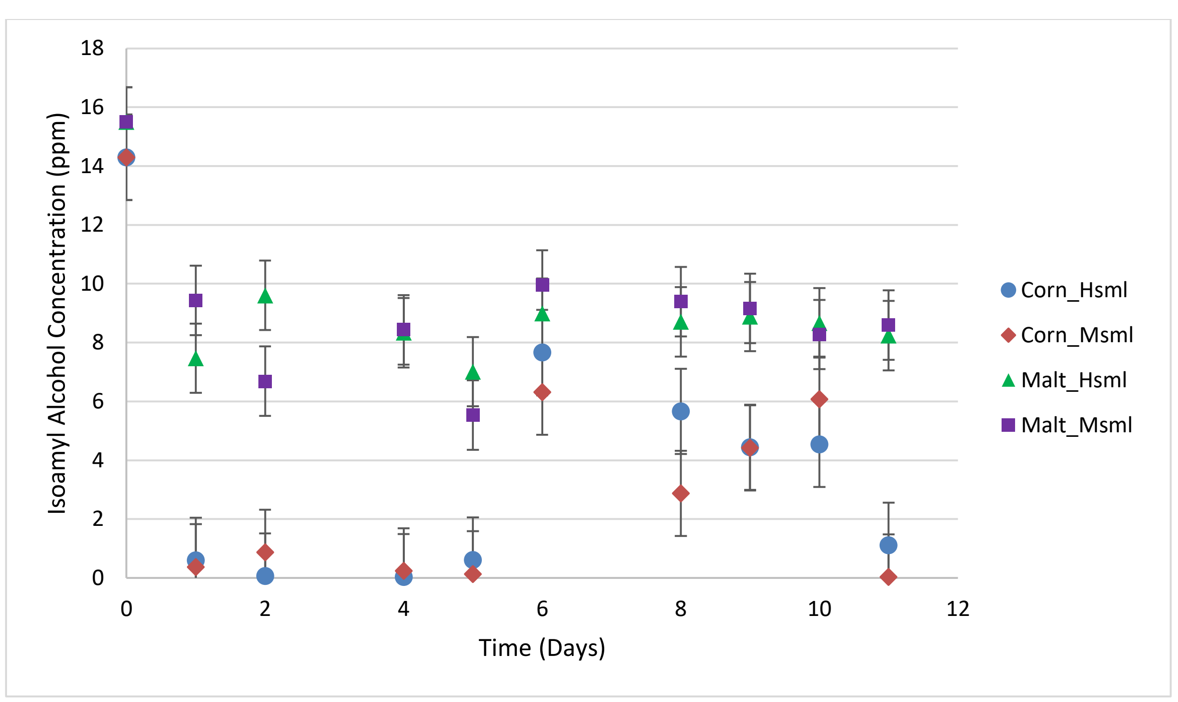

Figure 5.23: Isoamyl alcohol concentrations from day 0-11 in the corn and malt distillate samples. Error bars have been added using the standard deviation calculated in the corn and malt distillates for isoamyl alcohol (n = 3, section 4.1) as a proxy measurement of instrument variation overtime.

A compound often detected along with isoamyl alcohol is 2-methyl-1-butanol at a general ratio of 3:1 [1]. The ratio could not be calculated because only qualitative analysis was carried out for 2-methyl-1-butanol; future work would include quantitation of this compound to allow isoamyl alcohol and 2-methyl-1-butanol ratio calculations. Similar trends to isoamyl alcohol were seen for 2-methyl-1-butanol, the corn distillate varied considerably whereas the 2-methyl-1-butanol variation in the malt distillate was less pronounced. The peak area for 2-methyl-1-butanol in the corn and malt distillates before treatment was 0.62 and 1.2, respectively, and increased to a maximum peak area of 1.1 and 2.2, respectively. Rapp *et al.*, as cited in Dumitriu (Gabur) *et al.* [139] reported the increase in 2-methyl-1-butanol and isoamyl alcohol in samples with wood chips was due to acid catalysed ester hydrolysis. As previously mentioned 2-methyl-1-butanol along with several other higher alcohols are known to cause headaches and hangovers [21], therefore, low levels are desirable. The majority of results were within the commercial sample peak area range (0.04-1.9) with only the medium toast malt distillate sample prior to day 6 being above this.

Isobutanol peak areas increased from day 1-11 in the malt distillate samples; the medium toast (1.80) was higher than the heavy toast (1.55) sample on day 11. The increase was hypothesised to be from conversion of other butanol isomers (four isomers exist: *n*-butanol, *sec*-butyl alcohol,

tert-butyl alcohol and isobutanol) to the isobutanol isomer [21,28]. An increase over time was also seen in the heavy toast corn distillate sample whereas an overall decrease was seen in the medium toast sample. The decrease in isobutanol was suspected to be because of the increase in isobutyl acetate, formed by the reaction of isobutanol and acetic acid [146]. The peak area range for the corn distillate samples from day 1-11 (0.01-0.85) fell within the commercial sample range (0.03-1.36), however, only day 5 in the malt distillate samples (medium = 0.97; heavy = 1.35) were within the commercial sample range.

The concentration for 2-phenylethanol increased over time for all samples (corn and malt). Not only is 2-phenylethanol produced by the fermentation of yeast, it is also extracted from wood [28]. The corn distillate samples increased more rapidly than the malt distillate samples (Figure 5.24) because 2-phenylethanol was already present in the corn distillate (0.21 ppm) before treatment. On day 11, the concentration for 2-phenylethanol in the corn distillate was 0.21-0.25 ppm and in the malt distillate it was 0.20 ppm. The day 6 corn distillate results (0.14-0.15 ppm) were similar to two blended whiskies, Chivas Regal (0.15 ppm) and a New Zealand whiskey, Thompson (0.15 ppm). The concentrations for all samples (corn and malt) on day 11 (0.22-0.25 ppm) were similar to several commercial samples including; Wild Turkey, Glenrothes, Glenfiddich and Clynelish.

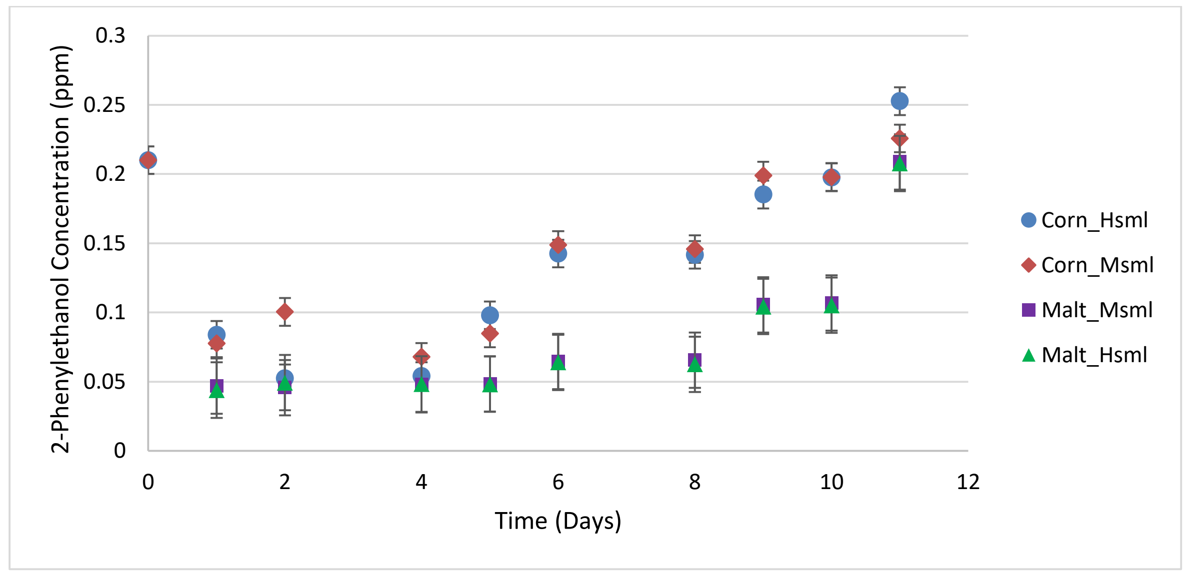

Figure 5.24: Concentration increase for 2-phenylethanol in the medium and heavy toast corn and malt distillate samples stored at 70 °C for 11 days. Error bars have been added using the standard deviation calculated in the corn distillate for 2-phenylethanol (n = 3, section 4.1.1) as a proxy measurement of instrument variation overtime. As 2-phenylethanol was not detected in the malt distillate (before treatment), the calculated standard deviation for 2-phenylethanol in the method development (n = 9, section 3.3.2) was used for error bars.

5.2.2.1. Small Chips Summary

The day 6 results for ethyl acetate, isoamyl alcohol, 2-phenylethanol, syringaldehyde, vanillin and 1,1-diethoxyethane in the corn distillate were at similar concentrations to several commercial samples; 21-year-old Glenfiddich, 10-year-old Jura, Ardbeg and 15-year-old Dalwhinnie. The day 6 results for isoamyl alcohol, ethyl octanoate, syringaldehyde and vanillin in the malt distillate had similar concentrations to 21-year-old Glenfiddich, 10-year-old Jura and Jim Beam. These results helped further narrow down the timeframe (to 6 days) for heating at 70 °C, however, it also showed that despite several compounds in each distillate having similar concentrations/peak areas to commercial samples, they still did not have the overall desired composition. To achieve the desired concentrations/peak areas for some compounds (e.g. furfural, isobutanol and esters) it meant low or undesirable concentrations/peak areas for other compounds (e.g. syringylacetone, 1,1-diethoxyethane, 2-methylbutyl acetate, 2-phenylethanol).

5.2.3. Wood Shavings in Corn Distillate

Singleton and Draper reported that extraction of low molecular weight compounds from wood in various grape neutral spirit solutions (wine alcohol, 0-95% ABV) at room temperature occurred faster using oak meal (ground up wood) as opposed to using oak chips [25,147]. An experiment was set up to determine whether the use of shavings extracted compounds faster than chips due to the higher surface area to volume ratio. Medium and heavy toast wood shavings submerged in corn distillate (malt distillate was unavailable at the time of the experiment) were stored at 70°C for 17 days. Sampling was carried out daily for the first 4 days and again on day 17 because it was expected that the extraction of compounds would occur rapidly then level off based on the results from section 5.2.1and 5.2.2. The experiment was carried out with two different quantities of shavings (0.40 g and 1.00 g); these samples had a 3 and 7.5-fold increase (0.40 g and 1.00, respectively) compared to traditional maturation (section 5.1.2). This was to compare the effect of different surface areas and determine if there was a difference in the concentration/peak area of extracted compounds when more shavings were used.

The GCMS chromatograms for all of the shaving samples did not detect many compounds for the duration of the experiment. There were two unidentified compounds (RT = 5.29 and 17.27 min) detected in the 1.00 g sample on day 17 but the peak areas were too small for the NIST library to provide a match. Table 5.2 summarises the compounds present at the beginning

and end of the experiment; these results are discussed in detail below. There was a mild whiskey aroma in the 1.00 g medium toast shavings at the end of the experiment, although there was an over-powering alcohol scent in all shavings samples.

Table 5.2: Summary of initial and final peak area or concentration (ppm) for compounds detected in 70 °C heat experiment with various masses of wood shavings at either medium or heavy toasting.

	Sample					
	0.40 g Medium Toast		**0.40 g Heavy Toast**		**1.0 g Medium toast**	
Compound	Day 0	Day 17	Day 0	Day 17	Day 0	Day 17
Peak area (ISTD corrected)						
Acetic Acid	n.d.	0.15	n.d.	0.14	n.d.	0.17
2-methyl-1-butanol	0.04	0.13	0.04	0.14	0.04	0.07
1,1-diethoxyethane	n.d.	0.03	n.d.	0.03	n.d.	0.03
Concentration (ppm)						
Ethyl Acetate	0.53	n.d.	0.53	0.07	0.53	0.19
Syringaldehyde	n.d.	n.d.	n.d.	0.54	n.d.	0.55
Vanillin	n.d.	n.d.	n.d.	n.d.	n.d.	0.30
Ethyl Decanoate	0.21	n.d.	0.21	n.d.	0.21	n.d.
Isoamyl Alcohol	14.3	0.58	14.3	1.3	14.3	1.92
2-phenylethanol	0.21	0.11	0.21	0.13	0.21	0.09

n.d. Not detected

Acetic Acid and Ethyl Acetate

The concentration of acetic acid increased over the 17 day period, however, the trend differed between the 0.40 g medium and heavy toast shavings as well as between the 0.40 g and 1.00 g medium toast shavings (Figure 5.25). Acetic acid in the 0.40 g medium toast sample increased rapidly from day 1-4 followed by a slower increase up to day 17 (logarithmic trend, $R^2 = 0.96$) which was thought to be due to the equilibrium of $acetic\ acid + ethanol \rightleftharpoons ethyl\ acetate$ and/or exhausted extraction from wood [28]. The trends seen in this experiment (for all samples) were similar to the trend seen in the ethanol and small chips heat experiments (section 5.2.1 and 5.2.2). The initial decrease in the 0.40 g heavy toast and 1.00 g medium toast samples was most likely due to the reaction of acetic acid with ethanol to form ethyl acetate, though the overall increasing trend followed a similar trajectory to traditional maturation [30]. The initial difference in acetic acid peak area between the 0.40 g medium and heavy shaving samples can be explained by the toasting temperature. Toasting temperatures of 100-200 °C (light-medium toast) generate acetic acid, while toasting temperatures of 200-280 °C (heavy toast) release carbon dioxide, acetic acid and water [148]. It was hypothesised that the toasting time

(30 minutes) was sufficient to generate acetic acid (for later extraction), however, the toasting time was not long enough for the release of acetic acid (prior to submerging into distillate). Comparison to the small chips results showed the acetic acid range overlapped but the shavings (0.14-0.17) were lower compared to the small chips (0.12-0.36). This comparison showed acetic acid was higher in the small chips but it took several days to surpass the shavings.

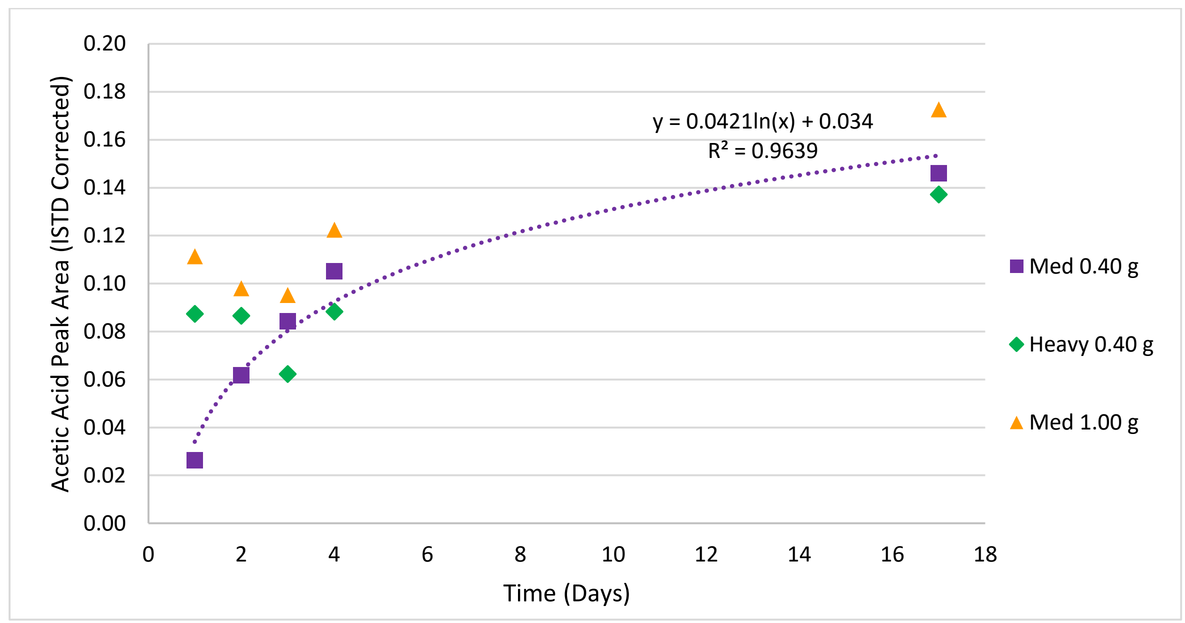

Figure 5.25: The change in acetic acid peak area for medium (0.40 and 1.00 g) and heavy toast (0.40 g) shavings over 17 days stored at 70 °C. The 0.40 g medium toast shavings showed a different trend to the other two samples. A logarithmic trend line was added for the 0.40 g medium toast shavings showing an R^2 of 0.96.

The decrease in acetic acid from day 1-3 in the 0.40 g heavy and 1.00 g medium toast could have be due to the formation of ethyl acetate but could not be confirmed because ethyl acetate was < LoD (1.45 ppm) throughout the experiment. These results showed the ethyl acetate LoD was too high for quantitation of ethyl acetate despite considerably higher concentrations reported in literature.

Phenolic Aldehydes

Syringaldehyde was detected in the 1.00 g medium toast sample on day 1 at 70 °C and the concentration stayed constant (0.54 ± 0.01 ppm) over time. The absence of syringaldehyde in the 0.40 g medium toast sample suggested the amount of shavings (surface area) was a factor in the extraction of syringaldehyde [20,21,28]. Syringaldehyde was detected on day 17 in the 0.40 g heavy toast sample at the same concentration as the 1.00 g medium toast sample (0.54 ppm) on day 1. It should be noted that it was unknown whether syringaldehyde was

present between day 4 and 17 in the heavy toast sample due to no sampling between these days. A toasting temperature around 200-220 °C (heavy toast) should extract more syringaldehyde than ~150 °C (medium toast) [28]. Therefore, the detection of syringaldehyde in the 1.00 g medium toast shavings prior to detection in the 0.40 g heavy toast shavings was due to the increased surface area. The syringaldehyde concentrations in the 0.40 g heavy toast and 1.00 g medium toast samples were similar to 50% of commercial samples analysed (0.54 ± 0.10 ppm, n = 19) but lower than the range reported in literature (0.67-5.96 ppm) [21,64].

Vanillin was only detected in the 1.00 g medium toast sample on day 17 (0.30 ppm), which was similar to the concentrations detected in commercial samples (0.28-0.34 ppm). The concentration on day 17 was similar to the day 1 concentration (0.34 ppm) in the small chips heat experiment (section 5.2.2) which suggested a larger quantity of wood was required to extract vanillin. The level of toasting also affected vanillin. Toasting wood releases more phenolic aldehydes compared to charring [28]; the heavy toast shavings were more charred than toasted due to the narrow dimensions, therefore, vanillin was higher in the medium toast.

The phenolic aldehydes were compared with the results from the small chips heat experiment. Syringaldehyde and vanillin were detected on day 4 in the small chips samples (0.71-0.73 and 0.35-0.36 ppm, respectively) whereas only syringaldehyde was detected in the 1.00 g medium toast shavings (0.54 ppm). On the final day of each experiment (shavings = day 17 and small chips = day 11) the syringaldehyde concentration in the shavings had not changed (0.55 ppm) whereas syringaldehyde was higher in the small chips (0.65-0.69 ppm). Vanillin was detected on day 17 in the 1.00 g medium toast shavings (0.30 ppm) which was lower than in the day 11 small chips samples (0.40-0.42 ppm). This demonstrated the wood size, surface area and toast level affected the extraction of phenolic aldehydes.

Miscellaneous Compounds

1,1-Diethoxyethane was detected in the 0.40 g heavy toast sample on day 2 and in the medium toast samples (0.40 and 1.00 g) on day 3; the peak area increased over the 17 day experiment for all samples. The peak areas for all samples from day 1-4 (0.003-0.026) were within the commercial sample peak area range (0.002-0.260) with similarity to a number of whiskies including, Glenlivet (0.003), Jameson (0.011, n = 2), Jack Daniels (0.011) and 12-year-old Chivas Regal (0.012). On day 17, the peak areas (0.03 ± 0.003) were only similar to Glengrant (0.035) and Laphroaig (0.030). This suggests 1-4 days was sufficient to obtain similar levels of 1,1-diethoxyethane to several well-known brands. The peak areas for 1,1-diethoxyethane on

day 4 in the 0.40 g shavings samples (0.004-0.01) were similar to the peak areas for the small chips samples (0.003-0.004) but the 1.00 g medium toast shavings was the highest (0.03). This suggested 1,1-diethoxyethane was easier to extract from shavings. On day 17 the peak areas in all shaving samples was 0.02-0.03, whereas it was much higher in the small chips (0.20). Based on these results, it was proposed that the equilibrium between acetaldehyde, hemiacetal and 1,1-diethoxyethane (section 5.2.1, Figure 5.8) was reached quicker with the shavings. Nevertheless, the shavings (0.004-0.03) were within the commercial sample range (0.002-0.260). A lighter amber colour was produced (compared to the small chips), therefore, shavings were preferable for 1,1-diethoxyethane extraction.

Esters

Ethyl decanoate was present in the corn distillate (0.21 ppm) prior to the shavings heat experiment. However, it was not detected during the experiment, the reason for this is unknown.

Alcohols

The isoamyl alcohol concentration remained constant for all samples in the shavings experiment (Figure 5.26) after the initial decrease. The decrease was likely due to its absorption into the wood [28,136] and/or reaction with acetic acid to form isoamyl acetate [9,28]. This reaction could not be confirmed because isoamyl acetate was not detected over the course of the experiment, potentially because of its LoD (0.26 ppm). The isoamyl alcohol concentrations for all of the shaving samples (0.60-1.92 ppm) were similar to several commercial samples (1.49 ± 0.34 ppm, n = 5).

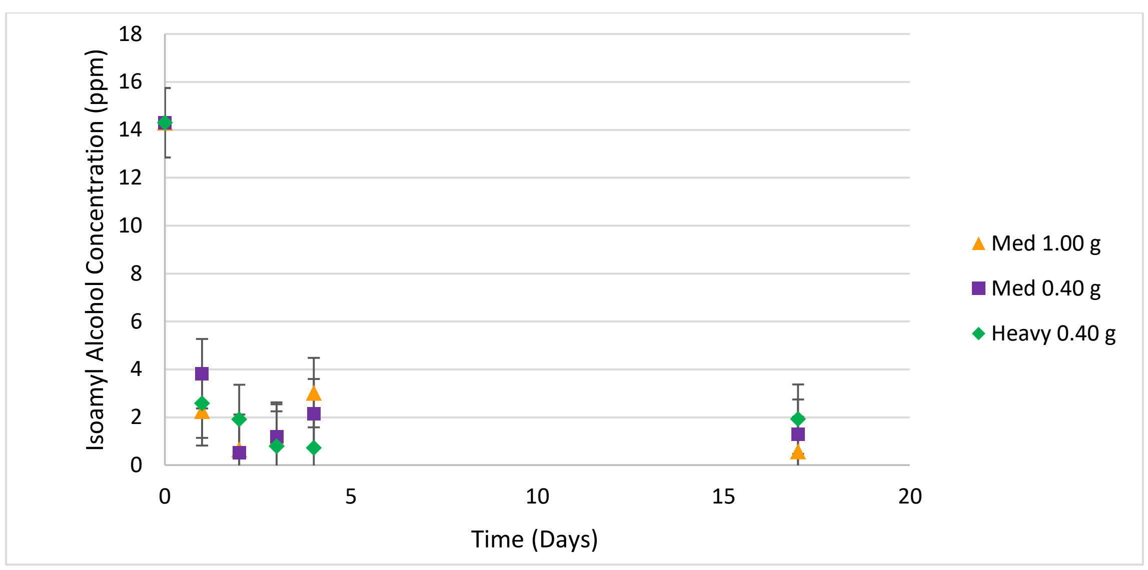

Figure 5.26: Isoamyl alcohol concentration over 17 days in the 0.40 g medium toast (purple square), 0.40 g heavy toast (green triangle) and 1.00 g medium toast (orange triangle) samples in the 70 °C shavings heat experiment. Error bars have been added using the standard deviation calculated in the corn distillate for isoamyl alcohol (n = 3, section 4.1.1) as a proxy measurement of instrument variation overtime.

An overall decrease in 2-phenylethanol (0.21 ppm to < 0.16 ppm) was seen in all shavings samples over the course of the experiment (Figure 5.27) but this trend was the opposite of the one seen in the small chips heat experiment (section 5.1.2). The decrease in concentration in the shavings samples aligned with what occurs during traditional ageing [28]. Besides the absorption of 2-phenylethanol into the wood [136], the decrease could also be due to 2-phenylethanol reacting with acetic acid to form phenethyl acetate [116]. Although, if phenethyl acetate was present, it was too low to detect. The initial decrease from day 0-2 followed by an increase in concentration on day 3 was the same pattern reported in a study by Martinez *et al.* [149] where naturally seasoned wood (exposed to climate changes) was stored in 12% ethanol solutions for 3 years and analysed yearly. Martinez *et al.* reported a decrease in 2-phenylethanol from 0-2 years followed by an increase in 2-phenylethanol after the third year. This indicated the changes seen in 2-phenylethanol during traditional maturation were occurring within 3 days of rapid maturation. The concentration of 2-phenylethanol in the shavings samples (0.09-0.13 ppm, n = 3) were lower than in the small chips samples at 11 days (0.23 and 0.25 ppm). Again the small chips were more similar to commercial samples which are ≥ 10 years old (0.23 ± 0.02 ppm, n = 3).

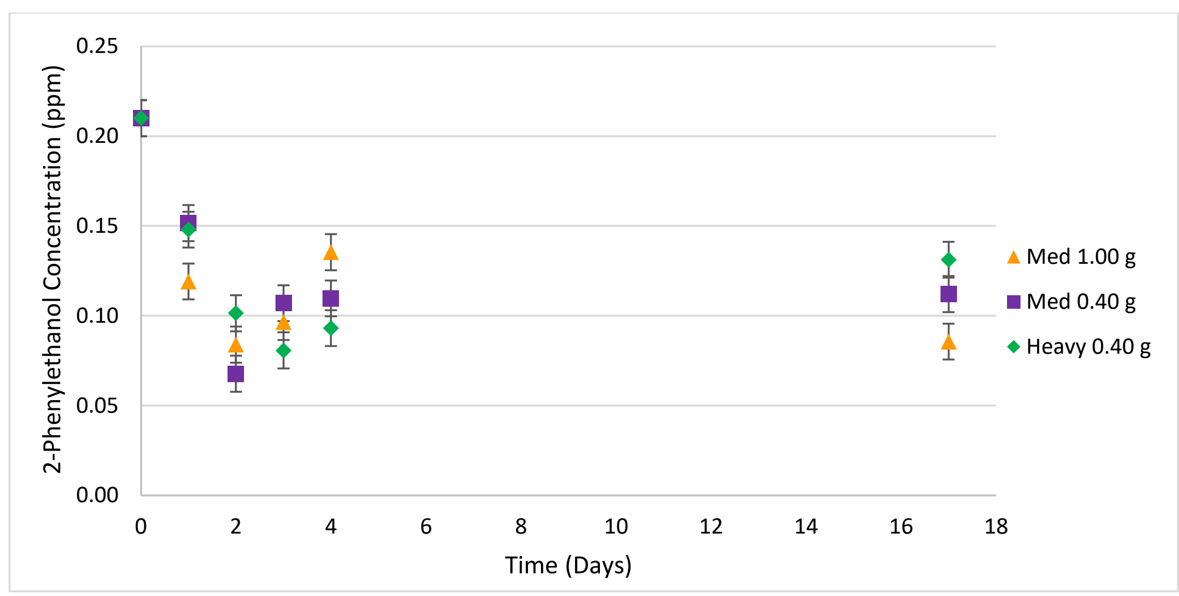

Figure 5.27: Concentration changes for 2-phenylethanol in all samples stored at 70 °C for 17 days. Error bars have been added using the standard deviation calculated in the corn distillate for 2-phenylethanol (n = 3, section 4.1.1) as a proxy measurement of instrument variation overtime.

5.2.3.1. Shavings Summary

The results showed there are only two advantages of using shavings; the extraction of 1,1-diethoxyethane was at similar levels to commercials samples (too high in small chips) and the colouration of the samples were similar to traditional whiskey (amber) whereas the small chips were darker. Overall, the shavings extracted desired compounds, however, the small chips extracted the compounds faster. Furthermore, it was difficult to remove the shavings from the solution because they disintegrated as the solution was heated; this would be even more difficult in the distillery with a large scale operation. It was thought that if shavings were used on a large scale that a filter/sieve could be used, however, there was no guarantee desired compounds would not absorb to the filter, further investigation would be required.

One of the objectives of this research (section 1.8) was to produce a chemical profile similar to ≥ 10-year-old whiskey but with its own unique flavours/aromas. Therefore, the absence of esters and smokey compounds (e.g. guaiacol) meant the shavings overall were not as effective as small chips at extracting compounds at desirable concentrations/peak areas (similar to commercial samples) in a short timeframe. Thus would not be used in further experiments.

5.2.4. Heat Summary

For the ethanol heat experiment, the samples stored at 70 °C extracted more of the desirable compounds in a shorter timeframe compared to the 50 °C. Ethyl acetate, syringaldehyde and 1,1-diethoxyethane had similar peak areas to several commercial samples including well-known brands like Glenfiddich, Laphroaig and Jim Beam.

In terms of toasting level, the medium toast provided the best results because it extracted desirable compounds with only a small number of unidentified compounds extracted. The colour of the medium toast samples were closer to the expected colour of whiskey (amber) compared to the heavy toast samples which showed the presence of wood breakdown. The light toasting was inadequate because it did not extract as many compounds, and for those it did extract, it took more than double the time compared to the medium and heavy toast samples.

The size of the wood affected the extraction of aroma and flavour compounds (e.g. vanillin and ethyl octanoate) as well as quality/age markers (e.g. vanillin, syringaldehyde and furfural) [122]. The small chips heat experiment with corn and malt distillate (section 5.1.2) had a faster increase in compound peak areas compared to ethanol. This was expected due to the more complex matrix and the presence of several compounds prior to treatment. Furthermore, many of the compounds detected in the small chips samples were only detected at the end of the shaving experiment, if at all, demonstrating that the shavings were not as suitable for use in rapid extraction. The small chips were better than the large blocks because the peak areas obtained for 1,1-diethoxyethane and furfural using the small chips were more similar to commercial samples.

The amount of time required to achieve the detection of desired compounds at concentrations/peak areas close to those found in commercial samples differed from compound to compound as well as between the corn and malt distillates. For example, the peak areas for 1,1-diethoxyethane in malt distillate samples with small chips (heavy and medium toasts) after 1 day at 70 °C were higher than commercial samples, whereas the corn distillate with small chips (heavy and medium toasts) samples from day 1-11 were within the commercial samples peak area range.

Overall, the medium toast small chips at 70 °C heat experiment with corn and malt distillates showed 6 days achieved concentrations similar to commercial samples for, ethyl acetate, syringaldehyde, vanillin, 1,1-diethoxyethane (excluding medium toast in malt distillate),

isoamyl alcohol, ethyl octanote (malt only), ethyl laurate (malt only) and ethyl decanoate as well as similar peak areas for acetic acid, 1,1-diethoxyethane (corn only), ethyl butyrate (malt only), ethyl hexanoate (malt only), 2-methyl-1-butanol (corn only) and isobutanol.

5.3. Light Experiment

Traditionally, prior to barrel construction the wood is left outdoors and is exposed to the varying weather conditions resulting in degradation of the wood's surface, this is termed weathering [25,68]. Solar radiation is the main environmental factor for weathering the surface of wood; water and heat (covered in section 5.2) are amongst several other environmental factors that affect the weathering of wood [68]. The focus of this experiment was to mimic solar radiation on wood within ethanol, corn distillate and a heat treated sample to determine the effects of light treatment. Exposure to a wavelength range of 450-600 nm for a given time was carried out for two purposes; firstly, to determine whether light would penetrate the wood and cause breakdown to extract lower molecular weight compounds such as phenolic aldehydes and secondly, to determine whether the light would also provide energy for reactions to occur (e.g. formation of esters). This treatment was of interest to the commercial company that the research was carried out for because Lost Spirits distillery have been successful (won awards) with producing whiskey using light exposure.

The rays from the sun penetrate the wood and are absorbed by polymeric components (cellulose, hemicellulose and lignin) or low molecular weight extractives (e.g. fatty acids and tannins) causing photodegradation by providing energy for photochemical reactions such as the cleavage of bonds within cellulose, hemicellulose and lignin. This results in the build-up of photodegradation products on the wood surface which is extracted when the wood comes into contact with liquid [68,150,151]. UV light (286-380 nm) degrades wood twice as fast as visible light (380-780 nm) because it is more energetic, therefore, it is able to cleave the bonds within the cellulose, hemicellulose and lignin. Visible light does not have the energy required to significantly degrade lignin, however, longer wavelengths of visible light can be used to degrade extractives of lower molecular weight such as fatty acids and tannins. A Growsaber LED tube light (40 W) with a wavelength range of 450-600 nm (visible spectrum) was provided by the commercial company and was used as the light source for this experiment. This wavelength range (450-600 nm) has been reported to increase the extraction of fatty acids and tannins which are precursors to desired compounds (e.g. esters) and also aid in lignin breakdown (releasing phenolic aldehydes) [68,152].

Lost Spirits [32] used light treatment as part of their maturation process and suggested samples should be exposed to a minimum of 2,280,000 lux hours. Lux hours based on the light used (40W) were calculated to estimate the minimum time required for exposure [32] – the light beam angle (120°), distance from the light (0.2 m) and light lumens (3200 lm) were used [153,154]. A total of 25,465 lux per hour could be achieved with the specified light. To achieve 2,280,000 lux hours, a minimum of 90 hours (specifically 89.5 hours) was required. The experiment was conducted over 15 days (360 hours) to monitor the changes in compound levels over an extended period to determine what length of exposure was required to achieve desired results. It should be noted that this experiment was conducted concurrently with the ethanol heat experiment and only qualitative analysis was carried out.

A maximum of five compounds (ethyl acetate, 1,1-diethoxyethane isobutanol, isoamyl alcohol and 2-methyl-1-butanol) were detected in all samples (ethanol and distillate) throughout the experiment. Review of the calibration standards and commercial samples which were analysed in the same batch as the light experiment samples revealed that there were instrument issues which caused problems with the detection of some compounds (e.g. esters). Recalibration (fresh standards) and re-analysis of the commercial samples (fresh aliquots) three days later showed more compounds were detected, further confirming instrument issues during the initial analysis. However, the light samples were not re-analysed due to volatile loss (samples were left over the weekend with pierced septa). Despite the detection issues, the samples were all analysed together under the same instrument conditions so qualitative analysis was carried out for the detected compounds and is briefly summarised below. The samples were compared to each other but not to the other maturation experiments or commercial samples.

Ethanol Samples

Light treatment had a minimal effect on all of the toast levels (light, medium and heavy) in both the large blocks and small chips in ethanol. Methylal (formaldehyde dimethyl acetal) was detected on day 1 of light treatment but after day 3 it was no longer detected. Methylal has a chloroform odour and has been found in alcoholic beverages but is undesirable [48,155]. In the heavy toasts samples (large blocks and small chips), 1,1-diethoxyethane was detected from day 4, but not until day 14 in the light and medium toast samples (large blocks and small chips). Ethyl acetate was detected in all samples on day 15.

Corn Distillate Sample

Corn distillate was exposed to light without wood blocks/chips to determine whether any reactions occurred in the presence of light and the absence of wood. Four compounds (ethyl acetate, isobutanol, isoamyl alcohol and 2-methyl-1-butanol) were detected in all of the corn distillate samples. All four compounds showed a linear increase ($R^2 = 0.82$-0.85) from 0-15 days (Figure 5.28) with isoamyl alcohol having a rapid increase (slope = 1.0926); day 8 results were excluded due to the absence of the ISTD. There were minor changes in the peak area for ethyl acetate whereas a large increase in peak area was observed for isoamyl alcohol and 2-methyl-1-butanol over time. The increase in isoamyl alcohol and 2-methyl-1-butanol was thought to be due to ester hydrolysis which was catalysed by light [139].

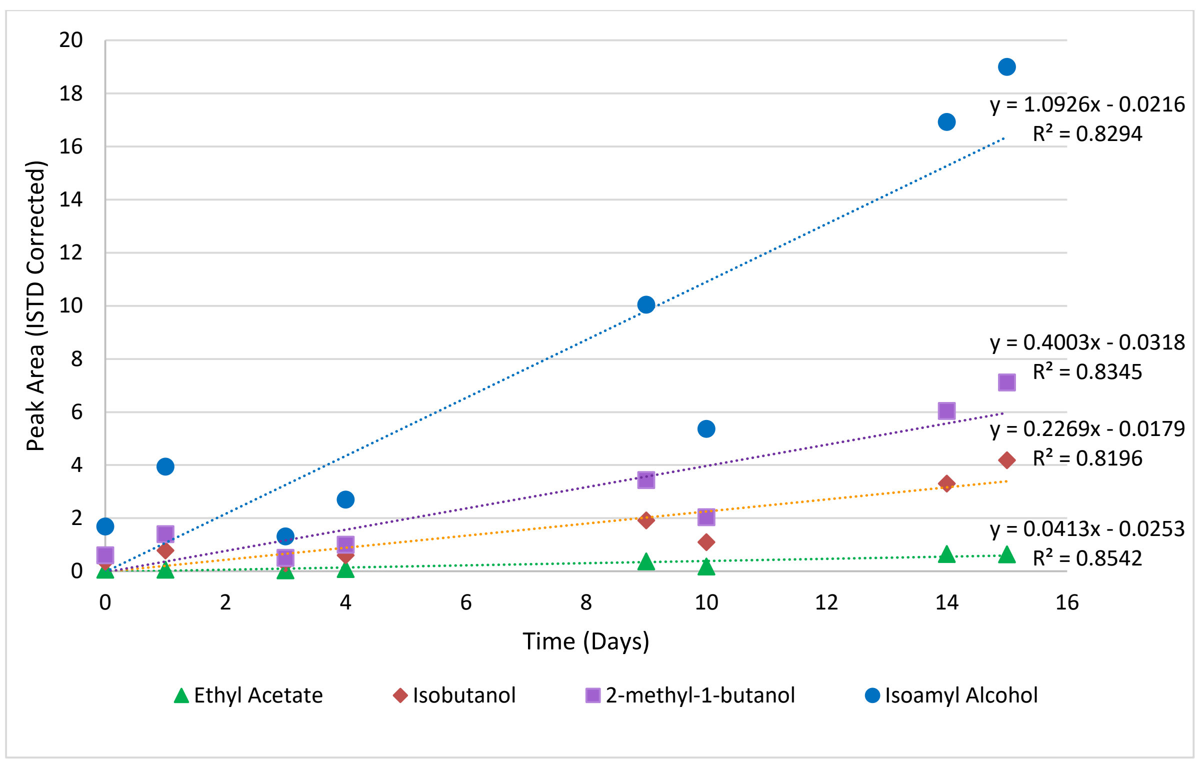

Figure 5.28: Changes in peak areas of corn distillate compounds over time in the light experiments. Day 8 was removed due to no ISTD addition. Increase in peak areas was linear for all compounds ($R^2 = 0.82 – 0.85$).

'Blended' Sample

A sample was prepared by mixing corn distillate and an ethanol heat treated sample (50 °C medium toast small chips) in a 1:1 ratio. The 'blended' sample was prepared to gain insight into the effects of light on a heat treated sample in the presence of fresh distillate compounds. Ideally, a 70 °C heat treated sample would have been used, however, due to evaporation during

the experiment (section 5.1.1), a 50 °C sample was used. The 'blended sample' was analysed before treatment and ethyl acetate, isobutanol, isoamyl alcohol and 2-methyl-1-butanol were detected. The absence of 2-phenylethanol in the 'blended' sample was due to the dilution of the fresh corn distillate (0.21 ppm 2-phenylethanol) which lowered the concentration to < LoD (0.10 ppm). Five compounds (ethyl acetate, isobutanol, isoamyl alcohol, 2-methyl-1-butanol and 1,1-diethoxyethane) were detected in the 'blended' sample from day 1 and showed similar trends to the corn distillate that was exposed to light. There was a small change in the peak areas of ethyl acetate and isobutanol. In contrast, isoamyl alcohol and 2-methyl-1-butanol showed a large increase, particularly from day 3-9 (Figure 5.29); this was the same trend seen in the corn distillate sample.

The absorption of light by compounds within the wood is reported to occur over many different wavelengths with a study conducted by Kataoka *et al.* [156] summarising that blue light (434-496 nm) penetrated the wood to a greater extent than violet light (specifically 403 nm), however, the energy was not sufficient to modify lignin. This means the samples would need to be exposed to a wider variety of wavelengths including ultra-violet light which is more energetic [66,68].

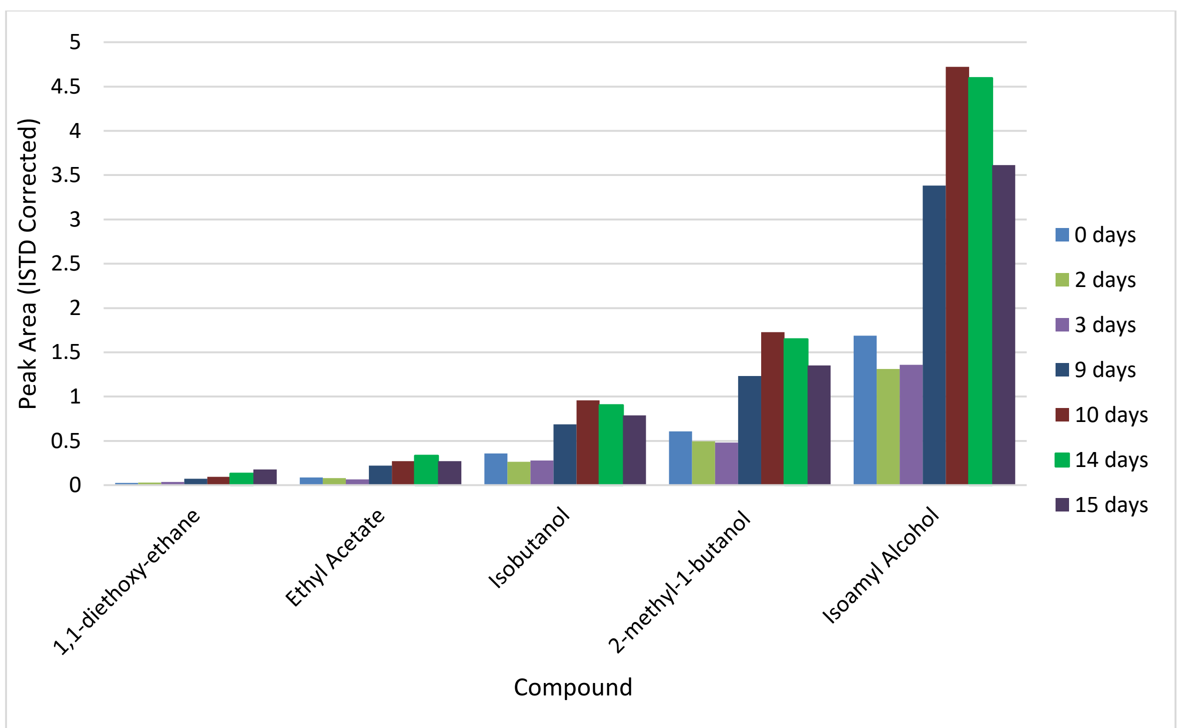

Figure 5.29: Results for the changes in compounds in the 'blended' sample (corn distillate and 50 °C heat treated sample blended at a 1:1 ratio) during 0-15 days of light exposure.

5.3.1. Light Summary

To reach the required 2,280,000 lux hours stated by Lost Spirits, samples needed to be exposed to the 40W GrowSaber light for 90 hours. The samples were exposed to light for 360 hours (15 days). There were minimal changes observed in the ethanol samples with only methylal, 1,1-diethoxyethane and ethyl acetate detected. The compounds which were detected in the corn distillate and the 'blended' sample showed a linear increase in peak area over time ($R^2 = 0.83 \pm 0.02$). Comparisons to commercial samples and other experiments could not be made due to the instrument issues. The instrument issues meant other compounds may have been present but were not detected. The light experiment should be repeated to confirm whether other compounds were, in fact, present, but due to time constraints, other experiments were perused over further investigation of light treatment. Furthermore, besides the instrument issues, the absence of other compounds may have also due to the wavelength range of the light (450-600 nm), compared to the wider range reported in literature (400-1000 nm) [32]. The Growsaber light used is aimed at growing plants (substitute for sunlight for photosynthesis to occur), therefore, might not have been suitable for this experiment.

5.4. Sonication Experiments

The number of articles exploring the use of sonication as a technique to speed up the ageing process has increased over the past 10 years. It has been shown to enhance polyphenol (volatile phenols, phenolic acids and ellagitannins) extraction. Sonication is economically advantageous over other methods such as gamma-irradiation and pulse electric field (which both inactivate microorganisms without detrimental effects to food). Sonication is environmentally friendly, has low energy consumption and is relatively affordable [34,73,74]. The use of ultrasound is simple and reduces extraction times by agitating particles in the matrix using sound waves [34,74]. The increase in polyphenolic extraction (specifically phenolic aldehydes) is desirable because, once extracted out of the wood, phenolic aldehydes undergo oxidation to form their respective acids and successive formation of ethyl esters (by reaction with ethanol) [34]. Tao *et al.* reported that sonication alone did not influence the yield of phenolics extracted from wood, however, the yield increased when temperature was applied during sonication. The application of temperature aided in wood swelling which in turn increased its porosity allowing faster diffusion and solubility of phenolic compounds (thermodynamic effect) [157]. The above information led to the next experiment, sonication with and without heat (50 °C) applied to determine whether sonication would extract wood compounds and whether reactions (e.g.

esterification) could be sped up by applying sonication. The temperature applied (50 °C) was chosen because Lost Spirits [150] maintained temperatures between 32-66 °C, with 32-49 °C being optimal, however, the sonicator used in the experiment did not allow fine temperature change. In terms of time and sonicator parameters, Lost Spirits stated a minimum of 1 hour sonication of a raw distillate solution containing oak chips at a minimum of 1 W L^{-1} was required, preferably 1-10 hours at 3 W L^{-1} [150]. The power output of the sonicator used in the sonication experiments within the current research was considerably higher (50 W L^{-1}). However, a study by Delgado-González *et al.* reported that ultrasound at higher powers (e.g. 40 W L^{-1}) as well as movement of oak chips (medium sized medium toast chips) within a solution (33.94% ABV) improved extraction of phenolic compounds after seven days [76]. This information led to an initial test with one hour intervals of sonication and one hour rest periods in-between. A cumulative sonication time of four hours for samples with medium toast small chips in corn and malt distillates was carried out for the experiment.

The ethanol results are not discussed because only 3-5 compounds (ethyl acetate, acetic acid and furfural) were detected in the ethanol samples in both of the sonication experiments (heat and non-heated).

Sonication of the corn and malt distillates over a period of 4 hours (non-consecutive cycles) showed a change in a number of compounds. Appendix M Table M.1 (page 200) and Table M.2 (page 202) summarise the compounds detected during the sonication experiments (non-heated and 50 °C respectively) in the corn and malt distillates, respectively. The QC performance (Appendix F, page 184, injection 11-12) was variable (for different compounds) when the corn distillate cycle 3 samples (non-heated and 50 °C) were analysed. Additionally, the QC that ran with the non-heated cycle 4 malt distillate samples (Appendix F, page 184, injection 15) were at or over the action limit so the data was also removed.

Acetic Acid and Ethyl Acetate

The acetic acid peak areas gradually increased in the corn and malt samples (Appendix M Table M.1 (page 200) and Table M.2 (page 202)) which was expected due to extraction from wood and formation by oxidation of ethanol. The peak areas in the 50 °C corn and malt samples (cycle 1-4) were similar to the 8 hour heat-freeze experiment samples (discussed in section 5.5) but acetic acid in the non-heated sonication samples were either not detected or were lower than the heat-freeze. These results illustrated the positive effect of heat on the extraction (from wood) and/or formation of acetic acid (acetaldehyde oxidation). There were decreases in acetic

acid at certain points (e.g. 50 °C malt distillate cycle 2-3), this was due to acetic acid further reacting to form ethyl esters [28].

The concentration of ethyl acetate in the corn distillate was < LoD (1.45 ppm) for the duration of the experiment. In comparison, the concentration of ethyl acetate in the malt distillate (non-heated and 50 °C sonication) for 1-4 cycles had opposite trends (Figure 5.30). The difference in trends could be linked to the temperature change during the experiment (Figure 5.31); the temperature increased over time in the non-heated sonication experiment whereas it was maintained (50-60 °C) in the 50 °C sonication experiment. The concentrations obtained for ethyl acetate for 0-3 cycles in the non-heated experiment (11.14-7.11 ppm, n = 3) and 0-4 cycles in 50 °C experiment (3.53-5.62 ppm, $n = 4$) showed temperature should be maintained between 50 and 60 °C to increase concentrations. If it was below 50 °C (e.g. cycle 1-3 in the non-heated experiment) it decreased the ethyl acetate concentration. The hypothesis for this was that temperatures between 50-60 °C must be sufficient to cause a reaction between acetic acid and ethanol to form ethyl acetate. The ethyl acetate concentrations in the malt distillate samples in both sonication experiments (3.53-11.14 ppm, n = 7) were considerably higher than commercial samples (1.49-2.71 ppm, n = 8). Despite the high concentration in the non-heated malt distillate samples, higher concentrations have been reported by Fitzgerald *et al.* [96] in four of the five Irish whiskies analysed (51-130 ppm) and both Scotch whiskies analysed (81-110 ppm) [64,96]. MacNamara *et al.* [158] measured the abundance of ethyl acetate (amongst other compounds) in fresh distillate, aged for 3 and 6 years and showed increasing concentration for ethyl acetate from 148 ppm (fresh distillate) to 523 ppm in the same whiskey aged for 6 years [28,158]. If an ethyl acetate concentration similar to the commercial samples analysed was desired (< LoD-2.70 ppm) the sonication temperature should be maintained below 50 °C (by addition of ice) to decrease ethyl acetate concentrations, although this would affect the extraction of some compounds (e.g. phenolic aldehydes).

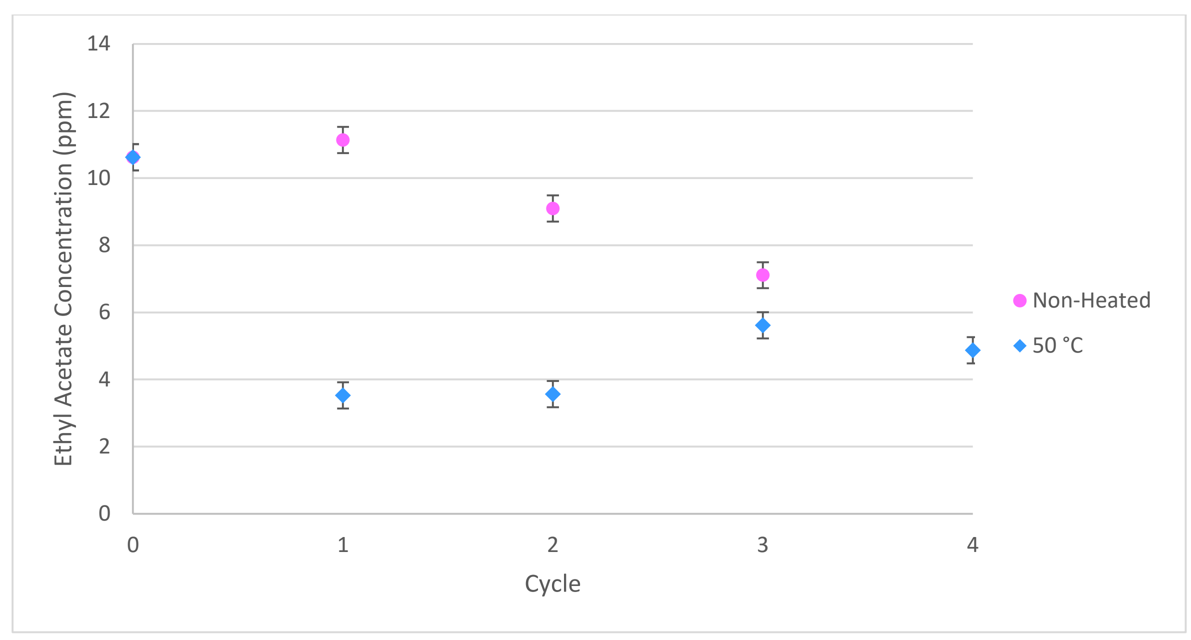

Figure 5.30: Change in the malt distillate ethyl acetate concentration from 0-3 sonication cycles in the non-heated samples and 0-4 sonication cycles in the 50 °C samples. Error bars have been added using the standard deviation calculated in the malt distillate for ethyl acetate (n = 3, section 4.1.2) as a proxy measurement of instrument variation overtime.

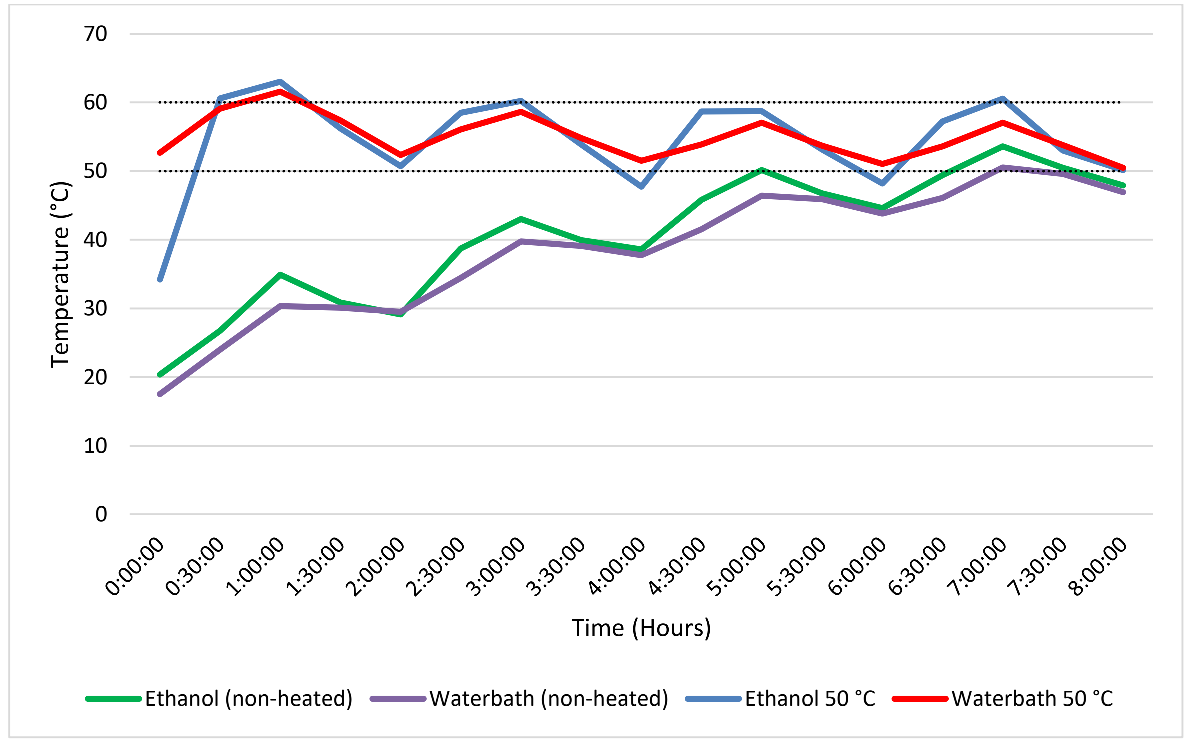

Figure 5.31: Temperature change of the waterbath and solutions (ethanol) during sonication experiment cycle 1 (0-2 hours) to cycle 4 (6-8 hours). The two reference lines (50 and 60 °C) show the optimal temperature region for several compounds.

Phenolic Aldehydes

Sinapaldehyde was not detected in the non-heated samples (corn and malt distillate). In the 50 °C experiment, it was detected from cycle 2 in the malt and cycle 3 in the corn distillate and remained constant for the remainder of the experiment.

Syringaldehyde was absent in the non-heated samples which can be explained by the absence of sinapaldehyde (oxidises to syringaldehyde). Syringaldehyde was detected in the 50 °C samples; in the corn distillate after 4 cycles (0.69 ppm) and in the malt distillate sample from cycle 1 with the concentration remaining constant (0.69 ± 0.005 ppm, n = 4) throughout the experiment. The results showed temperature was required because sonication alone could not extract syringaldehyde in a short period [31,40]. The syringaldehyde concentration in the sonication experiment was similar to one day of heating at 70 °C (0.68 ± 0.009 ppm, n = 4, section 5.2.2).

Coniferaldehyde was not detected in the majority of samples. For the samples it was detected in, it was very low, therefore, no coniferaldehyde was available to oxidise to vanillin. This was confirmed by the absence of vanillin.

Tao *et al.* [157] showed temperature was a factor in the release rate of phenolics (including syringaldehyde, coniferaldehyde and vanillin) with higher temperatures having a positive effect on extraction time. As the temperature of the sonicator was not controlled in the non-heated experiment, the temperature increased from ~20 °C to 47 °C (Figure 5.31) over the four cycles, however, syringaldehyde, vanillin, guaiacol and m-methoxyphenol were not extracted. This illustrated a temperature of > 50 °C was required for more than 1 hour.

Syringaldehyde was the only phenolic aldehyde detected in the sonication samples within this research. Moutounet *et al.* (1989) [159] agitated (250 rpm) oak chips (4 g/L) in water-alcohol solutions (12% v/v) for 8 hours and found that the concentrations of the detected wood extractives (vanillin, syringaldehyde, coniferaldehyde and sinapaldehyde) were similar to a solution stored in an oak barrel for 12 months. The difference between the results could be due to the difference in 'agitation', Moutounet *et al.* (1989) used mechanical agitation while the sonication experiments within this research used sound wave agitation (ultrasound). Also the exposure time would have affected the results; 4 hours in this research versus 8 hours in Moutounet *et al.* The absence of vanillin, guaiacol, *m*-methoxyphenol, coniferaldehyde and sinapaldehyde in all samples indicated more time, a higher temperature and/or heavy toast chips were required to extract these phenolic related compounds because several authors

experiments spanned 20-720 hours [45,74,159] and guaiacol and *m*-methoxyphenol were only present in heavy toast samples in the ethanol 70 °C experiment (section 5.2.1).

Miscellaneous Compounds

The peak areas for 1,1-diethoxyethane in the non-heated malt distillate samples decreased linearly (R^2 = 0.90). This was linked to the change in temperature; 1,1-diethoxyethane decreased with increasing temperature (~20-47 °C). Conversely, when the temperature was maintained between 50 and 60 °C, a minor linear increase ((R^2 = 0.98) was seen in the 50 °C malt distillate samples. The peak areas in the 50 °C malt distillate samples after 1 sonication cycle (0.33) was similar to 1 day (0.37) in the small chips heat distillate experiment (section 5.2.2).

The non-heated and 50 °C sonication experiments (cycle 1-4) achieved peak areas (0.08-0.33, n = 7) similar to the commercial samples (0.002-0.26). Due to the linear decrease/increase in the non-heated and 50 °C sonication samples respectively, the number of sonication cycles could be controlled to produce the desired amount of 1,1-diethoxyethane.

Furfural was detected from cycle 2 in all samples except for the 50 °C malt distillate where it was detected after the first cycle. The peak areas increased in all samples, although furfural was higher in the 50 °C samples. Two cycles of sonication (or 1 cycle for the malt at 50 °C) was necessary to achieve similar peak areas to those found in the analysed commercial samples (0.004-0.130). These results suggested temperature was not the only factor in furfural extraction. Time and %ABV were also factors because furfural was only detected in samples from cycle 2, except in the malt 50 °C where it was detected after 1 cycle. Furthermore, comparison to the small chips heat distillate experiment (section 5.2.2) showed the malt distillate peak areas (0.09-0.10) after 4 sonication cycles (non-heated and 50 °C) were lower than the peak areas on day 1 of the heat experiment (at 70 °C, 0.14).

Esters

Ethyl decanoate was constant in the non-heated corn samples (0.08 ± 0.001 ppm, n = 4) and in the 50 °C corn samples (0.15 ± 0.01 ppm, n = 3). Ethyl laurate and ethyl octanoate were not detected in the corn samples.

The change in concentration for ethyl decanoate, ethyl laurate and ethyl octanoate in the malt distillate showed the same trend; temperatures > 45 °C were required to form/increase esters as the concentrations decreased from cycle 1-3 in the non-heated sonication samples.

Furthermore, to further increase the concentrations, the temperature should be maintained between 50-60 °C as the 50 °C samples showed a general increase for all three esters. Nascimento *et al.* [97] found that the levels of ethyl esters in whiskey are generally low because ethyl esters are not extractive compounds from wood or from esterification reactions during maturation, but rather due to secondary metabolites of yeast. This is contradictory to many sources [9,15,21,28,31] and also the results obtained in the sonication experiment for this current research. Fermentation does produce esters, however, esters are also produced by reaction of acids with alcohols (e.g. octanoic acid and ethanol, Figure 1.9) [59,63,64].

Comparisons to commercial samples showed the concentration of ethyl decanoate in the corn distillate samples (non-heated and 50 °C) fell within the range of all commercial samples (0.002-0.163 ppm) but the malt distillate concentrations (0.35-0.42 ppm, n = 7) were higher. The concentration of ethyl laurate in the non-heated malt distillate samples (0.14 ± 0.01 ppm, n = 4) fell in the top concentration range for commercial samples (0.01-0.14 ppm), whereas the concentrations in the 50 °C malt distillate samples (0.24 ± 0.009 ppm, n = 4) was above the commercial sample concentration range.

Phenethyl acetate is formed by the condensation reaction between 2-phenylethanol and acetic acid [116]. This reaction was slow because phenethyl acetate was only detected in cycle 4 in the corn distillate samples (non-heated and 50 °C) and a slow increase in peak area (0.009-0.018) in the malt distillate samples (non-heated and 50 °C). These results suggested time and the temperature had a minimal effect on the formation of phenethyl acetate; the matrix and %ABV are more important factors because phenethyl acetate formed faster in the malt distillate (65% ABV).

Ethyl hexanoate, isoamyl acetate and isobutyl acetate were only detected in the malt distillate. Ethyl hexanoate showed a minor linear decrease (R^2 = 0.89, slope = −0.003) from cycle 1-3 in the non-heated malt distillate samples compared with a minor linear increase (R^2 = 0.94, slope = 0.004) from cycle 1-4 in the 50 °C malt samples. The isoamyl acetate concentration should decrease over time due to transesterification [28]; this was seen in the non-heated malt distillate samples from cycle 1-3 but remained constant from cycle 1-4 (0.46 ± 0.04, n = 4) in the 50 °C malt distillate samples. Isobutyl acetate remained steady in the non-heated and 50 °C malt distillate samples (0.016 ± 0.005, n = 7). Isobutyl acetate is formed by the reaction of isobutanol and acetic acid so it was expected that isobutanol would decrease with increasing isobutyl acetate. The peak areas did not confirm this because the change in peak area was small

for isobutyl acetate compared to isobutanol. This showed more time was required to convert isobutanol to isobutyl acetate and a higher temperature may be important in the formation. A linear increase (R^2 = 0.96) was observed for 2-methylbutyl acetate in the 50 °C malt distillate samples; the peak area remained constant from cycle 1-3 in the non-heated malt distillate. Thus, the above ester results illustrated temperature was a primary factor in the formation of esters and the temperature should be maintained between 50-60 °C to avoid hydrolysis.

Alcohols

Isoamyl alcohol was high in both the corn (14.30 ppm) and malt (15.50 ppm) distillates before starting the sonication experiments and corresponds to concentrations reported in commercial samples (1.04-23.00 ppm). There was a large decrease in concentration (14.30 ppm to ≤ 4.35 ppm) at the beginning of both experiments (cycle 0-1) for the corn distillate which is partially explained by absorption into the wood [136] as previously mentioned (section 5.1.3). The decrease could also arise from formation of isoamyl acetate, however, isoamyl acetate was not detected in the corn distillate samples most likely due to the LoD (0.26 ppm). The concentration of isoamyl alcohol in the corn distillate samples over 3 sonication cycles were similar in the non-heated (3.52-4.35 ppm) and 50 °C (2.48-4.22 ppm) experiment. The concentrations were also similar to some well-known commercial samples: Jack Daniels (2.20 ppm), 12-year-old Chivas Regal (3.14 ppm), 12-year-old Glen Dronach (4.27 ppm) and 10-year-old Glenfarclas (2.73 ppm).

The change in isoamyl alcohol concentration in the malt distillate was initially different between the non-heated and 50 °C sonication experiments (Figure 5.32) and they followed the same trend as ethyl acetate. The temperature appeared to affect the malt distillate more than the corn distillate, a suggested cause for this may be due to the difference in %ABV. High concentrations of isoamyl alcohol are undesirable because they are associated with headaches and hangovers [21], therefore, sonication could be used to decrease the isoamyl alcohol concentrations and thus reduce the hungover feeling. The concentration in the malt distillate for cycle 1-3 in the non-heated (7.12-12.35 ppm) and cycle 1-4 in the 50 °C samples (3.52-5.62 ppm) were similar to several commercial samples (Figure 5.33) including ≥ 12-year-old whiskies: 12-year-old Wild Turkey (10.30 ppm), 14-year-old Balvenie (7.93 ppm) and 15-year-old Dalwhinnie (3.81 ppm).

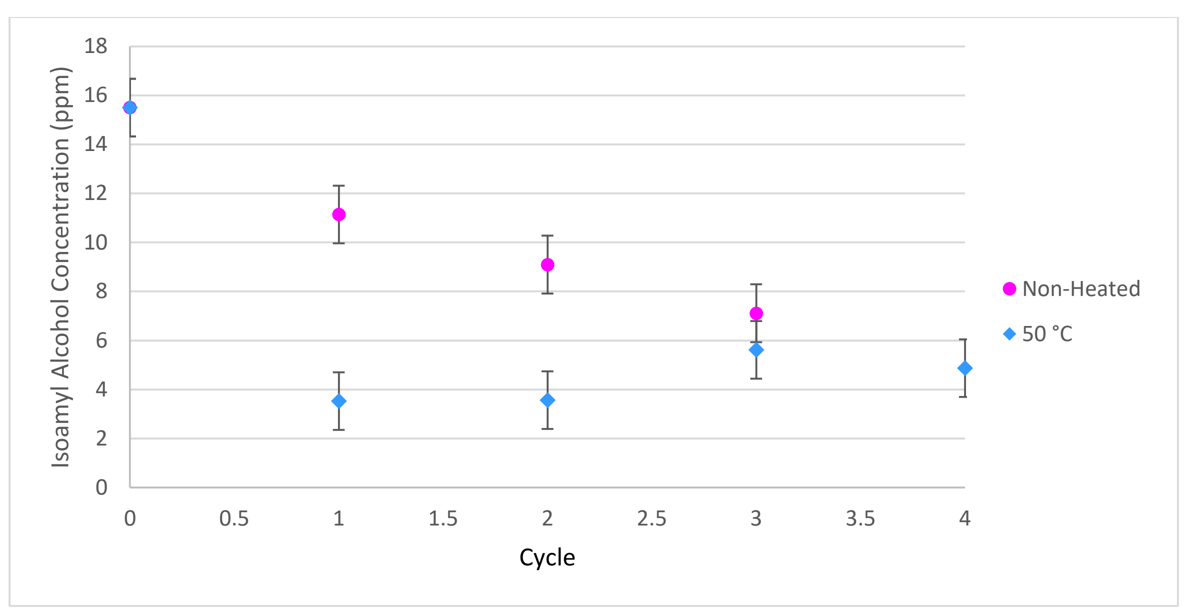

Figure 5.32: Isoamyl alcohol concentration (ppm) changes in the non-heated and 50 °C malt distillate sonication experiments. Error bars have been added using the standard deviation calculated in the malt distillate for isoamyl alcohol (n = 3, section 4.1.2) as a proxy measurement of instrument variation overtime.

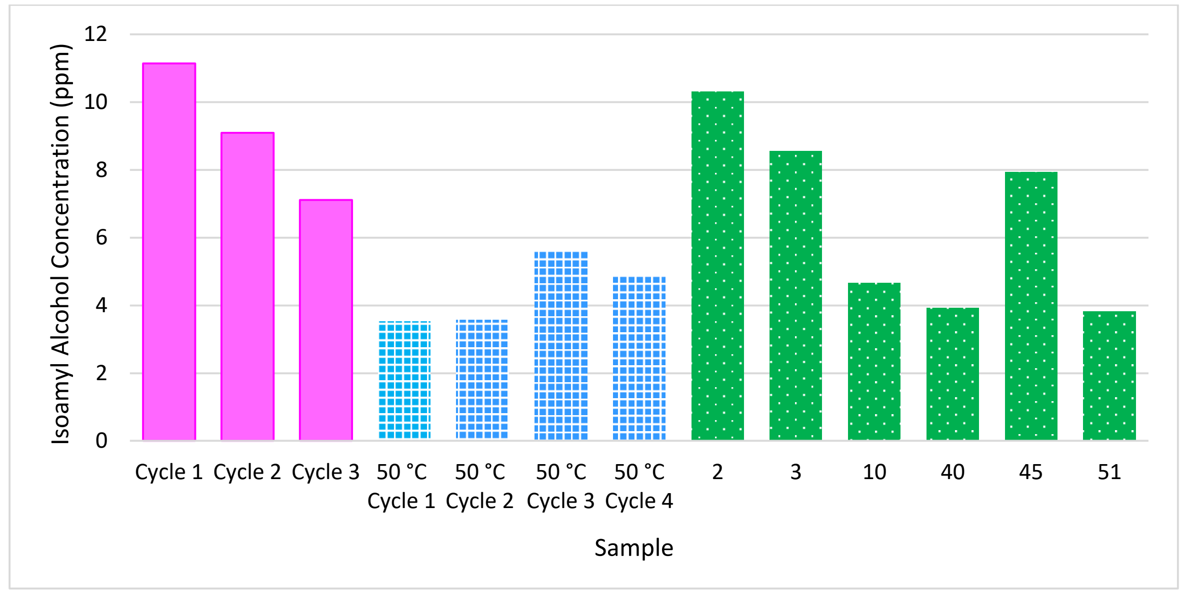

Figure 5.33: Isoamyl alcohol concentration (ppm) in the non-heated (pink) and 50 °C (checkered blue) malt distillate sonication samples and several commercial samples (dotted green).

Similar to other compounds, 2-methyl-1-butanol decreased in the non-heated samples (corn and malt). It was thought that the decrease was most likely due to the formation of 2-methylbutyl acetate, however, the peak areas for 2-methylbutyl acetate (in the malt distillate) had the same trend as 2-methyl-1-butanol (i.e. 2-methylbutyl acetate increased when

2-methyl-1-butanol increased). The peak areas remained constant in the 50 °C malt distillate samples (1.74 ± 0.15, n = 5), however, they increased in the 50 °C corn distillate samples (R^2 = 0.82) suggesting other factors (e.g. distillate composition) affected 2-methyl-1-butanol. High levels of 2-methyl-1-butanol are undesirable because they are associated with headaches and hangovers [21]. These results could be used to control the amount of 2-methyl-1-butanol; to decrease 2-methyl-1-butanol, the temperature has to be kept below 50 °C and to keep 2-methyl-1-butanol constant, the temperature should be maintained between 50-60 °C.

The isobutanol peak area decreased in the non-heated corn and malt distillate samples but remained constant in the 50 °C corn (0.34 ± 0.08, n = 4) and malt distillate (1.53 ± 0.13, n = 5) samples. As previously mentioned a higher temperature may be important in the reaction between isobutanol and acetic acid to form isobutyl acetate.

The concentration of 2-phenylethanol remained constant in both the non-heated corn distillate (0.26 ± 0.01 ppm, n = 3) and in the 50 °C corn distillate (0.14 ± 0.03 ppm, n = 3). 2-Phenylethanol was absent in the malt distillate before sonication and for the first 2 cycles. It was detected in the malt distillate samples (non-heated and 50 °C) after 3 cycles and the concentration remained the same in cycle 4 (0.19 ± 0.002 ppm and 0.097 ± 0.001 ppm, respectively). As previously mentioned (section 5.2.2), 2-phenyethanol is not only produced during fermentation but it is also extracted from wood [28], therefore, the detection of 2-phenylethanol in the malt samples showed sonication was effective at extracting 2-phenylethanol which is desired in the final product for its sweet and rose aroma [48]. The concentrations of the non-heated samples (corn and malt) and the 50 °C corn distillate were similar to commercial samples; for example, 21-year-old Glenfiddich (0.19 ppm), 12-year-old Highland Park (0.13 ppm), Johnnie Walker (0.17 ppm) and 12-year-old Glenfiddich (0.19 ppm). In contrast, the concentration in 50 °C malt distillate was too low compared to the commercial samples.

5.4.1. Sonication Summary

Sonication (non-heated and 50 °C) was effective at extracting desired compounds such as acetic acid, 1,1-diethoxyethane, furfural and 2-phenylethanol as well as the formation of esters in the malt distillate samples (50 °C only). Over the 4 sonication cycles, compound concentrations/peak areas did not change substantially; only minor changes were observed, although this experiment was only carried out over an 8 hour period. The biggest difference

between the non-heated and 50 °C results was the extraction of wood compounds (sinapaldehyde, syringaldehyde and coniferaldehyde) and the change in concentration/peak area. Only 1-2 cycles of 50 °C sonication was required for the malt distillate to achieve concentrations close to commercial samples (including ≥ 10 year olds) for several desired compounds (isoamyl acetate, isoamyl alcohol, 2-phenylethanol, syringaldehyde, ethyl octanoate), although, ethyl acetate was relatively higher than the analysed commercial samples.

Overall, some compounds suggested non-heated sonication was better, but other compounds suggested 50 °C was better; for example, the 2-phenylethanol concentrations in the non-heated sonication experiment (corn and malt samples) were similar to commercial samples compared to the 50 °C experiment where the concentrations were too high. However, 50 °C was the better temperature for extracting syringaldehyde after 1 sonication cycle in the malt distillate or 4 cycles in the corn distillate.

The non-heated sonication experiment showed sonicating for more than 1 cycle decreased all compound concentrations/peak areas whereas sonicating for more than 1 cycle in the 50 °C experiment increased all compound concentrations/peak areas. This meant desirable and undesirable compounds were affected the same rather than the preferred increase in desired compounds and decrease in undesirable compounds. Therefore, if only sonication was used, 50 °C would be preferential because compound concentrations and peak areas increased (and undesirable compounds were present at similar levels to commercial samples) and wood compounds (sinapaldehyde, syringaldehyde and coniferaldehyde) would be extracted. Although a mixture of non-heated and 50 °C (perhaps 1 cycle of each) would be ideal, not only to extract the wood compounds in both corn and malt distillates but also to lower the ethyl acetate and isoamyl alcohol concentrations. Also, vanillin, *m*-methoxyphenol, and guaiacol were not detected in any samples (corn and malt), therefore, sonication was useful in decreasing/increasing the concentrations/peak areas of compounds already present, however, extraction of additional desired compounds such as vanillin and smokey characteristics (*m*-methoxyphenol and guaiacol) did not occur. This indicated either a longer sonication time or a higher temperature was required to help extract these compounds or perhaps a previously heat treated sample could be sonicated.

These contrasting results prove the importance of carrying out sensory analysis, however, due to the experiments having been carried out in the laboratory, tasting was not possible. This meant if sonication was chosen for refinement experiments it would have to be carried out in

the distillery to allow sensory analysis to determine whether non-heated of 50 °C gave the whiskey a better overall flavour profile.

5.5. Heat-Freeze Experiment

Initially, a freeze-thaw experiment (−18 °C and room temperature) was carried out to gain insight into the effects of temperature variation on maturation. However, due to instrument issues (Appendix F, page 184, points 41-42), the data was not reliable so the method was refined based on the information presented below from literature and the results from the distillate heat experiment (section 5.2.2). Based on this information, a heat-freeze experiment (70 °C and −18 °C) was developed and carried out.

In traditional whiskey ageing, whiskey is matured over a number of years and the seasonal temperature variations, as well as the storage warehouse conditions, play an important role in the development of flavours and aromas in the final product. The temperature, humidity and ventilation within a warehouse cause changes in the internal temperature and pressure within the barrels which helps the distillate diffuse in and out of the wood [3,13–15,24]. Therefore, to simulate rapid seasonal change, samples were exposed to extreme temperatures to accelerate the extraction of compounds into the distillate. Samples were transferred between a 70 °C oven ("summer") and −18 °C freezer ("winter").

Heating the wood expands the pores (becoming more porous) allowing more liquid to move through it [67,68]. 70 °C was chosen because the ethanol (section 5.2.1) and distillate heat experiments (section 5.2.2) showed 70 °C extracted desirable compounds at concentrations/peak areas similar to commercial samples. Cold temperatures cause contraction of the wood pores forcing liquid out [67], −18 °C was chosen because this temperature simulates harsh winter temperatures and is easy to maintain in the distillery (using glycol). The experiment was carried out for a total of 9 days based on the results from the heat experiments (section 5.1.1); 6-8 days using medium toast small chips was found to be the optimal time for the extraction of desired compounds at 70 °C.

Initially, the samples were transferred between the oven and freezer every two hours (for eight hours) to rapidly simulate seasonal temperature variation (referred to as distress ageing by home-distillers) and then stored overnight at room temperature, for the first three days. This was to increase the expansion and contraction of the wood aiding diffusion of the distillate in a short time [68]. It was noted that the corn distillate samples were cloudy after removal from

the freezer. Due to the cold temperature, less soluble compounds such as lipids and large molecular weight lignin compounds (unwanted compounds) form a precipitate resulting in a hazy solution which can be filtered to remove the unwanted compounds, this is referred to as chill-filtration [28].

Three days was chosen to allow time for the distillate to diffuse in and out the wood pores. After three days the samples were stored in the oven for one day then in the freezer for one day, this was repeated once more.

Acetic Acid and Ethyl Acetate

The peak area for acetic acid in the corn distillate did not change overall. There was a minor linear increase (R^2 = 0.64, slope = 0.0005) in the malt distillate, particularly at 96 hours (≡ 3 days, Figure 5.34). The increase in the malt distillate was postulated to be from the presence of acetaldehyde in the malt distillate prior to age treatment which contributed to the formation of acetic acid. The stability of the peak area in the corn distillate suggested this precursor was not present prior to age treatment. This was similar to the trend seen in the results for the malt distillate samples in the heat experiment (section 5.2.2), however, the heat-freeze experiment took four times longer to achieve the same peak area as the distillate heat experiment on day 1. The day 1-9 peak area range in the corn distillate (0.04-0.10, n = 13) and malt distillate (0.02-0.13, n = 13) were similar to several of the analysed commercial samples (0.08 ± 0.03, n = 10).

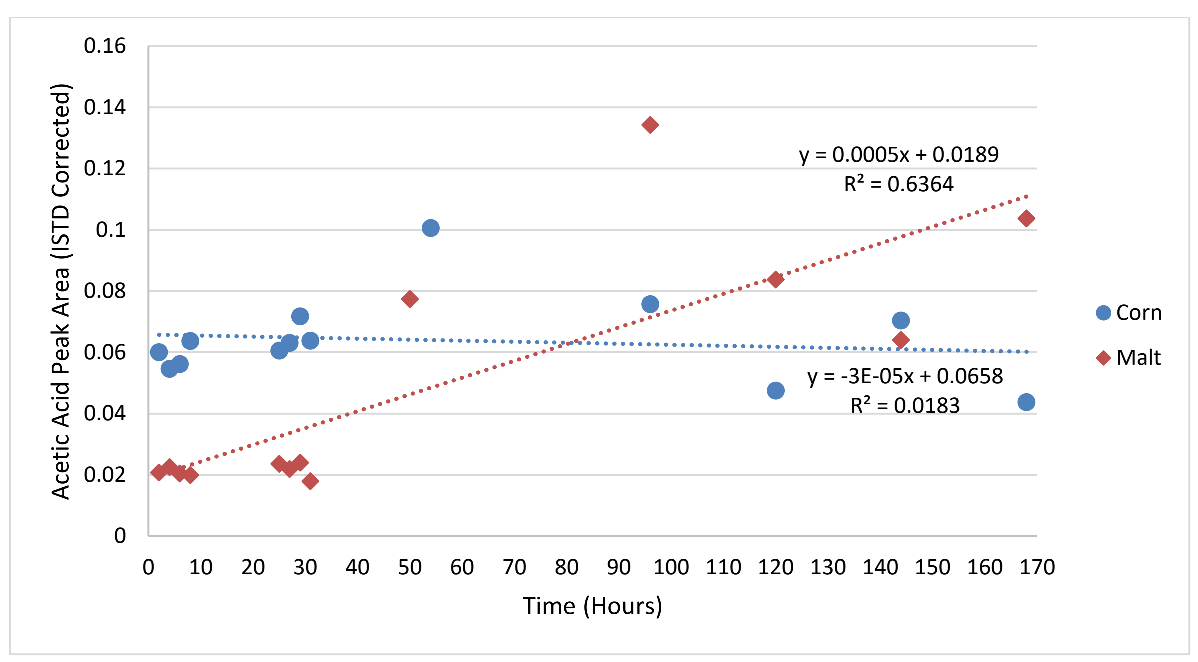

Figure 5.34: Minor acetic acid peak area increase ($R^2 = 0.64$) in the malt distillate samples in the heat-freeze experiment from 2-168 hours (7 days). The corn distillate did not change.

The ethyl acetate concentrations in the corn distillate were < LoD (1.45 ppm) for the duration of the experiment. In the malt distillate, the concentration remained constant (9.0 ppm ± 2.0, n = 17) compared to an increase when exposed to heat only (3.10-9.10 ppm, n = 12) (Figure 5.35). This is the opposite trend observed in traditional maturation where it increases with age. This suggests that there was an equilibrium occurring ($ethanol + acetic\ acid \rightleftharpoons ethyl\ acetate$) in the malt distillate samples [28]. As previously mentioned, ethyl acetate accounts for > 50% of the ester concentration in spirits and at low concentrations it aids in reducing the 'burn' associated with fresh distillate [1,9,45,64]. The concentration of ethyl acetate (9.0 ± 2.0 ppm, n = 17) was higher than commercial samples (2.07 ± 0.44 ppm, n = 8) but lower than what is commonly reported in literature (100-716 ppm) [20,28,63], therefore, the concentration was deemed acceptable.

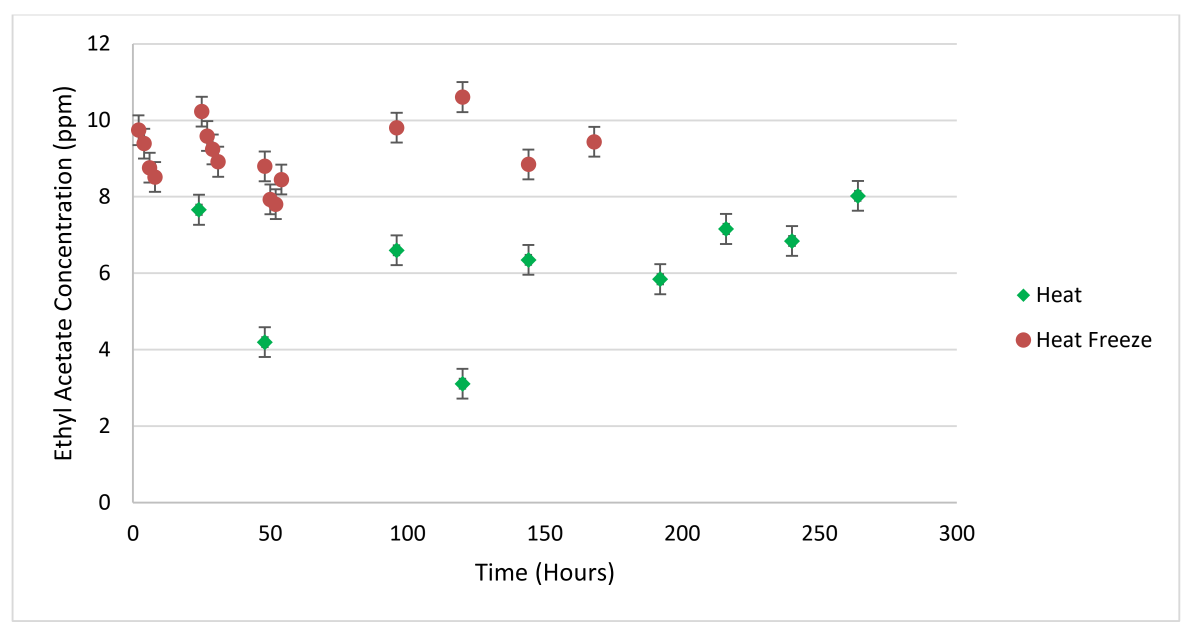

Figure 5.35: Comparison between the ethyl acetate concentrations in the malt distillate during the heat-freeze (orange circle) and heat only (green diamond) experiments. Ethyl acetate remained constant during the heat-freeze whereas the concentration increased and decreased in the heat only experiment. Error bars have been added using the standard deviation calculated in the malt distillate for ethyl acetate (n = 3, section 4.1.2) as a proxy measurement of instrument variation overtime.

Phenolic Aldehydes

The sinapaldehyde peak area continually increased in both the corn and malt distillate samples throughout the heat-freeze experiment whereas it fluctuated in the distillate heat experiment (section 5.2.2); the peak area range overlapped but results for heat-freeze were generally lower (0.01-0.16, n = 30) compared to the heat results (0.09-0.27, n = 32). This suggested that while sinapaldehyde continually increased during the heat-freeze experiment, constantly heating the distillate further increased the extraction of sinapaldehyde. Consequently, this meant there was more sinapaldehyde to oxidise to syringaldehyde in the heat only experiment.

Syringaldehyde was first detected in the 2 hour heat samples (corn and malt) and increased after 48 hours (2 days of transferring between the oven and freezer every 2 hours and leaving samples at room temperature overnight). The concentration of syringaldehyde after 24 hours was lower (0.29 ± 0.01 ppm, n = 10) than the distillate heat experiment day 1 results (0.68 ± 0.02 ppm, n = 4). This showed that varying the temperature did not speed up the extraction of syringaldehyde but slowed it down. The colour of the samples in the heat-freeze experiment were more similar to traditional whiskey than the colour of the heat experiment. This suggested heat-freeze might be more suitable even though it took a bit longer (9 days) to reach similar concentrations to the day 6 distillate heat results.

Coniferaldehyde was only detected from day 6-9 but increased linearly in the corn (R^2 = 0.98) and malt distillate (R^2 = 0.93) over this time period. The peak areas were lower than in the distillate heat experiment, again suggesting constantly heating the distillate extracted more coniferaldehyde which could subsequently oxidise to vanillin. Coniferaldehyde was not detected in the analysed commercial samples but has been reported as being present in the final whiskey product (0.6-1.2 ppm) [63,64].

Vanillin was detected in the malt distillate at the end of day 3 (54 hours) at a concentration of 0.38 ppm and increased to a maximum concentration of 0.43 ppm. The concentration of vanillin in the malt distillate samples (0.41 ± 0.03 ppm, n = 5) were similar to the heat distillate results (0.35 ± 0.05 ppm, n = 12) and both were higher than the analysed commercial samples (0.32 ± 0.02 ppm, n = 18). However, as previously mentioned (section 5.2.1), vanillin is a marker for age and quality with higher concentrations associated with older and better quality whiskies [1,23,37,46,53]. Vanillin was not detected in any of the corn distillate samples throughout the experiment and was presumed to be due to the lower coniferaldehyde peak areas in the heat-freeze experiment as well as the temperature change affecting the extraction of vanillin. Vanillin was detected on day 1 in the corn and malt distillate heat experiment, hence the heat-freeze combination was not a superior method for this compound.

Miscellaneous Compounds

The peak areas for 1,1-diethoxyethane (not detected in fresh distillate) were higher in the malt distillate samples compared to the corn distillate samples during the heat-freeze experiment. This was the same trend that was seen in the distillate heat experiment (section 5.2.2). 1,1-Diethoxyethane was detected at the first sampling point in both experiments (heat-freeze = 2 hours; heat = 24 hours) suggesting 1,1-diethoxyethane was easily formed from the intermediate hemiacetal. Longer heating periods produced higher levels of 1,1-diethoxyethane; the peak areas for the corn (0.03) and malt distillate (0.28) in the heat-freeze experiment at 144 hours (≡ 6 days) were lower when compared to the day 6 heat experiment results (0.09 and 0.58, respectively). The peak areas in the heat-freeze malt distillate samples (0.20-0.28) were similar to 6% of commercial samples which had a peak area range of 0.002-0.26, whereas the malt distillate results in the heat experiment (0.27-0.67) extracted more 1,1-diethoxyethane than what was found in the commercial samples. The peak areas in the heat-freeze corn distillate (0.007-0.03) samples were similar to 24% of commercial samples.

Furfural was detected after 2 hours in both the corn and malt distillate samples. The peak areas initially increased before levelling off; this suggested that the change in temperature every 2 hours (from 0-54 hours) was more effective at extracting furfural than transferring between the oven and freezer once a day (from 54-168 hours). However, the peak areas for furfural were higher in the ethanol and malt distillate heat experiments (section 5.2.1 and 5.2.2, respectively) suggesting the cold temperature had a negative effect on the extraction of furfural. The average peak areas in the heat-freeze experiment for furfural in the corn (0.05 ± 0.01, $n = 16$) and malt (0.05 ± 0.01, $n = 16$) distillates were similar to the average commercial sample peak area (0.03 ± 0.004, $n = 38$). Although the average peak area in the corn and malt distillates in the heat experiment were higher (0.10 ± 0.06 ($n = 9$) and 0.14 ± 0.01 ($n = 9$), respectively). These results illustrated the importance of temperature in the extraction of furfural; exposing samples to 70 °C for 1 day produced almost twice as much furfural in the corn distillate and almost three times more in the malt distillate compared to 24 hours in the heat-freeze experiment. It was postulated that placing the samples in the freezer and/or the length of time may have significantly closed the wood pores and, therefore, a large amount of time in the heat cycle was spent opening the pores which resulted in less diffusion of the distillate through the wood before entering the freezer again. Future experiments could include a longer heat cycle, fewer freezer cycles or using a higher temperature freezer (e.g. −4 °C).

Phenols

No phenols were detected in any of the heat-freeze samples whereas guaiacol, 2,6-dimethoxyphenol and *m*-methoxyphenol were detected in the medium and heavy toast samples in the heat distillate experiment (section 5.2.2). Two factors would have affected the extraction of phenols: the toast level and the treatment (heat-freeze). Higher toasting levels produce more guaiacol [28], this was observed in the heat distillate experiment (section 5.2.2) where guaiacol was detected from day 1 in the heavy toast samples and from day 9 in the medium toast samples. The ‘freezing’ of samples in the heat-freeze treatment would have contracted the wood pores making it harder for distillate to diffuse through and subsequently release phenols into the distillate.

Esters

Ethyl butyrate was < LoD (0.41 ppm) throughout the experiment. Ethyl hexanoate was detected in the malt distillate, however, it was not present consistently suggesting it was close to the LoD but this could not be confirmed due to the absence of standards.

The results for ethyl octanoate, ethyl decanoate and ethyl laurate showed the treatment did not have an effect on these three ester concentrations. The concentration of ethyl octanoate in the malt distillate was similar to 21-year-old Glenfarclas (0.13 ppm). The temperature, pH and %ABV affect the ester composition [139]. Ethyl octanoate was absent in the fresh corn distillate and was not detected in the heat-freeze corn samples which suggested octanoic acid was not present or was too low to form ethyl octanoate (by reaction with ethanol).

The concentrations of ethyl decanoate in the corn samples were within the concentration range for the analysed commercial samples (0.06-0.16 ppm), the corn distillate average (0.12 ± 0.04 ppm, n = 17) was similar to several 10-12 year-old samples whereas the malt distillate average (0.37 ± 0.03 ppm, n = 17) was over 2-fold higher than the analysed commercial samples but lower than those generally reported in literature (2-10 ppm) [11,37,64].

The concentration of ethyl laurate in the corn distillate (0.10 ± 0.02 ppm, n = 16) was similar to 20% of commercial samples including ≥ 14-year-old whiskies: 14 year-old Balvenie (0.08 ppm), 14-year-old Clynelish (0.13 ppm) and 15-year-old Dalwhinnie (0.08 ppm). The average concentration in the malt distillate (0.17 ± 0.04 ppm, n = 17) was similar to 15% of the commercial samples and included three samples which were ≥ 12 years old (0.19 ± 0.01 ppm, n = 3).

There was a minor decrease in the concentration of isoamyl acetate in the malt distillate (0.66 ppm to 0.54 ± 0.06 ppm) during the experiment, and it was not detected in the corn distillate. The concentration of isoamyl acetate was expected to increase because of the high abundance of isoamyl alcohol (precursor). This did not occur in the heat-freeze experiment which indicated a key factor to drive the reaction forward was missing. It was thought that the minute amount of acetic acid present in both distillates was the reason. It has been reported that acetate esters of higher alcohols decrease during ageing, although the reasons were not given as to why this is the case [28].

Minimal change in peak areas were observed over the duration of the experiment for isobutyl acetate (0.01 ± 0.003, n = 17) and 2-methylbutyl acetate (0.02 ± 0.009, n = 17). The peak areas were expected to increase; the low abundance of acetic acid was most likely the cause due to the high abundance of isobutanol (precursor to isobutyl acetate) and 2-methyl-1-butanol (precursor to 2-methylbutyl acetate). Future work would include quantitation of acetic acid and other acids using a suitable column.

Alcohols

The isoamyl alcohol concentration remained consistent in the heat-freeze experiment (Figure 5.36), the same was seen in the small chips heat experiment (section 5.2.2). Although the large decrease in concentration (14.30 ppm to 7.15 ppm) in the corn distillate and malt distillate (15.50 ppm to 10.50 ppm) was unexpected given that the acetic acid levels were low and the decrease was not thought to just be from the absorption into the wood. Therefore, it was speculated that sub-sampling may have caused some volatile loss if the time to cool down/warm-up was not sufficient. However, to minimise this a cool down time of 30 minutes was used.

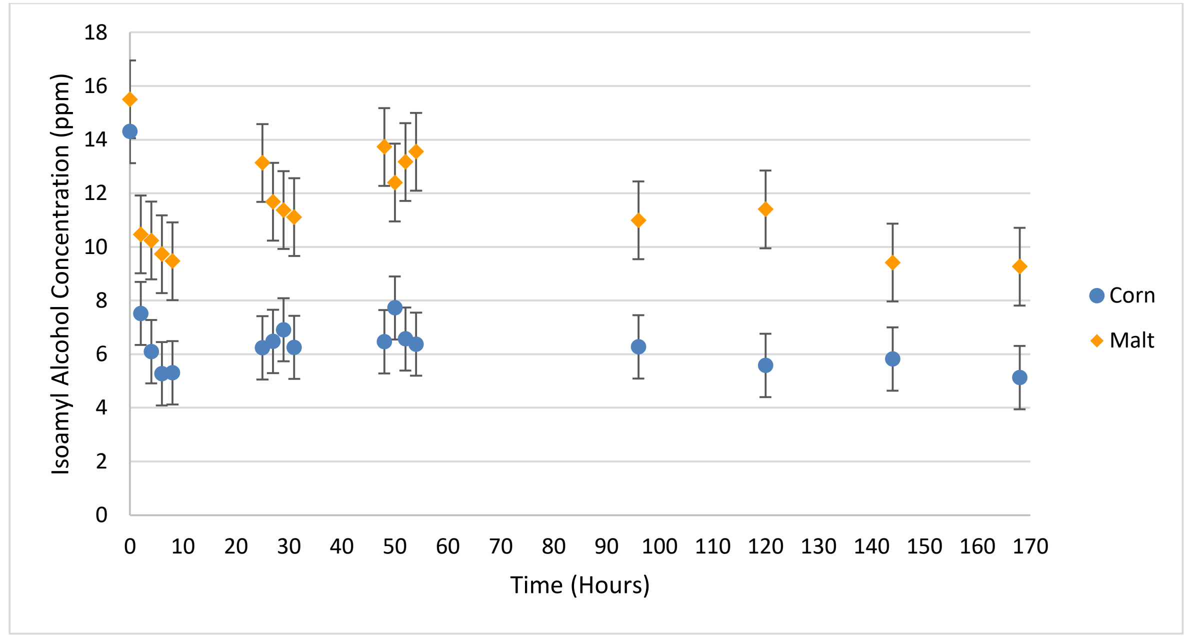

Figure 5.36: No change was observed in the isoamyl alcohol concentration (ppm) in the heat-freeze experiment for the corn and malt distillates. Error bars have been added using the standard deviation calculated in the corn (n = 3) and malt (n = 3) distillates for isoamyl alcohol (section 4.1) as a proxy measurement of instrument variation overtime.

The peak areas for 2-methyl-1-butanol were relatively consistent in the malt distillate during the experiment with a minor decrease (R^2 = 0.46, slope = −0.001) observed in the corn distillate (Figure 5.37). The slight decrease could be within experimental error, however, this could only be confirmed by quantitation and the determination of the LoD and LoQ. As previously mentioned (section 5.1.2), high levels of 2-methyl-1-butanol contribute to headaches and hangovers [21], therefore, the concentration should be quantified in future work. Nevertheless, the peak areas for 2-methyl-1-butanol in the corn distillate (0.39 ± 0.05, n = 16) were similar to a blended whiskey, Chivas Regal (0.45), two ≥ 10-year-old samples (15-year-old Dalwhinnie

(0.50) and 12-year-old Glenfiddich (0.48)) as well as two samples with no age statements: Talisker Skye (0.38) and Ardbeg (0.34). The 2-methyl-1-butanol peak areas in the malt distillate (0.96 ± 0.20) were similar to 25% of samples (0.97 ± 0.12).

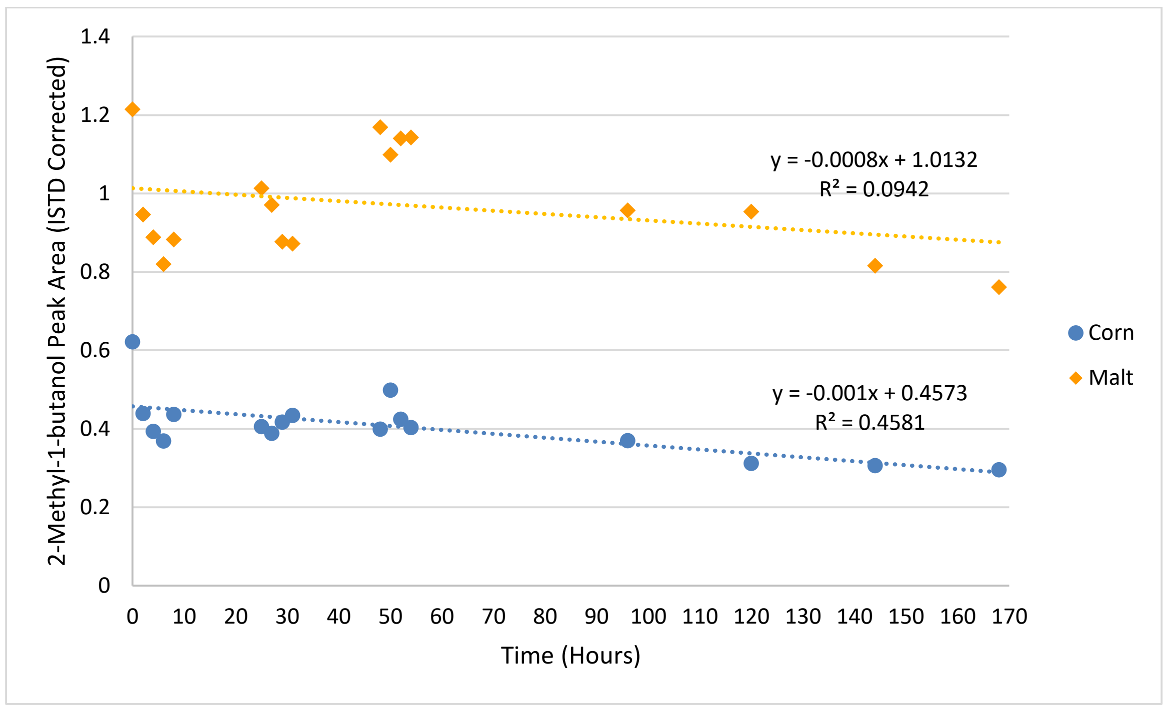

Figure 5.37: Peak area changes for 2-methyl-1-butanol in the corn (blue circle) and malt (orange diamond) distillate samples during the heat-freeze experiment.

Isobutanol had a gradual downward trend ($R^2 = 0.72$) in the malt distillate (Figure 5.38) during the experiment except for the 48 hour malt distillate sample; it was thought to be an outlier because it was the only sample to not fit within the trend and was subsequently removed. The downward trend was also observed in the distillate heat experiment (section 5.1.2), which was thought to be due to the reaction with acetic acid to form isobutyl acetate. The peak area for isobutanol in the corn distillate (0.41) remained relatively consistent (0.25 ± 0.05, n = 17), proposed to be because of the absence in the fresh distillate. The corn distillate peak areas were within the commercial samples peak area range (0.03-1.36, n = 39).

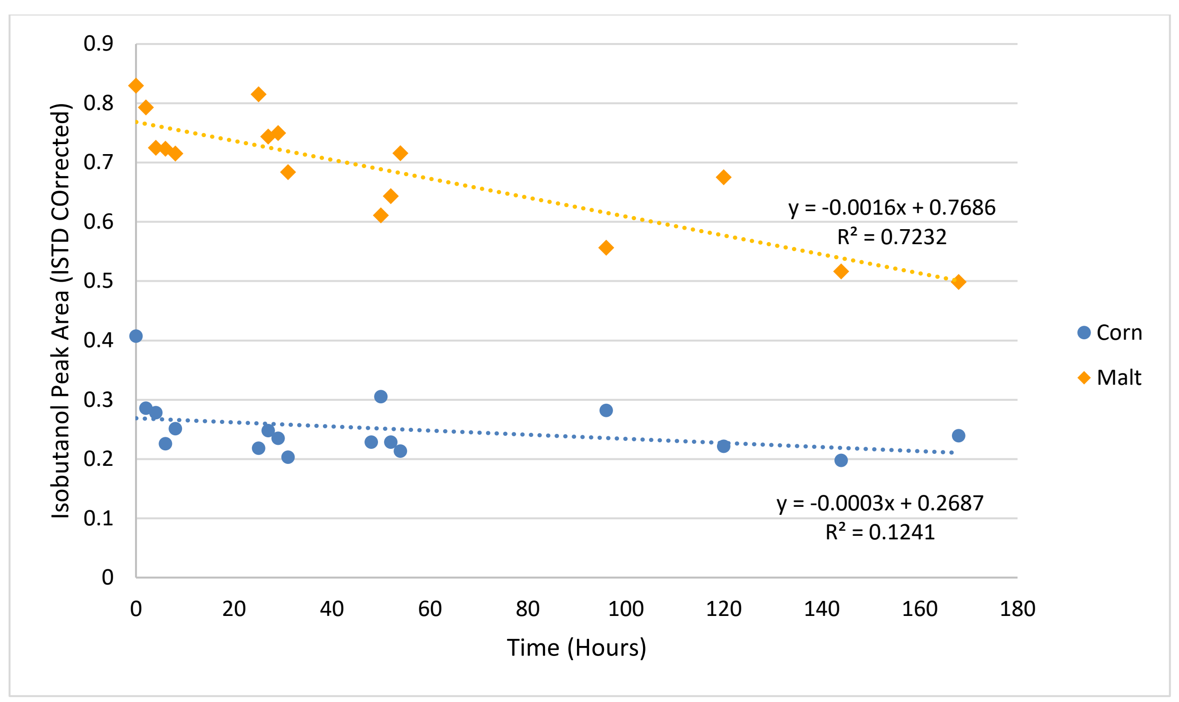

Figure 5.38: Isobutanol peak area change in the corn (blue circle) and malt (orange diamond) distillate samples. A gradual decrease ($R^2 = 0.72$) was observed in the malt distillate (48 hour data point removed) whereas the corn distillate remained constant.

In the malt distillate, 2-phenylethanol was only detected from 144 hours (0.13 ppm) and was close to the LoD (0.10 ppm). The detection of 2-phenylethanol in the malt distillate likely arose from the extraction of wood [28] and would also explain the minor increase in the 2-phenylethanol concentration (0.21 ppm to 0.28 ppm) in the corn distillate from 0-29 hours. At 96 hours (4 days), a decrease in concentration (0.20 ppm) was observed (Figure 5.39), however, the degree of concentration change from 31-96 hours was unknown because the 48-54 hour samples were removed from the graph since it was noted that the determination of 2-phenylethanol was poor in the QC (injection 42, Appendix F, page 184) when these samples were analysed. The minor decrease from 31-96 hours was suspected to be from reaction with acetic acid to form phenethyl acetate, however, if this did occur, phenethyl acetate was below the detection limit of the instrument (no standard was available to determine LoD).

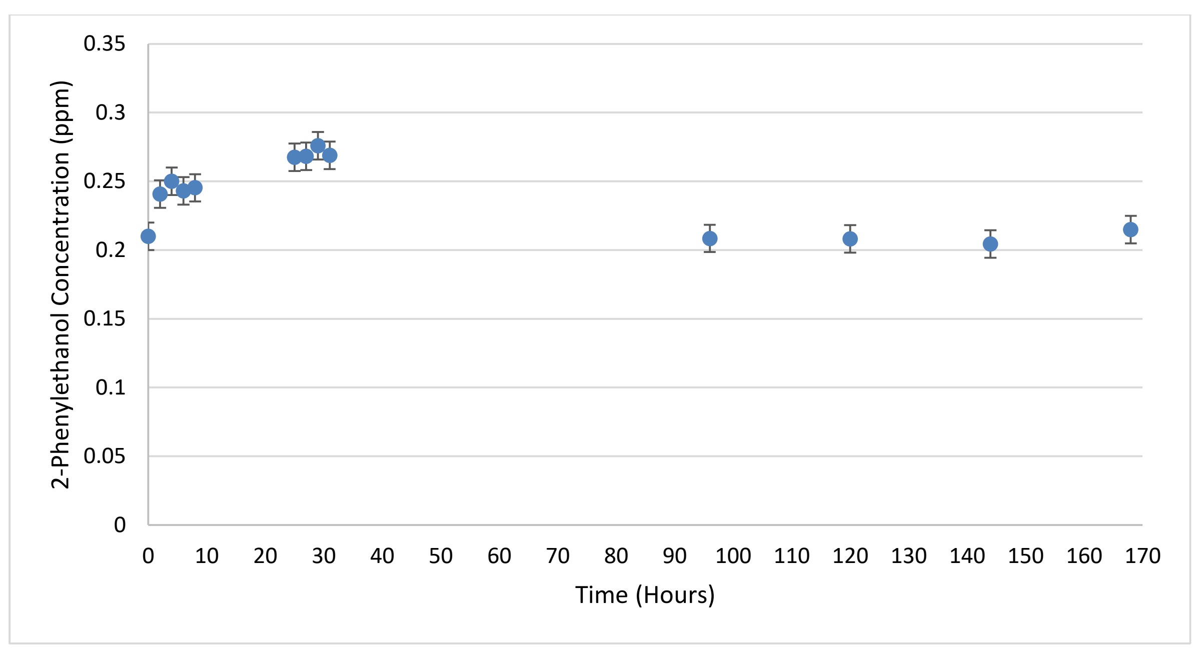

Figure 5.39: Change in concentration of 2-phenylethanol in the corn distillate over the course of the heat-freeze experiment. Error bars have been added using the standard deviation calculated in the corn distillate for 2-phenylethanol (n = 3, section 4.1.1) as a proxy measurement of instrument variation overtime.

5.5.1. Heat-Freeze Summary

The heat-freeze experiment was designed to simulate seasonal changes to gain insight into how the large temperature changes affected the extraction and reaction of compounds. The heat-freeze experiment produced several compounds (e.g. esters and furfural) at desired concentrations/peak areas but was slower compared to the heat only experiments. The heat-freeze experiment produced lower peak areas for 1,1-diethoxyethane and furfural which were closer to commercial samples compared to the peak areas in the heat only experiments. However, the concentrations for syringaldehyde and vanillin were lower in the heat-freeze experiment most likely due to the lower amounts of sinapaldehyde and coniferaldehyde. This was an issue because, as previously mentioned (section 5.2.1, phenolic aldehydes), vanillin and syringaldehyde are not only desired for flavour but also for age and quality indicators [1,23,37,46,53]. The aim of this research was not only to produce a chemical profile similar to a ≥ 10-year-old whiskey but also to produce a good quality whiskey.

Overall the peak areas throughout the heat-freeze experiment for 1,1-diethoxyethane and furfural were within the commercial sample peak area range whereas from day 6 in the heat distillate experiment (section 5.2.2) the peak areas in the malt distillate were above the

commercial sample peak area range. Furthermore, the heat-freeze samples were slightly lighter in colour compared to the heat experiment samples. The corn distillate was cloudy after removal from the freezer and did not look like the traditional amber colour associated with whiskey. An investigation into chill-filtration could be part of future work; this would need to be tested because chill filtration needs to be done correctly to avoid removing desired compounds. The concentrations of the esters were acceptable and similar to commercial samples. The absence of phenols suggested modification of the parameters were required to obtain smokey characteristics while keeping the concentration/peak areas at similar levels to commercial samples. The results of the heat-freeze experiment suggested that the heat only experiment was better at extracting/forming desired compounds in a short timeframe.

5.6. Comparisons Between Different Ageing Treatments

Comparison between the heat, heat-freeze and sonication results showed heat (70 °C) and sonication (50 °C) were the most promising ageing treatments. The majority of compounds detected in both experiments had similar concentrations/peak areas to commercial samples. It should be re-iterated that there were instrument issues during analysis of the light experiment and samples could not be re-analysed due to evaporation, therefore, the results could not be compared to other ageing treatments.

The heat-freeze day 9 results and the day 6 small chips distillate heat experiment (section 5.2.2) results for isoamyl alcohol, ethyl octanoate, syringaldehyde and vanillin in the malt distillate showed both heat-freeze and heat produced similar concentrations to several commercial samples. The peak areas throughout the heat-freeze experiment for 1,1-diethoxyethane and furfural were within the commercial sample peak area range whereas from day 6 in the heat distillate experiment (section 5.2.2) the peak areas in the malt distillate were above the commercial samples peak area range. The colour of the heat-freeze samples were more similar to traditional whiskey compared to the heat experiment samples. Hence, both the heat-freeze and heat experiments were able to extract desired compounds at levels similar to commercial samples, but heat alone was faster (6 days compared to 9 days).

Only 1-2 sonication cycles at 50 °C were required for the malt distillate to achieve concentrations close to commercial samples for several desired compounds (isoamyl acetate, isoamyl alcohol, 2-phenylethanol, syringaldehyde, ethyl octanoate) except for ethyl acetate which was relatively higher than the analysed commercial samples. These results showed

sonication was the quickest (amongst the ageing treatments tested) at increasing concentrations/peak areas of desired compounds.

Heating at 70 °C allowed extraction of several wood compounds (vanillin, syringaldehyde and 2,6-dimethoxyphenol), whereas the non-heated sonication experiment did not extract these wood compounds. Singleton (1962) [66] stated that temperature manipulation (heat) will be required in any rapid ageing system to achieve improvements to spirits in a shorter time frame, however, other techniques may be required too.

The heat experiment was the only experiment to produce phenols so depending on the client's preference for smokey characteristics this could be readdressed in future work.

The rapid maturation experiment data was added to the PCA and LDA datasets to observe clusters and investigate whether a similar compound profile to a ≥ 10-year-old whiskey was achieved with any of the ageing techniques. It was recognised that the results obtained for PCA and LDA were subjective to the compounds added to the multivariate treatment, therefore, if samples did cluster around ≥ 10-year-old samples it did not necessarily mean that a full whiskey profile was achieved. Furthermore, the large quantity of 10-12 year-old samples (18 out of 27 samples) used in the analysis was potentially skewing the PCA results.

5.6.1. Principal Component Analysis

Principal component analysis was carried out on the best results (toast level, wood size and day) for each experiment; this was determined by the minimal time to extract sufficient concentrations/peak areas of desired compounds compared to commercial samples. Six days for the heat only experiment (section 5.2.2), 9 days for the heat-freeze experiment (section 5.5) and one non-heated sonication cycle (section 5.4). Two separate PCAs were conducted, one with the corn distillate samples and another with the malt distillate samples; this was to separate the two types of distillate because throughout all the rapid maturation experiments they differed in their chemical composition (compounds present and their relative levels).

5.6.1.1. Corn Distillate Samples

The corn distillate experiment information and the key used in PCA are summarised in Table 5.3. The commercial samples used were the same as those in section 4.3.

Table 5.3: Experiment sample ID and information on the samples used in PCA with corn distillate samples and commercial samples.

Experiment Description	Wood Size	PCA Key
Heat, Heavy toast Corn, 6 days	Small	Heat6_ H
Heat, Medium toast Corn, 6 days	Small	Heat6_ M
Sonication, non-heated, 1 cycle, Corn	Small	Sonic1
Sonication, 50 °C, 1 cycle, Corn	Small	50 °C Sonic1
Heat-Freeze, Corn, 9 days	Small	Heat-Freeze9

The PCA for the corn distillate samples from various maturation treatments (Figure 5.40) showed all samples broadly clustered around the 10-12 year-old (group C) and > 12-year-old (group D) commercial samples. Although, one cycle of non-heated sonication and 9 days of heat-freeze were the closest to the bulk of the 10-12 year-old commercial samples. No outliers were found and the loading plot (Figure 5.41) showed many compounds had an influence on the variability in the samples. The eigenvectors (used to calculate the principal component scores) were all < 0.5 and > −0.5; this meant no compound in particular was influencing the variability in data. Overall all ageing treatments showed promise with corn distillate, especially the non-heated sonication and heat-freeze treatments.

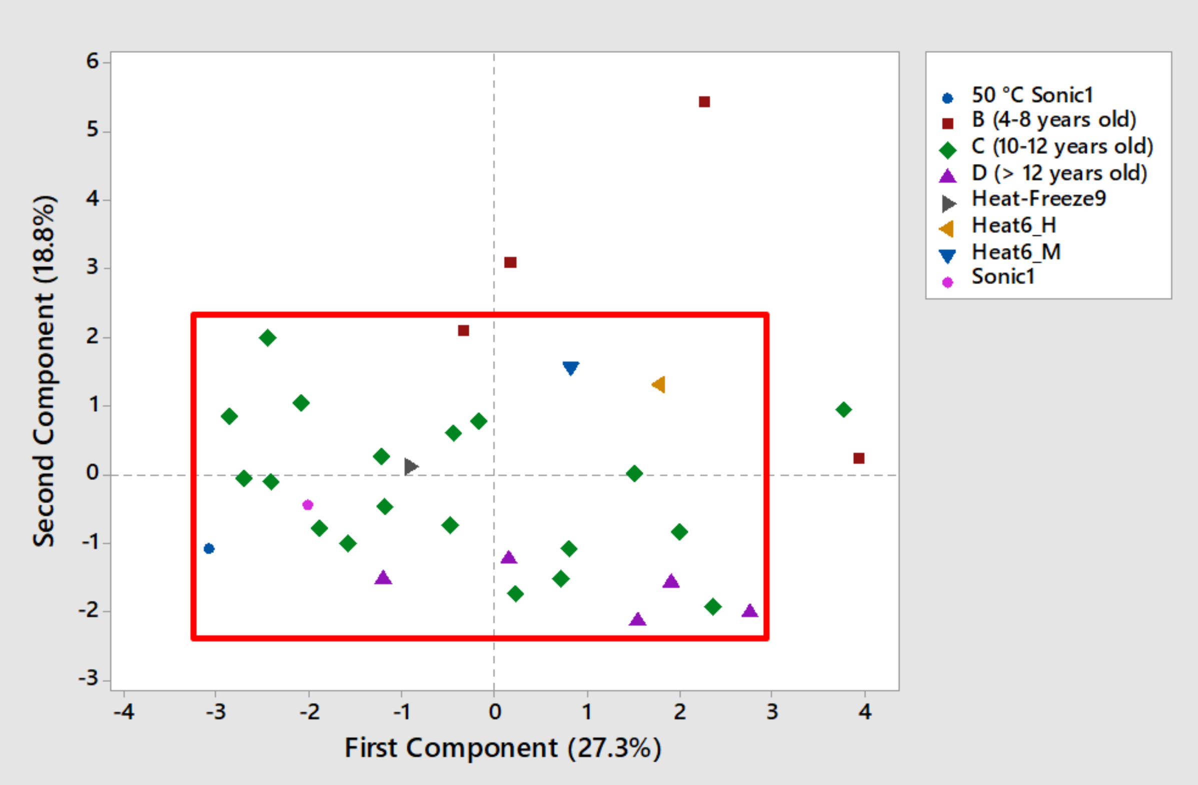

Figure 5.40: Score plot of commercial samples and results for corn distillate samples in the heat (6 days), sonication (one cycle) and heat-freeze (9 days) experiments. All corn samples appeared to broadly cluster (red square) with group C (10-12 years old) and D (> 12 years old) commercial samples.

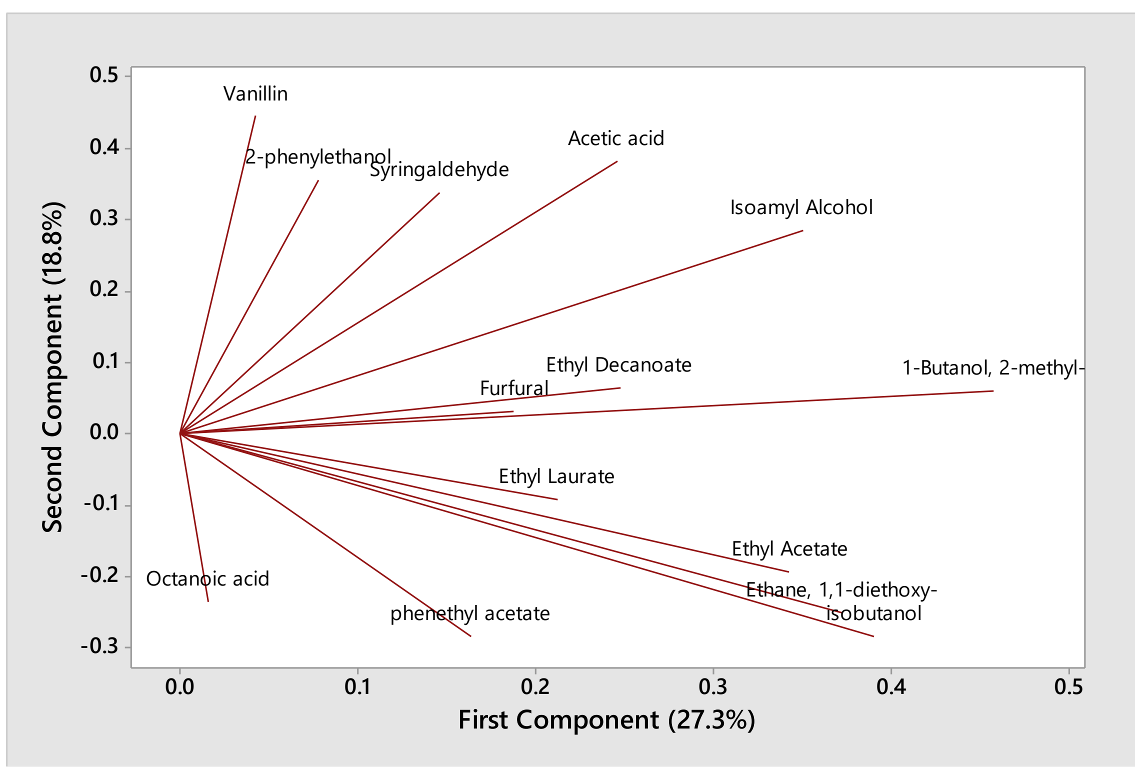

Figure 5.41: Loading plot for commercial samples and corn distillate samples in the heat (6 days), sonication (one cycle) and heat-freeze (9 days) experiments. All eigenvectors were < 0.5 and > -0.5 showing no compound severely influenced the variation in the samples.

5.6.1.2. Malt Distillate Samples

The malt distillate experiment information and the key used in PCA are summarised in Table 5.4. The commercial samples used were the same as those in section 4.3.

Table 5.4: Experiment sample ID and information on the samples used in PCA with malt distillate samples and commercial samples.

Experiment Description	Wood Size	PCA Key
Heat, Heavy toast Malt, 6 days	Small	Heat6_ H
Heat, Medium toast Malt, 6 days	Small	Heat6_ M
Sonication, non-heated, 1 cycle, Malt	Small	Sonic1
Sonication, 50 °C, 1 cycle, Malt	Small	50 °C Sonic1
Heat-Freeze, Malt, 9 days	Small	Heat-Freeze9

The PCA for the malt distillate samples from various maturation treatments did not cluster near any commercial samples (Figure 5.42). The loading plot (Figure 5.43) showed acetic acid, 2-phenylethanol and isoamyl alcohol were the only compounds to have eigenvectors < -0.4; furthermore, the eigenvectors showed furfural and octanoic acid were -0.565 and -0.513, respectively for PC4. These results suggested these five compounds were the only compounds

heavily contributing to the variation in data. Overall the PCA showed none of the ageing techniques had a similar profile to commercial samples despite the presence of desired compounds at desirable concentrations/peak areas.

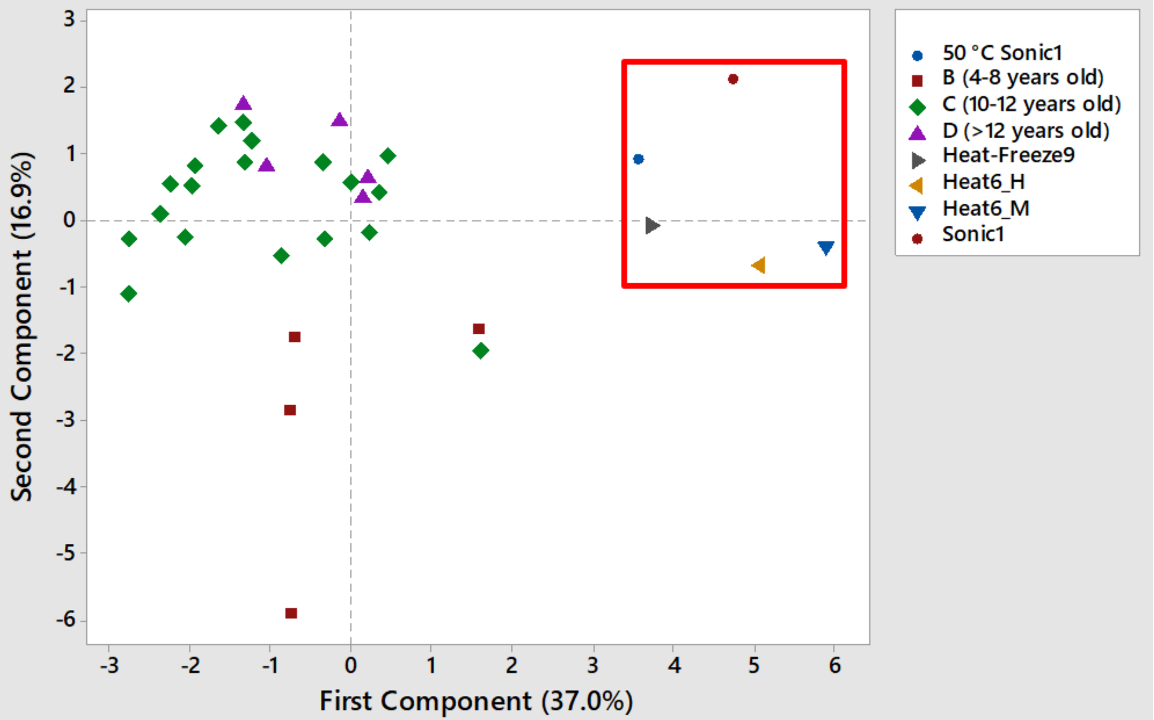

Figure 5.42: Score plot of commercial samples and results for malt distillate samples in the heat (6 days), sonication (one cycle) and heat-freeze (9 days) experiments. All maturation experiment samples broadly clustered around each other (red square) but did not cluster near any commercial samples.

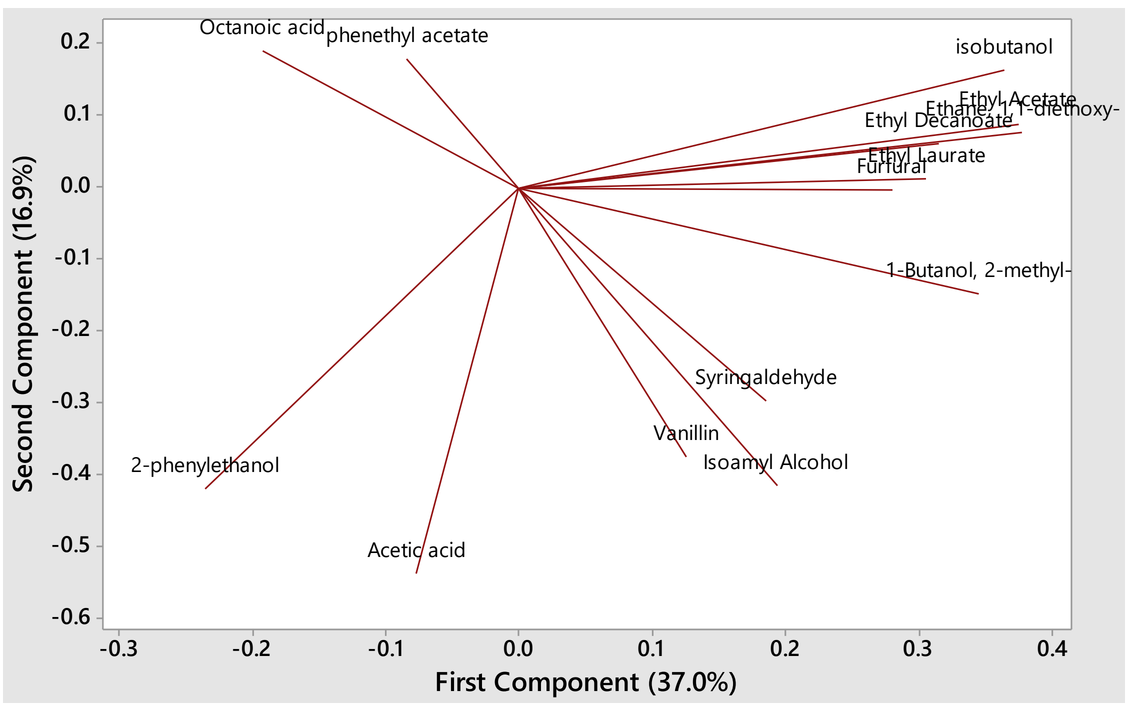

Figure 5.43: Loading plot for commercial samples and results for malt distillate samples in the heat (6 days), sonication (one cycle) and heat-freeze (9 days) experiments. Only acetic acid, 2-phenylethanol and isoamyl alcohol had eigenvectors < −0.4 showing these compounds had an influence on the variation in the samples.

5.6.2. Linear Discriminant Analysis

To investigate if a ≥ 10-year-old whiskey profile was achieved, the rapid maturation experimental data (the same samples used in PCA (Table 5.3 and Table 5.4)) were added to the LDA model generated in the analysis of commercial samples (section 4.4). The assessment involved looking at the probabilities of the experimental samples being categorised into group C (10-12 years old) and/or D (> 12 years old). The corn and malt distillate samples were not separated for LDA because they were assessed against the commercial samples and did not influence the probabilities.

Each of the experimental samples were categorised into a whiskey age group; group B (4-8 years old), C (10-12 years old) and D (> 12-years old). The raw data can be found in Appendix N, Table N.1, page 204. All probabilities were > 0.72 (Table 5.5), except for the 50 °C sonication corn distillate sample which had a relatively even probability between group C and D. These results indicated all samples matched the chemical profile of a ≥ 4-year-old whiskey. It was acknowledged that even though the majority of samples were 'predicted' to be ≥ 10 years old using the 14 compounds in this study, these results do not necessarily confirm

that the experimental samples had similar flavours and aromas to ≥ 10-year-old commercial samples. This would need to be confirmed by sensory analysis which would be carried out in future work at the distillery.

Table 5.5: LDA predicted age groups for experimental samples.

Toast level (small chips)	Age Treatment	Age Time	Distillate	Predicted group	Group Probability*		
					B†	C‡	D§
Heavy	Heat	6 days	Corn	C	0.00	**1.00**	0.00
Medium	Heat	6 days	Corn	C	0.00	**1.00**	0.00
Heavy	Heat	6 days	Malt	D	0.00	0.05	**0.95**
Medium	Heat	6 days	Malt	D	0.00	0.00	**1.00**
Medium	Sonication	cycle 1	Corn	C	0.00	**0.72**	0.28
Medium	Sonication	cycle 1	Malt	B	**1.00**	0.00	0.00
Medium	50 °C Sonication	cycle 1	Corn	D	0.00	0.47	0.53
Medium	50 °C Sonication	cycle 1	Malt	D	0.00	0.00	**1.00**
Medium	Heat-Freeze	9 days	Corn	C	0.00	**0.81**	0.20
Medium	Heat-Freeze	9 days	Malt	D	0.00	0.00	**1.00**

* The bold numbering highlights the highest group probability for a given sample

† Group B refers to commercial samples with age statements 10-12 years old

‡ Group C refers to commercial samples with age statements 4-8 years old

§ Group D refers to commercial samples with age statements > 12 years old

Chapter 6 - Conclusions and Future Work

The overall objectives of this thesis were achieved with several future work recommendations given below.

The objectives of this research were to:

1) Carry-out method development and validation for analysis of samples using GC-MS (Chapter 3)
2) Analyse a range of commercial whiskey samples of various ages and origins to determine the compounds present and their relative compositions (Chapter 4)
3) Conduct exploratory experiments to investigate the effects of ageing techniques on ethanol and distillate (Chapter 5)
4) Determine which ageing treatment is the most suitable to achieve a > 10-year-old whiskey profile (Chapter 5)

6.1. Conclusions

A gas chromatography mass spectrometry (GC-MS) method was set up and validated for the identification and quantification for 15 compounds present in whiskey. The method was deemed fit for purpose (sufficient resolution and sensitivity) in the analysis of samples (commercial and experimental) to determine the compound composition. Analysis of commercial samples revealed what compounds were desirable in a rapidly matured whiskey.

Four different ageing treatments (heat, light, sonication and heat-freeze) were investigated to determine which was the most suitable for the rapid maturation of corn and malt distillates. The use of different sized wood (large blocks, small chips and shavings) and toasting levels (light, medium and heavy) were investigated. Initially, different sized wood pieces were soaked in 65% ethanol and heated. The results revealed 70 °C to be the better temperature (compared to 50 °C). Furthermore, it was found that medium toast small chips were the best at extracting desired compounds (e.g. syringaldehyde, vanillin, furfural and 1,1-diethoxyethane) and precursors to desired compounds (e.g. acetic acid, sinapaldehyde and coniferaldehyde) in a short timeframe (≤ 14 days).

The different ageing treatments showed heat was a major factor in the extraction of compounds. Heating at 70 °C for 6 days proved to be the fastest at extracting and increasing the

concentrations/peak areas of desired compounds (acetic acid, esters, phenolic aldehydes, furfural and 1,1-diethoxyethane) as well as decreasing isoamyl alcohol and 2-methyl-1-butanol which are associated with causing headaches and hangovers. Despite the concentrations/peak areas being similar to many commercial samples, the malt distillate profile did not align with ≥ 10-year-old commercial samples on the PCA plot. Conversely, for the corn distillate one cycle of sonication (non-heated) showed promise because the chemical profile clustered in the same region as the 10-12 year-old samples on a PCA plot. Not all compounds present in the heat experiment were present in the sonication samples, therefore, further investigation was required.

6.2. Future Work

The results from this research have provided insight into the effects of different ageing techniques and wood chips (sizes and toasting levels) on corn and malt distillates. Future work could involve repeating the most successful experiments (70 °C and sonication) at the distillery to allow taste testing which is a key indicator as to whether a similar flavour and aroma profile to a commercial whiskey was achieved. Additional future work would involve further refinement of experiments to optimise the extraction of desired compounds while maintaining a profile similar to ≥ 10-year-old whiskies.

If the trials prove successful, this work will increase the economic viability by providing a more cost-effective alternative to the traditional maturation of whiskey once the desired profile is achieved.

6.2.1. Future GC-MS Method Development

Quantitation < 0.1 ppm was not possible in the present work. Many of the changes in compounds (extractions and reactions) are occurring at concentrations currently < LoD, therefore, further method development is required to improve detection limits. An easy way to improve sensitivity (i.e. lower the detection limit) is to acquire data using selected ion monitoring (SIM). The knowledge on what compounds were extracted from each ageing technique can be used to set up SIM to target these known compounds. Furthermore, standards could be purchased to quantify several compounds (furfural, 1,1-diethoxyethane, 2-methyl-1-butanol) to offer a more advanced summary of the compounds present in commercial and experimental samples.

Acids were not compatible with the column used in this research. The produced whiskey could undergo further testing on a different column for acids and other compounds which were not detected with the current column used in this research.

Quantitation of tannins could also be carried out because they are important in the perceived astringency of spirits. There are many ways to detect tannins including using Folin-Denis reagent (colour change due to metal reduction) [28] or Adams-Harbertson assay (protein precipitation) which is an inexpensive and reliable measurement of tannins [160]. Three ways to reduce tannin levels include: ultrafiltration after the maturation of whiskey is complete, reduce the amount of wood chips added or decrease the time the wood is left in solution.

6.2.2. Additional Maturation Experiments

Weathering of wood chips could be carried out to see whether the experiment time could be further reduced. Weathering wood outdoors (particularly in hot climates) has been reported to increase levels of volatile phenolic compounds (e.g. syringaldehyde, vanillin and sinapaldehyde).

If smokey characteristics are desired, 70 °C heat (with medium or heavy toast chips) would be required because the heat only experiment was the only experiment which produced phenols.

The sonication method would need to be refined because not all of the desired compounds were extracted (e.g. phenolic aldehydes) despite clustering with ≥ 10-year-old whiskies in the PCA plot. One potential adaption to the sonication method includes increasing the sonicator temperature to 70 °C as the heat experiment showed 70 °C extracted compounds faster than 50 °C. Another potential adaption is to combine the 70 °C heat experiment and the non-heated sonication experiment. The heat would extract the phenolic aldehydes and the sonication would provide further energy for reactions to occur.

The parameters from the heat-freeze experiment are simple to implement on a large scale, i.e. in a distillery. However, the corn distillate was cloudy after removal from the freezer and did not look like the traditional amber colour associated with whiskey. If heat-freeze treatment was pursued as a treatment, future heat-freeze experiments could include longer heat cycles and/or fewer freezer cycles and/or use of a higher temperature freezer (−4 °C). These changes would allow faster extraction of desired compounds and minimise cloudy solutions. Alternatively, chill filtration (cooling the whiskey before filtering) could be investigated as less soluble

compounds such as lipids and large molecular weight lignin compounds (unwanted compounds) form a precipitate when chilled. This results in a hazy solution which can be filtered to remove these undesirable compounds.

6.2.3. Future Modification to the Rapid Maturation Method

After distillery trials and/or additional experiments (section 6.2.2) have proved successful, the method can be further modified to produce a variety of different whiskey profiles. For example, to gain more smokey characteristics, medium toast chips could be substituted for heavy toast chips or a combination of medium and heavy toast chips could be used. To produce a sweeter whiskey the heavy toast oak chips can be switched out for fresh medium toast chips after a period of time to extract sufficient furfural and vanillin without adding too much smokey character.

Overall the results from this research have increased the understanding of the maturation process and produced one or more age treatments for further development and implementation in the distillery. Nevertheless, there are still many unexplored avenues to gain a complete understanding of the underlying science behind the maturation of whiskey.

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Appendix A. Wood Macromolecules

Table A.1: Summary of wood macromolecule derived compounds with flavour descriptors. Modified from [25,28,39,41,48,50].

Compound	Functional group and boiling point (B.P) [161]	Origin, notes	Flavour descriptor
cis-oak lactone (whiskey lactone) *trans*-oak lactone	Ester B.P = 246.5 °C	Oak lactone (whiskey lactone) from lipid oxidation of oak heartwood. Concentration may arise from low levels during toasting of wood.	Pleasing woody, earthy and herbaceous at low concentrations to coconut at high concentrations Coconut, herb and spice
Vanillin	Phenolic aldehyde B.P = 285 °C	Increased by toasting but decreased at high toast level.	Vanilla ice cream and vanilla bean
Eugenol & isoeugenol	Phenolic B.P = 256 °C Phenolic B.P = 266 °C	Arises from ethanolysis of lignin. Increased by thermal degradation of oak	Spicy-cloves, allspice, cinnamon and nutmeg with woody hints
Guaiacol	Phenolic B.P = 203-206 °C	Degradation of the lignin component of wood during toasting and charring (pyrolysis of lignin)	Char like, smokey and spicy
4-methylguaiacol	Phenolic B.P = 220-222 °C	Toasting of oak lignin	Smokey and char
4-ethylguaiacol	Phenolic B.P = 229-235 °C	From lignin breakdown	Carnation, smokey, spicy, medicinal
4-ethyl phenol	Phenolic B.P = 218-219 °C	From lignin breakdown	Horse-like/barnyard, medicinal

Compound	Functional group and boiling point (B.P) [161]	Origin, notes	Flavour descriptor
Furfural	Furan aldehyde B.P = 161-162 °C	From heat induced degradation of sugars and carbohydrates (hemicelluloses) – Caramalisation reactions and Maillard reactions.	Sweet, butterscotch, caramel, smokey and almond hints
5-methylfurfural	Furan aldehyde B.P = 187 °C		Caramel, fatty, musty, waxy
Syringaldehyde	Phenolic aldehyde B.P = 192-193 °C	From lignin breakdown	Plastic, woody, vanilla, sweet
Syringol	Phenolic B.P = 261-267 °C	From lignin breakdown	Toasted, coffee, balsamic, bacon
Sinapaldehyde	Phenolic aldehyde B.P = 335-409 °C	From lignin breakdown	Unknown
Coniferaldehyde	Phenolic aldehyde B.P = 175 °C	From lignin breakdown	Roasted oak, baked bread, toasted grain
Syringic acid	Phenolic Acid B.P = 363-379	From syringaldehyde oxidation (lignin breakdown)	Unknown

Compound	**Functional group and boiling point (B.P)** [161]	**Origin, notes**	**Flavour descriptor**
Vanillic acid	Dihydroxybenzoic acid B.P = 353 °C	From vanillin oxidation (lignin breakdown)	Vanilla bean, sweet, dairy

Appendix B. Chemicals

Table B.1: List of chemicals, CAS number, suppliers and purity for standard preparation

Chemical	CAS	Supplier	Batch	Purity %
1-Naphthol	90-15-3	AnalaR (British Drug Houses)		99.0*
2-Phenylethanol	60-12-8	Sigma-Aldrich	STBH1923	99.3
2-Propanol (IPA)	67-63-0	2-propanol EMPLURA		> 99.5*
Glacial Acetic Acid	64-19-7	Merck - 100% anhydrous for analysis EMSURE ACS, ISO, Reag. Ph Eur	K49860163802	100*
Acetone	67-64-1	Acetone for analysis EMSURE ACS, ISO, Reag. Ph Eur		> 99.8
Benzaldehyde	100-52-7	Sigma-Aldrich	B1334 MKBR2441V	99.7
Cinnamaldehyde	14371-10-9	M&B May and Baker LTD	c150/18/67	98.0*
Ethyl Acetate	141-78-6	Univar (Ajax Chemicals)	606638	99.0*
Ethyl Butyrate	105-54-4	Riedel-De Haen Ag Seelze-Hannover	60082 or 20069	unknown
Ethyl Decanoate	110-38-3	Sigma-Aldrich	STBH1307	99.2
Ethyl Laurate	106-33-2	Sigma-Aldrich	BCCB5060	99.7
Ethyl Octanoate	106-32-1	Sigma-Aldrich	MKCC4413	99.5
Gallic Acid	149-91-7	Sigma-Aldrich	100M0258V	99.7
Guaiacol	90-05-1	BDH Chemicals LTD (reagent)		99.9*
Isoamyl Acetate	123-92-2	Sigma-Aldrich	06714HC	99.4
Isoamyl Alcohol	123-51-3	Univar (a division of clyde industries limited)		98.5*
m-Cresol	108-39-4	The British Drug Houses LTD		99.0*
m-Methoxyphenol	150-19-6	K&K Laboratories and BDH Laboratory		98.0
n-Decanoic Acid	334-48-5	BDH LTD Biochemical		98.5
n-Pentanol	71-41-0	UniLab - Ajax Chemicals LTD	13676	98.0
Pentane	109-66-0	Univar Analytical Reagent		99.0
Succinic Acid	110-15-6	AnalaR BDH Chemicals LTD		99.0*

Chemical	CAS	Supplier	Batch	Purity %
Syringaldehyde	134-96-3	Sigma-Aldrich	07711BZ	98.0*
Syringic Acid	208-486-8	Sigma-Aldrich	77H5234	> 99.0
Vanillic Acid	121-34-6	Sigma-Aldrich	V-2250 23-F0762	98
Vanillin	121-33-5	AnalaR (British Drug Houses)		99.0*

* = Purity from bottle, no COA available

Appendix C. Preparation of Standards

Internal Standard (ISTD) Stock Solution and Working Solution

A stock solution (70 ppm) of the ISTD, 1-naphthol, was prepared by weighing out 0.10 g into a 10 mL volumetric and dissolved in 65% EtOH. A working ISTD solution (3.5 ppm) was prepared by weighing 2.5 g of the stock solution into 50 mL volumetric and filled to the line using 65% EtOH.

System Monitoring Compound (SMC)

The SMC, *n*-pentanol, was prepared by weighing out 0.017 g into a 20 mL volumetric and dissolved in 65% EtOH to give a concentration of 10 ppm.

Calibration Standards

To prepare the calibration standards (Table 2.4, section 2.4.1), individual stocks with concentrations of 200 ppm were prepared using the neat compounds and ethanol-water mix (65:35), with the exception of ethyl laurate (33 ppm) and ethyl decanoate (80 ppm) due to their low solubility in ethanol (1 mL in 9 mL of 80% EtOH and 1 mL in 4 mL of 80% EtOH respectively) [50]. The individual stocks (excluding ethyl laurate and ethyl decanoate) were combined to make two intermediate stocks (stock A and B) with concentrations of 25 ppm (Table C.1). Stocks A and B were combined to create mix 1 and 2 (high stock A concentration/low stock B concentration and vice versa, Table C.2) which were further diluted to create a calibration set. Table C.3 summarises the volumes of ISTD, SMC, mix 1 and mix 2 added to make the 10 calibration standards and Table 2.4 (section 2.4.1) summarises the concentration of mix 1 and 2 in each of the standards. Due to low solubility of ethyl laurate and ethyl decanoate these compounds were diluted to 25 ppm then made into an intermediate stock (C, Table C.1) with a concentration of 12.5 ppm. Stock C was used to create a set of calibration standards (Table C.4).

Table C.1: List of compounds present in stock A, B and C used to make calibration standards.

Stock A (esters + phenolic aldehydes)	Stock B (miscellaneous)	Stock C (2 esters)
Ethyl Acetate	Guaiacol	Ethyl Laurate
Ethyl Octanoate	*m*-Cresol	Ethyl Decanoate
Ethyl Butyrate	Isoamyl Alcohol	
Cinnamaldehyde	2-Phenylethanol	
Isoamyl Acetate	Vanillin	
Benzaldehyde	Syringaldehyde	
	m-Guaicol	

Table C.2: Volumes of Stock A and B added to make Mix 1 and 2.

Mix	**Stock A** 25 ppm stock (mL)	**Stock A** Overall concentration (ppm)	**Stock B** 25 ppm stock (mL)	**Stock B** Overall concentration (ppm)	**Total (mL)**
1	16	20	4	5	20
2	4	5	16	20	20

Table C.3: Volumes of mix 1 and 2 used to make calibration standards (10 mL total volume) ranging from 0.13-10 ppm.*

Cal Std	**Mix 1 (mL)**	**Mix 2 (mL)**
1	–	0.25
2	–	0.38
3	–	0.5
4	0.25	–
5	0.38	–
6	0.5	–
7	–	2.5
8	–	5.0
9	2.5	–
10	5.0	–

*2.5 mL ISTD and 0.5 mL SMC added to all standards

Table C.4: Volumes of stock C and to make calibration standards (10 mL total volume) ranging from 0.2-10 ppm.*

Cal Std	**Stock C (mL)**	**Overall Concentration (ppm)**
1	0.16	0.2
2	0.40	0.5
3	0.80	1.0
4	4.00	5.0
5	8.00	10.0

*2.5 mL ISTD and 0.5 mL SMC added to all standards

Appendix D. Whiskey Samples

Table D.1: Summary of commercial whiskey samples, brand, age, whiskey description and %ABV

No.	Brand	Age	Description	%ABV
1	Wild Turkey	7	101, Kentucky Straight Bourbon Whiskey	50.5
2	Wild Turkey	12	Kentucky Straight Bourbon Whiskey	50.5
3	Glenlivet	12	Single Malt Whisky	40
4	Bunnahabhain	12	Islay Single Malt Scotch Whisky, small batch distilled	46.3
5	Glenrothes	no age	Speyside Single Malt Scotch Whisky sherry cask reserve	40
6	Laphroaig	no age	Islay Single Malt Scotch Whisky - Legacy Edition	48
7	Jamieson	no age	Irish Whiskey	
8	Jack Daniels	no age	Jack Daniels Fire (American Bourbon) Cinnamon liqueur blended with Jack Daniels No.7 Tennessee Whiskey	35
9	Highland Park	12	Single Malt Scotch Whisky	40
10	Laphroaig	10	Islay Single Malt Scotch Whisky	40
11	Glenfiddich	12	Single Malt Scotch Whisky - signature malt	41
12	Ardbeg An Oa	no age	The Ultimate Islay Single Malt Scotch Whisky	46.6
13	Bunnahabhain	no age	Islay Single Malt Scotch Whisky - Cruach - Mhona limited edition release	50
14	Samuel Baker	no age	Black Label Reserve Bourbon, Kentucky Straight Bourbon Whiskey	37.2
15	Jameson	no age	Triple Distilled Irish Whiskey	40
16	Jack Daniels	no age	Jack Daniels No.7 Tennessee (sour mash) Whiskey	40
17	Jameson	no age	Triple Distilled Irish Whiskey	40
18	Booker's	7	Kentucky Straight bourbon whiskey	63.7
19	Wild Turkey	6-8	101, Kentucky Straight Bourbon Whiskey, barrel char no.4	50.5
20	Chivas Regal	12	Premium Scotch Whisky	40
21	Johnnie Walker	12	Black Label Blended Scotch Whisky	40
22	Jim Beam	no age	Devils Cut Kentucky Straight Bourbon Whiskey	40
23	Canadian Club	no age	Imported Blended Canadian Whisky	37
24	Glenfiddich	12	Single malt Scotch whisky	40
25	Jamieson	no age	Irish Whiskey Triple Distilled	40
26	Canadian Club	no age	Imported Blended Canadian Whisky	37
27	Jim Beam	no age	Kentucky Straight Bourbon Whiskey	37

No.	Brand	Age	Description	%ABV
28	Glengrant	no age	Major's Reserve – Rothes Speyside Single Malt Scotch Whisky	40
29	Glenfarclas	10	Highland Single Malt Scotch Whisky	40
30	Chivas Regal	12	Blended Scotch Whisky	40
31	Johnnie Walker	no age	Red Label Blended Scotch Whisky	40
32	Jack Daniels	no age	Old No.7 Tennessee Sour Mash Whiskey	40
33	Glenlivet	no age	Captains Reserve Single Malt Whisky - finished in cognac casks	40
34	Laphroaig	no age	Islay Single Malt Scotch Whisky - select cask	40
35	Canadian Club	no age	Imported Blended Canadian Whisky	37
36	Jim Beam	4	White Label	37
37	Glenfiddich		Cask Collection Single Malt Scotch Whisky	40
38	Laphroaig	10	Islay Single Malt Scotch Whisky	43
39	Glen Dronach	12	Original Highland Single Malt Scotch Whisky	43
40	Glenfarclas	12	Highland Single Malt Scotch Whisky	43
41	Aberfeldy	12	Highland Single Malt	40
42	Thompson	no age	New Zealand Whisky - two tone release (blend)	40
43	Sortilege	no age	Canadian Whisky + Maple Syrup	30
44	Clynelish	14	Single Malt Scotch Whisky	46
45	Balvenie	14	Single Malt Scotch Whisky, Caribbean Cask	43
46	Glenfarclas	15	Highland Single Malt Scotch Whisky	46
47	Glenfiddich	21	Single Malt Scotch Whisky, reserve rum cask finish	40
48	Talisker Skye	no age	Single Malt Scotch Whisky	45.8
49	Chivas Regal	12	Blended Scotch	40
50	Jura	10	Origin - Single Malt Scotch Whisky	40
51	Dalwhinnie	15	Highland Single Malt Scotch Whisky	43
52	K5	no age	Blended Scotch Whisky, Produce of Bhutan	40
53	Ardbeg	10	Single Islay Malt Scotch Whisky, non-chill filtered	46

Appendix E. Experiment Information

Table E.1: Summary of matrix, toasting, size and weight of chips for each ageing experiment.

Experiment	Matrix	Toasting Level	Wood Size	Weight of Chips (g)
Heat 70 °C	EtOH (65%)	Light	Large	6.2420
		Medium	Large	6.3797
		Heavy	Large	6.5210
		Light	Small	4.6081
		Medium	Small	4.7625
		Heavy	Small	4.6959
Heat 50 °C	EtOH (65%)	Light	Large	7.1089
		Medium	Large	7.2709
		Heavy	Large	6.8691
		Light	Small	4.7614
		Medium	Small	4.7419
		Heavy	Small	4.6333
Heat 70 °C - small chips	Corn (40%)	Medium	Small	4.0546
		Heavy	Small	3.9109
	Malt (65%)	Medium	Small	3.8424
		Heavy	Small	3.8686
Heat 70 °C - shavings	Corn (40%)	Medium	shavings	0.3858
		Heavy	shavings	0.3989
		Medium	shavings	1.0410
Light	EtOH (65%)	Light	Large	6.4782
		Medium	Large	6.3884
		Heavy	Large	7.0883
		Light	Small	4.6912
		Medium	Small	4.8769
		Heavy	Small	4.5586
Sonication	EtOH (65%)	Medium	Small	3.6745
	Corn (40%)	Medium	Small	3.7423
	Malt (65%)	Medium	Small	3.7211
Sonication - 50 °C	EtOH (65%)	Medium	Small	3.9198
	Corn (40%)	Medium	Small	3.8882
	Malt (65%)	Medium	Small	3.8563
Heat-freeze	EtOH (65%)	Medium	Small	3.6933
	Corn (40%)	Medium	Small	3.5011
	Malt (65%)	Medium	Small	3.5703

Appendix F. QC Plots

The following plots were generated for the compounds detected in the QC. It should be noted that the order of the QC data points does not reflect the order that the experiments are presented in within the thesis. The average (dashed black line), warning limits (orange line, ave ± 2SD) and action limits (red line, ave ± 3SD) are displayed on each plot. For the points that were near to the warning and action limits, general maintenance (liner change, syringe clean and new septa) was carried out on the instrument.

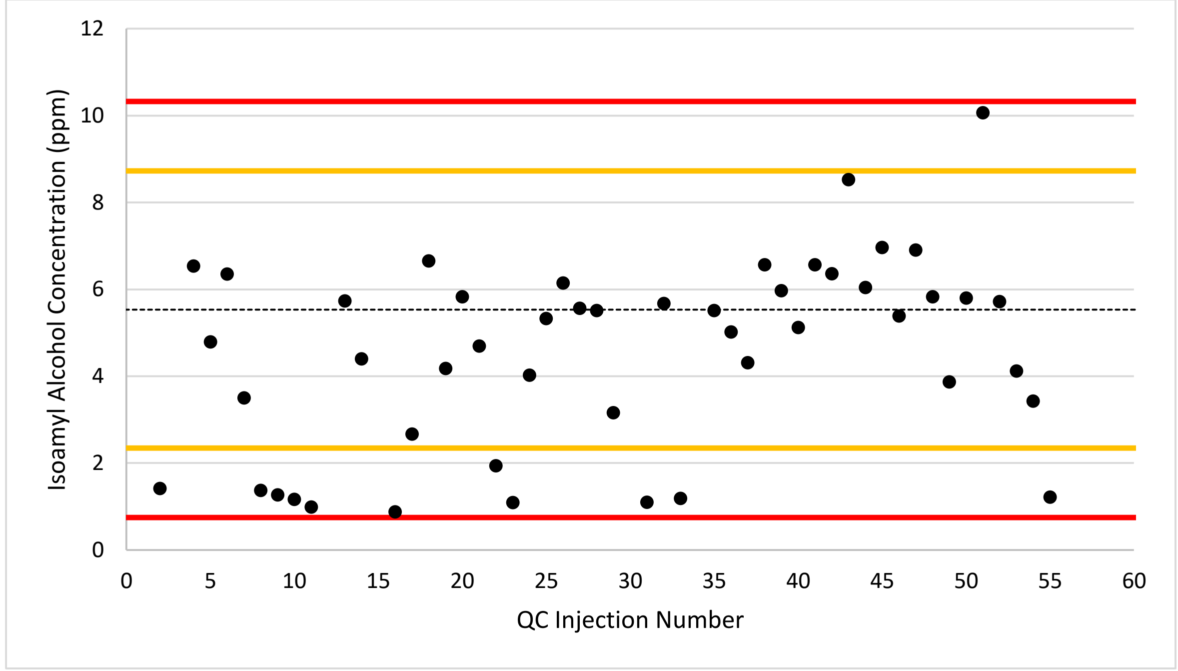

Figure F.1: Isoamyl alcohol concentration results for analysis of the QC with the average (dashed black line), warning limits (orange line, 5.53 ± 3.19 ppm, $n = 9$) and action limits (red line, 5.53 ± 4.79 ppm, $n = 9$) displayed. No sample data was used for any of the points which were on or below the low action limit.

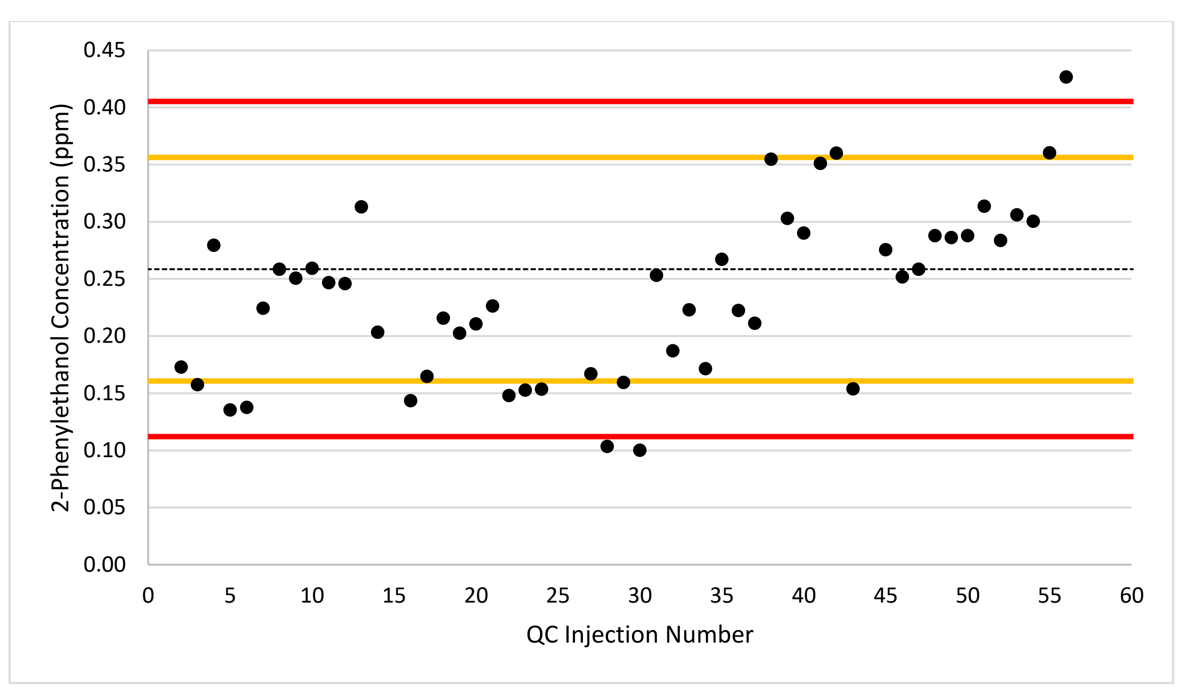

Figure F.2: 2-Phenylethanol concentration results for analysis of the QC with the average (dashed black line), warning limits (orange line, 0.26 ± 0.10 ppm, *n* = 9) and action limits (red line, 0.26 ± 0.15 ppm, *n* = 9) displayed. No sample data was used for any of the points which were below the low action limit or above the upper action limit.

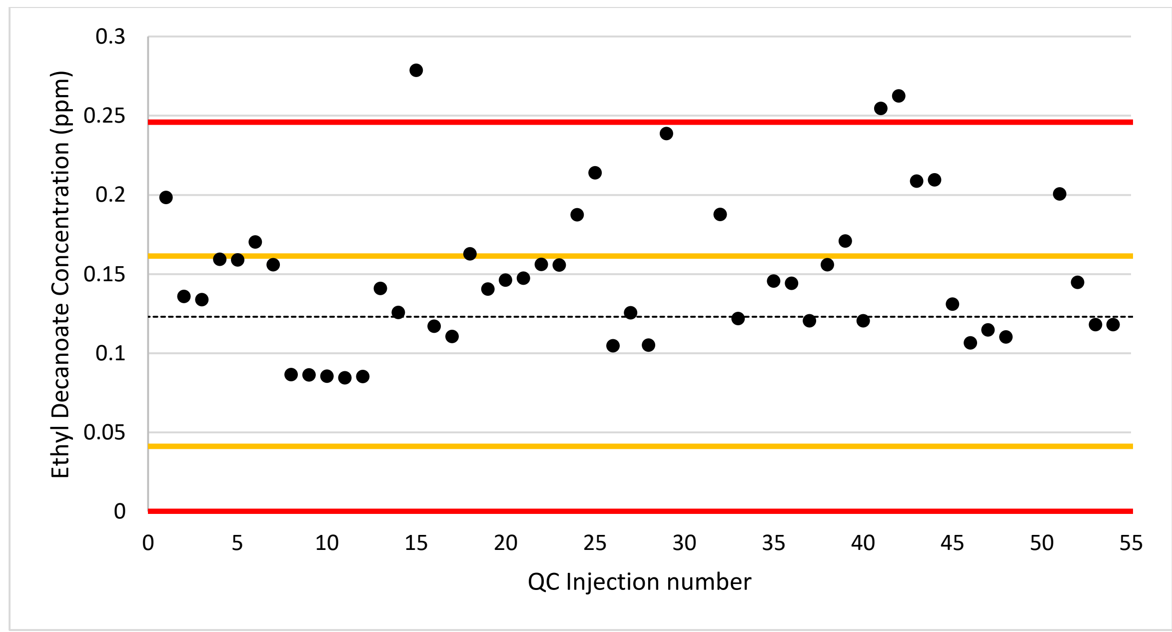

Figure F.3: Ethyl decanoate concentration results for analysis of the QC with the average (dashed black line), warning limits (orange line, 0.12 ± 0.08 ppm, *n* = 9) and action limits (red line, 0.12 ± 0.12 ppm, *n* = 9) displayed. No sample data was used for any of the points which were above the upper action limit.

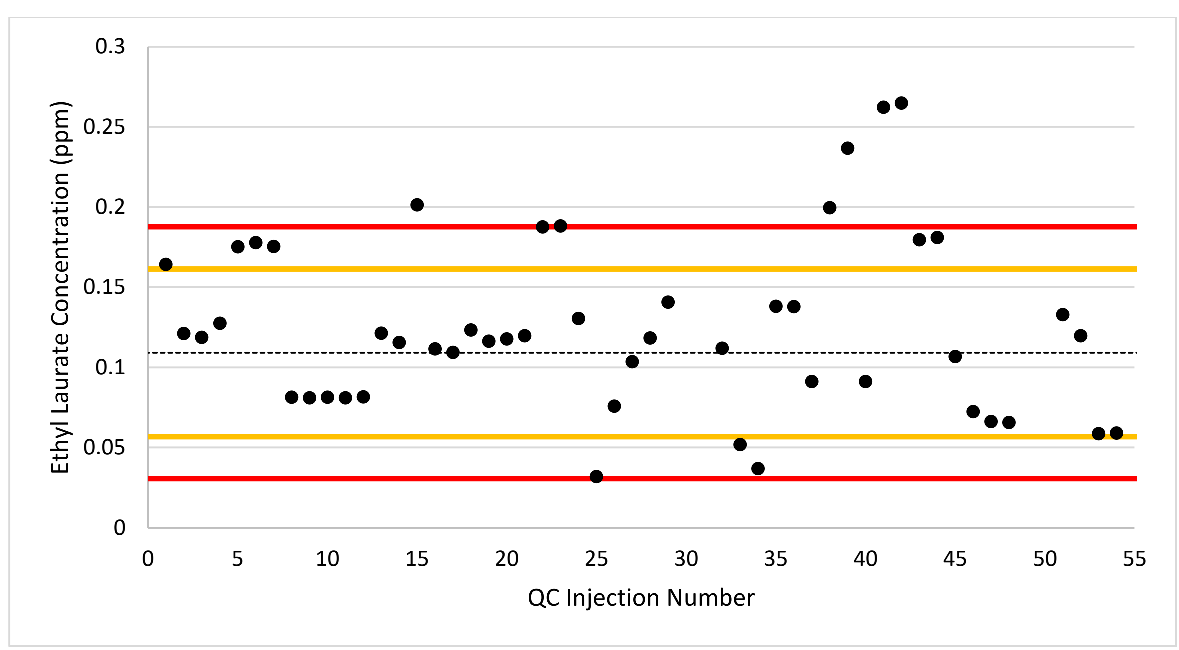

Figure F.4: Ethyl laurate concentration results for analysis of the QC with the average (dashed black line), warning limits (orange line, 0.11 ± 0.05 ppm, $n = 9$) and action limits (red line, 0.11 ± 0.08 ppm, $n = 9$) displayed. No sample data was used for any of the points which were below the low action limit or above the upper action limit.

Appendix G. Spike Recovery Data

Table G.1: Theoretical and calculated concentrations (ppm) for recovery of compounds at low (0.3 ppm) and high (5 ppm) concentrations in three whiskey samples and the corresponding calculated % recovery (n = 6).

Compound	Unspiked (ppm)			Low Spike (0.3 ppm)			High Spike (5 ppm)			Low Spike % Recovery			High Spike %Recovery		
	Sample (ppm)									Sample (% recovery)					
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Ethyl Acetate	2.79	1.63	1.29	N/A	N/A	N/A	7.39	7.12	6.78	N/A*	N/A	N/A	92	110	110
Isoamyl Alcohol	14.03	5.65	5.49	5.55	2.29	3.09	9.10	6.72	6.86	N/A	N/A	N/A	N/A	N/A	N/A
Ethyl Butyrate	0.03	0.03	0.03	0.35	0.32	0.34	5.08	4.60	4.66	106	96	103	101	91	93
Isoamyl Acetate	0.07	0.06	0.04	0.35	0.33	0.35	4.97	4.59	4.61	94	91	103	98	91	91
Benzaldehyde†	0.00	0.00	0.00	0.51	0.49	0.51	4.59	4.56	4.66	101	98	103	92	91	93
m-Cresol	0.00	0.00	0.00	0.31	0.28	0.30	4.88	4.87	4.79	103	92	101	98	97	96
Guaiacol	0.00	0.00	0.00	0.29	0.27	0.29	4.82	4.83	4.74	96	90	95	96	97	95
2-Phenylethanol†	0.23	0.32	0.30	0.70	0.79	0.78	4.94	4.98	4.90	93	94	96	95	94	94
Ethyl Octanoate†	0.01	0.01	0.01	0.55	0.47	0.53	4.89	4.67	4.68	106	93	103	96	92	92
m-Methoxyphenol	0.00	0.00	0.00	0.33	0.33	0.33	5.01	5.03	5.08	110	109	110	100	101	102
Cinnamaldehyde	0.00	0.00	0.00	0.31	0.28	0.31	4.60	4.64	4.53	102	95	102	92	93	91
Vanillin†	0.22	0.22	0.18	0.67	0.67	0.65	5.70	5.64	5.63	91	90	94	110	108	109
Syringaldehyde†	0.43	0.30	0.51	0.89	0.75	0.74	5.24	5.04	5.13	92	91	46 ‡	93	101	94

*N/A = No spike recovery calculated because endogenous concentration is over twice the spike concentration

† = 0.5 ppm low spike concentration

‡ high endogenous level affected spike recovery

Appendix H. Compounds Detected in Commercial Samples

Table H.1: Summary table of the common compounds detected (threshold ≥ 4×10^4) in the analysed commercial whiskey samples (n = 51) using qualitative analysis.

Compound	Samples that contain compound	Frequency (%)
Esters		
Ethyl Acetate	1-6, 10, 14-22, 24, 25, 28-39, 44-53	78
Ethyl Octanoate	1-6, 9, 18, 19, 21, 22, 24, 28, 30, 33, 39, 44-48, 50-53	49
Ethyl Decanoate	1-6, 13, 18, 19, 21, 22, 24, 25, 28, 30, 31, 33, 37, 39, 44-53	57
Ethyl Laurate	1-4, 6, 13, 18, 28, 30, 39, 40, 44-49, 51-53	39
Isoamyl Acetate	1-4, 6, 21, 24, 28, 30, 31, 44-48, 50-53	37
2-Methylbutyl Acetate	1, 44, 45	6
Ethyl Butyrate	1, 3, 28, 47	8
Phenethyl Acetate (Acetic Acid, 2-Phenylethyl Ester)	2-4, 12, 13, 24, 28, 29, 31-34, 37, 40-42, 44-48, 50, 52, 53	46
Ethyl Hexanoate	1-4, 6, 24, 44-47, 50-53	27
2-Hydroxyethyl Propionate (propanoic acid, 2-hydroxy- ethyl ester)	4-6, 13, 29, 34, 37, 39-41, 44, 46, 47, 51, 53	29
Diethyl Succinate (butanedioic acid, diethyl ester)	4-6, 12, 13, 24, 29, 37, 39, 40, 41, 46, 47, 50, 51, 53	31
Acids		
Acetic Acid	1-53*	100
n-Decanoic Acid	3-5, 9, 13, 28, 29, 33, 37, 39-42, 44-48, 50, 51, 53	41
Octanoic Acid	3-6, 9, 11, 13, 21, 24, 28, 29, 33, 37-42, 44-48, 50, 51, 53	51
Alcohols		
Isobutanol	1-7, 9-13, 21, 24, 25, 28-42, 44-48, 50-53	76
Isoamyl Alcohol	1-53*	100
2-Methyl-1-butanol	1-53*	100
2-Phenylethanol (Phenylethyl Alcohol)	1-6, 9-14, 18-21, 24, 26, 28-35, 37-42, 44-53	84
Diacetone Alcohol (2-Pentanone, 4-hydroxy-4-methyl-)	29, 31-38	18
$C_6H_{14}O$ (RT = 6.19 min)†	1, 2, 6, 32, 36, 44, 46	14

Compound	Samples that contain compound	Frequency (%)
Phenolic Aldehydes		
Vanillin	2, 4-6, 9, 12, 13, 16, 18, 19, 29, 32-34, 36-39	35
Syringaldehyde	1-6, 9, 10, 12, 13, 18, 19, 29, 32, 34, 36-39, 46	37
Phenols		
Phenol	6, 10, 12, 13, 34, 38, 53	14
Guaiacol	6, 10, 34, 38	8
m-Methoxyphenol	7, 10, 11, 47	8
Cresol (*o*-, *m*- and *p*-)‡	6, 10, 12, 13, 34, 38, 53	12
Miscellaneous		
Furfural	1-6, 10-13, 21, 24, 25, 28-42, 44-53	75
1,1-Diethoxyethane	1-6, 10, 14-22, 24, 25, 28, 30-33, 39, 44-53	65
Whiskey Lactone (*cis*- and *trans*- 3-Methyl-4-octanolide)	1-3, 6, 32, 34, 36, 38, 39	18
$C_6H_{12}O$ (RT = 2.04 min)†	1, 3-6, 10, 21, 28-32, 35-42, 44-48, 50-53	57

* = Excludes sample 8 and 43
† = Formula suggested by NIST
‡ = cresols combined

Appendix I. Minitab Printout for LDA

LDA Results For Scotch Versus American Whiskey

```
Group    Scotch    USA
Count        23      5

Summary of classification
                  True Group
Put into Group  Scotch    USA
Scotch              23      0
USA                  0      5
Total N             23      5
N correct           23      5
Proportion       1.000  1.000

N = 28           N Correct = 28     Proportion Correct = 1.000

Squared Distance Between Groups

         Scotch      USA
Scotch    0.000  110.821
USA     110.821    0.000

Linear Discriminant Function for Groups

                       Scotch      USA
Constant                 -7.6    -75.6
Ethyl Acetate            -0.9     19.2
Isoamyl Alcohol           0.6      6.7
2-phenylethanol          14.4    -32.8
Ethyl Octanoate           8.1   -196.0
Vanillin                 17.5    107.8
Syringaldehyde           -3.5    -13.5
Ethyl Decanoate          15.1    -22.0
Ethyl Laurate             1.6    -53.4
Acetic acid              21.7     54.1
isobutanol               26.9     91.4
Ethane, 1,1-diethoxy-   -37.1   -418.1
1-Butanol, 2-methyl-    -11.5     -9.4
Furfural                -90.6   -730.6
Octanoic acid             4.2    299.4
phenethyl acetate       -79.1  -2137.8
```

LDA Results for Year Group

Group	B	C	D
Count	4	19	5

Summary of classification

Put into Group	True Group B	C	D
B	4	0	0
C	0	18	1
D	0	1	4
Total N	4	19	5
N correct	4	18	4
Proportion	1.000	0.947	0.800

N = 28 N Correct = 26 **Proportion Correct = 0.929**

Squared Distance Between Groups

	B	C	D
B	0.0000	40.4347	43.4935
C	40.4347	0.0000	6.3048
D	43.4935	6.3048	0.0000

Linear Discriminant Function for Groups

	B	C	D
Constant	-26.65	-7.49	-7.38
Ethyl Acetate	-0.55	-3.77	-1.20
Isoamyl Alcohol	2.46	-0.16	0.20
2-phenylethanol	-6.38	20.84	15.82
Ethyl Octanoate	-158.85	29.82	36.60
Vanillin	18.26	11.37	2.39
Syringaldehyde	-0.55	-2.79	-1.91
Ethyl Decanoate	17.75	22.32	10.65
Ethyl Laurate	-79.09	10.93	5.94
Acetic acid	27.34	22.75	8.66
isobutanol	18.18	23.48	13.47
Ethane, 1,1-diethoxy-	-12.96	-6.56	12.83
1-Butanol, 2-methyl-	-3.26	-14.01	-7.23
Furfural	-285.22	-3.19	-47.63
Octanoic acid	295.49	-119.02	115.94
phenethyl acetate	-525.54	193.66	36.85

Summary of Misclassified Observations

Observation	True Group	Pred Group	Group	Squared Distance	Probability
Sample 24 (12**)	C	D	B	61.00	0.000
			C	13.87	0.161
			D	10.57	0.839
Sample 45 (22**)	D	C	B	49.659	0.000
			C	6.946	0.909
			D	11.556	0.091

Appendix J. Ethanol Heat Experiment Results Summary

Table J.1: Peak areas for each compound detected in large blocks and small chips with different toasting levels (light, medium and heavy) in 65% ethanol stored at 50 °C.

	Large Blocks						**Small Chips**					
	Light		**Medium**		**Heavy**		**Light**		**Medium**		**Heavy**	
Compound	**Day 1**	**Day 14**	**Day 1**	**Day 14**	**Day 1**	**Day 14**	**Day 1**	**Day 14**	**Day 1**	**Day 14**	**Day 1**	**Day 14**
Acetic Acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl Acetate	0.01	0.06	0.01	0.05	0.01	0.05	0.01	0.04	0.01	0.04	0.01	0.04
Sinapaldehyde	n.d.	n.d.	n.d.	0.00	n.d.	0.01	n.d.	n.d.	n.d.	0.07	n.d.	n.d.
Syringaldehyde	n.d.	n.d.	n.d.	0.03	n.d.	0.10	n.d.	n.d.	n.d.	0.04	n.d.	0.07
Vanillin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1,1-Diethoxyethane	n.d.	0.05	0.01	0.04	0.01	0.05	0.01	0.05	0.01	0.03	0.02	0.04
Furfural	n.d.	n.d.	0.11	0.08	0.13	0.09	n.d.	n.d.	0.04	0.03	0.05	0.03
2,6-Dimethoxyphenol	n.d.	n.d.	n.d.	n.d.	n.d.	0.02	n.d.	n.d.	n.d.	n.d.	n.d.	0.001

n.d. Not detected on specified day

Table J.2: Peak areas for each compound detected in large blocks and small chips with different toasting levels (light, medium and heavy) in 65% ethanol stored at 70 °C.

	Large Blocks						**Small Chips**					
	Light		**Medium**		**Heavy**		**Light**		**Medium**		**Heavy**	
Compound	**Day 1**	**Day 14**	**Day 1**	**Day 14**	**Day 1**	**Day 14**	**Day 1**	**Day 14**	**Day 1**	**Day 14**	**Day 1**	**Day 14**
Acetic Acid	0.20	0.06	0.07	n.d.	n.d.	0.03	0.06	0.03	0.08	n.d.	0.03	0.04
Ethyl Acetate	0.04	0.43	0.04	0.38	0.07	0.29	0.03	0.20	0.03	0.28	0.05	0.24
Sinapaldehyde	n.d.	0.03	0.02	0.09	n.d.	n.d.	n.d.	0.03	0.28	0.11	n.d.	n.d.
Syringaldehyde	n.d.	0.02	0.02	0.09	0.01	0.14	n.d.	0.02	0.03	0.19	0.02	0.13
Vanillin	n.d.	n.d.	n.d.	0.02	n.d.	0.01	n.d.	n.d.	n.d.	0.02	n.d.	0.01
1,1-Diethoxyethane	0.01	0.33	0.02	0.52	0.06	0.38	0.03	0.36	0.03	0.46	0.05	0.27
Furfural	0.07	0.03	0.23	0.12	0.15	0.09	0.04	0.02	0.19	0.10	0.20	0.13
2,6-Dimethoxyphenol	n.d.	n.d.	n.d.	n.d.	0.01	0.02	n.d.	n.d.	n.d.	n.d.	0.01	0.01

n.d. Not detected on specified day

Appendix K. Small Chips Heat Distillate Results Summary

Table K.1: Compounds detected in the medium and heavy toast corn distillate samples stored at 70 °C and the concentration (ppm) or peak area over time.

			Corn							
			Heavy Toast				Medium Toast			
Compound	**Measurement**	**LoD (ppm)**	**Day 0**	**Day 1**	**Day 6**	**Day 11**	**Day 0**	**Day 1**	**Day 6**	**Day 11**
Esters										
Ethyl Acetate	ppm	1.45	< LoD	< LoD	< LoD	< LoD	< LoD	< LoD	< LoD	< LoD
Isoamyl Acetate	ppm	0.26	n.d.	n.d.	< LoD	n.d.	n.d.	n.d.	< LoD	< LoD
Ethyl Octanoate	ppm	0.09	n.d.	n.d.	< LoD	< LoD	n.d.	n.d.	< LoD	n.d.
Ethyl Decanoate	ppm	0.06	0.21	n.d.	0.13	0.14	0.21	n.d.	0.13	0.13
Ethyl Laurate	ppm	0.03	n.d.	n.d.	< LoD	< LoD	n.d.	n.d.	< LoD	< LoD
Ethyl Hexanoate	peak area	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl Butyrate	ppm	0.41	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-Methylbutyl Acetate	peak area	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Isobutyl Acetate	peak area	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenylethyl Acetate	peak area	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alcohols										
Isobutanol	peak area	–	0.03	0.07	0.85	0.12	0.03	0.05	0.67	0.01
Isoamyl Alcohol	ppm	0.93	14.30	< LoD	7.66	1.10	14.30	< LoD	6.32	< LoD
2-Methyl-1-butanol	peak area	–	0.04	0.10	1.08	0.14	0.04	0.06	0.87	0.00
2-Phenylethanol	ppm	0.10	0.21	< LoD	0.14	0.25	0.21	< LoD	0.15	0.23
Phenolic Aldehydes										
Syringaldehyde	ppm	0.10	n.d.	0.69	0.51	0.65	n.d.	0.68	0.54	0.69
Vanillin	ppm	0.15	n.d.	0.34	0.38	0.39	n.d.	0.34	0.39	0.42
Sinapaldehyde	peak area	–	n.d.	n.d.	n.d.	0.003	n.d.	0.09	0.10	0.16
Coniferaldehyde	peak area	–	n.d.	n.d.	n.d.	0.01	n.d.	0.04	0.05	0.09

Compound	Measurement	LoD (ppm)	Corn							
			Heavy Toast				Medium Toast			
			Day 0	Day 1	Day 6	Day 11	Day 0	Day 1	Day 6	Day 11
Phenols										
m-Methoxyphenol	ppm	0.18	n.d.	n.d.	< LoD	< LoD	n.d.	< LoD	0.18	< LoD
Guaiacol	ppm	0.16	n.d.	n.d.	< LoD	0.18	n.d.	n.d.	n.d.	n.d.
2,6-dimethoxyphenol	peak area	–	n.d.	0.01	0.02	0.01	n.d.	0.01	0.02	0.03
Acids										
Acetic Acid	peak area	–	n.d.	0.15	0.35	0.36	n.d.	0.11	0.30	0.12
Miscellaneous										
1,1-Diethoxyethane	peak area	–	n.d.	n.d.	0.16	0.009	n.d.	n.d.	0.09	0.21
Furfural	peak area	–	n.d.	0.04	0.19	0.06	n.d.	0.03	0.17	0.04
$C_6H_{12}O$	peak area	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. Not detected

"–" denotes LoD not available due to no standards for quantitation

Table K.2: Compounds detected in the medium and heavy toast malt distillate samples stored at 70 °C and the concentration (ppm) or peak area over time.

			Malt							
			Heavy Toast				Medium Toast			
Compound	**Measurement**	**LoD (ppm)**	**Day 0**	**Day 1**	**Day 6**	**Day 11**	**Day 0**	**Day 1**	**Day 6**	**Day 11**
Esters										
Ethyl Acetate	ppm	1.45	10.62	5.39	5.61	5.98	10.62	7.66	6.35	8.02
Isoamyl Acetate	ppm	0.26	0.66	< LoD	< LoD	< LoD	0.66	0.39	< LoD	< LoD
Ethyl Octanoate	ppm	0.09	0.12	< LoD	0.12	< LoD	0.12	0.13	0.14	< LoD
Ethyl Decanoate	ppm	0.06	0.40	0.27	0.13	0.33	0.40	0.31	0.16	0.34
Ethyl Laurate	ppm	0.03	0.20	0.17	0.20	0.18	0.20	0.18	0.20	0.18
Ethyl Hexanoate	peak area	–	0.01	0.04	0.03	0.03	0.01	0.05	0.04	0.05
Ethyl Butyrate	ppm	0.41	n.d.	< LoD	< LoD	< LoD	n.d.	< LoD	< LoD	< LoD
2-Methylbutyl Acetate	peak area	–	0.03	0.05	0.04	0.03	0.03	0.06	0.05	0.03
Isobutyl Acetate	peak area	–	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.01
Phenylethyl Acetate	peak area	–	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Alcohols										
Isobutanol	peak area	–	1.22	1.37	1.60	1.55	1.22	1.83	1.76	1.79
Isoamyl Alcohol	ppm	0.93	15.50	7.47	8.99	8.24	15.50	9.44	9.96	8.60
2-Methyl-1-butanol	peak area	–	1.57	1.69	1.78	1.56	1.57	2.16	1.96	1.66
2-Phenylethanol	ppm	0.10	n.d.	< LoD	< LoD	0.21	n.d.	< LoD	< LoD	0.21
Phenolic Aldehydes										
Syringaldehyde	ppm	0.10	n.d.	0.70	0.57	0.73	n.d.	0.68	0.56	0.59
Vanillin	ppm	0.15	n.d.	0.35	0.40	0.42	n.d.	0.36	0.41	0.43
Sinapaldehyde	peak area	–	n.d.	0.01	0.01	0.03	n.d.	0.10	0.13	0.27
Coniferaldehyde	peak area	–	n.d.	0.01	0.01	0.03	n.d.	0.04	0.06	0.11
Phenols										
m-Methoxyphenol	ppm	0.18	n.d.	< LoD	0.18	< LoD	n.d.	< LoD	0.18	< LoD
Guaiacol	ppm	0.16	n.d.	n.d.	< LoD	0.18	n.d.	n.d.	< LoD	0.18

			Malt							
			Heavy Toast				Medium Toast			
Compound	**Measurement**	**LoD (ppm)**	**Day 0**	**Day 1**	**Day 6**	**Day 11**	**Day 0**	**Day 1**	**Day 6**	**Day 11**
2,6-dimethoxyphenol	peak area	–	n.d.	0.01	0.03	0.01	n.d.	0.009	0.02	0.02
Acids										
Acetic Acid	peak area	–	n.d.	0.13	0.34	0.34	n.d.	0.14	0.28	0.41
Miscellaneous										
1,1-Diethoxyethane	peak area	–	n.d.	0.21	0.37	0.31	n.d.	0.37	0.58	0.64
Furfural	peak area	–	n.d.	0.16	0.18	0.17	n.d.	0.14	0.15	0.13
$C_6H_{12}O$	peak area	–	n.d.	0.03	0.03	0.03	n.d.	0.04	0.03	0.03

n.d. Not detected

"–" denotes LoD not available due to no standards for quantitation

Appendix L. Syringaldehyde:Vanillin Ratio

The balanced decomposition of lignin is indicated by a syringaldehyde:vanillin ratio of 1.4:2.5. The syringaldehyde:vanillin ratio for the 70 °C small chips in distillate experimental data (Table L.1) was calculated using Equation 6. The resulting ratio was converted to the same format as literature for direct comparison. An example is shown in Equation 6 using the heavy toast corn distillate day 1 results.

$$\frac{syringaldehyde\ (ppm)}{syringaldehyde\ (ppm)} : \frac{vanillin\ (ppm)}{syringaldehyde\ (ppm)} \qquad \text{(Eq. 6)}$$

$$\frac{0.69}{0.69} : \frac{0.34}{0.69} = 1 : 0.49$$

$$1 \times 1.4 : 0.49 \times 1.4 = 1.4 : 0.69$$

Table L.1: Syringaldehyde and vanillin concentrations and the resulting syringaldehyde:vanillin ratio.

Matrix	Toast level	Day	Syringaldehyde (ppm)	Vanillin (ppm)	Syringaldehyde:Vanillin Ratio
Corn	Heavy	1	0.69	0.34	1.4 : 0.7
		2	0.73	0.35	1.4 : 0.7
		4	0.73	0.35	1.4 : 0.7
		5	0.73	0.35	1.4 : 0.7
		6	0.51	0.38	1.4 : 1.0
		8	0.54	0.39	1.4 : 1.0
		9	0.50	0.24	1.4 : 0.7
		10	0.52	0.24	1.4 : 0.7
		11	0.65	0.39	1.4 : 0.8
Corn	Medium	1	0.34	0.68	1.4 : 2.8
		2	0.35	0.69	1.4 : 2.8
		4	0.36	0.72	1.4 : 2.8
		5	0.36	0.74	1.4 : 2.8
		6	0.39	0.54	1.4 : 1.9
		8	0.41	0.57	1.4 : 2.0
		9	0.26	0.53	1.4 : 2.8
		10	0.27	0.56	1.4 : 2.9
		11	0.42	0.69	1.4 : 2.3

Matrix	Toast level	Day	Syringaldehyde (ppm)	Vanillin (ppm)	Syringaldehyde:Vanillin Ratio
Malt	Heavy	1	0.35	0.70	1.4 : 2.8
		2	0.36	0.72	1.4 : 2.8
		4	0.37	0.74	1.4 : 2.8
		5	0.36	0.76	1.4 : 2.9
		6	0.40	0.57	1.4 : 2.0
		8	0.41	0.60	1.4 : 2.1
		9	0.26	0.57	1.4 : 3.1
		10	0.26	0.59	1.4 : 3.1
		11	0.42	0.73	1.4 : 2.4
Malt	Medium	1	0.36	0.68	1.4 : 2.6
		2	0.36	0.70	1.4 : 2.7
		4	0.37	0.72	1.4 : 2.7
		5	0.37	0.73	1.4 : 2.8
		6	0.41	0.56	1.4 : 1.9
		8	0.42	0.59	1.4 : 2.0
		9	0.27	0.56	1.4 : 2.9
		10	0.28	0.57	1.4 : 2.9
		11	0.43	0.71	1.4 : 2.3

Appendix M. Sonication Experiment Results Summary

Table M.1: Compounds present in non-heated and 50 °C sonication experiments with corn distillate.

			Corn (Non-Heated)					Corn 50 °C			
Compound	**Measurement**	**LoD (ppm)**	**Cycle 0***	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**
Esters											
Ethyl Acetate	ppm	1.45	< LoD	< LoD	< LoD	n/a	< LoD	< LoD	< LoD	n/a	< LoD
Isoamyl Acetate	ppm	0.26	< LoD	< LoD	< LoD	n/a	< LoD	< LoD	< LoD	n/a	< LoD
Ethyl Octanoate	ppm	0.09	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a.	< LoD
Ethyl Decanoate	ppm	0.06	0.21	0.09	0.08	0.08	0.09	< LoD	0.15	0.15	0.16
Ethyl Laurate	ppm	0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ethyl Hexanoate	peak area	–	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a	n.d.
Ethyl Butyrate	ppm	0.41	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a	n.d.
2-Methylbutyl Acetate	peak area	–	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a	n.d.
Isobutyl Acetate	peak area	–	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a	n.d.
Phenylethyl Acetate	peak area	–	n.d.	n.d.	n.d.	n/a	0.02	n.d.	n.d.	n/a	n.d.
Alcohols											
Isobutanol	peak area	–	0.03	0.42	0.33	n/a	0.33	0.23	0.39	n/a	0.39
Isoamyl Alcohol	ppm	0.93	14.30	4.35	3.61	n/a	3.52	2.48	4.01	n/a	4.22
2-Methyl-1-butanol	peak area	–	0.04	0.57	0.49	n/a	0.46	0.29	0.47	n/a	0.54
2-Phenylethanol	ppm	0.10	0.21	0.25	0.25	0.24	0.26	n.d.	0.13	0.11	0.18
Phenolic Aldehydes											
Syringaldehyde	ppm	0.10	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a	0.69
Vanillin	ppm	0.15	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a	n.d.
Sinapaldehyde	peak area	–	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a	0.01
Coniferaldehyde	peak area	–	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a	0.004

Compound	Measurement	LoD (ppm)		Corn (Non-Heated)				Corn 50 °C			
			Cycle 0*	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Acids											
Acetic Acid	peak area	–	n.d.	0.01	0.03	n/a	0.06	0.04	0.07	n/a	0.11
Miscellaneous											
1,1-Diethoxyethane	peak area	–	n.d.	0.004	0.004	n/a	0.006	n.d.	n.d.	n/a	n.d.
Furfural	peak area	–	n.d.	n.d.	0.008	n/a	0.04	n.d.	0.04	n/a	0.08
$C_6H_{12}O$	peak area	–	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n/a	n.d.

* Concentration/peak area in fresh distillate prior to treatment

n.d. Not detected

"–" denotes LoD not available due to no standards for quantitation

n/a not available because data was removed due to poor QC performance

Table M.2: Compounds present in non-heated and 50 °C sonication experiments with malt distillate.

			Malt (Non-Heated)					Malt 50 °C			
Compound	**Measurement**	**LoD (ppm)**	**Cycle 0***	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**
Esters											
Ethyl Acetate	ppm	1.45	10.62	11.14	9.09	7.11	n/a	3.53	3.56	5.62	4.87
Isoamyl Acetate	ppm	0.26	0.66	0.60	0.52	0.43	n/a	0.41	0.46	0.46	0.50
Ethyl Octanoate	ppm	0.09	0.12	n.d.	n.d.	n.d.	n/a	0.15	0.17	0.16	0.18
Ethyl Decanoate	ppm	0.06	0.40	0.37	0.35	0.35	n/a	0.40	0.45	0.44	0.49
Ethyl Laurate	ppm	0.03	0.20	0.13	0.13	0.13	n/a	0.23	0.24	0.24	0.25
Ethyl Hexanoate	peak area	–	0.006	0.04	0.03	0.03	n/a	0.02	0.03	0.03	0.03
Ethyl Butyrate	ppm	0.41	n.d.	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n.d.
2-Methylbutyl Acetate	peak area	–	0.03	0.07	0.06	0.05	n/a	0.03	0.05	0.04	0.05
Isobutyl Acetate	peak area	–	0.009	0.02	0.02	0.01	n/a	0.01	0.01	0.02	0.02
Phenylethyl Acetate	peak area	–	n.d.	0.01	0.01	0.01	n/a	0.01	0.01	0.01	0.02
Alcohols											
Isobutanol	peak area	–	1.22	1.89	1.55	1.53	n/a	1.35	1.62	1.48	1.64
Isoamyl Alcohol	ppm	0.93	15.50	10.88	10.17	9.51	n/a	9.31	10.30	8.82	10.29
2-Methyl-1-butanol	peak area	–	1.57	2.10	1.92	1.78	n/a	1.72	1.88	1.63	1.90
2-Phenylethanol	ppm	0.10	n.d.	n.d.	n.d.	0.19	n/a	n.d.	n.d.	0.10	0.10
Phenolic Aldehydes											
Syringaldehyde	ppm	0.10	n.d.	n.d.	n.d.	n.d.	n/a	0.69	0.69	0.69	0.70
Vanillin	ppm	0.15	n.d.	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	n.d.
Sinapaldehyde	peak area	–	n.d.	n.d.	n.d.	n.d.	n/a	n.d.	0.01	0.01	0.02
Coniferaldehyde	peak area	–	n.d.	n.d.	n.d.	n.d.	n/a	n.d.	n.d.	n.d.	0.01
Acids											
Acetic Acid	peak area	–	n.d.	n.d.	0.01	n.d.	n/a	0.05	0.11	0.08	0.09

Compound	Measurement	LoD (ppm)	Cycle 0*	Malt (Non-Heated)				Malt 50 °C			
				Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Miscellaneous											
1,1-Diethoxyethane	peak area	–	n.d.	0.33	0.28	0.22	n/a	0.08	0.09	0.15	0.17
Furfural	peak area	–	n.d.	n.d.	0.02	0.05	n/a	0.04	0.07	0.08	0.09
$C_6H_{12}O$	peak area	–	n.d.	0.03	0.03	0.03	n/a	0.02	0.03	0.02	0.03

* Concentration/peak area in fresh distillate prior to treatment

n.d. Not detected

"–" denotes LoD not available due to no standards for quantitation

n/a not available because data was removed due to poor QC performance

Appendix N. LDA Output for Experiments

Table N.1: Calculated group probabilities and the overall group assignments for experiment samples.

Age Treatment	Age Time	Distillate	Predicted Group	Squared Distance	From Group	Probability
Heat	6 days	Corn	C	431.518	B	0.00
				322.09	C	1.00
				349.061	D	0.00
Heat	6 days	Corn	C	418.31	B	0.00
				307.587	C	1.00
				335.022	D	0.00
Heat	6 days	Malt	D	423.568	B	0.00
				306.626	C	0.05
				300.748	D	0.95
Heat	6 days	Malt	D	338.88	B	0.00
				244.108	C	0.00
				221.937	D	1.00
Sonication (non-heated)	1 cycle	Corn	C	42.71	B	0.00
				20.396	C	0.72
				22.307	D	0.28
Sonication (non-heated)	1 cycle	Malt	B	609.592	B	1.00
				693.719	C	0.00
				635.074	D	0.00
Sonication 50 °C	1 cycle	Corn	D	36.84	B	0.00
				15.621	C	0.47
				15.349	D	0.53
Sonication 50 °C	1 cycle	Malt	D	327.169	B	0.00
				266.014	C	0.00
				254.54	D	1.00
Heat-Freeze	9 days	Corn	C	177.144	B	0.00
				102.072	C	0.81
				104.908	D	0.19
Heat-Freeze	9 days	Malt	D	345.334	B	0.00
				309.947	C	0.00
				267.730	D	1.00