Flavour Interactions between the 'estery' and 'mature/woody' characters of whisky, bourbon & tequila

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ABSTRACT

Estery and woody flavour notes are important characteristics of distilled spirit flavour. It has been reported for malt whisky that the estery character of mature whiskies typically declines relative to that of the new make spirit, even though the analytical concentrations of esters remain broadly constant. One potential explanation for this observation would be a sensory interaction between mature and estery characters. The work described in this thesis was designed to test this hypothesis and to further explore the nature of the congeners responsible for eliciting these characteristics across different spirit types, as influenced by their maturation conditions (time, temperature, cask provenance etc.).

In the research described in Chapter 2, four pairs of non-mature and mature spirits (tequila, bourbon and 2 malt whiskies) were characterized by instrumental analysis with the aim of defining the key aroma compounds that determined the mature character in each spirit. According to PLS analysis of the full data set, concentrations of 17 congeners were positively correlated with ageing time and might thus influence the mature character of the aged spirits. In Chapter 3, the same eight spirit samples were analysed by GC-Olfactometry using the AEDA (aroma extraction dilution analysis) approach. Aged spirits presented a more complex aroma than new make spirits, and contained more compounds with the highest FD-factors. Whilst a full GC-O characterisation was completed, the main focus was on identifying compounds which contributed to the estery and woody/mature characters of each spirit. In Chapter 4 we attempted to reproduce these characters for each spirit through aroma recombination, based on blends of the odiferous compounds identified at high FD factors and their analytical concentrations in the actual samples as reported in Chapter 3. It soon became apparent that relatively simple mixtures of esters on the one hand and maturation-linked compounds on the other did not adequately reproduce the nature of these characteristics in the spirits themselves. This implied either that our analysis had missed some significant compounds contributing to these characteristics, or that the complexity of the full spirit matrix is required to give the groups of compounds the nuanced flavour that they lacked in isolation. The latter hypothesis was tested by adding in additional blends of compounds to increase the complexity of the recombinant aroma mixtures. It was concluded that the authenticity of the aroma blends overall was improved by both the addition of a cocktail of 'low boiling compounds' (those analysed by a separate direct injection GC technique) and the introduction of a 'structuring' compound (ethyl hexadecanoate) at a concentration that would cause agglomeration within the whisky (micellar structures) thus influencing aroma partitioning and release. It was concluded that these modifications produced recombinant aromas which were close enough to the authentic spirit characters to use them in sensory interaction studies (Chapter 5). As opposed to interaction effects there was simply a tendency for the woody/mature characters to suppress the corresponding estery character of mature spirits, particularly at the higher concentrations of added wood extractives.

Because the woody/mature compounds which characterised maturation were broadly similar across the spirit types, but differed in concentration according to the maturation conditions, we decided finally to investigate the extraction kinetics of wood-derived compounds from oak sticks as a function of ageing time, temperature, spirit type and alcohol content (Chapter 6). Temperature and alcohol content were the most significant factors that determined the extraction rate and final concentrations of all 18 wood-extractive compounds ($P < 0.05$) analysed. Not surprisingly, extraction rates increased with increasing temperature, but the trend in terms of alcoholic strength depended on the particular compound. Overall this thesis has improved knowledge of the chemical and sensory changes that accompany spirit maturation and has highlighted some of the factors that cause differences in mature character across spirit types. Moreover, it concludes that the sensory perception of woody/mature generally suppresses the fresh estery characteristic of new make spirits, even though analytically the esters are still there in similar concentrations.

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PUBLICATIONS

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ABREVIATIONS

GC-MS: gas chromatography-mass spectrometry

GC-O: gas chromatography olfactometry

AEDA: aroma extract dilution analysis

GC-FID: gas chromatography-flame ionization detector

HPLC-UV: high performance liquid chromatography-UV detector

FD: flavour dilution factor

LLE: liquid-liquid extraction

SPE: solid-phase extraction

LRI: linear retention index

ANOVA: analysis of variance

PCA: principal component analysis

PLSR: partial least squares regression analysis

DCM: dichloromethane

SWRI: Scotch whisky research institute

OAV: odour active values

QDA: quantitative descriptive analysis

C16: ethyl hexadecanoate

LBC: low boiling volatile compounds

ABV: alcohol by volume

NMSA: new make spirit 'A'

NMSB: new make spirit 'B'

MWA: malt whisky 'A'

MWB: malt whisky 'B'

IMB: non-mature bourbon

MB: mature bourbon

IMTEQ: non-mature tequila

MTEQ: mature tequila

1. INTRODUCTION

Estery and woody flavour notes are important characteristics of distilled spirit flavour. It has been reported for malt whisky that the estery character of mature whiskies typically declines relative to that of the new make spirit, even though the analytical concentrations of esters remain broadly constant. One potential explanation for this observation would be a sensory interaction between mature and estery characters. The work described in this thesis was designed to test this hypothesis and to further explore the nature of the congeners responsible for eliciting these characteristics across different spirit types, as influenced by their maturation conditions (time, temperature, cask provenance etc.). In this introductory chapter, the processes of malt whisky production will be summarised, with particular attention to the maturation process which could potentially give rise to reactions known to produce compounds that define the 'mature' character of ageing spirits. Also, consideration will be given to the analytical and sensory techniques applied to the analysis of distilled spirits, including methods of aroma extraction, detection, significant odorants analysis, and sensory evaluation. Finally, information concerning sensory interaction experiments and the chemical reactions which arise as a consequence of maturation of distilled beverages will be further discussed.

1.1 Scotch malt whisky production and origins of flavour active compounds

Whisky is the potable spirit obtained by distillation of an aqueous extract of an infusion of malted barley and other cereals that has been fermented with

strains of *Saccharomyces cerevisiae* yeast. Various types of whisky are produced in a number of different countries in the world. They differ principally in the nature and proportion of the cereals used as raw materials along with malted barley, and also in the type of still used for distillation (Bamforth et al. 2003; Lyons, 2003). Figure 1.1 illustrates a flow diagram of the principal stages involve in the production of Scotch malt whisky and Table 1.1 summarized the raw materials and unit processes used in its production.

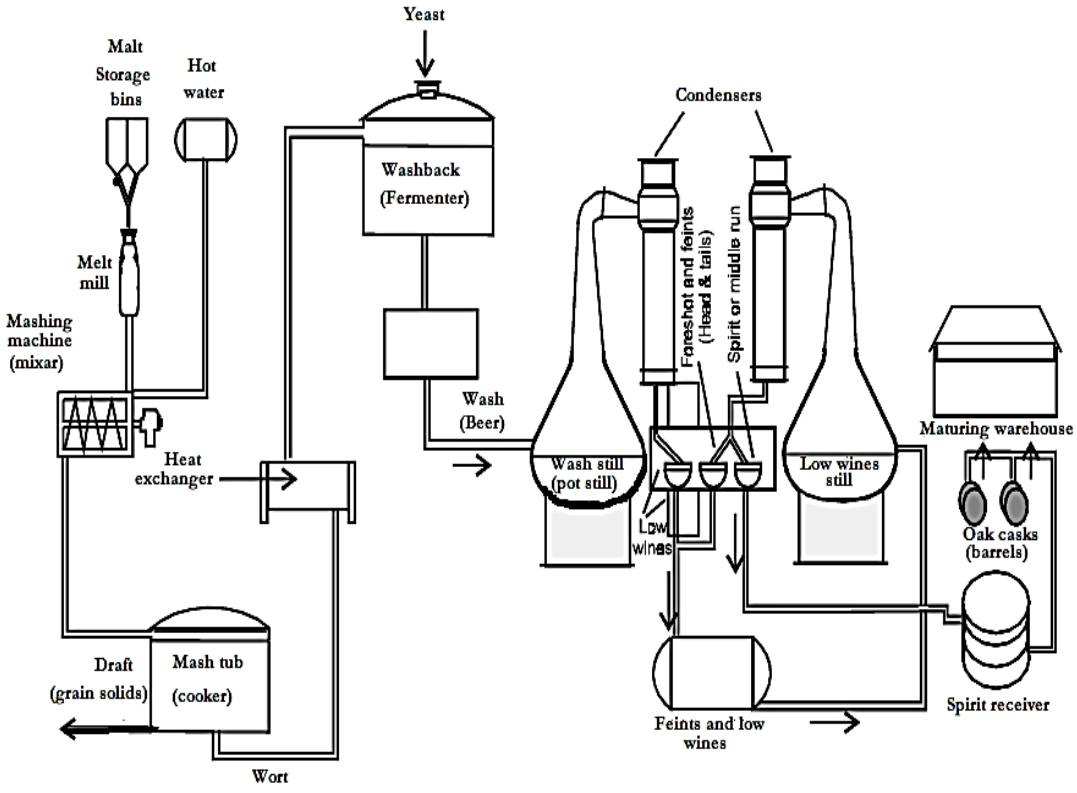

Figure 1.1. Flow diagram showing the principal operations of Scotch malt whisky production (Lyons, 2003)

Each processing stage has the potential to impact on the overall flavour quality of the spirit. The main processes involved will be reviewed, paying

particular attention to those during which the aroma active compounds are likely to be formed.

Table 1.1. Raw materials and unit processes involved on Scotch whisky production (Lyons, 2003).

Stage	Scotch malt	Scotch grain
Raw materials	Peated and unpeated malted barley	Wheat or corn and a small proportion of malted barley
Conversion	Infusion mash	Mash cook followed by conversion stand
Fermentation	Distillers yeast and brewer's yeast	Distillers yeast
Distillation	Two pot stills	Continuous still
Maturation	At 62º GL in used charred oak bourbon whiskey barrels or sherry casks for at least three years	Up to 67º GL in used charred oak bourbon whiskey barrels or sherry casks for at least three years

The product, known as Scotch malt whisky, is produced in small distilleries and marketed both as a straight malt whisky, and also as a blend with another type of whisky produced in Scotland known as Scotch grain whisky. Most Scotch whiskies available on the international market consist of blends with 20-30% malt whisky and 70-80% grain whisky. Within the blend, there may be as many as 20-30 individual malt whiskies and grain whiskies. These blends are, by law, matured for at least three years but in practice this period is much longer. Unblended Scotch malt whiskies are usually matured for a minimum of eight years (Lyons, 2003). (Table 1.1).

All whiskies are legally protected and defined, mainly because of the huge revenues that governments obtain from their sale, with legal definitions for Scotch being outlined in The Scotch Whisky Regulations (2009). A summary of these requirements is outlined in Table 1.2.

Table 1.2. Summary of legal requirements pertaining to Scotch whisky (The Scotch Whisky Regulations, 2009):

1. Produced from water and cereal
(a) Processed into a mash
(b) Converted to fermentable substrates by endogenous enzymes only
(c) Fermented by yeast
2. Distilled at < 94.8% strength
3. Matured in oak casks
(a) For a minimum of 3 years
(b) < 700 l capacity
4. Sold at a minimum strength of 40% alcohol
5. No additives permitted-other than water and caramel
6. Produced in Scotland

1.1.1 First steps in whisky production

A characteristic of Scotch malt whisky is that the only cereal used in its manufacture is malted barley (Table 1.1). However, in the manufacture of Scotch grain whisky, other cereals are used along with malted barley to provide additional starch in the mash tun (Table 1.1). The process of malting barley comprises steeping, germination and kilning (Bamforth et al. 2003; Boothroyd, 2013). During malt kilning, many flavour compounds are produced and destroyed simultaneously (Boothroyd, 2013). In addition to these compounds, peat can be used as an external source of flavour, which can be burned in the malt kiln, producing a smoke "peak reek" aroma into the final spirit (Bamforth et al. 2003; Boothroyd, 2013). After milling, the meal is mashed in a mash tun (Figure 1) similar to that used in breweries for beer production. During mashing or conversion, enzymes in the malt catalyse the hydrolysis of starch into fermentable sugars. In the manufacture of Scotch grain whisky, other cereals are used along with malted barley to provide

additional starch in the mash tub (Table 1.1). Owing to the high gelatinization temperature of their starches, unmalted cereals must be precooked before they are incorporated into the mash. The wort, or clear mash, leaving the mash tun is cooled and fed into a washbacks where it is mixed with yeast to conduct the fermentation (Bamforth et al. 2003; Lyons, 2003).

1.1.2 Fermentation

Fermentation is conducted with yeast strains of *Saccharomyces cerevisiae* that are usually propagated for this purpose, although Scotch malt whisky distillers may use some surplus brewer's yeast (Table 1.1). As the yeast metabolise the sugars in the wort to produce ethanol, many other flavour compounds are formed, which included amino acids, fatty acids and esters (Lee et al. 2001; Caldeira et al. 2007). Undoubtedly, many of these compounds are common to different whiskeys but differ analytically in terms of the relative amounts, which make the difference in their sensory appreciation and influences the overall quality of whisky flavour. Especially fatty acid esters form an essential group of aroma components in whisky. The Esters generally have a pleasant and some of them also a very intense odour and thus it may be assumed that they appear as important aroma components, even if they are present in relatively small amounts (Conner et al. 2001). The main ester component in alcoholic beverages is ethyl acetate, although ethyl, isobutyl and 3-methylbutyl esters of short-chain fatty acids, called ''fruit esters'' (due to their pleasant aromas), also appear (Lee et al. 2001). Quantitatively significant components are ethyl esters of octanoic, decanoic and dodecanoic acids. Of the higher esters, ethyl E-11-hexadecenoate is

interesting because significant amounts of this compound have been found mainly in Scotch whisky (Conner et al. 2001). The yeast used has a great influence on the production of esters in the fermentation process. The higher alcohols are also an important family, quantitatively and qualitatively speaking, being characterized by their strong and pungent smell and taste. It has been reported in wine that concentrations at levels lower than 300 mg/L have a positive contribution to the overall sensory properties of the beverage and in concentrations exceeding 400 mg/L can have a detrimental effect (Swiegers et al. 2005). Higher alcohol acetates are correlated with freshness and fruitiness character, while fatty acids can contribute with fruity, cheese, fatty and rancid notes to the whiskey sensory (Caldeira et al. 2007).

1.1.3 Malt whisky distillation

Batch or pot distillation using Copper stills is used for the production of highly flavoured malt spirit, whereas continuous distillation in a Coffey still is favoured for grain spirit manufacture, where a subtler flavour is preferred, as it is grain whisky which is used as the base for blended whiskies (Boothroyd, 2013). In pot still distilleries, the fermented mash or 'wash' (beer) is fed directly to a still known as the wash still, from which the distillates are redistilled in the second or low wines still (Figure 1.1). Some functions of distillation are: ethanol concentration, removal of solids, selection and elimination of specific flavour compounds, which are directed by cut points and formation of thermal reactions (Maillard and lipid oxidation, Bamforth et al. 2003; Lyons, 2003; Boothroyd, 2013). During distillation, the ester content of the beverage could be modified depending on whether or not

the yeast is present at the time of distillation. For example, if distillation occurs in the presence of yeast, the ethyl ester concentrations of certain esters could increase such as decanoic, dodecanoic and E-11-hexadecenoic acids (Demyttenaere et al. 2003).

1.1.4 Maturation

Finally, the freshly distilled whisky 'new make spirit at 70% ABV' is diluted to a cask strength of 60-65% ABV and stored in charred oak barrels for minimum periods of time that depend on the legislation in the producing country (Table 1.1). Wood-derived congeners will be transferred into the spirit and simultaneously subtractive reactions (reactions between wood components and constituents of the raw distilled, reactions involving only the wood extractives, reactions involving only the distillate components, and evaporation of volatile compounds through the cask) take place and the spirit meets the cask surface to reduce the harshness of the original spirit (Nishimura and Matsuyama 1989; Lyons, 2003; Boothroyd, 2013). Maturation process is important for the development of flavour; however, the final flavour depends mainly on the starting composition of the newly distilled spirit (Piggott et al. 1993; Boothroyd, 2013).

As previously mentioned distilled alcoholic spirits, such as Scotch whiskies rum, gin, and tequila are characterized by the presence of congeners, which arise from mashing, fermentation, distillation and ageing; some are oak derived, while others depend on processes technology factors (Fitzgerald et al. 2000). These components comprise over 1000 compounds, including alcohols and fusel alcohols, fatty acids and esters, lactones and other carbonyl

compounds, such as aldehydes, phenolic, sulphur and nitrogen compounds (Demyttenaere et al. 2003). Figure 2 shows a schematic representation of the transformation of flavour constituents of barley during whisky production and Table 1.3 and 1.4 shown a summary of the main characters, which have a significant contribution to whisky flavour.

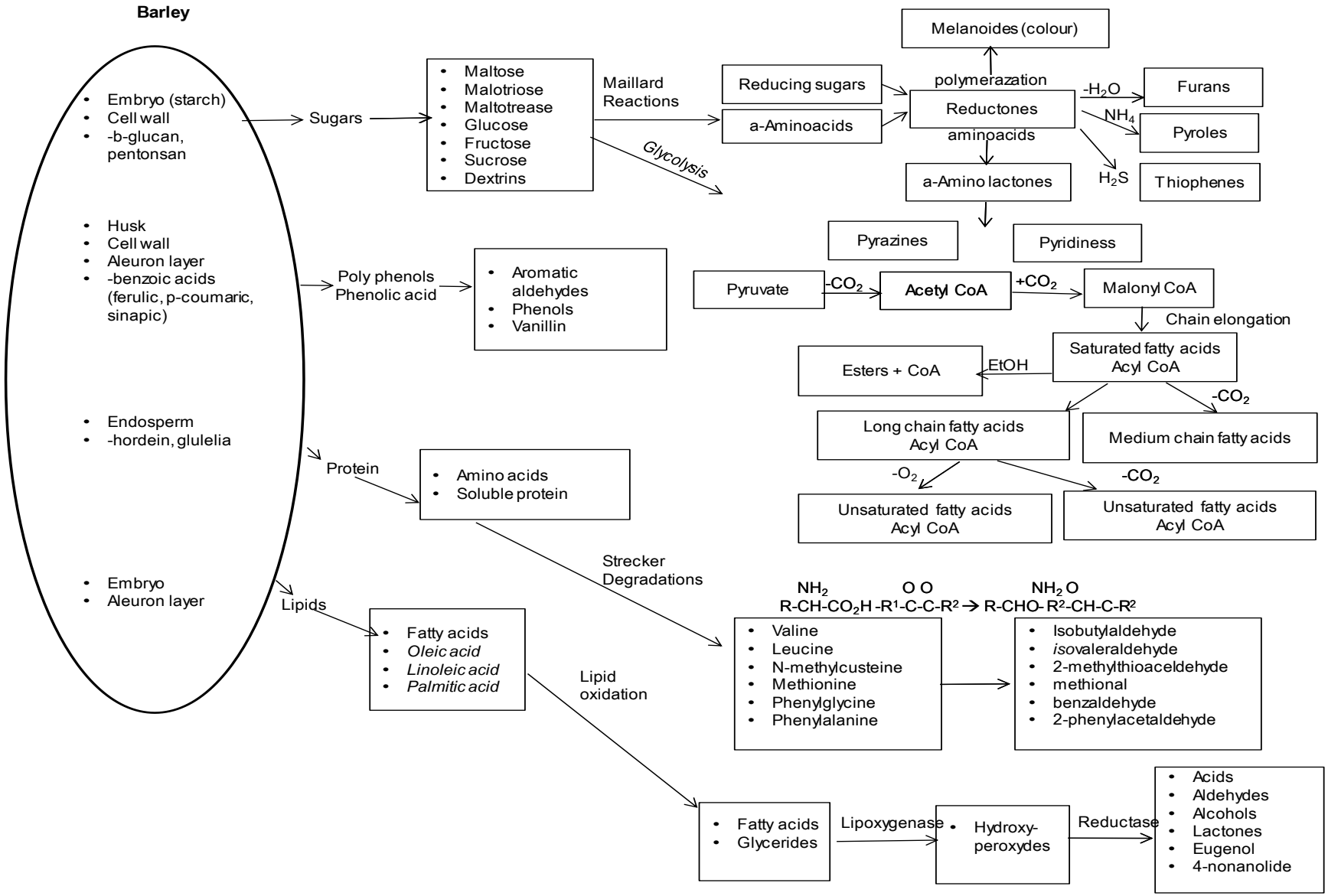

Figure 1.2 Transformation of flavour constituents of barley during whisky production (Lee et al. 2001).

Table 1.3 Summary of the main characters, which have a significant contribution to whisky flavour (Lee et al. 2001).

Flavour character	Main character	Descriptors associated	Origins	Compounds derived
Phenolic	Peaty	Burnt, smoky & medicinal notes	Smoke during kilning process	Phenolic compounds, sulphur & nitrogen congeners, pyridines & thiazoles
Fermentation	Grainy	Cereal, malt & mash, confer *fruity*, *hay-like* & *damp-straw* notes.	Formed during kilning, malting and distillation processes.	Furfural, aldehydes, enols, 2,3 butanediols, acetic acid and chloro- and bromomethoxybenzenes volatiles
Aldehydic	Grassy	*Fresh*, *green*, *dried* & leafy notes	From barley lipids	Hexanal, trans-2-hexenal, 2-and 3-hexenol
Estery	Estery	Solventy, fruity, floral and feinty notes	From esterification of fatty acids during fermentation and distillation	Ethyl esters of short and long chain such as ethyl acetate.
Fruity	Fruitiness	Solventy, orchard, tropical, citrus, berries and dried fruit	From fermentation and distillation	Short-chain alcohol esters, short ethyl esters and organic acids.
Floral	Natural & artificial	Fragrant notes such as *violet-like*	Autoxidation of vitamin A or lipids, from yeast or barley, and breakdown of oak norisoprenoids	Phenylethyl ethanol, β–damascenone, α– β–ionones.
Feints	Grainy, cheesy, oily and sulphury	Leathery, sweaty (piggery), stale, metallic and fish notes	Malt-derived phenols	*Iso*-valeric acid and dimethyl tri-sulphide (DMTS).
Maturation	Woody	Smoky and woody characters	White oak wood, containing cellulose (49-52%), lignin (31-33%), hemicellulose (22%) and extractable compounds.	Lipid-derived whisky lactones, and lignin breakdown compounds-vanillin, and related aromatic aldehydes, and derived acids, esters, tannins and sugars.
Sulphury	Stagnant, meaty, vegetable, sour, gassy and rubbery notes	Sulphur congeners confer light and neutral characters, such as: meaty, burnt, and thiamine-like attributes.	Thought *Stecker* degradation during yeast fermentation; however, during distillation sulphur congeners are reduced.	Methyl- (2-methyl-3-furyl)-disulphides (MMFD), bis-(2-methyl-3-furyl)-disulphides, and methyl (2-methyl-3-furyl)-sulphides and 2, 5-dimethyl-1-3-methyldihiofuran.
Cheesy	Rancid, sweaty and rancid notes		Bacterial action during mashing process and from yeast fermentation.	*n*-butyric acid, ethyl butyrate, propionic, *iso*-butyric, *iso*-valeric and *iso*-valeric acids
Oily	Soapy, buttery, lubricant, fat notes		Bacterial action and from yeast fermentations	Diacetyl, 3-hydroxybutanone and acetoin.

Table 1.4 Summary of mature characters, which contribute to woody attributes of whisky flavour (Lee et al. 2001).

Woody Characters				
Flavour character	**Main character**	**Descriptors associated**	**Origins**	**Compounds derived**
Maturation	Woody	Smoky and woody characters	White oak wood, containing cellulose (49-52%), lignin (31-33%), hemicellulose (22%) and extractable compounds.	Lipid-derived whisky lactones, and lignin breakdown compounds-vanillin, and related aromatic aldehydes, and derived acids, esters, tannins and sugars.
	New Woody Character	Sap, cedar, oak and pine characters	Sap originated from new barrels/cardboard in whiskies and other products; originate in linoleic acid oxidation in unsaturated barley lipids.	(E)-2-nonenal, 3-octen-1-one, (E)-2-octenal and 1-decanal
	Woody extractive	Nutty notes	Oak lipid oxidation	"Whisky lactone", "3-methyl-4-octanolides", "β–methyl-g-octalactone", "5-butyl-4-methyl-dihydro 2(3H)-furanone", or "Quercus lactone"
		Chocolate and cola/smooth, vanillin, sweet, malty, spicy, fruity and floral	Charring	Vanillin, vanillic acid, syringaldehyde and related compounds and their acids.
		Pungent, grainy, sour, oily, sulphury, catty, meaty, and fishy notes.	Uncharred casks	Higher contents of coniferaldehyde, sinapaldehyde and vanillic acid
		Sweet, smoky and spicy notes.	Lipid oxidation	Eugenol
		Caramel, sweet, burnt and smoky	Thermal breakdown of lignins	Guaiacol, 4-acetyl-guaiacol and syringol, homologues and derivates
		Burnt, roasted and matty notes	Maillard reactions	Furans, pyrazines, pyridines, thiazoles, aliphatic amines, quinolones and pyrans
	Defective wood	Mothball, musty, & vinegary notes	Fungi and actinomycetes on malts, defective casks and cork closures, notably Armillaria mella on corks	Methyl thiopyrazine, 2-acetyl piperidine and its isomer 2-acetyl tetra hydro pyridine, 1-octen-3-one (mushroom), 3-octen-1-one (musty, mouldy, earthy, mushroom)

1.1.5 Tequila and Bourbon Manufacturing Processes

Tequila and American Bourbon whiskey as Scotch malt whisky, are typical distilled spirits that undergo common step processes for spirit production (cooking, fermentation, distillation, and ageing). However, the specific conditions that each of them follow depends not only in their own general practices and techniques but also on legal standardized procedures (corresponding legislation). Table 1.5 shows a summary of the general steps that each of these spirits follow.

Table 1.5 Summary of Processes for Spirit Production. (Cedeño, 1995; Schieberle and Poisson, 2008; Russell, & Stewart, 2014)

Spirit type	Processes for spirit production					
	Raw material	**Preparation**	**Fermentation**	**Distillation**	**Ageing**	**Legislation**
Bourbon	Mixture of grains (corn 51%, and remaining rye, wheat, malted barley)	Grains are ground and mixed with warm water. The resulting mash is then mix with a previous distillation mash to create a sour mash.	The mash is fermented by inoculum of *S. cerevisiae.*	Must be at 80% pure alcohol using either a traditional alembic or continuous still.	Storage of the distilled in new, heat-charred oak casks.	Federal Standards of Identify for Distilled Spirits.
Tequila	*Agave tequilana Weber* blue variety	The agave plant is harvested (piña), then the head (core) is steamed to hydrolysed fructans, and finally cooked agave heads are milling to extract the juice for fermentation.	Spontaneous or by an inoculum of *Saccharomyces cerevisiae.*	Double distillation to a final alcohol content of 45% using copper still.	Maturation occurs inside white or holm oak containers for a length of 2 months to 3 years.	Norma Oficial Mexicana (NOM-006-SCFI-2012)
Scotch whisky	Malt or ground cereals	Grains are ground and cooked. Barley is malted, which is then dried and heated it. The cooked grain and malted barley are added to warm water, to obtain a liquid known as mash.	The mash is either fermented with yeast alone (sweet mash) or with a mixture of lactic acid bacteria, and yeast (sour mash)	Double distillation to a final alcohol content of 70% (new make spirit) using pot or continuous still.	New make spirits are matured for at least 3 years in re-used American wooden barrels.	The Scotch Whisky Regulations 2009 (SWR).

Although the steps of spirit production are broadly similar, the conditions for each of them are significantly different (Table 1.5). The primary source of difference is the raw material (Agave vs grains), accompanied by the necessary preparation steps

before fermentation (mashing for whisky and cooking and milling for tequila) and the parameters chosen for fermentation and distillation (yeast/bacteria selection, pot still used, and the final alcohol content of the distilled spirit). However, the main parameter in affecting the 'mature' character of the distilled spirits is the ageing process. In general, ageing practice differs for different products, with regard to factors such as time, temperature, cask origin, wood type and whether the cask is new or re-use. For example, during the ageing of tequila, the necessary time to acquire a product with a specific characteristic flavour might depend on the physiochemical and sensory characteristics that each company wishes to attribute to a given brand and by regulation; this may vary from two months to three years to achieve aged, extra aged and extra ultra-aged products. For aged tequilas, maturation occurs inside white oak containers (*Quercus alba*) or holm oak containers (*Quercus ilex*) (Lopez-Ramirez et al. ., 2013). In the case of American bourbon whiskey and Scotch malt whisky, both the type of cask and the duration of maturation vary widely and, as with tequila, are frequently defined by international or local statutes according to the product name. Thus, American bourbon whiskey must be matured in new, heat-charred oak casks for at least two years, whilst in the manufacture of Scottish whisky, maturation is for a legal minimum of three years but with many malts matured for 12 or more years, in reused casks (Mosedale, 1998; Conner et al. 1993).

1.2 Analytical techniques applied to the analysis of flavour volatiles in distilled spirits

In order to achieve a practical and reliable method for the analysis of volatiles in complex matrices such as whisky, rum, gin, and tequila samples, several extraction-concentration methods have been developed and used, including steam distillation, liquid-liquid extraction (LLE) (Ferreira et al. 2000; Ferreira et al. 2002; López et al. 2002), simultaneous distillation–solvent extraction (Nuñez and Bemelmans, 1984) solid phase extraction (Ferreira et al. 2002), supercritical fluid extraction (Blanch et al. 1995), microwave extraction (Razungles et al. 1993), and ultrasonic extraction (Cocito et al. 1995).

Although these analytical methods offer specific advantages under certain circumstances, they have some drawbacks such as the possibility of contamination with solvents, and later solvent concentration, generation of artefacts and the length of time analysis (Engel et al. 1999; Demyttenaere et al. 2003; Camara et al. 2007).

1.2.1 Solvent Extraction

Solvent extraction (SE) or liquid-liquid extraction (LLE) is the most common technique used in the first stages of most isolation strategies (Benn and Peppard 1996; Poisson and Schieberle 2008). However, in spite of its drawbacks, LLE continues to be the reference technique for the determination of volatile constituents from several beverages. An advantage of this method is that all volatile compounds (low, medium and high volatility) can be analysed in one extraction step, but the method may require solvent evaporation, which, in some cases, results in the loss or degradation of some compounds (Caldeira et al. 2007).

1.2.2 Solid Phase Extraction

Solid-phase extraction (SPE) is a separation process widely used for either sample extraction or sample clean up procedures, normally it is utilized to isolate analytes of interest from a wide variety of matrices, including urine, blood, water, beverages, soil, and animal tissue. During SPE analysis the compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties. Its principle is similar to LLE, involving a partitioning of compounds between two phases. However, in SPE the analytes to be extracted are partitioned between a solid and a liquid (rather than between two immiscible liquids as in LLE) and these analytes must have a greater affinity for the solid phase than for the sample matrix (retention or adsorption step). Compounds retained on the solid phase can be removed at a later stage by eluting with a solvent with a greater affinity for the analysis (elusion or desorption step) (Berrueta et al. 1995). SPE analysis offer several advantages such as: robustness, potential for automation, capacity for providing clean extracts, selective isolations and even a fractionation of the different sample components (Campo et al. 2007). For these reasons, SPE is a powerful pre-concentration technique, which can be easily adapted for routine analysis. Campo et al. (2007) extracted four ethyl esters in whisky, wine and brandy using SPE method and quantified them through GC-MS analysis. By choosing the appropriate SPE column and optimising the conditions to suit the elution of group of analytes required, the SPE can be a successful method of extraction, especially for the recovery of semi-volatiles (Boothroyd, 2013).

1.2.3 Solid phase micro-extraction (SPME)

In the early 1990's, a new variation of adsorption technique called solid phase micro-extraction (SPME) was developed (Arthur and Pawliszyn, 1990). Compared to conventional techniques this new technique offers many advantages such as high sensitivity, does not require solvent and combines extraction and pre-concentration in a single step without pre-treatment of samples (Kataoka et al. 2000). This technique, based on absorption (Caldeira et al. 2007) and/or adsorption mechanism, depending on the fibre coating, can be successfully applied for polar and non-polar compounds in gaseous, liquid and solid samples from environmental, biological and food samples and can be easily coupled with various analytical instruments such as GC, GC–MS, HPLC and LC–MS ((Fitzgerald et al. 2000; Conner et al. 2001; Caldeira et al. 2007). The type of fibre coating, extraction time and desorption time are the critical conditions to be optimized while developing a method using SPME. Fitzgerald, James et al. (2000) identified and quantified 17 congeners in scotch whiskies by using the following conditions: fibre [85 μm poly (acrylate)], extraction time of 35 min, and desorption time of 5 min. On the other hand, Camara et al. (2007) suggested that the highest enrichment of volatiles and the highest reproducibility of the peak areas in three whisky samples are obtained by using the Carboxen-poly (dimethylsiloxane) (CAR/PDMS), and Carbowax-divinylbenzene (CW/DVB) fibers, allowing the extraction of polar analytes and nonpolar and medium-polarity compounds.

1.3 Gas Chromatography-Olfactometry (GC-O) as a technique to evaluate/identify key flavour molecules.

In recent years, intensive studies have been carried out regarding the sensory activity of the individual components of the odours of various alcoholic beverages and the dependence between the odour and the chemical composition of the volatile fraction of these products, using gas chromatography with olfactometric detection (GC–O). GC-O detection is based on sensory evaluation of the eluate from the chromatographic column and is aimed at discovering the active odour compounds (Plutowska and Wardencki 2008). On leaving the GC column, the eluent is split between two detectors: one is a mass spectrometer (MS), the other is the human olfactory system. By sniffing at a specially designed nose port, the assessor can perceive the odour compounds leaving the column while the MS simultaneously detects them. Due to this synchronized method of odour and peak detection, it is possible to correlate the odours being perceived with the compounds being detected by the GC-MS chromatogram (Boothroyd, 2013). Figure 3 shows a scheme of a typical GC-O set up.

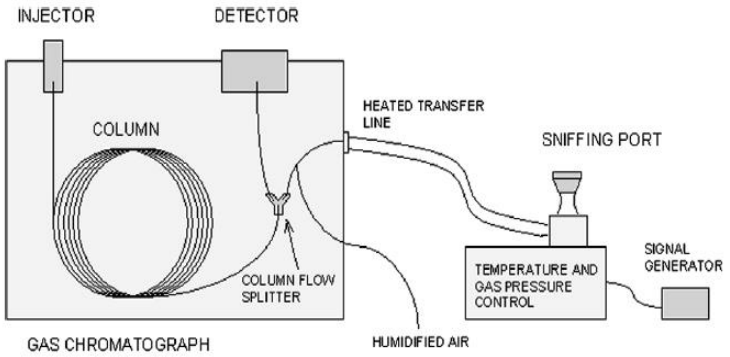

Figure 1.3 Scheme of the gas chromatograph equipped with the olfactometric detector (Plutowska and Wardencki 2008).

During GC-O analysis there are certain factors that need to be taken into account to assure the quality of results. For example, the volatile compound extraction technique is particularly important, as it affects the representativeness of the isolate and the composition of the eluate subject to sensory evaluation. Sample storage is also critical from the point of view of sample representativeness; to avoid damage the extract should be storaged at low temperature in deactivated GC vials. Since humans are the detector in the technique discussed all factors that influence the evaluator should be minimized (Delahunty et al. 2006; Plutowska and Wardencki 2008). For example, the environment in which olfactometric determination is being carried out is very important, for that reason the laboratory must be free of all foreign odours and sounds, and must preserve a consistent temperature and pressure. Chromatographic separation is also important; hence the conditions of the separation must be carefully optimized. Finally, one cannot avoid the effect of human involvement in this technique, and the limitations related to it. To minimize the deviations, one should strive to ensure constant analysis conditions for each evaluator, such as a random sample order for the samples analysed, or the same scale used for evaluating odour intensity (Delahunty et al. 2006).

1.3.1 Quantitative methods for GC-Olfactometry analysis.

Several quantitative methods for the evaluation of the intensity of odours and their relative influence on the odour of the sample have been reported (Pollien et al. 1999; Ferrari et al. 2004). These methods can be categorized into three groups based on the method of determination (Plutowska and Wardencki 2008).

1.3.2 Detection frequency methods

In frequency detection methods, a team consisting of 6-12 people analysed the same sample. The percentage of people who sensed the odour compound at a given retention time is counted. Compounds that are sensed more frequently than others are acknowledged as having the most important influence on the odour of the given sample. The results for each odour region are quantified using so-called olfactometric indices, such as NIF (Nasal Impact Frequency) values or SNIF (Surface of Nasal Impact Frequency). These methods do not required qualified evaluators, are repeatable, least time consuming, easy to conduct and the results reflect the differences in sensitivity between the evaluators (Pollien et al. 1999; Ferrari et al. 2004).

1.3.2.1 Dilution to threshold methods

Dilution to threshold methods offers a quantitative description of the odour potential of a given compound based on the ratio between its concentrations in the sample to the sensory threshold in air. These methods consist of preparing a series of dilutions of the extracts of odour compounds, most often using twofold, threefold, fivefold or 10-fold dilution levels (R) and then evaluating each sample using an odour detector. The evaluator states under which dilution the compound analysed can still be sensed, and usually describes the type of smell. The odour potential can be described as so-called aroma values or odour values, as well as odour units or flavour units (Delahunty et al. 2006). The most frequently counted are so-called odour activity values (OAVs), which represent the ratio of the concentration of a given substance in the sample to the sensory detection threshold (Plutowska and

Wardencki 2008). Methods include in this category are the combined hedonic aroma response measurement (CHARM) and aroma extract dilution analysis (AEDA). The AEDA method consists of measuring the highest sample dilution at which the odour of the analysed compound is still detectable. This value is used to calculate the so-called odour factor dilution (FD) (Delahunty et al. 2006). If the last dilution under which the analyte was still detectable is equal to P (P = 0, 1, 2, 3, n), then its dilution factor is R^P. It follows from the definition of FD that in order for the dilution factor to be treated as a quantitative measure, one should maintain the consistency of the conditions for the determination of the odour compounds in the analysed products, especially when it comes to the extraction process. The CHARM method requires an additional determination of the duration of the odour sensation in the column eluate, and allows for the determination of specific chromatographic peaks, which are expressed in dimensionless "CHARM" values and are proportional to the amount of the analyte in the extract, and inversely proportional to the sensory detection threshold (Plutowska and Wardencki 2008).

1.3.2.2 Direct intensity methods

Direct intensity methods the intensity of the stimuli and its duration are measured; using different kinds of quantitative scales. Depending on the method, the measurement can be performed in different ways. These include a single, time-averaged measurement, a measurement registered after the elution of the analyte (posterior intensity evaluation methods), or, most frequently, a dynamic measurement, where the appearance of an odour, its maximum intensity and decline are registered in a continuous manner. In the first case, the evaluator assigns each

compound appearing in the eluate an appropriate value from a previously defined intensity scale, while in the second case, peaks similar to chromatographic peaks are determined in response to the analytes. The olfactogram obtained is similar to chromatograms obtained with the use of conventional detectors and represents odour intensity as a function of the retention time. The height of the peak corresponds to the maximum odour intensity of a given analyte, while the width corresponds to odour duration (Plutowska and Wardencki 2008).

1.4 Application of GC-Olfactometry in the analysis of alcoholic beverages.

Further to GC-MS analysis, aroma research includes the use of molecular sensory science approach, which includes GC-O measurements and aroma extract dilution analysis (AEDA), which separate odour-active volatiles from the bulk of odourless food volatiles, and make possible determinate the potency of odorants in food and beverages (Grosch, 1994). Recently, only few studies using such techniques have been applied to study whisky and tequila flavour (Benn and Peppard, 1996; Conner et al. 2001; López Mercedes and Dufour, 2001; Poisson and Schieberle, 2008). Conner et al. (2001) applied GC-O on an extract obtained by SPME from Scotch whisky and particularly confirmed the importance of *cis*-whisky lactone and vanillin in the overall aroma of this type of whisky. On the other hand, Poisson and Schieberle (2008) characterised the most odour active compounds in an American Bourbon whiskey by application of the AEDA. This revealed 45 odour-active areas in the flavour dilution (FD) factor range of 32-4096 among which (E)-β-damascenone and *γ*–nonalactone showed the highest FD factors and (3*S*,4*S*)-*cis*-

whisky-lactone, γ-decalactone, eugenol and vanillin contributed to the overall vanilla-like, fruity, and smoky aroma note of the spirit. Furthermore, they applied GC-O to the headspace of the whisky revealing 23 aroma-active odorants, of which 3-methylbutanal, ethanol, and 2-methylbutanal were identified as additional important aroma compounds. With regard to tequila flavour, Benn and Peppard (1996) distinguished more than 175 volatile compounds in three brands of tequila; high concentrations of higher alcohols, along with low concentration of esters, acetals, terpenes, furans, acids, aldehydes, ketones, phenols and sulphurs compounds. Moreover, through sensory assays using GC-O-AEDA, they detected more than 60 odorants compounds, at least 30 of which could be correlated with specific GC peaks arising from components found in the extract. On the basis of their detection in the most dilute extracts analysed, five constituents were determined to be the most powerful odorants of tequila, these were: 3-methylbutanal, 3-methylbutanol, β–damascenone, 2-phenylethanol, and vanillin. Nevertheless, efforts to reconstitute tequila flavour by mixing together the various components identified were largely unsuccessful, indicating that at least some of the key contributors to tequila flavour have yet to be chemically characterized. In another study, Lopez and Dufour (2001) determined the qualitative and quantitative differences between three types of tequila (‘Blanco or Silver’, ‘Reposado or Aged’, ‘Añejo or Extra Aged’) by GC-Olfactometry of dichloromethane extracts obtained by liquid-liquid extraction. The most potent odorants were found to be phenyl ethanol and phenylethyl acetate in blanco tequila; phenyl ethanol, phenylethyl

acetate and vanillin in ‘reposado’ tequila; and phenyl ethanol, vanillin, and an unknown in ‘añejo’ tequila.

1.5 Sensory analysis of whisky flavour.

Analysis of flavour/aroma compounds is one of the most important steps in the evaluation of whisky quality. Being a combination of taste and olfaction senses, these characteristics are crucial factors in consumer acceptance of drinks and foods (Caldeira et al. 2007). Whisky flavour comprises over 1000 congeners from different chemical groups and families, which together are responsible for its unique aroma and flavour (Demyttenaere et al. 2003). Furthermore, combined instrumental and sensory analysis allows the correlation of sensory and chemical data (Piggott, 1990). This chemometric evaluation is a vital part of flavour congener identification, proving whether a correlation exists, linking the chemical composition with sensory data (Boothroyd, 2013). Sensory evaluation is also a major aspect of authenticity analysis (Jack and Steele, 2002). The major sensory characteristics of a whisky can be measured using Quantitative Descriptive Analysis (QDA) and a profile produced. This is carried out using a vocabulary based on the Scotch Whisky Flavour Wheel (Figure 1.4). The flavour wheel has been developed to cover all of the major typical characteristics and also atypical characteristics and off notes, that can be encountered in Scotch whisky (Shortreed et al. 1979). The Scotch whisky flavour wheel has recently been revised, and reference compounds have been defined for assessor training purposes against each flavour term (Table 1.6) (Lee et al. 2001). The primary tier terms of the Scotch whisky flavour include a range of common descriptors, including: peaty, grainy, grassy, fruity, feints,

woody, sweet, stale, sulphury, cheesy, and oily (Figure 1.4). Once these sensory profiles have been produced they must then be interpreted. Different Scotch whiskies can have very different characteristics, and hence different profiles (Jack and Steele, 2002).

Figure 1.4. The Scotch whisky flavour wheel (Shortreed et al. 1979).

Table 1.6 Whisky descriptors and reference compounds (Lee et al. 2001).

Attributes	Reference compounds	Concentration (mg/L)
Pungent	Formic acid	10×10^3
Burnt/smoky	Guaiacol [b]	27
Medicinal	o-Cresol [d]	1.75
Malty	Malted barley [a], 2-and 3-Methyl butanal, 4-Hydroxy-2(or 5)-ethyl-2 (or 5)-methyl-3(2H) furanone, 4-hydroxy-2,5-dimethyl-3 (2H) furanone	– 0.6 (2-methyl butanal)[e], 1.25 (3-methyl butanal)[e]
Grassy	Hexanal [b] cis-3-Hexen-1-ol[a]	5 $1{:}00 \times 10^3$
Solventy	Ethyl acetated, 2-Methyl propan-1-ol [a]	$1{:}12 \times 10^3$ $1{:}00 \times 10^3$
Fruity (apple)	Ethyl hexanoate [b]	2
Fruity (banana, pear-drop)	iso-Amyl acetate [b]	7
Berry Catty	Thiomenthone [d] Thiomenthone [d] Sodium sulphide + mesityl oxide [a]	3×10^3 1.26 100 each
Floral (Natural – rose– violet)	Phenyl ethanol [b]	$1{:}52 \times 10^3$; $>3 \times 10^3$
Floral (Artificial – scented, perfumed)	α–, β– Ionone [d] Geraniol [b]	 19
Nutty (coconut)	Whisky lactone [b]	266
Marzipan	Furfural [b]	839
Vanilla	Vanillin [b]	43
Spicy	4-Vinyl guaiacol [b]	71
Spicy (clove)	Eugenol [a,b]	1 to 55
Caramel (candy floss)	Maltol [b]	1.14×10^3
Mothball	Naphthalene	$>8 \times 10^3$
Mouldy	2,4,6-Trichlorocanisole [a]	10
Earthy, musty	Geosmin, 2-methyl iso-borneol	
Vinegary	Acetic acid [b]	5.32×10^3
Cardboard	2-Nonenal [d]	0.08
Stagnant, rubbery	Dimethyl tri-sulphide (DMTS) [c]	3
Yeasty Rotten egg meaty	Hydrogen sulphide (H2S) [c] Hydrogen sulphide [c] Methyl (2-methyl-3-furyl) disulphides [d]	 > 0.02 > 0.14
Vegetable (sweet corn, cooked cabbage)	Dimethyl sulphide (DMS) [c]	>0.6
Gassy	Ethanethiol [c]	>0.072
	3-Methyl-2-butene-1–thiol [c]	$>7.2 \times 10^4$
Rancid	n-Butyric acid/ethyl butyrate [d]	>2
Sweaty	Iso-Valeric acid [b]	2
Oily	Heptanol [a]	1
Soapy	Ethyl laurate [b]	12
	1-Decanol [a]	100
Buttery	Diacetyl [b]	0.1

[a] in 23% ethanol solution; [b] in 23% grain whisky; [c] in lager; [d]>threshold; [e] threshold in beer.

Several studies in this subject have been published (Piggott and Jardine 1979; Lee et al. 2000; Lee et al. 2001). Piggott et al. (1979) completed a descriptive vocabulary of thirty-five terms separated into the modalities of odour, flavour and after-flavour on five-point scales. Odour and flavour descriptors alone were sufficient to distinguish between the whiskies evaluated. On the hand, Lee et al. (2001) analysed whisky blends of 4 categories (Deluxe, standard, multiple retailer and West Highland), distinguishing their aroma characteristics using a sensory panel of 26 trained persons. Analysis of variance (ANOVA) and Principal component analysis (PCA) were implemented to determinate the most significant characteristics for discrimination between the whiskies, of which pungent, smoky/peaty, smooth, woody, vanilla and sulphury and rancid attributes were the most significant. Moreover, attributes such as fruity, solventy, and woody were considered important characters perceived in Deluxe, multiple retailer and West Highland blends. These studies showed the importance of sensory evaluation as a tool to distinguish and classify the main characteristics and distinctions between distilled products.

1.5.1 Sensory interactions in whisky

Whisky is a complex physicochemical system containing a range of compounds that influence both the static (equilibrium) and dynamic partitioning of aroma (Boothroyd, 2013). For the sensory assessment of flavour, distilled spirits are usually diluted to 23% ethanol by volume (% ABV) to reduce pungency (Conner et al. 1994). Dilution, however, changes the solubility of many volatile compounds that are more soluble in ethanol than water, such as ethyl esters of fatty acids.

Furthermore, these esters are amphiphilic, with a polar head and hydrophobic hydrocarbon chain, and may thus form agglomerates or micelles in aqueous ethanolic solutions (Conner et al. 1994). These micelle-like structures comprise of surface-active molecules such as long chain alcohols, aldehydes and esters (Boothroyd, 2013). They have the ability to incorporate other hydrophobic compounds such as shorter chain esters, alcohols and aldehydes from the solution and thus to decrease their free solution and consequently headspace concentrations, reducing their sensory impact and improving acceptability to consumers through marked changes in character (Piggott et al. 1996; Conner et al. 1999). Wood extracts have been shown to affect the size and stability of the ester agglomerates formed on dilution (Conner et al. 1999). Therefore, malt whisky, diluted for consumption, may be regarded as an emulsion but with a portion of the flavour molecules forming the disperse phase. However long chain-ethyl esters (ethyl dodecanoate & ethyl hexadecanoate) are the primary components of agglomerates formed in diluted distillate (Conner et al. 1994). But other compounds such as alcohols and long-chain aldehydes also influence ester activities, consequently, the activity or headspace concentration of a hydrophobic aroma compounds in an alcoholic beverage can be determined by the concentration and nature of other hydrophobic compounds present (Conner et al. 1994). Due to the complex nature of flavour congeners present in the whisky matrix, many reactions take place, some of which arise during the maturation and others are formed when the spirit is diluted. These reactions involve extraction of wood components, evaporation of low-boiling-point solutes from the distillate and interactions of wood and distillate components (Nishimura and

Matsuyama 1989; Bamforth et al. 2003). The origins of these interactions might be derived from extraction of cask wood, oxidation of ethanol and evaporation of ethanol and water (Conner et al. 1999). Nevertheless, few studies have analysed the physicochemical mechanisms that affect the release of flavour compounds in whisky or the effects associated with adding volatile compounds on the volatile partitioning in a whisky model system. Table 1.7 summarized the main studies performed until now.

Table 1.7 Studies performed in the field of whisky interactions.

Whisky Interactions				
Type of interaction	**Matrix**	**Study**	**Findings**	**Reference**
Physico-chemical	Brandy	The influence of non-volatile constituents on the extraction of ethyl esters from brandies	Non-volatile components including a wood extract caused a chain-length-dependent increase in the aqueous ethanol solubility of ethyl esters, which were shown to be correlated with immature odours in fractions of a brandy. It is likely that the increase in solubility, and corresponding reduction in headspace concentration, is partially responsible for the sensory characteristics of distilled beverages matured in oak casks.	(Piggott et al. 1992)
Physico-chemical	Whisky	Interactions between ethyl esters and aroma compounds in model spirit solutions	For hydrophobic aroma compounds in 23% ethanol, activity is determined by the presence of other hydrophobic compounds and its own concentration, also dissolution of wood extract during maturation influences the distribution of the compound between the agglomerate phase and the solution.	(Conner et al. 1994)
Physico-chemical	Whisky	Agglomeration of ethyl esters in model spirit solutions and malt whiskies.	Medium and long-chain ethyl ester form agglomerates at concentration above 23% ethanol, reducing the volatility of other esters. Addition of wood extractives changes the solubility parameters of organic compounds, decreasing the formation of agglomerates. These reductions have a direct effect on whisky flavour.	(Conner et al. 1994)
Physico-chemical	Whisky	Interactions between wood and distillate components in matured Scotch whisky.	Interactions between wood and distillate components reduced the perception of new distillate and immature characters of a mature whisky in the mouth.	(Conner et al. 1996)
Physico-chemical	Whisky	Role of organic acids in maturation of distilled spirits in oak casks.	Organic acids compounds influence the aggregation of ethanol in aqueous solution, reducing the activity coefficient of ethyl decanoate. This is a direct relationship between physico-chemical changes of distillate and concentrations of hydrophobic aroma compounds in headspaces above spirit samples.	(Conner et al. 1999)

Table 1.7 Studies performed in the field of whisky interactions (cont.)

Physico-chemical	Whisky	Release of distillate flavour compounds in Scotch malt whisky	The equilibration of hydrophobic compounds between a distilled spirit and the headspace occurs in two stages: first the activity is reduced by the aggregation of ethanol molecules and the second equilibrium is governed by: the vapour pressure of the solute, the ambient temperature and the pressure. Components of wood extract (ethanol lignin) acted in the same manner as high molecular weight esters and alcohols, displacing volatile components from the surface layer, and reducing the critical point for the aggregation of ethanol, which explain the decreased impact of undesirable, immature aromas when wood matured spirits are consumed.	(Conner et al. 1999)
Physico-chemical	Whisky	Solute effects on the interaction between water and ethanol in aged whiskey	The strength of the hydrogen bonding in aged whiskies is directly predominated by acidic and phenolic components gained in oak wood casks and not dependent on just the ageing times.	(Nose et al. 2004)
Physico-chemical	Whisky	Effects of ethanol and long-chain ethyl ester concentrations on volatile partitioning in a whisky model System	Aqueous concentrations of ethanol (5-40% ABV) and ethyl hexadecanoate (0-500 μg/L) significantly affected the partitioning behaviour of a test set of 14 whisky volatile compounds in aqueous model systems. These effects can be interpreted in terms of the physical chemistry of ethanolic solutions and the potential for agglomerate formation by long-chain fatty acid ethyl esters in spirit samples, which selectively diminish headspace concentrations of the more hydrophobic compounds. These structures can incorporate small hydrophobic flavour compounds, thus lowering their headspace concentrations and changing the balance of the nosed aroma toward more polar, hydrophilic compounds.	(Boothroyd, 2013)

Although these studies explain part of the interactions that affect the release of flavour compounds and the effects associated with adding volatile compounds to a model system, there have been no prior published studies dealing with the potential for sensory interactions between different flavour characters in whisky. However, some studies performed on wine flavour, have demonstrated the existence of strong perceptual interactions between the fruity and woody characters of the wine (Atanasova et al. 2004; Atanasova et al. 2005).

1.5.2 Odour quality and intensity interactions in binary mixtures

The variety of sensory perceptions observed when mixing several odorants could be the result of qualitative (odour quality) and quantitative (odour intensity) perceptual interactions between odorants in mixture (Atanasova et al. 2005). Perceptual interactions between volatile compounds in combination remain difficult to predict in alcoholic beverages, and even in synthetic solutions. In order to overcome this difficulty, several mathematical models have been developed to predict the quality and quantitative interactions in binary mixtures. According to these models, if both odorants have approximately equal unmixed intensities, both are perceived in the mixture, whose quality seems to be intermediate between the qualities of its unmixed components (Olsson, 1994). These observations were confirmed by using a predictive model for odour intensity and quality perception (Olsson 1994; Olsson 1998). This interaction model is the only model that predicts both the qualitative and quantitative characteristics of a mixture on the basis of the intensities of its unmixed compounds. Although, none of these studies included a

systematic evaluation of perceptual interactions induced by odorants found mixed 'naturally' in food or beverages. It was not only until the study of Atanasova et al. (2004, 2005) that the qualitative and quantitative perceptual interactions between woody odorants and fruity esters in wine were examined.

For quantitative or odour-intensity interactions, they suggest the use of:

$$\sigma = f(\tau) \text{ --------- (1)}$$

This parameter was first introduced by (Patte and Laffort, 1979). Where (τ) reflects the relative proportion of perceived intensity of fruity or woody unmixed odorant (I_F or I_W) in the binary mixture:

$$\tau = \frac{I_{FR}}{(I_{FR}+I_{WO})} \text{---------- (2)}$$

Where I_{FR} and I_{WO} are the perceived odour intensity of fruity and woody odorants not in a mixture. When τ = 0.5 the mixture is iso-intense. Sigma (σ) reflects the degree of overall intensity addition in the mixture:

$$\tau = \frac{I_{mix}}{(I_{FR}+I_{WO})} \text{---------- (3)}$$

Where I_{mix} is the overall perceived odour intensity of the mixture; I_{FR} and I_{WO} are the perceived odour intensity of fruity and woody components not in a mixture. Thus, when σ = 1, there is complete intensity addition, when σ > 1, there is hyper-addition and when σ < 1, there is hypo-addition (Atanasova et al. , 2004).

On the other hand for qualitative interactions, they suggest the use of Olsson´s interaction model and Linear logistic model.

1.5.2.1 Olsson´s interaction model

Olsson's model (1994), estimate the evolution of single component identification in the mixture when the intensity proportion of unmixed components varied. Specifically predicts identification probabilities such as:

$$P_{fruity=} {}^{I^2 fruity} \Big/ _{(I^2 fruity + I^2 woody)} \quad ----(4)$$

And,

$$P_{woody=} {}^{I^2 woody} \Big/ _{(I^2 fruity + I^2 woody)} \quad ----(5)$$

Where I_{fruity} and I_{woody} are the perceived intensities of the unmixed fruity and woody odorants respectively. According to this model, the probability of identifying a mixture as either fruity or woody is related to the perceived intensities of the components presented separately. Specifically, p_{fruity} equals p_{woody} when the component odours are iso-intense out of mixture.

1.5.2.2 Linear logistic model

A linear logistic model is used to model the probability of fruity (respectively woody) responses according to the fruity intensity proportion in the mixture; σ is the ratio between the perceived intensity of one of the odorants (fruity in our case) and the sum of the perceived intensities of each unmixed odorant:

$$\tau = {}^{I_{fruity}}\Big/_{(I_{fruity} + I_{woody})} \text{--------- (6)}$$

It reflects the relative intensity proportion of one odorant, compared with the sum of the intensities of both odorant. When σ = 0.5, the mixture is composed of iso-intense unmixed components. This linear logistic model is fitted using individual numbers of fruity (respectively woody) responses; assume to be distributed according to binomial distributions. It supposes that the relationship between the logit of fruity (respectively woody) response probability and σ is linear, and that the intercepts vary according to subjects.

1.6 Wood maturation of distilled beverages

Distilled beverages, such as whisky and tequila legally, bourbon, and cognac are matured for many years in oak barrels. Scotch whisky has to be stored for a period at least 3 years, bourbon for a period of 1 year (Mosedale, 1995), and tequila is matured for a period of 2 month to obtain ‘reposado or aged’ tequila and for 12 months or more to obtain ‘extra aged’ tequila (Cruz and Alvarez-Jacobs, 1999). During maturation a range of physical and chemical interactions take place between the barrel, the surrounding atmosphere and the maturing spirit, which transform both the flavour and composition of the drink. These changes during maturation are highly variable but primarily, depend on maturation time, species and geographic origin of wood used in the construction of the barrels, age of the barrel, toasting degree and barrel capacity, the storage conditions such as temperature, the disposition of the barrels, humidity, light, ventilation, as well as the beverage itself

(Mosedale and Puech 1998; Cruz and Alvarez-Jacobs 1999; López-Ramírez et al. 2013). However, ageing is the costliest factor influencing the quality of distilled beverages, due to the time of storage in the oak cask which is fundamental to the final flavour of whisky (Canaway, 1986; Mosedale, 1995). The type of oak cask chosen depends primarily on its effects on whisky flavour and the time required to reach a satisfactory condition, which is of financial and practical concern for the manufacturer and varies according to the type of cask used (Mosedale, 1995; Mosedale and Puech 1998). For example, American whiskies are matured in American oak barrels, which are predominantly *Quercus alba*, while the Scotch whisky shifted from using predominantly European (*Q. petraea and Q robur*) to American oak barrels (Mosedale, 1995).

Drying and heating are steps of cask manufacture that affect the compounds produced from the degradation of wood macromolecules, some of which include aromatic aldehydes, furan products and volatile phenols (Reid et al. 1993). The intensity of heating has led to increasing interest in more precisely controlling the process. Used casks release lower concentrations of most extractives and generally lead to much longer maturation times than found for new casks (Reazin, 1983). The use of oak chips is of particular use in compensating for the low levels of extractives remaining in old casks, that might be used to provide a 'cask-environment' and allow greater control and manipulation of the final taste (Mosedale and Puech, 1998). Temperature and humidity are the two most important environmental factors influencing the effects of maturation and, in particular, the losses in volume and alcoholic strength (Nishimura and Matsuyama, 1989). Attention has been focused

on limiting extreme fluctuations in temperature, either through better insulation or, in certain cases, through heating. Others have proposed as alternatives to accelerate maturation by speeding oxidation and other reactions by heating of the maturing distillate. However, these methods tend to result in rapid oxidation and do not produce the changes in concentration that occur due to evaporation during cask ageing, normally at low temperatures (Mosedale and Puech, 1998). For these reasons, any factor that causes variation of cask wood properties will thereby influence the maturation of whisky.

1.6.1 Flavour active congeners extracted from wood during maturation

The process of maturation and ageing is characterized by changes in the colour and flavour of the maturing spirit. However, the mechanisms by which maturation in oak casks occur are incompletely understood. Research has been carried out to identify compounds that contribute to the flavour or aroma of whisky, referred to as flavour congeners. Correlations between descriptive flavours and chemical analyses of mature whiskies, have identified over 400 flavour congeners (Philip, 1989). The principle ones are esters, carbonyls, sulphur compounds, lactones, phenols, and nitrogenous bases, including both desirable and undesirable components. In some cases, the origin and method of synthesis have been further studied and the involvement of the maturation stage confirmed. Changes in taste or aroma will be due to changes in these flavour congeners. The methods by which this may occur, during the maturation of whisky in oak casks, are listed below (Nishimura and Matsuyama, 1989).

1) Direct extraction of wood chemicals.
2) Decomposition of wood macromolecules and extraction of these into the distillate.
3) Reactions between wood components and constituents of the raw distillate.
4) Reactions involving only the wood extractives.
5) Reactions involving only the distillate components.
6) Evaporation of volatile compounds through the cask.

The extraction and subsequent transformation of compounds from the oak cask is believed to be of prime importance to the final flavour. Figure 1.5 describes the variety of extractive and structural compounds that make up oak heartwood (Mosedale, 1995), and Table 1.8 summarized these compounds and its contribution to whisky flavour.

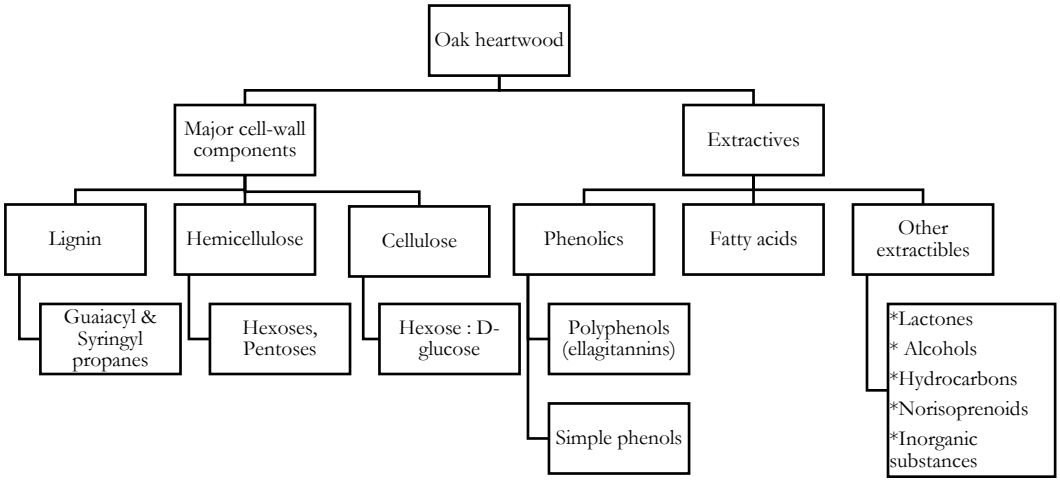

Figure 1.5 Components of oak heartwood (Mosedale and Puech, 1998).

Table 1.8 Summary of extractive oak compounds and their contribution to whisky flavour (Mosedale, 1995).

Extractives oak compounds	Group associated	Contribution to whisky flavour
Tannins and their derivatives	Phenolic compounds	Its impact is unknown.
Lignin degradation products	Aromatics (Fig 5)	Uncertain but may have a less influence flavour.
Oak lactones	γ-lactone isomers (cis and trans)	Unknown, although relatively high levels in mature whisky are considered desirable.
Fatty acids	Steroids and triglycerides, as well as palmic, steric and oleic acids.	Unknown
Carbohydrates	Glucose, arabinose, and protoquercitol,	Not thought to have a major influence on flavour
Nitrogenous compounds	Pyrazines and pyridines	
Terpenes	Monoterpenes, sesquiterpenes and various norisoprenoids	Important contribution but unknown yet.

Piggott et al. (1992) emphasized that it is the concentration in the 'headspace' (the air space in the cask or container) rather than in the mature spirit that determines the influence on flavour of many volatiles. Some extractive compounds such as ellagitannins or polyphenols, influence the aroma of whisky by reducing the headspace concentration of volatile compounds, and other compounds serve as reference of high quality (eugenol and whisky lactone) and poor quality (glycerol and fatty acid derivatives) in whisky flavour (Reid et al. 1993). Furthermore, the concentration of volatiles in the headspace will be influenced by any factors affecting their solubility in the distillate, including the concentrations of non-volatile compounds. Canaway (1983) described how the variation of samples from different casks could equal the variation between samples of differing age. Such variation between casks could be due to differences in the raw distillate, and the conditions of maturation or the cask wood. Although both the raw distillate and particularly the conditions of maturation may play an important role in determining

the result of maturation, the oak cask in which maturation takes place appears to be of prime importance to the final flavour of whisky (Canaway, 1983; Mosedale, 1995).

1.6.2 Flavour change through maturation

As previously stated, maturation plays an important stage in the development of flavour congeners, aimed to bring about sensory changes in whisky flavour and improve its final quality. However, maturation involves a complex series of reactions including those which occur naturally in the spirit and those that are influenced by the cask environment (Clyne et al. 1993). The initial sensory characteristics of the distillate, and the type of cask used for maturation, can vary greatly and affect the sensory characteristics of the resulting whisky. The effect of the cask is determined by the type of oak employed (American or Spanish), the treatment of the wood surface (charred or wine treated) and the number of times the cask has been used (Philp, 1989). Thus, the maturation process can be controlled to an extent to produce a whisky with the desired sensory characteristics. At any point in the maturation process the maturing spirit has a unique flavour profile resulting from flavour congeners derived from the distillate 'base' flavour, flavour congeners removed from the spirit, flavour congeners added to the spirit by extraction from cask wood or by reactions within the spirit, and flavour congeners produced by interaction of the wood surface with the spirit (Philp, 1989). Some authors have studied the chemical changes brought about by charring, the majority of whom have found increased levels of the aromatic aldehydes vanillin, syringaldehyde and

increased levels of other aldehydes and esters (Reazin, 1983; Philp, 1989; Conner, Paterson et al. 1993). Conner et al. (1993) suggested that after charring and pyrolysis of the wood through maturation, the successive whisky maturation resulted in extractions of low-molecular weight phenolic compounds, followed by progressive ethanolysis of susceptible lignin bonds from deeper regions of the wood such as acetovanillone.

Although, these studies described the changes associated with cask charring, Conner et al. (1993) studied such changes by descriptive sensory analysis of whisky distillates maturing in uncharred and charred American oak cask over a period of 3 years. Charred cask samples were associated with mature characteristics such as 'smooth, vanilla and sweet', and cask charring enhanced levels of syringaldehyde and total phenols and decreased levels of coniferaldehyde, sinapaldehyde and vanillic acid. These differences could be related to changes in the sensory characteristics of the distillate. Furthermore, other studies have demonstrated that cooperage, wood origin region, and maturation time, affect compounds found in matured alcoholic beverages (Masuda and Nishimura, 1982; Mosedale and Puech, 1998; Fernandez et al. 2003). Nishimura and Masuda (1981) reported that many sulphur compounds decrease during ageing of malt whiskies and even some of them disappear after three years (DMS, 3-(methylthio) propyl acetate, dihydro-2-methyl3(2H)-thiophenone and ethyl 3-(methylthio)propanoate decrease. Bujake (1992) reported a high correlation between maturation time and esters, aldehydes, total colour, solids, ethyl acetate, esters, 2-methylpropan-1-ol, and 3-methylbutan-1-ol concentrations in whisky samples (Bujake, 1992). Moreover, López-Ramírez

et al. (2013) identified the influence of barrel origin and maturation time over the physicochemical parameters regulated by the Official Mexican Standard of tequila. These findings clearly state the importance and influence of maturation on the development of flavour congeners of distilled beverages such as whisky and tequila.

1.7 Composition and formation of the estery component of new make spirits

New make spirits are distilled products obtained from Scotch malt and grain whisky that have not undergone the minimum maturation term. In other words, they are produced from nothing other than yeast, water and malted barley and distilled in copper pot stills, without any ageing step (Bamforth et al. 2003). Since new make spirits do not required maturation, the flavour congeners presented at this stage are derived mainly from:

1) The malt, and the cereal of grain distilling,
2) Volatile structural components of the yeast
3) Metabolic products of yeast growth
4) Microbial contaminants of fermentation

Although the more efficient rectification of continuously distilled spirit reduces the flavour contribution of these four factors in comparison with the effect of subsequent maturation, both the yeast itself and the progress of fermentation are important contributors to the character of these two types of Scotch whisky (Bamforth et al. 2003).

During Scotch whisky fermentation, *S. cerevisiae* is the yeast commonly used to conduct this stage. As a rule, *S. cerevisiae* produced ethanol, CO_2 and important minor compounds including alcohols and fusel alcohols, fatty acids, and esters,

lactones, and other carbonyl compounds (Demyttenaere et al. 2003), which make important contributions to whisky flavour (Table 1.9). However fatty acid esters form an essential group of aroma components in whisky. The esters generally are formed during non-enzymatic esterification of acids and alcohols during maturation of whisky, however that reaction is too slow to account for the esters produced during fermentation (Hough et al. 1982). Production of esters is related to the recycling of the enzyme cofactor, coenzyme A (CoA). Acetic acid and longer-chain fatty acids are important intermediates in biosynthetic activities, but a proportion of these compounds are lost to the culture medium, to appear as flavour congeners (Table 9).

Table 1.9 Products of yeast fermentation (Bamforth et al. 2003).

Alcohols	**Acids**	**Esters**	**Others**
Ethanol	Acetic	Ethyl acetate and any other combination of acids and alcohols on the left	Carbon oxide
Propanol	Caproic		Acetaldehyde
Butanol	Caprylic		Diacetyl
Amyl alcohol	Lactic		Hydrogen sulphide
Glycerol	Pyruvic		
Phenylethanol	Succinic		

Acetyl CoA and its higher homologues, collectively indicated as R-CO~S.CoA in Figure 1.6, are important intermediates in the biosynthesis of enzyme proteins, nucleic acids and lipids. Note, incidentally, the unusual removal of two phosphate groups from ATP (to form adenosine monophosphate) in transferring the energy-rich bond from ATP to the CoA complex. If, however, the acetyl or higher acyl CoA is not required, it must be recycled to recover the energy conventionally represented by the ~ bond, and maintain the limited intracellular supply of CoA itself. On removal of the CoA the acid group is stabilized by esterification, with the

involvement of an esterase enzyme (esterases catalyse the reaction in either direction) (Bamforth et al. 2003).

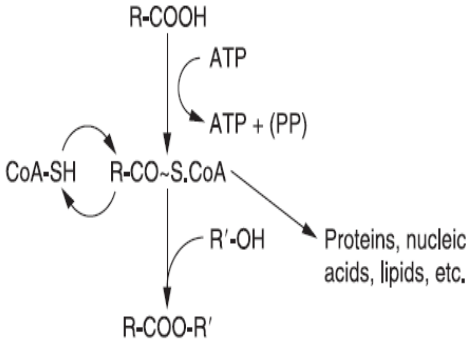

Figure 1.6 Formation of esters by recycling of coenzyme A (Bamforth et al. 2003).

Since acetate and ethanol are the acid and alcohol formed in greatest amounts during fermentation, naturally ethyl acetate is the principal ester in quantity, although other esters with lower aroma threshold have more effect on the wash, spirit and final whisky and thus it may be assumed that they appear as important aroma components, contributing the 'estery' character of the beverage even if they are present in relatively small amounts (Demyttenaere et al. 2003). The wider implications of recycling CoA are shown in Figure 1.7, which indicates the sources of ethanol, higher alcohols and the various acid groups involved in ester production.

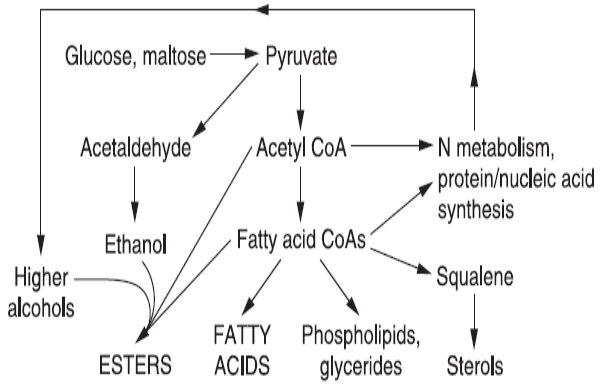

Figure 1.7 Formation of esters and fatty acids as by-products of yeast growth (Bamforth et al. 2003).

Due to many of the flavour congeners are produced during Scotch whisky fermentation, several factors are known to affect its production such as (Hough et al. 1982).

1) Genetic properties of yeast strains
2) The conditions of the yeast at pitching (viability and vitality)
3) The amount of yeast inoculum
4) Initial aeration of the wort
5) The temperature profile during fermentation
6) Microbial contamination

Furthermore, Table 1.10 shows a summary of the effects of fermentation conditions on the production of flavour congeners, mainly higher alcohols and esters.

Table 1.10 Factors affecting ester and higher alcohol production by yeast (Makinen et al. 1970).

Cultural condition	Effect on higher alcohol production	Effect on ester production
Increased growth of yeast (higher temperature, increased O2)	Increase (lees available-NH2)	Decrease (less recycled CoA)
Decreased growth of yeast (lower temperature, decreased O2)	Decrease (more available-NH2)	Increase (more recycled CoA)
Deficiency of amino N	Increase (less available NH2)	Usually decrease if less growth, less recycled CoA
Redox effects:		
a) Deficiency of NAD+	Increase (to generate NAD)	Neutral, or increase if less growth
b) Sufficiency of NAD+	Neutral or decrease	Neutral, or decrease if more growth
Genetic properties of yeast	Increase or decrease, according to properties of yeast strain	

Fermentation temperature has an important influence, as a general rule, increasing fermentation temperature increases the amount of yeast growth in proportion to the available amino nitrogen, resulting in increased production of higher alcohols (Makinen et al. 1970). Conversely, since increased growth means less recycling of CoA, ester production is increased. Although it is not distillery practice to maintain a constant temperature as in breweries, consistent levels of flavour congeners in the wash require consistent temperature profiles throughout successive fermentations, achieved by judging the correct initial temperature of the wort for the ambient temperature. The genetic properties of the yeast also affect production of flavour congeners. No two strains have identical responses to fermentation temperature and the dissolved oxygen and amino nitrogen content of the wort. Therefore, changing to a different culture yeast may affect the flavour of the wash and ultimately the spirit and whisky (Bamforth et al. 2003). Since an ‘estery’ aroma has become a desirable quality, Hay et al. (1994) developed a higher-yielding distilling yeast by genetic manipulation. However, with the present public antagonism to genetically manipulated organisms, it is unlikely that such yeast will be used commercially in

the near future to generate higher ester levels in whisky, even though it complies with the Scotch whisky regulations (Hay et al. 1995).

1.8. Overall aims

In Chapters 2 and 3 four pairs of non-mature and mature spirits (tequila, bourbon, and 2 malt whiskies) will be characterized by instrumental and sensory analysis, with the main objective to identify the key aroma compounds that define the mature character in each spirit, and those that comprise the estery and woody/mature characters of each spirit type. In Chapter 4 we will attempt to reproduce these characters for each spirit throught aroma recombination, considering the odiferous compounds identified at high FD factors (GC-O/AEDA) and by both the addition of a cocktail of low boiling compounds (those identified by GC-FID analysis) and the introduction of a 'structuring' compound (ethyl hexadecanoate) at a concentration that could influence aroma partitioning and release. In Chapter 5, these recombinant aromas will be use as aroma bases to test potential sensory interactions between these characters (estery and woody) for each spirit type. Finally, because the woody/mature compounds which characterised maturation were broadly similar across the spirit types, but differed in concentration according to the maturation conditions, we decided to investigate the extraction kinetics of wood-derived compounds from oak sticks as a function of ageing time, temperature, spirit type and alcohol content (Chapter 6).

2. CHARACTERIZATION OF KEY AROMA COMPOUNDS IN SPIRIT SAMPLES OF BOURBON, TEQUILA AND MALT WHISKIES.

2.1 AIM

Aroma characterization by means of instrumental analysis of non-mature and mature spirit samples of bourbon, tequila and malt whiskies of the same brand and provenance, with the purpose to select or define the key aroma compounds that define and influence the mature character in each of the spirit samples as a consequence of the maturation process.

2.2 INTRODUCTION

The aroma of most alcoholic beverages consists of hundreds of volatile compounds, which belong to a great variety of families such as: ethyl esters, higher alcohols, fatty acids, higher alcohols, acetates, and carbonyl compounds, such as aldehydes and ketones, sulphur compounds, furanic compounds, lactones, volatile phenols, among others. This great diversity of compounds are produced through metabolic pathways and their genesis depends on many factors related to raw materials and the subsequent processes of mashing, fermentation, distillation and ageing, others are oak derived, while others depend on the type of technological treatment. Many of these compounds are common to different spirits but differ analytically in terms of the relative amount (Caldeira et al. 2007). Particularly important is the maturation in oak barrels, in which the fresh distillates change their colour and flavour as a result of complex reactions which included those which

occur naturally and those that are influenced by the cask environment (Clyne et al. 1993). Studies carried out on the contribution of oak to the olfactory characteristics of wine and whisky have shown that these are mainly influenced by compounds such as furfural, guaiacol, whisky lactone, eugenol, vanillin and syringaldehyde (Clyne et al. 1993; Withers et al. 1995; Arapitsas et al. 2004).

Furthermore, in order to achieve a practical and reliable method for the analysis of volatiles in complex matrices such as whisky samples, several extraction methods have been developed and used, including the conventional liquid-liquid extraction (LLE), solid-phase extraction (SPE), and solid-phase microextraction (SPME) among others (Caldeira et al. 2007; Boothroyd et al. 2013). Although these methods offer specific advantages under specific circumstances, they have some drawbacks such as the possibility of contamination with solvents, and later solvent concentration, generation of artefacts and the length of time analysis. In spite of these inconvenient, LLE methods continue to be the reference technique for determination of volatile constituents from several beverages, mainly because it allows the extraction of low, medium and high volatile compounds in one single step (Caldeira et al. 2007). SPE has been applied previously to elucidate the odour active regions in which nutty, nutty-cereal or nutty-oily descriptors were perceived along 35 new make spirits (Boothroyd et al. 2013). Therefore, the main aims of this chapter were: firstly to evaluate the use of solid phase extraction (SPE) alongside the more traditional liquid-liquid extraction (LLE) to see if this offered selectivity, or better sensitivity for particular groups of compounds among the spirit samples, with the intention to select the better extraction process to further characterize the

aroma extracts by means of GC-O/AEDA analysis, and secondly, to use the results from instrumental analysis (GC-MS, GC-FID and HPLC) to select or define the key aroma compounds that define and influence the mature character in each of the spirit samples as a consequence of the ageing period.

2.3 MATERIAL AND METHODS

2.3.1 Samples

Eight spirit samples (non-mature and mature exemplars of each kind), consisting of two commercial brands of Scotch malt whiskies from two distilleries (A and B), tequila and bourbon samples were confidentially sourced from the industry by the Scotch Whisky Research Institute (SWRI) and were used to carry out each experiment. The mature bourbon sample had been matured for a period of 5 years; mature tequila for 29 months (2.4 years) and Scotch malt whiskies from distilleries A and B were matured for a period of 4 years.

2.3.2 Reagents and Chemicals

The standards of the aroma compounds studied were supplied by Sigma-Aldrich (Poole, Dorset, UK), VWR International (Lutterworth, Leicestershire, UK), Fisher (Loughborough, Leicestershire, UK) and Merck (Merck KGaA, Darmstadt, Germany) (Appendix 1). All other chemicals and regents used were analytical grade.

2.3.3 Gas Chromatography analysis of spirit samples

2.3.3.1 Direct injection method

When performing GC analysis of extracts in dichloromethane, the solvent front of the chromatogram always obscures some highly volatile compounds in the sample. To analyse these compounds, such as methanol and acetaldehyde, we used a direct injection GC method, without prior extraction and concentration. This technique enabled the analysis of the major volatile compounds of spirit samples which include acetaldehyde, ethyl acetate, acetal, methanol, *n*-propanol, isobutanol, amyl and isoamyl alcohols, ethyl lactate, acetic acid, and furfural. These compounds were analysed by gas chromatography (GC) using a Bruker Scion 456-GC gas chromatograph, coupled to a flame ionization detector (FID). Spirit sample (0.5 μL) was injected into the chromatograph in split mode. Separations were performed using a ZB-Wax capillary column (60 m × 0.25 mm i.d., 1.0 μm film thickness; Phenomenex, Macclesfield, UK). Operating conditions were as follows: carrier gas (helium) at 1.5 mL min^{-1}; initial oven temperature was 35ºC, and then the temperature was raised at 6 ºC/min to 120 ºC and held for 0 min. Finally, the temperature was increased at 100 °C /min to 220 °C and held for 4 min. Injector and detector temperatures were maintained at 200 ºC and 210 ºC, respectively. Quantification was achieved following normalization to the internal standard (250 μg mL^{-1}, *n*-pentanol) of eight diluted solutions in the range of 5 – 1250 μg mL^{-1}. Calibration curves reported a correlation coefficient (R^2) greater than or equal to 0.99 for each compound.

2.3.3.2 Extraction of volatile compounds from spirit samples

Volatile compounds were extracted from spirit samples using solid-phase extraction and liquid-liquid extraction following method previously described by Boothroyd et al. (2014).

a) Liquid-liquid extraction (LLE): spirit samples (100 mL) were spiked with an internal standard (2-acetylthiazole; 10 μg mL^{-1}), diluted with 400 mL water and subsequently extracted with two successive aliquots of dichloromethane (200 mL) in a 1 L separating funnel. The two dichloromethane extracts were combined and dried with anhydrous magnesium sulphate before the concentration step. The solvent was then decanted into a conical flask, which was heated in a water bath at 37 °C. Finally, DCM extracts were concentrated down to 1 mL under a stream of nitrogen and transferred to a glass vial ready for GC analysis.

b) Solid-phase extraction of aroma compounds: spirit samples (5 mL) were diluted with water (25 mL). An internal standard was added to the samples to achieve a final concentration of 10 mg/L of 2-acetylthiazole, and then shacked and allowed to equilibrate for a period of 4 h. LiChrolut EN SPE columns (Merck KGaA, Darmstadt, Germany; sorbent bed 500 mg) were placed on a SPE vacuum manifold, conditioned with 8 mL methanol and equilibrated with 8 mL aqueous ethanol (12% ABV). Spirit samples were loaded onto individual columns and allowed to fully saturate the sorbent bed for 1 min before vacuum was applied. Once the samples had been loaded, care was taken to not allow the bed to run dry until after the washing step, during which water (5 mL) was run through the cartridge.

The sorbent bed was dried by applying a vacuum (10 kPa) for 30 min. Aroma compounds were eluted from the cartridge into dichloromethane (6 mL). Each spirit sample was extracted in triplicate in a randomized order. Dichloromethane extracts were dried with anhydrous magnesium sulphate (Sigma Aldrich) and concentrated to a final volume of 1 mL under a stream of nitrogen prior to analysis.

2.3.4 Gas Chromatography analysis of spirit extracts

Aroma extracts from spirit samples were analysed by gas chromatography employing two methods of detection: i) mass spectrometry (MS): and simultaneous MS and ii) odour port evaluation using the technique of aroma extract dilution analysis (AEDA) (Poisson and Schieberle, 2008).

2.3.4.1 Gas chromatography-Mass Spectrometry (GC-MS)

Analysis was performed following the conditions used by Boothroyd et al. (2014) and included analysis of spirit extracts in dichloromethane using a ThermoScientific TraceGC Ultra with a DSQ II mass spectrometer and an AS 3000 Auto sampler (Thermo Electron Corporation). Compounds were separated on a Zebron ZB-WAX column (30 m × 0.25 mm i.d., 1.0 μm film thickness; Phenomenex, Macclesfield, UK) starting at an oven temperature of 40 °C (1 min hold) followed by a ramp to 250 °C at 4 °C min^{-1}. The helium carrier gas flow rate was 1.6 mL min^{-1} and injection (1 μL; temperature 240 °C) was splitless. The transfer line from the oven to the mass spectrometer was maintained at 250 °C. The mass spectrometer was operated in full scan mode over the range m/z 35–250. Identification and

quantitation of compounds was achieved using the Qual and Quan Browser applications of Xcalibur Software (Thermo Electron Corporation, Altrincham, Cheshire, UK). Identification was based upon: (a) EI-MS library matching; (b) measurement and confirmation against literature sources of the linear retention index (LRI) against alkanes (C8 to C22); and, when possible, (c) confirmation of the retention time of authentic standards run under identical chromatographic conditions. Quantification was achieved following normalization to the internal standard (10 μg mL^{-1}, 2-acetylthiazole) of six diluted solutions in the range of 0.05 to 5 μg mL^{-1} containing the minor compounds listed in Appendix 1. However, for major compounds such as isoamyl acetate, ethyl hexadecanoate, ethyl decanoate, and 2-phenylethanol six diluted solutions in the range 2 to 64 μg mL^{-1} were prepared. Calibration curves reported a correlation coefficient (R^2) greater than or equal to 0.99 for each compound. Furthermore, for those compounds that could not be quantified following internal standardization of the compounds listed in Appendix 1, the quantification was based upon: a) following normalization to the internal standard (10 μg mL^{-1} 2-acetylthiazole) and b) by using the calibration curve of the chemical compound with similar composition belonging to the same family of compounds.

2.3.5 Liquid Chromatography analysis of spirit samples

Non-volatile compounds which include gallic acid, vanillic acid, vanillin, syringic acid, syringaldehyde, coniferaldehyde, sinapaldehyde, ellagic acid and 5-hydroxymethyl furfural, were measured by HPLC using a Water Alliance 2695 and

996 PDA UV detector comprised the chromatographic system. The separations were done using a Grace C18 endcapped column (150 mm x 3 mm) with 3 μm spherical particles, which was preceded by a Security Guard cartridge guard system (Phenomenex, Macclesfield, UK) containing a C18 cartridge. The elution solvents used were: 2% v/v acetic acid in water (phase A) and 70% v/v methanol and 2% v/v acetic acid in water (phase B). The gradient program was as follows: 100% (A) from 0 to 6 min, then 60-40% (A, B) from 6 to 35 min, after that 40-60% (A, B) from 35 to 43 min, and finally 100% (B) from 43 to 50 min. Flow rate was 0.75 ml min^{-1} and run time was 60 min. The UV detector scanned the wavelengths of 260–350 nm. The run was performed at 45 °C. The injection volume of the sample was 10 μL. Identification of the compounds was achieved by comparing their retention time with those of the authentic compounds. Quantification was done using external standards (0.3-30 mg/L). The data was acquired and handled by Empower software. Each sample was assayed in triplicate.

2.3.6. Data treatment and statistical analysis

Chromatograms obtained from the Instrumental analysis were integrated and the area ratio of each compound against its internal standard recorded. Analysis of variance (ANOVA) and Tukey's test (HSD) tests were performed using Statgraphics plus software Version 16.1.11. Analysis of variance (ANOVA) and Tukey's test was carried out to establish which compound concentrations were significantly different among the samples according to both provenances (non-mature v mature) and extraction method (LLE v SPE). Finally, Principal component

analysis (PCA) and Partial Least Squares Regression (PLS) were carried out using Simca software – P7.01. PCA was performed to depict variability in the compound concentration data set as related to the sample provenance and the extraction technique used. PLS analysis was performed to investigate the relationship between aroma compounds (X) and ageing period (Y).

2.4. RESULTS AND DISCUSSION

2.4.1 Analysis of volatile compounds in aroma extracts of non-mature and mature spirit samples

The isolation of volatile compounds and chemical characterization of spirit samples (mature and non-mature) was performed using the previous extractions techniques (LLE and SPE) and GC-MS conditions used by Boothroyd et al. (2013).

The aroma compounds that were identified and quantified in the aroma extracts of the spirit samples are present in Table 2.1. Sixty compounds were identified in the extracts by LLE and SPE. The compounds were drawn from a variety of chemical classes including acetals, acids, alcohols, esters, furans, ketones, phenols and terpenes. These compound classes have been reported previously as important contributors of Spirits flavour such as whisky and tequila (Benn and Peppard, 1996; Caldeira et al. 2007). There were significant differences in the concentrations of all quantified compounds between the non-mature and mature spirit samples extracted either by LLE and SPE ($P < 0.05$). Compounds that belong to acetals, acids, furans, oak-lactones, terpenes, and volatile-phenols were significantly increased in the

mature spirits, mainly because of the strong influence of the maturation process (Tables 2.1 and 2.2)

Wood-derived compounds (oak lactones/whisky lactones) and volatile polyphenols (such as eugenol, guaiacol, 4-ethyl guaiacol, and vanillin) were volatile markers of maturation, identified only in the mature spirits (Table 2.1). These compounds are strong indicators of oak maturation, which influence the taste and aroma of maturing spirits (Mosedale and Puech, 1998). Particularly important are the sensory effects caused by acids, aldehydes, and phenolic compounds including, whisky lactones, eugenol, and vanillin (Chatonnet et al. 1990; Conner et al. 1993; Mosedale and Puech, 1998). Some of these are used as markers or ageing indicators, since their quantification during the ageing process can be used to estimate the time required to age a distilled beverage (De Aquino et al. 2006). Lignin hydrolysis is the major chemical process which occurs, and it is through this that several phenolic compounds are extracted. Oxidation of these compounds yields aldehydes, acids, vanillin, and syringaldehyde (Martinez et al. 1996). Furanic aldehydes are also important contributors of the ageing character; however, other conditions affect their concentrations such that they cannot be taken as ageing markers (Schieberle et al. 1995). Their presence has been attributed to physicochemical reactions that arise during maturation, including the extraction of wood components, evaporation of volatile compounds and interactions between wood and distillate components (Nishimura and Matsuyama, 1989; Mosedale and Puech, 1998).

Table 2.1. Concentrations (mg/L[a]) of aroma compounds determined in aroma extracts of spirit samples of new make spirits and malt whiskies A and B

Compound	*LRI*	ID	NMSA	MWA	NMS B	MWB	NMSA	MWA	NMS B	MWB
			LIQ-LIQ EXT (LLE)				SOLID-PHASE EXTRACTION (SPE)			
Isovaleraldehyde diethyl acetal	1083	B	ND	ND	ND	ND	ND	ND	ND	ND
Isobutanal diethyl acetal	1274	B	ND	0.71±0.01	ND	0.52±0.009	ND	0.83±0.21	ND	0.612±0.08
β-Ethoxypropionaldehyde diethyl acetal	1321	B	0.051±0.003	0.25±0.02	0.12±0.08	0.29±0.03	0.13±0.01	0.21±0.007	0.045±0.00	0.311±0.03
Σ ACETALS[£]			**0.051±0.00**	**0.97±0.03**	**0.12±0.08**	**0.81±0.04**	**0.13±0.01**	**1.04±0.22**	**0.05±0.003**	**0.92±0.102**
Acetic acid	1504	A, B	ND	ND	ND	ND	ND	ND	ND	ND
Hexanoic acid	1883	A, B	3.35±0.28	3.14±0.39	5.52±0.95	7.53±1.00	0.29±0.005	3.89±0.57	0.3±0.008	6.66±0.94
Octanoic acid	2088	A, B	19.18±0.65	18.1±2.41	23.9±1.79	33.56±2.05	1.25±0.13	19.45±1.02	0.22±0.024	31.1±2.35
Decanoic acid	2268	A, B	17.95±0.38	22.9±1.95	22.47±1.17	29.58±2.42	1.66±0.30	23.72±2.2	0.36±0.006	30.64±2.86
Dodecanoic acid	2437	A, B	5.80±0.33	4.50±10.6	3.58±0.44	4.59±0.51	0.45±0.04	8.17±0.64	0.18±0.02	7.60±0.65
Σ ACIDS[£*]			**46.3±1.64**	**48.7±5.30**	**55.5±4.34**	**75.3±5.98**	**3.64±0.47**	**55.2±4.42**	**1.05±0.06**	**76.0±6.80**
n-Propanol	1037	A, B	10.2±1.05	7.86±0.32	9.5±0.17	4.89±0.24	3.66±0.08	3.75±0.35	3.65±0.05	2.92±0.25
Isobutanol	1102	A, B	85.6±8.42	69.6±3.35	88.0±1.09	51.9±1.79	86.7±3.1	81.8±13.7	78.4±2.96	69.0±9.17
n-Butanol	1158	B	2.10±0.17	2.62±0.12	1.99±0.31	1.71±0.05	2.15±0.11	3.26±0.42	2.07±0.13	2.41±0.25
3-Penten-2-ol	1190	B	ND	ND	ND	ND	ND	ND	ND	ND
Isoamyl alcohol	1226	A, B	609±20.2	571±23.8	569±8.72	486±2.55	619±25.1	667±9.73	673±22.5	604±12.9
n-Pentanol	1268	B	0.53±0.046	0.54±0.01	0.66±0.02	0.45±0.01	0.70±0.03	0.64±0.09	0.53±0.05	0.57±0.04
n-Hexanol	1370	A, B	3.39±0.28	5.51±0.24	7.56±0.13	3.57±0.03	6.96±0.1	5.67±0.33	3.21±0.14	3.79±0.001
3-Ethoxy-1-propanol	1397	B	0.41±0.05	0.28±0.03	0.35±0.006	0.22±0.02	0.21±0.01	0.21±0.03	0.26±0.04	0.18±0.01
n-Heptanol	1486	A, B	ND	ND	ND	ND	ND	ND	ND	ND
3-Ethyl-4-methyl-1-pentanol	1529	B	ND	ND	ND	ND	ND	ND	ND	ND
Methionol	1747	B	0.19±0.02	ND	0.22±0.01	ND	0.068±0.00	ND	0.04±0.00	ND
n-Decanol	1779	B	0.31±0.03	1.06±0.03	0.35±0.03	0.16±0.002	0.34±0.04	1.08±0.04	0.30±0.03	0.19±0.02
4-Dodecenol	1813	B	ND	ND	ND	ND	ND	ND	ND	ND
Phenethyl alcohol	1949	A, B	69.4±6.05	68.0±1.48	61.3±0.42	39.7±1.47	59.0±1.00	68.9±4.24	68.4±2.51	39.5±1.38
n-Undecanol	1984	B	0.23±0.03	0.34±0.06	0.15±0.015	0.15±0.02	0.27±0.02	0.63±0.02	0.29±0.02	0.20±0.02
n-Hexadecanol	2169	B	1.26±0.19	1.06±0.05	0.41±0.06	0.61±0.07	1.81±0.08	4.40±0.29	1.95±0.16	1.66±0.03
(9E)-9-Hexadecen-1-ol	2341	B	2.77±0.38	1.23±0.24	1.23±0.08	0.68±0.02	1.39±0.05	2.11±0.05	0.96±0.02	0.77±0.04
Σ ALCOHOLS[£]			**785.3±36.9**	**729±29.7**	**741±10.7**	**589±6.26**	**782±29.7**	**840±29.3**	**833±28.6**	**725±24.1**
Benzaldehyde	1561	A, B	0.35±0.03	0.16±0.00	0.33±0.01	0.17±0.00	0.34±0.01	0.170±0.01	0.37±0.00	0.18±0.01
Σ ALDEHYDES[£]			**0.35±0.026**	**0.16±0.00**	**0.33±0.01**	**0.17±0.00**	**0.34±0.01**	**0.170±0.01**	**0.37±0.00**	**0.18±0.01**
Isoamyl acetate	1138	A, B	7.25±0.55	6.57±0.23	8.03±0.08	4.45±0.14	8.06±0.30	6.96±0.97	6.82±0.37	5.12±0.74
Ethyl hexanoate	1253	A, B	1.22±0.14	1.15±0.01	1.28±0.03	1.79±0.07	1.29±0.04	1.16±0.13	1.11±0.03	1.96±0.24
Ethyl lactate	1367	A, B	5.19±0.63	5.88±0.28	34.0±0.27	4.48±0.11	24.7±0.52	5.03±0.52	3.67±0.25	3.97±0.16

Table 2.1. Concentrations (mg/L[a]) of aroma compounds determined in aroma extracts of spirit samples of new make spirits and malt whiskies A and B (cont.).

Ethyl octanoate	1452	A, B	13.8±2.64	10.9±0.54	12.1±0.06	18.4±0.88	18.1±0.53	15.0±0.33	15.3±0.54	24.2±0.75
Ethyl nonanoate	1555	A, B	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl decanoate	1655	A, B	69.0±8.31	46.9±6.9	44.1±2.11	59.7±3.46	188.1±2.12	177.5±9.55	165.9±1.27	200.5±3.10
Diethyl succinate	1698	A, B	0.19±0.00	0.30±0.01	0.19±0.003	0.12±0.002	0.183±0.00	0.29±0.01	0.18±0.01	0.13±0.01
Ethyl benzoate	1702	A, B	1.45±0.063	1.13±0.03	1.18±0.09	1.08±0.04	1.19±0.04	1.14±0.05	1.42±0.02	1.16±0.05
Phenethyl acetate	1851	A, B	6.51±0.31	8.08±0.22	8.13±0.06	4.14±0.09	7.97±0.16	7.81±0.38	6.70±0.17	4.16±0.16
Ethyl dodecanoate	1860	B	25.3±3.98	11.2±1.93	11.0±0.48	10.4±1.62	47.8±0.80	49.0±4.52	35.1±0.46	37.1±4.24
Isoamyl decanoate	1877	B	0.54±0.02	0.26±0.03	0.33±0.02	0.22±0.04	0.96±0.03	1.11±0.12	0.61±0.02	0.64±0.09
Ethyl tetradecanoate	2059	B	3.05±0.27	1.54±0.12	0.99±0.07	0.76±0.05	1.09±0.04	2.63±0.45	0.93±0.05	1.14±0.08
Ethyl 4-ethoxybenzoate	2188	B	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl hexadecanoate	2238	A, B	14.7±0.67	12.1±0.76	12.9±1.32	5.22±0.32	2.77±0.11	3.96±0.38	1.1±0.06	1.71±0.15
Ethyl 9-hexadecenoate	2263	A, B	8.83±0.42	3.27±0.66	4.29±0.4	3.25±0.30	3.02±0.13	3.38±0.5	1.32±0.02	3.60±0.30
Methyl oleate	2397	B	ND	ND	ND	ND	ND	ND	ND	ND
Σ ESTERS[£*]			157±18.0	109±11.7	138±4.97	114±7.11	305.2±4.81	275±17.91	240±3.26	286±10.05
Dihydro-2-methyl-3(2H)-furanone	1291	B	ND	ND	ND	ND	ND	ND	ND	ND
Furfural	1494	A, B	9.51±2.15	12.5±0.48	19.9±0.35	9.56±0.28	20.4±0.48	13.6±0.80	10.3±0.34	10.3±0.13
2-Acetyl furan	1539	B	0.20±13.6	0.15±0.01	0.58±0.01	0.15±0.02	0.55±0.02	0.16±0.02	0.18±0.01	0.15±0.01
5 Methyl Furfural	1605	A, B	0.20±6.55	0.41±0.02	0.39±0.03	0.30±0.03	0.35±0.02	0.41±0.00	0.18±0.01	0.30±0.01
Σ FURANS[£]			9.90±0.24	13.1±0.51	20.8±0.34	10.0±0.33	21.3±0.52	14.1±0.81	10.6±0.35	10.8±0.15
Diacetyl	993	B	ND	ND	ND	ND	ND	ND	ND	ND
β-Damascenone	1854	A, B	ND	ND	ND	ND	ND	ND	ND	ND
Benzophenone	2456	B	ND	ND	ND	ND	ND	ND	ND	ND
Σ KETONES[£*]			ND	ND	ND	ND	ND	ND	ND	ND
Trans-whisky lactone	1929	A, B	ND	0.25±0.00	ND	0.25±2.17	ND	0.26±0.01	ND	0.25±0.01
Cis-whisky lactone	2002	A, B	ND	2.02±0.03	ND	1.70±4.32	ND	2.15±0.23	ND	1.69±0.03
γ-nonalactone	2084	B	ND	ND	ND	ND	ND	ND	ND	ND
Σ LACTONES[£]			ND	2.27±0.04	ND	1.94±0.08	ND	2.41±0.24	ND	1.94±0.04
Cis Linalool oxide	1465	B	ND	ND	ND	ND	ND	ND	ND	ND
Linalool	1566	B	ND	ND	ND	ND	ND	ND	ND	ND
α-Terpineol	1724	B	ND	ND	ND	ND	ND	ND	ND	ND
Citronellol	1786	B	ND	ND	ND	ND	ND	ND	ND	ND
Σ TERPENES[£*]			ND	ND	ND	ND	ND	ND	ND	ND
Vanillin	2517	A, B	0.04±0.00	2.09±0.01	0.03±0.00	1.91±0.00	0.013±0.00	2.15±4.21	0.03±0.00	2.06±0.07
Guaiacol	1898	A, B	ND	0.14±0.01	ND	0.14±0.00	ND	0.136±0.14	ND	0.14±0.00
4 Ethyl guaiacol	2064	A, B	ND	0.19±0.00	ND	0.18±0.00	ND	0.198±1.60	ND	0.18±0.00
Eugenol	2187	A, B	ND	0.26±0.05	ND	0.23±0.06	ND	0.26±7.48	ND	0.22±0.00
Σ VOLATILE PHENOLS[£]			0.041±4.30	2.67±0.06	0.03±0.00	2.45±0.07	0.01±0.00	2.75±0.10	0.03±0.00	2.60±0.07

[a]: Quanfication was achived following normalization to the internal standard (10 μg mL^{-1}, 2-acetylthiazole) using multilevel calibration curves (Appendix 1)
LRI: Experimental Linear Retention Index.
LLE: Liquid-Liquid Extraction; SPE: Solid Phase Extraction;
ND: not detected under the conditions of analysis. NA: information not available in the literature.
A, B: Compounds were identified by EI-MS library matching (NIST), comparison against authentic standards and confirmation of their linear retention index (LRI) against published values for a DB-Wax column. http://www.pherobase.com/database/kovats/kovats-index.php
B: Compounds were identified by EI-MS library matching (NIST), and confirmation of their linear retention index (LRI) against published values for a DB-Wax column (http://www.pherobase.com/database/kovats/kovats-index.php)
Data represent the average of three independent extractions ± standard deviation.
£ Indicates statistically significant difference between results for the samples in the aroma compounds groped by chemical group extracted by LLE and SPE (P<0.05).
* Indicates statistically significant difference between results for the same sample in each of the chemical group extracted by LLE and SPE (P<0.05).

Table 2.2. Concentrations (mg/L[a]) of aroma compounds determined in aroma extracts of spirit samples of non-mature and mature Bourbon and Tequila

Compound	*LRI*	ID	NMB	MB	NMTEQ	MTEQ	NMB	MB	NMTEQ	MTEQ
			LIQ-LIQ EXTRACTION (LLE)				SOLID PHASE EXTRACTION (SPE)			
Isovaleraldehyde diethyl acetal	1083	B	ND	ND	0.32±0.03	0.12±0.02	ND	ND	0.23±0.02	ND
Isobutanal diethyl acetal	1274	B	ND	0.52±0.01	ND	2.75±0.03	ND	0.47±0.03	ND	2.86±0.01
β-Ethoxypropionaldehyde diethyl acetal	1321	B	ND	ND	ND	2.38±0.11	ND	ND	ND	2.43±0.01
Σ ACETALS[£]			**ND**	**0.52±0.01**	**0.32±0.03**	**5.25±0.24**	**ND**	**0.47±0.03**	**0.23±0.02**	**5.29±0.02**
Acetic acid	1504	A, B	0.07±0.009	7.00±1.22	ND	ND	0.28±0.072	0.19±0.06	ND	ND
Hexanoic acid	1883	A, B	0.90±0.01	2.29±0.21	ND	ND	0.39±0.1	1.13±0.13	ND	ND
Octanoic acid	2088	A, B	0.63±0.09	2.70±0.24	0.27±0.02	1.84±0.35	ND	1.57±0.36	ND	0.57±0.08
Decanoic acid	2268	A, B	0.88±0.26	6.23±0.48	0.70±0.13	6.14±1.45	ND	4.04±0.92	ND	2.07±0.22
Dodecanoic acid	2437	A, B	ND	ND	ND	ND	ND	ND	ND	ND
Σ ACIDS[£*]			**2.48±0.37**	**18.2±2.16**	**0.97±0.15**	**7.98±1.80**	**0.66±0.171**	**6.92±1.47**	**ND**	**2.64±0.30**
n-Propanol	1037	A, B	28.4±1.76	8.95±1.98	9.02±2.18	17.1±4.65	15.5±1.97	15.62±1.06	3.93±0.87	6.91±1.28
Isobutanol	1102	A, B	396.7±25.06	374.2±28.5	119.9±21.3	169.2±33.6	383.3±9.66	360.5±25.14	150.8±14.6	190.8±14.5
n-Butanol	1158	B	ND	ND	0.42±0.09	1.41±0.24	ND	ND	0.52±0.04	1.51±0.08
3-Penten-2-ol	1190	B	6.94±0.35	0.39±0.07	ND	ND	0.24±0.03	0.151±0.04	ND	ND
Isoamyl alcohol	1226	A, B	783±13.4	833±16.3	381±4.38	801±12.6	769±27.6	852±17.3	431±12.7	796±24.4
n-Pentanol	1268	B	0.14±0.01	7.64±0.01	ND	ND	1.81±0.22	1.92±0.19	ND	ND
n-Hexanol	1370	A, B	4.29±0.18	3.47±0.10	ND	ND	4.23±0.17	3.47±0.17	ND	ND
3-Ethoxy-1-propanol	1397	B	ND	ND	ND	ND	ND	ND	ND	ND
n-Heptanol	1486	A, B	0.02±0.002	0.027±0.005	ND	ND	0.03±0.03	0.40±0.04	ND	ND
3-Ethyl-4-methyl-1-pentanol	1529	B	ND	ND	0.89±0.01	2.28±0.21	ND	ND	0.91±0.05	2.15±0.04
Methionol	1747	B	ND	ND	ND	ND	ND	ND	ND	ND
n-Decanol	1779	B	ND	ND	ND	ND	ND	ND	ND	ND
4-Dodecenol	1813	B	0.81±0.02	0.72±0.09	ND	ND	0.98±0.09	0.75±0.13	ND	ND
Phenethyl alcohol	1949	A, B	15.2±0.46	20.3±0.19	1.22±0.05	1.61±0.32	15.6±0.20	20.8±0.64	1.10±0.03	1.75±0.03

Table 2.2. Concentrations (mg/L[a]) of aroma compounds determined in aroma extracts of spirit samples of non-mature and mature Bourbon and Tequila (cont.)

n-Undecanol	1984	B	ND	ND	ND	ND	ND	ND	ND	ND
n-Hexadecanol	2169	B	ND	ND	ND	ND	ND	ND	ND	ND
(9E)-9-Hexadecen-1-ol	2341	B	ND	ND	ND	ND	ND	ND	ND	ND
Σ ALCOHOLS[£]			**1235±41.24**	**1241±47.27**	**513±27.9**	**993±51.7**	**1191±40.0**	**1263±46.2**	**589±28.3**	**999.4±40.4**
Benzaldehyde	1561	A, B	0.02±0.00	0.03±0.01	ND	ND	0.03±0.007	0.04±0.01	ND	ND
Σ ALDEHYDES[£]			0.02±0.003	**0.03±0.01**	ND	**ND**	**0.03±0.007**	**0.039±0.01**	**ND**	**ND**
Isoamyl acetate	1138	A, B	41.2±1.07	26.8±1.29	2.40±0.29	3.13±0.21	39.3±1.46	27.9±1.38	2.38±0.04	2.65±0.14
Ethyl hexanoate	1253	A, B	2.22±0.06	2.27±0.05	0.09±0.01	0.25±0.02	2.15±0.05	2.28±0.07	0.06±0.02	0.23±0.01
Ethyl lactate	1367	A, B	5.67±0.23	5.07±0.16	1.36±0.09	1.97±0.22	4.78±0.07	3.93±0.19	1.21±0.03	1.77±0.04
Ethyl octanoate	1452	A, B	11.7±0.78	8.57±0.17	4.83±0.49	8.32±1.34	11.7±0.55	8.52±0.16	5.35±0.41	7.85±0.24
Ethyl nonanoate	1555	A, B	0.07±0.01	0.08±0.003	ND	ND	0.09±0.01	0.11±0.02	ND	ND
Ethyl decanoate	1655	A, B	16.4±1.38	13.5±0.61	2.06±0.13	6.32±0.80	25.6±0.34	20.5±0.53	1.35±9.01	10.11±0.16
Diethyl succinate	1698	A, B	0.81±0.02	0.92±0.01	0.10±0.01	0.41±0.06	0.85±0.04	0.99±0.11	0.08±13.96	0.42±0.01
Ethyl benzoate	1702	A, B	0.04±0.02	0.026±0.00	0.01±0.00	0.012±0.00	0.04±0.00	0.03±0.01	<0.01±0.00	0.01±0.00
Phenethyl acetate	1851	A, B	1.42±0.03	0.60±0.01	0.36±0.02	0.15±0.01	1.45±0.033	0.64±0.02	0.30±0.02	0.16±0.003
Ethyl dodecanoate	1860	B	6.48±0.6	3.57±0.53	0.42±0.04	0.68±0.12	10.9±0.53	13.6±0.32	0.42±0.05	4.73±0.19
Isoamyl decanoate	1877	B	ND	ND	ND	ND	ND	ND	ND	ND
Ethyl tetradecanoate	2059	B	1.31±0.29	0.58±0.03	ND	0.02±0.01	0.35±0.05	1.70±0.22	ND	0.16±0.01
Ethyl 4-ethoxybenzoate	2188	B	0.02±0.01	0.01±0.00	0.66±0.10	0.07±0.01	ND	0.01±0.00	0.02±0.00	0.02±0.00
Ethyl hexadecanoate	2238	A, B	19.8±1.86	6.52±0.72	0.03±0.00	0.08±0.01	1.27±0.06	3.63±0.48	ND	0.12±0.01
Ethyl 9-hexadecenoate	2263	A, B	ND	ND	ND	ND	ND	ND	ND	ND
Methyl oleate	2397	B	ND	0.20±0.008	ND	ND	0.09±0.02	0.40±0.09	ND	ND
Σ ESTERS[£*]			**107±6.35**	**68.6±3.59**	**12.3±1.19**	**21.4±2.81**	**98.5±3.21**	**84.3±3.61**	**11.2±0.71**	**28.2±0.81**
Dihydro-2-methyl-3(2H)-furanone	1291	B	ND	ND	0.79±0.08	1.50±0.10	ND	ND	0.75±0.07	1.44±0.02
Furfural	1494	A, B	0.05±0.00	4.73±0.17	0.74±0.05	2.29±0.12	0.07±0.01	5.19±0.37	0.77±0.01	2.24±0.04
2-Acetyl furan	1539	B	ND	ND	0.25±0.01	0.75±0.05	ND	ND	0.23±0.03	0.77±0.01
5 Methyl Furfural	1605	A, B	0.01±0.00	0.26±0.01	0.40±0.01	0.61±0.06	0.01±0.00	0.29±0.03	0.35±0.05	0.65±0.01
Σ FURANS[£]			**0.06±0.00**	**4.99±0.18**	**2.18±0.15**	**5.15±0.32**	**0.08±0.01**	**5.47±0.39**	2.10±0.15	**5.10±0.08**
Diacetyl	993	B	ND	ND	0.75±0.22	0.15±0.02	ND	ND	0.14±0.03	0.15±13.4
β-Damascenone	1854	A, B	ND	ND	0.33±0.09	0.03±0.00	ND	ND	0.03±0.01	0.07±9.79
Benzophenone	2456	B	ND	ND	ND	ND	0.31±0.01	0.26±0.04	ND	ND
Σ KETONES[£*]			**ND**	**ND**	**1.08±0.31**	**0.18±0.03**	**0.31±0.01**	**0.26±0.04**	**0.17±0.04**	**0.22±0.03**
Trans-whisky lactone	1929	A, B	1.02±0.14	2.96±0.48	ND	0.31±0.08	0.22±0.01	1.19±0.21	ND	0.33±0.01
Cis-whisky lactone	2002	A, B	ND	8.67±0.24	ND	1.50±0.36	ND	9.66±0.57	ND	1.66±0.01
γ-nonalactone	2084	B	0.02±0.001	0.032±0.001	ND	ND	0.073±0.01	0.17±0.03	ND	ND
Σ LACTONES[£]			**1.04±0.15**	**11.8±0.72**	**ND**	**1.81±0.43**	0.29±0.02	**11.0±0.81**	**ND**	**1.98±0.02**
Cis Linalool oxide	1465	B	ND	ND	0.36±0.01	0.84±0.011	ND	ND	0.30±3.50	0.79±1.80

Table 2.2. Concentrations (mg/L[a]) of aroma compounds determined in aroma extracts of spirit samples of non-mature and mature Bourbon and Tequila (cont.)

Linalool	1566	B	ND	ND	0.41±0.01	1.46±0.04	ND	ND	ND	ND
α-Terpineol	1724	B	ND	ND	2.44±0.16	6.28±0.4	ND	ND	2.20±5.26	6.48±0.06
Citronellol	1786	B	ND	ND	0.26±0.04	0.53±0.01	ND	ND	0.23±8.42	0.48±0.04
Σ TERPENES[£*]			**ND**	**ND**	**3.48±0.23**	**9.11±0.46**	**ND**	**ND**	3.07±0.17	**9.18±0.13**
Vanillin	2517	A, B	0.02±0.00	2.68±0.08	ND	0.88±0.04	ND	2.68±0.09	ND	0.77±0.01
Guaiacol	1898	A, B	ND	0.04±0.00	ND	0.01±0.00	0.01±0.00	0.04±0.01	ND	0.011±0.00
4 Ethyl guaiacol	2064	A, B	ND	0.02±0.00	ND	ND	ND	0.02±0.006	ND	ND
Eugenol	2187	A, B	ND	0.02±0.004	ND	0.02±0.04	ND	0.36±0.09	ND	0.07±0.02
Σ VOLATILE PHENOLS[£]			**0.02±0.00**	**2.76±0.09**	**ND**	**0.91±0.04**	**0.01±0.00**	**3.11±0.19**	**ND**	**0.85±0.03**

[a]: Quanfication was achived following normalization to the internal standard (10 μg mL^{-1}, 2-acetylthiazole) using multilevel calibration curves (Appendix 1)
LRI: Experimental Linear Retention Index.
LLE: Liquid-Liquid Extraction; SPE: Solid Phase Extraction;
ND: not detected under the conditions of analysis. NA: information not available in the literature.
A, B: Compounds were identified by EI-MS library matching (NIST), comparison against authentic standards and confirmation of their linear retention index (LRI) against published values for a DB-Wax column. http://www.pherobase.com/database/kovats/kovats-index.php
B: Compounds were identified by EI-MS library matching (NIST), and confirmation of their linear retention index (LRI) against published values for a DB-Wax column (http://www.pherobase.com/database/kovats/kovats-index.php)
Data represent the average of three independent extractions ± standard deviation.
£ Indicates statistically significant difference between results for the samples in the aroma compounds groped by chemical group extracted by LLE and SPE (P<0.05).
* Indicates statistically significant difference between results for the same sample in each of the chemical group extracted by LLE and SPE (P<0.05).

The presence of terpene compounds such as α-terpineol, linalool and citronellol is characteristic of tequila. Concentrations of these compounds were greatly increased through maturation (Table 2.1). The concentrations of terpene compounds are determined both biochemically (via raw materials and fermentation) and chemically (through distillation and ageing) (Marais, 1986; Peña-Alvarez et al. 2006; Prado-Jaramillo et al. 2015). In wine, terpene compounds from grapes have been reported to be sensitive to acidic conditions and to increase with maturation temperature and storage time (Marais, 1986).

Some acetals have been reported to appear after fermentation and others after distillation where they are concentrated (Kłosowski and Czupryński, 2006). Their formation in spirits depends on the raw material and normally is by addition of an alcohol to the carbonyl group of an aldehyde (Kłosowski and Czupryński, 2006). Isobutanal diethyl acetal and β-ethoxypropionaldehyde diethyl acetal were two of the acetals, which were only detected or increased in concentration in the mature spirits, and were therefore affected by the maturation process (Tables 2.1 and 2.2).

Furthermore, Tables 2.1 and 2.2 summarise the analysed concentrations of volatile compounds in the aroma extracts by chemical class. Extracts of mature spirits in general contained higher concentrations of the majority of volatile compounds detected, as compared to the corresponding extracts of non-mature spirits (Tables 2.1 and 2.2). The most abundant classes of aroma compounds analysed were alcohols and esters (Table 2.1). Isobutanol, isoamyl alcohol and phenethyl alcohol were among the higher alcohols presented in higher concentration in the new make

spirit samples. Ethyl octanoate, ethyl decanoate and ethyl dodecanoate were the esters present in the highest concentrations among the aroma extracts (Tables 2.1 and 2.2). Interestingly, a considerable increase in alcohols and esters concentrations were detected in the mature tequila as comparison with its non-mature version, however for the rest of the samples different concentrations were observed, especially when comparing among non-mature and mature spirits of the same batch (Tables 2.1 and 2.2).

Additionally, esters are produced by yeast during fermentation by condensation between Acyl-CoA catalysed by intracellular enzymes, normally they confer a pleasant 'fruity-notes' to alcoholic beverages (Swiegers et al. 2005). Nevertheless, the increase in ester content observed in the extracts of mature tequilas could be due to esterification reactions that arise during the maturation process (Reazin, 1981). Particularly important is the presence of ethyl acetate, which has been associated to increase its concentration along other major congeners during the maturation process (Reazin, 1981), however at this stage its concentration was not estimated mainly because of loses during the extraction process. As well as esters, higher alcohols are secondary yeast metabolites, and their presence has been associated to highly influence the aroma and flavour of alcoholic beverages (Swiegers et al. 2005). They confer a strong pungent taste and odour to alcoholic drinks. At concentrations less than 300 mg/L, they contribute to desired complexity but if they are present in concentrations greater than 400 mg/L they may confer negative attributes to spirit aroma (Díaz-Montaño et al. 2008). The concentration of higher alcohols depends on several factors, including the type of yeast strain, fermentation

temperature, pH, and amino acid composition of the culture medium (Swiegers et al. 2005).

Overall (Tables 2.2 and 2.3), analytical data for the various chemical classes were quite similar across the two extraction techniques used. However, the asterisked compound groups in Table 2.3 (acids, esters and ketones) are those for which the method of extraction caused a significant difference in recovery from the same sample. For organic acids it is apparent that LLE was a superior method of extraction, recovering greater amounts of these compounds. The acids are secondary yeast metabolites, which can have both negative and positive impacts on aroma and flavour, depending on their concentration in the final spirit (Swiegers et al. 2005).

In the case of esters and ketones, SPE was the superior method of extraction with higher amounts recovered in the aroma extracts of the spirit samples (Tables 2.1 and 2.2).

2.4.1.2 Principal component analysis (PCA) of compound concentration data

PCA was conducted on the analytical data for mature and non-mature spirit samples extracted by both SPE and LLE techniques. A bi-plot for PC1 and PC2 (Figure 2.1) accounted for over 59.3% of variation in the data set. Furthermore, PC1, which accounted for the majority of the variation, represented the separation between spirit samples, which were mainly separated by their provenance in three regions. Non-mature and mature bourbons were positioned in the second region of the analysis,

while non-mature and mature tequilas were found in the third region and new make spirits and malt whiskies from both distilleries (A & B) in the fourth region of PCA plot (Figure 2.1). The concentrations of ethyl benzoate, phenethyl acetate, 3-ethyl-1-propanol, isoamyl acetate, (9E)-9-Hexadecen-1-ol were negatively correlated with PC1, indicating that these compounds were more prevalent in the new make spirits and malt whiskies. Positive correlated with PC1 were cis-linalool oxide, citronellol, dihydro-2-methyl-3(2H)-furanone and 5-methyl furfural, which were in overall mostly associated with non-mature and mature tequilas. PC2 was driven by differences in concentration due to non-mature and mature bourbon composition. Positive correlated to PC2 were; 4-dodecanol, isoamyl alcohol, γ-nonalactone, ethyl nonanoate, and 1-heptanol respectively (Figure 2.1).

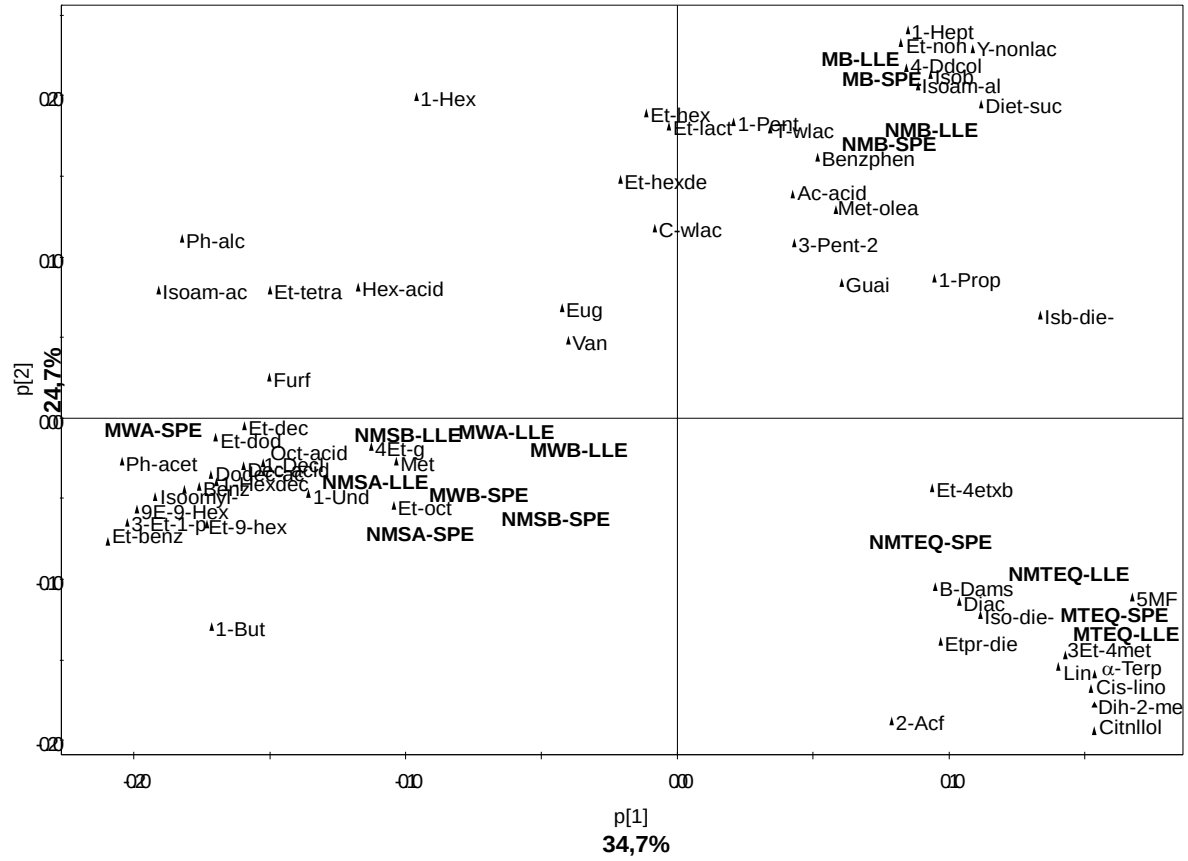

Figure. 2.1. Principal Component analysis (PCA) bi-plot of the analytical data for volatile compound concentrations, with the spirit samples overlaid (bourbon, tequila and malt whiskies).

Figure. 2.1. Principal Component analysis (PCA) bi-plot of the analytical data for volatile compound concentrations, with the spirit samples overlaid (bourbon, tequila and malt whiskies) (cont.).

Key: diac: diacetyl; 1-prop: 1-propanol; iso-die: isovaleraldehyde diethyl acetal; isob: isobutanol; isoam-ace: isoamyl acetate; 1-but: 1-butanol; 3-pent-2: 3-Penten-2-ol; isoam-alc: isoamyl alcohol; et-hex: ethyl hexanoate; 1-pent: 1-pentanol; isb-die: isobutanal diethyl acetal; dih-2-met: dihydro-2-methyl-3 (2H)-furanone; etprop-die: β-ethoxypropionaldehyde diethyl acetal; et-lact: ethyl lactate; 1-hex:1-hexanol; 3-et-1-prop: 3-ethoxy-1-propanol; et-oct: ethyl octanoate; cis-linoxd: cis-linalool oxide. 1-hept: 1-heptanol; furf: furfural; ac-acid: acetic acid; 3Et-4met-1pet: 3-ethyl-4-methyl-1-pentanol. 2-acf: 2-acetylfuran; et-non: ethyl nonanoate; benz: benzaldehyde; lin: linalool; 5MF: 5-methyl furfural; et-dec: ethyl decanoate; diet-suc: diethyl succinate; et-benz: ethyl benzoate; α-terp: α-terpineol; met: methionol; 1-decl: 1-decanol; citnllol: citronellol; 4-ddcol: 4-dodecenol; ph-acet: phenethyl acetate; β-dam: β-damascenone; et-dod: ethyl dodecanoate; isoamyl-dec: isoamyl decanoate; hex-acid: Hexanoic acid; guai: guaiacol; t-wlac: trans-whisky lactone; ph-alc: phenylethyl alcohol; 1-und: 1-undecanol; c-wlac: cis-whiskey lactone; et-tetradec: ethyl tetradecanoate; 4et-g: 4-ethylguaiacol; y-nonlac: y-nonalactone; oc-acid: octanoic acid; et-4etxbenz: ethyl 4-ethoxybenzoate; 1-hexdec: 1-hexadecanol; eug: eugenol, et-hexdec: ethyl hexadecanoate; et-9-hexdec: ethyl 9-hexadecenoate; dec-acid: decanoic acid; 9e-9-hex: (9E)-9-hexadecen-1-ol; met-olea: methyl oleate; dodec-ac: dodecanoic acid; benzphen: benzophenone and van:vanillin. NMSA-LLE: extract of new make spirit from distillery 'A' obtained by LLE; NMSA-SPE: extract of new make spirit from distillery 'A' obtained by SPE; NMSB-LLE: extract of new make spirit from distillery 'B' obtained by LLE; NMSB-SPE: extract of new make spirit from distillery 'B' obtained by SPE; MWA-LLE: extract of malt whisky from distillery 'A' obtained by LLE; MWA-SPE: extract of malt whisky from distillery 'A' obtained by SPE; MWB-LLE: extract of malt whisky from distillery 'B' obtained by LLE; MWB-SPE: extract of malt whisky from distillery 'B' obtained by SPE; NMSB-LLE: extract of non-mature bourbon obtained by LLE; NMSB-SPE: extract of non-mature bourbon obtained by SPE; MSB-LLE: extract of mature bourbon obtained by LLE; MB-SPE: extract of mature bourbon obtained by SPE; NMTEQ-LLE: extract of non-mature tequila obtained by LLE; NMTEQ-SPE: extract of non-mature tequila obtained by SPE; MTEQ-LLE: extract of mature tequila obtained by LLE; MTEQ-SPE: extract of mature tequila obtained by SPE.

The fact that the majority of the compounds no matter the extraction process or the spirit type (non-mature or mature) are located in separated groups in the PCA plot indicates that the origin or provenance of the samples is the main factor that has the highest significant effect during PCA analysis. However, based on the results of Table 2.1, LLE was better in terms of the extraction of a broad cross-section of volatile compounds, SPE can be a useful complementary technique for the analysis of certain compounds. Both the SPE phase and extraction protocol employed were based on earlier studies by Boothroyd et al. 2013 in malt whisky and further optimisation for tequila or bourbon was not carried out. Therefore, by choosing the appropriate SPE column and optimizing the conditions to suit the elution of the groups of analytes required, SPE can be a successful method of extraction, especially for the recovery of semi-volatiles (Boothroyd et al. 2013).

2.4.2 Analysis of major and non-volatile compounds in spirit samples

In like manner as with aroma extracts, major and non-volatile compounds were determined without any pre-treatment in the spirit samples following GC-FID and HPLC-UV determinations (Table 2.3). Statistical analysis showed significant differences among the samples in the amount of the aroma compounds determined ($P < 0.05$). Amongst the compounds, acetaldehyde, ethyl acetate, acetal, acetic acid, furfural, 5-hydroxy-methyl furfural, vanillic acid, syringic acid, syringaldehyde, coniferaldehyde, sinapaldehyde and ellagic acid were highly increased in the mature spirits, and were therefore produced or affected by the maturation process (Table 2.3). Prior studies of the chemical mechanisms involved in the maturation of

whiskey showed that the formation of acetaldehyde, acetic acid, and ethyl acetate originates in the distillation, whilst some acetic acid is produced by interactions between the distilled and wood components. However, an increase in those congeners have been observed mainly because of mechanisms of ethanol transformation during ageing. Initially oxidation of ethanol into acetic acid yields acetaldehyde as intermediate, and condensation of ethanol and acetic acid produce ethyl acetate (Reazin, 1981). Particularly important is the ageing period, which contributes greatly to an increase in the concentration of all components. These concentrations, however, depend on other factors such as the evaporation, and also the formation and depletion reactions of these compounds such as oxidation, esterification, hydrolysis, and rearrangements (Watts et al. 2003). The concentration of any specific volatile component in an ageing spirit is therefore the net result of these processes, and positive or negative correlations with age should be expected. Therefore, the volatile concentrations of minor and major congeners of the spirits samples were selected as X variables and modelling against the maturation time (Y variable) of each of the samples using partial-least- squares (PLS) regression to predict sample age and separate spirit samples of different ages (Figure 2.2). PLS model generated with the concentrations of 73 compounds (X variables) and maturation time (Y variable) for the 8 spirit samples, explained 98.4% of the variance in age, and a graph of predicted age versus real age had a slope of 0.9842 and a correlation coefficient of 0.9842 (Figure 2.3), indicating a satisfactory age prediction. From the score plot, it was determined that the 8 spirits were separated according to their ages along PC1 and PC2 (Figure 2.2).

Table 2.3. Concentrations (mg/L[a]) of aroma compounds determined in Spirit Samples using the direct injection method.

No	Odorant	LRI	Determination	NMSA	MWA	NMSB	MWB	NMB	MB	NMTEQ	MTEQ
1	Acetaldehyde*	781	GC-FID	15.8±0.20	16.2±1.15	5.27±0.34	15.3±2.02	2.62±0.32	23.9±0.8	11.4±2.49	39.1±1.23
2	Ethyl acetate*	888	GC-FID	277±30.5	250±1.51	196±3.38	232±8.77	43.7±2.25	305±29.4	82.7±2.51	112±1.68
3	Acetal*	895	GC-FID	204±22.6	183±2.40	131±3.71	167±7.01	26.2±1.82	221±21.3	48.8±2.49	81.2±1.53
4	Methanol*	909	GC-FID	23.5±1.41	25.9±3.01	17.4±0.57	15.6±0.63	23.4±0.93	39.2±3.65	919±11.18	653±3.85
5	*n*-Propanol*	1061	GC-FID	284±1.47	280±1.92	304±0.44	204±1.15	103±1.02	148±0.8	183±0.55	275±0.37
6	Isobutanol*	1119	GC-FID	555±2.47	520±0.23	546±0.88	416±0.75	390±2.19	464±1.6	309±2.27	358±0.75
7	2-methyl-1-butanol*	1229	GC-FID	643±30.6	702±21.5	669±19.4	590±47.2	678±29.9	708±26.8	329±14.4	551±14.9
8	3-methyl-1-butanol*	1234	GC-FID	769±28.4	824±28.9	813±19.8	758±55.0	883±32.8	931±35.6	482±18.6	768±19.6
9	Ethyl lactate*	1382	GC-FID	18.0±0.21	6.37±0.35	3.56±0.62	4.36±0.21	10.1±0.36	10.5±0.5	10.6±0.32	16.3±0.26
10	Acetic acid*	1481	GC-FID	36.1±2.47	231±4.24	42.3±3.61	332±6.06	69.9±12.1	435±11.6	94.9±18.3	281±10.4
11	Furfural*	1504	GC-FID	15.6±0.02	11.8±0.40	8.3±0.31	8.42±0.25	0.05±0.00	7±0.24	0.74±0.05	2.29±0.12
12	5Hidroxy-methyl furfural*	ND	HPLC-UV	ND	0.74±8.11	ND	0.37±7.59	ND	2.06±0.60	ND	0.05±24.1
13	Gallic acid*	ND	HPLC-UV	47.1±3.24	35.5±4.38	46.0±7.52	23.7±5.84	0.69±0.28	28.3±2.63	3.46±4.41	9.95±6.97
14	Vanillic acid*	ND	HPLC-UV	ND	0.90±6.30	ND	1.17±8.04	ND	1.74±2.57	ND	0.64±3.10
15	Syringic acid*	ND	HPLC-UV	ND	2.06±4.64	ND	1.53±6.91	ND	4.10±1.32	ND	1.22±2.30
16	Syringaldehyde*	ND	HPLC-UV	ND	5.62±6.83	ND	5.53±3.89	ND	10.1±6.12	ND	2.52±2.85
17	Coniferaldehyde*	ND	HPLC-UV	ND	1.05±6.72	ND	0.77±2.06	ND	2.77±6.52	ND	0.33±0.48
18	Sinapaldehyde*	ND	HPLC-UV	ND	1.82±7.08	ND	1.29±2.06	ND	4.57±7.13	ND	0.37±2.94
19	Ellagic acid*	ND	HPLC-UV	ND	49.3±1.69	ND	42.9±1.28	ND	125±1.69	ND	16.0±1.19

[a]: Quanfication was achived following normalization to the internal standard (250 μg mL^{-1}, *n*-pentanol) for GC-FID analysis and following external standards for HPLC-UV analysis, in all cases using multilevel calibration curves (Appendix 1)

For GC-FID determinations, compounds were identified by comparison of their retention times (R.I) against those of authentic standards and confirmation based on their linear retention index (LRI).

For HPLC-UV determinations, compounds were identified by comparison of their retention times (R.I) against those of authentic standards and when possible by comparing their UV spectrums.

LRI: Linear Retention Index.

Data represent the average of three independent injections into the GC-FID and HPLC ± standard deviation.

ND: not detected under the conditions of analysis.

* Indicates statistically significant difference between the non-mature and mature spirits ($P<0.05$).

Non-mature spirits were located in the opposite part of the analysis particularly in the first and fourth region of the PLS plot. However, mature spirits were situated in the second and third region of the PLS plot, where mature bourbon was the spirit with the strongest correlation over PC1. The most significant variables (a total of 17) were identified as the ones with the strongest correlations loading on PC1 and PC2. These compounds included acetaldehyde, ethyl acetate, acetal, acetic acid, volatile phenols, oak lactones, furanic aldehydes, phenolic acids, and phenolic aldehydes respectively (Table 2.3). Interestingly, these 17 compounds had positive loading on PC1; that is, they correlated positively with sample age. A possible reason for this is that volatiles that show negative correlations with age may be produced in early stages of spirits production, such as fermentation and distillation (Prado-Jaramillo, et al. 2015). Of these compounds showing negative loading on PC1 include ethyl esters, alcohols, acids, acetals, terpenes, and furanic compounds. Overall these compounds were located in the regions where non-mature spirits were situated; therefore, indicating their formation might arise or be developed in the early stages of spirits production. Mature bourbon was the sample showing the highest correlation with these mature congeners, mainly because it was the sample with the longest period of maturation (5 years) among the mature spirits (Figure 2.2).

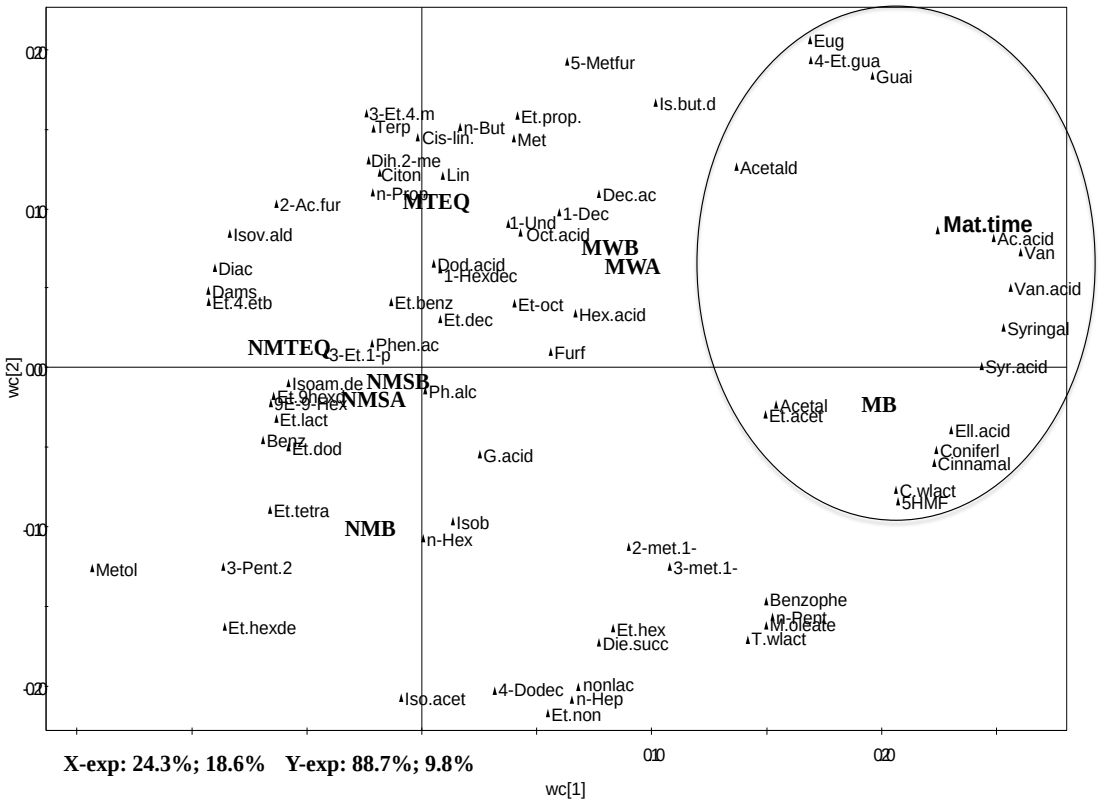

Figure 2.2. Partial least-squares (PLS) regression analysis of volatile and non-volatile compounds (X-variables) and maturation time (Y-variables); scores and loadings for the first two principal components.

Key: *Flavour compounds:* Acetald: acetaldehyde; Et.acet: ethyl acetate; Acetal: acetal; Met: methanol; Diac: diacetyl; n-Prop: *n*-propanol; Iso.alddieac: isovaleraldehyde diethyl acetal; Isob: isobutanol; Iso.acet: isoamyl acetate; n-But: *n*-butanol; 3-Pent.2-ol: 3-Penten-2-ol; 2-met-1-but: 2-methyl-1-butano; 3-met-1-but: 3-methyl-1-butanol; Et.hex: ethyl hexanoate; n-Pent: *n*-pentanol; Is.but.dietac: isobutanal diethyl acetal; Dih.2-met: dihydro-2-methyl-3 (2H)-furanone; Et.prop.ald: β-ethoxypropionaldehyde diethyl acetal; n-Hex: *n*-hexanol; Et.lact: ethyl lactate; 3-Et.1-prop: 3-ethoxy-1-propanol; Et-oct: ethyl octanoate; Cis-lin.ox: cis-linalool oxide; Ac.acid: acetic acid; n-Hep: *n*-heptanol; Furf: furfural; 3-Et.4.met.1.p: 3-ethyl-4-methyl-1-pentanol; 2-Ac.fur: 2-acetylfuran; Et.non: ethyl nonanoate; Benz: benzaldehyde; Lin: linalool; 5MF: 5-methyl furfural; Et.dec: ethyl decanoate; Die.succ: diethyl succinate; Et.benz: ethyl benzoate; Terp: α–terpineol; Metol: methionol; 1-Dec: *n*-decanol; Citon: citronellol; 4-Dodec: 4-dodecenol; Phen.ac: phenethyl acetate; Dams: β-damascenone; Et.dod: ethyl dodecanoate; Isoam.dec: isoamyl decanoate; Hex.acid: hexanoic acid; Guai: guaiacol; T.wlact: trans-whisky lactone; Ph.alc: phenylethyl alcohol; 1-Und: *n*-undecanol: C.wlact: *cis*-whiskey lactone; Et.tetradec: ethyl tetradecanoate; 4-Et.guai: 4-ethylguaiacol; nonlac: γ-nonalactone Oct.acid: octanoic acid; 1-Hexdec: *n*-hexadecanol; Eug: eugenol; Et.4.etben: ethyl 4-ethoxybenzoate; Et.hexdec: ethyl hexadecanoate; Et.9hexdec: Ethyl 9-hexadecenoate; Dec.ac: decanoic acid; 9E-9-Hexd-1-ol: (9E)-9-Hexadecen-1-ol: M.oleate: methyl oleate; Dod.acid: dodecanoic acid; Benzophe: benzophenone; Van:vanillin; 5HMF: 5-hidroxy-methyl furfural; G.acid: gallic acid; Van.acid: vanillic acid; Syr.acid: syringic acid; Syringald:

syringaldehyde; Coniferl: coniferaldehyde; Cinnamald: sinapaldehyde; Ell.acid: ellagic acid. Mat. Time: maturation time. *Spirits:* NMSA: new make spirit from distillery 'A'; NMSB: new make spirit from distillery 'B'; NMB: non-mature bourbon; NMTEQ: non-mature tequila; MWA: malt whisky from distillery 'A'; MWB: malt whisky from distillery 'B'; MB: mature bourbon; MB: mature bourbon; MTEQ: mature tequila.

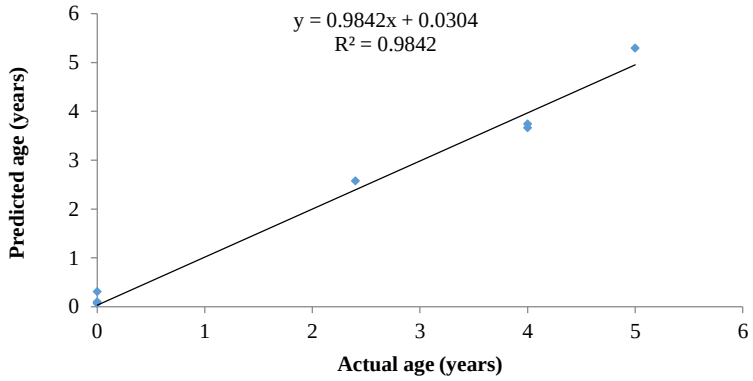

Figure 2.3. Predicted age versus actual age (n = 8) using PLS model with all 73 variables

Table 2.4. Optimum volatile compounds for age prediction of the 8 spirit samples according to PLS analysis

Volatile compound	Chemical group	Correlation with PC1 (age)
Acetaldehyde	Aldehyde	+
Ethyl acetate	Ester	+
Acetal	Acetal	+
Acetic acid	Acid	+
Guaiacol	Volatile-phenol	+
Trans-whisky lactone	Oak-lactone	+
Cis-whisky lactone	Oak-lactone	+
4-ethyl guaiacol	Volatile-phenol	+
Eugenol	Volatile-phenol	+
Vanillin	Volatile-phenol	+
5-Hydroxymethyl furfural	Furanic aldehyde	+
Vanillic acid	Phenolic acid	+
Syringic acid	Phenolic acid	+
Syringaldehyde	Phenolic aldehyde	+
Coniferaldehyde	Phenolic aldehyde	+
Sinapaldehyde	Phenolic aldehyde	+
Ellagic acid	Phenolic acid	+

Our data confirm the compounds, which arise or are affected by the maturation process, and illustrates differences among spirit samples mainly because of the strong influence of this important parameter. However, different stages shape the complex processes of each spirit production, and therefore different concentrations of congeners among spirits could be found. For example, tequila is an alcoholic beverage obtained from fermentation and distillation of the juice of cooked heads of blue Agave (*A. tequilana Weber*), which is then matured for at least two months in oak containers (*Quercus alba, Quercus ilex*) (Lopez-Ramirez et al. 2013). For whisky and bourbon production, malt and/or ground cereals are used as the source of sugars for fermentation, and the resulting beer is then distilled, which is finally stored in oak barrels. However, for bourbon whiskey, maturation occur in new charred barrels for a period of at least two years, while for scotch whisky maturation occurred in used charred oak bourbon whiskey barrels or sherry casks for at least three years (Conner et al. 1993). Undoubtedly, among spirits production stages, barrel ageing is amongst the key processing steps in the generation of the unique aroma of spirits samples. Furthermore, the time of storage and the oak cask type during ageing were the factors in showing a higher impact in the mature flavour of the ageing spirits of the present study. For bourbon whiskey, the ageing period was five years in new charred barrels, for scotch malt whiskies it was for a period of four years in used charred oak bourbon whiskey barrels and for mature tequila a period of 2.4 years in oak containers. The time of residence inside the barrels is reflected in the amount of congener concentrations; in this sense mature bourbon was the spirit containing the highest amount of ageing congeners, among the mature spirits

(Figure 2.1). Interestingly, these ageing congeners include wood-derived compounds (oak lactones/whisky lactones), volatile polyphenols (eugenol, guaiacol, 4-ethyl guaiacol, and vanillin), phenolic compounds (vanillic acid, syringic acid, syringaldehyde, coniferaldehyde and sinapaldehyde) and furanic aldehydes (5-hydroxymethyl-furfural) (Tables 2.1, 2.2, 2.3). Some of them are used as markers or ageing indicators, and therefore their presence could influence the taste and aroma of matured spirits (Mosedale and Puech, 1998). Their presence has been attributed to physicochemical reactions that arise during maturation, including the extraction of wood components, evaporation of volatile compounds and interactions between wood and distilled components (Nishimura, et al. 1983; Mosedale and Puech, 1998). Particular important were guaiacol, 4-ethyl guaiacol, *cis/trans*-whisky lactones, eugenol and vanillin, among other non-volatile compounds which were strongly affected by the ageing period (Figure 2.1, Table 2.4). Furthermore, different concentrations of the ageing congeners were found among the spirits, indicating significant differences as a consequence of the ageing period employed and the unique characteristics of the cask used for ageing (e.g. new versus re-use) (Tables 2.1, 2.2, 2.3). *Cis*-whisky lactone was the congener presented in higher concentration among spirits; follow by *trans*-whisky lactone, and vanillin. Previous reports have assessed the importance of Z-whisky lactone; eugenol and vanillin to spirit flavour and their presence has been found to be directly correlated to assessments of whisky quality (Mosedale and Puech, 1998) and is considerable desirable for most distilled beverages.

2.5 CONCLUSIONS

Our data indicate that whilst LLE was better in terms of the extraction of a broad cross-section of spirits volatiles, SPE can be a useful complementary technique for the analysis of certain compounds. Therefore, by choosing the appropriate SPE column and optimizing the conditions to suit the elution of the groups of analytes required, SPE can be a successful method of extraction, especially for the recovery of semi-volatiles (Boothroyd et al. 2013). Furthermore, the instrumental data from volatile and non-volatile compounds and analysed with PLS regression was a suitable method to distinguish between non-mature and mature spirits of bourbon, tequila and malt whiskies. The subset of 17 congeners (acetaldehyde, ethyl acetate, acetal, acetic acid, 4 volatile phenols, 2 oak lactones, 1 furanic aldehyde, 3.phenolic acids, and 3 phenolic aldehydes) with positive loading in the PLS plot, showed the compounds that were highly influence by the ageing period (maturation time) and possibly the ones that define the mature character in the spirit samples, however further work is needed to determine which of the compounds that have been highly correlated with age are in fact significant at sensory level. This will be discussed further in Chapter 3.

3. IMPACTS OF WOOD AGEING ON THE SENSORY AND ANALYTICAL PROFILES OF MATURE SPIRITS

3.1. AIM

The main objectives of the work described in this Chapter were:

1. To identify the significant odorants which contribute to the estery and mature/woody characters of four different spirits (bourbon, tequila and 2 distinct malt whisky samples)
2. To compare and contrast the impacts of wood ageing on the aroma and sensory properties of these four different spirits, with the hypothesis that some commonalities exist, both in terms of the extracted wood-derived components and their impacts on the overall aroma profile of the spirits.

The results obtained in this Chapter were then used as a basis for sensory studies in which sensory interactions between these two important spirit characters (estery and woody) were evaluated by varying the concentrations of the relevant congeners (Chapter 5).

3.2. INTRODUCTION

Tequila, bourbon and Scotch malt whisky, are typical distilled spirits that are aged in wooden barrels to develop the desirable sensory properties associated with ‘mature’ character. The change in flavour of the maturing spirit results from changes in the composition and concentration of compounds influencing the taste and aroma. Among these changes, the best documented, is that of aroma extracted from the

wood (Nishimura et al. .1983). It is well known that oak wood releases significant amounts of some aroma compounds, which have a profound impact on the aroma profile and on the sensory characteristics of the spirit (Chatonnet and Boidron, 1990; De Aquino et al. 2006). The sensory impacts of acids, aldehydes, and phenolic compounds including, whisky lactones, eugenol, and vanillin (Mosedale, 1998; Conner et al. 1993, Chatonnet and Boidron, 1990) are particularly notable. Some of these compounds can be used as markers of ageing, whereby their quantification during the ageing process can be used to estimate the time required to age a distilled beverage (De Aquino et al. 2006). Lignin hydrolysis is a major chemical process during wood ageing which generates a range of phenolic compounds in the mature spirit. Oxidation of these compounds yields aldehydes, acids, vanillin, and syringaldehyde (Martinez et al. 1996). Furanic aldehydes are also important; however, they are not believed to have a direct effect on the final sensory characteristics of the beverage (Quesada-Granados et al. 1996; Escalona et al. 2002).

The aroma of an alcoholic beverage is typically complex and consists of hundreds of volatile compounds; however only a small proportion of the aroma compounds contribute significantly to the spirit flavour (Grosh, 1994). These are the so-called key odorants, which can be characterized by GC-Olfactometry. In this context, gas chromatography olfactometry (GC-O) and aroma extract dilution analysis (AEDA) are significant techniques because they enable the odours experienced by a panellist to be traced to compounds eluting at the times aroma is experienced (Schieberle, 1995). GC-O thus enables the identification of odour-active volatiles from the bulk

of odourless volatiles and AEDA then determines the relative odour potency of compounds present in a sample extract (by successively diluting the extract and identifying which aromas are detected orthonasally at the highest dilution factors; Grosh, 1994; Martí, 2003). Relatively few studies have reported the compounds responsible for the odour of aged spirits such as bourbon whiskey, malt whisky or tequila. Conner et al. (2001) showed that cis-whisky lactone and vanillin were important in the aroma of Scotch whisky, whilst Poisson and Schieberle (2008) reported that (3S,4S)-cis-whisky lactone, γ-decanolactone, eugenol, and vanillin contributed the overall vanilla-like, fruity, and smoky aroma notes of an American Bourbon whiskey. Whereas for tequila, 3-methylbutanal, isoamyl alcohol, β-damascenone, 2-phenyethanol, phenylethyl acetate, and vanillin have been shown to be important to the overall aroma of different classes of spirit (Benn and Peppard, 1996; López and Dufour, 2001; González-Robles and Cook, 2016).

Whilst previous studies have reported the key aroma compounds present in various spirit samples, currently there are no published studies focused on understanding the impact of wood ageing on the aroma and sensory characteristics of distilled spirits as a category.

3.3. MATERIAL AND METHODS

3.3.1. Samples

Eight spirit samples (non-mature and mature exemplars of each kind), consisting of two commercial brands of Scotch malt whiskies from two distilleries (A and B), tequila and bourbon samples were utilized to carry out the experiments of this results chapter

3.3.2. Reagents and Chemicals

As described in Section 2.3.2.

3.3.3. Extraction of volatile compounds from spirit samples

Volatile compounds were extracted from spirit samples using liquid-liquid extraction following the method of Boothroyd et al. (2014) as detailed in Section 2.3.3.2.

3.3.4. Gas chromatography-Mass Spectrometry/Olfactometry (GC-MS/O)

GC-MS and odour port evaluation were carried out using the conditions for GC-MS analysis as described in Section 2.3.4. For odour port evaluation a splitter was fitted to the end of the ZB-Wax column (30m× 0.25mm i.d., 1.0 μm film thickness; Phenomenex, Macclesfield, UK), such that approximately half of the flow was diverted to an ‘odour sniffing port’ via a fused silica capillary passing within a heated transfer line, set at a temperature of 200 °C. A panel of four panellists (3

female and 1 male aged between 24 and 30 years) were used to carry out the GC-O work. During each GC run, a panellist placed his/her nose close to and above the top of the sniffing port and evaluated the odour of the chromatographic effluent and recorded the time at which they perceived an odour and gave an appropriated odour descriptor. As the GC runs were 52 min long, two assessors were used to sniff each chromatogram, swapping over halfway, in order to avoid fatigue. The GC-O analysis was performed following the aroma extract dilution analysis (AEDA) approach, for which spirit extracts were stepwise diluted using dichloromethane as the solvent to obtain dilutions of 1:3, 1:9, 1:27, 1:81, 1:243, 1:729; 1:2187 and 1:6561 of the original extract (Gonzalez-Robles et al. 2016). Sniffing of each dilution was performed in triplicate until no odorant was perceived and then each odorant was assigned a flavour dilution factor (FD factor). A preliminary training session with the panellists was done by GC-O using a standard mixture of several important spirit flavour compounds (based on Poisson and Schieberle 2008). A further GC-O analysis was done to confirm the influence of the highly volatile, early eluting compounds of spirit samples (which are obscured by the solvent front in DCM extracts). The spirit direct injection method described at Section 2.3.3.1 was replicated using the GC-MS/O set-up, such that a 20-minute run-time was enough to evaluate the influence of these compounds sensorially. To reduce the time of analysis a flavour dilution factor of 10-fold was implemented, such that only 4 dilutions per sample were analysed (dilutions: 10, 100 and 1000). The analysis of each sample and dilution was performed in duplicate.

3.3.5. Sensory evaluation

The aroma of the spirit samples was assessed by the SWRI's expert sensory panel, which has extensive experience in the evaluation of whiskies and related spirits. The samples were diluted to 20% ABV and presented (30 mL) in clear nosing glasses, covered with watch glasses. Panellist were asked to score the intensities of the following sixteen aroma attributes: 'cereal', 'dried fruit', 'feinty', 'floral', 'fresh fruit', 'green/grassy', 'oily', 'pungent', 'soapy', 'solventy', 'sour', 'spicy', 'stale', 'sulphury', 'sweet', and 'woody' aromas in each of the spirit samples, using an intense scale of 0-3. The panel has previously been trained to recognize these attributes using a range of production samples, spirits with the key flavour characters and, where available, individual flavour compounds. The sensory assessments were carried out over a number of sessions, with a minimum of 10 panellists participating in each session. Mean panel scores were calculated for each attribute.

3.3.6. Data treatment and statistical analysis

The sensory and instrumental data were analysed using Statgraphics plus software Version 16.1.11 and Simca software – P7.01. To test significant differences among spirit composition, analysis of variance (ANOVA) was applied. In sensory analysis, Tukey´s HSD test was performed to establish which flavour profiles were significantly different among the samples compared into two category groups (non-mature and mature). Furthermore, Pearson correlations among all sensory and instrumental data was calculated, basically by measuring the strength of the linear

association between these two variables in a positive or negative way. In addition, to show the relationship between the analysed concentrations of aroma compounds (X), and the spirit sensory properties (Y), partial least squares regression (PLSR) was applied. PLSR create, starting from a table with several observations described by several variables, a set of components. This is a data reduction technique in that it reduces the Xvariables to a set of noncorrelated factors that describe the variation in the data. Thus, PLSR regression is of particular interest because it can analyse correlated data and redundant variables (X-variables) and can also model several characteristics (Y-values) at the same time (Vilanova et al. 2010).

3.4. RESULTS AND DISCUSSION

3.4.1 Preliminary GC-O studies: screening and training

In order to carry out the GC-O analysis of spirit samples, preliminary GC-O studies were performed which included the selection of the most sensitive assessors and the screening of a training mixture solution.

3.4.2 Odour screening recognition test

A solution containing 10 compounds (Table 3.1) was prepared and evaluated by 7 assessors (5 female and 3 males between 24 and 30 years, all of them students from the University of Nottingham). These compounds were selected because they have been reported as important compounds of whisky flavour (Schieberle et al. 2008, Boothroyd et al. 2013).

From Table 3.1, ethyl butanoate and ethyl 2-methylpropanoate showed the same

retention time, as did ethyl propanoate and ethyl (S)-2-methylbutanoate. Considering this behaviour when assessors analysed the solution, these pairs of compounds were considered as mixtures, due to their similarity in terms of molecular weight and EI mass spectrum.

Table 3.1: Chemical compounds present in the solution at 10 ppm.

Compound	Odour description	Odour Threshold (µg/L)	LRI
Ethanol	Solvent-like	0.01	958
Ethyl butanoate	Fruity-like	9.5	1080
Ethyl 2-methylpropanoate	Fruity-like	4.5	1080
Ethyl propanoate	Fruity-like	3452	1097
Ethyl (S)-2-methylbutanoate	Fruity-like	0.2	1097
Ethyl 3-methylbutanoate	Fruity-like	1.6	1101
3-Heptanone	Fresh, herbal, lavender	140-3000	1191
Ethyl octanoate	Fruity	147	1464
2-Acetylthiazole	Nutty popcorn roasted peanuts hazelnut	10	1712
Eugenol	Clove-like	7.1	2219

Once the assessors examined the solution the percentage of frequency detection for each compound was calculated (Figure 3.1). From Figure 3.1, ethanol and 2-acetylthiazole scored 100% of detection among assessors, followed by the mixture of ethyl propanoate and ethyl (S)-2-methylbutanoate and eugenol (85.7%). The rest of the compounds were detected at lower frequencies; 71.4% for ethyl 3-methylbutanoate and 3-heptanone, 57.1% for ethyl octanoate and only 28.6% for the mixture of ethyl butanoate and ethyl 2-methylbutanoate (Figure 3.1). The variation observed could be explained by the specific odour threshold of each compound and for the sensitivity of perception that each assessor had for each compound.

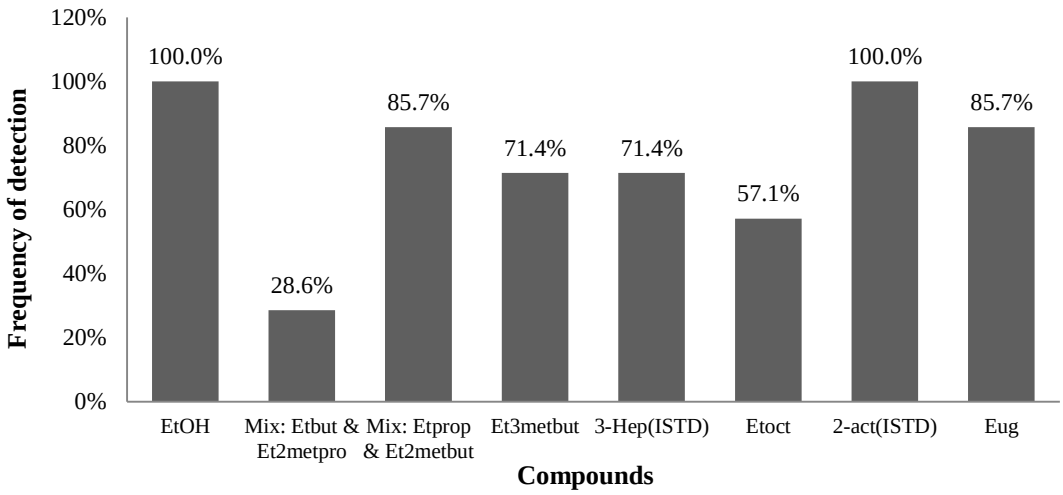

Figure 3.1. Panellist screening for GC-O using a spirit flavour training mixture: Detection frequency (%) by compound. Results are the average of detection obtained with 7 assessors.

Moreover, the percentage of individual detection obtained by the assessors was also calculated and is shown in Figure 3.2. Six assessors detected more than 60% of the compounds in the solution, with just assessor 6 detecting less than 60% of compounds.

From these results, assessors 1, 2, 3, 4, 5 and 7 were selected as the potential candidates to assess the upcoming GC-O-AEDA trials using a training mixture solution containing some of the most odour active compounds found by Schieberle et al. (2008) in an American Bourbon Whiskey.

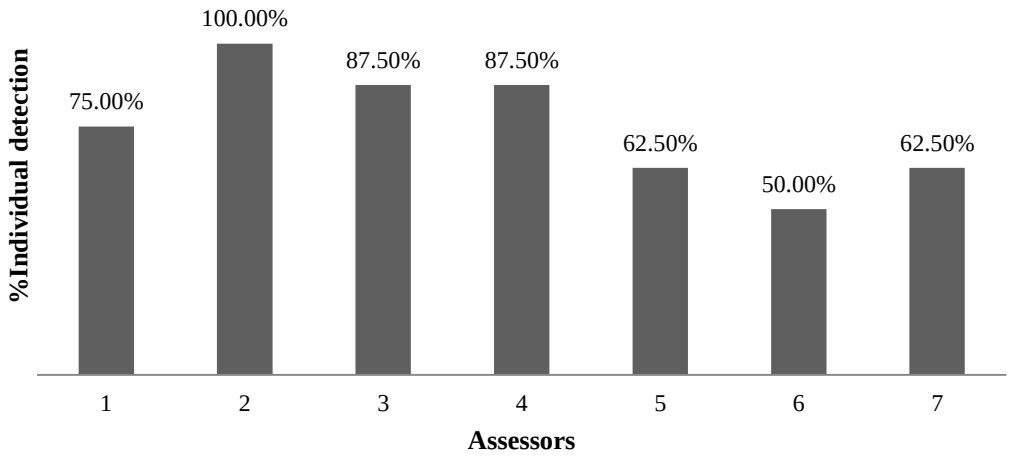

Figure 3.2. Percentage of individual detection for the compounds

3.4.3 Training in AEDA

Table 3.2 summarizes the results of the GC-O-AEDA trial performed with the training mixture solution. 5 assessors evaluated the original solution and 3 of its dilutions (1:2, 1:8 and 1:32). These dilutions were selected with the purpose of allowing the assessors to get familiar with the GC-O-AEDA approach and to evaluate their general performance. Moreover, the GC run was split between 2 assessors in each sample because the GC run time was too long (54 min).

Table 3.2. Summary of the GC-O-AEDA results using a training mixture solution and five panellists.

No	Compound	LRI	Odour threshold (µg/L)	Conc. (mg/L)	Assessors				Consensus Odour descriptor	Odour descriptor Literature Reference	Frequency of Detection			FD-factor
					1 & 2	1 & 3	1 & 4	1 & 5			1:2	1:8	1:32	FD
1	Ethanol	946	10,000	----	Solvent	Solvent	Solvent	Yeast, dough	Solvent	Solvent	100%	100%	100%	> 32
2	Ethyl butanoate	1070	9.5	13.702	Peach	Sweet, gum	Fruity	Bubble gum	Fruity	Fruity	100%	100%	75%	> 32
3	Ethyl 2-methylpropanoate	1070	4.5	12.355	Peach	Sweet, gum	Fruity	Bubble gum	Fruity	Fruity	100%	100%	75%	> 32
4	Ethyl propanoate	1085	3452	9.467	Sweet, flower	Bubble gum	Fruity	Fruity	Fruity	Fruity	100%	100%	100%	> 32
5	Ethyl (S)-2-methylbutanoate	1085	0.2	8.158	Sweet, flower	Bubble gum	Fruity	Fruity	Fruity, sweet	Fruity	100%	100%	100%	> 32
6	Ethyl 3-methylbutanoate	1100	1.6	10.427	Fruity	Fruity	Apple	Strawberry	Fruity	Fruity	100%	100%	100%	> 32
7	Ethyl hexanoate	1238	30	9.108	Artificial fruit	Peach	Fruity	Strawberry	Fruity	Fruity	100%	100%	100%	> 32
8	3-Methylbutanol	1262	56100	9.441	Cereal	Cereal	Malty grainy	Musty, cheesy	Cereal, malty	Malty	100%	100%	100%	> 32
9	Ethyl octanoate	1446	147	8.395	Fruity	Cider	Fruity	Fruity	Fruity	Fruity	100%	0%	0%	2
10	(E)-2-Nonenal,	1567	0.6	8.769	Green, fresh	Cucumber	Green tree	Grassy, oil, fatty	Green, cucumber, grassy	Green-like	100%	100%	100%	> 32
11	(E, Z)-2,6-nonadienal	1622	0.9	8.971	Fresh cucumber, raspberry, leafs	Cucumber	Cucumber	Cucumber	Cucumber	Green-like	100%	100%	100%	> 32
12	(E)-2-decenal	1675	1.8	13.68	Oil	Oil	Oily, Fatty	Fatty, oily, skin	Oily, fatty	Fatty	75%	100%	100%	> 32
13	2-Acethyl thiazole	1696	10	10	Popcorn	Burned, nut	Burned, roasted	Popcorn, rice,	Popcorn, burned, nut	Popcorn roasted	100%	75%	75%	> 32
14	(E, E)-2,4-nonadienal	1741	2.6	8.971	Herbs, grassy	Oil, fatty	Butter	Grassy, fatty	Grassy, fatty	Fatty	100%	100%	100%	> 32
15	(E, E)-2,4-decadienal	1956	1.1	7.191	Oily, Fatty	Oily	Fatty	Fatty, oily	Fatty	Fatty	100%	50%	50%	> 32
16	E-Damascenone	1958	0.1	8.208	Flowery	Flowers	Flowery	Rose	Flowery	Fruity, rose,	100%	25%	100%	> 32
17	Phenyl ethyl acetate	1965	108	9.187	Flowery	Flowers	Flowery	Rose	Flowery	Flowery	100%	25%	100%	> 32

Table 3.2. Summary of the GC-O-AEDA results using a training mixture solution and five panellists (cont.).

18	Guaiacol	2015	9.2	17.326	Phenol, Flower	Phenol, solvent	Phenol, Flower	Polyphenol, dry leaves	Phenol, solvent	Phenolic	100%	100%	100%	> 32
19	2-Phenylethanol	2065	2600	8.712	Flowers	Flowers	Flowers	Rose petal, perfume	Flowers	Flowery	100%	100%	100%	> 32
20	4-Ethyl-guaiacol	2079	6.9	10.819	Phenol, varnish	Phenol, solvent, clove	Phenol	Nuts, oil	Phenol, clove	Phenolic, clove-like	25%	0%	0%	2
21	γ -Nonalactone	2081	21	12.074	Fruity	Fresh, fruity	Phenol	Nuts, oil	Fruity	Peach-like	100%	100%	100%	> 32
22	Trans-ethyl cinnamate	2165	0.7	7.92	Fruity	Fruity	Fruity	fruity	Fruity	Fruity	100%	75%	0%	32
23	γ-Decalactone	2183	21	24.382	Fruity	Fruity	Fruity	Fruity	Fruity	Peach-like	100%	75%	25%	> 32
24	Eugenol	2201	7.1	20.038	Clove	Clove	Clove	Clove,	Clove	Clove-like	100%	100%	100%	> 32
25	Vanillin	2532	22	13.068	Vanilla	Chocolate	chocolate	Sugar syrup	Sugar, chocolate	Vanilla-like	100%	50%	0%	32

According to the results summarized in Table 3.2; 15 compounds were detected at the greatest dilution factor (1:32), indicating that their flavour dilution factor (FD) is higher than the one tested. Four compounds were not detected in the highest dilution showing that their flavour dilution factor (FD) was equal to or less than 32. Furthermore, regarding the odour descriptors associated with the 25 compounds detected, it can be seen that most of the descriptors given by the panellists are in accordance with widely accepted odour descriptors for each compound reported in the literature. However, some compounds such as β-damascenone and phenethyl acetate co-eluted under the chromatographic conditions, making it difficult to correctly assign odour qualities attributed by the assessors to specific compounds. At this stage, it was possible to make correct assignment of odour qualities to odorants because the analysis was made using solutions of known compounds, but when working with spirit samples of such complexity as whisky it is necessary to take other factors into account in order to establish a correct identification. Thus, in subsequent experiments with authentic spirit samples, identification of compounds responsible for noted odours was made using: confirmation of odour qualities using pure compounds, comparison based on linear retention index (LRI), identification based on MS of pure compounds and fractionation of the sample (if necessary).

Further, more detailed GC-O-AEDA trials were necessary to establish definitive FD factors for key odorants. However, this first trial enabled assessors to improve their performance and to become familiar with the AEDA approach.

3.4.4 GC-O studies of non-mature and mature Spirit samples

Following on from the analytical characterisation of the spirit samples reported in Chapter 2 (Section 2.4), the most odour active volatile compounds were detected using Gas Chromatographic-Olfactometry (GC-O) and Aroma Extract Dilution Analysis (AEDA) analysis.

3.4.4.1 Identification of the most odour active compounds in non-mature and mature Bourbon

Due to the broader cross-section of compounds, which were extracted efficiently by liquid-liquid extraction (LLE), this method was selected to perform the GC-O/AEDA analysis of the Spirit samples. Furthermore, the aroma impact of the major volatile compounds (Table 2.2) was also assayed using the GC-O/AEDA approach with direct injection of spirit samples. GC-O identified 38 odour-active regions in the chromatograms of non-mature or mature bourbon whiskey samples, taken across both the extract and direct spirit injection GC-O analyses. Table 3.3 presents data for each of these odour active regions, sorted by the flavour dilution factor obtained from AEDA analysis of the mature bourbon sample. In theory this orders the compounds according to their likely impact on the aroma of mature bourbon. As with all GC-O studies it must be noted that since odorants are sniffed individually during GC-O, this technique takes no account of potential interactions (*e.g.* synergy or masking) between odourants, which can influence perceived aroma. It is also not possible to account for factors such as sub-threshold enhancement or modification of aroma, whereby the perceived quality or intensity of an aroma can be modified

by compounds which individually are present beneath their odour threshold. However, GC-O/AEDA remains a popular approach because it highlights compounds, which are likely to play a major part in determining the overall perception; namely those present in substantial excess of their sensory threshold, such that they are still sensed even at the highest dilution factors.

Non-mature bourbon was characterized by 25 odour-active regions with flavour dilution factors (FD) ≥ 27. The compounds with the highest FD factors (≥ 1000) were ethyl hexanoate and several low-boiling compounds such as acetaldehyde, acetic acid, and ethyl acetate among others (Table 3.3). The individual aroma descriptors associated with these compounds (Table 3.3) include qualities such as fruity, flowery, pungent, solvent-like, malty, green-like, vinegar among others.

Table 3.3. The most odour-active (FD ≥ 27) volatile compounds identified in non-mature and mature bourbons, and their estimated odour-activity values (OAV). Data are sorted by FD factor in the mature bourbon.

LRI (ZB-Wax)	Odour quality	Odorant	Identity	Flavour dilution factor (FD)		Odour threshold (mg/L)	OAV		Earlier reported as compound in bourbon whiskey
				Non-mature bourbon	Mature bourbon		Non-mature bourbon	Mature bourbon	
1462	Green, fruity, floral-like	Ethyl octanoate	A, B	243	6561	0.15[b]	80	58	Schieberle et al. 2008
1718	Potato, celery, green, maize	Ethyl benzoate*	A, B	ND	6561	0.06[a]	0.7	0.4	Boothroyd, 2013
1718	Potato, celery, green, maize	Diethyl succinate*	A, B	ND	6561	1.20[e]	0.7	0.8	Boothroyd, 2013
1920	Phenolic	Guaiacol*	A, B	ND	6561	0.0092[b]	ND	4.2	Schieberle et al. 2008
2084	Flowery	4-Ethylguaiacol*	A, B	ND	6561	0.01[b]	ND	3.3	Schieberle et al. 2008
2088	Fruity, flowery	γ-nonalactone*	A, B	ND	6561	0.021[b]	ND	1.5	Schieberle et al. 2008
2286	Fatty, oily	Decanoic acid*	B	ND	6561	6.00[e]	0.1	1.0	Camara et al. 2007
1260	Fruity, flowery	Ethyl hexanoate	A, B	6561	2189	0.03[b]	74	76	Schieberle et al. 2008
788	Pungent, sweet	Acetaldehyde[$]	A, B	1000	1000	19.2[h]	0.2	1.2	Schieberle et al. 2008
893	Varnish, leafy	Ethyl acetate[$]	A, B	1000	1000	12[e]	3.64	25.42	Schieberle et al. 2008
900	Earthy, solvent	Acetal[$]	A, B	1000	1000	0.719[h]	34	308	Camara et al. 2007
919	Solvent-like	Methanol[$]	A, B	1000	1000	100[b]	0.2	0.4	Conner, 1993
954	Ethanol, solvent-like, sweet	Ethanol[$]	A, B	1000	1000	24.9[b]	----	----	Schieberle et al. 2008
1073	Solvent-like, fruity, sweet	*n*-Propanol[$]	A, B	1000	1000	9[b]	11	16	Schieberle et al. 2009
1124	Winey, sweet, solventy	Isobutanol[$]	A, B	1000	1000	40[b]	10	12	Schieberle et al. 2010
1241	Malty, solvent, sweet, fruity	2-methyl-1-butanol[$]	A, B	1000	1000	4[b]	165	177	Schieberle et al. 2008
1241	Malty, solvent, sweet, fruity	3-methyl-1-butanol[$]	A, B	1000	1000	56.1[b]	15	17	Schieberle et al. 2008
1404	Green-like, grass	Ethyl lactate[$]	A, B	1000	1000	14[a]	0.8	0.7	Camara et al. 2007
1515	Vinegar	Acetic acid[$]	A, B	1000	1000	75.5[h]	0.8	6	Camara et al. 2007
1148	Banana	Isoamyl acetate*	A, B	243	729	0.03[c]	476	109	Schieberle et al. 2008
1668	Fruity	Ethyl decanoate	A, B	729	729	0.50[e]	33	27	Boothroyd, 2013
1946	Flowery, tobacco	Trans-whiskey lactone*	A, B	ND	729	0.79[b]	ND	3.7	Schieberle et al. 2008
1966	Flowery-like	Phenylethyl Alcohol*	A, B	243	729	2.60[b]	5.8	7.8	Schieberle et al. 2008
2021	Flowery, coconut	Cis-Whiskey lactone*	A, B	ND	729	0.79[b]	ND	11	Schieberle et al. 2008
2204	Spicy, sweet, clove-like	Eugenol*	A, B	ND	729	0.0071[b]	ND	2.5	Schieberle et al. 2008
1574	Caramel, sweet	Benzaldehyde*	A, B	27	243	2.00[e]	0.01	0.01	Boothroyd, 2013
1868	Fruity, flowery, sweet	Phenethyl acetate	A, B	729	243	0.11[b]	13	5.6	Schieberle, 2008
2100	Fatty, oil	Octanoic acid*	B	81	243	10[e]	0.1	4.3	Camara et al. 2007
1622	Caramel, coffee-like	5 Methyl furfural*	A, B	27	81	16[d]	<0.01	0.02	Boothroyd, 2013

Table 3.3. The most odour-active (FD ≥ 27) volatile compounds identified in non-mature and mature bourbons, and their estimated odour-activity values (OAV). Data are sorted by FD factor in the mature bourbon (cont.).

2247	Fatty-like	Ethyl hexadecanoate	A, B	243	81	2.00[g]	9.9	0.3	Boothroyd, 2013
2414	Fresh	Methyl oleate*	B	ND	81	NA	ND	NA	Camara et al. 2007
1380	Solvent, fruity, sweet	*n*-Hexanol	A, B	243	27	2.5[a]	2	1.4	Camara et al. 2007
1565	Tropical, fruity, waxy, sweet	Ethyl nonanoate	A, B	ND	27	NA	NA	NA	Boothroyd, 2013
1876	Fruity, flowery, sweet	Ethyl dodecanoate*	B	ND	27	5.9[g]	1	0.6	Camara et al. 2007
2533	Caramel, sweet, vanillin	Vanillin	A, B	729	729	0.02[b]	0.9	141	Schieberle et al. 2008
1082	Sweet, fruity	Unknown	C	81	ND	NA	NA	NA	NA
1201	Green-like	3-Penten-2-ol	B	243	ND	NA	NA	NA	NA
1562	Almond, caramel, burnt sugar	Furfural*	A, B	ND	243	15[d]	<0.01	0.5	Boothroyd, 2013

ND: not detected under the condition of analysis. NA: Odour threshold data not available in the literature.
A, B: Compounds were identified by EI-MS library matching (NIST), comparison against authentic standards and confirmation of their linear retention index (LRI) against published values for a DB-Wax column.
B: Compounds were identified by EI-MS library matching (NIST), and confirmation of their linear retention index (LRI) against published values for a DB-Wax column
Odour threshold references: [a]: http://www.leffingwell.com/odorthre.htm; [b]: Schieberle et al. (2008); [c]: Ferreira et al. (2002); [d]: Franco et al. (2004); [e]: Peinado et al. (2004); [f]: Lopez et al. (2002) [g]: Pino et al. (2011). [h]: Uselmann et al. (2015).
Odour thresholds from most of the references were determined in hydroalcoholic solutions (Ethanol 10 and 40%), with the exception of ref [a] which was determined in water.
* Compounds with a significant difference in odour potency between non-mature and mature bourbon samples.
[$] Flavour dilution factors (FD) optimized for the analysis of the influence of major volatile compounds into the overall aroma of spirits flavour.
---: data not available since the concentration was not determined
Note: an average delay of 18 s was found between the detection of the compounds by GC-MS and panellist's nose during the GC-O analysis

Since non-mature bourbon is a freshly distilled product, the most potent odorants detected in the AEDA study are important markers of the preparation, mashing, fermentation and/or distillation steps of bourbon production.

Mature bourbon was characterized by the presence of 36 odour-active regions with FD ≥ 27. Comparisons of FD factors among non-mature and mature bourbon whiskies reveals the increased complexity of mature bourbon aroma, resulting both from the presence of maturation-derived components with high FD factors and from the increase in concentration of many other components, as already noted in Chapter 2, across maturation. The compounds with the highest FD factors (6561) were ethyl octanoate, ethyl benzoate, diethyl succinate, guaiacol, 4-ethylguaiacol, γ-nonalactone, and decanoic acid (Table 3.3). A further group of important odorants (FD factor of 1000) consisted of the low boiling volatile compounds acetaldehyde, acetic acid, and ethyl acetate among others. Interestingly, these compounds were detected at similar (FD) values among the bourbon samples, indicating that their formation originates in the distilled, whilst some other amounts might arise by interactions between the distilled and wood components during the maturation process (Reazin, 1981). Prior studies have revealed the importance of (E)-β-damascenone, γ-nonalactone, *cis*-whisky lactone, γ–decanolactone, eugenol and vanillin in the overall aroma of an American bourbon whiskey (Schieberle et al. 2008). Our results confirmed the significance of many of these compounds, although naturally there were differences between the studies in terms of FD/CHARM values, particularly because of the complexity of bourbon flavour and the individual brands selected for analysis in each case. Besides FD factors, a further way to consider the likely impact of individual compounds to the overall aroma

of a system is to consider ‘dose over threshold’. In this approach the analysed concentration of the compound is divided by its published odour threshold (where available) to produce an odour activity value (OAV; Table 3.3). A total of 15 and 24 components were present at concentrations higher than their reported odour thresholds, across non-mature and mature bourbon samples respectively. According to these OAVs, the most important odorants considered across both non-mature and mature bourbon samples (OAV > 20) were ethyl octanoate, ethyl hexanoate, ethyl acetate, acetal, 2 methyl-1 butanol, isoamyl acetate, and ethyl decanoate (Table 3.3). It is however probably more accurate to rank the significance of odorants in terms of the AEDA FD factors (Table 3.3), because this is consistent with the panellists and samples used in this study. Whereas, the calculation of odour thresholds is subject to a number of factors including the sensory methodology adopted, the number and identity of the panellists used in the study and how/ in which matrix samples are presented. Hence reported odour thresholds can vary substantially according to source; this is probably the major reason why the ranking according to OAV in Table 3.3 would be very different to that which is presented according to FD factor. Having said that, within a particular FD band (Table 3.3), the OAV provides further evidence of the likely significance of a particular odorant – in particular at the upper end of the study, where there is no information in the FD value over and above the fact that compounds were detected at the 6561-fold dilution factor. Amongst such compounds, ethyl acetate was noteworthy as being present at very high OAV’s, in both spirit types.

3.4.4.2 Identification of the most odour active compounds in non-mature and mature Tequila

As with bourbon samples, non-mature and mature tequila samples were analysed using the GC-O/AEDA approach. Results of the GC-O work identified 47 odour-active regions in the chromatograms of non-mature or mature tequila samples, taken across both the extract and direct spirit injection GC-O analyses. Table 3.4 presents data for each of these odour active regions, sorted by the flavour dilution factor obtained from AEDA analysis of the mature tequila sample. Non-mature tequila was characterized by 38 odour-active regions with flavour dilution factors (FD) $\geq$ 27.

Table 3.4. The most odour-active (FD ≥ 27) volatile compounds identified in non-mature and mature tequila, and their estimated odour-activity values (OAV). Data are sorted by FD factor in the mature tequila.

LRI (ZB-Wax)	Odour quality	Odorant	Identity	Flavour dilution factor (FD)		Odour threshold (mg/L)	OAV		Earlier reported as compound in tequila
				Non-mature tequila	Mature tequila		Non-mature tequila	Mature tequila	
1266	Strawberry, sweet	Ethyl hexanoate	B	6561	6561	0.03[b]	3	8.33	
1470	Fruity, leafy, mint	Ethyl octanoate	A, B	6561	6561	0.147[b]	33	56.60	
1863	Rose-like, fruity	2-phenylethyl acetate	A, B	6561	6561	0.108[b]	3	1.39	
1872	Rose-like, fruity	β-Damascenone	A, B	6561	6561	0.0001[b]	3320	270.00	
1911	Phenolic, smoky, flowery, green-like	Guaiacol*	A, B	729	6561	0.0092[b]	2.17	1.52	
1963	Rose-like	Phenethyl alcohol	A, B	6561	6561	2.6[b]	0.47	0.62	
1956	Flowery, lactone-like	Trans-whisky lactone*	A, B	ND	6561	0.79[b]	ND	0.39	
2033	Flowery, lactone-like	Cis-whisky lactone*	A, B	ND	6561	0.067[b]	ND	22.39	
2097	Fresh, cheese	Octanoic acid	B	6561	6561	10[e]	0.03	0.18	
2088	Flowery	4-ethyl-guaiacol*	A, B	ND	6561	0.0069[b]	ND	1.45	
2523	Caramel, vanillin, sweet	Vanillin*	A, B	729	6561	0.022[b]	0.45	40	
1547	Balsamic	2-Acetylfuran	B	2189	2189	10[a]	0.03	0.08	
1572	Lavender, flowery	Linalool	B	2189	2189	0.006[a]	68	243.33	
1704	Celery, musty, potato baked	Ethyl benzoate	A, B	2189	2189	0.06[a]	0.18	0.20	
1713	Celery, musty, potato baked	Diethyl succinate	A, B	2189	2189	1.2[e]	0.083	0.342	Benn & Peppard 1996; Prado-Jaramillo et al. 2015
1794	Rose petals	Citronellol*	B	729	2189	0.04[a]	6.5	13.25	
788	Sweet, fresh, balsamic, humidity	Acetaldehyde*[$]	A, B	1000	1000	19.2[a]	1	2	
893	Solvent-like, pungent, musty, fruity, varnish	Ethyl acetate[$]	A, B	1000	1000	12[e]	6.89	9.33	
900	Earthy, musty, fresh, solvent-like, ethereal	Acetal[$]	A, B	1000	1000	0.719[h]	68	113	
919	Alcoholic, pungent, solvent-like	Methanol[$]	A, B	1000	1000	100[b]	9	7	
954	Solvent-like, pungent, varnish, ethanol, sweet	Ethanol[$]	A, B	1000	1000	24.9[b]	----	----	
1073	Solvent-like, varnish, fruity, alcoholic, pungent	*n*-Propanol[$]	A, B	100	1000	9[b]	20	31	
1124	Solvent-like, pungent, wine	Isobutanol[$]	A, B	1000	1000	40[b]	8	9	
1515	Vinegar, sweaty, musty	Acetic acid[$]	A, B	1000	1000	75.52[h]	1	4	
1134	Fruity-like	Isoamyl acetate[$]	A, B	729	729	0.0[3c]	104.33	80	
1733	Anise, mint	α-Terpineol*	B	243	729	0.25[f]	9.76	25.12	
2209	Clove-like, anaesthesia, numb odour	Eugenol*	A, B	ND	729	0.0071[b]	ND	2.82	
2249	Sweet, waxy	Ethyl hexadecanoate	A, B	729	729	2[g]	0.02	0.04	

Table 3.4. The most odour-active (FD ≥ 27) volatile compounds identified in non-mature and mature tequila, and their estimated odour-activity values (OAV). Data are sorted by FD factor in the mature tequila (cont.).

2282	Soapy, clean	Decanoic acid	A, B	729	729	6[e]	0.12	3.07	
1300	Sweet, caramel	Dihydro-2-methyl-3(2H)-furanone	B	729	243	NA	NA	NA	
1489	Leafy	*Cis*-Linalool oxide	B	243	243	0.006[a]	60	140.00	
1624	Burnt sugar, caramel	5-Methyl furfural	A, B	243	243	16[d]	0.02	0.04	
1179	Green, fresh, solvent	*n*-Butanol*[$]	B	10	100	0.5[a]	0	2.82	
1241	Cereal, malty, musty, stale, bubble gum, solvent-like	2-methyl-1-butanol[$]	A, B	1000	100	4[b]	82	138	
1241	Cereal, malty, musty, stale, bubble gum, solvent-like	3-methyl-1-butanol[$]	A, B	1000	100	56.1[b]	9	14	
1404	Nutty, almond, sweet, fruity, green-like	Ethyl lactate[$]	A, B	100	100	14[a]	0.8	1.2	
1404	Nutty, almond, sweet, fruity, green-like	*n*-Hexanol[$]	B	100	100	8[h]	0.1	0.1	
1107	Fruity, fatty, herbaceous	Isovaleraldehyde, diethyl acetal*	B	ND	81	NA	ND	NA	
1340	Solvent-like, varnish	β-Ethoxypropionaldehyde diethyl acetal*	A, B	ND	81	NA	ND	NA	
1509	Flowery, almond	Furfural	A, B	81	81	15[d]	0.05	0.15	
1684	Fruity, grape	Ethyl decanoate	A, B	81	81	0.5[e]	4	12.64	
1880	Fruity, mango	Ethyl dodecanoate*	B	27	81	5.9[g]	0.07	0.12	
2084	Solvent-like	Ethyl tetradecanoate*	B	ND	81	4[g]	ND	0.01	
1295	Solvent-like	Isobutanal diethyl acetal*	B	ND	27	NA	ND	NA	
1555	Sweet, solvent-like	3-Ethyl-4-methyl-1-pentanol *	B	ND	27	NA	ND	NA	NA
976	Butter	Diacetyl	A, B	27	ND	0.0023 - 0.0065[a]	ND	115	Benn & Peppard 1996; Prado-Jaramillo et al. 2015
2185	Solvent-like, fresh, fatty	Ethyl 4-ethoxybenzoate	B	81	ND	NA	ND	NA	

ND: not detected under the condition of analysis. NA: Odour threshold data not available in the literature.

A, B: Compounds were identified by EI-MS library matching (NIST), comparison against authentic standards and confirmation of their linear retention index (LRI) against published values for a DB-Wax column.

B: Compounds were identified by EI-MS library matching (NIST), and confirmation of their linear retention index (LRI) against published values for a DB-Wax column

Odour threshold references: [a]: http://www.leffingwell.com/odorthre.htm; [b]: Schieberle et al. (2008); [c]: Ferreira et al. (2002); [d]: Franco et al. (2004); [e]: Peinado et al. (2004); [f]: Lopez et al. (2002) [g]: Pino et al. (2011). [h]: Uselmann et al. (2015).

Odour thresholds from most of the references were determined in hydroalcoholic solutions (Ethanol 10 and 40%), with the exception of ref [a] which was determined in water.

* Compounds with a significant difference in odour potency between non-mature and mature tequila samples.

[$] Flavour dilution factors (FD) optimized for the analysis of the influence of major volatile compounds into the overall aroma of spirits flavour.

---: data not available since the concentration was not determined

Note: an average delay of 18 s was found between the detection of the compounds by GC-MS and panellist's nose during the GC-O analysis

The compounds with the highest FD factors (6561) were ethyl hexanoate, ethyl octanoate, 2-phenethyl acetate, phenethyl alcohol, octanoic acid and β-damascenone. The individual aroma descriptors associated with these compounds (Table 3.4) include qualities such as fruity, rose-like, flowery, or cheese-like. A second important group of components (FD of 2187) consisted of isoamyl alcohol, the combined contribution of two co-eluting esters (ethyl benzoate/diethyl succinate), linalool and 2-acetylfuran. As noted for non-mature bourbon, some of the most potent odorants detected in the AEDA study in the non-mature tequila are likely to be due to the previous stages of tequila production such as the cooking, fermentation and/or distillation steps (Benn & Peppard, 1996). For example, β-damascenone is more likely degradation product of carotenoids (Mordi, 1993).

Mature tequila was characterized by the presence of 45 odour-active regions with FD ≥ 27 (Table 3.4). Comparison between results for non-mature and mature tequilas reveals an increased complexity of mature tequila aroma, resulting both from the presence of maturation-derived components with high FD factors and from the increase in concentration of many other components across maturation. The compounds with the highest FD factors (6561) were isoamyl alcohol, phenethyl alcohol, ethyl hexanoate, ethyl octanoate, 2-phenylethyl acetate, β-damascenone, guaiacol, 4-ethyl-guaiacol, vanillin, *cis* & *trans*-whisky lactone, and octanoic acid. A further group of odorants (FD factor of 2187 and 1000) consisted of the combined contribution of two co-eluting esters (ethyl benzoate, diethyl succinate), 2-acetylfuran, isobutanol, linalool, citronellol and several low boiling volatile compounds (Table 3.4). Lopez-Ramirez, et al. (2013) studied the physicochemical changes that arise as a consequence of tequila barrel maturation, and included a considerable increase

in higher alcohols, methanol, esters, acetaldehyde, and furan-2-carboxaldehyde (furfural) content in the first weeks of maturation. Our results are in accordance with these findings; since increases in the concentrations of these major compounds were observed in the matured tequila (Table 2.2). Furthermore, 3-methylbutanal, isoamyl alcohol, β-damascenone, 2-phenyethanol, phenylethyl acetate, and vanillin have been shown to be important to the overall aroma of different classes of tequila (Been & Peppard, 1998 and López & Dufour, 2001), particulally to the ageing types. These compounds were also identified in the present study as key odorants of tequila flavour (Table 3.4), albeit with differing FD/CHARM values in each case. In addition, other important congeners were newly introduced during this study, including some ethyl esters, mature relate compounds (oak lactones), furans (2-acetyl furan), and terpens (linalool). This gives futher evidence of the likely complexity of tequila production and how different technological treatmets can derive in completely different sensorially products. Additionally, Table 3.4 illustrates the potential odour impact of individual compounds in terms of odour activity value (OAV). In this aspect, 27 components were present at concentrations higher than their reported odour thresholds, across both tequila samples. According to these OAVs, the most important odorants in the non-mature and mature tequila samples (OAV > 20) were diacetyl, *cis*-linalool oxide, isoamyl acetate, *n*-propanol, 2-methyl-1-butanol, ethanol, acetal, ethyl acetate, linalool, β-damascenone and ethyl octanoate. Furthermore, α-terpineol, vanillin and *cis*-whisky lactone presented OAVs higher than 20 only in mature tequila (Table 3.4). Interestingly, β-damascenone was significant as being present at very high OAV's, particularly in the non-mature tequila, therefore providing evidence of its likely significance to tequila flavour.

3.4.4.3 Identification of the most odour active compounds in a non-mature and mature Malt whiskies (Distillery A and B).

As with the other spirit samples, GC-O/AEDA analysis was conducted on the new make spirits and malt whiskies from distilleries 'A' and 'B' and the results of the most odour-active compounds (FD ≥ 27) are shown in Tables 3.5 and 3.6. The samples yielded between 38 and 48 odour-active regions among the new make and malt whiskies from both distilleries (A and B) with flavour dilution factors ranging from 27 to 6561; this data has been sorted by flavour dilution factors (FD) in the mature spirits. Furthermore, other important parameters are also displayed such as the odour-activity values (OAV's). In terms of the number of odour active regions/areas (OAA), malt whiskies spirits were more complex than new make spirits, due to the presence of a higher number of OAA (Tables 3.7). Interestingly, these same spirits were characterized by presenting the higher number of the most odour-active compounds (OAC) in the most diluted sample (FD ≥ 6561). Although both sets of spirit samples (new make spirits and malt whiskies) were characterized by presenting alcohols, acids, aldehydes and ethyl esters in the odour-active areas (OAA), the compounds that differentiated them were mainly volatile-phenols (e.g. guaiacols, vanillin) and oak lactones (*cis/trans* whisky lactones). These ageing congeners are known as important contributors of mature character in aged spirits such as bourbon and Scotch whisky (Escalona et al. 2002). Regarding the individual components, which were the most powerful odorants (FD ≥ 6561) in each spirit type; new make spirits were characterized by presenting fewer odorants in comparison to the malt whiskies (Tables 3.5 and 3.6). The compounds with the highest FD factor (6561) among new make spirits and malt whiskies included compounds such as

ethyl hexanoate, ethyl octanoate, ethyl benzoate/diethyl succinate, among others. Interestingly, at this FD factor (6561) is where the ageing congeners (volatile phenols and oak lactones) are detected (Tables 3.5 and 3.6).

Table 3.5. The most odour-active (FD ≥ 27) volatile compounds identified in new make spirit and malt whisky from Distillery A, and their estimated odour-activity values (OAV). Data are sorted by FD factor in the malt whisky 'A'

LRI (ZB-Wax)	Odour quality	Odorant	Identity	Flavour dilution factor (FD)		Odour threshold (mg/L)	OAV		Earlier reported as compound in whisky
				NMSA	MWA		NMSA	MWA	
1268	Strawberry, fruity, sweet	Ethyl hexanoate	A, B	6561	6561	0.03[b]	41	38	Schieberle et al. 2008
1385	Resin, grass, fresh, flowery, green-like	*n*-Hexanol	A, B	6561	6561	2.5[a]	1.4	2.2	Camara et al. 2007
1468	Green leafy, grass, anise, wet bricks, menthol	Ethyl octanoate	A, B	6561	6561	0.147[b]	94	74	Schieberle et al. 2008
1640	Sweet, caramel, burnt sugar	5-Methyl furfural*	A, B	729	6561	16[d]	0.01	0.03	Camara et al. 2007
1725	Musty, flowery, sweaty potato, fruity, flowery	Ethyl benzoate	A, B	6561	6561	0.056[a]	26	20	Boothroyd, 2013
1725	Musty, flowery, sweaty potato, fruity, flowery	Diethyl succinate	A, B	6561	6561	1.2[e]	0.2	0.2	Boothroyd, 2013
1867	Rose-like, sweet	2-phenylethyl acetate*	A, B	27	6561	0.108[b]	60	75	Schieberle et al. 2008
1901	Sweaty, cheesy	Hexanoic acid	A, B	6561	6561	3[e]	1.1	1	Conner, 1993
1912	Lactone-like, phenolic, flowery, smoky	Guaiacol*	A, B	ND	6561	0.0092[b]	ND	14.8	Schieberle et al. 2008
1944	Flowery, lactone-like, coconut, sweet	Trans-whisky lactone*	A, B	ND	6561	0.79[b]	ND	0.3	Schieberle et al. 2008
1979	Rose-like, flowery	Phenethyl alcohol	A, B	6561	6561	2.6[b]	27	26	Schieberle et al. 2008
2014	Flowery, lactone-like, lilac	Cis-whisky lactone*	A, B	ND	6561	0.067[b]	ND	30	Schieberle et al. 2008
2074	Phenolic, flowery, lilac, rose-like, sweet	4-ethyl-guaiacol*	A, B	ND	6561	0.0069[b]	ND	27	Schieberle et al. 2008
2091	Cheese, sweaty, fatty	Octanoic acid	B	6561	6561	10[g]	1.9	1.8	Camara et al. 2007
2195	Honey, anaesthesia, sweet, clove-like	Eugenol*	A, B	ND	6561	0.0071[g]	ND	37	Schieberle et al. 2008
1539	Almond, caramel, burnt sugar	Furfural*	A, B	6561	6561	15[d]	0.6	0.8	Boothroyd, 2013
2273	Soapy, freshly bathed	Decanoic acid	B	6561	6561	6[e]	3	3.8	Camara et al. 2007
1555	Balsamic, humidity	2-Acetylfuran	A, B	6561	2187	10[a]	0.02	0.02	Boothroyd, 2013
1574	Almond, burnt sugar, caramel, sweet	Benzaldehyde	A, B	6561	2187	2[e]	0.2	0.1	Boothroyd, 2013
1671	Fruity, grape, wine, sweet	Ethyl decanoate*	A, B	81	2187	0.5[e]	138	94	Boothroyd, 2013
1794	Scented wax, flowery, sweet	*n*-Decanol	B	2187	2187	0.4[c]	0.8	2.7	Conner, 1993
2174	Scented candle, flowery	*n*-Hexadecanol	B	6561	2187	0.75[g]	1.7	1.4	Camara et al. 2007
2244	Scented candle, waxy	Ethyl hexadecanoate*e	A, B	243	2187	2[g]	7.4	6.1	Boothroyd, 2013
2266	Scented candle, waxy	Ethyl 9-hexadecenoate*	B	27	2187	NA	NA	NA	Camara et al. 2007
2348	Scented candle, flowery	(9E)-9-Hexadecen-1-ol	B	6561	2187	NA	NA	NA	Camara et al. 2007
788	Sweet, fresh, balsamic, humidity	Acetaldehyde[$]	A, B	1000	1000	19.2[a]	0.82	0.8	Schieberle et al. 2008
893	Solvent-like, pungent, musty, fruity, varnish	Ethyl acetate[$]	A, B	1000	1000	12[e]	23.08	20.83	Schieberle et al. 2008
900	Earthy, musty, fresh, solvent-like, ethereal	Acetal[$]	A, B	1000	1000	0.72[h]	284	255	Camara et al. 2007
919	Alcoholic, pungent, solvent-like	Methanol[$]	A, B	1000	1000	100[b]	0.2	0.3	Conner, 1993
954	Solvent-like, pungent, varnish, ethanol, sweet	Ethanol[$]	A, B	1000	1000	24.9[b]	----	----	Schieberle et al. 2008

Table 3.5. The most odour-active (FD ≥ 27) volatile compounds identified in new make spirit and malt whisky from Distillery A, and their estimated odour-activity values (OAV). Data are sorted by FD factor in the malt whisky 'A' (cont.).

1073	Solvent-like, varnish, fruity, alcoholic, pungent	*n*-Propanol[$]	A, B	1000	1000	9[b]	32	31	Schieberle et al. 2008
1124	Solvent-like, pungent, wine	Isobutanol[$]	A, B	1000	1000	40[b]	14	13	Schieberle et al. 2008
1140	Fruity, sweet, musty	Isoamyl acetate*[$]	A, B	100	1000	0.245[h]	102	106	Camara et al. 2007
1183	Alcohol, fruity, sweet, cough syrup, fresh	*n*-Butanol*[$]	A, B	10	1000	0.5[b]	13	21	Schieberle et al. 2008
1241	Cereal, malty, musty, stale, bubble gum, solvent-like	2-methyl-1-butanol[$]	A, B	1000	1000	4[b]	161	176	Schieberle et al. 2008
1241	Cereal, malty, musty, stale, bubble gum, solvent-like	3-methyl-1-butanol[$]	A, B	1000	1000	56.1[b]	14	15	Schieberle et al. 2008
1404	Nutty, almond, sweet, fruity, green-like	Ethyl lactate[$]	A, B	1000	1000	14[a]	1.3	0.2	Camara et al. 2007
1515	Vinegar, sweaty, musty	Acetic acid[$]	A, B	1000	1000	75.52[h]	0.5	3.1	Camara et al. 2007
1404	Fresh, fatty, flowery, sweet	3-Ethoxy-1-propanol	B*	ND	729	NA	NA	NA	NA
1282	Humidity, cardboard wet	Isobutanal diethyl acetal	B*	ND	243	NA	NA	NA	NA
1883	Mango-like, sweet, fruity	Ethyl dodecanoate	B*	27	243	5.9[g]	4	2	Boothroyd, 2013
2442	Fatty, soapy	Dodecanoic acid	B	729	243	10[a]	0.6	0.5	Camara et al. 2007
2521	Sweet, caramel, vanilla	Vanillin*	A, B	243	6561	0.022[b]	1.9	95	Schieberle et al. .2008
1891	Fruity-like	Isoamyl decanoate	B*	ND	81	NA	ND	NA	Camara et al. 2007
2003	Mandarin, fruity, sweet	*n*-Undecanol	A, B*	27	81	6.25[a]	0.04	0.05	Camara et al. 2007
1275	Strawberry, fruity	*n*-Pentanol	A, B*	ND	27	0.147[a]	4	3.7	NA
1769	Sweet, potato baked	Methionol	B	6561	ND	1[c]	0.2	ND	Boothroyd, 2013

ND: not detected under the condition of analysis. NA: Odour threshold data not available in the literature.
A, B: Compounds were identified by EI-MS library matching (NIST), comparison against authentic standards and confirmation of their linear retention index (LRI) against published values for a DB-Wax column.
B: Compounds were identified by EI-MS library matching (NIST), and confirmation of their linear retention index (LRI) against published values for a DB-Wax column
Odour threshold references: [a]: http://www.leffingwell.com/odorthre.htm; [b]: Schieberle et al. (2008); [c]: Ferreira et al. (2002); [d]: Franco et al. (2004); [e]: Peinado et al. (2004); [f]: Lopez et al. (2002) [g]: Pino et al. (2011). [h]: Uselmann et al. (2015).
Odour thresholds from most of the references were determined in hydroalcoholic solutions (Ethanol 10 and 40%), with the exception of ref [a] which was determined in water.
* Compounds with a significant difference in odour potency between non-mature and mature tequila samples.
[$] Flavour dilution factors (FD) optimized for the analysis of the influence of major volatile compounds into the overall aroma of spirits flavour.
---: data not available since the concentration was not determined
Note: an average delay of 18 s was found between the detection of the compounds by GC-MS and panellist's nose during the GC-O analysis

Table 3.6. The most odour-active (FD ≥ 27) volatile compounds identified in new make spirit and malt whisky from Distillery B, and their estimated odour-activity values (OAV). Data are sorted by FD factor in the malt whisky 'B'

LRI (ZB-Wax)	Odour quality	Odorant	Identity	Flavour dilution factor (FD)		Odour threshold (mg/L)	OAV		Earlier reported as compound in whisky
				NMSB	MWB		NMSB	MWB	
1258	Bubble gum, strawberry, fruity	Ethyl hexanoate	A, B	6561	6561	0.03[b]	43	60	Schieberle et al. 2008
1463	Green leafy, herbaceous odour, menthol	Ethyl octanoate	A, B	6561	6561	0.147[b]	82	125	Schieberle et al. 2008
1569	Burnt sugar, sweet, almond	Benzaldehyde	A, B	6561	6561	2[e]	0.16	0.09	Boothroyd, 2,013
1726	Musty, sweaty, fabric, potato	Ethyl benzoate	A, B	6561	6561	0.056[a]	21	19	Boothroyd, 2013
1726	Musty, sweaty, fabric, potato	Diethyl succinate	A, B	6561	6561	1.2[e]	0.16	0.10	Boothroyd, 2013
1791	Flowery, waxy	*n*-Decanol	A, B	6561	6561	0.4[c]	0.87	0.40	Conner, 1993
1530	Almond, caramel, burnt sugar	Furfural	A, B	6561	6561	15[d]	0.6	0.6	Boothroyd, 2013
1908	Lactone-like, flowery, phenolic, smoky	Guaiacol*	A, B	ND	6561	0.0092[b]	ND	15	Schieberle et al. 2008
1935	Flowery, lactone-like, coconut	Trans-whisky lactone*	A, B	ND	6561	0.79[b]	ND	0.31	Schieberle et al. 2008
1959	Flowery, rose-like, honey	Phenethyl alcohol	A, B	6561	6561	2.6[b]	24	15	Schieberle et al. 2008
2010	Lactone-like, coconut, sweet, flowery	Cis-whisky lactone*	A, B	ND	6561	0.067[b]	ND	25	Schieberle et al. 2008
2075	Flowery, lactone-like, phenolic	4-ethyl-guaiacol*	A, B	ND	6561	0.0069[b]	ND	26	Schieberle et al. 2008
2094	Cheese, moss, fresh	Octanoic acid	A, B	6561	6561	10[g]	2.40	3.36	Camara et al. 2007
2176	Flowery, waxy, sweet	*n*-Hexadecanol*	B	729	6561	0.75[g]	0.54	0.81	Camara et al. 2007
2193	Flowery, honey, clove-like	Eugenol*	A, B	ND	6561	0.0071[g]	ND	33	Schieberle et al. 2008
2351	Flowery, waxy	(9E)-9-Hexadecen-1-ol*	B	729	6561	NA	ND	NA	Camara et al. 2007
2448	Soapy, fatty	Dodecanoic acid*	B	729	6561	10[a]	0.36	0.46	Camara et al. 2007
2525	Caramel, vanillin, sweet	Vanillin*	A, B	243	6561	0.022[b]	1.41	87	Schieberle et al. 2016
1272	Fruity	*n*-Pentanol*	B	27	2187	0.147[a]	4.50	3.09	NA
1391	Resin, green-like, herbaceous odour, flowery	*n*-Hexanol*	A, B	1000	2187	2.5[a]	0.2	1.43	Camara et al. 2007
1546	Balsamic, wet brick, humidity	2-Acetylfuran	A, B	6561	2187	10[a]	0.058	0.01	Boothroyd, 2013
1614	Burnt sugar, caramel, sweet	5-Methyl furfural*	A, B	81	2187	16[d]	0.02	0.02	Camara et al. 2007
1892	Sweaty, cheese	Hexanoic acid	A, B	6561	2187	3[e]	1.84	2.51	Conner, 1993
2246	Cheese, mild sweet	Ethyl hexadecanoate*	A, B	729	2187	2[g]	6.43	2.61	Boothroyd, 2013
2277	Soapy, fatty	Decanoic acid	A, B	2187	2187	6[e]	3.74	4.93	Camara et al. 2007
788	Sweet, fresh, balsamic, humidity	Acetaldehyde[$]	A, B	1000	1000	19.2[a]	0.3	0.8	Schieberle et al. 2008
893	Solvent-like, pungent, musty, fruity, varnish	Ethyl acetate[$]	A, B	1000	1000	12[e]	16.3	19.33	Schieberle et al. 2008
900	Earthy, musty, fresh, solvent-like, ethereal	Acetal[$]	A, B	1000	1000	0.72[h]	182	232	Camara et al. 2007
919	Alcoholic, pungent, solvent-like	Methanol[$]	A, B	1000	1000	100[b]	0.2	0.2	Conner, 1993
954	Solvent-like, pungent, varnish, ethanol, sweet	Ethanol[$]	A, B	1000	1000	24.9[b]	---	----	Schieberle et al. 2008
1073	Solvent-like, varnish, fruity, alcoholic, pungent	*n*-Propanol[$]	A, B	1000	1000	9[b]	34	23	Schieberle et al. 2009

Table 3.6. The most odour-active (FD ≥ 27) volatile compounds identified in new make spirit and malt whisky from Distillery B, and their estimated odour-activity values (OAV). Data are sorted by FD factor in the malt whisky 'B' (cont.).

1124	Solvent-like, pungent, wine	Isobutanol[$]	A, B	1000	1000	40[b]	14	10	Schieberle et al. 2010
1141	Fruity, sweet, musty	Isoamyl acetate[$]	A, B	243	1000	0.245[h]	268	68	Camara et al. 2007
1241	Malty, solvent, sweet, fruity	2-methyl-1-butanol[$]	A, B	1000	1000	4[b]	167	148	Schieberle et al. 2008
1241	Malty, solvent, sweet, fruity	3-methyl-1-butanol[$]	A, B	1000	1000	56.1[b]	14	14	Schieberle et al. 2008
1404	Nutty, almond, sweet, fruity, green-like	Ethyl lactate[$]	A, B	1000	1000	14[a]	0.3	0.3	Camara et al. 2007
1515	Vinegar, sweaty, musty	Acetic acid[$]	A, B	1000	1000	75.52[h]	1	4	Camara et al. 2007
1280	Wet brick, humidity, old house	Isobutanal diethyl acetal[$]*	B	243	729	NA	NA	NA	Camara et al. 2007
1404	Wet brick, humidity, green-like	3-Ethoxy-1-propanol	B	6561	729	NA	NA	NA	NA
1665	Grape, fruity, wine, sweet	Ethyl decanoate	A, B	729	729	0.5[e]	88	119	Boothroyd ,2013
2268	Fatty-like, mild sweet	Ethyl 9-hexadecenoate	B	729	729	NA	ND	NA	Camara et al. 2007
1327	Fruity, sweet, balsamic, humidity	β-Ethoxypropionaldehyde diethyl acetal*	B	ND	243	NA	NA	NA	NA
1864	Fruity, sweet, rose-like	2-phenylethyl acetate	A, B	243	243	0.108[b]	75	38	Schieberle et al. 2008
1172	Alcohol, fruity, sweet, cough syrup, fresh	*n*-Butanol	A, B	243	100	0.5[b]	4	14	Schieberle et al. 2008
1874	Fruity, sweet	Ethyl dodecanoate	B	243	81	5.9[g]	2	1.76	Boothroyd, 2013
1906	Rose-like, flowery	Unknown*	C	ND	81	NA	NA	NA	NA
1991	Fruity, citric, mandarin	*n*-Undecanol	B	243	81	6.25[a]	0.02	0.02	Camara et al. 2007
2061	Varnish, solvent-like	Ethyl tetradecanoate*	B	ND	81	4[g]	ND	0.19	Camara et al. 2007
1753	Potato, sweet	Methionol	B	6561	ND	1[c]	0.22	ND	Conner, 1993
1884	Fruity, sweet, honey, flowery	Isoamyl decanoate	B	729	ND	NA	NA	NA	Camara et al. 2007

ND: not detected under the condition of analysis. NA: Odour threshold data not available in the literature.

A, B: Compounds were identified by EI-MS library matching (NIST), comparison against authentic standards and confirmation of their linear retention index (LRI) against published values for a DB-Wax column.

B: Compounds were identified by EI-MS library matching (NIST), and confirmation of their linear retention index (LRI) against published values for a DB-Wax column

C: Compounds which were not identified by any of the identification methods (A, B) available.

Odour threshold references: [a]: http://www.leffingwell.com/odorthre.htm; [b]: Schieberle et al. (2008); [c]: Ferreira et al. (2002); [d]: Franco et al. (2004); [e]: Peinado et al. (2004); [f]: Lopez et al. (2002) [g]: Pino et al. (2011). [h]: Uselmann et al. (2015)

Odour thresholds from most of the references were determined in hydroalcoholic solutions (Ethanol 10 and 40%), with the exception of ref [a] which was determined in water.

* Compounds with a significant difference in odour potency between non-mature and mature tequila samples.

[$] Flavour dilution factors (FD) optimized for the analysis of the influence of major volatile compounds into the overall aroma of spirits flavour.

---: data not available since the concentration was not determined

Note: an average delay of 18 s was found between the detection of the compounds by GC-MS and panellist's nose during the GC-O analysis

Further contributors to spirits aroma were the low boiling volatile compounds (e.g. acetaldehyde, ethyl acetate, acetal, and methanol), which were present at similar flavour intensities (FD ≥ 1000) in mature and non-matured spirit samples, indicating that their formation most likely originated in the distilled or possibly in alternative transformations during the ageing process (Reazin, 1981). These compounds confer wide odour descriptors to spirits aroma such as fruity, solventy, phenolic, flowery, and fatty notes respectively (Tables 3.5 and 3.6). Also, among the odourants, which were exclusive to a particular spirit type, methionol was a compound only detected in the new make spirits at high odour potency, basically because it is a yeast derived product originated during fermentation and with a very low odour threshold (0.005–0.0002 μg/mL) (Swiegers et al. 2005). Furthermore, this sulphur compound has been reported to increase its removal by charred wood during maturation (Nishimura & Matsuyama, 1989), this could explain its absence from the mature products (Tables 3.5 and 3.6). Although compounds of low odour potency (FD ≤ 6561) might not have the same impact on the flavour perception as the odourants of high potency (FD ≥ 6561), their presence is undoubtedly indispensable for achieving the right balance in aroma and sensory properties in the alcoholic beverage. Previous studies (Conner, et al. 2001; Poisson and Schieberle, 2008) have suggested the high importance of *cis*-whisky lactone, vanillin, β-damascenone, γ-nonalactone, γ-decanolactone, and eugenol to whisky flavour. Some of these compounds were identified during this study, however others were newly identified including compounds such ethyl hexanoate, phenethyl alcohol, octanoic acid, furfural, among others (Table 3.5 and 3.6). These results as with tequila, give evidence of the highly impact of the technological

treatments (cooking, distilling, ageing) during spirit production and their effect on the flavour and sensory characteristics of the final product.

Tables 3.5 and 3.6 indicate the likely sensory impact of individual compounds in terms of odour activity value (OAV). Around 20 to 29 components were present at concentrations higher than their reported odour thresholds, across both new make spirits and malt whiskies from distilleries A and B. According to these OAVs, the most important odorants (OAV $\geq$ 20) which were common to both spirits types (new makes and malt whiskies) were ethyl hexanoate, ethyl octanoate, ethyl benzoate, 2-phenethyl acetate, phenethyl alcohol, ethyl decanoate, ethyl acetate, *n*-propanol, isoamyl acetate, and 2-methyl-*1*-butanol. Interestingly *cis*-whisky lactone, 4-ethyl guaiacol, eugenol, *n*-butanol, and vanillin were significant as being present at very high OAV's, particularly in the malt whiskies. Undoubtedly, because of the highly influence of the ageing process. However, it is worth noting to highlight that the novelty of our results relies in the full comparison and contrast of the impacts of wood ageing on the aroma and sensory properties of these four different spirits, including GC-O characterization. Therefore, these findings are highly valuable in the field of food and flavour analysis of spirit samples.

Making comparisons across the set of four non-mature and mature spirits, non-mature spirits were less complex than mature ones, basically by presenting a reduction in terms of odour active regions (Table 3.7). Additionally, compounds that were associated with ageing were; guaiacol, 4-ethyl guaiacol, *cis/trans*-whisky lactones, eugenol and vanillin, which were consistently increased in odour potency or flavour dilution factor (FD) or only detected in mature spirits (Table 3.7). Results obtained from the GC-O/AEDA work enabled us to propose

groups of congeners that comprised the woody and estery characters of each of the spirit samples. In general, the woody fraction was defined by the increased in FD factor of volatile phenols and oak-lactones in the mature spirits, and indeed earlier studies have highlighted them as important contributors of the mature character in ageing spirits (Clyne et al. 1993; Withers et al. 1995; Mosedale, 1998). The estery fraction was basically defined by the presence of ethyl esters and acetate esters of varying FD factors such as ethyl acetate, ethyl hexanoate and ethyl octanoate, however their contribution in each of the spirit types was significantly different and varied according to the individual production processes (raw materials, fermentation, distillation etc.). The composition of these fractions, based on the work in this Chapter, will be described in detail in Chapter 4.

Table 3.7. Summary of the number of odour active regions/areas (OAA) and maturation markers detected along the set of spirits obtained from GC-O/AEDA approach.

Maturation Marker/ No. OAA	**NON-MATURE SPIRITS**				**MATURE SPIRITS**			
	Borb.	***Teq.***	***NMSA***	***NMSB***	***Borb.***	***Teq.***	***MWA***	***MWB***
	25	38	38	43	36	45	46	48
Guaiacol	nd	nd	nd	nd	*	*	*	*
4-Ethyl guaiacol	nd	nd	nd	nd	*	*	*	*
Eugenol	nd	nd	nd	nd	*	*	*	*
Vanillin	nd	nd	nd	nd	*	*	*	*
cis/trans-whisky lactone	nd	nd	nd	nd	*	*	*	*
γ-nonalactone	nd	nd	nd	nd	*	nd	nd	nd

Nd: not detected; *: detected.

3.4.5 Sensory evaluation of spirit samples

The aroma profile of the non-mature and mature versions of all spirit samples were nosed by a trained sensory panel and scored for each of 16 aroma characters (Figure 3.3). Non-mature tequila (Figure 3.3A) was predominantly *pungent*, *green/grassy*, and *woody*, whilst the mature tequila showed the expected reduction in *pungent* and *feinty* characters, with strong enhancement of *green/grassy*, *floral* and *spicy* attributes. The panel scored *feinty* as the major attribute in the non-mature bourbon (Figure 3.3B), followed by *cereal, pungent,* and *stale* impressions. In contrast, mature bourbon was characterised by *woody, spicy*, *floral* and *dried fruit* notes, followed by *sweet*. New make malt whisky spirit 'A' (Figure 3.3C) was perceived to have a strong *feinty, sulphury* and *pungent* aroma character, followed by a *cereal* note, in contrast to the matured product which had a more balanced character with *spicy*, *oily*, *woody*, *sweet*, *floral* and *fresh fruit* characteristics all being enhanced relative to the non-mature version. Lastly, new make spirit 'B' (Figure 3.3D) was predominantly *feinty* and *pungent* whereas the mature malt whisky 'B' showed enhancements in the same attributes noted for malt whisky A on maturation (Figure 3.3D) with reduced *feinty, cereal* and *green/grassy* notes.

Comparing across spirit types (Figure 3.3), *feinty, cereal, woody*, *spicy*, and *sulphury* aroma qualities were the attributes that were scored significantly differently between the 4 non-mature spirit samples ($P < 0.05$). For mature spirits, *cereal, green/grassy, floral, solventy, dried fruit, sweet, woody, spicy, sour,* and *sulphury* were rated significantly differently dependent on spirit type ($P < 0.05$). Mature bourbon whiskey was the spirit which presented the highest

intensity of mature attributes (*woody*, *spicy*, *sweet* and *dried fruit*), most likely because it was the spirit with the longest period of maturation (five years) among the samples. Additionally, these aroma differences could be associated with the technology of maturation implemented and the characteristics of the cask used for ageing (e.g. new versus re-use) in each spirit production. However, the ageing time is among the key factors affecting the flavour of the maturing spirit, which will vary according to the characteristics of the raw distillate, the size, wood origins and treatment of the cask and the environment in which the spirit is matured (Reazin, 1981).

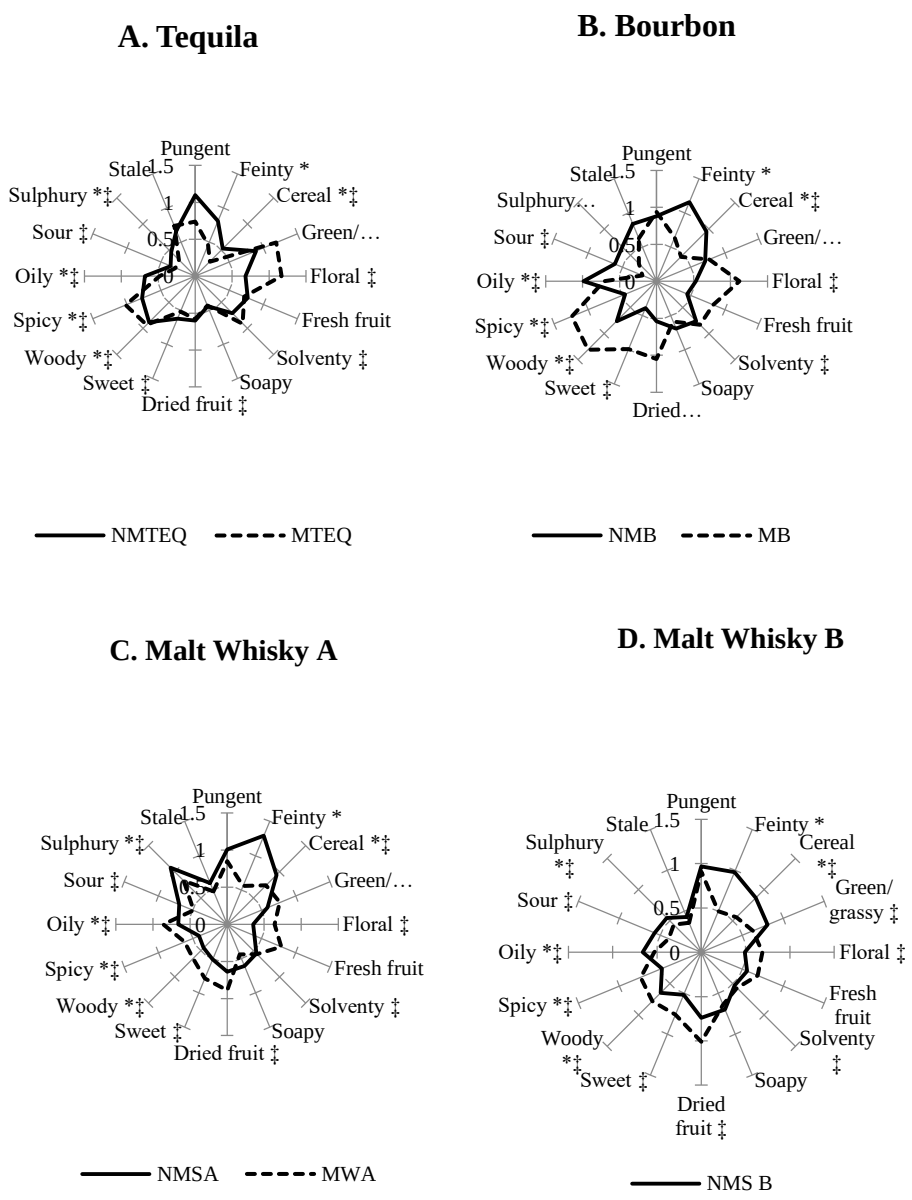

Figure 3.3. Comparison of aroma profiles among non-mature and matures spirit samples of (A) bourbon whiskey, (B) tequila, (C) malt whisky A and (D) malt whisky B.

Key: NMSA: new make spirit from distillery ‘A’; NMSB: new make spirit from distillery ‘B’; NMB: non-mature bourbon; NMTEQ: non-mature tequila; MWA: malt whisky from distillery ‘A’; MWB: malt whisky from distillery ‘B’; MB: mature bourbon; MB: mature bourbon; MTEQ: mature tequila. *Indicates statistically significant difference among sensory attributes between non-mature spirits ($P < 0.05$). +indicates statistically significant difference among sensory attributes between mature spirits ($P < 0.05$).

3.4.5.1 Correlation between sensory and instrumental data

We decided to carry out a correlation analysis between the quantitive analytical date set and sensory scores for the spirit aroma characters to see if this would highlight potential associations between them and help identify the main congeners resposible for the mature character in the spirit samples. In the first instance, Pearson correlation analysis was performed, and the correlation coefficients are reported in Table 3.7. Fifty compounds showed significant correlations between their spirit concentrations and the sensory data. Some correlations were positive whereas others were negative, which suggests, that the perception of an aromatic note is influenced not only by the presence of a few components which combine to generate the perceived aroma, but also by the presence of other odourants that affect negatively the perception of such aromas (Aznar, Lopez, Cacho, & Ferreira, 2003). However, it must be noted before discussing these in any depth that correlation merely shows co-variation between concentrations of a compound and a particular spirit character, but does not prove a causal link. This can be observed for example with the woody sensory character, which, sensibly enough, was positively correlated with both cis and trans whisky lactone concentrations (r = 0.82, 0.81 respectively). However, other compounds which increased in concentration with maturation time, and thus broadly co-varied with woody compound concentrations, would not be expected to contribute a woody sensory character (e.g. acetic acid, r = 0.75, which increases through maturation due to oxidation). Likewise, compounds which correlated negatively with woody character were mainly those which were present at higher concentrations in the new make spirits, and showed high FD factors (e.g. 3-Ethoxy-1-propanol), although one cannot

completely rule out there being a sensory basis to some of the noted correlations. Interestingly there were no strong positive correlations with *pungency*, although isobutanal diethyl acetal showed a negative correlation (r = -0.80) to this attribute. During the GC-O analysis this compound was described with a solvent-like and related notes, which to some extend correlated with the previous sensory attribute. However, its perception (FD-factor) varied among the spirits in which it was detected (tequila and malt whiskies). This is to be expected, since the concentrations in those products was different.

Table 3.8. *Significant Pearson Correlations between aroma compounds and sensory attributes.

No.	Aroma compounds	Sensory attributes															
		Pungent	Feinty	Cereal	Green/ grassy	Floral	Fresh fruit	Solventy	Soapy	Dried fruit	Sweet	Woody	Spicy	Oily	Sour	Sulphury	Stale
1	Acetaldehyde	---	---	-0.81	---	0.81	---	---	---	---	---	---	0.72	---	---	---	---
2	Ethyl acetate	---	---	---	---	---	---	---	---	0.77	---	---	---	---	---	---	---
3	Acetal	---	---	---	---	---	---	---	---	0.76	---	---	---	---	---	---	---
4	Methanol	---	---	---	0.75	---	---	---	---	---	---	---	---	---	---	---	---
5	Isoamyl acetate	---	---	---	---	---	---	---	---	---	---	---	---	0.72	---	---	---
6	3-Penten-2-ol	---	---	---	---	---	---	---	---	---	---	---	---	0.73	---	---	---
7	Ethyl hexanoate	---	---	---	---	---	---	---	0.74	---	---	---	---	---	---	---	---
8	*n*-Pentanol	---	---	---	---	---	---	---	---	---	0.75	0.76	---	---	---	---	---
9	Isobutanal diethyl acetal	-0.80	---	-0.73	0.80	0.75	---	---	---	---	---	---	---	---	---	---	---
10	Dihydro-2-methyl-3(2H)-furanone	---	---	-0.71	0.91	---	---	---	---	---	---	---	---	---	---	---	---
11	β-Ethoxypropionaldehyde diethyl	-0.74	---	---	0.83	---	---	---	---	---	---	---	---	---	---	---	---
12	3-Ethoxy-1-propanol	---	---	---	---	---	---	-0.88	---	---	---	-0.77	---	---	---	---	---
13	Cis Linalool oxide	---		-0.71	0.92	---	---	---	---	---	---	---	---	---	---	---	---
14	Acetic acid	---	-0.81	-0.73	---	0.81	0.74	---	---	0.75	0.81	0.75	0.85		-0.82	---	---
15	3-Ethyl-4-methyl-1-pentanol	---	---	-0.71	0.92	---	---	---	---	---	---	---	---	---	---	---	---
16	2-Acetyl furan	---	---	---	0.74	---	---	---	---	---	---	---	---	---	---	---	---
17	Benzaldehyde	---	---	---	---	---	---	-0.84	---	---	---	-0.72	---	---	---	---	---
18	Linalool	---	---	---	0.92	---	---	---	---	---	---	---	---	---	---	---	---
19	5 Methyl Furfural	---	---	---	0.77	---	---	---	---	---	---	---	---	---	---	---	---
20	Ethyl decanoate	---	---	---	---	---	---	-0.84	---	---	---	---	---	---	---	---	---
21	Ethyl benzoate	---	---	---	---	---	---	-0.90	---	---	---	-0.76	---	---	---	---	-0.76
22	α–Terpineol	---	---	-0.71	0.92	---	---	---	---	---	---	---	---	---	---	---	---
23	Citronellol	---	---	-0.72	0.91	---	---	---	---	---	---	---	---	---	---	---	---
24	Phenethyl acetate	---	---	---	---	---	---	-0.88	---	---	---	-0.73	---	---	---	---	---
25	Ethyl dodecanoate	---	---	---	---	-0.72	---	-0.72	---	---	---	-0.75	---	---	0.78	0.87	---
26	Isoamyl decanoate	---	---	---	---	---	---	-0.82	---	---	---	-0.78	---	---	0.71	0.77	---
27	Hexanoic acid	---	---	---	---	---	---	-0.74	---	---	---	---	---	---	---	---	-0.89
28	Guaiacol	---	---	---	---	---	---	---	---	0.73	---	---	---	---	---	---	---
29	Trans-whisky lactone	---	---	---	---	---	---	---	---	---	---	0.81	---	---	---	---	---
30	Phenethyl alcohol	---	---	---	---	---	---	-0.85	---	---	---	---	---	---	---	0.73	---
31	*n*-Undecanol	---	---	---	---	---	---	-0.80	---	---	---	---	---	---	---	0.71	---

**Table 3.8. ***Significant Pearson Correlations between aroma compounds and sensory attributes (cont.).

32	Cis-whisky lactone	---	---	---	---	---	---	---	---	0.72	0.85	0.82	0.80	---	---	---	---
33	Ethyl tetradecanoate	---	---	0.78	---	---	---	---	---	---	---	---	---	---	0.81	0.94	---
34	Octanoic acid	---	---	---	---	---	---	-0.83	---	---	---	---	---	---	---	---	-0.91
35	*n*-Hexadecanol	---	---	---	---	---	---	-0.77	---	---	---	-0.74	---	---	---	0.83	---
36	Ethyl hexadecanoate	---	0.71	0.92	---	---	-0.72	---	---	---	---	---	-0.72	0.73	0.81	---	---
37	Ethyl 9-hexadecenoate	---	---	---	---	---	---	-0.77	---	---	---	-0.77	---	---	---	0.79	---
38	Decanoic acid	---	---	---	---	---	---	-0.79	---	---	---		---	---	---	---	-0.92
39	(9E)-9-Hexadecen-1-ol	---	---	---	---	---	---	-0.74	---	---	---	-0.78	---	---	0.72	0.85	---
40	Methyl oleate	---	---	---	---	---	---	---	---	---	0.73	0.81	0.72	---	---	---	---
41	Dodecanoic acid	---	---	---	---	---	---	-0.88	---	---	---	-0.74		---	---	---	-0.74
42	Benzophenone	---	---	---	---	---	---	---	---	---	0.73	0.81	0.72	---	---	---	---
43	Vanillin	---	-0.72	---	---	---	---	---	---	0.88	0.91	---	---	---	---	---	---
44	5Hidroxy-methyl furfural	---	---	---	---	---	---	---	---	0.78	0.89	0.73	---	---	---	---	---
45	Vanillic acid	---	-0.72	---	---	---	---	---	---	0.86	0.89	---	0.76	---	-0.71	---	---
46	Syringic acid	---	---	---	---	---	---	---	---	0.80	0.90	0.73	0.78	---	---	---	---
47	Syringaldehyde	---	---	---	---	---	---	---	---	0.88	0.93	0.67	0.72	---	---	---	---
48	Coniferaldehyde	---	---	---	---	---	---	---	---	0.81	0.91	0.75	0.74	---	---	---	---
49	Sinapaldehyde	---	---	---	---	---	---	---	---	0.82	0.91	0.73	0.72	---	---	---	---
50	Ellagic acid	---	---	---	---	---	---	---	---	0.83	0.92	0.74	0.74	---	---	---	---

*Only compounds with significant correlations are included. ($P < 0.05$).

3.4.4.2 Regression analysis between volatile compounds and sensory descriptors using partial least squares analysis (PLSR)

Further associations between aroma compounds and sensory data were visualised using PLSR analysis. Among multivariate techniques, PLSR has been used to investigate the relationship between sensory and instrumental data (Martens & Martenes, 2011). In this work the correlation loading from PLSR of 73 compounds determined by GC-MS, GC-FID, and HPLC-UV, and 16 sensory descriptors from bourbon whiskey, malt whisky and tequila spirit samples were analysed (Figure 3.4). Sensory descriptors that contribute to the aroma were chosen as Y-variables and volatile and non-volatile compounds as X-variables. A biplot of the products and their sensory characteristics is shown in Figure 4. PLSR developed a model with two optimal components (PC1 and PC2) which accounted for 59.2% and 66.1% of variation in the analytical data and sensory properties respectively (Figure 3.4).

Through PLSR analysis (Figure 3.4) it was possible to locate the position of the samples relative to the sensory descriptors and compound concentrations most associated with them. For reasons already discussed, this does not imply a causal link between compounds and nearby sensory characteristics, even where it might appear plausible (e.g. methionol locates next to '*sulfury*' character). However, the PLS plot in Figure 3.4 is a very powerful representation of a lot of experimental data, which gives good separation of the products and enables some rapid conclusions to be drawn about the sensory and analytical properties of each spirit. Generally, the mature-correlated characters and compounds are located in the upper right quadrant, which is logically dominated by the longest aged sample utilizing new oak casks, namely bourbon. The analysis clearly

shows that the non-mature and mature tequila samples were more similar to one another than to the whisky samples (unsurprisingly) and they are pushed together in the lower right quadrant co-located with sensory attributes *green/grassy* and *solventy* and the concentration of components such as linalool, citronellol, cis-linalool oxide, α-terpineol, 3-ethyl-4-methyl-1-pentanol and dihydro-2-methyl-3(2H)-furanone (Figure 3.4). Interestingly, these associations (PLSR analysis) correlated with some of the sensory attributes assigned to the aroma compounds during the GC-O analysis. For example, terpene compounds such as linalool, citronellol, and α-terpineol were described or associated with leafy, or green-related characters in the tequila sample. This gives strong evidence of the most likely aroma character that defines the congeners evaluated. However, for other compounds (mosly those located in the upper left quadrant of the PLS plot) a different pattern might apply since stronger links between these variables were not found.

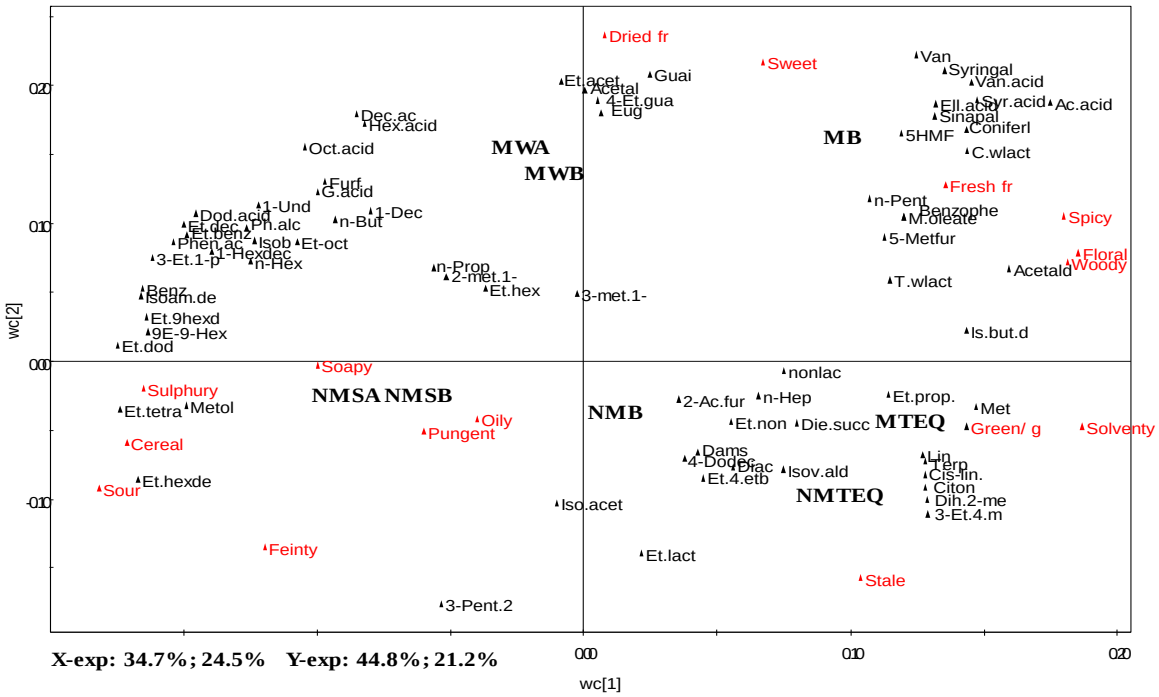

Figure 3.4. Partial Least-Squares Regression analysis (PLS) of volatile and non-volatile compounds (X-variables) and sensory descriptors (Y-variables); scores and loadings for the first two principal components.

KEY: *Flavour compounds*: Acetald: acetaldehyde; Et.acet: ethyl acetate; Acetal: acetal; Met: methanol; Diac: diacetyl; n-Prop: *n*-propanol; Iso.alddieac: isovaleraldehyde diethyl acetal; Isob: isobutanol; Iso.acet: isoamyl acetate; n-But: *n*-butanol; 3-Pent.2-ol: 3-Penten-2-ol; 2-met-1-but: 2-methyl-1-butano; 3-met-1-but: 3-methyl-1-butanol; Et.hex: ethyl hexanoate; n-Pent: *n*-pentanol; Is.but.dietac: isobutanal diethyl acetal; Dih.2-met: dihydro-2-methyl-3 (2H)-furanone; Et.prop.ald: β–ethoxypropionaldehyde diethyl acetal; n-Hex: *n*-hexanol; Et.lact: ethyl lactate; 3-Et.1-prop: 3-ethoxy-1-propanol; Et-oct: ethyl octanoate; Cis-lin.ox: cis-linalool oxide; Ac.acid: acetic acid; n-Hep: *n*-heptanol; Furf: furfural; 3-Et.4.met.1.p: 3-ethyl-4-methyl-1-pentanol; 2-Ac.fur: 2-acetylfuran; Et.non: ethyl nonanoate; Benz: benzaldehyde; Lin: linalool; 5MF: 5-methyl furfural; Et.dec: ethyl decanoate; Die.succ: diethyl succinate; Et.benz: ethyl benzoate; Terp: α–terpineol; Metol: methionol; 1-Dec: *n*-decanol; Citon: citronellol; 4-Dodec: 4-dodecenol; Phen.ac: phenethyl acetate; Dams: β–damascenone; Et.dod: ethyl dodecanoate; Isoam.dec: isoamyl decanoate; Hex.acid: hexanoic acid; Guai: guaiacol; T.wlact: trans-whisky lactone; Ph.alc: phenylethyl alcohol; 1-Und: *n*-undecanol: C.wlact: *cis*-whiskey lactone; Et.tetradec: ethyl tetradecanoate; 4-Et.guai: 4-ethylguaiacol; nonlac: γ-nonalactone Oct.acid: octanoic acid; 1-Hexdec: *n*-hexadecanol; Eug: eugenol; Et.4.etben: ethyl 4-ethoxybenzoate; Et.hexdec: ethyl hexadecanoate; Et.9hexdec: Ethyl 9-hexadecenoate; Dec.ac: decanoic acid; 9E-9-Hexd-1-ol: (9E)-9-Hexadecen-1-ol: M.oleate: methyl oleate; Dod.acid: dodecanoic acid; Benzophe: benzophenone; Van:vanillin; 5HMF: 5-hidroxy-methyl furfural; G.acid: gallic acid; Van.acid: vanillic acid; Syr.acid: syringic acid; Syringald: syringaldehyde; Coniferl: coniferaldehyde; Sinapald: sinapaldehyde; Ell.acid: ellagic acid; *Flavour attributes:* Pungent; Feinty; Cereal; Green/grassy; Floral; Fresh fruit; Solventy; Soapy; Dried fruit; Sweet; Soapy; Woody; Spicy; Oily; Sour; Sulphury; Stale. *Spirits:* NMSA: new make spirit from distillery 'A'; NMSB: new make spirit from distillery 'B'; NMB: non-mature bourbon; NMTEQ: non-mature tequila; MWA: malt whisky from distillery 'A'; MWB: malt whisky from distillery 'B'; MB: mature bourbon; MB: mature bourbon; MTEQ: mature tequila.

3.5 OVERALL DISCUSSION AND CONCLUSIONS

Non-mature and mature spirits of bourbon, tequila and malt whiskies are undoubtedly complex matrices with hundreds of components that significantly contribute to spirit aroma. Findings from this Chapter showed that mature spirits presented a more complex aroma than the non-mature one, especially in terms of odour active regions detected. Key ageing odourants were volatile phenols (e.g. guaiacols) and oak lactones (*cis/trans*-whisky lactone), which increased significantly in FD factor in the mature spirits and were linked to mature-relate attributes during the PLSR analysis. These odourants, along with ethyl esters and acetate esters, were used as the basis to define the 'estery' and 'mature/woody' character in each of the spirits types. These fractions were then used to study and understand potential flavour interactions that occur between these important characters in each of the spirits, and clarify whether there is a significant overlap in their congeneric origins and the resulting effect in flavour perception. Furthermore, results from this Chapter enabled the effect of wood ageing to be linked to changes in sensory character and spirit composition. Instrumental analysis (GC-FID, GC-MS, HPLC and GC-O) highlighted the compounds that were mostly affected by the ageing period and results from sensory evaluation (nosing) of the spirits showed strong correlations between concentrations of several ageing congeners and the perceived mature attributes (*dried fruit*, *sweet*, *spicy*, and *woody*). Also, ageing enhanced the floral characters of all four spirits (by breakdown of oak norisoprenoids) and the fresh fruit character of all whiskies. Feinty and cereal characters declined on ageing across all samples. Thus, a strong influence of the ageing process on the sensory character of mature spirits was demonstrated. In particular it is argued that the

ageing period and the characteristics of the cask used for ageing (e.g. new versus re-use) are important factors governing the development of the aged character of these spirits. Results from the present study provide a better understanding of the effects of maturation in wood across different spirit categories and emphasise its significance to the perceived aroma of matured spirits.

4. RECONSTITUTION EXPERIMENTS IN SPIRIT SAMPLES OF BOURBON, TEQUILA AND MALT WHISKIES.

4.1 AIM

The objective of this chapter was to attempt to reproduce the ‘estery’ and ‘woody’ characters for each spirit through aroma recombination, based on blends of the odorant compounds identified at high FD factors and their analytical concentrations in the actual samples as reported in Chapter 3.

4.2 INTRODUCTION

By applying the molecular sensory science approach on food aromas (Usulmann et al. 2015), it has been shown for a considerable number of foods that not all of the volatiles present in a food are able to interact with the human olfactory receptors, but only a sub-set of so-called key odourants are selectively detected by the receptors and thus stimulate aroma perception in the brain (Dunkel, 2014). To identify such key odourants amongst the bulk of odourless volatile compounds, the method of choice is GC-O/AEDA coupled with calculation of odour activity values (OAVs) in the so-called Sensomics approach (Schieberle and Hoffman, 2012). Furthermore, the results from GC-O/AEDA identified the key odourants which were the most odour-active and define the overall aroma of the spirit samples, thus in this chapter by using the results from such systematic approach we evaluated the effect of adding the exact quantitative data of aroma compounds in the overall aroma of the authentic spirit samples by means aroma recombinants. However, the results from these first experiments did not prove a close enough match for the sensory characteristics of the spirit

samples, so the chapter subsequently moves on to consider other factors in distilled spirits which can influence the release and perception of spirit aroma. Some of these factors include the presence of ethyl esters of fatty acids (e.g. ethyl hexadecanoate). These compounds generally do not contribute to aroma but rather form agglomerates in aqueous ethanolic solutions (Conner et al. 1994; 1994), which can subsequently incorporate other hydrophobic compounds (shorter chain esters, alcohols and aldehydes) from the solution and thus to decrease their free solution and consequently headspace concentrations, reducing their sensory impact and improving acceptability to consumers through marked changes in character (Piggott et al. 1996; Conner et al. 1999). This phenomenon explained why in our next recombined experiments we added in ethyl hexadecanoate into the spirit model solutions. In addition, the strong correlation that the non-volatile wood extractives (e.g. *cis/trans*-whisky lactone) and the early boiling compounds (methanol, acetic acid, acetaldehyde, etc) showed to the ageing period (PLSR analysis), make them strong candidates to be considered into the following spirit formulations. In general, we wanted to evaluate potential interactions with the core congeners analysed by GC-O, and to examine physicochemical interaction with aroma release (by inclusion of non-volatile wood extractives) and to understand if these modulate the perception of the key odorants mainly by the presence of low boiling volatile compounds (methanol, acetic acid, etc) and a long chain-ethyl ester (ethyl hexadecanoate).

4.3 MATERIAL AND METHODS

4.3.1. Samples

Eight spirit samples (non-mature and mature exemplars of each kind), consisting of two commercial brands of Scotch malt whiskies from two distilleries (A and B), tequila and bourbon samples were utilized to carry out the experiments of this results chapter. Spirits were diluted with RO water to an alcohol strength of 23% ABV for 'nosing' purposes.

4.3.2. Reagents and Chemicals

As described in Section 2.3.2.

4.3.3. Formulation of recombined spirit blends and sensory evaluation

For each of the eight spirit samples in the study, an aroma simulant was created in pure ethanolic solution (23% ABV) by adding the same concentrations of flavour active compounds which had been analysed in the corresponding spirit sample (Chapter 3) and which had a FD factor greater than or equal to 27 in the GC-O/AEDA study (Appendices 2 and 3). A series of sensory triangle tests were then used to see whether panellists could readily differentiate the authentic spirit samples from their respective simulants. Furthermore, panellists were asked during the same test to evaluate the difference by using a degree of difference scale from 0 (not at all similar) to 3 (practically identical). The individual fractions which represent the estery and woody character (Appendix 4) of each of the spirit samples were further characterized by SWRI's expert sensory panel following the conditions described in Section 3.3.5.

4.3.3.1 Formulation of improved spirit aroma blends and sensory evaluation

In addition to the eight spirit simulants, another series of aroma recombinants were prepared and consisted of five simulants per mature spirit, so that 20 model spirits of different aroma composition were recreated (Appendices 5 to 8). These were evaluated following the above triangle sensory methodology using between 23 to 28 panellists. The first simulants, consisted of the most odour active-compounds (FD > 27) determined by GC-O analysis; the second simulants consisted besides the most odour-active compounds of the low boiling volatile compounds (LBC) determined by GC-FID (Table 2.4); the third simulants consisted besides the previous compounds of the presence of ethyl hexadecanoate (C16) at 500 mg/L (optimal concentration to improve the perceived aroma character of the spirits, according to Boothroyd, 2013). The fourth simulant consisted of the presence of most odour active compounds, low boiling volatile compounds (LBC) and the non-volatile compounds (NBC) determined by HPLC (Table 2.4). Finally, the fifth simulant comprised of all the previous compounds plus the presence of ethyl hexadecanoate (C16) at 500 mg/L.

4.3.3.2 Sensory evaluation of improved estery and woody fractions

A new attempt was made to improve the current existing estery and woody fractions, by incorporating the same congeners (low boiling volatile compounds, non-volatile compounds and ethyl hexadecanoate) as in the simulants in each of the model spirits (Appendices 9 and 10). These new fractions were evaluated in informal sensory evaluations using the expertise in spirit flavour of Dr. David

Cook (main supervisor of this PhD thesis). Sensory comments were obtained after sniffing each of the fractions (estery and woody) and comparing against their original spirit sample. This procedure was repeated for each of the spirits and their respective simulants. Once these fractions were evaluated, we selected the fractions that best represented the overall aroma character of each of the spirits. These fractions, were then evaluated by the SWRI expert panel using quantitative descriptive analysis (QDA) as described in Section 3.3.5.

4.3.4. Addition of 'mature' ageing congeners into new make spirits– experiments and sensory evaluation

Further sensory experiment was conducted to test the authenticity of the improved aroma blends for mature character by adding them into authentic new make spirit samples (Table 4.1). The congeners added into the non-mature spirits, were basically those identified as important congeners correlated with ageing time in the mature samples, according to the results from PLS analysis reported in Chapter 2. These aroma blends were analysed by quantitative descriptive analysis (QDA) using the SWRI expert panel and the conditions described in section 3.3.5.

Table 4.1. Improved aroma blends for mature character added into authentic new make spirits (concentrations mg/L)

Odorant	Group	BOUBON	TEQUILA	MWA	MWB
γ-nonalactone	*Woody*	0.03	0.00	0.00	0.00
Cis/trans-whisky lactone	*Woody*	11.63	1.81	2.27	2.08
Guaiacol	*Woody*	0.04	0.01	0.14	0.14
4-Ethylguaiacol	*Woody*	0.02	0.01	0.19	0.18
Eugenol	*Woody*	0.02	0.02	0.26	0.23
Vanillin	*Woody*	2.68	0.88	2.09	1.91
Acetaldehyde[a]	LBC	21.28	27.70	0.08	2.00
Acetal[a]	LBC	194.80	32.40	0.00	7.20
Acetic acid[a]	LBC	365.10	186.10	38.98	57.94
5HMF	NVC	2.06	0.05	0.15	0.07

Table 4.1. Improved aroma blends for mature character added into authentic new make spirits (cont.).

Gallic acid	NVC	27.56	9.95	7.10	4.74
Vanillic acid	NVC	1.74	0.64	0.18	0.23
Syringic acid	NVC	4.10	1.22	0.41	0.31
Syringaldehyde	NVC	10.06	2.52	1.12	1.11
Coniferaldehyde	NVC	2.77	0.33	0.21	0.15
Cinnamaldehyde	NVC	4.57	0.37	0.36	0.26
Ellagic acid	NVC	125	16.00	9.86	8.58

LBC: low boiling volatile compounds, NVC: non-volatile compounds. [a]: concentrations of compounds presented in both non-mature and mature spirits, which were adjusted to the difference required to simulate the final aged product.

4.3.5. Data treatment and statistical analysis

Results from triangle tests were evaluated using Meilgaard (1987) Table T8 (Appendix 11). For the analysis and interpretation of results, the number of correct responses were counted (correctly identified odd samples) and the number of total responses. Then in the Table T8 (Appendix 11), entries were the minimum number of correct responses required for significance at the stated α-level (0.05, column) for the corresponding number of respondents, n (23 to 26 panellists, row). We rejected the assumption of 'no difference' if the number of correct responses was greater than or equal to the tabled value. The similarity in the overall flavour of the spirit models was scored according to the degree difference scale previously mentioned (Section 4.3.3) and the results from panellists' performance were averaged to obtain its mean values and expressed accordingly with the standard deviation.

4.4. RESULTS AND DISCUSSION

4.4.1 Reconstitution experiments and sensory evaluation of spirit model systems

The non-mature and mature spirit samples of bourbon, tequila and malt whiskies were compared sensorially against their aroma simulants (mixtures containing all the compounds in Appendices 2 and 3.) and the results of the corresponding triangle tests are presented in Table 4.2. All of the aroma simulants were found to be sensorially significantly different ($P > 0.05$) relative to the spirit samples themselves, showing only a weak or medium degree of similarity. This showed that simply mixing the most odour active volatiles (GC-O) from each spirit in the ratios they were analysed in the authentic samples was insufficient to re-create the original aroma of each sample. Other unidentified factors clearly add to the complexity, release and perception of aroma from the authentic samples in a way which was not present in our simulants. The flavour of the reconstituted non-mature 'bourbon' was found to be more fragrant and sweet than the genuine product. Furthermore, it lacked the feinty, smoky, malty and balsamic notes of the authentic product. Regarding the mature version, the reconstituted 'bourbon' was defined as having a sweet, woody, vanilla, almond and fruity aroma relative to the authentic bourbon, which was more woody/oaky, smoky, and vanilla. The remaining non-mature spirits of tequila, and new make spirits (A and B) were defined basically with a feinty and alcoholic character, whilst the aroma simulants were more herbal/grassy, fruity, and flowery. The mature tequila and malt whiskies, by their own were defined with an oaky/woody, vanilla, and pungent character, while the model spirits were recognized with herbal, fruity, vanilla, and flowery descriptors (Table 4.3).

Table 4.2 Results of triangle tests to evaluate whether sensorially significant differences were identified between authentic spirit samples and their corresponding aroma simulant (all nosed at 23% ABV)

Triangular test	Number of compounds added	Correct response/total response	Critical number of correct responses[a]	*P value*	Level of similarity
NMB vs SM	14	20/23	12	< 0.05	1.13±1
MB vs SM	25	17/23	12	< 0.05	2.17±0.4
NMTEQ vs SM	27	22/26	14	< 0.05	1.67±0.71
MTEQ vs SM	32	22/26	14	< 0.05	1.71±0.62
NMSA vs SM	26	21/24	13	< 0.05	1.50±0.67
MWA vs SM	33	21/24	13	< 0.05	1.55±0.70
NMSB vs SM	26	17/24	13	< 0.05	1.63±0.83
MWB vs SM	35	18/24	13	< 0.05	1.69±0.91

The similarity in the overall flavour of the model was scored on the following scale: 0, not at all; 1, weak similarity; 2, medium similarity; 3, practical identical. The results obtained by 23-26 panellists were averaged and expressed with the standard deviation. NMB: non-mature bourbon; MB: mature bourbon; NMTEQ: non-mature tequila; MTEQ: mature tequila; NMSA: new make spirit 'A'; MWA: malt whisky 'A'; NMSB: new make spirit 'B'; MWB: malt whisky 'B'. SM: simulate model. [a]: minimun number of correct responses required for significance at the state α-level (0.05) for the corresponding number of responses, *n* (23 to 26). We rejected the assumption of 'no difference' if the number of correct responses is greater than or equal to the tabled value.

Table 4.3 Characteristic aroma qualities defined for the authentic spirits and their spirit models during reconstitution experiments

Non-mature Spirits				**Mature Spirits**			
Bourbon	**Tequila**	**NMSA**	**NMSB**	**Bourbon**	**Tequila**	**MWA**	**MWB**
Feinty, smoky, malty, balsamic, pungent/alcoholic	Pungent/alcoholic, feinty, smoky, fermented	Feinty, smoky, alcoholic, astringent, sweet/caramel, burnt	Alcohol/pungent, feinty, fermented, fruity, leafy, flowery	Woody, burnt, smoky, alcoholic vanilla, fermented	Woody, vanilla, sweet, herbal, earthy, agave, fruity, pungent	Smoky, woody, burnt, vanilla, caramel, flowery, alcoholic/pungent	Woody, smoky, vanilla, coconut, fruity, alcoholic, burnt, caramel
Non-mature model spirits				**Model spirits**			
Fragrant, penetrating odour, sweet, alcoholic	Herbal, grassy, fruity, sweet, alcoholic flowery, vanilla, woody	Fruity, sweet, green-like, alcoholic, flowery, humidity, citric	Vanilla, alcoholic, woody, fruity, flowery, citric, feinty, sweet	Sweet, marzipan aroma, woody, vanilla, green-like, almond, woody, fermented	Herbal, sweet, flowery, fruity, vanilla, alcoholic, smoky, caramel, woody, and anise	Fruity, vanilla, woody, coconut, sweet, flowery	Vanilla, coconut, fruity, sweet, woody, flowery, honey/caramel

4.4.1.1 Sensory characteristics of the estery and woody fractions

Whilst previous model spirit solutions were somewhat different to the spirit they aimed to re-create, we suspected that further contributors to spirit flavour remain yet unidentified. Therefore, the effect of adding other important congeners to the model spirits was further investigated. However, first we formulated the individual fractions that define the estery and woody fractions in each of the spirits (Appendix 4), considering for this the results obtained from the recombinants experiments. The main purpose of this experiment was to fully understand the sensory characteristic that define each of the fractions (in order to optimise their authenticity prior to use in subsequent flavour interaction experiments), and to see if these terms accordingly fit with the attributes defined as estery and woody in the whisky flavour wheel (Lee et al. 2001). Also, the appreciation of other underlying aroma complexities besides estery and woody were analysed. For achieving this goal, the SWRI's expert panel carried out a full flavour profiling of each of the simulants and the original spirits. Figures 4.1 and 4.2 show the comparison in aroma profile between the estery and woody fractions of the non-mature and mature spirits against the original products. Prior studies have reported that the descriptive terms that define the mature character of aged spirits, are mainly defined by smooth, vanilla and sweet aroma characters (Clyne et al. 1998); however, the SWRI's expert panel have defined the 'mature' character of the spirits to be described by the aroma qualities of spicy, woody, dry fruit and sweet, and the 'fruity/estery' character by the aroma qualities of soapy, solventy and fresh fruit respectively. As we compare along the spirit sets, it can be seen that simulants that recreate the estery character of

each spirit samples were described with an intense fresh fruit, and solvent character. In fact, in the vast majority of these simulants an underlying sweet aroma was detected, possibly because of the influence of ethyl esters present. These compounds contributed to spirit aroma by incorporating fruity and sweet aromas (Conner et al. 1994). In addition, the aroma balance of the estery character in the new make spirits was subtler with distinctive fresh fruit, solventy and soapy character.

Indeed, the estery character of the new make spirit models lacked the characteristic attributes that define this character, however the information gathered from this experiment allowed us to balance the following model spirits to achieved if possible more authentic products. The estery character in the mature model spirts lacked balance in the fresh fruit, solventy and soapy characters, which were presented in the original mature spirits.

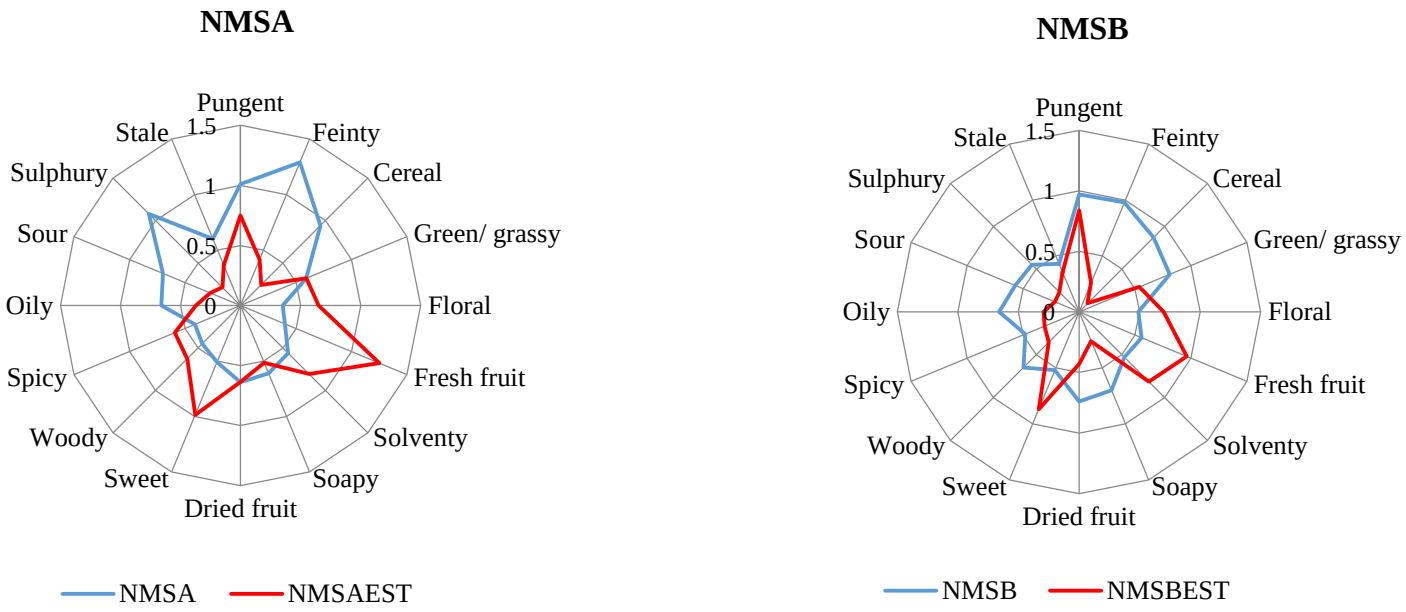

Figure 4.1 Aroma profiles of the estery fractions of non-mature simulants as compared to the original samples. NMSA: new make spirit 'A'; NMSB: new make spirit 'B'; NMB: non-mature bourbon; NMTEQ: non-mature tequila; NMSAEST: new make spirit 'A' estery fraction; NMSBEST: new make spirit 'B' estery fraction; NMBEST: non-mature bourbon estery fraction; and NMTEQEST: non-mature tequila estery fraction.

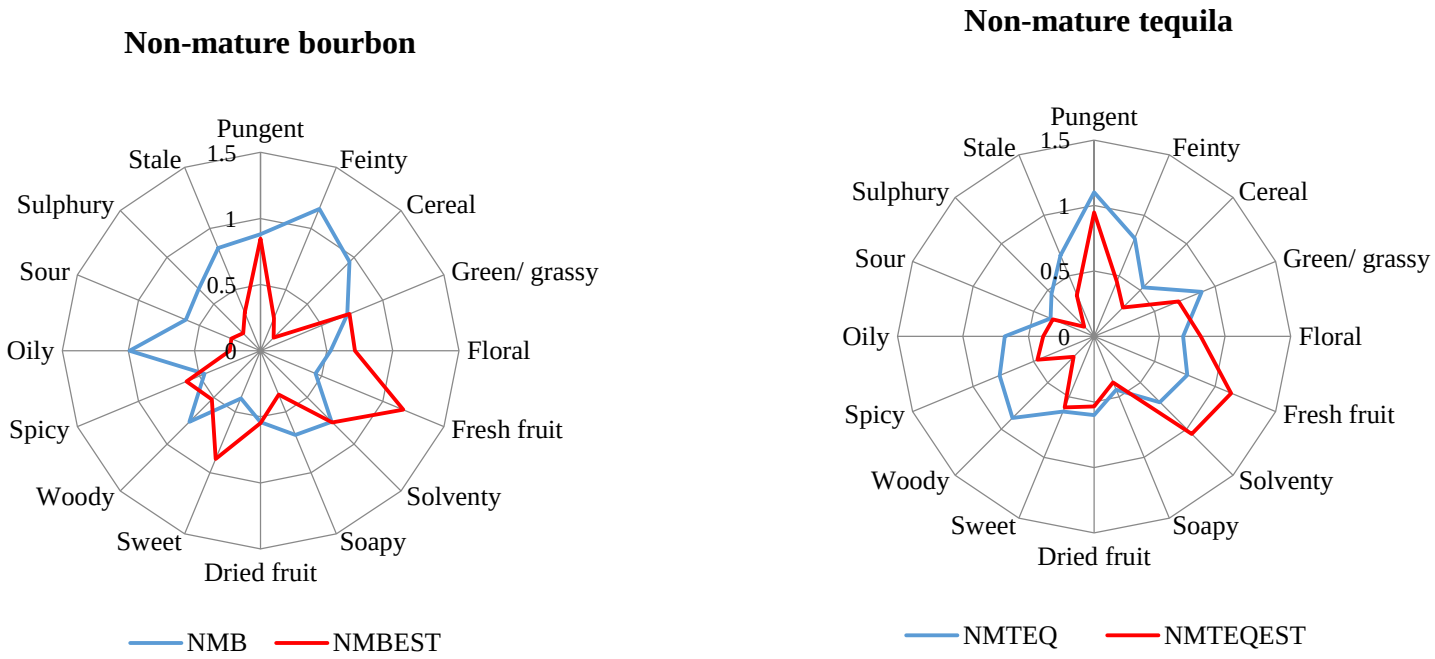

Figure 4.1 Aroma profiles of the estery fractions of non-mature simulants as compared to the original samples. NMSA: new make spirit 'A'; NMSB: new make spirit 'B'; NMB: non-mature bourbon; NMTEQ: non-mature tequila; NMSAEST: new make spirit 'A' estery fraction; NMSBEST: new make spirit 'B' estery fraction; NMBEST: non-mature bourbon estery fraction; and NMTEQEST: non-mature tequila estery fraction (cont.).

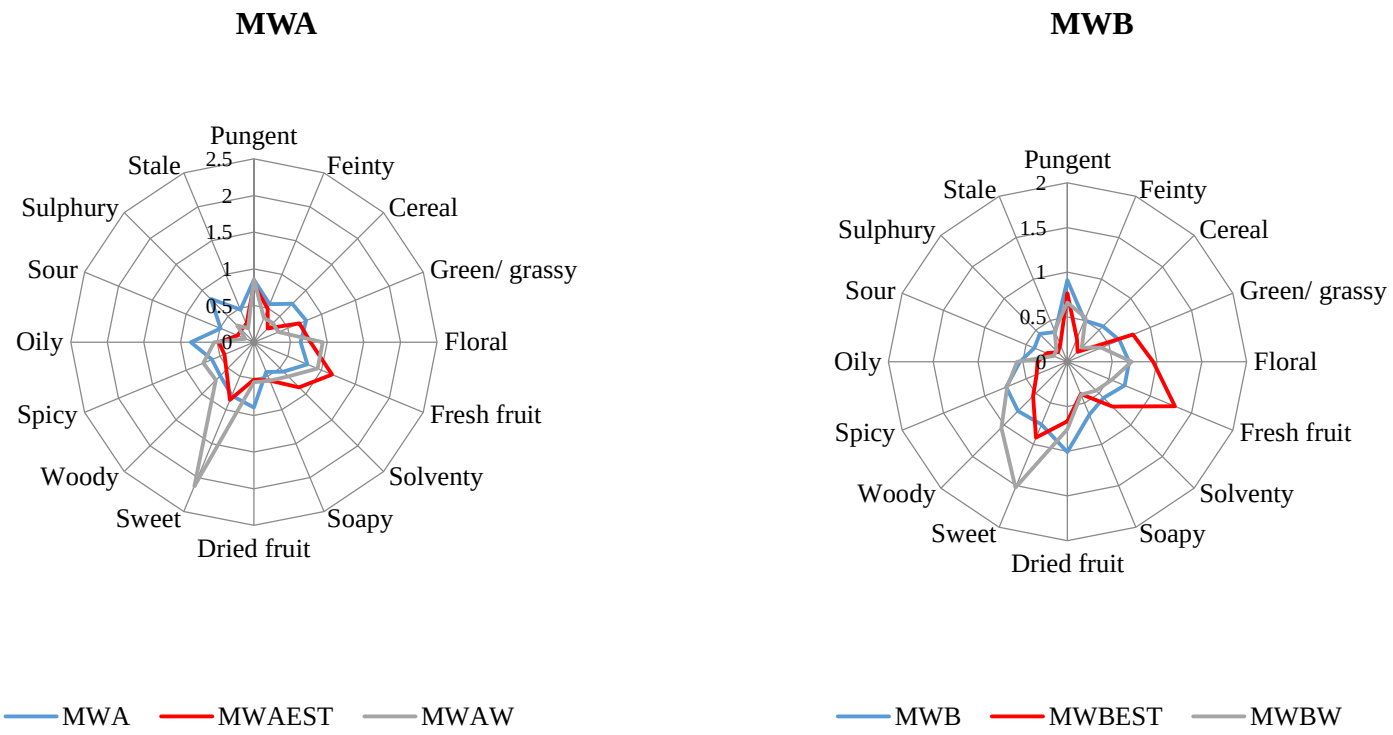

Figure 4.2 Aroma profiles of the estery and woody fractions of mature simulates as compare to the original samples. MWA: malt whisky 'A'; MWB: malt whisky 'B'; MB: mature bourbon; MTEQ: mature tequila; MWAEST: malt whisky 'A' estery fraction; MWBBEST: malt whisky 'B' estery fraction; MBEST: mature bourbon estery fraction; and MTEQEST: mature tequila estery fraction. MWAW: malt whisky 'A' woody fraction; MWBW: malt whisky 'B' woody fraction; MBW: mature bourbon woody fraction; and MTEQW: mature tequila woody fraction.

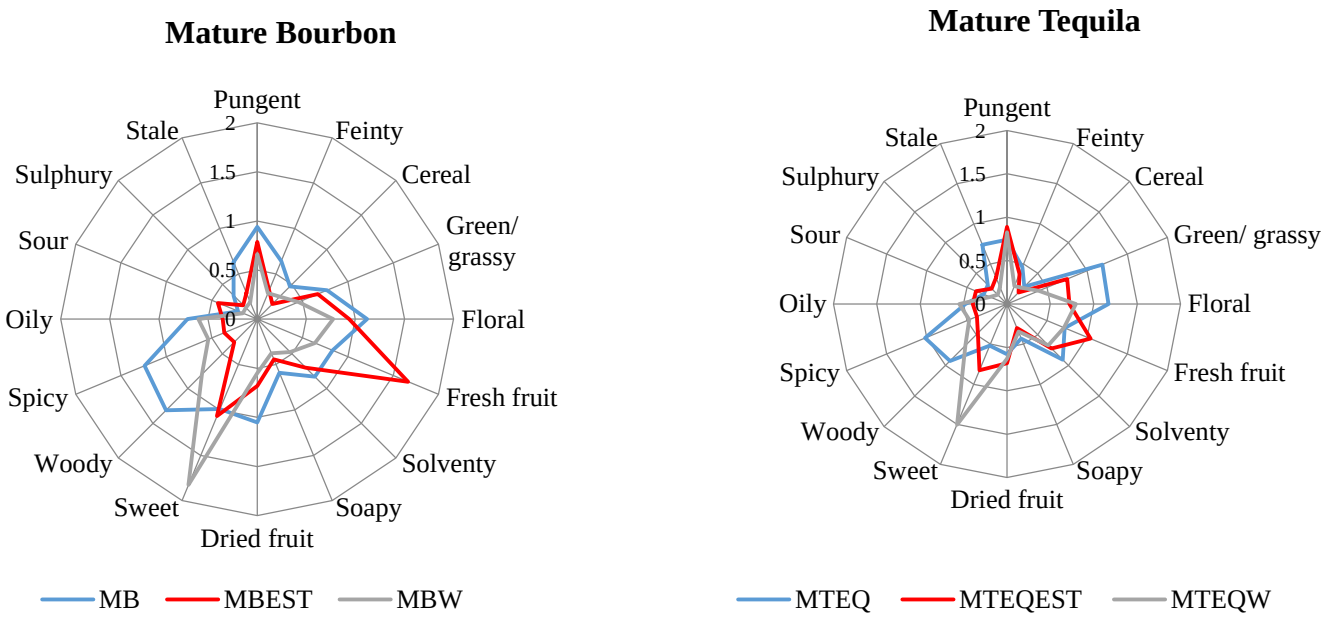

Figure 4.2 Aroma profiles of the estery and woody fractions of mature simulates as compare to the original samples. MWA: malt whisky 'A'; MWB: malt whisky 'B'; MB: mature bourbon; MTEQ: mature tequila; MWAEST: malt whisky 'A' estery fraction; MWBBEST: malt whisky 'B' estery fraction; MBEST: mature bourbon estery fraction; and MTEQEST: mature tequila estery fraction. MWAW: malt whisky 'A' woody fraction; MWBW: malt whisky 'B' woody fraction; MBW: mature bourbon woody fraction; and MTEQW: mature tequila woody fraction (cont.).

On the other hand, the woody character in the authentic products was defined by a balance between woody, spicy, sweet and dried fruit notes, as opposed to the simulants which were distinguished with an overly sweet aroma character. Amongst the mature spirits, bourbon was rated as the most intense in terms of woody attributes, mainly due to the longest maturation and the use of new-American oak casks for maturation. In general, the mature character of the simulants lacked in some extent of the sensory characters that characterized aged spirits (spicy, woody, and dried fruit). Possible reasons are due to complex sensory interactions between these odourants and other compounds (FD ≤ 27), and to synergic effects that modulate or mask important sensory characteristics (Conner et al. 1996; Atanasova et al. 2004, 2005; Chaput et al. 2012). Figure 4.3 shows the aroma compounds that were used to define the estery and woody fraction compositions. The compounds which defined the estery fractions were essentially ethyl and acetate esters, among which ethyl decanoate was the compound present at the highest concentrations, followed by ethyl octanoate and ethyl hexadecanoate. Regarding the 'mature/woody' fractions, *cis/trans*-whisky lactone was the compound present in higher amounts in the simulants, followed by vanillin and eugenol. Although these were the key odourants identified as the most odour-active compounds in the aroma extracts by GC-O analysis, some other congeners (e.g. C16) which were not added in these simulant mixtures might played an important role while defining or improving the aroma qualities of these model spirits to a similar extent as those found in the original products. Therefore, the impact of further aroma contributors to spirit flavour was investigated, including long chain ethyl ester (ethyl hexadecanoate), low boiling

volatile compounds (e.g. acetaldehyde) and non-volatile compounds (e.g. vanillic acid).

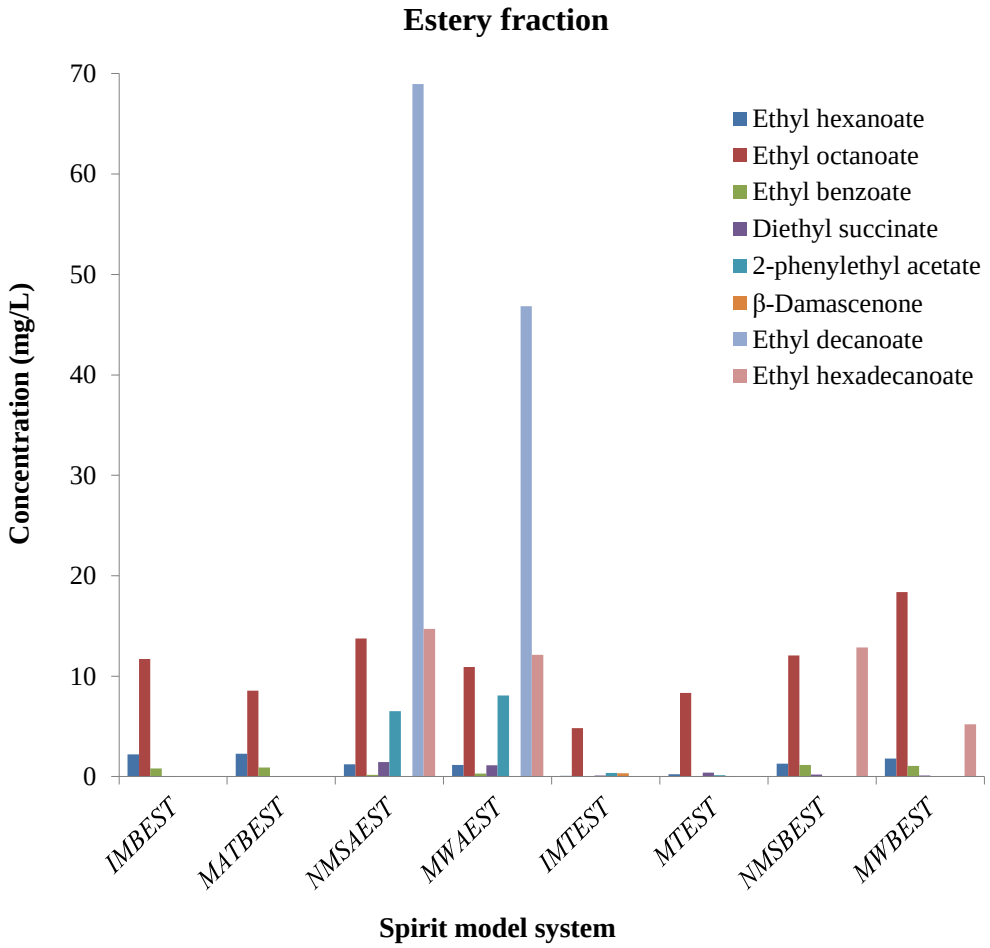

Figure 4.3 Estery and woody fraction compositions of the non-mature and mature simulants of bourbon, tequila and malt whiskies (A and B).

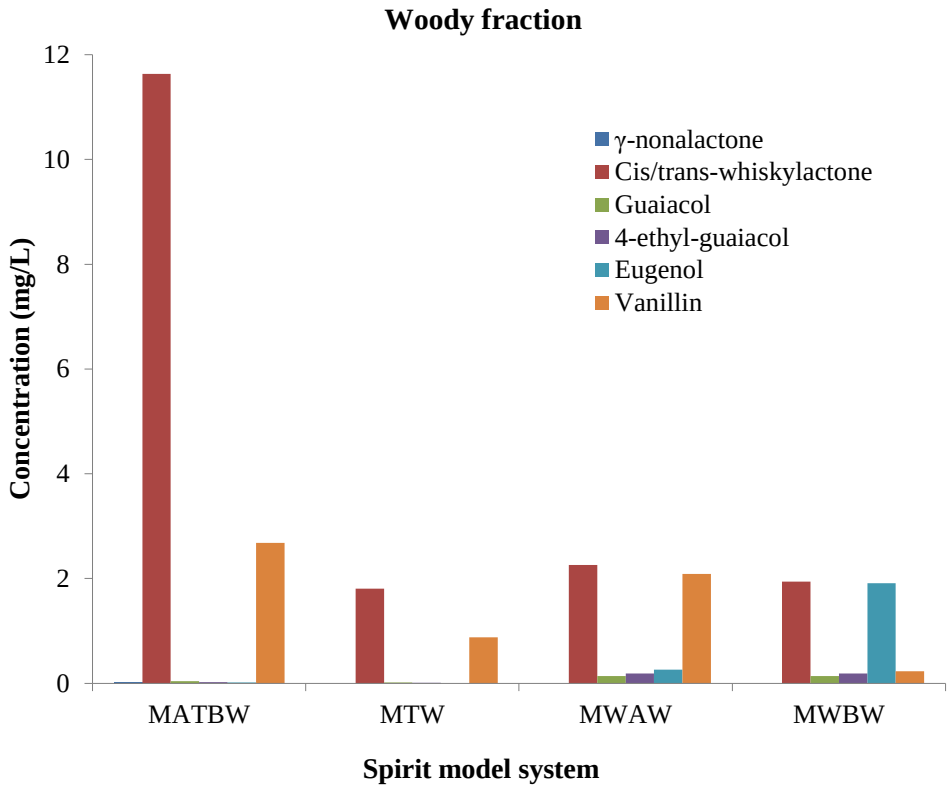

Figure 4.3 Estery and woody fraction compositions of the non-mature and mature simulants of bourbon, tequila and malt whiskies (A and B) (cont.).

IMBEST: non-mature bourbon estery fraction; MATBEST: mature bourbon estery fraction; NMSAEST: new make spirit 'A' estery fraction; MWAEST: malt whisky 'A' estery fraction; IMTEST: non-mature tequila estery fraction; MTEST: mature tequila estery fraction; NMSBEST: new make spirit 'B' estery fraction; MWBEST: malt whisky 'B' estery fraction; MATBW: mature bourbon woody fraction; MTW: mature tequila woody fraction; MWAW: malt whisky 'A' woody fraction; and MWBW: malt whisky 'B' woody fraction.

4.4.2 Attempts to improve the authenticity of spirit aroma simulants by increasing the complexity of the mixtures in varied ways.

Because of the lack of 'mature/woody' character in the spirit aroma simulants of bourbon, tequila and whisky, further experiments were performed to identify the effect of key aroma compound additions on the aroma perception of these simulants, with particular attention to the 'mature' character of aged spirits. Assessment of whisky flavour was conducted at an alcoholic strength of 20-23% ABV. Dilution of the spirit affects the solubility of many volatile compounds, for example, the ethyl esters of fatty acids. These compounds behaved as the primary components of agglomerates or micelles in the diluted distillate (Conner et al. 1994; 1994). However, other compounds such as alcohols, and long-chain aldehydes also influence ester activity, and therefore, headspace concentration, which lead to changes in the perceived aroma character of the spirit (Boothroyd et al. 2013). Additionally, because of the unsuccessful aroma recreation of the previous simulants, it was suggested (Dr. John Conner, SWRI; personal communication) that the addition of a fraction of low boiling volatile compounds (present at high concentrations in spirits) might help to bring the aroma of the simulants closer to their respective authentic products. In addition, the presence of non-volatile compounds (e.g. vanillic acid, syringic acid, etc) showed strong correlation with ageing time, therefore we wanted to evaluate if their presence could improve the aroma release of the aged character in these new simulants. For the above reasons, we performed further recombined model spirits (reconstitution experiments), in which we added in ethyl hexadecanoate, low boiling volatile compounds and non-volatile compounds (Appendices 5 to 8).

The impact of each of them was assessed individually employing five model systems per spirit (Appendices 5 to 8), so that 20 simulants were evaluated against their respective original mature spirit in a tringle test as described in section 4.3.3.

Table 4.4 shows the results of the reconstitution experiments carried out with the mature bourbon. It can be seen that of the five aroma simulants, the second and fifth system were most similar to the authentic mature bourbon ($P > 0.05$). The second simulant consisted basically of compounds which showed FD ≥ 27 (GC-O results), and low boiling volatile compounds; and the fifth simulant consisted of the compounds with FD ≥ 27, plus low boiling volatile compounds, ethyl hexadecanoate, and non-volatile compounds (Table 4.4). Although the remaining simulants showed significant differences ($P < 0.05$) in comparison with the authentic bourbon, the level of similarity increased (> 2) as comparison with the previous aroma recombination experiments (Table 4.2). Furthermore, the presence of key compounds such as acetaldehyde, acetic acid and ethyl acetate (in the low boiling volatile compounds fraction), definitely influenced the aroma balance of these simulants, providing a more authentic mature character in keeping with the original spirit. In fact, prior studies have shown that during maturation key odourants such as acetaldehyde, acetic acid, and ethyl acetate undergo chemical reactions with wood components and thus an increase in their concentration is expected along with a strong influence on the mature character of ageing spirits (Reazin, 1981).

Table 4.4. Reconstitution experiments MATURE BOURBON

Triangle test	Comp´s add in	Aroma impression	Correct responses/total responses	Critical number of correct responses[a]	*P value*	Conclusions	Level of similarity
MB/1st SM	Without LBC	Pungent/alcoholic, solventy, varnish, vanilla, honey and slightly woody character	20/28	15	< 0.05	Sig. differences between samples	0.94 ± 0.4
MB/2nd SM	LBC	Pungent/alcoholic, fragrant, fruity-like, caramel, vanillin, honey, musty and strong woody-relative character	14/28	15	> 0.05	**No differences between samples**	2.29 ± 0.53
MB/3nd SM	LBC + C16	Similar impressions as with model 2 but with an accentuated smoky character	18/28	15	< 0.05	Sig. differences between samples	2.02 ± 0.75
MB/4th SM	LBC + Non-volatile compounds	Similar to 2nd reconst. model solution, but a little bit spicier	19/28	15	< 0.05	Sig. differences between samples	1.95 ± 0.63
MB/5th SM	LBC + Non-volatile compounds + C16	Similar to the authentic bourbon character, however is still a small difference in aroma character. An additional sourness is present in the simulant.	13/28	15	> 0.05	**No differences between samples**	2.22 ± 0.60

The similarity in the overall flavour of the spirit models was scored on the following scale: 0, not at all; 1, weak similarity; 2, medium similarity; 3, practical identical. The results obtained by 28 panellists were averaged and expressed with the standard deviation. LBC: low boiling volatile compounds, C16: ethyl hexadecanoate. [a]: minimun number of correct responses required for significance at the state α-level (0.05) for the corresponding number of responses, *n* (28). We rejected the assumption of 'no difference' if the number of correct responses is greater than or equal to the tabled value.

In addition, non-volatile compounds such as phenolic compounds and furanic aldehydes have been shown to be important congeners that arise or are produced during the ageing process, influencing thus the composition of the mature spirit (Clyne et al. 1993). According to the level of similarity between simulants, we can observe that the mean values for samples 2 – 5 were similar, indicating a certain order of similarity between these samples as opposed to sample 1, which showed the lower mean value and therefore was considered as the most different sample in the pool of simulants examined (Table 4.4).

Likewise, as with mature bourbon, reconstitution experiments of mature tequila were performed, and the results are presented in Table 4.5. In terms of the level of similarity between the simulants and the mature tequila, significant differences were observed among them *($P < 0.05$)*. However, the fourth simulant presented the greatest level of similarity (1.94) to the original product. Although none of the products matched the genuine tequila closely in terms of the overall aroma character, all of them kept some of the essential sensory attributes that define the mature tequila, such as green/grassy, floral, and woody (Table 4.5). Whilst the presence of low boiling volatile compounds, non-volatile compounds and ethyl hexadecanoate highly influenced the aroma perception of the simulants of mature tequila, the simulant blends still lacked some compounds which are apparently important to the overall perception.

Table 4.5. Reconstitution experiments MATURE TEQUILA

Triangule test	Comp´s added	Aroma impressions	Correct responses/total responses	Critical number of correct responses[a]	*P value*	Conclusion	Level of similarity
TEQ/1st SM	Without LBC	Green/grassy, floral, sweet/vanilla, solventy, oaky, medicinal, stale, alcoholic, buttery, fruity.	23/26	14	< 0.05	Sig. differences between samples	0.87 ± 0.53
TEQ/2nd SM	With LBC	Green/grassy, floral/fragrant, sweet/vanilla, medicinal/smoky, solventy, pungent/alcoholic, nutty, sulphury, stale, woody, earthy, dried fruit, fruity, buttery	21/26	14	< 0.05	Sig. differences between samples	1.44 ± 0.69
TEQ/3rd SM	LBC + C16	Green/grassy, sweet/vanilla, smoky, pungent/alcoholic, spicy, floral/fragrant, woody, solventy, fruity, cinnamon, nutty	22/26	14	< 0.05	Sig. differences between samples	1.38 ± 0.69
TEQ/4th SM	LBC + Non-volatile compounds	Green/grassy, floral/fragrant, pungent/alcoholic, sweet/vanilla, musty, solventy, smoky/medicinal, woody, nutty, sulphury, metallic, cinnamon, fruity, stale	14/26	14	< 0.05	Sig. differences between samples	1.94 ± 0.65
TEQ/5th SM	LBC + Non-volatile compounds + C16	Fragrant/floral, smoky, pungent/alcoholic, sweet/vanilla, solventy, woody, stale, sulphury cinnamon, leafy, fresh and dried fruit	20/26	14	< 0.05	Sig. differences between samples	1.26 ± 0.81

The similarity in the overall flavour of the spirit models was scored on the following scale: 0, not at all; 1, weak similarity; 2, medium similarity; 3, practical identical. The results obtained by 26 panellists were averaged and expressed with the standard deviation. LBC: low boiling volatile compounds, C16: ethyl hexadecanoate. [a]: minimun number of correct responses required for significance at the state α-level (0.05) for the corresponding number of responses, *n* (26). We rejected the assumption of 'no difference' if the number of correct responses is greater than or equal to the tabled value.

Benn and Peppard (1996) characterized tequila flavour by instrumental and sensory analysis, and although they were able to identify the most powerful odorants of tequila (highest FD factors), efforts at reconstituting tequila flavour from its component parts were not successful, indicating the presence of complex sensory interactions within the spirit matrix, which tend to dominate the overall aroma perception in a way not experience when the 'key' odorants are recombined. These observations correlated with our findings, and suggested the complexity of tequila flavour and states that further work is needed to fully understand how the different fractions (tequila flavour wheel) interact with each other and modify the sensory character of the final product. Some results are discussed in this manner and present in Chapter 5.

In addition to mature bourbon and mature tequila, Table 4.6 shows the results from reconstitution experiments of malt whisky 'A'. Significant differences ($P < 0.05$) in terms of flavour perception were observed between the majority of simulants and the authentic malt whisky 'A'. The third simulant was the exception to this observation, since this model presented the highest level of similarity (2.11) to malt whisky 'A' relative to the remaining products. As with tequila and bourbon, the simulants which had the greater degree of similarity with the original products were those with the addition of low boiling volatile compounds and ethyl hexadecanoate. These results confirmed the high impact of these congeners in modulating the perception of key odourants which define the mature character of aged spirits. The simulants and malt whisky 'A' shared common sensory attributes that included pungent/alcoholic, fruity, sweet, vanilla, and woody respectively (Table 4.6). Nevertheless, the third simulant was the product with the strongest woody character among the model spirits and the characteristic 'smoky' character of the malt whisky 'A' was not found in any of the simulants.

Table 4.6. Reconstitution experiments MALT WHISKY 'A'

Triangle test	Comp´s added	Aroma impressions	Correct responses/total responses	Critical number of correct responses[a]	*P value*	Conclusion	Level of similarity
MWA/1st SM	Without LBC	Pungent/alcoholic, estery, sweet/vanilla and fatty attributes	20/26	14	< 0.05	Sig. differences between samples	0.88 ± 0.45
MWA/2nd SM	With LBC	Pungent/alcoholic, estery, sweet/vanilla, and a slightly woody character	20/26	14	< 0.05	Sig. differences between samples	1.75 ± 0.66
MWA/3rd SM	LBC + C16	Pungent/alcoholic, floral/fragrant, estery, sweet/vanilla, fatty, and an increase of woody character	13/26	14	> 0.05	**No differences between the samples**	2.11 ± 0.63
MWA/4th SM	LBC + Non-volatile-compounds	Pungent/alcoholic, smoky, feinty, floral/fragrant, estery, sweet/vanilla, woody-character, fatty and sulphury character	18/26	14	< 0.05	Sig. differences between samples	1.85 ± 0.63
MWA/5th SM	LBC + Non-volatile compounds + C16	Pungent/alcoholic, burnt, floral/fragrant, estery, sweet/vanilla, woody-like character and fatty.	18/26	14	< 0.05	Sig. differences between samples	1.78 ± 0.65

The similarity in the overall flavour of the spirit models was scored on the following scale: 0, not at all; 1, weak similarity; 2, medium similarity; 3, practical identical. The results obtained by 26 panellists were averaged and expressed with the standard deviation. LBC: low boiling volatile compounds, C16: ethyl hexadecanoate. [a]: minimun number of correct responses required for significance at the state α-level (0.05) for the corresponding number of responses, *n* (26). We rejected the assumption of 'no difference' if the number of correct responses is greater than or equal to the tabled value.

As with malt whisky ‘A’, Table 4.7 shows the results from the reconstitution experiments carried out in the malt whisky ‘B. In this case, for the third and fifth simulants, they were both somewhat similar and did not differ from each other in their degree of similarity to the authentic whisky (*P < 0.05).* Among the sensory characteristics that were common among these simulants and could also be found as well in the original product were pungent, smoky, fragrant, sweet, and woody character, and fatty-like aroma. These results further confirmed our previous observations in malt whisky ‘A’ regarding the impact of low boiling volatile compounds and ethyl hexadecanoate in the flavour perception of aged spirits. Whilst the presence of low boiling volatile compounds and ethyl hexadecanoate could modify the aroma perception of mature spirits of bourbon, and malt whiskies (A and B) to more closely match the characteristics of the original spirits, for mature tequila their presence definitely modified the aroma balance of the spirit, however did not significantly improve the similarity to the authentic tequila. Possible reasons for this observation could be that the contribution of other underlying aroma compounds (FD ≤ 27) was significant, or to the fact that during GC-O analysis we did not consider potential interactions (e.g. synergy or masking) between odourants, which can influence perceived aroma (Conner et al. 1996; Atanasova et al. 2004, 2005; Chaput et al. 2012). In addition, it is possible that the differences were due to the arbitrary cut-off of using compounds with FD > 27 and thus the omission of some odour-active compounds from the simulant blends.

Table 4.7. Reconstitution experiments MALT WHISKY 'B'

Triangle test	Comp´s added	Aroma impressions	Correct responses/total responses	Critical number of correct responses[a]	*P value*	Conclusion	Level of similarity
MWB/1st SM	Without LBC	Pungent/alcoholic, smoky, floral, estery-like, sweet/vanilla, fatty, musty and slightly woody character	15/26	14	< 0.05	Sig. differences between samples	1.46 ± 0.68
MWB/2nd SM	With LBC	Pungent/alcoholic, smoky, feinty, grassy, floral, estery-like, sweet/vanilla, woody characters, and fatty-like aroma	16/26	14	< 0.05	Sig. differences between samples	1.94 ± 0.68
MWB/3rd SM	LBC + C16	Pungent/alcoholic, smoky, floral/fragrant, sweet/vanilla, woody character, and fatty-like aroma	13/26	14	> 0.05	**No differences between samples**	2.46 ± 0.51
MWB/4th SM	LBC + Non-volatile-compounds	Pungent/alcoholic, smoky, floral/fragrant, estery-like, musty and a sweet overtone	18/26	14	< 0.05	Sig. differences between samples	1.87 ± 0.85
MWB/5th SM	LBC + Non-volatile compounds + C16	Smoky, floral/fragrant, estery-like, sweet/vanilla, woody character, fatty, sulphury and earthy aroma	13/26	14	> 0.05	**No differences between samples**	2.22 ± 0.57

The similarity in the overall flavour of the spirit models was scored on the following scale: 0, not at all; 1, weak similarity; 2, medium similarity; 3, practical identical. The results obtained by 26 panellists were averaged and expressed with the standard deviation. LBC: low boiling volatile compounds, C16: ethyl hexadecanoate. [a]: minimun number of correct responses required for significance at the state α-level (0.05) for the corresponding number of responses, *n* (26). We rejected the assumption of 'no difference' if the number of correct responses is greater than or equal to the tabled value.

4.4.3 Sensory evaluation of the improved estery and woody fractions of spirit aroma simulants

In general, the presence of low boiling volatile compounds and ethyl hexadecanoate had a significant effect on the overall aroma of the simulants of mature spirits, which resulted in higher levels of reported similarity as compared with the authentic products, particularly in the mature character. Additionally, these compounds were also used as background congeners to improve the authenticity of the existing estery and woody fractions by adding them into each of the fractions of the model spirits. In this sense, improved estery and woody fractions were prepared (Appendices 9 and 10) and tested in informal sensory sessions as reported in Section 4.3.3 and the results are summarized in Appendix 12. The best fractions were found in the fourth model systems for the estery fraction and in the third model systems for the woody fraction in all spirits evaluated. The presence of ethyl hexadecanoate (C16) and low boiling volatile compounds were the main congeners in affecting the overall aroma balance of these new fractions. However, C16 has been reported as the key compound in altering the aroma balance of aqueous alcoholic solutions (Boothroyd et al. 2013). Particularly, by allowing the incorporation of other hydrophobic compounds (e.g. alcohols and aldehydes) into the micelle-like structure that they formed during dilution of spirit and thus impacting directly on the sensory properties of the final product (Piggott et al. 1996; Conner et al. 1999; Boothroyd et al. 2013). Because of the significant effect of the addition of low boiling volatile compounds and ethyl hexadecanoate in altering the aroma quality of the majority of the estery and woody fractions, we decided to select these compositions as the aroma base for developing blends of flavour compounds

responsible of estery and woody characters and determining whether there were significant sensory interactions between them and how they influence the perception of the mature character in each of the spirits (Chapter 5).

Furthermore, the SWRI performed a full aroma profile (QDA) of some selected fractions, with the intention to evaluate the impact of ethyl hexadecanoate (C16) and low boiling volatile compound addition in the flavour perception of new model estery and woody fractions, and compared them against their previous simulants and the authentic mature spirit (Figure 4.4). Bourbon fractions were selected as the ideal to characterize, since in the first attempts to improve the overall aroma authenticity of bourbon model spirits, these were the ones in showing the higher level of similarity to the original spirit. The new estery fractions were defined with an intense fresh fruit and solventy character relative to the earlier simulant version which was predominantly characterized by a higher intensity of fresh fruit aroma. Although the new estery fraction increased its fresh fruit and solventy character relative to the original spirit, in general it contained the key sensory properties that defined the estery character of this spirit (fresh fruit, solventy, and soapy aromas). On the other hand, the revised mature/woody fraction showed a better balance in woody attributes (spicy, woody, sweet and dried fruit) relative to the earlier simulant which was essentially sweeter. In fact, this new simulant was closer in mature character to the original spirit (Figure 4.4).

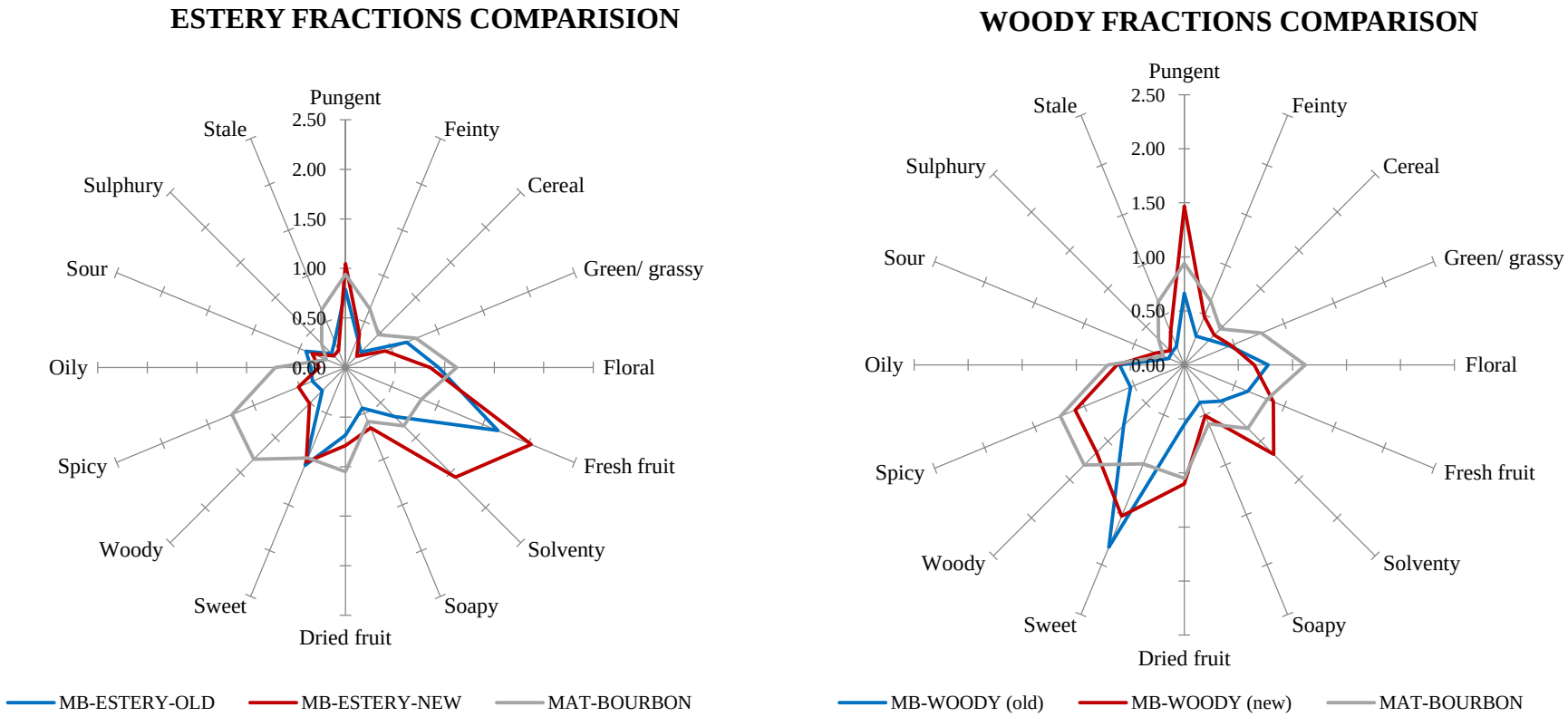

Figure 4.4 Aroma profiles comparison of old and new estery and woody fractions of mature bourbon simulates and the original spirit. MB-ESTERY-OLD: old estery version of mature bourbon; MB-ESTERY-NEW: new estery version of mature bourbon; MAT-BOURBON: mature bourbon; MB-WOODY-OLD: old woody version of mature bourbon; MB-WOODY-NEW: new woody version of mature bourbon.

These results confirm our previous observations regarding the strong influence of the additional components (low boiling volatile compounds and ethyl hexadecanoate) which were added to increase the degree of sophistication/ authenticity of the overall aroma of these mature simulants and supported the findings of Boothroyd et al. (2013) with regard ethyl hexadecanoate (C16) addition and its effect on the headspace concentrations of whisky aroma volatiles and thus on the perceived aroma character of whisky. In addition, several authors have reported the success of mimicking the overall aroma of different spirit matrices by aroma recombination experiments. Particularly important are those made by Peter Schieberle's group employing the full Sensomics approach (Dunkel, et al. 2014), which consists of i) aroma extraction by SAFE analysis, ii) identification of most odour active compound by means of AEDA analysis, iii) quantification by stable isotope dilution assay (SIDA), iv) determination of the importance of each odorant on the basis of OAVs, and v) validation of the results though aroma reconstitution experiments and sensory evaluation. These have been successfully applied to American Bourbon Whiskey (Poisson and Schieberle, 2008), Cognac and German Brandy (Uselmann and Schieberle, 2014) and Sherry Wine (Marcq and Schieberle, 2015). Other authors have also reported application of this approach in Rose Wine (Ferreira et al. 2002). Although in the majority of the cases, the main application of Sensomics approach is the identification of the key odorants that define the overall aroma or flavour of alcoholic beverages or foodstuffs, for the present study it was the selection of key odourants that define the estery and woody character of aged spirits. This information was then used to develop blends of flavour compounds responsible of these characters and to determine whether there are significant

sensory interactions between them and the resulting effects in flavour perception (Chapter 5).

4.4.4 Modification of sensory perception by addition of 'mature' ageing compounds into the non-mature spirits.

Although a considerable improvement in the mature character of the simulants was achieved in the work described thus far, we decided to test the authenticity of our developed aroma blends for mature character by adding them into authentic new make spirit samples. The aim was to use a more complex/authentic base for the mature aroma blend to be assessed in, to see if the resulting spirit closely resembled the actual mature spirit derived from wood ageing. This was a good way to accurately assess how close our maturation blend was to mimicking the chemical/sensory changes actually brought about by wood ageing. For this purpose, a full sensory screening (QDA) of these 'new' simulants against the non-mature and mature spirits was performed using the SWRI expert panel. Compounds added into the new make spirits were the ones representing the woody character plus non-volatile and some low-boiling compounds (e.g. acetaldehyde, acetal and acetic acid) that were highly associated to the maturation process according to results from Chapter 2. The concentrations spiked were the ones identified in the original aged spirits and displayed in Table 4.1. Figure 4.5 and 4.6 shows the sensory profiles of these 'new' simulants compared with their non-mature and mature counter-parts. In general, the incorporation of 'mature' congeners into new make spirits definitely modified the aroma perception to something approaching the aroma profile of the respective mature spirits. However, for some sensory properties, the aroma

perception was still significantly different ($P < 0.05$). For example, in bourbon samples, the simulated matured product presented a similar aroma to that of mature bourbon in the perception of 'pungent', 'feinty', 'cereal', 'green/grassy', 'solventy', 'dried fruit', 'spicy', 'sour', 'sulphury', and 'stale' sensory characters, whilst significant differences were recorded in the sensory detection of 'floral', 'soapy', 'sweet', 'woody', and 'oily' notes. A visual comparison of the 3 aroma profiles for each spirit (Figures 4.5 & 4.6) indicates differences in terms of aroma profiles, particularly in those sensory descriptors that define the 'mature' character of aged spirits (*dried fruit, sweet, woody,* and s*picy*). For example, simulated mature bourbon was rated as having a more 'sweet' character relative to the other aged simulants and the original mature spirits. However, a better balance in terms of mature character of the simulated products was observed in the malt whiskies 'A and B' (Figures 4.5 & 4.6). In general, the addition of ageing congeners into the new-make spirits resulted in a modification of the overall aroma of these spirit blends, mimicking to some extent the sensory changes derived as consequence of wood maturation. In most of the cases, the added maturation blends resulted in simulants which were oversweet and less woody relative to the mature character of the aged spirits. In fact, it appears that spiking ageing congeners into non-mature spirits (of same brand and provenance) could not closely recreate the sensory profile of the mature product obtained from cask maturation. However, this approach helped us to have a better understanding of the effects of adding ageing-congeners into the distillate matrix and confirms the complexity of wood maturation and the difficulty of re-creating its sensory impacts on spirit aroma through a 'sensomics' type of approach.

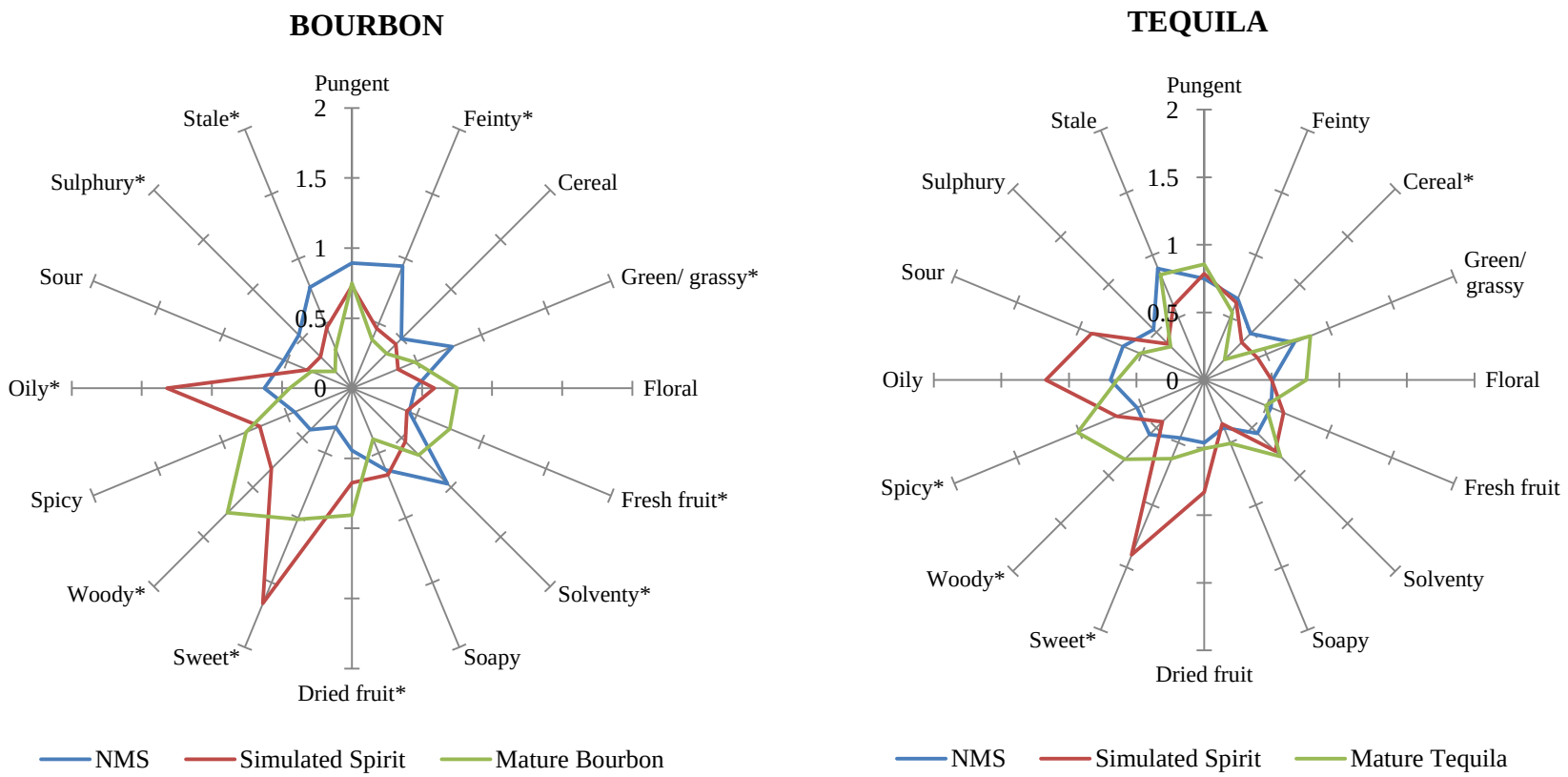

Figure 4.5 Aroma profiles of non-mature and mature spirits of bourbon and tequila and their respective simulated spirits consisting of the 'mature character' spiked into the non-mature spirit. NMS: new make spirit. * Indicate a statistical difference between the samples in their respective sensory character. ($P < 0.05$)

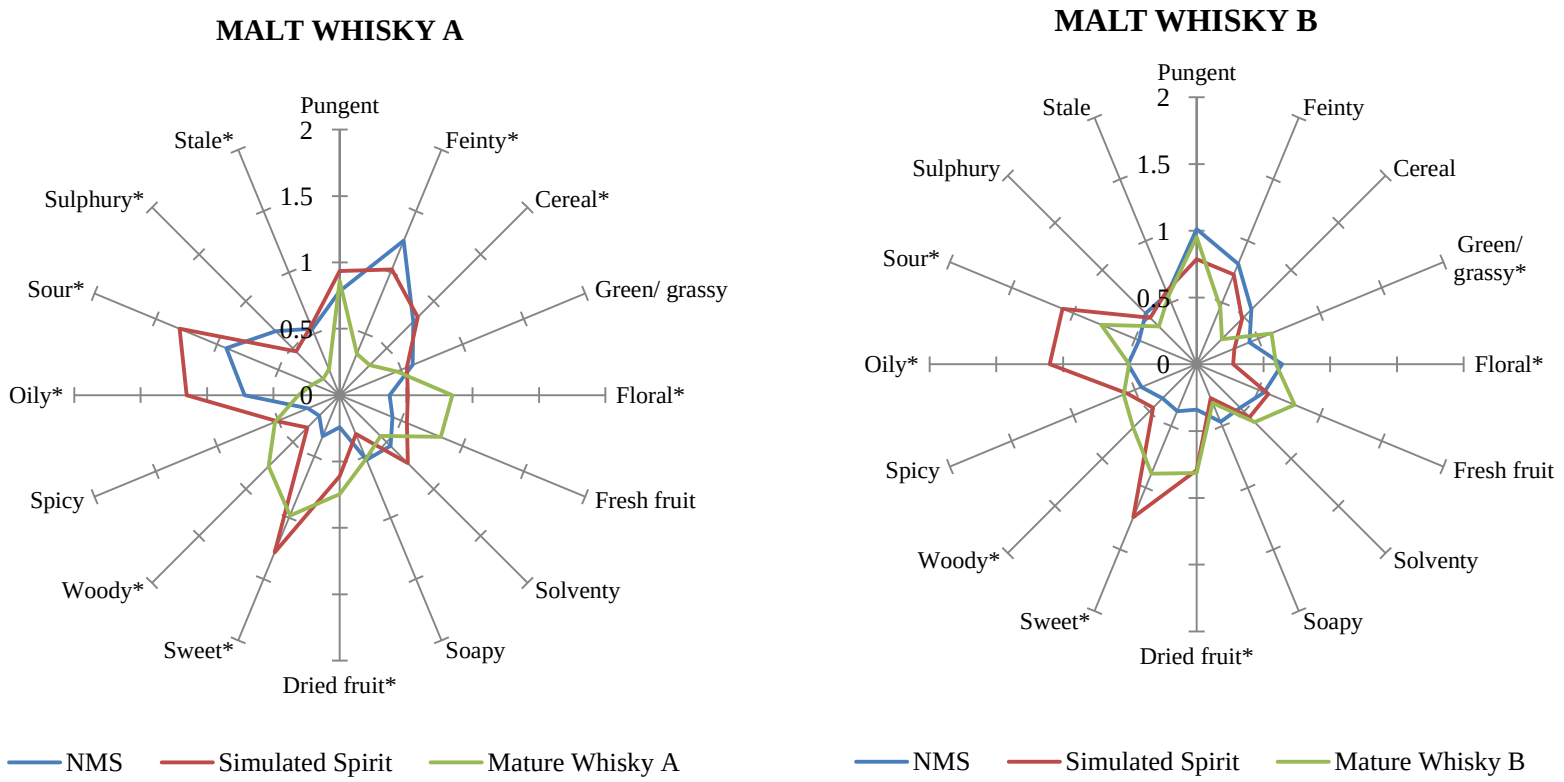

Figure 4.6 Aroma profiles of new make spirits and malt whiskies A and B and their respective simulated spirits consisting of the 'mature character' spiked into the new make spirit. NMS: new make spirit. * Indicate a statistical difference between the samples in their respective sensory character. ($P < 0.05$)

4.5. CONCLUSIONS

The aroma reconstitution of four different spirits (bourbon, tequila and malt whiskies) was attempted by means of recombination experiments in which the most odour active compounds (FD > 27) were mixed according to the proportions analysed in the original products and compared with the authentic samples. In the first attempts, the flavours imparted by the aroma simulant blends were easily differentiated from the authentic samples (using sensory triangle tests). Thus, it was speculated that the contribution of other underlying aroma compounds remains yet unidentified (e. g. compounds with FD ≤ 27 or presented before solvent front during GC-O analysis). In further experiments, the impacts of long chain ethyl ester (ethyl hexadecanoate), non-volatile compounds and low boiling volatile compounds addition were evaluated. The presence of ethyl hexadecanoate and low boiling volatile compounds significantly affected the overall aroma perception of these new simulants by giving a more authentic aroma balance as compared with the real products. Possible reasons for this are that the presence of ethyl hexadecanoate in the model spirits induced agglomerate formation that affected the partition of aroma and hence the perception of spirit flavour (Boothroyd et al. 2013). In addition, the impact of these compounds was further investigated when defining the estery and mature/woody characters of each of the spirits. In general, the presence of low boiling volatile compounds and ethyl hexadecanoate particularly increased the authenticity of the estery character of each of the fractions, by achieving a more balanced and fruity character, similar to the ones found in the original products. In the case of the woody fractions, their presence also impacted the overall ‘mature’ character such that it was closer to that of the real products.

This was further confirmed by the SWRI sensory panel which defined the simulated woody fraction of mature bourbon with similar sensory characters (spicy, woody, sweet and dried fruit) as for the authentic mature bourbon. Additionally, spiking of the developed blends of ageing congeners into new make spirits of same brand and provenance, resulted in modifications to scores for maturation related sensory properties. However, the resulting sensory profiles still did not entirely match those of the authentic spirits following cask maturation. Based on these results, we formulated the blends that recreated, as closely as was possible, the estery and woody fractions of each of the spirits. These fractions were then used as the basis to investigate potential interactions between the perception of woody and estery characters in mature spirits in Chapter 5.

5. FLAVOUR INTERACTIONS BETWEEN THE 'ESTERY' AND 'MATURE/WOODY' CHARACTERS OF WHISKY, BOURBON AND TEQUILA.

5.1. AIM

Based on the results of Chapters 3 and 4, which identified the most odour active compounds (GC-O/AEDA work) that contribute to the estery and woody fractions in each of the spirits, as well as the improvement of these fractions by incorporation of ethyl hexadecanoate and low boiling volatile compounds. The main objectives of this Chapter were: i) investigate the impacts of varying levels of 'estery' character in each spirit on the perceived mature/woody character and ii) investigate the impacts of varying levels of 'mature/woody' character in each spirit on the perceived 'estery' character. The background to this study was the observation (Dr John Conner, SWRI: personal communication) that the estery character of mature whiskies typically declines relative to the new make spirit, even though the analytical concentrations of esters remain broadly constant. One potential explanation for this observation would be a sensory interaction between mature and estery characters and the experiments in this Chapter were designed to test that hypothesis.

5.2. INTRODUCTION

One of the major challenges of today's aroma research is to understand the rationale determining the qualitative and quantitative characteristics of the sensory perception elicited by complex mixtures of odorants. Whisky aroma is not just the sum of individual constituents, but the result of complex interactions between a large numbers of chemical compounds. Odorants can interact,

showing either additive or competitive effects, which may turn even into synergistic or antagonistic effects (Ferreira, 2012). Interactions that influence the aroma properties of whisky have been the object of whisky research for decades, although most of these studies have focused on aroma interactions of simple mixtures (Piggott et al. 1992; Conner et al. 1994; 1994; Conner et al. 1996; Conner et al. 1999; Nose et al. 2004; Boothroyd et al. 2013).

In wine, several studies have reported that when compounds are present at concentrations below their detection threshold, an odour can be perceived as a result of perceptual synergism. More specifically, these works highlighted the existence of perceptual interactions for woody versus fruity notes in wine (Atanasova et al. 2004; Atanasova et al. 2005), proving that woody notes tended to dominate over the fruity ones in binary mixtures containing sub- and peri-threshold levels of woody odourants. Ethanol can change some of those effects and itself be masked (Atanasova, et al. 2007). The former is the reason why whisky is diluted to alcohol strength of 20-23% ABV for nosing purposes (Boothroyd et al. 2013).

While all these studies highlight important features about the perceptual interaction between odourants, some of them are using simple working schemes, such as synthetic solutions, simple spirit models or single odorants solutions, which may limit the generalisation of their results. In addition, in most cases researchers have studied the interaction between two odours, when the fact is that in whisky and any other spirits there are many odorants creating different odours and competing simultaneously. Finally, and to the best of our knowledge, there is no study in which the ‘estery’ and ‘woody/mature’ character of such complex matrices as tequila, bourbon and malt whisky have been systematically

assessed for their contribution and sensory interaction. Therefore, the main objective of this chapter was to assess the aromatic sensory interactions of these characters in each of the spirits and their resulting effects on flavour perception.

5.3. MATERIAL AND METHODS

5.3.1. Samples

For each spirit set, nine model spirit solutions were prepared according to a multi-level factorial experimental design (Tables 5.2, 5.4, 5.6, 5.8) in which the 'estery' and 'mature/woody' fractions were varied using an ethanolic solution at 23% ABV and ethyl hexadecanoate at 500 mg/L (concentration found in Chapter 4 as the ideal to modify the balance of volatile compounds in the headspace to an extend that improve the perceived character of the aged spirits). The concentrations of the aroma compounds in each of the fractions as well as the compounds that form the common aroma base of these spirits models are presented in Tables 5.1, 5.3, 5.5, and 5.7.

Table 5.1. Concentration (mg/L) of compounds that formed the 'estery' and 'woody' fractions in Bourbon spirit models and the pool of compounds comprising the common aroma base.

Odorant	Chemical group	Fraction	FD	Concentration (mg/L)
Ethyl acetate*	Ester		1000	305.35
Isoamyl acetate*	Ester		729	26.67
Ethyl hexanoate	Ester		2189	2.27
Ethyl lactate*	Ester		1000	10.45
Ethyl octanoate	Ester		6561	8.57
Ethyl nonanoate	Ester		27	0.08
Ethyl decanoate	Ester	**Estery**	729	13.49
Diethyl succinate	Ester		6561	0.92
Ethyl benzoate	Ester		6561	0.03
Phenethyl acetate	Ester		243	0.60
Ethyl dodecanoate	Ester		27	3.57
Ethyl hexadecanoate	Ester		81	6.52
	Total estery content			**379**
γ-nonalactone	Ketone		6561	0.03
Cis/trans-whisky lactone	Oak-lactone		729	11.63
Guaiacol	Volatile-phenol	**Woody**	6561	0.04
4-ethyl-guaiacol	Volatile-phenol		6561	0.02
Eugenol	Volatile-phenol		729	0.02
Vanillin	Volatile-phenol		729	2.68
	Total woody content			**14**
Octanoic acid	Acid		243	0.6
Decanoic acid	Acid		6561	0.9
n-Hexanol	Alcohol		27	3
Phenylethyl Alcohol	Alcohol		729	15
Benzaldehyde	Aldehyde		243	0.03
Acetaldehyde*	Aldehyde	**Pool of**	1000	24
Acetal*	Acetal	**compounds**	1000	25
Acetic acid*	Acid	**comprising the**	1000	435
Furfural*	Furan	**common aroma**	243	7
5MF	Furan	**base**	81	0.01
Methanol*	Alcohol		1000	39
n-Propanol*	Alcohol		1000	148
Isobutanol*	Alcohol		1000	464
2-methyl-1-butanol*	Alcohol		1000	708
3-methyl-1-butanol*	Alcohol		1000	931

*LBC: low boiling volatile compounds. Concentrations of pool of compounds that create the common aroma base were estimated from the original concentrations in the non-mature spirits with the exception of LBC whose concentrations are as analysed in the mature spirit.

Table 5.2 Levels of the 'estery' and 'woody' fractions used in the 3-level factorial experimental design of bourbon model spirit solutions.

	Levels		Amount (Σ (mg/L))	Amount (Σ (mg/L))
Solution	**Estery Fraction**	**Woody Fraction**	**Estery Fraction**	**Woody Fraction**
1	100%	200%	379	28
2	50	100%	189	14
3	50	200%	189	28
4	50	0%	189	0
5	100%	100%	379	14
6	150%	200%	568	28
7	100%	0%	379	0
8	150%	0%	568	0
9	150%	100%	568	14

Table 5.3. Concentration (mg/L) of compounds that formed the 'estery' and 'woody' fractions of Tequila model spirit solutions and the pool of compounds comprising the common aroma base.

Odorant	Chemical group	Fraction	FD	Concentration (mg/L)
Ethyl acetate*	Ester		1000	111.7
Isoamyl acetate*	Ester		1000	3.1
Ethyl hexanoate	Ester		6561	0.3
Ethyl lactate*	Ester		1000	16.3
Ethyl octanoate	Ester		6561	8.3
Ethyl decanoate	Ester	**Estery**	729	6.3
Diethyl succinate	Ester		6561	0.4
Ethyl benzoate	Ester		6561	0.0
Phenethyl acetate	Ester		243	0.2
Ethyl dodecanoate	Ester		81	0.7
Ethyl tetradecanoate	Ester		81	0.02
Ethyl hexadecanoate	Ester		2187	0.1
	Total estery content			**147**
Cis/trans-whisky lactone	Oak-lactone		6561	1.81
Guaiacol	Volatile-phenol		6561	0.014
4-ethyl-guaiacol	Volatile-phenol	**Woody**	6561	0.01
Eugenol	Volatile-phenol		6561	0.02
Vanillin	Volatile-phenol		6561	0.88
	Total woody content			**2.7**
Acetaldehyde*	Aldehyde		1000	39
Acetal*	Acetal		1000	81
Acetic acid*	Acid		1000	281
Furfural*	Furan		81	2
Methanol*	Alcohol		1000	653
n-Propanol*	Alcohol		1000	275
Isobutanol*	Alcohol		1000	358
2-methyl-1-butanol*	Alcohol		1000	551
3-methyl-1-butanol*	Alcohol		1000	768
n-Butanol*	Alcohol		100	1.4
Diacetyl	Alcohol	**Pool of**	ND	0.4
β-Damascenone	Alcohol	**compounds**	6561	0.2
Linalool	Alcohol	**comprising**	2189	0.9
Citronellol	Alcohol	**the common**	2189	0.4
α-Terpineol	Alcohol	**aroma base**	729	4.4
Dihydro-2-methyl-3(2H)-furanone	Alcohol		243	1.1
2-Acetylfuran	Furan		2189	0.5
5-Methyl furfural	Furan		243	0.5
cis-Linalool oxide	Aldehyde		2189	0.6
n-Hexanol	Alcohol		100	0.9
Octanoic acid	Acid		6561	1.1
Decanoic acid	Acid		729	3.4
Phenethyl alcohol	Alcohol		6561	1.4
Isobutanal diethyl acetal	Acetal		27	2.8
β-Ethoxypropionaldehyde diethyl acetal	Acetal		81	2.4

*LBC: low boiling volatile compounds. Concentrations of pool of compounds that create the common aroma base were estimated from the original concentrations in the non-mature spirits with the exception of LBC whose concentrations were as analysed in the mature spirit.

Table 5.4 Levels of the 'estery' and 'woody' fractions used in the 3-level factorial experimental design of tequila model spirit solutions

	Levels		**Amount (Σ (mg/L))**	**Amount (Σ (mg/L))**
Solution	Estery Fraction	Woody Fraction	Estery Fraction	Woody Fraction
1	50	0%	147	0
2	100%	200%	295	5.5
3	50	100%	147	2.75
4	150%	0%	442	0
5	150%	100%	442	2.75
6	50	200%	147	5.5
7	100%	100%	295	2.75
8	100%	0%	295	0
9	150%	200%	442	5.5

Table 5.5. Concentration (mg/L) of compounds that formed the 'estery' and 'woody' fractions in MWA model spirit solutions and the pool of compounds comprising the common aroma base

Odorant	Chemical group	Fraction	FD	Concentration (mg/L)
Ethyl acetate*	Ester		1000	250.40
Isoamyl acetate*	Ester		1000	25.90
Ethyl hexanoate	Ester		6561	1.15
Ethyl lactate*	Ester		1000	3.30
Ethyl octanoate	Ester		6561	10.92
Ethyl decanoate	Ester	**Estery**	2187	46.85
Diethyl succinate	Ester		6561	1.13
Ethyl benzoate	Ester		6561	0.30
Phenethyl acetate	Ester		6561	8.08
Ethyl dodecanoate	Ester		243	11.26
Ethyl hexadecanoate	Ester		2187	12.12
	Total estery content			**371**
Cis/trans-whisky lactone	Oak-lactone		6561	2.27
Guaiacol	Volatile-phenol		6561	0.136
4-ethyl-guaiacol	Volatile-phenol	**Woody**	6561	0.185
Eugenol	Volatile-phenol		6561	0.262
Vanillin	Volatile-phenol		6561	2.090
	Total woody content			**4.9**
Acetaldehyde*	Aldehyde		1000	16
Acetal*	Acetal		1000	184
Acetic acid*	Acid		1000	231
Furfural*	Furan		6561	12
Methanol*	Alcohol		1000	27
n-Propanol*	Alcohol		1000	280
Isobutanol*	Alcohol		1000	520
2-methyl-1-butanol*	Alcohol		1000	702
3-methyl-1-butanol*	Alcohol		1000	824
n-Butanol*	Alcohol		1000	10
n-Pentanol	Alcohol	**Pool of**	27	1
n-Hexanol	Alcohol	**compounds**	6561	4
n-Decanol	Alcohol	**comprising**	2187	1
n-Hexadecanol	Alcohol	**the**	2187	1
n-Undecanol	Alcohol	**common**	81	0.2
(9E)-9-Hexadecen-1-ol	Alcohol	**aroma base**	2187	2
2-Acetylfuran	Furan		2187	0.2
5-Methyl furfural	Furan		6561	0.4
Benzaldehyde	Aldehyde		2187	0.3
Hexanoic acid	Acid		6561	3
Octanoic acid	Acid		6561	19
Decanoic acid	Acid		6561	20
Dodecanoic acid	Acid		243	5
Methionol	Alcohol		6561	0.2
Phenethyl alcohol	Alcohol		6561	69
Isobutanal diethyl acetal	Acetal		243	0.7

*LBC: low boiling volatile compounds. Concentrations of pool of compounds that create the common aroma base were estimated from the original concentrations in the non-mature spirits with the exception of LBC which were as analysed in the mature spirit

Table 5.6 Levels of the 'estery' and 'woody' fractions used in the 3-level factorial experimental design of MWA model spirit solutions

	Levels		Amount (Σ (mg/L))	Amount (Σ (mg/L))
Solution	Estery Fraction	Woody Fraction	Estery Fraction	Woody Fraction
1	150%	200%	556	9.8
2	100%	200%	371	9.8
3	100%	0%	371	0
4	50%	100%	186	4.9
5	100%	100%	371	4.9
6	150%	100%	556	4.9
7	150%	0%	556	0
8	50%	200%	186	9.8
9	50%	0%	186	0

Table 5.7. Concentration (mg/L) of compounds that formed the 'estery' and 'woody' fractions in MWB model spirit solutions and the pool of compounds comprising the common aroma base

Odorant	Chemical group	Fraction	FD	Concentration (mg/L)
Ethyl acetate*	Ester	Estery	1000	232
Isoamyl acetate*	Ester	Estery	1000	16.6
Ethyl hexanoate	Ester	Estery	6561	1.79
Ethyl lactate*	Ester	Estery	1000	4.40
Ethyl octanoate	Ester	Estery	6561	18.4
Ethyl decanoate	Ester	Estery	729	59.7
Diethyl succinate	Ester	Estery	6561	0.12
Ethyl benzoate	Ester	Estery	6561	1.08
Phenethyl acetate	Ester	Estery	243	4.14
Ethyl dodecanoate	Ester	Estery	81	10.4
Ethyl tetradecanoate	Estery	Estery	81	0.76
Ethyl hexadecanoate	Ester	Estery	2187	5.22
	Total estery content			**356**
Cis/trans-whisky lactone	Oak-lactone		6561	2.08
Guaiacol	Volatile-phenol		6561	0.136
4-ethyl-guaiacol	Volatile-phenol	**Woody**	6561	0.177
Eugenol	Volatile-phenol		6561	0.231
Vanillin	Volatile-phenol		6561	1.911
	Total woody content			**4.5**
Acetaldehyde*	Aldehyde		1000	15
Acetal*	Acetal		1000	167
Acetic acid*	Acid		1000	333
Furfural*	Furan		6561	8.4
Methanol*	Alcohol		1000	16
n-Propanol*	Alcohol	**Pool of**	1000	204
Isobutanol*	Alcohol	**compounds**	1000	416
2-methyl-1-butanol*	Alcohol	**comprising the**	1000	590
3-methyl-1-butanol*	Alcohol	**common aroma**	1000	758
n-Butanol*	Alcohol	**base**	100	7.0
n-Pentanol	Alcohol		2187	0.6
n-Hexanol	Alcohol		2187	2.4
n-Decanol	Alcohol		6561	0.3
n-Hexadecanol	Alcohol		6561	0.5
n-Undecanol	Alcohol		81	0.2
(9E)-9-Hexadecen-1-ol	Alcohol		6561	1.0
2-Acetylfuran	Furan		2187	0.4
5-Methyl furfural	Furan		2187	0.3
Benzaldehyde	Aldehyde		6561	0.3
Hexanoic acid	Acid		2187	6.5
Octanoic acid	Acid		6561	29
Decanoic acid	Acid		2187	26
Dodecanoic acid	Acid		6561	4.1
Methionol	Alcohol		6561	0.2
Phenethyl alcohol	Alcohol		6561	50
Isobutanal diethyl acetal	Acetal		729	0.5
β-Ethoxypropionaldehyde diethyl acetal	Acetal		243	0.3

*LBC: low boiling volatile compounds. Concentrations of pool of compounds that create the common aroma base were estimated from the original concentrations in the non-mature spirits with the exception of LBC whose concentrations were as analysed in the mature spirit

Table 5.8 Levels of the 'estery' and 'woody' fractions used in the 3-level factorial experimental design of MWB model spirit solutions

	Levels		Amount (Σ (mg/L))	Amount (Σ (mg/L))
Solution	Estery Fraction	Woody Fraction	Estery Fraction	Woody Fraction
1	150%	200%	534	9
2	100%	200%	356	9
3	100%	0%	356	0
4	50%	100%	178	4.5
5	100%	100%	356	4.5
6	150%	100%	534	4.5
7	150%	0%	534	0
8	50%	200%	178	9
9	50%	0%	178	0

5.3.2. Reagents and Chemicals

As described in section 2.3.2.

5.3.3. Flavour interaction sensory evaluation

According to the multi-level factorial experimental design (Tables 5.2, 5.4, 5.6, 5.8), nine model spirit solutions per spirit were recreated to evaluate potential flavour interactions between the 'estery' and 'mature/woody' fraction for each spirit type. A panel consisting of 16 panellists (13 women and 3 men, ranging in age between 28 and 50 years old) were involved in the sensory evaluation of these spirit blends. All of them were research staff or students of the University of Nottingham. Some members of this panel performed the previous sensory evaluations of the reconstitution experiments. Furthermore, the same panel carried out the preliminary sensory interaction experiments in which they evaluated 36 model spirit solutions (9 for each spirit type) as obtained from the multi-level factorial experimental design. A total of 4 sessions were necessary to complete the evaluation of the model spirit solutions, in order to avoid sensory fatigue. The samples (20 mL) were assessed in individual booths at 20-22 ºC using random 3 digit-coded standard amber glasses. During the sessions, panellists were asked to evaluate the model spirits solutions orthonasally, by rating the intensity of the 'estery' and 'mature/woody' attributes using a 15-cm unstructured line scale with anchors of 'low' and 'high' intensity placed at 10% and 90% of the line respectively. Reference standards of the 'estery' and 'mature/woody' attributes that represent the low and high intensity of the scale were provided to the panellists during the sensory sessions. The 'estery' reference composition in each of the spirits models consisted of the low (50%)

and high (150%) levels of the multi-level factorial experimental design and the 'mature/woody' reference composition consisted of 25 and 200% of the levels of the multifactorial level experimental design. In this way it was clear to the panellists what the definition of 'mature/woody' and 'estery' characteristics were which they should be scoring for intensity. Based on the results from the preliminary sensory work at Nottingham, only the model spirit solutions that showed the potential for flavour interactions were selected for evaluation by the SWRI expert panel following the above sensory methodology but a reduced unstructured line scale of 3 cm.

5.3.4. Data treatment and statistical analysis

Estery and mature/woody ratings of all judges were analysed using ANOVA with judges considered as random factors and all other variables as fixed factors (Statgraphics plus software Version 16.1.11). Significant factor effects and interactions between factors are reported at $P < 0.05$.

5.4. RESULTS AND DISCUSSION

5.4.1. Preliminary flavour interaction experiments in spirit models of Bourbon Whiskey

The effect of the estery fraction in the perception of the estery and mature/woody attributes is summarized in the ANOVA Table 5.9. According to this table, the estery fraction did not have a significant effect on the perception of the estery character ($P > 0.05$), however the mature/woody fraction had a significant effect on the perception of this attribute ($P < 0.05$). Furthermore, no clear interaction

between these two factors (*P > 0.05*) was observed in the perception of the 'estery' character.

Table 5.9. Analysis of Variance for Estery flavour perception in bourbon model spirits

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
A: Estery fraction	2.87042	1	2.87042	0.16	0.6908
B: Woody fraction	193.802	1	193.802	10.73	**0.0014**
AA (Estery*Estery)	3.16681	1	3.16681	0.18	0.6761
AB (Estery*Woody)	33.0625	1	33.0625	1.83	0.1785
BB (Woody*Woody)	113.753	1	113.753	6.30	**0.0134**
blocks	373.542	15	24.9028	1.38	0.1680
Total error	2221.5	123	18.0609		
Total (corr.)	2941.69	143			

Numbers in bold indicate significant effects of the factors in the perception of the 'estery' character

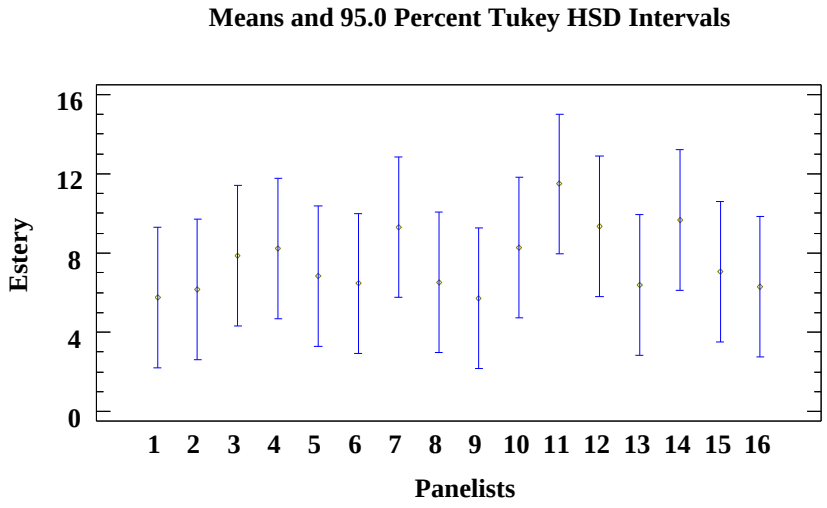

Figure 5.1. Means and 95% Percent Tukey HSD Intervals among panellists in the perception of estery character in spirit bourbon models.

In addition to the ANOVA table, Figure 5.1 shows the means and 95% Percent Tukey HSD Intervals variation among panellists in the perception of estery character. Since *P > 0.05*, there was not significant differences among the panellists in the perception of the estery character.

This pattern was to be expected since panellists were giving aroma references while evaluating the estery and woody attributes in the spirit models during the sensory evaluation. Thus, the quality of the data obtained was sufficient to support preliminary conclusions about the effects of adding certain amounts of estery congeners on the perception of the estery and woody characters in the spirit models. In this sense, Figure 5.2-A shows how the perpection of the estery aroma changes as a function of the estery content, in general the increase in the estery fraction did not seem to affect the perception of the estery character since similar variations were observed, indicating as a result an insignificant effect of this fraction in the perception of this character ($P > 0.05$). Furthermore, Figure 5.2-B confirmed the strong influence of the mature/woody fraction in the perception of the estery character ($P < 0.05$), specifically when the concentration of the fraction increased (14 and 28 ppm), there was a noticeable reduction in the perception of the this character. In fact, the estery character was only strongly perceived when the mature/woody fraction was ommited in the spirit models. Consequently, this data confirmed the strong influence of mature/woody character versus fruity/estery notes while evaluating them in spirit bourbon models.

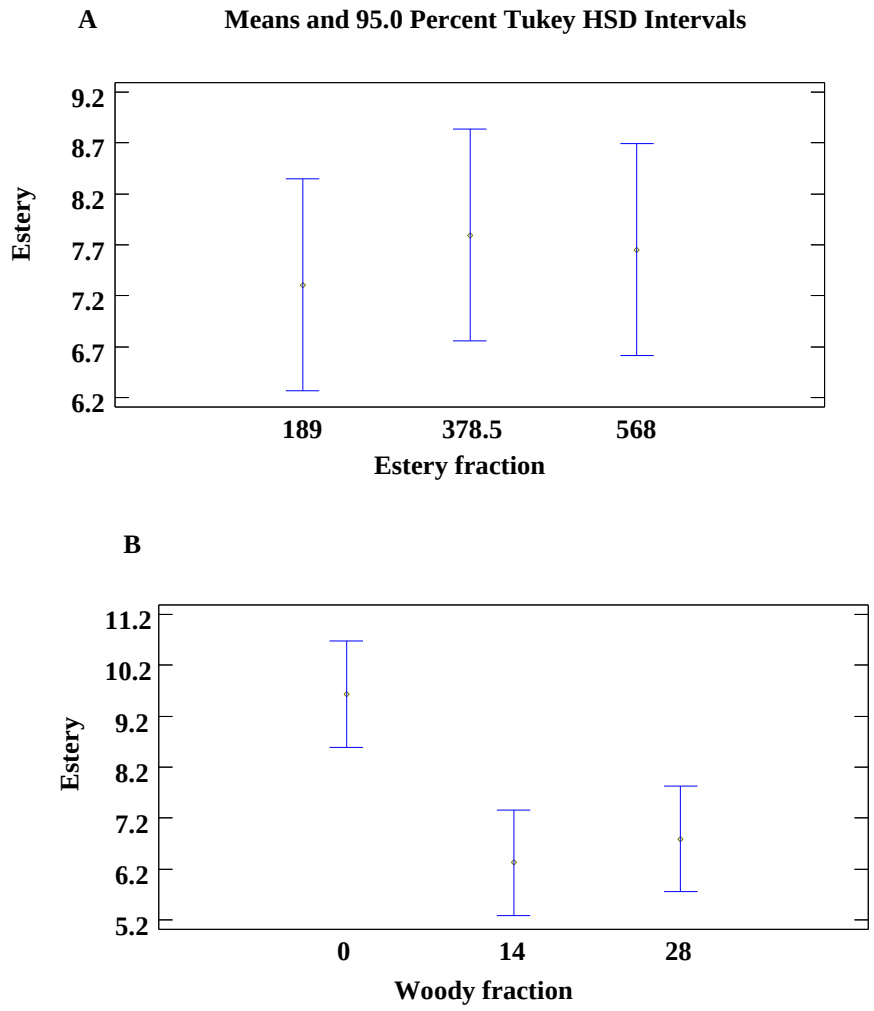

Figure 5.2. Means and 95% Percent Tukey HSD Intervals showing the impacts of the estery (A) and woody (B) fractions on the perception of estery character in model bourbon spirits.

Although significant sensory interactions were not found between the factors in the perception of the estery character ($P > 0.05$), interaction plots were valuable tools used to understand potential flavour interactions between these fractions (Figure 5.3A-B). In general, no parallel lines were presented, which would be indicative of interactions between the factors. For example, as we increased the concentration of the estery fraction in the absence of mature/woody fraction, the perception of the estery character increased as its fraction increased, however,

as the concentration of the mature/woody fraction increased the perception of the estery character decreased, indicating a strong suppressive effect of the mature/woody fraction on the perception of this character ($P < 0.05$).

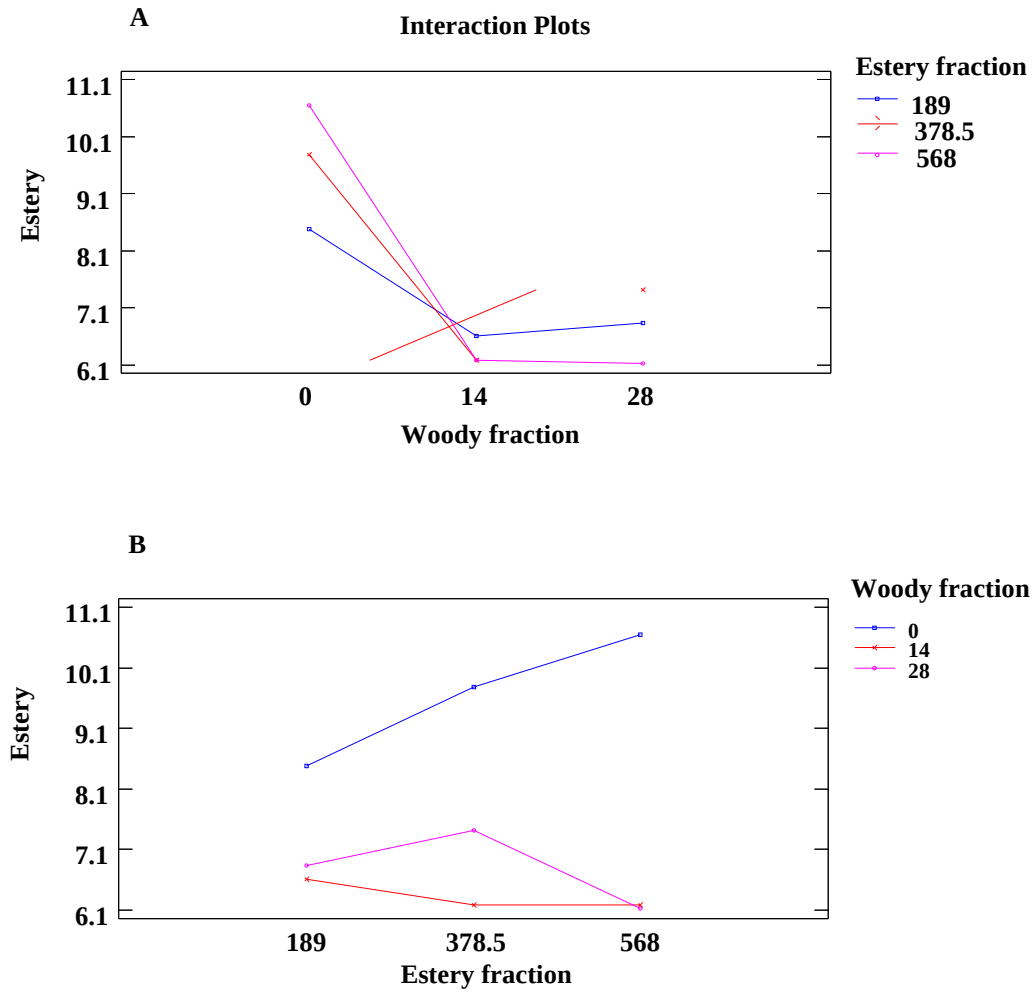

Figure 5.3. Interaction plots for the perception of estery character as influenced by concentrations of the woody fraction (A) and estery fraction (B) in spirit bourbon models.

Further to the effects of the estery fraction, Table 5.10 summarizes the effects of the mature/woody fraction on the perception of estery and woody characters. According to this ANOVA table, the mature/woody fraction had a significant effect on the perception of mature/woody character ($P < 0.05$); however, the

estery fraction had an insignificant effect on the perception of this attribute ($P > 0.05$). Once again, no clear interaction between these factors was observed ($P > 0.05$) in the perception of the woody character.

Table 5.10 Analysis of Variance for Woody in bourbon model spirits

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
A: Estery fraction	3.3376	1	3.3376	0.24	0.6234
B: Woody fraction	618.135	1	618.135	44.87	**0.0000**
AA (Estery*Estery)	31.535	1	31.535	2.29	0.1329
AB (Estery*Woody)	25.7556	1	25.7556	1.87	0.1740
BB (Woody*Woody)	66.8939	1	66.8939	4.86	**0.0294**
blocks	341.02	15	22.7347	1.65	0.0701
Total error	1694.49	123	13.7764		
Total (corr.)	2781.17	143			

Numbers in bold indicate significant effects of the factors in the perception of the 'woody' character

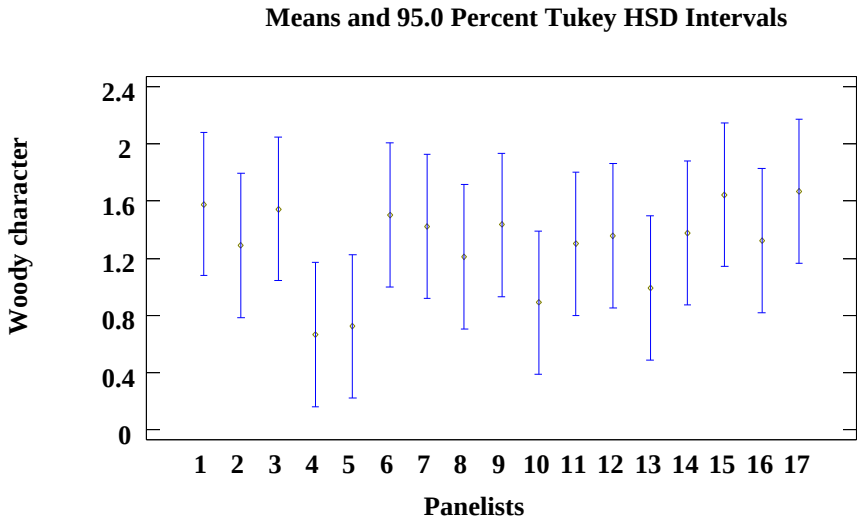

Figure 5.4. Means and 95% Percent Tukey HSD Intervals among panellists in the perception of woody character in spirit bourbon models.

Similarly, as with the estery fraction, significant differences were not observed among the panellists in the perception of the mature/woody character ($P > 0.05$; Figure 5.4). Also, Figure 5.5 A-B shows how the aroma perception of the woody character is affected or modified as consequence of the woody and estery

fractions. In general, a greater intensity of the woody character was observed at lower estery contents (189 and 378.5 ppm) and a reduction was perceived when the concentration of the estery fraction increased (568 ppm). In terms of the influence of the woody fraction, a significant increase of the woody character was perceived as the concentration in the spirit blends increased (Figure 5.5-B). Overall these results confirmed the strong suppression of the estery character by the presence of the mature/woody compounds of the spirit matrix.

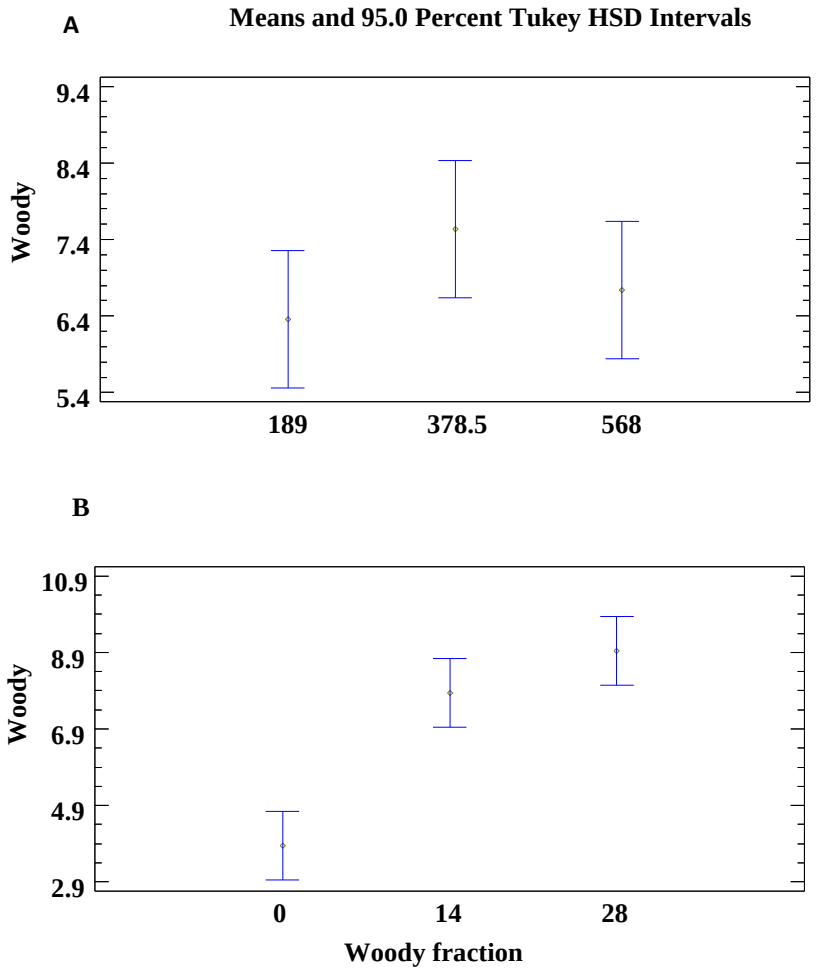

Figure 5.5. Means and 95% Percent Tukey HSD Intervals showing the impacts of the estery (A) and woody (B) fraction on the perception of woody character in model bourbon spirits.

|As with the estery character, no clear interactions between the fractions were observed in the perception of the woody character ($P > 0.05$). For example, as we increased the concentration of the mature/woody fraction, no matter what the concentration of the estery fraction, the perception of the woody character increased (Figure 5.6-A). This pattern can be further confirmed with the second graphic (Figure 5.6-B), for example in the blue line (0 content of woody) the perception of the woody character was reduced, and no apparent change occur as the concentration of the estery fraction increased. However, when the woody fraction was increased (14 and 28 ppm) the perception of the woody character increased independently of the concentration of estery. This data clearly confirmed a suppression of estery character by mature/woody congeners. This is in accordance with previous observations in wine, which had highlighted the existence of perceptual interaction for woody versus fruity/estery notes (Atanasova et al. 2004). This suggests a masking effect attributed to woody aroma over fruity character in binary mixtures, even when present at lower values of their respective odour thresholds of the woody congeners (Atanasova et al. 2007). During the sensory evaluations, the panellists suggested that the use of 'anchors' for the attributes were very useful for rating the samples. Furthermore, some of them reported other sensory characteristics during the evaluation of the reference samples, in particular some of them referred to a 'solventy/varnish/nail/polish/pear drops' odour found in the estery character and 'sweet/vanilla/nutty/almond' odour in the woody character.

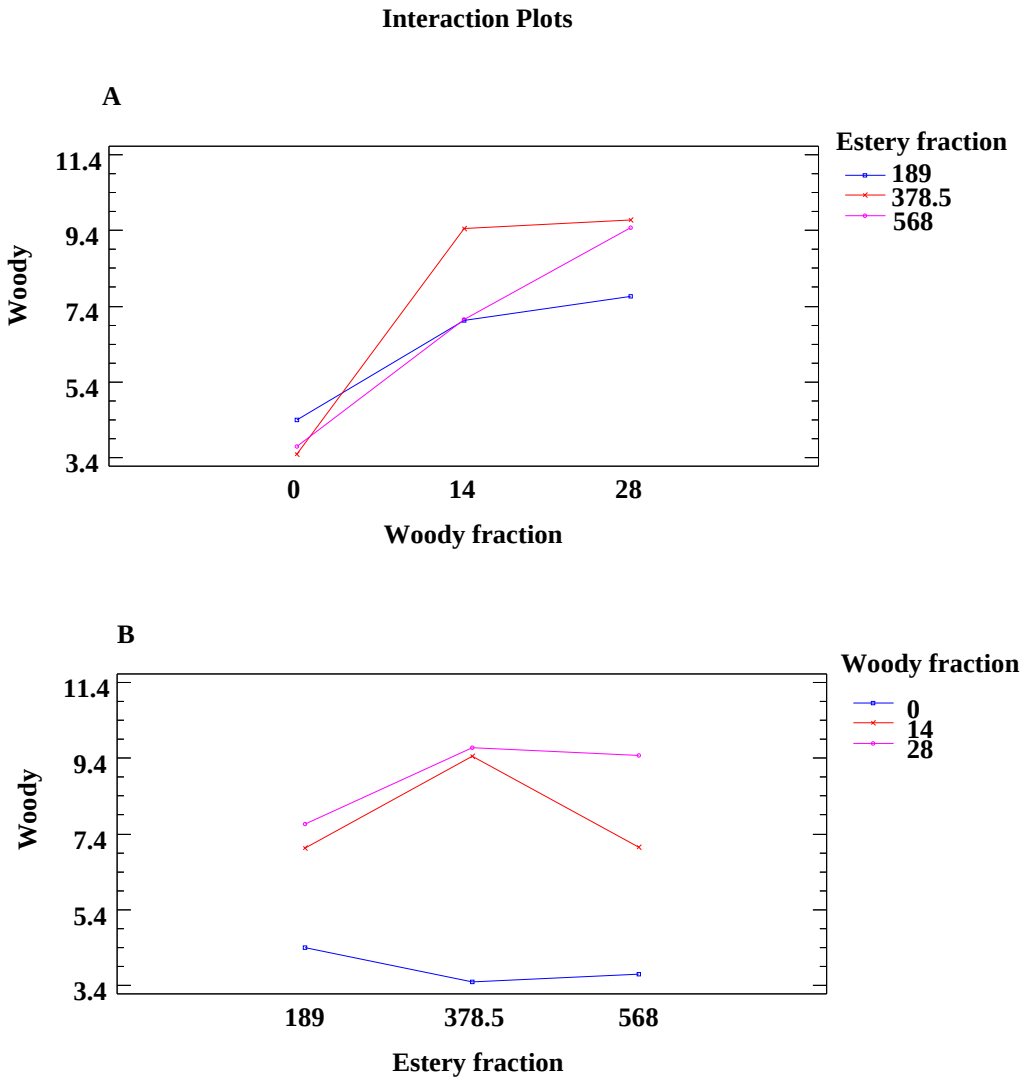

Figure 5.6. Interaction plots for the perception of estery character as influenced by concentrations of the woody fraction (A) and estery fraction (B) in spirit bourbon models.

Since a strong suppression of the estery character by mature/woody character in the model bourbon spirit was observed, we wanted to further confirm these observations and potential flavour sensory interactions between these fractions by assessing these samples using the SWRI expert panel.

5.4.2 Confirmation of the suppression of estery character by mature/woody congeners using SWRI expert panel

Table 5.11 shows the results from the analysis of variance (ANOVA) in which the effects of the estery and mature/woody fraction concentrations on the perception of the estery character can be found. According to this table, the estery and mature/woody fractions have significant effects on the perception of the estery character ($P < 0.05$). However, no clear interaction between these two factors was observed ($P > 0.05$) in determining the perception of the estery character. Contrary to the results found by the untrained panel at Nottingham, the estery fraction had a significant effect on the perception of the estery character; an explanation for this difference could be the greater ability of the trained panel to recognise this attribute in complex mixtures. Although a better performance was found by the SWRI panel in the perception of this attribute, significant differences ($P > 0.05$) were identified among the panellists when rating this attribute (Figure 5.7).

Table 5.11 Analysis of Variance for perception of Estery character in model bourbon spirits (SWRI panel data).

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
A: Estery fraction	2.83333	1	2.83333	7.09	**0.0087**
B: Woody fraction	11.0025	1	11.0025	27.52	**0.0000**
AA (Estery*Estery)	2.1417	1	2.1417	5.36	**0.0222**
AB (Estery*Woody)	0.941176	1	0.941176	2.35	0.1274
BB (Woody*Woody)	0.203954	1	0.203954	0.51	0.4764
blocks	13.3111	16	0.831944	2.08	0.0127
Total error	52.3774	131	0.399827		
Total (corr.)	82.8111	152			

Numbers in bold indicate significant effects of the factors in the perception of the 'estery' character

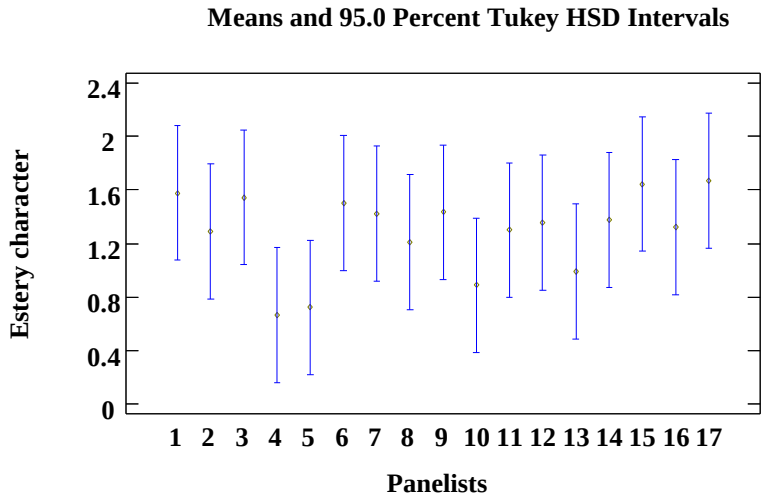

Figure 5.7. Means and 95% Percent Tukey HSD Intervals among panellists in the perception of estery character in spirit bourbon models. (SWRI panel data).

Figure 5.8A-B shows the perception of estery character as a function of the different levels of estery and woody fractions. The estery character increased at the highest level of the estery fraction, however at the lowest values (189 and 378.5 ppm) there was not much difference in the perception of this character. This was mainly due to the suppresive effect of the woody content on the estery character as previously determined by the untrained panel (Figure 5.3 A-B). Although there were not clear interactions between these two factors in the perception of the estery character ($P > 0.05$), interaction plots were an informative depiction of the data (Figure 5.9). As the concentration of the estery fraction increased in the absence of the mature/woody component, the perception of the estery character increased, however, as the concentration of the woody fraction increased the perception of the estery character decreased, thus confirming a strong suppression of this character by the mature/woody fraction.

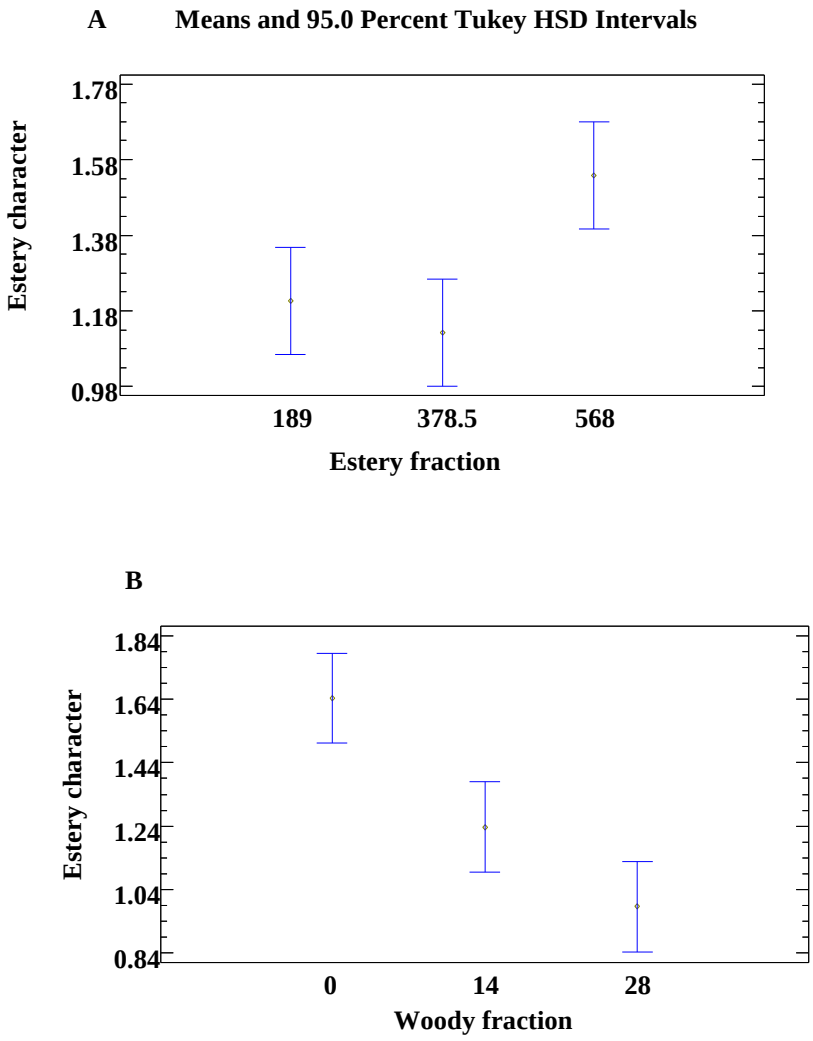

Figure 5.8. Means and 95% Percent Tukey HSD Intervals showing the impacts of the estery fraction (A) and woody fraction (B) on the perception of estery character in model bourbon spirits (SWRI trained panel data).

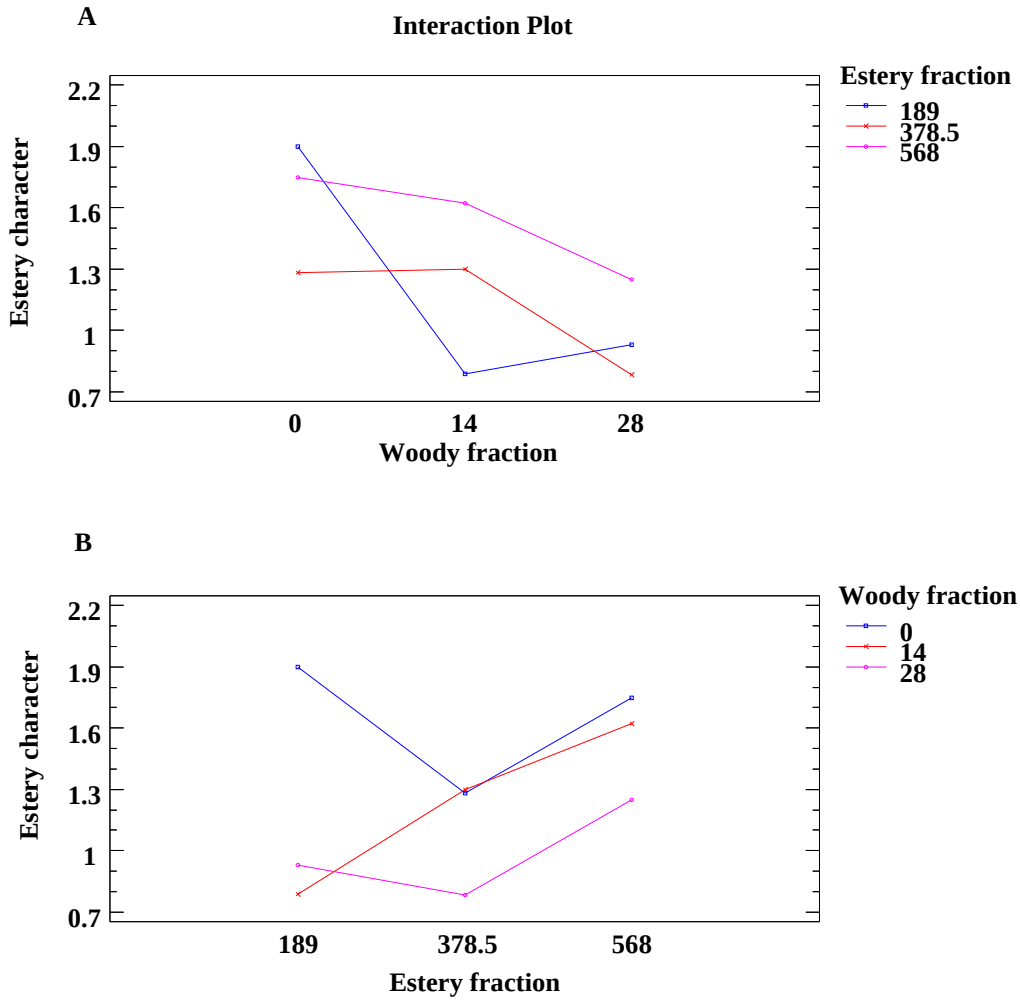

Figure 5.9. Interaction plots between the woody (A) and estery (B) fractions in determining perception of the estery character of model bourbon spirits (SWRI trained panel data).

Table 5.12 shows the corresponding ANOVA output for perception of woody character of the model bourbon spirit samples. The mature/woody fraction had a significant effect on the perception of woody character ($P < 0.05$), however the estery fraction did not have a significant impact on the perception of this attribute ($P > 0.05$). Once again, no clear interaction between these two factors was observed ($P > 0.05$) in the perception of mature/woody character.

Table 5.12 Analysis of Variance for Woody character in bourbon model spirits.

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
A: Estery fraction	0.401569	1	0.401569	0.95	0.3328
B: Woody fraction	7.2001	1	7.2001	16.94	**0.0001**
AA (Estery*Estery)	0.0256209	1	0.0256209	0.06	0.8064
AB (Estery*Woody)	0.190588	1	0.190588	0.45	0.5042
BB (Woody*Woody)	0.203954	1	0.203954	0.48	0.4897
blocks	11.5167	16	0.719796	1.69	0.0552
Total error	55.6671	131	0.424939		
Total (corr.)	75.2056	152			

Numbers in bold indicate significant effects of the factors in the perception of the 'woody' character

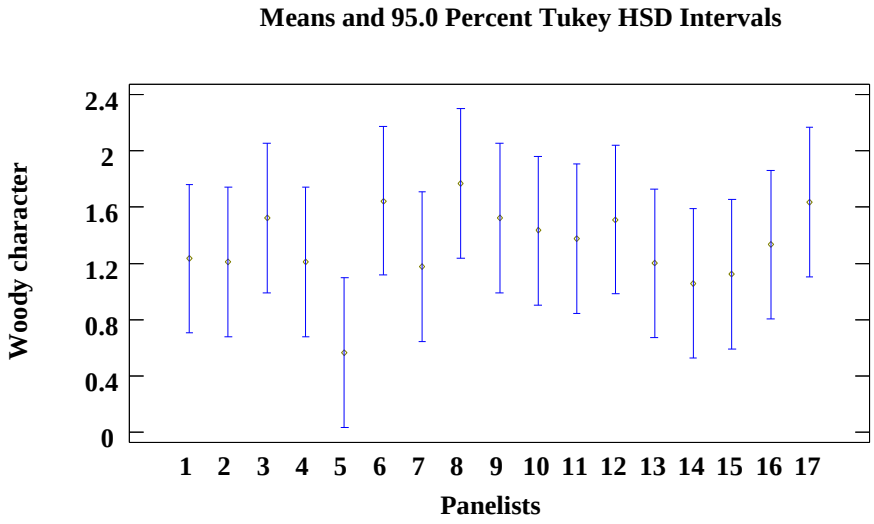

Figure 5.10. Means and 95% Percent Tukey HSD Intervals among panellists in the perception of woody character. (SWRI trained panel data).

Figure 5.10 shows the mean perception of the panel whilst rating the woody character in the spirit models, and overall no statistical differences were observed among them ($P > 0.05$). This data confirmed the reliability of the sensory panel and therefore the data obtained. In addition, Figures 5.11 shows the effect of different levels of the estery and mature/woody fractions on the perception of the woody character. The estery fraction had no significant effect on the perception of this character ($P > 0.05$), whilst the woody fraction did ($P < 0.05$). This data confirmed our previous observations, namely that

woody/mature content generally drives the perception of its respective woody/mature character and suppresses the perception of estery character in these model bourbon spirits.

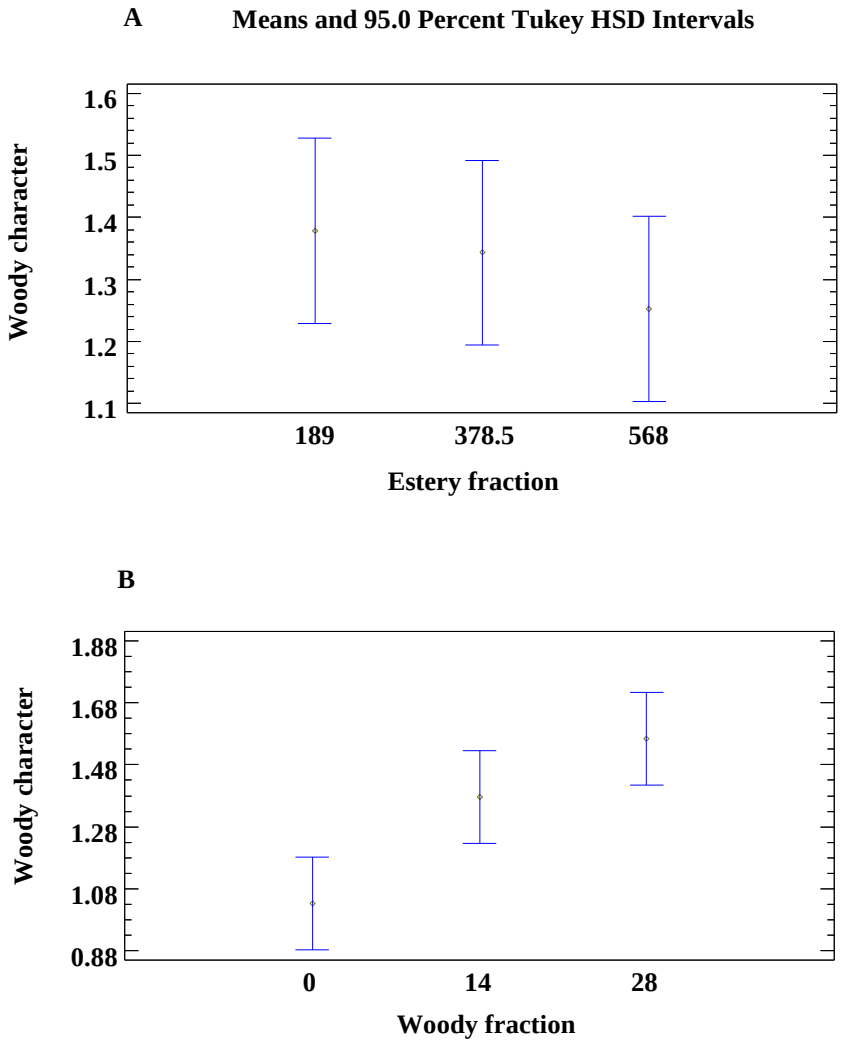

Figure 5.11. Means and 95% Percent Tukey HSD Intervals of the estery fraction (A) and woody fraction (B) in the perception of woody character. (SWRI trained panel data).

Although there were no significant interactions ($P > 0.05$), inspection of the interaction plots (Figure 5.12-A) shows that the intensity of the woody character increased as the concentration of the fraction increased, however at some levels

of the estery fraction (378.5 ppm) the perception of this character followed a linear increase with concentration as compare to the other two levels of the estery fraction (189, 568 ppm). Which showed a steady intensity in the perception of this character particularly when the concentration of the woody content was situated at the highest concentrations of the spirit blends (14 and 28 ppm). This could suggest a suppression of the woody character at the highest woody content by the presence of higher amounts of estery fraction. Yet other implications could be presented, since the SWRI expert panel evaluated the same products as the untrained panel following the same sensory methodology, however they identified differences in the estery character as consequence of the estery fraction that the other panel did not, giving evidence of a higher sensitivity and the ability to recognise sensory differences in complex mixtures (e.g. bourbon whiskey models).

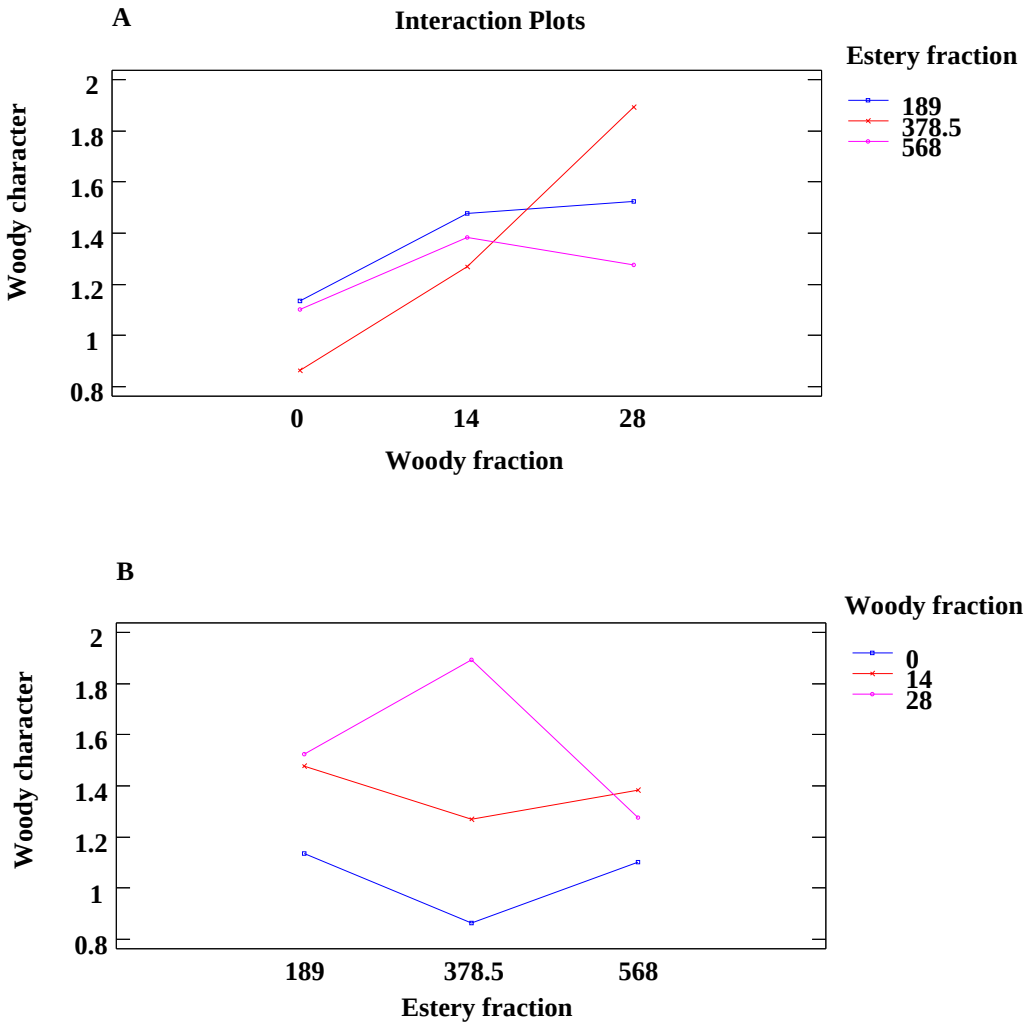

Figure 5.12 Interaction plots showing the impacts of the woody (A) and estery (B) fractions on the perception of the woody character (SWRI trained panel data).

On the other hand, in the second graphic (Figure 5.12-B) it is apparent that perception of woody character was independent of the estery fraction concentration, since a marked reduction of its character was observed.

These results (SWRI expert panel) confirmed our previous observations obtained from the untrained panel, which are in accordance with the masking

effect attributed to woody character over estery character in complex mixtures such as spirits (Atanosova, et al. 2004, 2005). In addition, studies investigating fruity aromas in red wines over the past decade have revealed a certain number of compounds that are potentially involved. Perceptual interactions have been described involving furanones (furaneol and homofuraneol) (Kotseridis, 2000), C13-norisoprenoids, such as β–damascenone, (Kotseridis, 2000; Escudero et al. 2007; Pineau et al. 2007), sulphur compounds, such as dimethyl sulphide (Anocibar-Beloqui et al. 1996; Segurel et al. 2004), or diacetyl, and acetoin, acetic acid, and γ-butyrolactone (Lytra et al. 2012), which may contribute indirectly to fruity expression in red wines. These examples emphasize the importance of perceptual interactions in determining the intensity and quality of fruity aromas. Pineau et al. (2009) demonstrated that, in some complex mixtures in dearomatized red wine, very small variations in the concentrations of some ethyl esters were perceived, even at concentrations far below their individual olfactory thresholds, and affected their red- and blackberry aromas. According to Berglund et al. (1976) perceptual interactions may have different origins. These authors proposed four levels of possible interactions. The first level of interactions is pre-sensory, involving chemical or physicochemical interactions between components in the mixture, reflecting changes in physical stimuli properties (Walker et al. 1999, 2003). The second level of interaction is peripheral sensory, involving interactions at the receptor level (Holley et al. 1974; Brodin et al. 2009; Chaput et al. 2012). The third level of interaction is electrophysiological, at the peripheral level of the nervous system. Signals from a particular receptor may interact with signals from other receptors on the way to the olfactory bulb. Interactions occur through the convergence of many

primary neurons to a specific glomerulus or via lateral connections among neurons (Moulton et al. 1964; MacLeod et al. 1971; Valova et al. 2007). The fourth level of interaction occurs in the central nervous system (Le Berre, et al. 2008).

5.4.3 Sensory interaction experiments in model spirits of Malt Whiskies 'A and B' and Tequila

As with bourbon model spirits, we applied the Flavour Interaction Sensory Methodology to the remaining model spirits including two Malt Whiskies (A & B) and Tequila. Table 5.13 summarizes the statistical significance in terms of *P values* of the fractions (factors) on the perception of estery and woody characters for each of the model spirits. It is worth noticing that those results were obtained from the sensory evaluation of the model spirits using the untrained panel, as the use of the SWRI expert panel was limited to these model spirits that presented a preliminary suppression of the estery character by the presence of the woody fraction or other significant sensory observations (as bourbon did). However, interesting and important sensory results were derived from this evaluation using the untrained panel. As we can observe from Table 5.13 the concentration of woody/mature fractions significantly ($P < 0.05$) determined the woody mature character in all three spirit blends. A similar pattern was observed with the concentration of the estery fraction, which significantly ($P < 0.05$) defined the estery character in all model spirits. Examination along the four spirit blends regarding the estery and woody compositions, allow us to make conclusions concerning the impact of each of them while defining the estery and woody aroma characters. In general, bourbon model spirits were the blends with the

highest amount of woody fraction (more aged/woody samples), this pattern could explain the fact that the woody fraction significantly modulated or supressed the estery character of these spirt blends (Figures 5.2 and 5.8). However, for the remaining model spirits (malt whiskies and tequila), the level of suppression presented was noteworthy reduced; basically because of the lower amounts of woody content presented (Tables 5.4, 5.6 and 5.8). Even though their concentrations were still significant to modulate and define the woody character of the spirit blends. For example, tequila models presented the lower amount of woody content (2.75 ppm) along the four set of model spirits.

Table 5.13. Summary of the statistical significance (*P values*) of fractions (factors) on the perception of 'estery' and 'woody' characters of the MWA, MWB and TEQUILA model spirit solutions.

Model spirit	Character	Factor significance (*P value*)					Interaction Effect (From Plots)	
		Fractions (factors)		Interactions				
		Estery (A)	Woody (B)	A*B	A*C	B*C	Estery character (EC)	Woody character (WC)
MWA	Estery	**0.0083**	0.1705	0.9387	0.9628	0.4726	EC was affected by WF, since a reduction in its perception was observed as the concentration of WF increased, and in all cases the perception of the EC increased as the concentration of the EF increased. In fact, in both cases, there was an overdose effect of WF on EC.	At the lowest WF the intensity of this character was reduced. However, as the concentration increased (WF) the perception (WC) increased no matter the EF present in both malt whiskies.
	Woody	0.0853	**0.0000**	0.7109	0.2819	0.0598		
MWB	Estery	**0.0054**	0.1757	0.7803	0.8350	0.7894		
	Woody	0.5643	**0.0003**	0.4659	0.2726	0.5222		
TEQUILA	Estery	**0.0129**	0.3457	0.0773	0.6370	0.5451	As EF increased its perception (EC) also did it, however reached a steady point as the concentration increased, indicating a synergic effect due to the presence WF.	WC was marked reduce as consequence EF, however its concentration was still significant to affect the overall perception of this character in the spirit blends.
	Woody	0.1613	**0.0295**	0.6480	0.4555	0.1083		

Numbers in bold indicate significant effects of the factors in the perception of the ‘estery’ and ‘woody’ characters. Abbreviations: EC: estery character; EF: estery fraction; WC: woody character; WF: woody fraction.

In terms of the estery fraction composition along the four spirit blends (Tables, 5.2, 5.4, 5.6, and 5.8), bourbon and malt whiskies presented concentrations in the range of 356 to 371 ppm and Tequila presented the lower quantities (295 ppm). In most of the cases, the estery character was generally driven by the concentrations of their respective estery fractions and the perception of this character was somehow supressed as the concentration of the woody fraction increased, irrespective of the amount of estery fraction present. However, the higher level of suppression was observed in the bourbon model spirits, where the higher concentrations of woody content were present unlike the other spirit blends (Figures 5.3-A & 5.9-A).

Furthermore, as with the bourbon model spirits no significant interaction terms between the estery and woody fractions ($P > 0.05$) were observed along the three spirit blends, however interaction plots were useful to visualise the effect of different levels of each stimulus (Appendices 15, 18, 21, and 24). A summary of these results is presented in Table 5.13. For MWA and MWB, the estery character was suppressed by the presence of the woody fraction, since a depletion of its perception was observed as the concentration of this fraction increased. For Tequila, the estery character increased as the concentration of the fraction increased, however reached a steady stete as the concentration increased, giving indication of a synergic effect due to the presence of the woody content. Regarding the woody character, in both malt whiskies, the intensity of this character was reduced at the lowest woody content, nevertheless as the concentration increased also its perception did (Appendices 15, and 18). In the case of Tequila, the perception of the woody character was significantly reduced by the presence of the estery fraction, yet its concentration was still significant

to modulate and define this character (Appendices 21 and 24). Previously, it has been reported that the estery character of aged spirits generally declined, in the presence of the same concentrations of esters as in the new make spirit (Piggott et al. 1992; Conner et al. 1994; 1994; Conner et al. 1996). These observations agree with our results, since a significant reduction of the estery character was perceived as consequence of the woody fraction in the model spirits, particularly in the case of the most aged spirit blends (bourbon). Additionally, it is well known that whisky is a complex system with hundreds of compounds that influence both the static (equilibrium) and dynamic partitioning of aroma (Boothroyd, 2013). Therefore, during whisky flavour assessment, samples are diluted to an alcoholic strength of 20-23% ABV (Conner et al. 1994). Dilution, however, changes the solubility of many volatile compounds that are more soluble in ethanol than water, such as ethyl esters of fatty acids. In addition, these esters are amphiphilic, with a polar head and hydrophobic hydrocarbon chain, and may thus form agglomerates or micelles in aqueous ethanolic solutions (Conner et al. 1994, 1994). These micelle-like structures comprise of surface-active molecules such as long chain alcohols, aldehydes and esters (Boothroyd, 2013). They have the ability to incorporate other hydrophobic compounds such as shorter chain esters, alcohols and aldehydes from the solution and thus to decrease their free solution and consequently headspace concentrations, reducing their sensory impact and improving acceptability to consumers through marked changes in character (Piggott et al. 1996, Conner et al. 1999). Wood extracts have been shown to affect the size and stability of the ester agglomerates formed on dilution (Conner et al. 1999). Therefore, malt whisky, diluted for consumption, may be regarded as an emulsion but with a

portion of the flavour molecules forming the disperse phase. However long chain-ethyl esters (ethyl dodecanoate & ethyl hexadecanoate) are the primary components of agglomerates formed in diluted distillate (Conner et al. 1994), but other compounds such as alcohols, long-chain aldehydes also influence ester activities, consequently, the activity or headspace concentration of a hydrophobic aroma compound in an alcoholic beverage can be determined by the concentration and nature of other hydrophobic compounds present (Conner et al. 1994). Due to the complex nature of flavour congeners present in the whisky matrix, many reactions take place, some of which arise during the maturation and others are formed when the spirit is diluted. These reactions involve extraction of wood components, evaporation of low-boiling-point solutes from the distillate and interactions of wood and distillate components (Nishimura and Matsuyama 1989, Bamforth et al. 2003). The origins of these interactions might be derived from extraction of cask wood, oxidation of ethanol and evaporation of ethanol and water (Conner et al. 1999). Nevertheless, few studies have analysed the physicochemical mechanisms that affect the release of flavour compounds in whisky or the effects associated with adding volatile compounds on the volatile partitioning in a whisky model system (Conner et al. 1998; Boothroyd et al. 2012). Thus, results from this chapter have helped to have a better understanding of the impacts of adding different levels of estery and woody fractions in a wide range of spirit blends (which have maintained the pool of compounds comprising the common aroma base) and the resulting effects on flavour perception of the estery and mature/woody character. And also, how their presence can enhance or supressed the perception of each stimulus. The use of interaction plots gave evidence of strong perceptual interactions between

these factors, which have been reported as the main cause of most aroma changes (Ferreira et al. 2006). In addition, these interactions have been reported among wine polyphenols and aroma substances (Dufour et al. 1999), in binary mixtures of wine odourants (Atanasova et al. 2004; Atanasova et al. 2005), between major white wine components (Jones et al. 2008), and amongst six common aroma vectors explaining four main red wine aroma nuances (Ferreira et al. 2016).

5.5 OVERALL DISCUSSION AND CONCLUSIONS

Sensory perceptions of woody/mature and estery characters were generally driven by concentrations of the respective fractions. Overall, higher levels of woody content led to a higher degree of suppression in the estery character as exemplified by the bourbon model spirits. In addition, although the woody content in the model spirits of malt whiskies and tequila were three and five times lower than bourbon models, these concentrations were still significant for defining and influencing the woody character in each of these models and to supress to some extent the estery character (especially at the highest levels of woody fraction). These findings were observed among both trained and untrained panels, particularly for the bourbon spirit models which were the only samples compared by both panels. In terms of panel's performance, the SWRI expert panel presented a better sensitivity than the untrained panel. Mostly at recognizing significant differences in the estery character as consequence of the estery fraction in bourbon model spirits, probably because they evaluate spirit samples on a daily basis and are able to recognize common aroma attributes in complex spirit matrices. Nevertheless, the untrained panel provided a full insight of the impacts of adding different levels of estery and woody content in a broad

of range of model spirits and the resulting effects on the flavour perception of their respective characters. Interestingly, during the first sensory sessions, the untrained panel presented nasal fatigue, due to the alcohol content of the samples and the initial number of samples evaluated (nine samples per set of model blends). Therefore, the sessions were split to twice a week (four to five each time) and two minutes break between samples were then introduced. In a future work, it will be recommended to provide full training to the panel at Nottingham, even though this represents an extra cost and time, this will facilitate the process it obtaining results and will reduce the dependence on external panels (e.g. SWRI).

Overall there was little evidence of interaction effects between estery and woody characters, rather, there was a tendency for woody/mature character to suppress the estery character of mature spirits and that is likely the cause of the previous observations by Conner and co-workers (Piggott et al. 1992; Conner et al. 1994; 1994; Conner et al. 1996). Additionally, early in Chapters 2 and 3 the key maturation congeners that are affected as consequence of the ageing process was reported. Based on this data, in Chapter 6 we wanted to evaluate how different ageing conditions affected the extraction kinetics of these maturation compounds.

6. **EXTRACTION OF WOOD-DERIVED CONGENERS INTO SPIRITS AS A FUNCTION OF AGEING TIME, TEMPERATURE, SPIRIT TYPE AND ALCOHOL CONTENT: A KINETIC STUDY.**

6.1. AIM

Results reported elsewhere in this thesis have emphasised the sensory and chemical changes resulting from maturation in American Oak casks across different spirit categories (tequila, bourbon and 2 malt whiskies). Furthermore, the profile of flavour active extractives resulting from ageing depends not only on the wood and its provenance (e.g. new v re-use cask) but also on factors such as the duration and temperature conditions for maturation and the alcohol content (%ABV) of the new make spirit. Therefore, the objective of the research described in this chapter was to investigate the extraction kinetics of wood-derived compounds from oak sticks as a function of ageing time, temperature, spirit type and alcohol content.

6.2. INTRODUCTION

Wood ageing is a common practice in distilled spirit production. Different types of oak are used to produce casks to store whisky, bourbon and tequila as part of the ageing process. For aged tequilas, maturation occurs inside white oak containers (*Q. alba*) or holm oak containers (*Q. ilex*) (Lopez-Ramirez et al. ., 2013). In the case of American bourbon whiskey and Scotch malt whisky, maturation occurs inside American oak containers of different species including *Q. alba, Q. bicolor, Q. macracarpa* and small amounts of *Q. lyrata* (Conner et al. 2003). Ageing contributes to the mature character of these spirits by the

extraction of volatile and non-volatile compounds that produce complex interactions with other spirit components. The environment developed inside the cask also provides the conditions for further reactions such as oxidation, hydrolysis and polymerisation which further modify the balance of flavour congeners present (Mosedale, 1998; Conner et al. 1993). Although ageing conditions (humidity, temperature, and time) are significant factors which affect the sensory characteristics of aged spirits, other factors are equally important and include the raw material of the barrel -oak- and its treatment, cask size and the environment in which the spirit is matured (Reazin, 1981).

Studies carried out on the contribution of oak to the olfactory characteristics of wine and spirits have shown that these are mainly influenced by compounds such as furfural, guaiacol, whisky lactone, eugenol, vanillin and syringaldehyde (Reazin, 1981, Conner et al. 1993; Conner et al. 1994; 1994; Conner et al. 1996; Conner et al. 1999; Escalona, et al. ., 2002; Arapitsas et al. 2004). Some of these compounds can be used as markers of ageing, whereby their quantification during the ageing process can be used to estimate the time required to age a distilled beverage (De Aquino et al. 2006). Among the most important are furfural which basically originates from degradation of monosaccharides produced by partial hydrolysis of hemicellulose. It contributes to the character of dried fruits, and particularly of burned almonds (Arapitsas et al. 2004). Guaiacol by its own is produced by lignin breakdown during wood toasting and is responsible for the burnt/smoky notes of whisky aroma (Lee et al. 2001). On the other hand, oak lactones (*cis* and *trans* isomers of β-methyl-γ-octalactone), are often referred to as whiskey lactones, since they were first discovered in bourbon (Suomalainen and Nykanen, 1970), and originate from

oak lipids and directly influence the character of aged spirits (Mosedale et al. 1998). This is why their concentrations are very significant to spirit quality and consumers acceptance of whisky (Conner et al. 1993). The main odour qualities associated with the whisky lactones are woody and coconut character (Lee et al. 2001). Other sensorially important aroma compounds include eugenol, vanillin and syringaldehyde, which are mainly produced from lignin breakdown during wood toasting and influence the aroma by conferring clove, smoke and vanilla notes to the aged spirit (Conner et al. 1993; Lee et al. 2001; Aquino et al. 2006). Although, syringaldehyde and vanillin are the most predominant wood-derived congeners in aged spirits, other important congeners are also present and include syringic acid, sinapic acid, vanillic acid and ferulic acid, as well as their esters (De Aquino et al. 2006). Even though, these compounds have not been found to have a directly significant impact on whisky aroma their presence could influence the body and viscosity of the aged spirits.

Previous studies conducted in wine have suggested the use of oak chips or inner staves to artificially age wine. Such procedures have been proposed as valuable alternatives to oak barrel ageing for obtaining, in a short period of time, wines with particular characteristics given by oak wood. Alternatively, they can be used as a tool to discriminate artificially aged from barrel-aged wine (Escalona et al. 2002; Arapitsas et al. 2004; Morales et al. 2004; Tesfaye et al. 2004). However, in the case of spirits, ageing using oak chips or inner staves has not been successfully implemented as an alternative to traditional long-term warehoused maturation ageing. Furthermore, the profile of flavour active extractives resulting from ageing depends not only on the wood and its provenance (e.g. new v re-use cask) but also on factors such as the dimensions

of the barrel, its surface area and depth of penetration of spirit into the cask, the duration and temperature conditions for maturation and the alcohol content (%ABV) of the new make spirit. Considering this we formulated the objective (above) of this chapter and conducted the study based on a multi-level factorial experimental design.

6.3. MATERIAL AND METHODS

6.3.1. Samples

Four non-mature authentic spirits (tequila, bourbon and 2 new make spirits) of different alcohol content (40-63.5% ABV) were used as the new make spirits and were the same spirits samples as extensively characterised earlier in this thesis. In a parallel experiment pure ethanolic solutions of different alcohol content (40-64% ABV) were used as the model spirit solutions. Thus, from comparison of the two sets of data it would be possible to see whether % ABV alone was the driver of extraction rate, or whether the remaining spirit matrix (be it bourbon, whisky or tequila) has a significant impact.

6.3.2. Wood chips

American Oak chips were provided by the Scotch Whisky Research Institute (SWRI) and were sourced from an authentic bourbon cask. They had been subsequently toasted at 220°C for 20 min to simulate charring. The chips had average dimensions of 0.8 cm x 0.8 cm x 9 cm and were cut down to 3 cm lengths using a manual hacksaw. The 3 cm chips were then weighed into 100 mL Duran flasks at a rate approximately proportionate to cask ageing conditions with regards to the ratio of wood to spirit (5% w/v).

6.3.3. Reagents and Chemicals

As described in Appendix 1.

6.3.4. Artificial ageing experiments

A multi-level factorial experimental design was developed to analyse the effect of the factors (temperature, alcohol content (% ABV) and ageing time) on the kinetic development of 18 maturation congeners using pure aqueous ethanolic solutions and new make spirits. The time-course of extraction was monitored over a 28-day period at 4 different alcohol concentrations (40, 48, 56 & 64 %ABV) and four maturation temperatures (10, 20, 30 & 40 °C) for the aqueous ethanolic solutions. In a parallel study, we repeated this design, but using the authentic new make spirits (tequila [40% ABV], bourbon [57% ABV] and 2 malt whiskies [63.4 & 63.5 % ABV]). Spirit samples (50 mL) containing 5% w/v of oak sticks were incubated at 4 different ageing temperatures (10, 20, 30 & 40 °C) and analysed for extracted maturation compounds at intervals of 2, 7, 10, 14, 21 and 28 d. A total of 224 samples were obtained and essayed in duplicate.

6.3.5 Gas and Liquid Chromatography analysis

Spirit samples were extracted in duplicate as proposed by Boothroyd et al. (2014). Then, each spirit extract was subjected to GC analysis, using a Bruker Scion 456-GC gas chromatograph, coupled to a flame ionization detector (FID). The sample (1 μL) was injected into the chromatograph in splitless mode. Separations were performed using a ZB-Wax capillary column (60 m × 0.25 mm i.d., 1.0 μm film thickness; Phenomenex, Macclesfield, UK). Operating

conditions were as follows: carrier gas (helium) was 1.5 mL/min; initial oven temperature was 75 °C; then the temperature was raised at 6 °C/min to 240 °C and held for 15 min; injector and detector (flame ionization) temperatures were maintained at 200 and 220 °C, respectively. Quantification was achieved following normalization to the internal standard (10 μg/mL, 2-acetylthiazole) of nine diluted solutions in the range of 0.05–50 μg/mL, containing the following compounds, cis/trans-whisky lactone, γ-nonalactone, vanillin, eugenol, guaiacol, 4-ethyl guaiacol, furfural, 5-methyl furfural, and 2-acetyl furan. Calibration curves reported a correlation coefficient (R^2) ≥ 0.99 for each compound. Non-volatile compounds, including gallic acid, vanillic acid, vanillin, syringic acid, syringaldehyde, coniferaldehyde, sinapaldehyde, ellagic acid and 5-hydroxymethyl furfural, were measured and quantified by HPLC-UV following the conditions propose by Oladukun et al. (2016).

6.3.6. Data treatment and statistical analysis

The simultaneous effect of variation of the factors (temperature, alcohol content, and ageing-time) on the response variables (data set composed of the values obtained from GC and HPLC analysis) were analysed using a multifactorial experimental design (Statgraphics Centurion XVI software). Kinetics were established using nonlinear regression (Statgraphics Centurion XVI software) between compounds concentrations and *t* values. All correlations were established at least at a 95% significance level ($P < 0.05$). Principal component analysis was performed using Simca software P7.01.

6.4. RESULTS AND DISCUSSION

6.4.1. Evolution of mature-related congeners as function of ageing time, temperature and alcohol content.

Spirit systems, comprising i) aqueous ethanolic solutions and ii) authentic new make spirits were used to investigate the effects of ageing time, temperature, spirit type and alcohol content (% ABV) on the extraction of wood-derived congeners. For this purpose, the extraction of congeners from the oak sticks into the spirit systems, was monitored over a period of 28 days. By sampling at predetermined intervals (2, 4, 7, 10, 14, 21, and 28) a set of 18 maturation compounds were monitored and plotted against time (*t*). Because of the complexity of the data set, we decided to group the 18 maturation compounds into five family groups (lactones, volatile phenols, phenolic acids, phenolic aldehydes, and furanic aldehydes), in such a way that we could observe the changes in flavour profile of these compounds as a consequence of the factors. Table 6.1 shows the composition of these five family groups.

Table 6.1. Mature related congeners that conform each of the five family groups.

Lactones	Volatile Phenols	Phenolic Acids	Phenolic Aldehydes	Furanic Aldehydes
Cis-whisky lactone	Guaiacol	Gallic acid	Syringaldehyde	Furfural
Trans-whisky lactone	4-Ethyl guaiacol	Vanillic acid	Coniferaldehyde	2-Acetyl furan
γ-nonalactone	Eugenol	Syringic acid	Sinapaldehyde	5-Methyl furfural
---	Vanillin	Ellagic acid	---	5-Hydroxymethyl furfural

Figures 6.1a – c and 6.2a – c show the evolution with time of concentrations of these groups of congeners in both model spirit systems. Of the lactones, *Cis*-whisky lactone was the compound extracted in greatest amounts, with maximum

concentrations of 5.50 mg/L (40 °C and 64% ABV) for aqueous ethanolic solutions and 3.01 mg/L (40 °C and 63.4% ABV) for new make spirits respectively. Ageing time, temperature and alcohol content were significant factors ($P < 0.05$), that influenced the evolution of these compounds. The concentrations of *trans*-whisky lactone in both spirit systems were below its sensory threshold level (0.79 mg/L; Poisson and Schieberle, 2008), with maximum concentrations of 0.40 and 0.69 mg/L presented at highest temperature and alcohol content (63.5/64 % ABV and 40 °C) in aqueous ethanolic solutions and new make spirits. Concerning γ-nonalactone maximum concentrations in excess of 0.49 ppm were attained in both spirit model systems, relative to its reported threshold of 0.29 mg/L (Poisson and Schieberle, 2008). It would therefore likely contribute to the perceived aroma of these spirits. Undoubtedly oak lactones have been reported to be of significant value for oak-matured spirits such as whisky and tequila (Conner et al. 2003; Gonzalez-Robles et al. 2016), particularly for the strong contribution of wood-related aromas to the beverage as previously demonstrated during the olfactometry and sensory evaluation of the spirits. In addition, whisky lactones, have been directly correlated to assessment of whisky quality (Mosedale and Puech, 1998) and are considered desirable in most categories of aged distilled beverages.

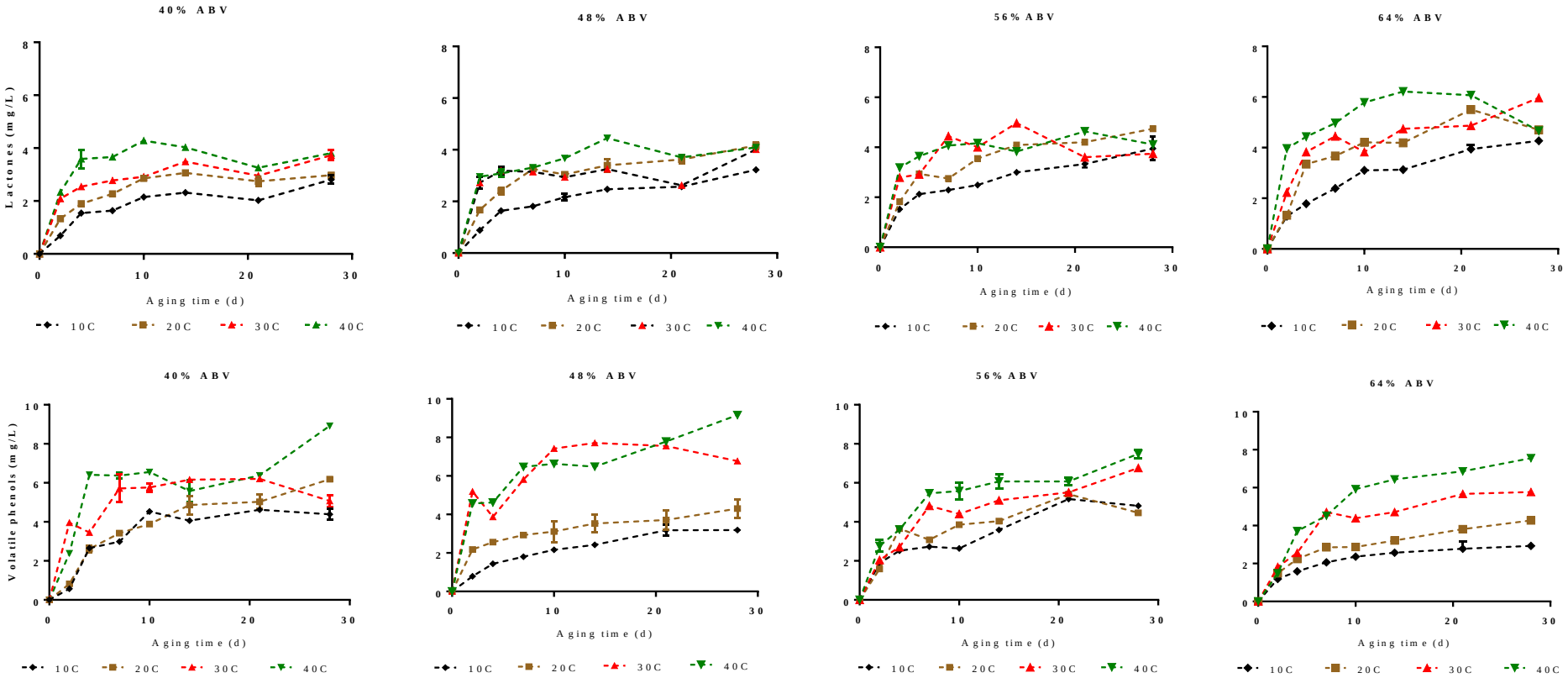

Figure 6.1a. Evolution of the concentration of lactones and volatile phenols (mg/L) in model ethanol solutions. Each point is the mean ± standard deviation of two replicate.

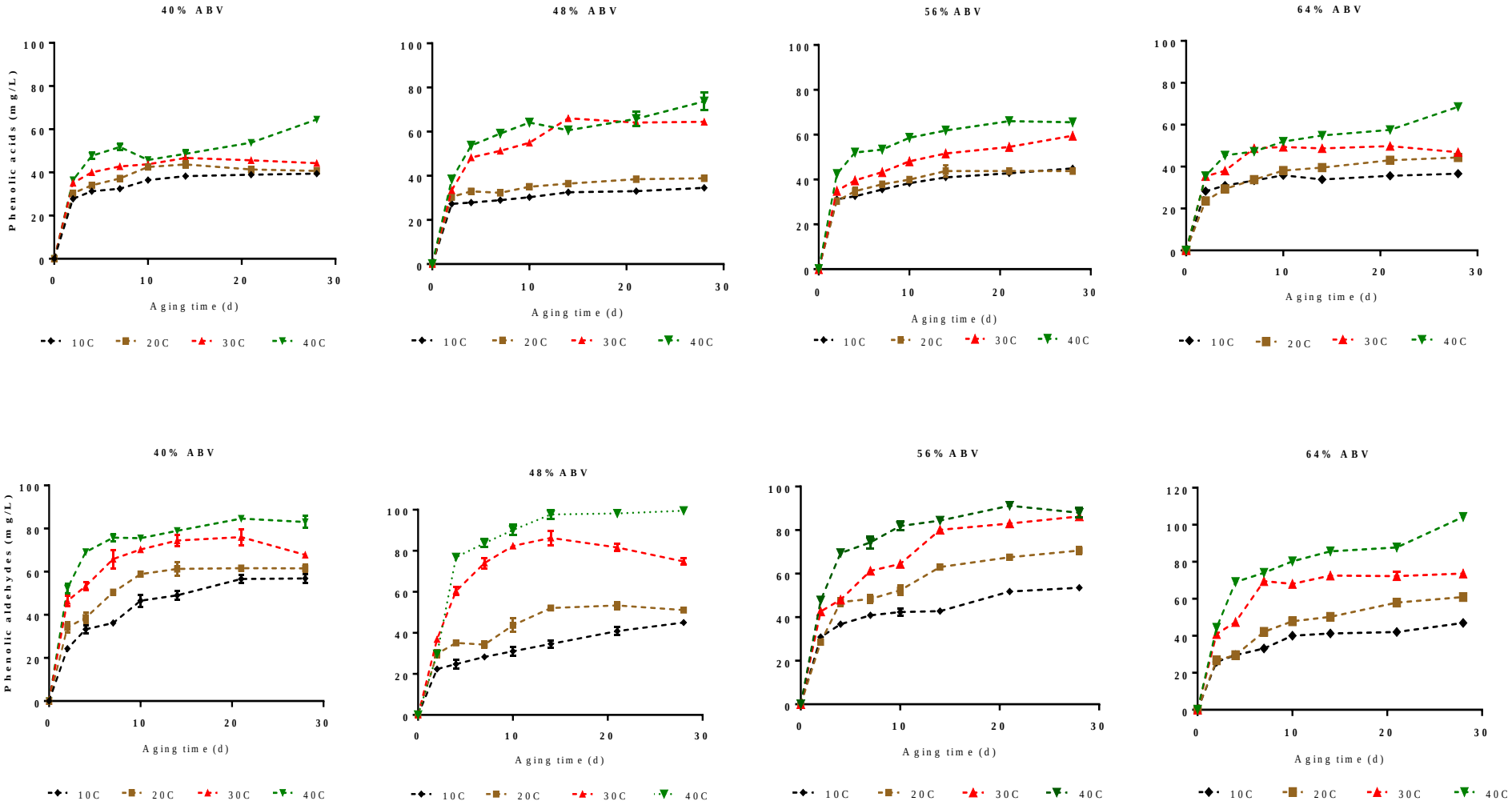

Figure 6.1b. Evolution of the concentration of phenolic acids and phenolic aldehydes (mg/L) in model ethanol solutions. Each point is the mean ± standard deviation of two replicate.

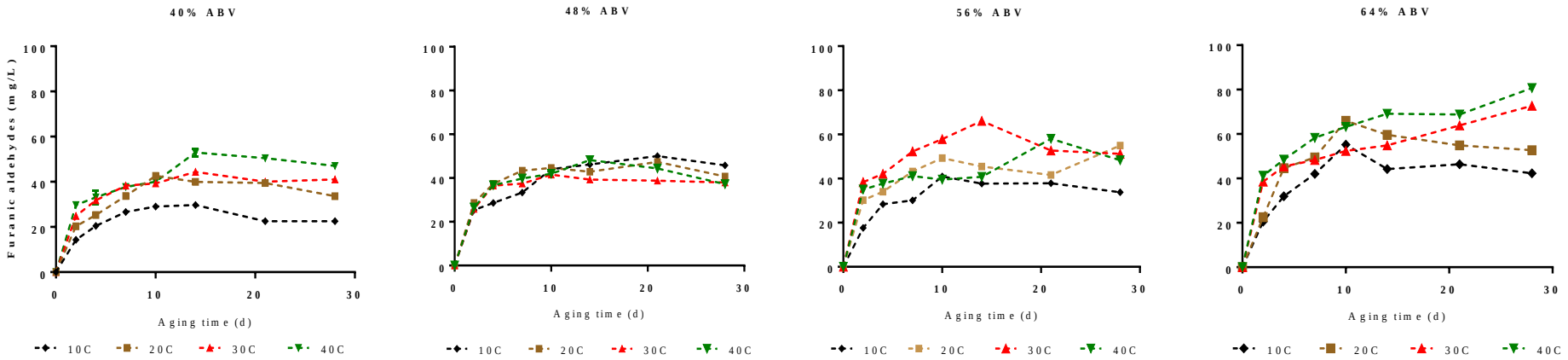

Figure 6.1c. Evolution of the concentration of furanic aldehydes (mg/L) in model ethanol solutions. Each point is the mean ± standard deviation of two replicate.

Volatile phenols are another important group of compounds, which are known to be formed from the thermal degradation of lignin at high temperatures (Cerdan et al. 2004). Interestingly, vanillin was the compound produced in higher concentrations in this group of compounds, with maximum concentrations of 7.92 and 5.75 mg/L (presented in both cases at 40%ABV and 40°C) in the aqueous ethanol systems and new make spirits respectively. Ageing time, temperature and alcohol content were significant factors ($P < 0.05$), that influenced the evolution of these compounds, however the extraction of eugenol was not affected by %ABV in the pure aqueous ethanol solutions (Table 6.3). Basically, because the higher extraction was observed at lower alcohol content (40% ABV) and not significant changes occurred at higher values (48 – 64% ABV) (95% Tukey HSD intervals). The presence of eugenol has been related as a typical characteristic of oak-matured drinks, and provides a clove-like flavour (Mosedale and Puech 1998). Vanillin and other aromatic aldehydes have also been identified as largely responsible for the 'bouquet' of cognac (Ledauphin et al. 2004). Furthermore, volatile phenols, have often been identified as contributing to the smokey characters of many alcoholic beverages, although some of these compounds may derived from sources other than charred oak wood (Mosedale and Puech 1998; Karvela et al. 2008). The temperature certainly affected the extracted concentrations of volatile phenols (Fig. 6.1 & 6.2), since the higher concentrations of these compounds were observed particularly at the highest temperature (40 °C) in both spirit systems. Alcohol content was a significant factor ($P < 0.05$), however the maximum amount of volatile phenols was observed at 48 and 57.8% ABV in the aqueous ethanolic solutions and new make spirits respectively. Phenolic acids and aldehydes are

another important group of compounds which were extracted from oak sticks into the model spirit systems and have been reported to arise into the aged spirit from the thermal degradation of lignin (Mosedale and Puech 1998).

Table 6.2 Effect of ageing time, temperature and alcohol content on the flavour profile of 18 maturation compounds in the new make spirits

Compound	Factor significance (*P value*)							Interaction Effect
	Factors			Interactions			Model R^2	
	Temp.	% ABV	Time	Temp*% ABV	Temp* Time	%ABV* Time		
Furfural	**<0.0001**	**0.0174**	**<0.0001**	0.2644	**0.0207**	**<0.0001**	78.62	Its concentration increases as temperature and time increase
2-acetylfuran	**0.0018**	**<0.0001**	**<0.0001**	**0.0043**	0.4813	**0.0457**	65.12	Its concentration increases with temperature and time and decrease as % ABV increase.
5-methyl furfural	**<0.0001**	**<0.0001**	**<0.0001**	**0.0851**	0.132	**0.0441**	64.24	Its concentration increases with temperature and time and decrease as % ABV increase
Guaiacol	**0.0023**	0.0611	**<0.0001**	0.2241	**0.0045**	**0.6374**	54.47	Its concentration increases with temperature and decrease as % ABV increase
Trans-whisky lactone	**0.0087**	**<0.0001**	**<0.0001**	**<0.0001**	0.3084	**<0.0001**	61.14	Its concentration increases with temperature, time and % ABV.
Cis-whisky lactone	**<0.0001**	**<0.0001**	**<0.0001**	0.4008	**<0.0001**	**<0.0001**	67.72	Its concentration increases as temperature and time increase, and decrease as % ABV increase.
4-ethyl guaiacol	**<0.0001**	**<0.0001**	**<0.0001**	**0.004**	0.4339	0.7708	79.06	Its concentration increases as temperature, time and % ABV increase.
γ−nonalactone	**<0.0001**	**<0.0001**	**<0.0001**	**0.0406**	**0.0102**	**0.0077**	86.34	Its concentration increases as the factors increase.
Eugenol	**<0.0001**	**<0.0001**	**<0.0001**	0.9207	0.9327	0.5528	69.75	There is not a strong interaction between the factors.
Vanillin	**<0.0001**	**<0.0001**	**<0.0001**	0.6929	**<0.0001**	0.0409	83.21	Its concentration increases as the temperature and time increase, and decrease as the % ABV increase.
Gallic acid	**<0.0001**	0.0781	**<0.0001**	**<0.0001**	0.5055	**0.0015**	64.27	Its concentration increases as temperature and time increase, and not clear effect due to % ABV is observed.
5 HMF	**<0.0001**	0.3128	**<0.0001**	**0.0894**	**0.0589**	0.2678	66.75	Its concentration increases as temperature and time increase, and not clear effect due % ABV is observed.

Table 6.2 Effect of ageing time, temperature and alcohol content on the flavour profile of 18 maturation compounds in the new make spirits (cont.).

Vanillic acid	**<0.0001**	0.2317	**<0.0001**	**0.0087**	**0.0321**	0.155	72.16	Its concentration increases as the temperature and time increase, and decrease as the %ABV increase.
Syringic acid	**<0.0001**	0.333	**<0.0001**	**0.0339**	0.3705	0.0962	62.14	Its concentration increases as the temperature and time increase, however not clear effect due to %ABV is observed.
Ellagic acid	**<0.0001**	**<0.0001**	**<0.0001**	**0.0014**	**<0.0001**	**<0.0001**	92.25	Its concentration increases as the factors increase.
Syringaldehyde	**<0.0001**	0.0907	**<0.0001**	0.4382	**0.015**	0.7695	67.2	Its concentration increases as the temperature and time increase, and not apparent effect due to %ABV is observed.
Coniferaldehyde	**<0.0001**	**0.0023**	**<0.0001**	0.4072	0.1397	0.1598	69.85	Not apparent interaction between the factors occurs.
Sinapaldehyde	**<0.0001**	**<0.0001**	**<0.0001**	0.9889	0.9662	0.1966	77.68	Not apparent interaction between the factors occurs.

Table 6.3 Effect of ageing time, temperature and alcohol content on the flavour profile of 18 maturation compounds in the aqueous ethanolic solutions.

Compound	Factor significance (*P value*)							Interaction Effect
	Factors			Interactions			Model R^2	
	Temp	% ABV	Time	Temp*% ABV	Temp* Time	%ABV*Time		
Furfural	**<0.0001**	**<0.0001**	**<0.0001**	0.1963	**0.0263**	**0.002**	70.34	Its concentration increases as these parameters increase over time
2-acetylfuran	**0.0063**	**<0.0001**	**<0.0001**	0.7222	**0.0014**	**<0.0001**	76.9	Its concentration increases as these parameters increase over time
5-methylfurfural	**<0.0001**	**<0.0001**	**<0.0001**	0.7211	0.146	**<0.0001**	68.57	Its concentration increases as these parameters increase over time
Guaiacol	**0.0198**	**<0.0001**	**<0.0001**	0.525	**<0.0001**	0.0604	60.94	Its concentration increases as the % ABV, temperature and time increase over time
Trans-whisky lactone	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.2343	**0.0064**	65.77	Its concentration increases as these parameters increase over time
Cis-whisky lactone	**<0.0001**	**<0.0001**	**<0.0001**	**0.0022**	**<0.0001**	**<0.0001**	82.31	Its concentration increases as these parameters increase over time
4-ethyl guaiacol	**<0.0001**	**0.0034**	**<0.0001**	0.2783	0.0631	0.1899	66.59	Not evident interaction between the factors occurred.
γ- nonalactone	**0.0144**	**<0.0001**	**<0.0001**	0.171	**0.0206**	0.0789	57.42	Only a clear interaction between time and temperature.
Eugenol	**<0.0001**	0.2195	**<0.0001**	**<0.0001**	0.1048	0.6823	72.5	There is a strong interaction between temperature and % ABV.
Vanillin	**<0.0001**	**<0.0001**	**<0.0001**	0.5504	**<0.0001**	0.646	76.36	Its concentration increases as the temperature and time increase, and drops as the % ABV increase.
Gallic acid	**<0.0001**	**<0.0001**	**<0.0001**	0.4049	0.5773	0.6169	58.95	Not clear interaction between the factors occurs.
5 HMF	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0621**	**0.0243**	73.87	Its concentration increases as the factors increase over time
Vanillic acid	**<0.0001**	**<0.0001**	**<0.0001**	0.587	**0.0234**	0.4027	61.28	Its concentration increases as the temperature and time increase, and decrease as the % ABV increase.

Table 6.3 Effect of ageing time, temperature and alcohol content on the flavour profile of 18 maturation compounds in the aqueous ethanolic solutions (cont.).

Syringic acid	**<0.0001**	**<0.0001**	**<0.0001**	0.4668	**0.0005**	0.9009	63.13	Its concentration increases as the temperature and time increase, and decrease as the % ABV increase.
Ellagic acid	**<0.0001**	**<0.0001**	**<0.0001**	0.8242	**<0.0001**	0.0527	86.06	Its concentration increases as the factors increase.
Syringaldehyde	**<0.0001**	**<0.0001**	**<0.0001**	0.8588	**<0.0001**	0.3906	75.7	Its concentration increases as the temperature and time increase, and decrease as the % ABV increase.
Coniferaldehyde	**<0.0001**	0.1652	**<0.0001**	0.6449	0.2663	0.4117	85.97	Not clear interactions between the factors occurred.
Sinapaldehyde	**<0.0001**	**<0.0001**	**<0.0001**	**0.002**	0.6091	**0.0043**	91.12	Its concentration increases as the factors increase.

Figures 6.1b & 6.2b shows the evolution of these group of compounds in the model spirit systems. For phenolic acids, temperature and time were decisive factors that influenced the evolution of these congeners in the authentic and aqueous ethanol solutions ($P < 0.05$). However, alcohol content (% ABV) just had a significant effect in the evolution of these compounds in the aqueous ethanol solutions (Tables 6.2 & 6.3) as opposed to the authentic new make spirit systems. Interestingly, the highest concentrations of this group of compounds were achieved at 40 °C in each of the alcohol contents evaluated in both spirit models, nevertheless the higher amount of phenolic acids was obtained at 63.5% ABV in the new make spirits and 48% ABV in the aqueous ethanolic solutions (Figures 6.1b & 6.2b). Ellagic acid was found to be the compound extracted at the highest concentrations in this group of compounds in both spirit model systems. Also, phenolic aldehydes were affected by temperature and time ($P < 0.05$) in both spirit systems (Tables 6.2 & 6.3). However, alcohol content (% ABV) only affected the evolution over time of some congeners of this group (Tables 6.1 & 6.2). Sinapaldehyde was the compound produced in greatest concentration in this group and its extraction was significantly affected by temperature, since the higher amounts were observed at higher temperature (> 30 °C) in the aqueous ethanolic solutions and in the new make spirits (Figure 6.1 & 6.2).

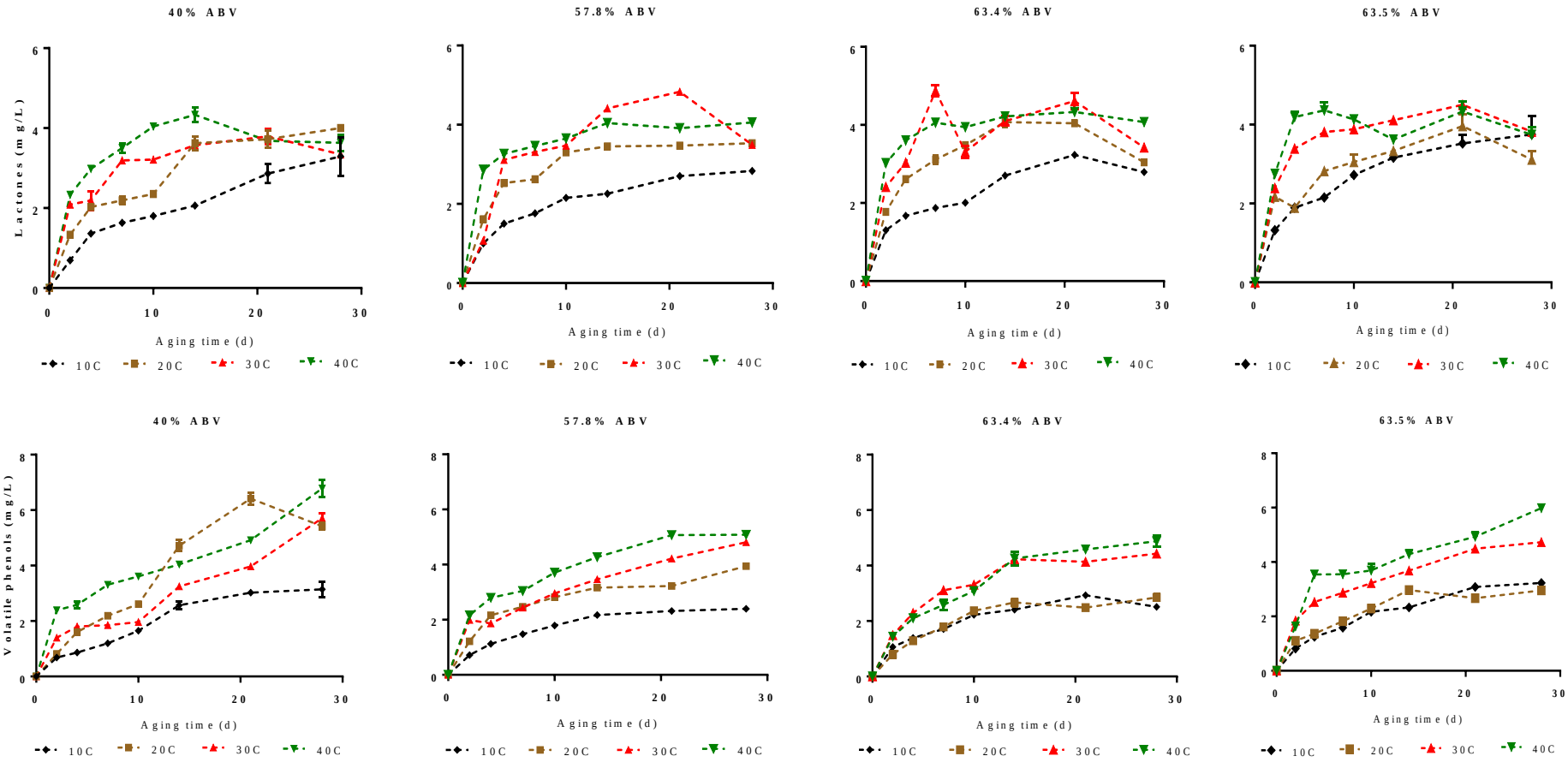

Figure 6.2a. Evolution of the concentration of phenolic acids and phenolic aldehydes (mg/L) in new make spirits. Each point is the mean ± standard deviation of two replicate.

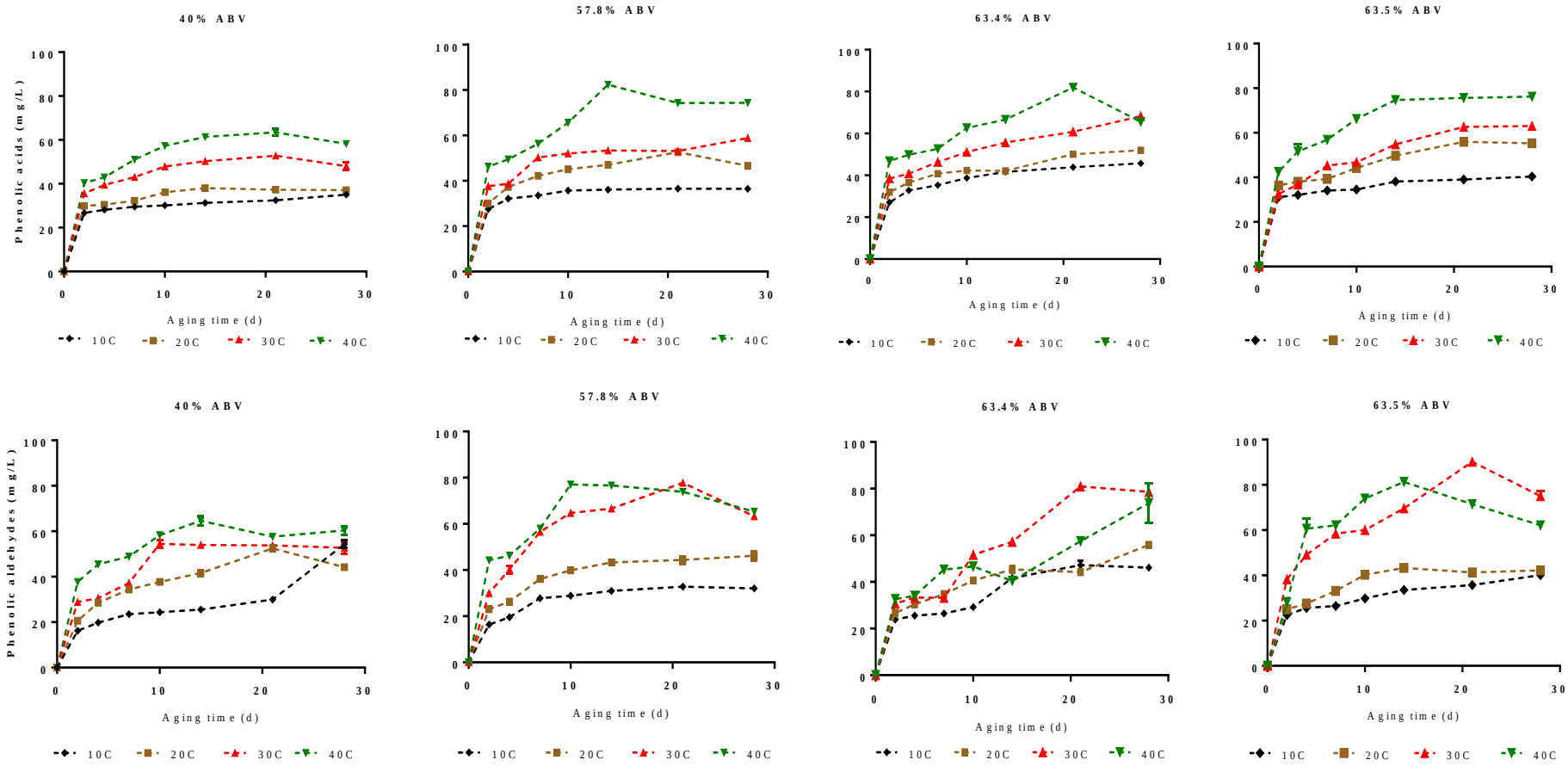

Figure 6.2b. Evolution of the concentration of wood-derived compounds and volatile phenols (mg/L) in new make spirits. Each point is the mean ± standard deviation of two replicate.

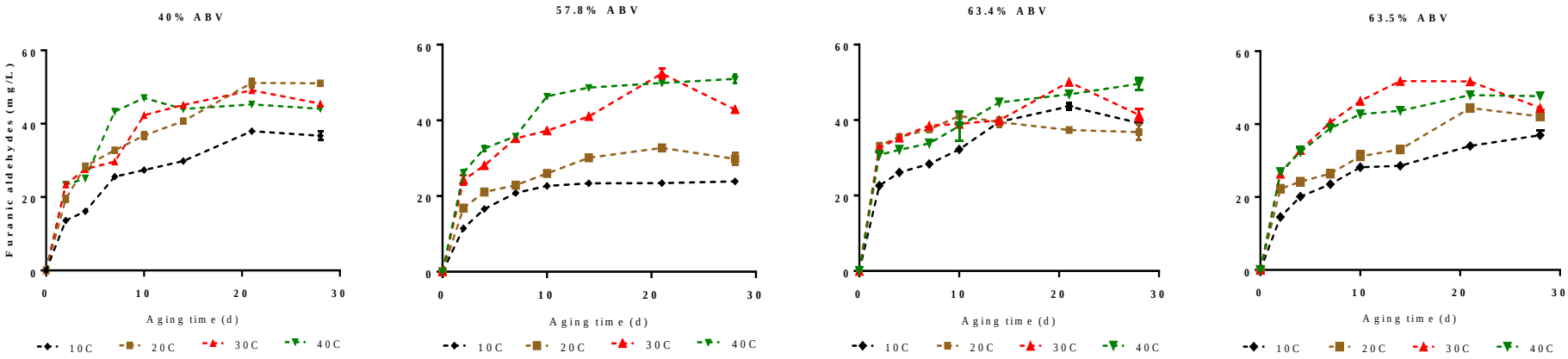

Figure 6.2c. Evolution of the concentration of furanic aldehydes (mg/L) in new make spirits. Each point is the mean ± standard deviation of two replicate.

Finally, but no less important, are the furanic compounds, which are formed during the toasting of the wood through degradation of carbohydrates (Mosedale and Puech 1998). Among these congeners, furfural was extracted at the highest concentrations, with maximum concentrations of 57.9 and 34 mg/L in the aqueous ethanol solutions and new make spirits respectively. Their concentrations were positively influenced by increasing temperature, ageing time and alcohol content ($P < 0.05$), since the highest amounts of this set of compounds were observed at the highest values of these parameters in both spirit systems (40 °C, 63.5 and 64% ABV). Only furfural reached a significantly higher concentration than its sensory threshold level (15 mg/L) reported by Franco et al. (2004). However, these compounds have been reported to synergistically enhance the aroma of oak lactones, even at low concentrations (Reazin, 1981).

In general, the extraction rate of the majority of compounds increased at elevated temperatures and alcohol contents, confirming their significance to the concentrations of wood extractives derived from the maturation processes. Previous studies have reported the effect of various factors on the extraction rate of several oak-relate compounds in aged-spirits such as whisky. Clyne et al. (1993) reported the effect of cask charring on Scotch whisky maturation, showing that the charred cask samples were significantly different in terms of non-volatile compounds (syringaldehyde, coniferaldehyde, sinapaldehyde, vanillic acid, and total phenols) relative to the uncharred cask samples, with a particular increase of these compounds over a total maturation period of 3 years ($P < 0.001$). The same authors (1993) confirmed the effects of maturation in oak casks on Scotch whisky composition and flavour for spirits with varying

histories. Their work demonstrated clear differences in mature flavour congeners as a consequence of maturation time and use of oak casks of different histories (previously used for bourbon whiskey, subsequently used for Scotch malt whisky and use several times and with little maturation potential).

6.4.1.1 Principal components analysis

In order to compare and contrast the effect of the factors (temperature, ageing time and alcohol content) in the flavour development of 18 maturation volatiles, PCA analysis was applied to both model spirit systems using for this the final concentrations obtained for each condition at the end of the 28 days of maturation (Figures 6.3a and 6.3b). PCA in the pure aqueous ethanol solutions accounted for over 73.7% of variation in the data in two principal components (Figure 3a). PC1, which accounted for the majority of the variation, represent the separation between the samples mainly due to the effect of the factors ($P < 0.05$, multifactor ANOVA). In general, at lower temperatures (10 and 20 ºC) and alcohol contents (40 and 48% ABV) was where the lower extraction rate of the wood ageing congeners was presented (factors loaded positively on PC1). Contrary, at higher values of these parameters (> 30 ºC and > 56 %ABV) was where the higher extraction rate occurred (factors loaded negatively on PC1). These observations confirmed the actual locations of the wood ageing congeners into PCA plot, with positions in the second and fourth quadrant of the analysis (Figure 6.3a). These locations indicate where the highest rate of extraction occurred, for example for compounds such as *cis/trans*-whisky lactone, 5-methyl furfural, 5-hydroxymethyl furfural, furfural, and sinapaldehyde was derived at just 64% ABV and temperatures higher than 30 ºC. Conversely, for

other compounds such as ellagic acid and coniferaldehyde the higher extraction rate was presented between 40 – 48% ABV and 40 °C (Figure 6.3a). These results, confirmed how the different factors affect the extraction rate of different chemical groups, to an extension of some of them being more susceptible to the effects of temperature and others more to alcohol content.

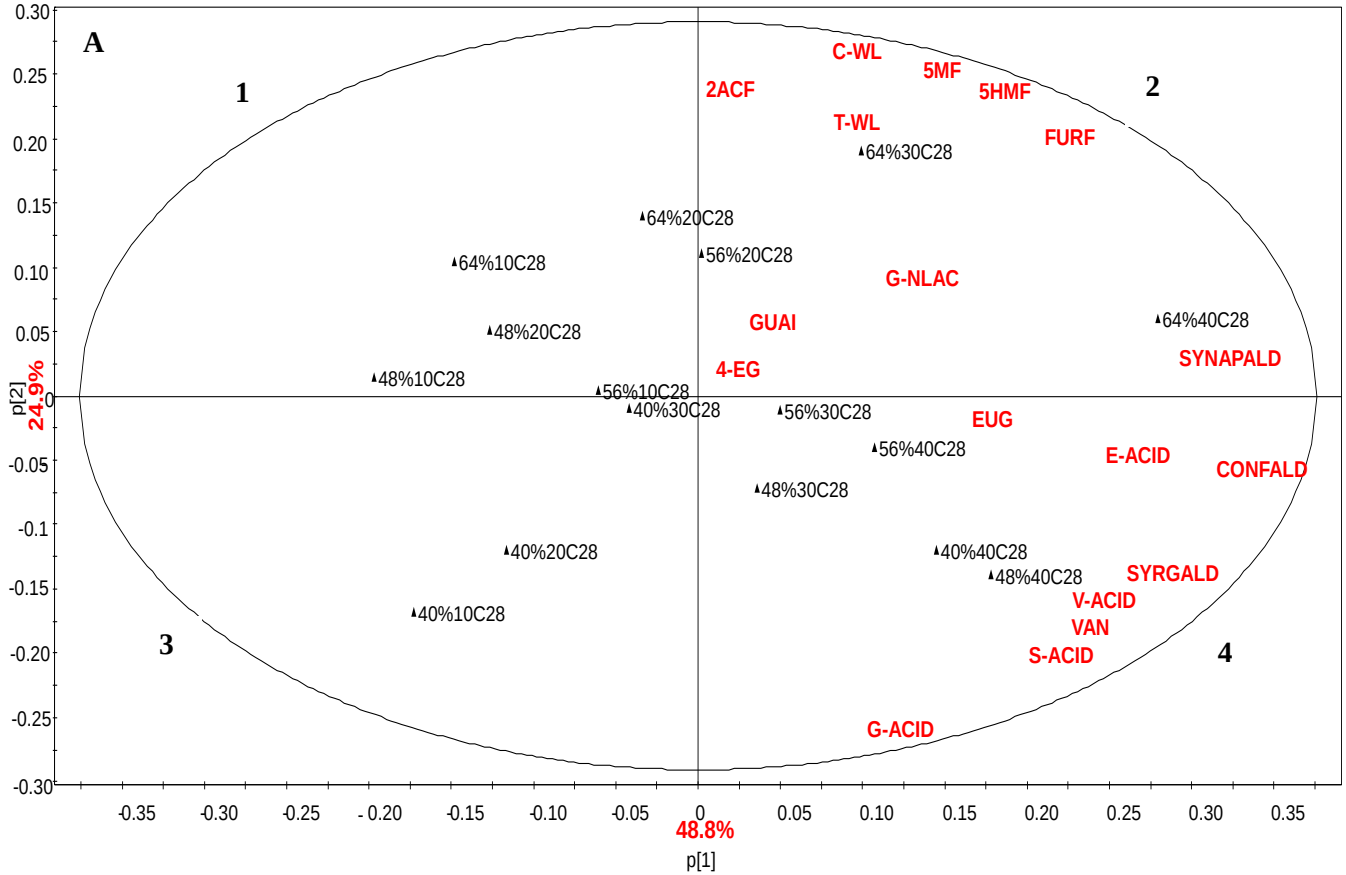

Figure 6.3a. Principal component analysis (PCA) bi-plot of the analytical data for volatile compound concentrations, with the pure aqueous ethanol from different conditions overlaid.

Abbreviations used for each of the conditions represent different alcohol contents (40, 48, 56, 64% ABV); and temperatures (10, 20, 30 and 40 ºC) at the final maturation time (28 days). For example, 64%40C28d represent 64%ABV, 40ºC and 28 days. *Key compounds*: FURF: furfural; 2ACF: 2-acetyl furan; 5MF: 5-methyl furfural; 5HMF: 5-hydroxymethyl furfural; GUIA: guaiacol; 4-EG: 4-ethyl guaiacol; EUG: eugenol; VAN: vanillin; T-WL: trans-whisky lactone; C-WL: cis-whisky lactone; G-NLAC: γ-nonalactone; G-ACID: gallic acid; V-ACID: vanillic acid; S-ACID: syringic acid; E-ACID: ellagic acid; SYRGALD: syringaldehyde; CONFALD: coniferaldehyde; SYNAPALD: sinapaldehyde.

Regarding new make spirits, PCA accounted 51.7% of explained variance in two principal components (Figure 6.3b). In which, PC1 accounted for the majority of the variation, and showing significant differences due to the effect of the factors ($P < 0.05$, multifactor ANOVA). However, alcohol content and temperature were the main factors in allowing the separation of the samples in the PCA plot (Figure 6.3b). Precisely, the higher concentrations of majority of these ageing-congeners were derived at high alcohol contents (> 57% ABV) and elevated temperatures (> 30 °C). For example, compounds such as vanillin, *cis*-whisky lactone, furfural and guaiacol presented their higher concentrations at 57% ABV and 40 °C. In fact, between pure aqueous solutions and new makes spirits, the extraction rate of majority of mature compounds were driven by increasing spirit content and elevated temperatures. Likewise, the chemical effects of temperature were straightforward, higher temperatures increased the rates of extraction and reaction and increased the rate of diffusion. Under controlled conditions, the non-volatile content extracted during maturation significantly increases with temperature (Philp, 1989). Spirits matured at higher temperature have been described as sweeter but less clean, whilst smoother and more pleasant whiskies are produced at lower temperatures (Philp, 1989). This suggests that the influence of temperature conditions is subtle, and can be masked by cask type and distillate character. Comparisons have shown that the physical and chemical reactions typical of maturation proceed at a greater rate in places with the warmest temperature. However, no optimal temperature has been determined for producing the desired product quality (Reazin, 1981), and the effects of different conditions are averaged out during blending. Interesting, the conditions that influenced the higher extraction of compounds were

different, for example vanillin was enhanced at 40% ABV in the pure aqueous ethanol solutions and at 57% ABV in the new make spirits. These differences are to be expected since the matrix composition of both model spirits were different, particularly in the new make spirits where important interactions between the distillate and wood components occurred during oak chips maturation.

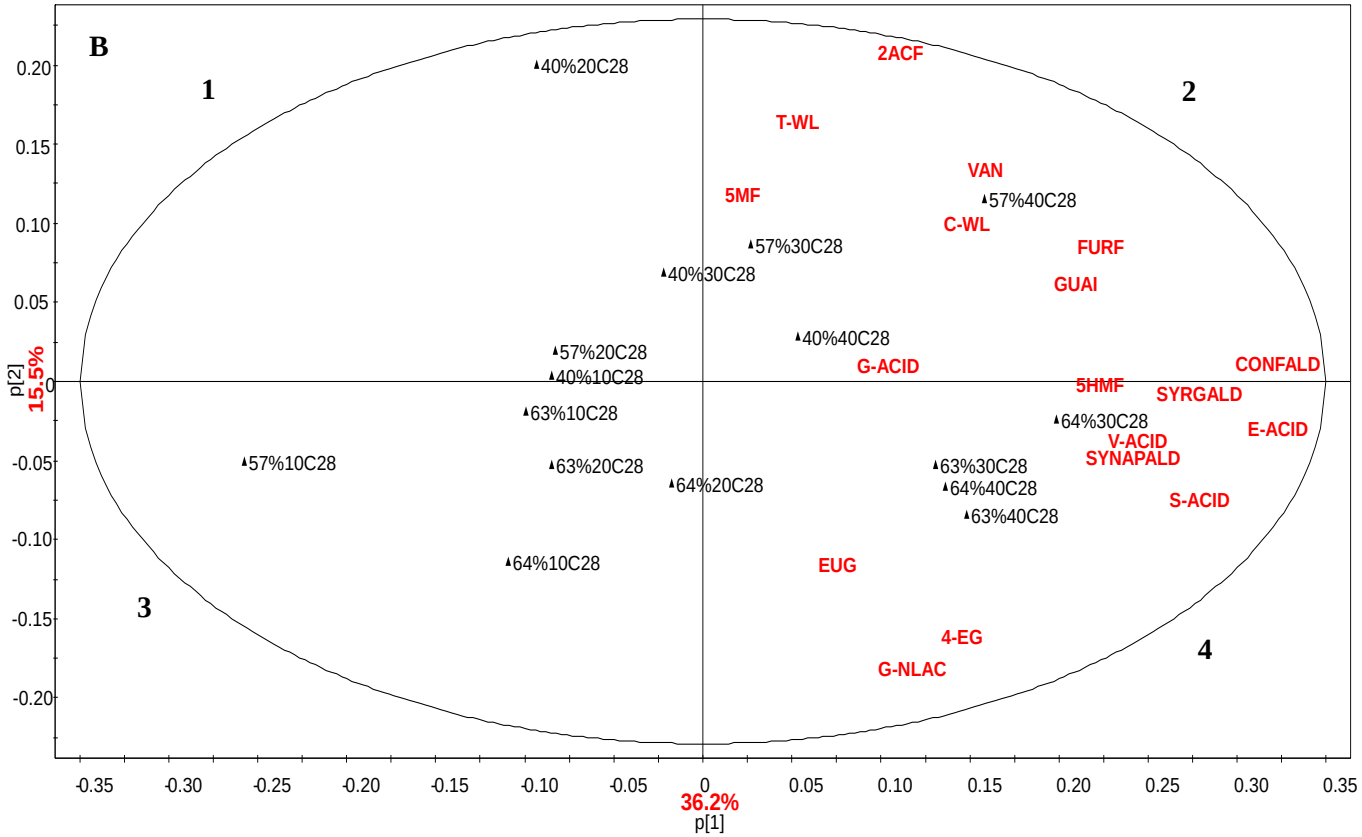

Figure 6.3b. Principal component analysis (PCA) bi-plot of the analytical data for volatile compound concentrations, with the new make spirits from different conditions overlaid.

Abbreviations used for each of the conditions represent different alcohol contents (40, 48, 56, 64% ABV); and temperatures (10, 20, 30 and 40 ºC) at the final maturation time (28 days). For example, 64%40C28d represent 64% ABV, 40 ºC and 28 days. *Key compounds*: FURF: furfural; 2ACF: 2-acetyl furan; 5MF: 5-methyl furfural; 5HMF: 5-hydroxymethyl furfural; GUIA: guaiacol; 4-EG: 4-ethyl guaiacol; EUG: eugenol; VAN: vanillin; T-WL: trans-whisky lactone; C-WL: cis-whisky lactone; G-NLAC: g-nonalactone; G-ACID: gallic acid; V-ACID: vanillic acid; S-ACID: syringic acid; E-ACID: ellagic acid; SYRGALD: syringaldehyde; CONFALD: coniferaldehyde; SYNAPALD: sinapaldehyde.

6.4.2. Effect of spirit matrix on the extraction of mature-relate congeners as a consequence of temperature and alcohol content

In this section differences in the amount of maturation related congeners developed as a consequence of temperature and alcohol content in spirit models of different matrix composition were discussed. For this purpose, we compared the final concentrations achieved after 28 days of maturation for each of the treatments in both model spirits. Figures 6.4 to 6.7 shows the flavour development of minor and major ageing compounds in both model spirits as consequence of alcohol content (% ABV) and temperature (10 and 40 °C respectively). At 10 °C, the extraction rate of all compounds was significantly lower as opposed to the one observed at 40 °C. Generally, in the model ethanol solutions the concentration increased 1.03 to 3.34 times more for temperatures of 10 and 40 °C respectively. For the case of the new make spirits, the concentration increased 1.07 to 2.98 times more. This tendency suggests a significant effect of temperature in the flavour development of these key ageing congeners, particularly increasing as temperature increase. At 10 and 40 °C some compounds such as 5-methyl furfural, eugenol, trans-whisky lactone, cis-whisky lactone, 5-hydroxy methyl furfural, gallic acid, vanillic acid, syringic acid, ellagic acid, and coniferaldehyde increase their concentrations as alcohol content increase. However, for some other compounds such as vanillin and syringaldehyde, the greatest concentration was derived at just 40% ABV in both spirit models (Fig. 6.4 – 6.7). When comparing model ethanol solution with spirits of equivalent strength, minor effects due to ‘matrix’ were apparent. For example, at lower temperatures (10 °C), 5-methyl furfural, vanillin and cis-whisky lactone were more efficiently extracted in model ethanol solutions than

in new make spirits. However, as temperature increase to 40 °C, other compounds were further extracted in model ethanol solutions, including furfural, syringic acid, syringaldehyde, coniferaldehyde, and sinapaldehyde. Contrary, ellagic acid was the only compound more efficiently extracted in new make spirits than model ethanol solutions, particularly at 40 °C.

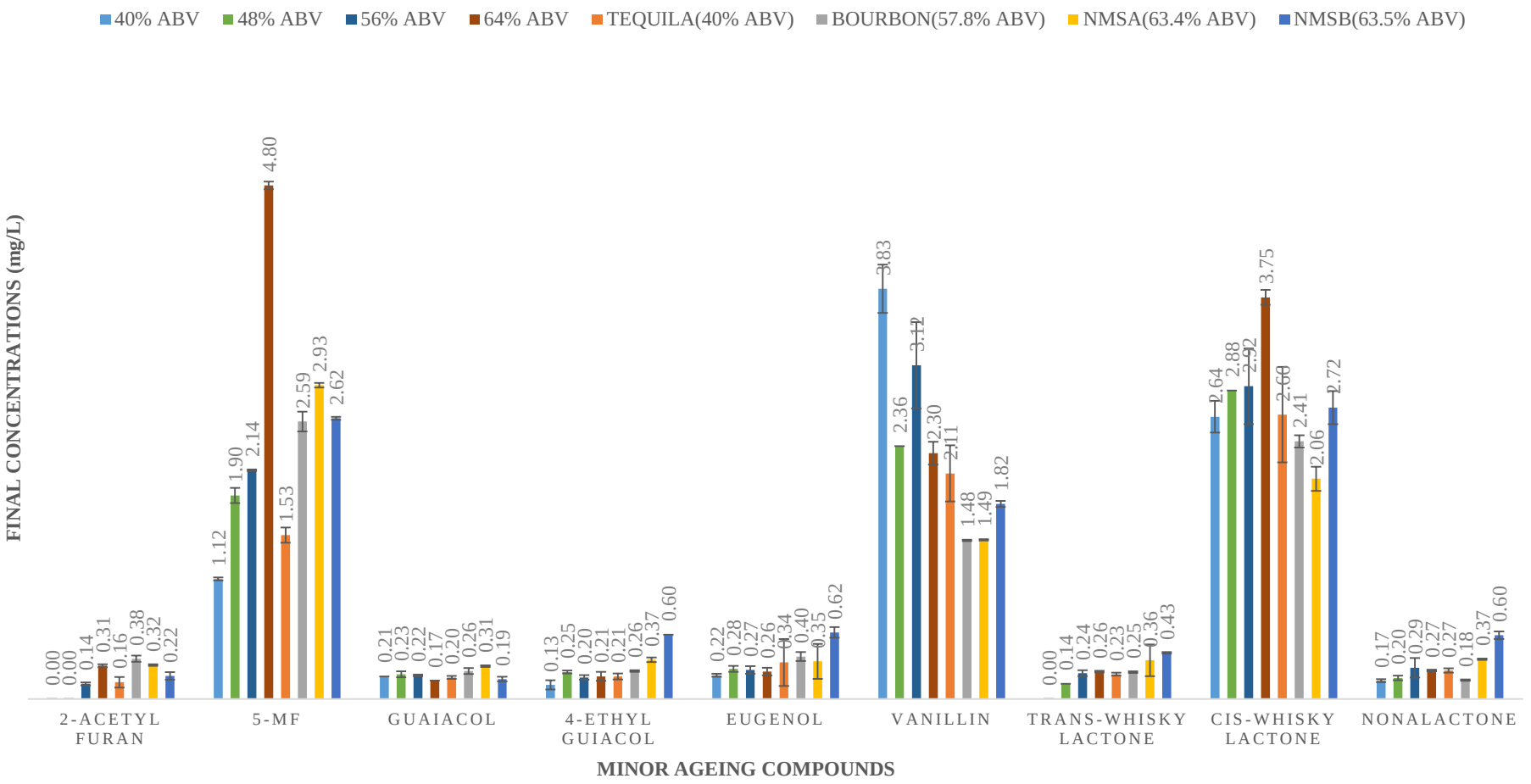

Figure 6.4 Extraction of minor ageing compounds as a function of alcohol content and spirit matrix. Values are the final concentrations (28d) obtained at **10°C**.

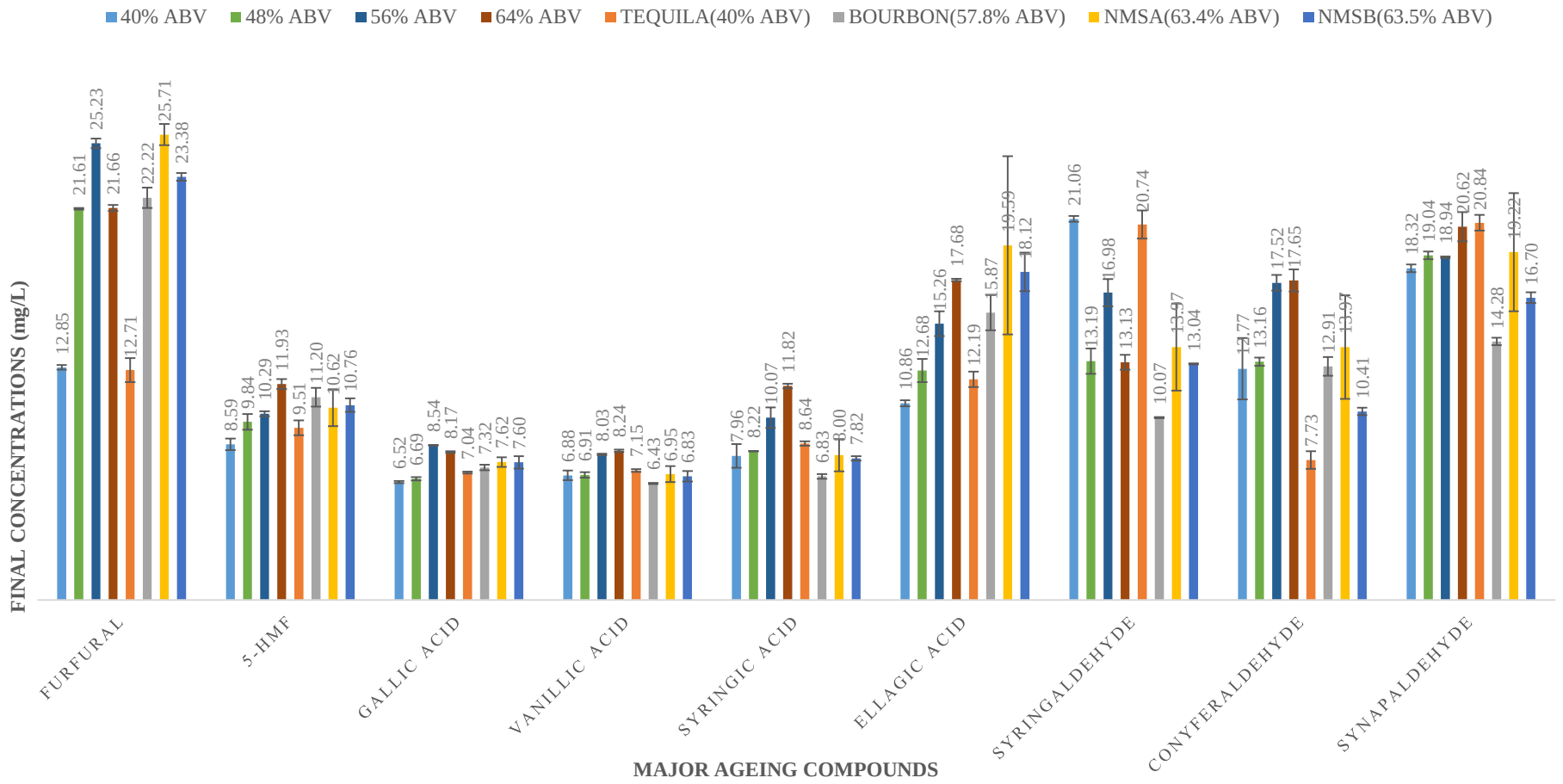

Figure 6.5 Extraction of major ageing compounds as a consequence of alcohol content and spirit matrix. Values are the final concentrations (28d) obtained at **10°C**.

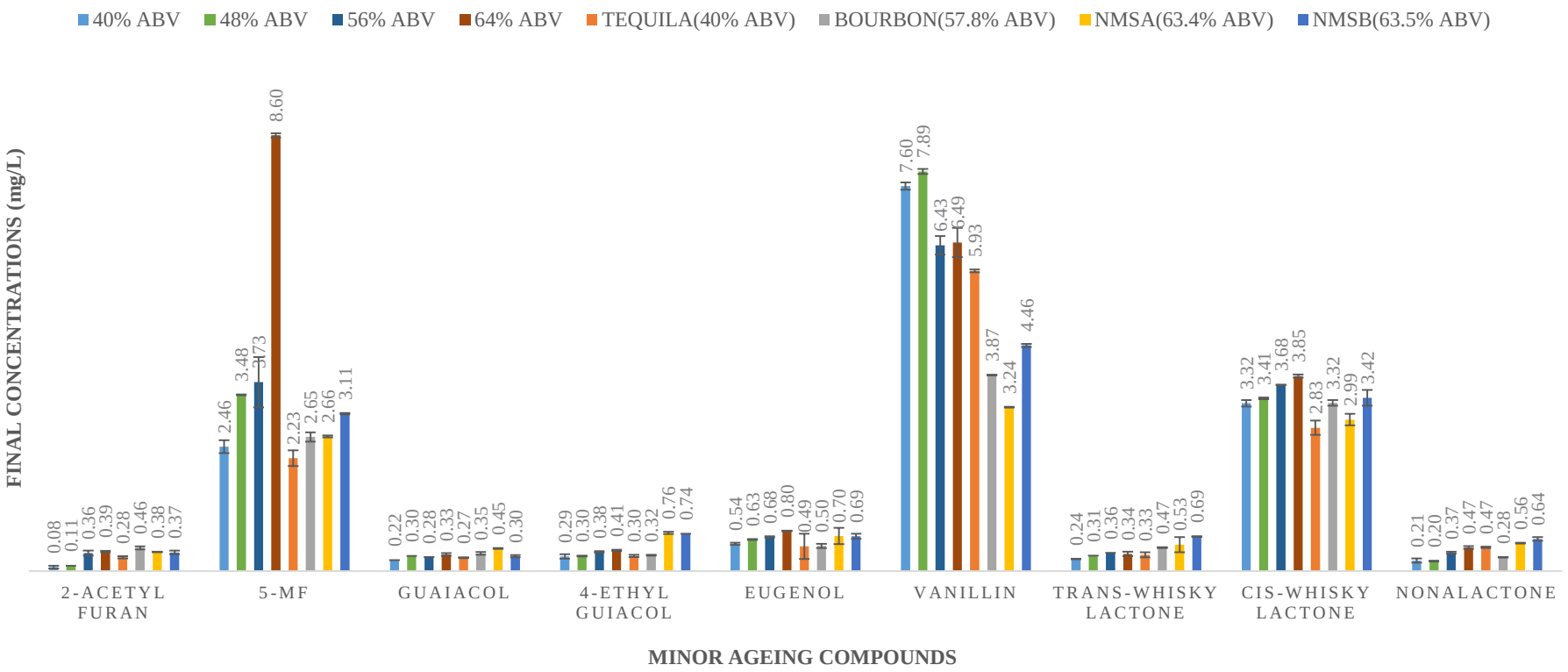

Figure 6.6 Extraction of minor ageing compounds as a consequence of alcohol content and spirit matrix (type). Values are the final concentrations (28d) obtained at **40°C**.

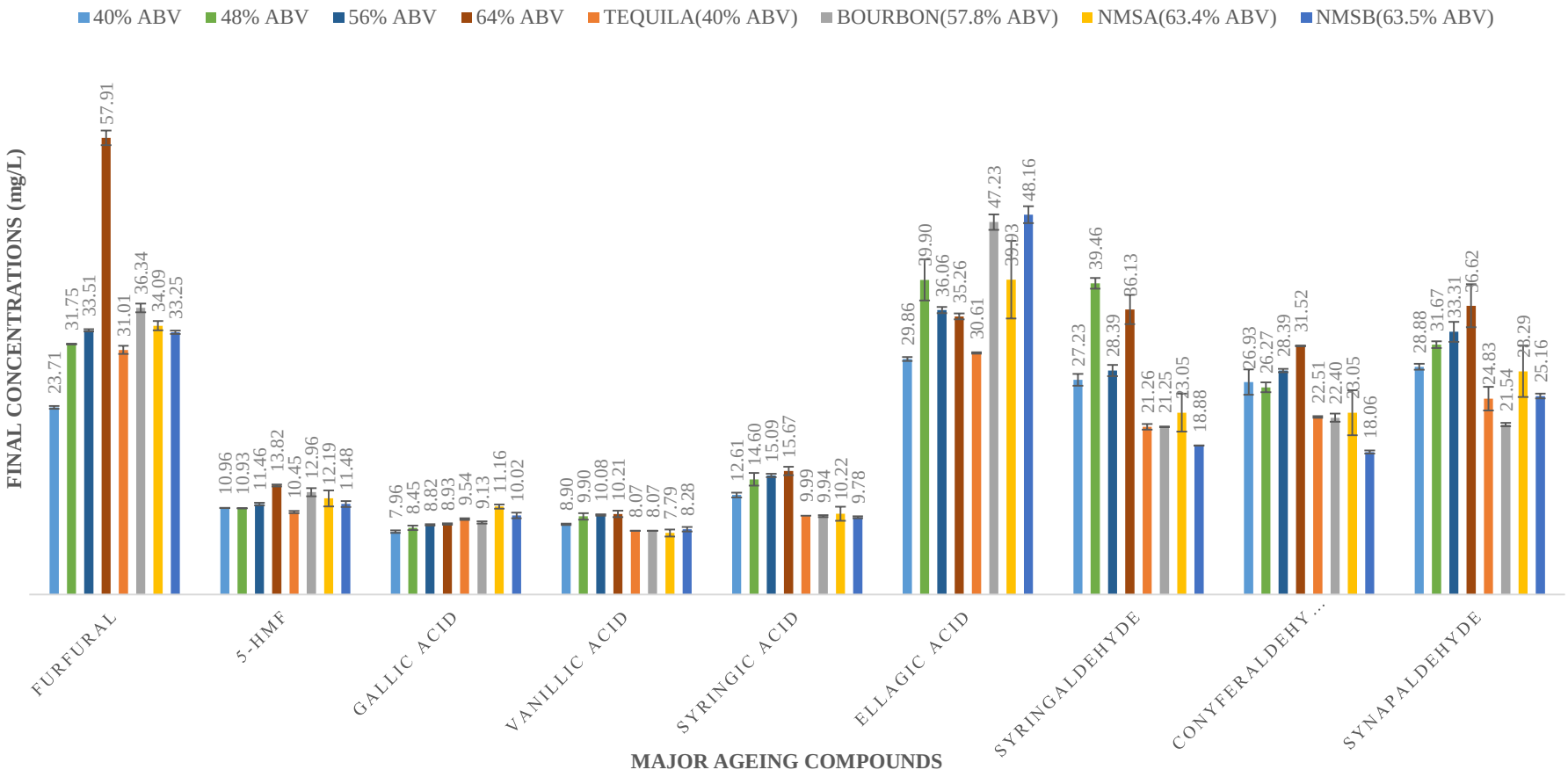

Figure 6.7 Extraction of major ageing compounds as a consequence of alcohol content and spirit matrix. Values are the final concentrations (28d) obtained at **40°C**.

Remaining compounds exhibited minor effect due to ‘matrix’ composition. These differences are to be expected since the composition between new make spirits and model ethanol solutions is clearly evident, relying on not only in the alcohol content (% ABV) but more in the composition of the alcoholic matrix. New make spirits are well known products consisting of hundreds of compounds derived mainly from the fermentation process and ultimately concentrated during the distillation process (Campbell, 2003; Nicol, 2003). These compounds experience then the ultimate ageing process before commercialization, during which a range of physical and chemical interactions take place between the barrel, the surrounding atmosphere and the maturing spirit which transform both the flavour and composition of the drink (Mosedale & Puech, 1998). Because of the presence of flavour compounds already in the new make spirits, the subsequent reactions taking place during maturation (e.g. oxidation, hydrolysis and polymerisation) could explaine the reduction of some of the ageing congeners as compared with the model ethanol solutions, where the major compounds before ageing is basically ethanol.

6.4.3. Extraction kinetics of wood-derived congeners into spirits as a function of ageing, temperature, spirit type and alcohol content

After evaluating the evolution of 18 maturation compounds, the kinetics of extractions was studied in order to understand variations with temperature and what that tells us about the activation energy for extraction of particular compounds.

The model chosen to display the best fitting of the data was the one described by Psarra et al. (2015), which follows a hyperbola of second-order kinetics, described by eqn 1.

$$C = \frac{Csat^2(kt)}{1+Csat(kt)} \text{---- (1)}$$

Where *C* represents the concentration, and *Csat* and *k* represent the concentration of saturation and the extraction rate constant, at *t* time respectively. Transformation of eqn 1 yields the following linearized form:

$$\frac{t}{C} = \frac{1}{KCsat^2} + \frac{1}{Csat} \text{ ---- (2)}$$

When *t* approaches 0, the initial extraction rate, *h*, given as *C/t*, is defined as:

$$h = kCsat^2 \text{--- (3)}$$

Plotting *t*/*C* vs *t* would give a straight line in the form of $y = ax + b$, where *a* = $1/C_{sat}$ and $b=1/h$. Thus, for each case tested, C_{sat}, *h* and *k* could be determined graphically. Once the extraction rate constant (*k*) was obtained from eqn 3, *Ea* (activation energy) from Arrhenius Law eqn 4 was determined:

$$k = Ae^{-Ea/(RT)} \text{--- (4)}$$

Where *k* is the extraction rate constant, *T* is the absolute temperature (K), *A* is the pre-exponential factor, E_a is the activation energy (J) and *R* is the universal gas constant (8.314 J/ K-moL). Accordingly, when a reaction has a rate constant that obeys Arrhenius's eqn 4, a plot of *Ln(k)* versus *1/T* gives a straight line, whose gradient and intercept can be used to determine E_a and *A* (Aquilanti et al. 2010). Thus, Appendix 25 shows the kinetic parameters determined for 18 maturation compounds, including R^2, *h, k,* and *Csat.* In addition, Table 6.4 summarizes the activation energies of extraction of these compounds and Figure 6.8 shows an example of the fitting of second-order kinetic model (eqn 1) used to determine the kinetic parameters for extraction of *cis*-whisky lactone.

In general, for *cis*-whisky lactone the model implemented for nonlinear correlation fitted the majority of the spirit models, including model ethanol solutions and new make spirits, with correlation coefficients (R^2) that were statistically significant ($P < 0.05$) and in the range of 0.86 – 0.98 (Fig. 6.8, appendix 25). The nonlinear regressions between *C* and *t* for *cis*-whisky lactone in bourbon spirit models (57.8% ABV) at different temperatures (10 – 40 ºC) shows how the evolution of this flavour compound was affected as a consequence of time, and temperature ($P < 0.05$). In the majority of cases the flavour profile follows a hyperbolic fitting with an increase in concentration as the temperature increases (Fig. 6.8). For the remaining compounds (Appendix 25), the model gave regression coefficients, $R^2 \geq 0.87$. The variation of *k* and *h* values as a function of temperature (T) was not consistently increased as temperature increased for all spirit systems (Appendix 25). However, for some compounds such as *cis*-whisky lactone, *k* and *h* increased positively as T increased as long as the C_{sat} increased (Fig. 6.8). This parameter (C_{sat}) was

significant different among model spirits and for *cis*-whisky lactone was in the range 2.44 to 5.04 mg/L. Other related compounds such as guaiacol, eugenol, and 4-ethyl guaiacol showed $C_{sat} \leq 1$ mg/L. An important kinetic parameter was the activation energy (*Ea*), which is defined as the minimum energy required to start a chemical reaction (Schleicher et al. 1988). For *cis*-whisky lactone, *Ea* was positive and situated in the range of 13.05 – 42.22 KJ/moL based on Arrhenius plots at each % ABV (Table 6.4).

Table 6.4. Activation energies (*Ea*) in KJ/moL determined for the extraction of wood-derived congeners from oak sticks into pure aqueous ethanolic solutions and authentic new make spirits

Volatile compound	Pure aqueous ethanolic solutions (% ABV)				New make spirits (% ABV)			
	40%	48%	56%	64%	Tequila (40%)	Bourbon (57.8%)	NMSA (63.4%)	NMSB (63.5 %)
Furfural	9.85	14.57	13.71	16.00	5.54	33.02	18.59	5.43
2-Acetyl furan	nd	nd	nd	nd	12.76	13.95	25.57	25.15
5-Methyl furfural	29.94	9.59	29.99	39.92	7.80	8.06	8.16	9.32
5-Hidroxy-methyl furfural	25.59	21.95	32.61	11.80	5.49	8.19	12.83	11.70
Guaiacol	37.57	33.49	9.46	7.73	20.14	38.92	9.50	19.16
4-Ethyl guaiacol	4.94	5.43	10.74	20.62	17.08	9.28	35.11	17.76
Eugenol	20.84	20.14	27.55	18.83	13.74	7.34	30.50	28.44
Vanillin	0.99	14.15	21.23	31.34	14.24	15.74	19.19	9.40
Trans-whisky lactone	14.39	11.12	25.65	27.77	0.56	0.32	0.98	10.25
Cis-whisky lactone	13.05	36.98	31.24	28.85	31.10	24.49	36.52	42.22
γ-nonalactone	6.34	8.39	61.41	20.76	1.79	17.18	43.64	4.71
Gallic acid	29.43	11.87	13.86	44.28	27.63	36.09	46.66	18.97
Vanillic acid	14.61	7.28	3.05	28.61	9.40	29.06	20.86	15.20
Syringic acid	3.60	18.27	21.91	40.43	15.18	13.78	32.49	33.94
Ellagic acid	31.18	35.18	13.49	41.23	38.09	29.62	47.50	44.75
Syringaldehyde	26.73	34.64	60.72	24.28	41.53	48.62	43.70	22.14
Coniferaldehyde	24.18	24.65	16.04	4.23	11.99	23.26	40.74	18.30
Sinapaldehyde	21.91	29.23	27.98	26.14	16.63	10.09	2.82	19.73

nd: not determined during the conditions of analysis, therefore the *Ea* was not estimated.

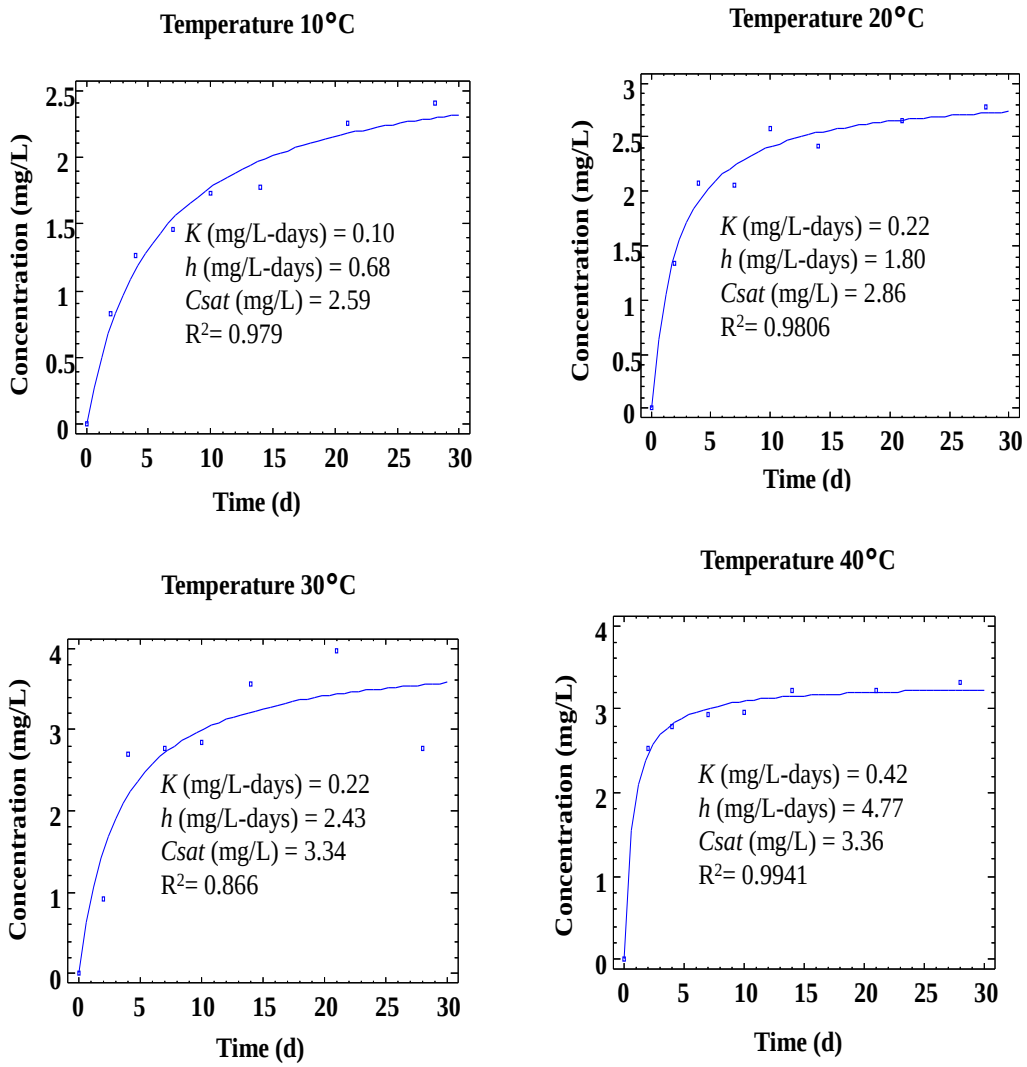

Figure. 6.8. Estimated kinetic parameters (R^2, *K*, *h* and C_{sat}) obtained during the modelling (non-linear regressions) of the extraction of *cis*-whisky lactone from American oak sticks in a bourbon model solution (57.8%ABV).

Kinetic parameters; R^2, coefficient correlation, *h*, initial extraction rate, *k*, extraction rate constant, *Csat*, concentration at saturation of *cis*-whisky lactone.

However, there were not exclusive tendencies along the date set of the spirit systems evaluated, which could be used to link the activation energy for extraction with the % ABV of the spirit. Compounds such as 5-methyl furfural, eugenol, and trans-whisky lactone, presented higher E_a values in the aqueous

ethanolic solutions than in the authentic new make spirits. In general, the activation energy (*Ea*) provides information about how extraction rate changes with temperature, therefore if there are changes in the concentration extracted they are a function of this and not the cause of the different activation energies (Schleicher et al. 1988). Normally higher temperatures, would lead to higher *Ea* values. Regarding the effect of alcohol content (% ABV) while defining *Ea,* this parameter had not significant impact since different values were observed along the data set in both the aqueous ethanolic solutions and new make spirits (Table 6.4). When comparing pure aqueous ethanolic solutions with spirits of equivalent strength, in terms of kinetic parameters significant differences were observed ($P < 0.05$). Particularly in terms of *k* and *h* values (Appendix 25), e. g. *cis*-whisky lactone and vanillin presented higher values in pure aqueous ethanolic solutions than in the new spirits, possibly because of a higher extraction rate in those model spirits than in the authentic new spirits. Remaining compounds exhibited different trends that change from model spirits to its equivalent of similar alcohol strength.

6.4.3 General discussion

Extraction of 18 maturation congeners (including wood-derived compounds, volatile phenols, phenolic acids, phenolic aldehydes, and furanic aldehydes) from charred oak chips into spirits were significantly affected ($P < 0.05$) by ageing time, temperature, spirit type and alcohol content. In general, results show that the alcoholic degree had a major impact on the extraction of the compounds studied during the 28 days of extraction. For example, compounds such as furfural, *cis*-whisky lactone and ellagic acid were enhanced as spirit alcohol content was increased, whereas the greatest concentrations of others, such as vanillin and syringaldehyde, were derived at just 40% ABV. Previous studies have shown the influence of the alcohol content of model wine solutions on the total and individual ellagic tannin and polyphenols extraction, confirming thus that this factor cannot be an important factor for the extraction/degradation of these compounds during wine oak ageing (Jordão et al. 2005; Vivas and Glories, 1996). Conversely, in the case of the spirits evaluated in this study, alcohol contents were much higher and therefore significantly impact the rate of extraction of wood derived compounds. This observation was confirmed through the PCA analysis, where wood congeners were separated based on their alcohol content, with the highest concentrations of the majority of compounds being extracted into aqueous ethanol and new make spirit systems at higher alcohol contents (> 63.4% ABV) respectively. Furthermore, different new make spirits and aqueous ethanolic solutions extracted different profiles of compounds, however, such differences were mainly attributable to differences in alcohol content (% ABV) and the remaining spirit matrix appeared to exert a lesser effect. For example, the flavour profile resulting from ageing in new make

whisky spirits (63.4 and 63.5% ABV) were significantly different to that in 64% aqueous ethanol, even though the experimental conditions were the same. Another important factor was the temperature, which consistently influenced the extraction rate of all compounds, increasing as the temperature increased (Figures 6.1 & 6.2). In fact, temperature was a decisive factor influencing the extractability of these compounds from oak wood, mostly by allowing the oxidation, polymerisation or hydrolysis reactions between the distillate and the surrounding oak barrel to occur (Mosedale & Puech, 1998). Nevertheless, the higher rate of extraction of all compounds were presented at elevated temperatures that at lower temperatures. For example, key ageing congeners such as *cis*–whisky lactone, guaiacol, eugenol, and vanillin increased their concentrations on average 1 to 3 times more as temperature raised from 10 to 40 ºC (Figures 6.4–6.7). These results confirmed the higher impact of temperature on the extraction rate of all ageing congeners. Additionally, the kinetic behaviour reported during diffusion of wood-related congeners from oak sticks into ethanolic matrices was efficiently described by a second order model (Psarra et al. 2014). The parameters that were calculated graphically (nonlinear regressions) suggested that extraction might be accelerated in systems with higher ethanol concentration and temperature. Also, the activation energy (E_a) for all maturation compounds was estimated, and suggested significant differences in the extraction rate of all compounds as a consequence of the temperature (Table 6.3). For example, at different alcohol contents (% ABV) there were not specific trends that showed the effect of this factor on the *Ea* of 18 maturation compounds. However, the analysis of key congeners at specific % ABV suggest different behaviours. For example, at 40% ABV in both model

spirits the *Ea* of guaiacol and eugenol were higher in the aqueous ethanol solutions than in the new make spirits, and for vanillin and *cis*–whisky lactone the values were higher in the new make spirits than in the model ethanol solutions (Table 6.3). These differences are to be expected since different extraction rate constants (*k*) were observed along the model spirits and new make spirits at each of the temperatures evaluated. Normally, higher extraction rates constants (*k*) would lead to higher *Ea* values and therefore to higher extraction rates of the compounds in the spirit system. For example, guaiacol was efficiently extracted in the model ethanol solutions than in the new make spirit. This is corroborated with the higher *Ea* presented in the model ethanol solution. Same observations could be addressed for other compounds in Table 6.3. Generally, extraction rate constant (*k*) increases with temperature, suggesting higher extraction rates at elevated ageing temperatures. This observation is confirmed with *cis* – whisky lactone and other ageing congeners (Appendix 25). Overall, after 21 days of maturation for the majority of compounds the ageing profiles reached a saturation point in their concentrations (C_{sat}), which from this moment did not exhibit significant changes according to the second-order modelling of the compounds analysed. If the time course of maturation would have lasted longer (as in practice), possibly the kinetic differences would be less important, however, they could still impact on the final product flavour since the kinetics will control the composition. Yet, the results of the main differences presented were the consequence of the different ageing conditions implemented, and the chemical mechanism that arise as outcome of the ageing process and influence the extraction of wood-derived congeners into the spirt and affect the

flavour composition of the aged product (Reazin, 1981; Mosedale & Puech, 1998).

6.5. CONCLUSIONS

The profile analysis of 18 flavour active extractives compounds confirmed the significant effect of ageing time, temperature, spirit type and alcohol content ($P < 0.05$) in flavour evolution. In general, the higher rate of extraction was favoured at elevated temperatures (> 40 ºC) and higher alcohol contents (> 63.4% ABV) in both model spirits. These observations were further confirmed by PCA analysis. Comparison among ethanol solution with spirit of equivalent strength, showed minor effects due to spirit 'matrix' composition, however for some compounds such as vanillin and syringaldehyde the higher concentrations extracted were derived at 40% ABV in both spirit systems and for ellagic acid this was at 40 ºC in new make spirits. These differences suggest different extraction behaviours as consequence of the factors, nevertheless the higher extraction rate for all of compounds was observed at elevated temperatures. Additionally, kinetic analysis showed that the extraction process followed a second order model (Psarra et al. 2014) and the parameters calculated graphically (*e.g. k, Ea*), showed that the extraction of majority of ageing congeners exhibited different extraction behaviours with changing temperatures (different *Ea* values). For example, syringaldehyde presented higher *Ea* values, indicating a greater impact of increasing temperature on extraction rate, than for compounds such as vanillin and furfural. This study demonstrated that the use of oak chips of inner staves during spirit ageing was a useful model system that enabled the impact of different ageing conditions to be studied; such as the duration, the temperature of ageing and the alcohol content of the new make

spirit. In addition, results from this chapter complemented observations arising from previous Chapters, in which, there was a significant impact of maturation on the flavour development of key maturation compounds across different spirit types.

7. OVERALL DISCUSSION, CONCLUSIONS & RECOMMENDATIONS FOR FUTURE WORK

7.1 Overall Discussion and Conclusions.

Distilled spirits are frequently aged in wooden casks (of varying provenance) as a part of the production process to enhance their sensory properties and value. The authenticity of aged spirits can be corroborated using instrumental and sensory analysis, which can also reveal the significant contribution of selected groups of compounds to spirit flavour. Previous studies conducted in aged spirits, have highlighted a reduction of the sensory estery character in the mature spirits, even though the ester concentrations before and after maturation were roughly similar (Piggott et al. 1992; Conner et al. 1994; 1994; Conner et al. 1996). One potential explanation for this observation would be a sensory interaction between mature and estery characters. Testing this hypothesis was one of the objectives of this PhD thesis, which aimed to improve knowledge of the potential for sensory interactions in complex multi-component systems as represented by the spirits used in the study, namely malt whisky, tequila and bourbon whiskey. Likewise, due to the high impact of the maturation process on the aroma composition of these products, we decided to investigate the effect of selected ageing conditions on the extraction kinetics of wood-derived congeners. Four pairs of non-mature and mature spirits of the same brand and provenance (tequila, bourbon and 2 malt whiskies) were utilized throughout the studies described in this thesis.

The work reported in Chapters 2 and 3 compared and contrasted the impacts of wood ageing on the aroma composition and sensory properties of these four pairs of spirits, with the hypothesis that some commonalities exist, both in terms

of the extracted wood-derived compounds and their impacts on the overall aroma profiles of the spirits. Correlation analysis (Pearson and PLSR) enabled us to distinguish spirits in terms of aroma composition and sensory properties. In general, the compounds that differentiated the spirits were oak lactones, volatile phenols, phenolic compounds and furanic aldehydes. These compounds were reported with high FD factors (≤ 6561) in the mature spirits, and their concentrations were positively correlated with the time of the ageing process used in manufacture of these spirit samples. Additionally, sensory analysis further confirmed strong associations between mature characters (*dried fruit, sweet, spicy, and woody*) and these ageing congeners, such as, ethyl-acetate, acetal, guiaiacol, 4-ethyl guaiacol, and eugenol to name a few.

The degree of these characteristics developed in each spirit was evidently related to the ageing period employed and the characteristics of the cask used for ageing (*e.g*. new versus re-use). Moreover, GC-O/AEDA analysis enabled the identification of the most significant flavour compounds which defined or contributed to the 'mature/woody' and 'estery' character of the spirit samples. These odorants were volatile phenols and oak lactones (mature fraction), ethyl and acetate esters (estery fraction).

The precise fraction compositions required to recreate the estery and woody characters of each authentic spirit sample were further assessed in Chapter 4. Here, the analytical and GC-O data of Chapter 3 were used to create recombinant aroma blends based on the analysis of each spirit type with regard to the estery and woody/mature congeners. The aim was to mimic specified characteristics (estery and mature) of the authentic spirit samples as closely as possible. Particular attention was given to the core congeners analysed by GC-O/AEDA,

to the non-volatile wood extractives, and to the presence of low boiling volatile compounds and long chain-ethyl esters (ethyl hexadecanoate). The groups of compounds that were found to substantially modulate spirit flavour (and thus make the simulated aroma mixtures more authentic) were the relatively high concentration of low boiling volatile compounds (analysed by direct GC method) and ethyl hexadecanoate (a 'structuring' ester with minimal aroma of its own). Addition of these compounds greatly improved the authenticity of the final simulants, such that their aroma characters were similar to those of the authentic aged spirits. The effect of ethyl hexadecanoate in the model spirits, is thought to result from formation of agglomerates that incorporated relatively hydrophobic compounds, thus removing them from the headspace and altering the perceived aroma (Boothroyd et al. 2013). In general, the presence of low boiling volatile compounds and ethyl hexadecanoate particularly improved the estery character authenticity of each of the fractions, by achieving a more balanced and fruity character which better represented the original products.

In the case of the mature/woody fractions, their presence also modified the overall mature/woody character by providing a more authentic and closer aroma as with the genuine products. This was further confirmed by the SWRI expert panel, which defined the mature/woody character of the recombined mature bourbon with similar sensory characters as were present in the authentic mature bourbon. Findings from these Chapters provided enough information to adequately formulate the spirit blends that define each of the fractions and were a significant output of this PhD work. The recombined fractions which were optimised in Chapter 4 were used in Chapter 5 to investigate the impacts of varying levels of estery and mature/woody fractions on the perceived estery and

mature character in each of the spirits, to explore potential sensory interactions between these fractions. Overall, sensory perception of the woody/mature and estery characters were generally driven by concentrations of the respective fractions. In most spirit simulants, higher levels of the woody fraction led to a higher degree of suppression in the estery character as exemplified by the bourbon model spirits. However, for malt whiskies and tequila model spirits, which contained analytically lower amounts of ageing congeners than bourbon, suppression of the estery character was still observed, to varying degrees. These findings supported the initial hypothesis of this PhD work and are most likely the cause of the previous observations by Conner and co-workers (Piggott et al. 1992; Conner et al. 1994; 1994; Conner et al. 1996). Additionally, it is worth notice, that the spirit systems developed here were complex in terms of their flavour composition and took substantial amounts of detailed analysis and considerable persistence to develop the analytical knowledge to firstly devise and then test interactions between blends of congeners responsible for these precepts in four different spirit systems. Nevertheless , attempts were made to improve the authenticity of these fractions by increasing their complexity through the addition of other spirit components thought to impact on their perception through physico-chemical or sensory mechanisms. This led to obtain novel findings, which have not been published elsewhere, especially in such complex spirit systems as the ones described here.

Finally, because of the significant contribution of maturation to spirit quality, we proceeded in Chapter 6 to physico-chemical studies investigating the extraction kinetics of selected wood-derived congeners (e.g. cis/trans-whisky lactones) from oak sticks as a function of temperature, time and alcohol content

(%ABV) and spirit type. In summary, the extraction rates of 18 maturation compounds were significantly affected by temperature, time and alcohol content ($P < 0.05$). However, the extraction rates for these compounds were more rapid at elevated temperatures and alcohol contents (≥ 40 ºC and ≥ 63.4 % ABV) in both spirit matrices (pure ethanolic solutions versus authentic non-mature spirit samples over a similar range of % ABV). Interestingly, some exceptions were observed, particularly for vanillin and syringaldehyde, which resulted in the greatest extracted concentrations at just 40% ABV, possibly due to a higher affinity to this spirit matrix. Comparison of results for pure aqueous ethanol solutions with those for new-make spirits of equivalent strength, showed only minor effects due to new-make spirit components other than ethanol. Additionally, kinetic analysis showed that the extraction process followed a second order model (Psarra et al. 2014) and the parameters calculated graphically (nonlinear regressions) indicated that different ageing congeners exhibited different extraction behaviours with changing temperatures (different activation energies calculated from Ahrrenius plots). This finding suggests that temperature of maturation does not simply change the rate of extraction yielding a similar product flavour; rather it suggests that the relative proportions of wood-derived congeners, and thus the mature character, will change according to maturation temperature. We would thus predict based on this study that the mature/woody character imparted by the relatively warm and rapid maturation of tequila would be different relative to that which results from the longer, cooler maturation employed in the Scotch whisky industry. Thus, the mature spirit characteristics would vary both as a result of differences in cask provenance and maturation conditions.

In conclusion, findings across the thesis have highlighted the significant influence of the maturation process on the mature aroma character of different spirit categories (bourbon, tequila, and malt whiskies). The suppression of the non-mature estery character by the presence of the mature wood-derived congeners was demonstrated through sensory interaction studies, even at the lower concentrations of wood-derived congeners which result from the relatively short and warm maturation employed for the anejo tequila used in this study. Moreover, this study enabled the effects of physical parameters to be linked to changes in spirits composition across different model spirits. This has provided a better understanding of the factors that affect the development of key mature components in aqueous model ethanol solution and new make spirits. Undoubtedly, results from the present PhD work have led to significant new scientific knowledge that could be use by the Tequila and Whisky Industries to understand the effects of maturation in wood and how its impacts significantly modulate the perceived aroma of these matured spirits. Also, the data reported in this thesis could be used as a basis to define specific storage maturation conditions for the extraction of selected wood maturation congeners.

7.2 Recommendations for future work

For future work, I suggest a more comprehensive and detailed aroma characterization. In addition, to look for further methods of isolation, concentration and identification besides the ones used in the present study, such as the ones described by Schieberle's group and which comprise the well-known Sensomics methodology (Schieberle and Hoffman, 2012). Typically, this includes isolation of the volatile fraction by extraction/solvent assisted flavour evaporation (SAFE), identification of the key aroma compounds by aroma

extract dilution analysis (AEDA), the quantitation of these compounds by stable isotope dilution assay (SIDA) and the determination of the importance of each odorant based on OAVs, and validation of the results through aroma reconstitution experiments and sensory evaluation. Although in the present study we followed some steps of this approach regarding identification and validation essays, it has been reported (Schieberle et al. 2011) that the use in detail of the whole approach helps to obtain the complete aroma profile of the authentic product. This will be useful for further assessing specific groups of sensory characters that are so important to spirit flavour as defined by their flavour wheels, such as the ones described in this work (estery and woody).

Ideally, I would like to extend the principle of sensory interactions to other important fractions of the whisky flavour wheel such as sweet or feinty and beyond. This would allow us to obtain a complete fingerprint as to how each of the major fractions modulate the perception of spirit flavour.

Since the ageing process was an important parameter in determining the overall aroma character of the spirit samples, it would be ideal to know in more detail the exact ageing conditions of these products. This would allow us to trace the exact parameters that defined the mature character of the products, and would be useful also in defining the specific parameters that we used to design the experimental of the kinetics extractions using oak chips (Chapter 6).

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APPENDICES

Appendix 1. Chemical Standards Used in the Study

Compound	Other names	CAS	Purity	Source
	Minor compounds analysed by GC-MS			
ethyl hexanoate	ethyl caproate	123-66-0	99%	Fisher
ethyl octanoate	ethyl caprylate	106-32-1	99%	Fisher
2-phenylethyl acetate	acetic acid, 2-phenylethyl ester	103-45-7	99%	Sigma Aldrich
ethyl lactate	lactic acid ethyl ester	97-64-3	98%	Sigma Aldrich
ethyl nonanoate	---	123-29-5	97%	Sigma Aldrich
diethyl succinate	butanedioic acid diethyl ester	123-25-1	99%	Sigma Aldrich
ethyl benzoate	benzoic acid ethyl ester	93-89-0	99%	Sigma Aldrich
benzaldehyde	---	100-52-7	98%	Sigma Aldrich
2,3-butandione	diacetyl	431-03-8	97%	Sigma Aldrich
E-damascenone	β-damascenone	23726-93-4	90%	Sigma Aldrich
γ-nonalactone	2(3H)-furanone, dihydro-5-pentyl-	104-61-0	98%	Sigma Aldrich
cis, trans-whisky lactone	----	39212-23-2	98%	Sigma Aldrich
4-allyl-2-methoxyphenol	eugenol	97-53-0	99%	VWR International
2-methoxyphenol	guaiacol	90-05-1	98%	VWR International
4-ethyl-2-methoxyphenol	4-ethyl-guaiacol	2785-89-9	98%	VWR International
n-hexanol	---	117273	98%	Sigma Aldrich
hexanoic acid	---	142621	100%	Sigma Aldrich
decanoic acid	---	334-48-5	98%	Sigma Aldrich
furfural	furan-2-carboxaldehyde	98-01-1	99%	Sigma Aldrich
n-butanol	Butyl alcohol	71-36-3	99	Sigma Aldrich
5-methyl furfural	5-methyl-2-furancarboxaldehyde	620-02-0	99%	Sigma Aldrich
2-acetylthiazole	---	24295-03-2	99%	Sigma Aldrich
	Major compounds analyzed by GC-MS			
isoamyl acetate	3-methyl-1-butyl acetate	123-92-2	99%	Merck
ethyl hexadecanoate	ethyl palmitate	628-97-7	95%	Sigma Aldrich
ethyl decanoate	ethyl caprate	110383	99%	Sigma Aldrich
2-phenylethanol	phenethyl alcohol	60-12-8	99%	Sigma Aldrich
	Major compounds analyzed by GC-FID			
n-propanol	propionyl alcohol	71238	99%	Sigma Aldrich
isobutanol	isobutyl alcohol	78831	99%	Fisher
acetaldehyde	Ethanal	75-07-0	99.5	Sigma Aldrich
ethyl lactate	Propanoic acid, 2-hydroxy-, ethyl ester	97-64-3	98	Sigma Aldrich
ethyl acetate	Ethyl ester of acetic acid	141-78-6	98	Sigma Aldrich
acetal	Acetaldehyde, diethyl acetal	105-57-7	98	Sigma Aldrich
methanol	Methyl alcohol	67-56-1	98	Sigma Aldrich
n-pentanol	Pentyl alcohol	71-41-0	99	Sigma Aldrich
2-methyl-1-butanol	Active amyl alcohol	137-32-6	99	Sigma Aldrich
3-methyl-1-butanol	Isoamyl alcohol, primary	123-51-3	98	Sigma Aldrich
acetic acid	Ethanoic acid	64-19-7	98	Sigma Aldrich
	Major compounds analyzed by HPLC-UV			
gallic acid	3,4,5-Trihydroxybenzoic acid	149-91-7	97	Sigma Aldrich
vanillic acid	2-Methoxy-4-carboxyphenol	121-34-6	99	Sigma Aldrich
4-hydroxy-3-methoxybenzaldehyde	vanillin	121-33-5	99%	Sigma Aldrich
syringic acid	Gallic acid 3,5-dimethyl ether	530-57-4	98	Sigma Aldrich
syringaldehyde	4-Hydroxy-3,5-dimethoxybenzaldehyde	134-96-3	99	Sigma Aldrich
coniferaldehyde	(Z)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enal	458-36-6	98	Sigma Aldrich
sinapaldehyde	3-(4-Hydroxy-3,5-dimethoxyphenyl)prop-2-enal	4206-58-0	98	Sigma Aldrich
ellagic acid	2,3,7,8-Tetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione	476-66-4	98	Sigma Aldrich
5-hidroxymethyl furfural	5-(hydroxymethyl)-2-furaldehyde	67-47-0	99	Sigma Aldrich

Appendix 2. Concentrations of key odorants in the aroma recombinants of non-mature spirits

NMSA			NMSB			Non-mature Bourbon			Non-mature Tequila		
Odorant	**FD**	**Conc (mg/L)**	**Odorant**	**FD**	**Conc (mg/L)**	**Odorant**	**FD**	**Conc (mg/L)**	**Odorant**	**FD**	**Conc (mg/L)**
Ethyl hexanoate	≥6561	1.22	Isoamyl alcohol	≥6561	569	Isoamyl alcohol	6561	783	Ethyl hexanoate	≥6561	0.09
n-Hexanol	≥6561	3.39	Ethyl hexanoate	≥6561	1.28	Ethyl hexanoate	6561	2.22	2-Phenylethyl acetate	≥6561	0.36
Ethyl octanoate	≥6561	13.8	3-Ethoxy-1-propanol*	≥6561	0.35	Isobutanol	729	397	β-Damascenone	≥6561	0.33
Furfural	≥6561	9.51	Ethyl octanoate	≥6561	12.1	Vanillin	729	0.02	Phenethyl alcohol	≥6561	1.22
2-Acetylfuran	≥6561	0.20	Furfural	≥6561	19.8	Isoamyl acetate	243	14.3	Octanoic acid	≥6561	0.27
Benzaldehyde	≥6561	0.35	2-Acetylfuran	≥6561	0.58	3-Penten-2-ol*	243	6.94	Ethyl octanoate	≥6561	4.83
Ethyl benzoate	≥6561	1.45	Benzaldehyde	≥6561	0.33	*n*-Hexanol	243	4.29	Isoamyl alcohol	2189	381
Diethyl succinate		0.19	Ethyl benzoate	≥6561	1.18	Ethyl octanoate	243	11.7	2-Acetylfuran	2189	0.25
Methionol	≥6561	0.19	Diethyl succinate		0.19	Furfural	243	0.05	Linalool	2189	0.41
Hexanoic acid	≥6561	3.35	Methionol	≥6561	0.22	Phenylethyl Alcohol	243	15.2	Ethyl benzoate	2189	0.01
Phenethyl alcohol	≥6561	69.4	*n*-Decanol	≥6561	0.35	Ethyl hexadecanoate	243	19.8	Diethyl succinate	2189	0.10
Octanoic acid	≥6561	19.2	Hexanoic acid	≥6561	5.52	*n*-Propanol	81	28.4	Isobutanol	729	120
Decanoic acid	≥6561	18.0	Phenethyl alcohol	≥6561	61.3	Octanoic acid	81	0.63	Isoamyl acetate	729	2.40
Isoamyl alcohol	2187	609	Octanoic acid	≥6561	24.0	Benzaldehyde	27	0.03	Dihydro-2-methyl-3(2H)-furanone	729	0.79
n-Decanol	2187	0.31	Isobutanol	2187	88.0	5MF	27	0.01	Citronellol	729	0.26
n-Hexadecanol	2187	1.26	*n*-Pentanol	2187	0.66	---	---	---	Guaiacol	729	0.02
(9E)-9-Hexadecen-1-ol	2187	2.77	Decanoic acid	2187	22.47	---	---	---	Ethyl hexadecanoate	729	0.03
n-Propanol	729	10.2	*n*-Propanol	729	9.50	---	---	---	Decanoic acid	729	0.70
Isobutanol	729	85.6	*n*-Hexanol	729	7.56	---	---	---	Vanillin	729	<0.01
Isoamyl acetate	729	7.25	Ethyl decanoate	729	44.1	---	---	---	Cis-Linalool oxide	243	0.36
Ethyl hexadecanoate	243	14.7	Isoamyl decanoate	729	0.33	---	---	---	5-Methyl furfural	243	0.40
Dodecanoic acid	243	5.80	*n*-Hexadecanol	729	0.41	---	---	---	α-Terpineol	243	2.44
Vanillin	243	0.04	Ethyl hexadecanoate	729	12.9	---	---	---	*n*-Propanol	81	9.02
Ethyl decanoate	81	69.0	Ethyl 9-hexadecenoate	729	4.29	---	---	---	Furfural	81	0.74
2-phenylethyl acetate	27	6.51	(9E)-9-Hexadecen-1-ol	729	1.23	---	---	---	Ethyl decanoate	81	2.06
Ethyl dodecanoate	27	25.3	Dodecanoic acid	729	3.58	---	---	---	Ethyl 4-ethoxybenzoate*	81	0.66
n-Undecanol	27	0.23	Isoamyl acetate	243	8.03	---	---	---	Diacetyl	27	0.75

Ethyl 9-hexadecenoate	27	8.83	*n*-Butanol	243	1.99	---	---	---	Ethyl dodecanoate	27	0.42
---	---	---	2-phenylethyl acetate	243	8.13	---	---	---	---	---	---
---	---	---	Ethyl dodecanoate	243	11.0	---	---	---	---	---	---
---	---	---	*n*-Undecanol	243	0.15	---	---	---	---	---	---
---	---	---	Vanillin	243	0.03	---	---	---	---	---	---
---	---	---	5-Methyl furfural	81	0.39				---	---	---

Appendix 3. Concentrations of key odorants in the aroma recombinants of mature spirits

MWA			MWB			Mature Bourbon			Mature Tequila		
Odorant	**FD**	**Conc (mg/L)**	**Odorant**	**FD**	**Conc (mg/L)**	**Odorant**	**FD**	**Conc (mg/L)**	**Odorant**	**FD**	**Conc (mg/L)**
Isoamyl alcohol	≥6561	571	Isoamyl alcohol	≥6561	486	Isoamyl alcohol	≥6561	833	Isoamyl alcohol	≥6561	801
Ethyl hexanoate	≥6561	1.15	Ethyl hexanoate	≥6561	1.79	Ethyl octanoate	≥6562	8.57	Ethyl hexanoate	≥6561	0.3
n-Hexanol	≥6561	5.51	Ethyl octanoate	≥6561	18.4	Diethyl succinate	≥6563	0.92	Ethyl octanoate	≥6561	8.3
Ethyl octanoate	≥6561	10.9	Furfural	≥6561	9.55	Ethyl benzoate	≥6564	0.03	2-phenylethyl acetate	≥6561	0.2
Furfural	≥6561	12.5	Benzaldehyde	≥6561	0.17	Guaiacol	≥6565	0.04	β-Damascenone	≥6561	0.0
5-Methyl furfural	≥6561	0.41	Ethyl benzoate	≥6561	1.07	4-Ethylguaiacol	≥6566	0.02	Guaiacol	≥6561	0.0
Ethyl benzoate	≥6561	1.13	Diethyl succinate	≥6561	0.12	γ-nonalactone	≥6567	0.03	Trans-whisky lactone	≥6561	0.3
Diethyl succinate	≥6561	0.30	*n*-Decanol	≥6561	1.27	Decanoic acid	≥6568	6.23	Phenethyl alcohol	≥6561	1.6
2-phenylethyl acetate	≥6561	8.08	Guaiacol	≥6561	0.13	Ethyl hexanoate	2189	2.27	Cis-whisky lactone	≥6561	1.5
Hexanoic acid	≥6561	3.14	Trans-whisky lactone	≥6561	0.25	*n*-Propanol	729	8.95	4-ethyl-guaiacol	≥6561	0.0
Guaiacol	≥6561	0.14	Phenethyl alcohol	≥6561	39.6	Isobutanol	729	374	Octanoic acid	≥6561	1.8
Trans-whisky lactone	≥6561	0.25	Cis-whisky lactone	≥6561	1.60	Isoamyl acetate	729	26.7	Vanillin	≥6561	0.9
Phenethyl alcohol	≥6561	68.1	4-ethyl-guaiacol	≥6561	0.17	Ethyl decanoate	729	13.5	Isobutanol	2189	169
Cis-whisky lactone	≥6561	2.02	Octanoic acid	≥6561	33.6	Trans-whiskey lactone	729	2.96	2-Acetylfuran	2189	0.8
4-ethyl-guaiacol	≥6561	0.19	*n*-Hexadecanol	≥6561	0.61	Phenylethyl Alcohol	729	20.3	Linalool	2189	1.5
Octanoic acid	≥6561	18.1	Eugenol	≥6561	0.23	Cis-Whiskey lactone	729	8.67	Ethyl benzoate	2189	0.0
n-Hexadecanol	≥6561	1.06	(9E)-9-Hexadecen-1-ol	≥6561	0.67	Eugenol	729	0.02	Diethyl succinate	2189	0.4
Eugenol	≥6561	0.26	Dodecanoic acid	≥6561	4.59	Vanillin	729	2.68	Citronellol	2189	0.5
Decanoic acid	≥6561	22.9	Vanillin	≥6561	1.91	Furfural	243	4.73	Isoamyl acetate	729	3.1
(9E)-9-Hexadecen-1-ol	≥6561	1.23	Isobutanol	2187	51.9	Benzaldehyde	243	0.03	α-Terpineol	729	6.3
Vanillin	≥6561	2.09	*n*-Butanol	2187	1.71	Phenethyl acetate	243	0.60	Eugenol	729	0.0
Isobutanol	2187	69.6	*n*-Hexanol	2187	3.57	Octanoic acid	243	2.69	Ethyl hexadecanoate	729	0.1
2-Acetylfuran	2187	0.15	2-Acetylfuran	2187	0.15	5MF	81	0.26	Decanoic acid	729	6.1
Benzaldehyde	2187	0.16	5-Methyl furfural	2187	0.30	Ethyl hexadecanoate	81	6.52	*n*-Propanol	243	17.1
Ethyl decanoate	2187	46.8	Hexanoic acid	2187	7.53	Methyl oleate	81	0.20	Dihydro-2-methyl-3(2H)-furanone	243	1.5
n-Decanol	2187	1.07	Ethyl hexadecanoate	2187	5.23	*n*-Hexanol	27	3.47	Cis-Linalool oxide	243	0.8
Ethyl hexadecanoate	2187	12.1	Decanoic acid	2187	29.6	Ethyl nonanoate	27	0.08	5-Methyl furfural	243	0.6
Ethyl 9-hexadecenoate	2187	3.27	*n*-Propanol	729	4.89	Ethyl dodecanoate	27	3.57	Isovaleraldehyde, diethyl acetal	81	0.1
3-Ethoxy-1-propanol	729	0.28	Isoamyl acetate	729	4.45	----	----	----	β-Ethoxypropionaldehyde diethyl acetal	81	2.4

Dodecanoic acid	729	4.51	Isobutanal diethyl acetal	729	0.52	----	----	----	Ethyl lactate	81	2.0
n-Propanol	243	7.86	3-Ethoxy-1-propanol	729	0.22	----	----	----	Furfural	81	2.3
Isoamyl acetate	243	6.57	Ethyl decanoate	729	59.7	----	----	----	Ethyl decanoate	81	6.3
n-Butanol	243	2.62	Ethyl 9-hexadecenoate	729	3.25	----	----	----	Ethyl dodecanoate	81	0.7
Isobutanal diethyl acetal	243	0.71	β-Ethoxypropionaldehyde diethyl acetal	243	0.29	----	----	----	Ethyl tetradecanoate	81	0.0
Ethyl dodecanoate	243	11.3	2-phenylethyl acetate	243	4.14	----	----	----	*n*-Butanol	27	1.4
Isoamyl decanoate	81	0.26	Ethyl dodecanoate	81	10.4	----	----	----	Isobutanal diethyl acetal	27	2.4
n-Undecanol	81	0.34	*n*-Undecanol	81	0.15	----	----	----	3-Ethyl-4-methyl-1-pentanol	27	2.3
n-Pentanol	27	0.54	Ethyl tetradecanoate	81	0.76	----	----	----	----	----	----
			n-Pentanol	27	0.45	----	----	----	----	----	----
----	----	----	Ethyl lactate	27	4.48	----	----	----	----	----	----

Appendix 4. Individual fraction compositions of the estery and woody components in bourbon, tequila, and malt whiskies (A, B).

		Concentration (mg/L)				
		Estery fractions				
No	**Compound**	**Chemical Group**	**Bourbon**	**Tequila**	**MWA**	**MWB**
1	Ethyl hexanoate	Estery	2.27	0.25	1.14	1.79
2	Ethyl octanoate	Estery	8.57	8.32	10.92	18.38
3	Ethyl benzoate	Estery	0.03	0.01	1.12	1.08
4	Diethyl succinate	Estery	0.92	0.41	0.29	0.12
5	Phenylethyl acetate	Estery	X	0.15	8.08	X
6	Ethyl decanoate	Estery	X	X	46.85	X
7	Ethyl hexadecanoate	Estery	X	X	12.12	5.22
		Woody fractions				
1	γ-nonalactone	Lactone	0.03	X	X	X
2	Cis/trans-whisky lactone	Lactone	11.6	1.81	2.27	1.94
3	Guaiacol	Volatile-phenol	0.04	0.01	0.14	0.14
4	4-ethyl-guaiacol	Volatile-phenol	0.02	0.01	0.19	0.18
5	Eugenol	Volatile-phenol	0.02	0.02	0.26	0.23
6	Vanillin	Volatile-phenol	2.68	0.88	2.09	1.91

X: compounds not detected in the samples during the GC-O analysis, therefore were not added into the fractions. Compounds added into the fractions were the most odour active compounds in each of the spirit samples obtained from the GC-O/AEDA analysis.

Appendix 5. Concentrations of key odorants in five aroma recombinants that recreated the overall aroma of mature bourbon.

Odorant	Simulants composition (concentration mg/L)				
	1 (FD ≥ 27)	**2 (FD ≥ 27 + LBC)**	**3 (FD ≥ 27 + LBC + C16)**	**4 (FD ≥ 27 + LBC + NVC)**	**5 (FD ≥ 27 + LBC + NVC + C16)**
Ethyl octanoate	8.57	8.57	8.57	8.57	8.57
Diethyl succinate	0.92	0.92	0.92	0.92	0.92
Ethyl benzoate	0.03	0.03	0.03	0.03	0.03
Guaiacol	0.04	0.04	0.04	0.04	0.04
4-Ethylguaiacol	0.02	0.02	0.02	0.02	0.02
γ-nonalactone	0.03	0.03	0.03	0.03	0.03
Decanoic acid	6.23	6.23	6.23	6.23	6.23
Ethyl hexanoate	2.27	2.27	2.27	2.27	2.27
Isoamyl acetate	26.7	26.7	26.7	26.7	26.7
Ethyl decanoate	13.5	13.5	13.5	13.5	13.5
Trans-whiskey lactone	2.96	2.96	2.96	2.96	2.96
Phenylethyl Alcohol	20.3	20.3	20.3	20.3	20.3
Cis-Whiskey lactone	8.67	8.67	8.67	8.67	8.67
Eugenol	0.02	0.02	0.02	0.02	0.02
Vanillin	2.68	2.68	2.68	2.68	2.68
Furfural	4.73	4.73	4.73	4.73	4.73
Benzaldehyde	0.03	0.03	0.03	0.03	0.03
Phenethyl acetate	0.6	0.6	0.6	0.6	0.6
Octanoic acid	2.69	2.69	2.69	2.69	2.69
5MF	0.26	0.26	0.26	0.26	0.26
Ethyl hexadecanoate	6.52	6.52	6.52	6.52	6.52
Methyl oleate	0.2	0.2	0.2	0.2	0.2
n-Hexanol	3.47	3.47	3.47	3.47	3.47
Ethyl nonanoate	0.08	0.08	0.08	0.08	0.08
Ethyl dodecanoate	3.57	3.57	3.57	3.57	3.57
Acetaldehyde	---	23.9	23.9	23.9	23.9
Ethyl acetate	---	305	305	305	305
Acetal	---	221	221	221	221
Methanol	---	39.2	39.2	39.2	39.2
n-Propanol	---	148	148	148	148
Isobutanol	---	464	464	464	464
2-methyl-1-butanol	---	708	708	708	708
3-methyl-1-butanol	---	931	931	931	931
Ethyl lactate	---	10.5	10.5	10.5	10.5

5Hidroxy-methyl furfural	---	---	---	2.06	2.06
Gallic acid	---	---	---	28.3	28.3
Vanillic acid	---	---	---	1.74	1.74
Syringic acid	---	---	---	4.1	4.1
Syringaldehyde	---	---	---	10.1	10.1
Coniferaldehyde	---	---	---	2.77	2.77
Sinapaldehyde	---	---	---	4.57	4.57
Ellagic acid	---	---	---	125	125
Ethyl hexadecanoate	---	---	500	---	500

LBC: Low boiling volatile compounds; NVC: non-volatile compounds; C16: ethyl hexadecanoate. ---: compounds not-added into the spirit model system.

Appendix 6. Concentrations of key odorants in five aroma recombinants that recreated the overall aroma of mature tequila

Odorant	Simulant number composition (concentration mg/L)				
	1 (FD ≥ 27)	2 (FD ≥ 27 + LBC)	3 (FD ≥ 27 + LBC + C16)	4 (FD ≥ 27 + LBC + NVC)	5 (FD ≥ 27 + LBC + NVC + C16)
Ethyl hexanoate	0.3	0.3	0.3	0.3	0.3
Ethyl octanoate	8.3	8.3	8.3	8.3	8.3
2-phenylethyl acetate	0.2	0.2	0.2	0.2	0.2
β-Damascenone	0	0	0	0	0
Guaiacol	0	0	0	0	0
Trans-whisky lactone	0.3	0.3	0.3	0.3	0.3
Phenethyl alcohol	1.6	1.6	1.6	1.6	1.6
Cis-whisky lactone	1.5	1.5	1.5	1.5	1.5
4-ethyl-guaiacol	0	0	0	0	0
Octanoic acid	1.8	1.8	1.8	1.8	1.8
Vanillin	0.9	0.9	0.9	0.9	0.9
2-Acetylfuran	0.8	0.8	0.8	0.8	0.8
Linalool	1.5	1.5	1.5	1.5	1.5
Ethyl benzoate	0	0	0	0	0
Diethyl succinate	0.4	0.4	0.4	0.4	0.4
Citronellol	0.5	0.5	0.5	0.5	0.5
Isoamyl acetate	3.1	3.1	3.1	3.1	3.1
a-Terpineol	6.3	6.3	6.3	6.3	6.3
Eugenol	0	0	0	0	0
Ethyl hexadecanoate	0.1	0.1	0.1	0.1	0.1
Decanoic acid	6.1	6.1	6.1	6.1	6.1
Dihydro-2-methyl-3(2H)-furanone	1.5	1.5	1.5	1.5	1.5
Cis-Linalool oxide	0.8	0.8	0.8	0.8	0.8
5-Methyl furfural	0.6	0.6	0.6	0.6	0.6
Isovaleraldehyde, diethyl acetal	0.1	0.1	0.1	0.1	0.1
β-Ethoxypropionaldehyde diethyl acetal	2.4	2.4	2.4	2.4	2.4
Ethyl lactate	2	2	2	2	2
Furfural	2.3	2.3	2.3	2.3	2.3
Ethyl decanoate	6.3	6.3	6.3	6.3	6.3
Ethyl dodecanoate	0.7	0.7	0.7	0.7	0.7

Ethyl tetradecanoate	0	0	0	0	0
Isobutanal diethyl acetal	2.4	2.4	2.4	2.4	2.4
3-Ethyl-4-methyl-1-pentanol	2.3	2.3	2.3	2.3	2.3
Acetaldehyde	---	39.1	39.1	39.1	39.1
Ethyl acetate	---	112	112	112	112
Acetal	---	81.2	81.2	81.2	81.2
Methanol	---	653	653	653	653
n-Propanol	---	275	275	275	275
Isobutanol	---	358	358	358	358
n-Butanol	---	1.41	1.41	1.41	1.41
2-methyl-1-butanol	---	551	551	551	551
3-methyl-1-butanol	---	768	768	768	768
Ethyl lactate	---	16.3	16.3	16.3	16.3
5Hidroxy-methyl furfural	---	---	---	0.05	0.05
Gallic acid	---	---	---	9.95	9.95
Vanillic acid	---	---	---	0.64	0.64
Syringic acid	---	---	---	1.22	1.22
Syringaldehyde	---	---	---	2.52	2.52
Coniferaldehyde	---	---	---	0.33	0.33
Sinapaldehyde	---	---	---	0.37	0.37
Ellagic acid	---	---	---	16	16
Ethyl hexadecanoate	---	---	500	---	500

LBC: Low boiling volatile compounds; NVC: non-volatile compounds; C16: ethyl hexadecanoate. ---: compounds not-added into the spirit model system.

Appendix 7. Concentrations of key odorants in five aroma recombinants that recreated the overall aroma of malt whisky 'A'

Odorant	Simulant number composition (concentration mg/L)				
	1 (FD ≥ 27)	2 (FD ≥ 27 + LBC)	3 (FD ≥ 27 + LBC + C16)	4 (FD ≥ 27 + LBC + NVC)	5 (FD ≥ 27 + LBC + NVC + C16)
Ethyl hexanoate	1.15	1.15	1.15	1.15	1.15
n-Hexanol	5.51	5.51	5.51	5.51	5.51
Ethyl octanoate	10.9	10.9	10.9	10.9	10.9
Furfural	12.5	12.5	12.5	12.5	12.5
5-Methyl furfural	0.41	0.41	0.41	0.41	0.41
Ethyl benzoate	1.13	1.13	1.13	1.13	1.13
Diethyl succinate	0.3	0.3	0.3	0.3	0.3
2-phenylethyl acetate	8.08	8.08	8.08	8.08	8.08
Hexanoic acid	3.14	3.14	3.14	3.14	3.14
Guaiacol	0.14	0.14	0.14	0.14	0.14
Trans-whisky lactone	0.25	0.25	0.25	0.25	0.25
Phenethyl alcohol	68.1	68.1	68.1	68.1	68.1
Cis-whisky lactone	2.02	2.02	2.02	2.02	2.02
4-ethyl-guaiacol	0.19	0.19	0.19	0.19	0.19
Octanoic acid	18.1	18.1	18.1	18.1	18.1
n-Hexadecanol	1.06	1.06	1.06	1.06	1.06
Eugenol	0.26	0.26	0.26	0.26	0.26
Decanoic acid	22.9	22.9	22.9	22.9	22.9
(9E)-9-Hexadecen-1-ol	1.23	1.23	1.23	1.23	1.23
Vanillin	2.09	2.09	2.09	2.09	2.09
2-Acetylfuran	0.15	0.15	0.15	0.15	0.15
Benzaldehyde	0.16	0.16	0.16	0.16	0.16
Ethyl decanoate	46.8	46.8	46.8	46.8	46.8
n-Decanol	1.07	1.07	1.07	1.07	1.07
Ethyl hexadecanoate	12.1	12.1	12.1	12.1	12.1
Ethyl 9-hexadecenoate	3.27	3.27	3.27	3.27	3.27
3-Ethoxy-1-propanol	0.28	0.28	0.28	0.28	0.28
Dodecanoic acid	4.51	4.51	4.51	4.51	4.51
Isoamyl acetate	6.57	6.57	6.57	6.57	6.57
Isobutanal diethyl acetal	0.71	0.71	0.71	0.71	0.71
Ethyl dodecanoate	11.3	11.3	11.3	11.3	11.3

Isoamyl decanoate	0.26	0.26	0.26	0.26	0.26
n-Undecanol	0.34	0.34	0.34	0.34	0.34
n-Pentanol	0.54	0.54	0.54	0.54	0.54
Acetaldehyde	16.2	16.2	16.2	16.2	16.2
Ethyl acetate	250	250	250	250	250
Acetal	183	183	183	183	183
Methanol	25.9	25.9	25.9	25.9	25.9
n-Propanol	280	280	280	280	280
Isobutanol	520	520	520	520	520
n-Butanol	2.62	2.62	2.62	2.62	2.62
2-methyl-1-butanol	702	702	702	702	702
3-methyl-1-butanol	824	824	824	824	824
Ethyl lactate	6.37	6.37	6.37	6.37	6.37
5Hidroxy-methyl furfural	---	---	---	0.74	0.74
Gallic acid	---	---	---	35.5	35.5
Vanillic acid	---	---	---	0.9	0.9
Syringic acid	---	---	---	2.06	2.06
Syringaldehyde	---	---	---	5.62	5.62
Coniferaldehyde	---	---	---	1.05	1.05
Sinapaldehyde	---	---	---	1.82	1.82
Ellagic acid	---	---	---	49.3	49.3
Ethyl hexadecanoate	---	---	500	---	500

LBC: Low boiling volatile compounds; NVC: non-volatile compounds; C16: ethyl hexadecanoate. ---: compounds not-added into the spirit model system.

Appendix 8. Concentrations of key odorants in five aroma recombinants that recreated the overall aroma of malt whisky 'B'

Odorant	Simulant number composition (concentration mg/L)				
	1 (FD ≥ 27)	2 (FD ≥ 27 + LBC)	3 (FD ≥ 27 + LBC + C16)	4 (FD ≥ 27 + LBC + NVC)	5 (FD ≥ 27 + LBC + NVC + C16)
Ethyl hexanoate	1.79	1.79	1.79	1.79	1.79
Ethyl octanoate	18.4	18.4	18.4	18.4	18.4
Furfural	9.55	9.55	9.55	9.55	9.55
Benzaldehyde	0.17	0.17	0.17	0.17	0.17
Ethyl benzoate	1.07	1.07	1.07	1.07	1.07
Diethyl succinate	0.12	0.12	0.12	0.12	0.12
n-Decanol	1.27	1.27	1.27	1.27	1.27
Guaiacol	0.13	0.13	0.13	0.13	0.13
Trans-whisky lactone	0.25	0.25	0.25	0.25	0.25
Phenethyl alcohol	39.6	39.6	39.6	39.6	39.6
Cis-whisky lactone	1.6	1.6	1.6	1.6	1.6
4-ethyl-guaiacol	0.17	0.17	0.17	0.17	0.17
Octanoic acid	33.6	33.6	33.6	33.6	33.6
n-Hexadecanol	0.61	0.61	0.61	0.61	0.61
Eugenol	0.23	0.23	0.23	0.23	0.23
(9E)-9-Hexadecen-1-ol	0.67	0.67	0.67	0.67	0.67
Dodecanoic acid	4.59	4.59	4.59	4.59	4.59
Vanillin	1.91	1.91	1.91	1.91	1.91
n-Hexanol	3.57	3.57	3.57	3.57	3.57
2-Acetylfuran	0.15	0.15	0.15	0.15	0.15
5-Methyl furfural	0.3	0.3	0.3	0.3	0.3
Hexanoic acid	7.53	7.53	7.53	7.53	7.53
Ethyl hexadecanoate	5.23	5.23	5.23	5.23	5.23
Decanoic acid	29.6	29.6	29.6	29.6	29.6
Isoamyl acetate	4.45	4.45	4.45	4.45	4.45
Isobutanal diethyl acetal	0.52	0.52	0.52	0.52	0.52
3-Ethoxy-1-propanol	0.22	0.22	0.22	0.22	0.22
Ethyl decanoate	59.7	59.7	59.7	59.7	59.7
Ethyl 9-hexadecenoate	3.25	3.25	3.25	3.25	3.25
β-Ethoxypropionaldehyde diethyl acetal	0.29	0.29	0.29	0.29	0.29
2-phenylethyl acetate	4.14	4.14	4.14	4.14	4.14

Ethyl dodecanoate	10.4	10.4	10.4	10.4	10.4
n-Undecanol	0.15	0.15	0.15	0.15	0.15
Ethyl tetradecanoate	0.76	0.76	0.76	0.76	0.76
n-Pentanol	0.45	0.45	0.45	0.45	0.45
Ethyl lactate	4.48	4.48	4.48	4.48	4.48
Acetaldehyde	15.3	15.3	15.3	15.3	15.3
Ethyl acetate	232	232	232	232	232
Acetal	167	167	167	167	167
Methanol	15.6	15.6	15.6	15.6	15.6
n-Propanol	204	204	204	204	204
Isobutanol	416	416	416	416	416
n-Butanol	1.71	1.71	1.71	1.71	1.71
2-methyl-1-butanol	590	590	590	590	590
3-methyl-1-butanol	758	758	758	758	758
Ethyl lactate	4.36	4.36	4.36	4.36	4.36
5Hidroxy-methyl furfural	---	---	---	0.37	0.37
Gallic acid	---	---	---	23.7	23.7
Vanillic acid	---	---	---	1.17	1.17
Syringic acid	---	---	---	1.53	1.53
Syringaldehyde	---	---	---	5.53	5.53
Coniferaldehyde	---	---	---	0.77	0.77
Sinapaldehyde	---	---	---	1.29	1.29
Ellagic acid	---	---	---	42.9	42.9
Ethyl hexadecanoate	---	---	500	---	500

LBC: Low boiling volatile compounds; NVC: non-volatile compounds; C16: ethyl hexadecanoate. ---: compounds not-added into the spirit model system.

Appendix 9. Improved estery fractions, as consequence of the incorporation of estery congeners with FD ≥ 27, low boiling volatile compounds (LBC), and ethyl hexadecanoate (C16).

1. Estery mix fraction with compounds with FD ≥≥ 6561						
			Concentration (mg/L)			
No	**Compound**	**Chemical Group**	**Bourbon**	**Tequila**	**MWA**	**MWB**
1	Ethyl hexanoate	Ester	2.27	0.25	1.14	1.79
2	Ethyl octanoate	Ester	8.57	8.32	10.92	18.38
3	Ethyl benzoate	Ester	0.03	0.01	1.12	1.08
4	Diethyl succinate	Ester	0.92	0.41	0.29	0.12
5	Phenylethyl acetate	Ester	X	0.15	8.08	X
6	Ethyl decanoate	Ester	X	X	46.85	X
7	Ethyl hexadecanoate	Ester	X	X	12.12	5.22
2. Estery mix fraction containing compounds with FD ≥ 27						
1	Isoamyl acetate	Ester	26.67	3.13	25.9	16.61
2	Ethyl hexanoate	Ester	2.27	0.25	1.14	1.79
3	Ethyl octanoate	Ester	8.57	8.32	10.92	18.38
4	Ethyl nonanoate	Ester	0.08	X	X	X
5	Ethyl decanoate	Ester	13.49	6.32	46.85	59.66
7	Ethyl benzoate	Ester	0.03	0.01	1.12	1.08
6	Diethyl succinate	Ester	0.92	0.41	0.29	0.12
8	Phenethyl acetate	Ester	0.6	0.15	8.08	4.14
9	Ethyl dodecanoate	Ester	3.57	0.68	11.26	10.36
9	Ethyl tetradecanoate	Ester	X	0.02	X	0.76
10	Ethyl hexadecanoate	Ester	6.52	0.04	12.12	5.22
3. Estery mix fraction containing LBC and compounds with FD ≥ 27						
1	*Ethyl acetate*	*Ester*	*305.35*	*111.7*	*250.4*	*232*
2	Isoamyl acetate	Ester	26.67	3.13	25.9	17
3	Ethyl hexanoate	Ester	2.27	0.25	1.14	2
4	*Ethyl lactate*	*Ester*	*10.45*	*16.3*	*3.3*	*4*
5	Ethyl octanoate	Ester	8.57	8.32	10.92	18
6	Ethyl nonanoate	Ester	0.08	X	X	X

7	Ethyl decanoate	Ester	13.49	6.32	46.85	60
8	Ethyl benzoate	Ester	0.03	0.01	1.12	1
9	Diethyl succinate	Ester	0.92	0.41	0.29	0.12
10	Phenethyl acetate	Ester	0.6	0.15	8.08	4
11	Ethyl dodecanoate	Ester	3.57	0.68	11.26	10
12	Ethyl tetradecanoate	Ester	X	0.02	X	0.76
13	Ethyl hexadecanoate	Ester	6.52	0.04	12.12	5
	4. Estery mix fraction containing LBC, compounds with FD ≥ 27 and C16					
1	*Ethyl acetate*	*Ester*	*305.35*	*111.7*	*250.4*	*232.21*
2	Isoamyl acetate	Ester	26.67	3.13	25.9	16.61
3	Ethyl hexanoate	Ester	2.27	0.25	1.14	1.79
4	*Ethyl lactate*	*Ester*	*10.45*	*16.3*	*3.3*	*4.4*
5	Ethyl octanoate	Ester	8.57	8.32	10.92	18.38
6	Ethyl nonanoate	Ester	0.08	X	X	X
7	Ethyl decanoate	Ester	13.49	6.32	46.85	59.66
8	Ethyl benzoate	Ester	0.03	0.01	1.12	1.08
9	Diethyl succinate	Ester	0.92	0.41	0.29	0.12
10	Phenethyl acetate	Ester	0.6	0.15	8.08	4.14
11	Ethyl dodecanoate	Ester	3.57	0.68	11.26	10.36
12	Ethyl tetradecanoate	Ester	X	0.02	X	0.76
13	Ethyl hexadecanoate	Ester	500	500	500	500

Compounds in italics define the congeners that belong to the low boiling volatile compounds group (LBC). X: not added into the fractions.

Appendix 10. Improved woody fractions, as consequence of the incorporation of woody congeners with FD ≥ 27, low boiling volatile compounds (LBC), non-volatile compounds and ethyl hexadecanoate (C16).

			Concentration (mg/L)			
No	**Compound**	**Chemical Group**	**Bourbon**	**Tequila**	**MWA**	**MWB**
		1. Woody mix fraction with compounds FD ≥ 6561				
1	γ-nonalactone	Lactone	0.03	X	X	X
2	Cis/trans-whisky lactone	Lactone	11.6	1.81	2.27	1.94
3	Guaiacol	Volatile-phenol	0.04	0.01	0.14	0.14
4	4-ethyl-guaiacol	Volatile-phenol	0.02	0.01	0.19	0.18
5	Eugenol	Volatile-phenol	0.02	0.02	0.26	0.23
6	Vanillin	Volatile-phenol	2.68	0.88	2.09	1.91
		2. Woody mix fraction with compounds FD ≥ 27 and LBC				
1	γ-nonalactone	Lactone	0.03	X	X	X
2	Cis/trans-whisky lactone	Lactone	11.6	1.81	2.27	1.94
3	Guaiacol	Volatile-phenol	0.04	0.01	0.14	0.14
4	4-ethyl-guaiacol	Volatile-phenol	0.02	0.01	0.19	0.18
5	Eugenol	Volatile-phenol	0.02	0.02	0.26	0.23
6	Vanillin	Volatile-phenol	2.68	0.88	2.09	1.91
7	*Acetaldehyde*	*Aldehyde*	*24*	*39*	*16*	*15*
8	*Acetal*	*Acetal*	*221*	*81*	*184*	*167*
9	*Acetic acid*	*Acid*	*435*	*282*	*231*	*333*
10	*Furfural*	*Furan*	*7*	*2*	*12*	*8*
12	*Methanol*	*Alcohol*	*39.2*	*653*	*25.9*	*15.6*
13	*n-Propanol*	*Alcohol*	*148*	*275*	*280*	*204*
14	*Isobutanol*	*Alcohol*	*464*	*358*	*520*	*416*
15	*2-methyl-1-butanol*	*Alcohol*	*708*	*551*	*702*	*590*
16	*3-methyl-1-butanol*	*Alcohol*	*931*	*768*	*824*	*758*
17	*n-Butanol*	*Alcohol*	*X*	*1.41*	*X*	*7.00*
		3. Woody mix fraction with compounds FD ≥ 27, LBC and C16				
1	γ-nonalactone	Lactone	0.03	X	X	X

2	Cis/trans-whisky lactone	Lactone	11.6	1.81	2.27	1.94
3	Guaiacol	Volatile-phenol	0.04	0.01	0.14	0.14
4	4-ethyl-guaiacol	Volatile-phenol	0.02	0.01	0.19	0.18
5	Eugenol	Volatile-phenol	0.02	0.02	0.26	0.23
6	Vanillin	Volatile-phenol	2.68	0.88	2.09	1.91
7	*Acetaldehyde*	*Aldehyde*	*24*	*39*	*16*	*15*
8	*Acetal*	*Acetal*	*221*	*81*	*184*	*167*
9	*Acetic acid*	*Acid*	*435*	*282*	*231*	*333*
10	*Furfural*	*Furan*	*7*	*2*	*12*	*8*
11	*Methanol*	*Alcohol*	*39.2*	*653*	*25.9*	*15.6*
12	*n-Propanol*	*Alcohol*	*148*	*275*	*280*	*204*
13	*Isobutanol*	*Alcohol*	*464*	*358*	*520*	*416*
14	*2-methyl-1-butanol*	*Alcohol*	*708*	*551*	*702*	*590*
15	*3-methyl-1-butanol*	*Alcohol*	*931*	*768*	*824*	*758*
16	*n-Butanol*	*Alcohol*	*X*	*1.41*	*X*	*7*
16	Ethyl hexadecanoate	Ester	500	500	500	500
17	**4. Woody mix fraction with compounds FD ≥ 27, LBC and non-volatile compounds**					
	γ-nonalactone	Lactone	0.03	X	X	X
1	Cis/trans-whisky lactone	Lactone	11.6	1.81	2.27	1.94
2	Guaiacol	Volatile-phenol	0.04	0.01	0.14	0.14
3	4-ethyl-guaiacol	Volatile-phenol	0.02	0.01	0.19	0.18
4	Eugenol	Volatile-phenol	0.02	0.02	0.26	0.23
5	Vanillin	Volatile-phenol	2.68	0.88	2.09	1.91
6	*Acetaldehyde*	*Aldehyde*	*24*	*39*	*16*	*15*
7	*Acetal*	*Acetal*	*221*	*81*	*184*	*167*
8	*Acetic acid*	*Acid*	*435*	*282*	*231*	*333*
9	*Furfural*	*Furan*	*7.01*	*2.29*	*11.80*	*8.42*
11	*Methanol*	*Alcohol*	*39.2*	*653*	*25.9*	*15.6*
12	*n-Propanol*	*Alcohol*	*148*	*275*	*280*	*204*
13	*Isobutanol*	*Alcohol*	*464*	*358*	*520*	*416*
14	*2-methyl-1-butanol*	*Alcohol*	*708*	*551*	*702*	*590*
15	*3-methyl-1-butanol*	*Alcohol*	*931*	*768*	*824*	*758*
16	*n-Butanol*	*Alcohol*	*X*	*1.41*	*X*	*7*

16	5HMF	Non-volatile compound	2.06	0.05	0.7	0.4
17	Gallic acid	Non-volatile compound	28.3	9.95	35.5	23.7
18	Vanillic acid	Non-volatile compound	1.74	0.64	0.90	1.17
19	Syringic acid	Non-volatile compound	4.10	1.22	2.06	1.53
20	Syringaldehyde	Non-volatile compound	10.1	2.52	5.62	5.53
21	Coniferaldehyde	Non-volatile compound	2.77	0.33	1.05	0.77
22	Sinapaldehyde	Non-volatile compound	4.57	0.37	1.82	1.29
23	Ellagic acid	Non-volatile compound	125	16	49	43
	5. Woody mix fraction with compounds FD ≥ 27, LBC, non-volatile compounds and C16					
	γ-nonalactone	Lactone	0.03	X	X	X
1	Cis/trans-whisky lactone	Lactone	11.6	1.81	2.27	1.94
2	Guaiacol	Volatile-phenol	0.04	0.01	0.14	0.14
3	4-ethyl-guaiacol	Volatile-phenol	0.02	0.01	0.19	0.18
4	Eugenol	Volatile-phenol	0.02	0.02	0.26	0.23
5	Vanillin	Volatile-phenol	2.68	0.88	2.09	1.91
6	*Acetaldehyde*	*Aldehyde*	*24*	*39*	*16*	*15*
7	*Acetal*	*Acetal*	*221*	*81*	*184*	*167*
8	*Acetic acid*	*Acid*	*435*	*282*	*231*	*333*
9	*Furfural*	*Furan*	*7.01*	*2*	*12*	*8*
11	*Methanol*	*Alcohol*	*39.2*	*653*	*25.9*	*15.6*
12	*n-Propanol*	*Alcohol*	*148*	*275*	*280*	*204*
13	*Isobutanol*	*Alcohol*	*464*	*358*	*520*	*416*
14	*2-methyl-1-butanol*	*Alcohol*	*708*	*551*	*702*	*590*
15	*3-methyl-1-butanol*	*Alcohol*	*931*	*768*	*824*	*758*
16	*n-Butanol*	*Alcohol*	*X*	*1.41*	*X*	*7*
16	5HMF	Non-volatile compound	2.1	0.05	0.74	0.37
17	Gallic acid	Non-volatile compound	28.3	9.95	35.5	23.7
18	Vanillic acid	Non-volatile compound	1.7	0.64	0.90	1.17
19	Syringic acid	Non-volatile compound	4.1	1.22	2.06	1.53
20	Syringaldehyde	Non-volatile compound	10.1	2.52	5.62	5.53
21	Coniferaldehyde	Non-volatile compound	2.8	0.33	1.05	0.77
22	Sinapaldehyde	Non-volatile compound	4.6	0.37	1.82	1.29
23	Ellagic acid	Non-volatile compound	125	16.0	49.3	42.9

24	Ethyl hexadecanoate	Ester	500	500	500	500

Compounds italics define the congeners that belong to the low boiling compounds group (LBC). X: not added into the fractions.

Appendix 11. Critical Number of Correct Responses in a Triangle Test

(Meilgaard, 2006)

TABLE T8
Critical Number of Correct Responses in a Triangle Test
(Entries are $x_{\alpha,n}$)

Entries are the minimum number of correct responses required for significance at the stated α-level (i.e., column) for the corresponding number of respondents, n (i.e., row). Reject the assumption of "no difference" if the number of correct responses is greater than or equal to the tabled value.

	α						
n	0.40	0.30	0.20	0.10	0.05	0.01	0.001
3	2	2	3	3	3	—	—
4	3	3	3	4	4	—	—
5	3	3	4	4	4	5	—
6	3	4	4	5	5	6	—
7	4	4	4	5	5	6	7
8	4	4	5	5	6	7	8
9	4	5	5	6	6	7	8
10	5	5	6	6	7	8	9
11	5	5	6	7	7	8	10
12	5	6	6	7	8	9	10
13	6	6	7	8	8	9	11
14	6	7	7	8	9	10	11
15	6	7	8	8	9	10	12
16	7	7	8	9	9	11	12
17	7	8	8	9	10	11	13
18	7	8	9	10	10	12	13
19	8	8	9	10	11	12	14
20	8	9	9	10	11	13	14
21	8	9	10	11	12	13	15
22	9	9	10	11	12	14	15
23	9	10	11	12	12	14	16
24	10	10	11	12	13	15	16
25	10	11	11	12	13	15	17
26	10	11	12	13	14	15	17
27	11	11	12	13	14	16	18
28	11	12	12	14	15	16	18
29	11	12	13	14	15	17	19
30	12	12	13	14	15	17	19
31	12	13	14	15	16	18	20
32	12	13	14	15	16	18	20
33	13	13	14	15	17	18	21
34	13	14	15	16	17	19	21
35	13	14	15	16	17	19	22
36	14	14	15	17	18	20	22
42	16	17	18	19	20	22	25
48	18	19	20	21	22	25	27
54	20	21	22	23	25	27	30
60	22	23	24	26	27	30	33
66	24	25	26	28	29	32	35
72	26	27	28	30	32	34	38
78	28	29	30	32	34	37	40
84	30	31	33	35	36	39	43
90	32	33	35	37	38	42	45
96	34	35	37	39	41	44	48
102	36	37	39	41	43	46	50
108	38	40	41	43	45	49	53
114	40	42	43	45	47	51	55
120	42	44	45	48	50	53	57
126	44	46	47	50	52	56	60
132	46	48	50	52	54	58	62
138	48	50	52	54	56	60	64
144	50	52	54	56	58	62	67
150	52	54	56	58	61	65	69
156	54	56	58	61	63	67	72
162	56	58	60	63	65	69	74
168	58	60	62	65	67	71	76
174	61	62	64	67	69	74	79
180	63	64	66	69	71	76	81

Note: For values of n not in the table, compute $z = (k - 1(1/3)n)/\sqrt{(2/9)n}$, where k is the number of correct responses. Compare the value of z to the α-critical value of a standard normal variable, i.e., the values in the last row of Table T3 ($z_\alpha = t_{\alpha,\infty}$).

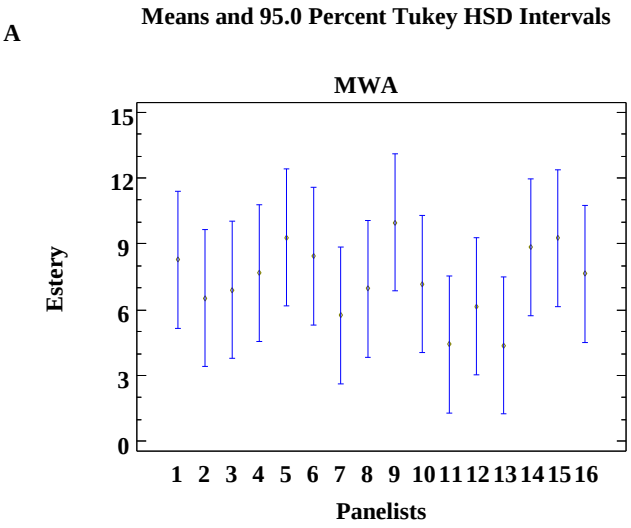
Appendix 12. Sensory comments regarding the contribution of each of the new improved estery and woody fractions to mature spirits

		Sensory comments*		
Estery fraction blend	**Bourbon**	**Tequila**	**MWA**	**MWB**
1. FD ≥ 6561	Apple/fruity	Have a pronounced green apple/fruity character which is missing in the actual spirit.	Not even close to the authentic spirit	Artificial fruity flavoured. Too light and fruity, not heavy enough somehow
2. FD ≥ 27	More isoamyl acetate character (banana/pear)	Better (more complex) but still fruitier than the actual spirit.	Closer although there is wrong ester emphasis, perhaps to pear-drops	Feels to simplistic, not sophisticated enough (missing some notes)
3. FD ≥ 27 + LBC	More solventy character	Similar comments as with blend 2, however this one is more 'solventy'.	Slightly less balance than blend 4 also has a more dominated estery character.	Nail polish, solventy notes, dominated too much, however it was fruity enough.
4. FD ≥ 27 + LBC + C16	Interesting, the C16 round this off a bit so it feels more convincing or less dominated by other characters. **More authentic balance**	**Best blend** among them, however rounds off the ester character somewhat so that ethyl acetate (or solventy notes) is less pronounced relative to blend 3.	**Best blend** and also the closer approach. Remain the main estery character of the original spirit.	Slightly better than blend 3, seems to **have the right balance** and character, almost as the authentic spirit.
Woody fraction	**Bourbon**	**Tequila**	**MWA**	**MWB**
1. FD ≥ 6561	A little 'marzipan' dominated aroma like benzaldehyde. Not quite correct character of bourbon	Has a 'green' character which is not very close to the woody character of the original spirit.	Somewhat sweeter than the original spirit.	Seems nothing like the woody character of the MWB, has a slightly musty note, not particularly fragrant
2. FD ≥ 27 + LBC	Interestingly more authentic, provided the mature character of bourbon	Much better approximation than blend 1, suddenly has a mature type of character, however is still very low in intensity.	Contain some nutty notes, it doesn't contain the mature character of the original spirit	Much better than blend 1 but still a considerable different in character. Too much almond/benzaldehyde.
3. FD ≥ 27 + LBC + C16	C16 provided an overdose sweet character to the spirit blend emphasizing the mature character of the original bourbon. **In general the best approach.**	Very similar to blend 2. However has some fatty aroma. So far, **the best approach.**	**Very similar to the original spirit**, it has a more authentic character.	**Very close to the original spirit**. A remarkable imitation of the woody character of the authentic spirit.
4. FD ≥ 27 + LBC + Non-Vol-Comp's	There is a slightly 'off'/acrid character here. Otherwise the mature character hasn't changed much relative to mature blends 2 and 3.	The presence of non-volatile compounds doesn't make too much impact in the aroma.	Very similar to blend 3, perhaps is a little bit more authentic.	The non-volatile compounds don't make a hush impact in aroma perception. The aroma is similar to blend 3.

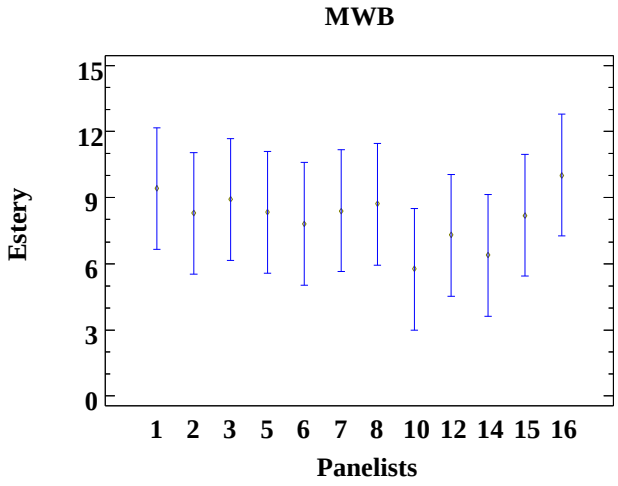
5. FD ≥ 27 + LBC + Non-Vol-Comp's+C16	No too much difference from blends 2 and 3. It seems that the non-volatile make an insignificant impact in the aroma.	Similar to blend 4.	Somewhat emphasised marzipan/almond notes. Seems to keep the essential mature character of the original spirit; however blends 3 and 4 are better.	The same as with blend 4, non-volatile compounds plus C16 do not create too much difference as regard blend 3

*Sensory comments were obtained by individual consensus of David Cook (PhD supervisor). In all cases the comparison in each of the fraction was performed against their original spirit sample. LBC: low boiling volatile compounds; C16: ethyl hexadecanoate.

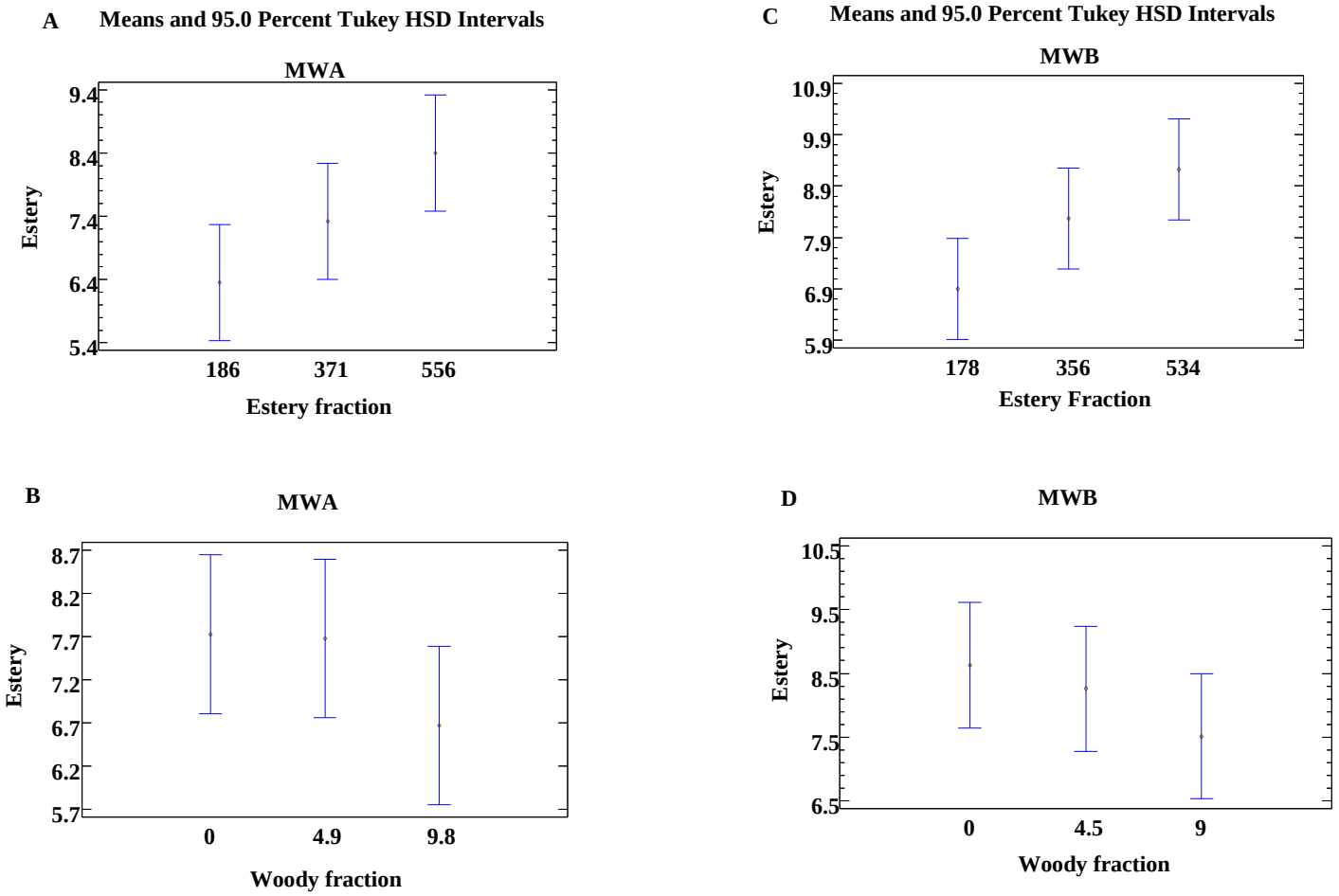
A



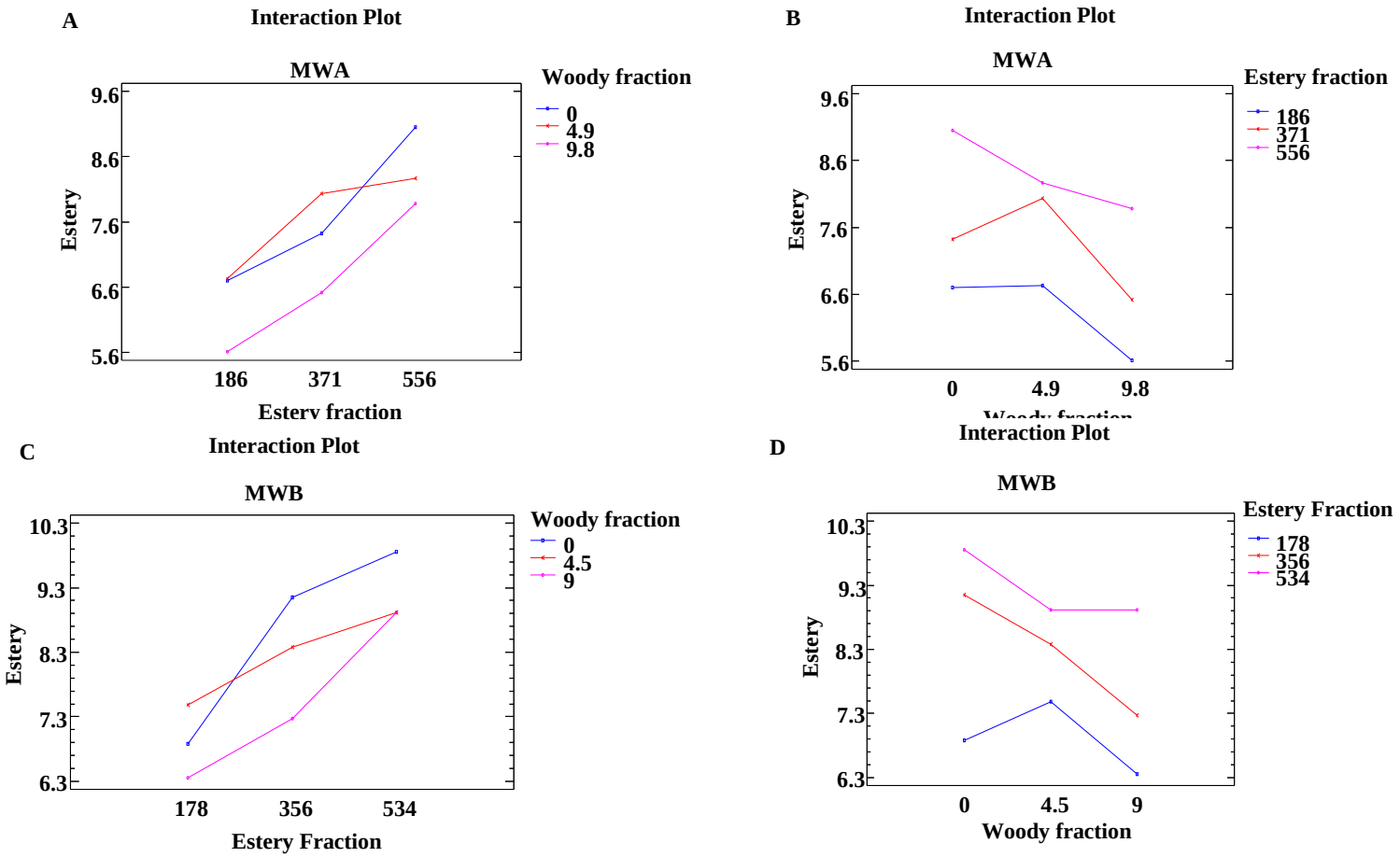
B



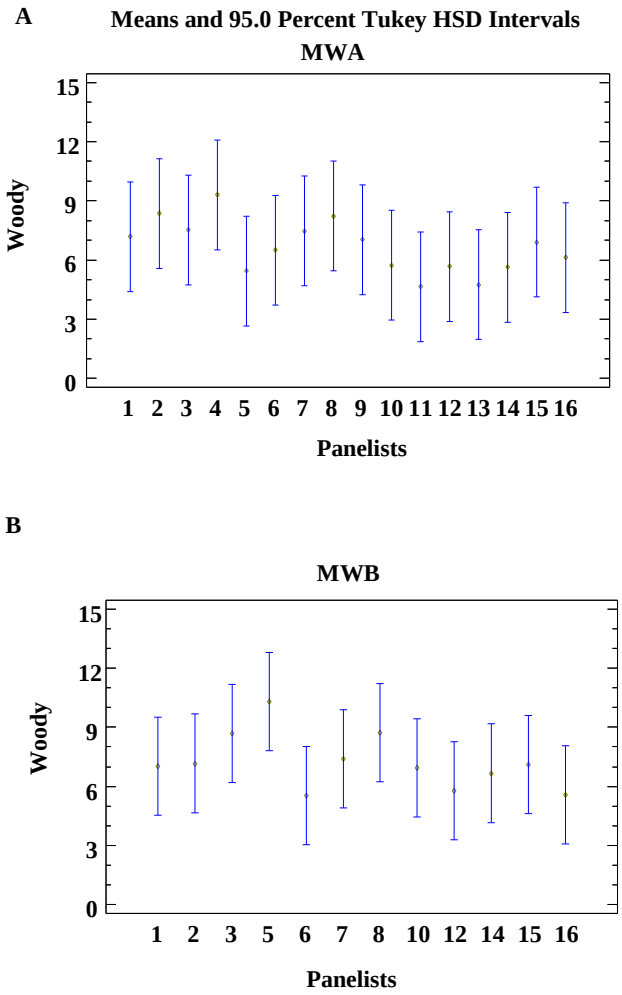
Appendix 13. Means and 95% Percent Tukey HSD Intervals among panellists in the perception of estery character in the spirit models of MWA (A) and MWB (B).



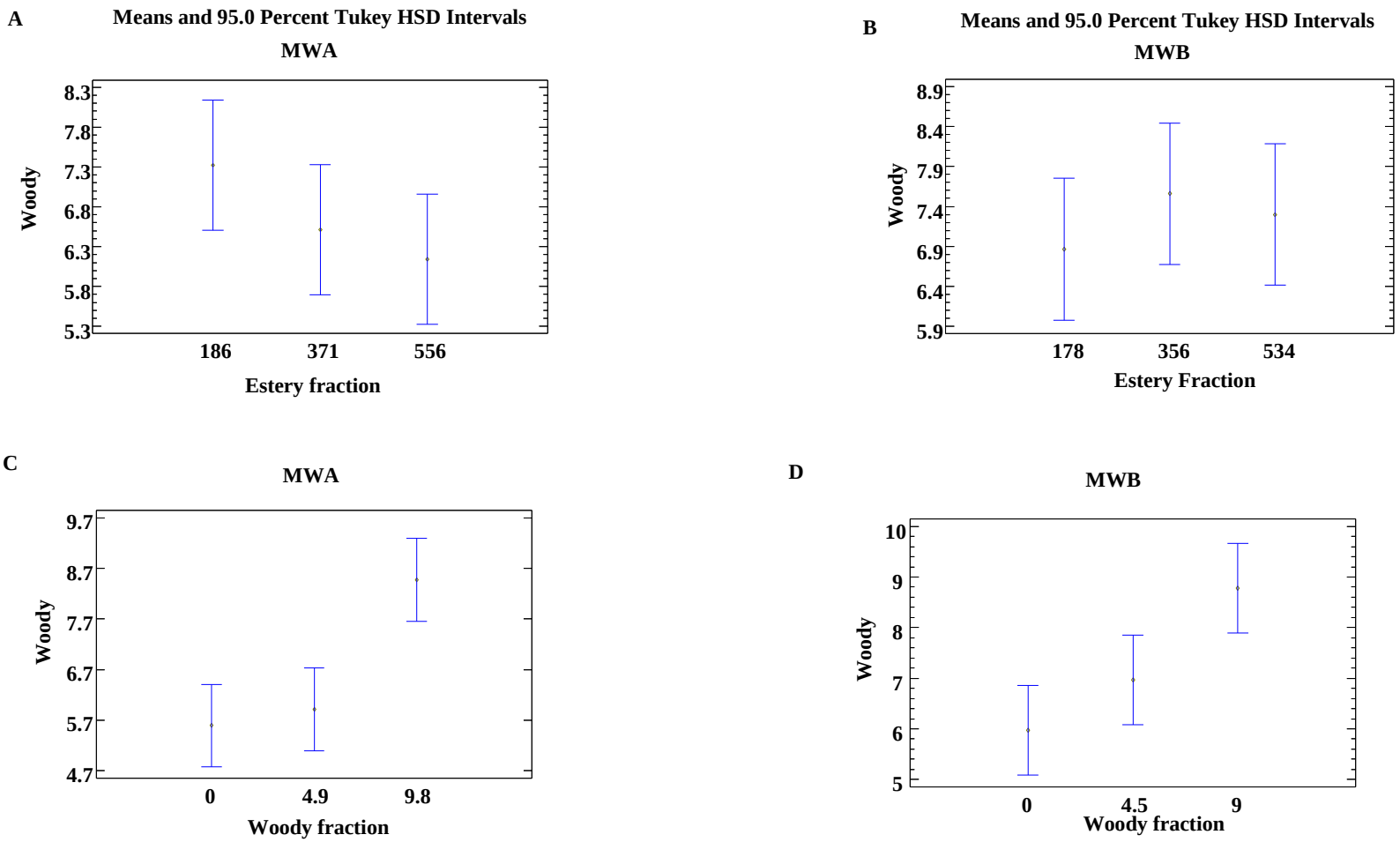
Appendix 14. Means and 95% Percent Tukey HSD Intervals showing the impact of the estery (A and C) and woody (B and D) fractions in the perception of estery character in model spirits of MWA and MWB.



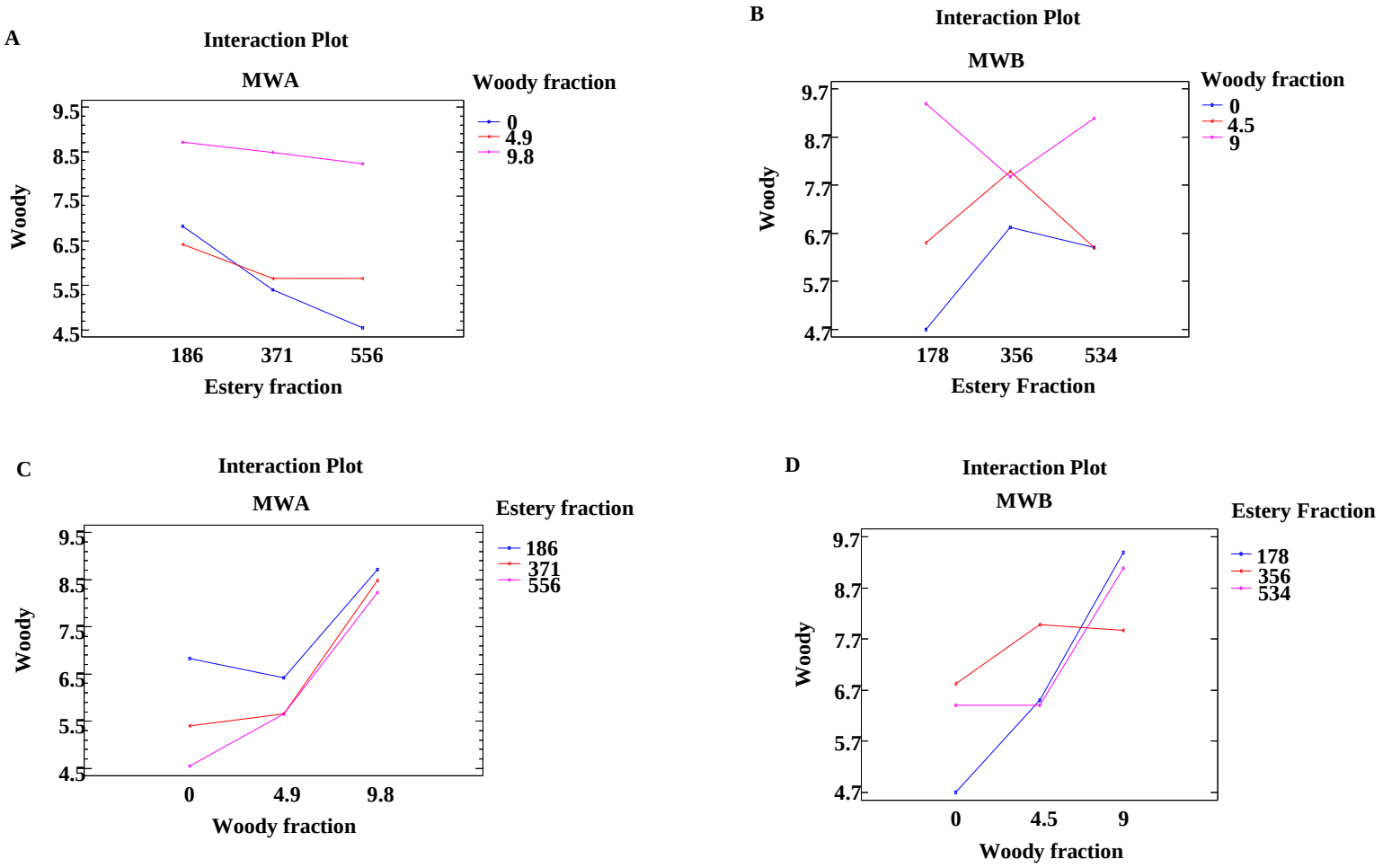
Appendix 15. Interaction plots for the perception of estery character as influenced by concentration of the estery (A and C) and woody (B and D) fractions in models spirits of MWA and MWB.



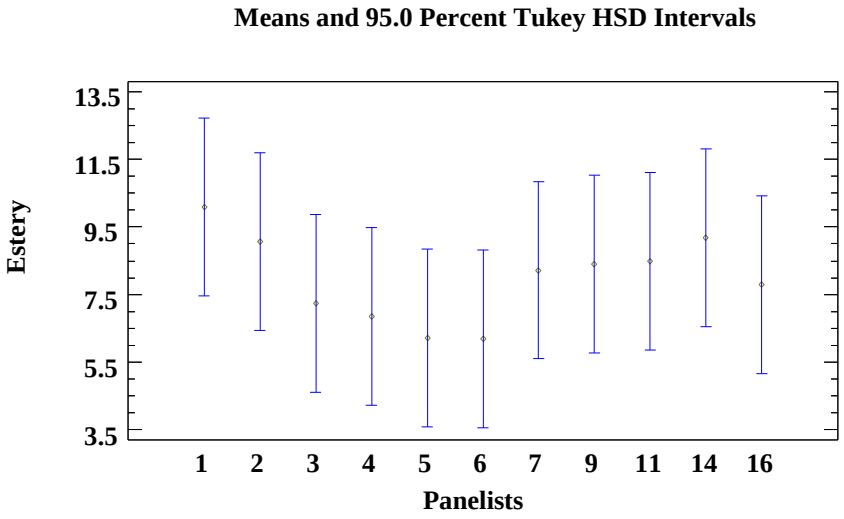
Appendix 16. Means and 95% Percent Tukey HSD Intervals among panellists in the perception of woody character in model spirits of MWA (A) and MWB (B).



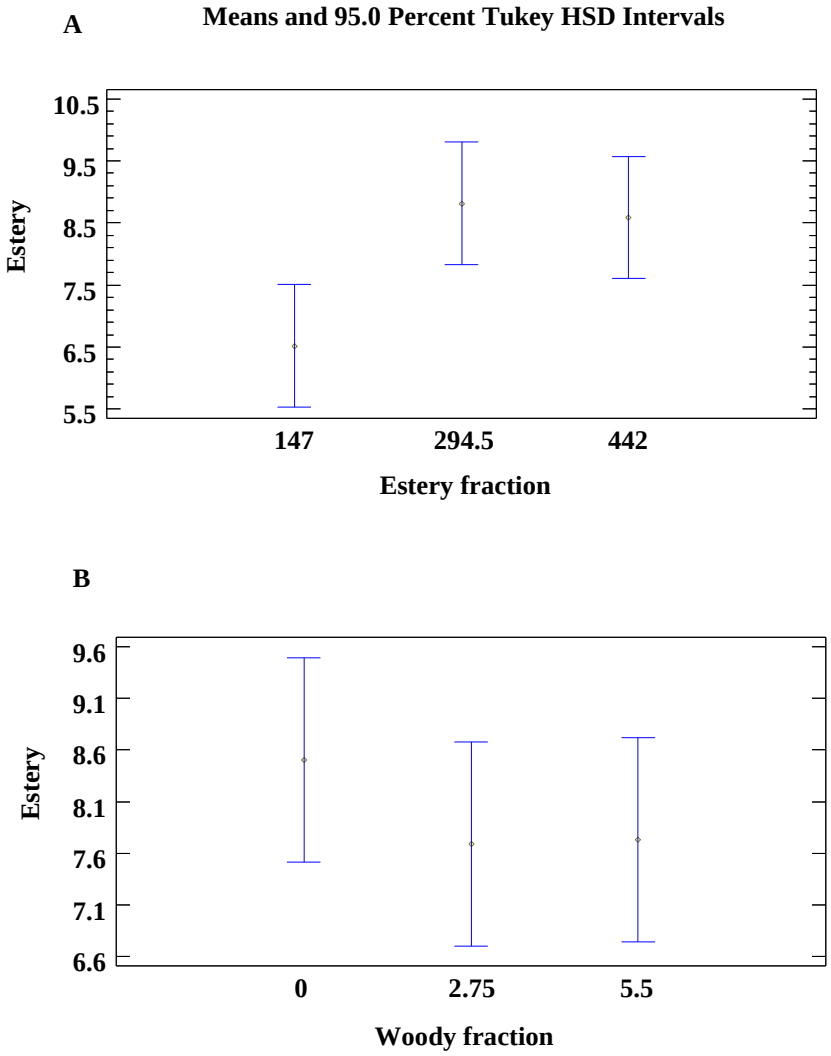
Appendix 17. Means and 95% Percent Tukey HSD Intervals showing the impact of the estery (A and B) and woody (C and D) fractions in the perception of woody character in model spirits of MWA and MWB.



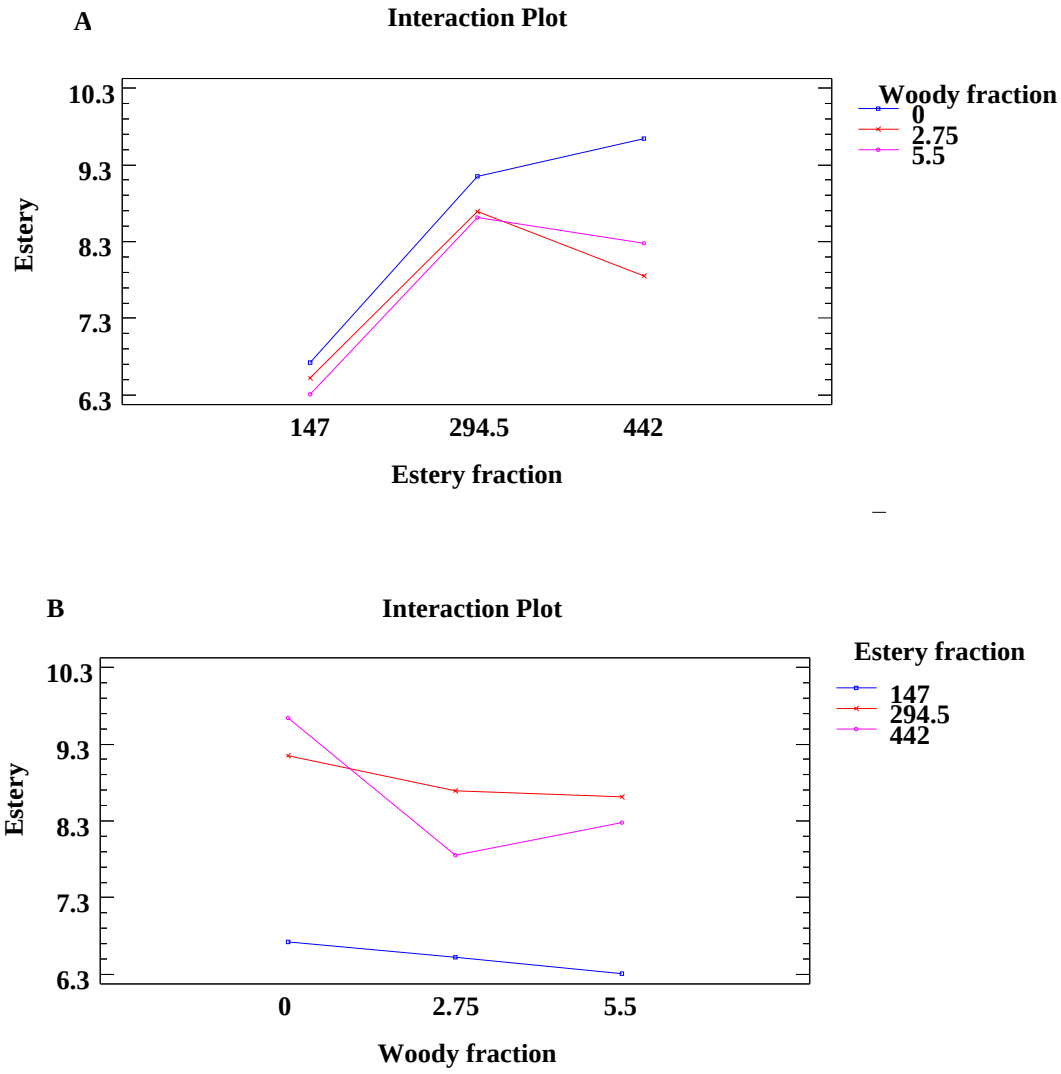
Appendix 18. Interaction plots for the perception of woody character as influenced by concentration of the estery (A and C) and woody (B and D) fractions in models spirits of MWA and MWB.



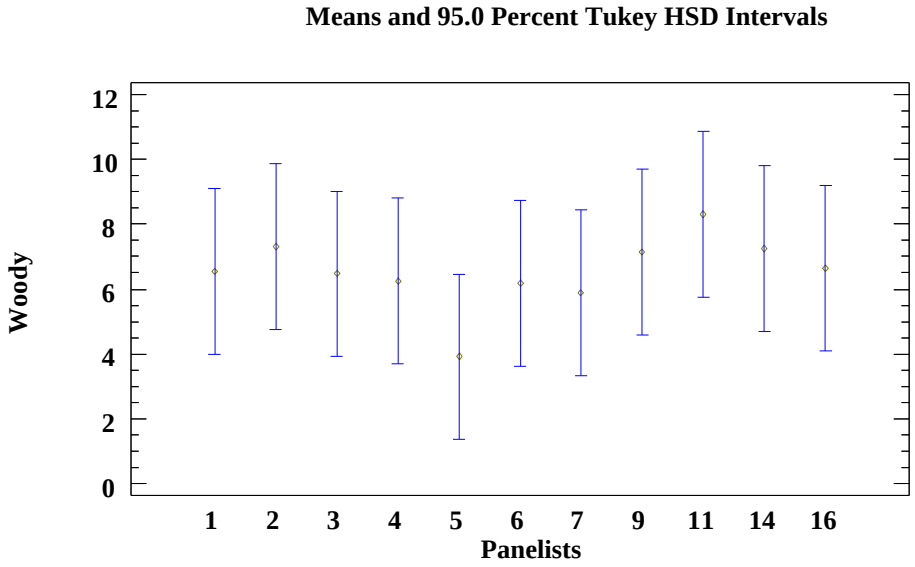
Appendix 19. Means and 95% Percent Tukey HSD Intervals among panellists in the perception of estery character in the model spirit of Tequila.



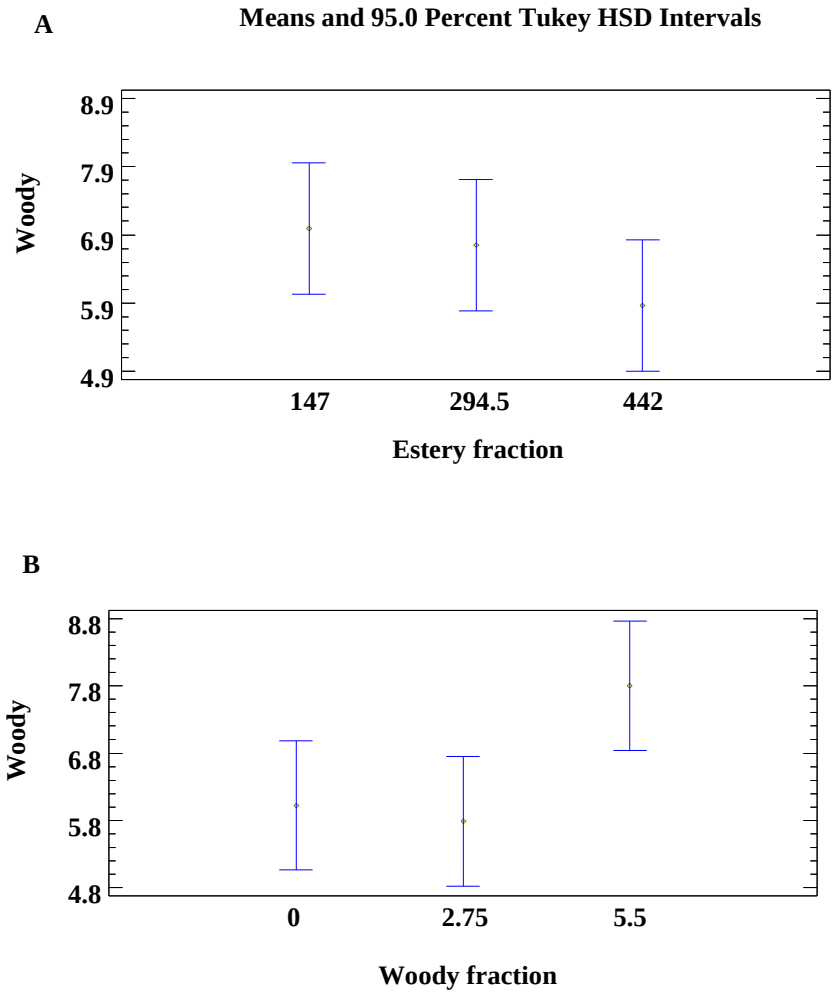
Appendix 20. Means and 95% Percent Tukey HSD Intervals showing the impact of the estery (A) and woody (B) fractions in the perception of estery character in model spirits of Tequila.



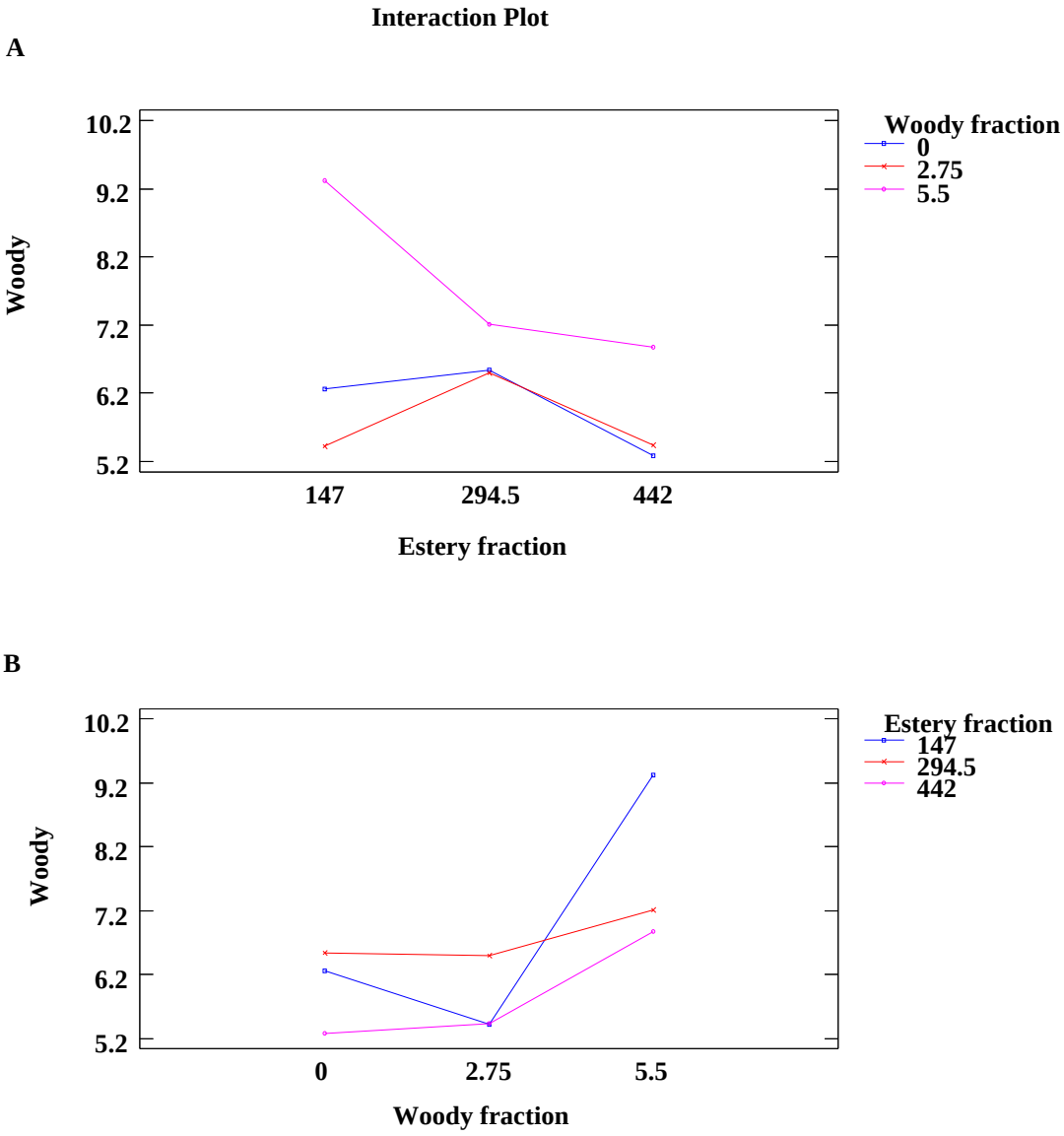
Appendix 21. Interaction plots for the perception of the estery character by concentration of the estery (A) and woody (B) fractions in models spirits of Tequila.



Appendix 22. Means and 95% Percent Tukey HSD Intervals among panellists in the perception of woody character in model spirits of Tequila.



Appendix 23. Means and 95% Percent Tukey HSD Intervals showing the impact of the estery (A) and woody (B) fractions in the perception of woody character in model spirits of Tequila.



Appendix 24. Interaction plots for the perception of the woody character by concentration of the estery (A) and woody (B) fractions in models spirits of Tequila.

Appendix 25. Parameters of second-order kinetics, determined for the extraction of wood-derived congeners into spirit models as a function of ageing time, temperature, and alcohol content

	FURFURAL							2-ACETYL FURAN						
Spirit	**% ABV**	**Temp °C**	**R2**	***p***	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**	**% ABV**	**Temp °C**	**R2**	***p***	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**
Spirit model	40.00	10°C	0.77	<0.05	0.08	15.03	13.28	40.00	10°C	ND	ND	ND	ND	ND
	40.00	20°C	0.91	<0.05	0.03	21.11	26.02	40.00	20°C	ND	ND	ND	ND	ND
	40.00	30°C	0.96	<0.05	0.03	24.10	27.25	40.00	30°C	ND	ND	ND	ND	ND
	40.00	40°C	0.94	<0.05	0.03	31.14	34.72	40.00	40°C	ND	ND	ND	ND	ND
	48.00	10°C	0.96	<0.05	0.02	19.15	34.76	48.00	10°C	ND	ND	ND	ND	ND
	48.00	20°C	0.94	<0.05	0.03	21.74	27.96	48.00	20°C	ND	ND	ND	ND	ND
	48.00	30°C	0.94	<0.05	0.05	26.89	23.10	48.00	30°C	ND	ND	ND	ND	ND
	48.00	40°C	0.92	<0.05	0.05	34.44	26.26	48.00	40°C	ND	ND	ND	ND	ND
	56.00	10°C	0.82	<0.05	0.01	14.82	27.68	56.00	10°C	ND	ND	ND	ND	ND
	56.00	20°C	0.82	<0.05	0.02	15.02	35.47	56.00	20°C	ND	ND	ND	ND	ND
	56.00	30°C	0.92	<0.05	0.01	18.62	50.66	56.00	30°C	ND	ND	ND	ND	ND
	56.00	40°C	0.96	<0.05	0.01	21.27	57.86	56.00	40°C	ND	ND	ND	ND	ND
	64.00	10°C	0.89	<0.05	0.05	28.86	24.34	64.00	10°C	ND	ND	ND	ND	ND
	64.00	20°C	0.88	<0.05	0.02	24.33	33.08	64.00	20°C	ND	ND	ND	ND	ND
	64.00	30°C	0.95	<0.05	0.03	38.97	37.08	64.00	30°C	ND	ND	ND	ND	ND
	64.00	40°C	0.87	<0.05	0.02	38.28	36.19	64.00	40°C	ND	ND	ND	ND	ND
Tequila	40.00	10°C	0.96	<0.05	0.01	4.83	27.42	40.00	10°C	0.93	<0.05	1.53	0.23	0.39
	40.00	20°C	0.97	<0.05	0.00	7.24	41.67	40.00	20°C	0.93	<0.05	1.09	0.28	0.51
	40.00	30°C	0.95	<0.05	0.01	9.58	34.34	40.00	30°C	0.90	<0.05	2.12	0.32	0.39
	40.00	40°C	0.91	<0.05	0.01	11.13	32.75	40.00	40°C	0.82	<0.05	4.05	0.35	0.30
Bourbon	57.80	10°C	0.97	<0.05	0.03	5.84	13.82	57.80	10°C	0.90	<0.05	2.34	0.06	0.16
	57.80	20°C	0.96	<0.05	0.02	7.35	18.80	57.80	20°C	0.94	<0.05	0.27	0.08	0.55
	57.80	30°C	0.92	<0.05	0.01	10.74	30.86	57.80	30°C	0.92	<0.05	1.19	0.16	0.36
	57.80	40°C	0.98	<0.05	0.01	11.14	39.96	57.80	40°C	0.88	<0.05	0.31	0.12	0.63
NMSA	63.40	10°C	0.95	<0.05	0.02	14.69	28.70	63.40	10°C	0.96	<0.05	2.26	0.22	0.31
	63.40	20°C	0.98	<0.05	0.10	57.32	23.72	63.40	20°C	0.96	<0.05	0.45	0.07	0.41
	63.40	30°C	0.96	<0.05	0.06	52.44	28.57	63.40	30°C	0.92	<0.05	1.09	0.17	0.39
	63.40	40°C	0.94	<0.05	0.01	13.61	35.95	63.40	40°C	0.97	<0.05	1.22	0.10	0.29
NMSB	63.50	10°C	0.97	<0.05	0.01	6.35	24.88	63.50	10°C	0.92	<0.05	2.71	0.14	0.22
	63.50	20°C	0.91	<0.05	0.01	6.82	32.63	63.50	20°C	0.96	<0.05	0.85	0.11	0.36
	63.50	30°C	0.94	<0.05	0.01	9.94	26.75	63.50	30°C	0.95	<0.05	0.78	0.12	0.39
	63.50	40°C	0.98	<0.05	0.01	15.01	35.36	63.50	40°C	0.94	<0.05	1.35	0.20	0.39

	5-METHYL FURFURAL							5-HYDROMETHYL FURFURAL						
Spirit	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**
Spirit model	40.00	10°C	0.77	<0.05	1.02	1.58	1.24	40.00	10°C	97.78	<0.05	0.82	62.60	8.74
	40.00	20°C	0.72	<0.05	0.32	0.47	1.22	40.00	20°C	0.97	<0.05	0.57	57.09	9.98
	40.00	30°C	0.89	<0.05	0.23	2.38	3.22	40.00	30°C	0.97	<0.05	0.07	9.83	11.86
	40.00	40°C	0.89	<0.05	0.08	1.37	4.05	40.00	40°C	0.98	<0.05	0.04	5.99	12.03
	48.00	10°C	0.93	<0.05	0.05	1.08	4.69	48.00	10°C	0.95	<0.05	0.08	9.88	11.02
	48.00	20°C	0.90	<0.05	0.30	5.25	4.18	48.00	20°C	0.97	<0.05	0.07	8.30	10.69
	48.00	30°C	0.89	<0.05	0.17	2.84	4.14	48.00	30°C	0.98	<0.05	0.08	11.13	11.73
	48.00	40°C	0.75	<0.05	0.24	1.72	2.66	48.00	40°C	0.99	<0.05	0.13	16.58	11.16
	56.00	10°C	0.81	<0.05	1.32	7.09	2.32	56.00	10°C	0.99	<0.05	0.19	18.93	9.89
	56.00	20°C	0.91	<0.05	0.06	1.72	5.22	56.00	20°C	1.00	<0.05	0.08	13.89	13.10
	56.00	30°C	0.96	<0.05	0.30	6.98	4.86	56.00	30°C	0.89	<0.05	0.12	14.69	10.89
	56.00	40°C	0.84	<0.05	0.09	1.40	4.00	56.00	40°C	0.97	<0.05	0.15	15.89	11.66
	64.00	10°C	0.95	<0.05	0.09	2.71	5.37	64.00	10°C	0.98	<0.05	0.06	9.22	12.09
	64.00	20°C	0.93	<0.05	0.05	2.29	6.67	64.00	20°C	0.97	<0.05	0.04	8.52	14.05
	64.00	30°C	0.95	<0.05	0.03	1.51	7.52	64.00	30°C	0.99	<0.05	0.07	15.44	14.58
	64.00	40°C	0.98	<0.05	0.03	2.50	9.07	64.00	40°C	0.97	<0.05	0.06	14.37	14.14
Tequila	40.00	10°C	0.98	<0.05	0.03	0.44	3.92	40.00	10°C	0.97	<0.05	0.05	6.25	11.01
	40.00	20°C	0.91	<0.05	0.07	1.26	4.22	40.00	20°C	0.98	<0.05	0.04	5.90	10.78
	40.00	30°C	0.75	<0.05	0.38	4.03	3.27	40.00	30°C	0.97	<0.05	0.16	26.67	12.74
	40.00	40°C	0.74	<0.05	0.62	4.87	2.80	40.00	40°C	0.88	<0.05	0.11	15.74	11.76
Bourbon	57.80	10°C	0.97	<0.05	0.15	0.45	1.75	57.80	10°C	1.00	<0.05	0.22	20.88	9.68
	57.80	20°C	0.95	<0.05	0.12	0.39	1.72	57.80	20°C	0.99	<0.05	0.12	16.35	11.48
	57.80	30°C	0.91	<0.05	0.03	0.73	4.80	57.80	30°C	0.95	<0.05	0.41	66.47	12.79
	57.80	40°C	0.78	<0.05	0.34	0.59	1.32	57.80	40°C	0.99	<0.05	0.05	10.35	13.15
NMSA	63.40	10°C	0.96	<0.05	0.04	0.43	3.34	63.40	10°C	0.97	<0.05	0.07	8.74	11.12
	63.40	20°C	0.99	<0.05	0.74	2.54	1.85	63.40	20°C	0.99	<0.05	0.13	17.26	11.33
	63.40	30°C	0.83	<0.05	0.60	1.51	1.59	63.40	30°C	0.95	<0.05	0.05	10.36	13.89
	63.40	40°C	0.90	<0.05	0.04	0.63	3.97	63.40	40°C	0.98	<0.05	0.21	29.30	11.74
NMSB	63.50	10°C	0.99	<0.05	0.03	0.30	3.26	63.50	10°C	1.00	<0.05	0.07	8.15	11.16
	63.50	20°C	0.87	<0.05	0.22	0.77	1.86	63.50	20°C	0.93	<0.05	0.40	49.18	11.14
	63.50	30°C	0.97	<0.05	0.26	2.38	3.05	63.50	30°C	0.98	<0.05	0.04	12.30	18.72
	63.50	40°C	0.93	<0.05	0.28	2.47	2.97	63.50	40°C	0.98	<0.05	0.08	8.90	11.70

	GUAIACOL							4-ETHYL GUIACOL						
Spirit	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**
Spirit model	40.00	10°C	0.94	<0.05	0.10	0.02	0.43	40.00	10°C	0.73	<0.05	2.07	0.06	0.17
	40.00	20°C	0.97	<0.05	0.12	0.02	0.44	40.00	20°C	0.95	<0.05	0.33	0.04	0.35
	40.00	30°C	0.95	<0.05	0.50	0.05	0.33	40.00	30°C	0.94	<0.05	0.70	0.13	0.44
	40.00	40°C	0.89	<0.05	5.87	0.35	0.24	40.00	40°C	0.95	<0.05	0.47	0.06	0.36
	48.00	10°C	0.99	<0.05	0.53	0.06	0.35	48.00	10°C	0.97	<0.05	0.36	0.04	0.34
	48.00	20°C	0.95	<0.05	7.31	0.43	0.24	48.00	20°C	0.98	<0.05	0.99	0.09	0.30
	48.00	30°C	0.99	<0.05	1.44	0.17	0.35	48.00	30°C	0.95	<0.05	1.48	0.12	0.29
	48.00	40°C	0.95	<0.05	2.82	0.24	0.29	48.00	40°C	0.97	<0.05	0.64	0.12	0.43
	56.00	10°C	0.97	<0.05	0.08	0.05	0.82	56.00	10°C	0.99	<0.05	0.20	0.07	0.60
	56.00	20°C	0.97	<0.05	0.38	0.06	0.41	56.00	20°C	0.98	<0.05	0.60	0.13	0.47
	56.00	30°C	0.89	<0.05	3.10	0.21	0.26	56.00	30°C	0.97	<0.05	3.11	0.29	0.31
	56.00	40°C	0.88	<0.05	5.91	0.31	0.23	56.00	40°C	0.99	<0.05	2.30	0.22	0.31
	64.00	10°C	0.90	<0.05	1.02	0.05	0.22	64.00	10°C	0.96	<0.05	1.11	0.06	0.24
	64.00	20°C	0.97	<0.05	0.82	0.06	0.27	64.00	20°C	0.95	<0.05	1.79	0.16	0.30
	64.00	30°C	0.96	<0.05	0.76	0.09	0.35	64.00	30°C	0.97	<0.05	0.52	0.09	0.41
	64.00	40°C	0.95	<0.05	1.39	0.18	0.36	64.00	40°C	0.99	<0.05	0.81	0.16	0.45
Tequila	40.00	10°C	0.75	<0.05	0.32	0.03	0.29	40.00	10°C	0.96	<0.05	0.05	0.01	0.46
	40.00	20°C	0.87	<0.05	0.09	0.02	0.45	40.00	20°C	0.89	<0.05	0.74	0.07	0.32
	40.00	30°C	0.77	<0.05	0.14	0.02	0.40	40.00	30°C	0.95	<0.05	0.90	0.10	0.33
	40.00	40°C	0.89	<0.05	0.04	0.02	0.64	40.00	40°C	0.91	<0.05	0.35	0.06	0.43
Bourbon	57.80	10°C	0.94	<0.05	1.61	0.13	0.28	57.80	10°C	1.00	<0.05	0.29	0.03	0.34
	57.80	20°C	0.92	<0.05	0.35	0.05	0.40	57.80	20°C	0.94	<0.05	0.18	0.04	0.49
	57.80	30°C	0.86	<0.05	0.03	0.02	0.93	57.80	30°C	0.94	<0.05	0.00	0.01	1.88
	57.80	40°C	0.93	<0.05	0.02	0.03	1.29	57.80	40°C	0.94	<0.05	0.00	0.01	11.00
NMSA	63.40	10°C	0.95	<0.05	0.74	0.08	0.32	63.40	10°C	0.89	<0.05	2.80	0.44	0.40
	63.40	20°C	0.86	<0.05	0.92	0.04	0.20	63.40	20°C	0.93	<0.05	0.89	0.26	0.54
	63.40	30°C	0.96	<0.05	1.40	0.21	0.39	63.40	30°C	0.89	<0.05	0.81	0.31	0.62
	63.40	40°C	0.89	<0.05	2.01	0.11	0.24	63.40	40°C	0.98	<0.05	0.33	0.20	0.79
NMSB	63.50	10°C	0.98	<0.05	0.57	0.03	0.22	63.50	10°C	0.99	<0.05	0.68	0.29	0.65
	63.50	20°C	0.99	<0.05	0.11	0.02	0.45	63.50	20°C	0.95	<0.05	2.36	0.38	0.40
	63.50	30°C	1.00	<0.05	0.16	0.06	0.60	63.50	30°C	0.97	<0.05	0.56	0.23	0.64
	63.50	40°C	0.97	<0.05	0.07	0.02	0.56	63.50	40°C	0.98	<0.05	0.87	0.55	0.79

	EUGENOL							VANILLIN						
Spirit	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**
Spirit model	40.00	10°C	0.96	<0.05	0.73	0.04	0.23	40.00	10°C	0.91	<0.05	0.04	0.89	4.81
	40.00	20°C	0.80	<0.05	0.78	0.05	0.24	40.00	20°C	0.97	<0.05	0.02	0.86	6.34
	40.00	30°C	0.89	<0.05	0.42	0.18	0.65	40.00	30°C	0.92	<0.05	0.04	0.73	4.28
	40.00	40°C	0.85	<0.05	0.34	0.31	0.96	40.00	40°C	0.79	<0.05	0.05	2.20	6.80
	48.00	10°C	0.98	<0.05	0.27	0.04	0.38	48.00	10°C	0.98	<0.05	0.07	0.53	2.68
	48.00	20°C	0.90	<0.05	0.32	0.07	0.46	48.00	20°C	0.95	<0.05	0.13	1.49	3.36
	48.00	30°C	0.97	<0.05	0.38	0.11	0.54	48.00	30°C	0.86	<0.05	0.21	8.09	6.26
	48.00	40°C	0.89	<0.05	0.26	0.09	0.59	48.00	40°C	0.92	<0.05	0.05	2.91	7.89
	56.00	10°C	0.95	<0.05	0.10	0.05	0.74	56.00	10°C	0.82	<0.05	0.08	1.04	3.55
	56.00	20°C	0.94	<0.05	0.32	0.07	0.46	56.00	20°C	0.86	<0.05	0.14	1.93	3.77
	56.00	30°C	0.97	<0.05	0.81	0.17	0.45	56.00	30°C	0.94	<0.05	0.03	1.14	6.12
	56.00	40°C	0.97	<0.05	0.90	0.27	0.54	56.00	40°C	0.97	<0.05	0.05	1.96	6.55
	64.00	10°C	0.85	<0.05	0.48	0.05	0.33	64.00	10°C	0.98	<0.05	0.17	0.94	2.37
	64.00	20°C	0.88	<0.05	0.73	0.13	0.43	64.00	20°C	0.96	<0.05	0.07	0.81	3.49
	64.00	30°C	0.95	<0.05	0.70	0.16	0.48	64.00	30°C	0.95	<0.05	0.05	1.40	5.17
	64.00	40°C	0.96	<0.05	0.33	0.18	0.73	64.00	40°C	0.98	<0.05	0.02	1.15	7.60
Tequila	40.00	10°C	0.96	<0.05	0.08	0.03	0.61	40.00	10°C	0.95	<0.05	0.05	0.33	2.59
	40.00	20°C	0.95	<0.05	0.43	0.09	0.46	40.00	20°C	0.91	<0.05	0.01	0.51	6.91
	40.00	30°C	0.93	<0.05	0.23	0.08	0.58	40.00	30°C	0.89	<0.05	0.02	0.42	4.97
	40.00	40°C	0.99	<0.05	5.48	0.67	0.35	40.00	40°C	0.92	<0.05	0.03	0.96	5.54
Bourbon	57.80	10°C	0.98	<0.05	0.69	0.13	0.43	57.80	10°C	0.99	<0.05	0.13	0.39	1.71
	57.80	20°C	0.91	<0.05	2.20	0.32	0.38	57.80	20°C	0.93	<0.05	0.10	0.81	2.91
	57.80	30°C	0.90	<0.05	2.61	0.28	0.33	57.80	30°C	0.94	<0.05	0.05	0.79	3.98
	57.80	40°C	0.95	<0.05	1.57	0.24	0.39	57.80	40°C	0.95	<0.05	0.08	1.48	4.19
NMSA	63.40	10°C	0.96	<0.05	0.58	0.08	0.38	63.40	10°C	0.90	<0.05	0.21	0.67	1.76
	63.40	20°C	0.90	<0.05	1.76	0.34	0.41	63.40	20°C	0.96	<0.05	0.09	0.43	2.18
	63.40	30°C	0.93	<0.05	1.86	0.35	0.44	63.40	30°C	0.97	<0.05	0.07	0.85	3.37
	63.40	40°C	0.94	<0.05	0.19	0.15	0.87	63.40	40°C	0.95	<0.05	0.05	0.67	3.62
NMSB	63.50	10°C	0.99	<0.05	0.22	0.12	0.74	63.50	10°C	0.96	<0.05	0.05	0.24	2.23
	63.50	20°C	0.94	<0.05	0.56	0.13	0.47	63.50	20°C	0.95	<0.05	0.14	0.60	2.04
	63.50	30°C	0.96	<0.05	0.25	0.10	0.63	63.50	30°C	0.96	<0.05	0.10	1.08	3.28
	63.50	40°C	0.98	<0.05	0.64	0.20	0.56	63.50	40°C	0.88	<0.05	0.04	0.90	4.50

	TRANS-WHISKY LACTONE							CIS-WHISKY LACTONE						
Spirit	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**
Spirit model	40.00	10°C	ND	ND	nd	nd	nd	40.00	10°C	0.94	<0.05	0.11	0.73	2.62
	40.00	20°C	0.99	<0.05	nd	nd	nd	40.00	20°C	0.97	<0.05	0.42	2.80	2.59
	40.00	30°C	0.92	<0.05	0.43	0.08	0.42	40.00	30°C	0.95	<0.05	0.31	2.79	3.01
	40.00	40°C	0.96	<0.05	2.35	0.35	0.38	40.00	40°C	0.91	<0.05	0.35	3.43	3.12
	48.00	10°C	0.75	<0.05	6.66	0.19	0.17	48.00	10°C	0.97	<0.05	0.07	0.64	3.12
	48.00	20°C	0.98	<0.05	1.69	0.17	0.32	48.00	20°C	0.97	<0.05	0.10	1.34	3.65
	48.00	30°C	0.94	<0.05	4.35	0.25	0.28	48.00	30°C	0.90	<0.05	0.33	2.75	2.87
	48.00	40°C	0.96	<0.05	7.47	0.83	0.33	48.00	40°C	0.93	<0.05	0.39	4.46	3.39
	56.00	10°C	0.98	<0.05	0.59	0.13	0.47	56.00	10°C	0.95	<0.05	0.12	1.03	2.96
	56.00	20°C	0.98	<0.05	0.89	0.13	0.38	56.00	20°C	0.96	<0.05	0.09	1.51	4.17
	56.00	30°C	0.82	<0.05	4.03	0.23	0.24	56.00	30°C	0.86	<0.05	2.30	26.42	3.39
	56.00	40°C	0.83	<0.05	3.17	0.20	0.25	56.00	40°C	0.97	<0.05	0.44	6.48	3.85
	64.00	10°C	0.79	<0.05	nd	nd	nd	64.00	10°C	0.98	<0.05	0.06	0.95	4.12
	64.00	20°C	0.96	<0.05	0.26	0.05	0.45	64.00	20°C	0.94	<0.05	0.09	1.90	4.61
	64.00	30°C	0.99	<0.05	0.59	0.12	0.45	64.00	30°C	0.93	<0.05	0.08	2.02	5.04
	64.00	40°C	0.97	<0.05	2.22	0.29	0.36	64.00	40°C	0.93	<0.05	0.40	7.48	4.30
Tequila	40.00	10°C	0.92	<0.05	nd	nd	nd	40.00	10°C	0.92	<0.05	0.06	0.45	2.81
	40.00	20°C	0.96	<0.05	nd	nd	nd	40.00	20°C	0.86	<0.05	0.02	0.69	6.40
	40.00	30°C	0.85	<0.05	nd	nd	nd	40.00	30°C	0.94	<0.05	0.68	5.95	2.95
	40.00	40°C	0.87	<0.05	2.83	nd	0.34	40.00	40°C	0.94	<0.05	1.32	11.38	2.94
Bourbon	57.80	10°C	0.92	<0.05	1.63	0.12	0.27	57.80	10°C	0.98	<0.05	0.10	0.68	2.59
	57.80	20°C	0.89	<0.05	7.59	1.30	0.41	57.80	20°C	0.98	<0.05	0.22	1.80	2.86
	57.80	30°C	0.94	<0.05	0.77	0.21	0.52	57.80	30°C	0.87	<0.05	0.22	2.43	3.34
	57.80	40°C	0.95	<0.05	3.17	0.75	0.49	57.80	40°C	0.99	<0.05	0.42	4.77	3.36
NMSA	63.40	10°C	0.96	<0.05	1.35	0.22	0.40	63.40	10°C	0.93	<0.05	0.09	0.54	2.45
	63.40	20°C	0.79	<0.05	1.70	0.43	0.51	63.40	20°C	0.91	<0.05	0.56	3.39	2.46
	63.40	30°C	0.98	<0.05	0.66	0.24	0.61	63.40	30°C	0.85	<0.05	1.40	10.30	2.71
	63.40	40°C	0.95	<0.05	2.80	0.85	0.55	63.40	40°C	1.00	<0.05	0.99	9.37	3.07
NMSB	63.50	10°C	0.81	<0.05	0.19	0.50	0.44	63.50	10°C	0.96	<0.05	0.06	0.55	3.05
	63.50	20°C	0.98	<0.05	3.37	0.83	0.50	63.50	20°C	0.86	<0.05	0.33	1.95	2.44
	63.50	30°C	0.92	<0.05	3.18	0.86	0.52	63.50	30°C	0.97	<0.05	0.57	5.35	3.05
	63.50	40°C	0.95	<0.05	1.41	0.71	0.71	63.50	40°C	0.87	<0.05	0.56	3.55	2.52

	γ-NONALACTONE							GALLIC ACID					
Spirit	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**
Spirit model	10°C	0.75	<0.05	nd	nd	nd	40.00	10°C	0.98	<0.05	0.13	10.40	8.91
	20°C	0.86	<0.05	nd	nd	nd	40.00	20°C	0.99	<0.05	0.15	12.07	8.96
	30°C	0.95	<0.05	nd	nd	nd	40.00	30°C	1.00	<0.05	0.25	17.57	8.43
	40°C	0.81	<0.05	0.36	0.03	0.23	40.00	40°C	0.99	<0.05	0.30	24.20	9.04
	10°C	0.96	<0.05	0.26	0.02	0.35	48.00	10°C	0.99	<0.05	0.29	12.69	6.66
	20°C	0.97	<0.05	0.28	0.29	3.71	48.00	20°C	1.00	<0.05	2.14	89.23	6.46
	30°C	0.90	<0.05	0.29	0.13	1.55	48.00	30°C	1.00	<0.05	0.56	50.51	9.49
	40°C	0.97	<0.05	0.39	0.17	1.14	48.00	40°C	1.00	<0.05	0.83	65.61	8.90
	10°C	0.99	<0.05	1.43	0.03	0.01	56.00	10°C	0.97	<0.05	0.08	7.25	9.49
	20°C	0.98	<0.05	0.49	0.05	0.19	56.00	20°C	1.00	<0.05	0.47	17.93	6.20
	30°C	0.91	<0.05	0.19	0.06	1.62	56.00	30°C	0.99	<0.05	0.24	14.19	7.69
	40°C	0.94	<0.05	0.21	0.09	1.94	56.00	40°C	0.99	<0.05	0.91	63.66	8.35
	10°C	0.98	<0.05	0.29	0.08	0.96	64.00	10°C	1.00	<0.05	0.65	27.67	6.51
	20°C	0.90	<0.05	0.69	0.03	0.05	64.00	20°C	0.99	<0.05	0.30	14.60	7.02
	30°C	0.97	<0.05	0.47	0.08	0.38	64.00	30°C	0.99	<0.05	0.14	9.52	8.35
	40°C	0.95	<0.05	0.46	0.21	0.99	64.00	40°C	0.99	<0.05	0.21	15.72	8.61
Tequila	10°C	0.96	<0.05	nd	nd	nd	40.00	10°C	1.00	<0.05	0.68	35.28	7.18
	20°C	0.90	<0.05	nd	nd	nd	40.00	20°C	0.97	<0.05	0.51	28.55	7.49
	30°C	0.82	<0.05	nd	nd	nd	40.00	30°C	1.00	<0.05	0.21	19.85	9.71
	40°C	0.95	<0.05	nd	nd	nd	40.00	40°C	0.99	<0.05	0.21	21.24	10.02
Bourbon	10°C	0.9164	<0.05	2.031	0.09	0.21	57.80	10°C	0.99	<0.05	0.41	22.39	7.40
	20°C	0.9895	<0.05	0.364	0.08	0.47	57.80	20°C	0.98	<0.05	0.28	20.93	8.68
	30°C	0.9085	<0.05	1.238	0.13	0.32	57.80	30°C	0.98	<0.05	0.13	12.06	9.79
	40°C	0.9398	<0.05	0.547	0.05	0.31	57.80	40°C	0.98	<0.05	1.37	115.07	9.16
NMSA	10°C	0.918	<0.05	0.06	0.40	0.39	63.40	10°C	0.98	<0.05	0.04	5.44	11.76
	20°C	0.958	<0.05	2.815	0.64	0.48	63.40	20°C	0.99	<0.05	0.19	14.08	8.68
	30°C	0.9714	<0.05	3.86	0.89	0.48	63.40	30°C	0.99	<0.05	0.11	10.61	9.84
	40°C	0.9381	<0.05	2.718	0.98	0.60	63.40	40°C	0.94	<0.05	2.62	175.75	8.19
NMSB	10°C	0.989	<0.05	0.524	0.23	0.66	63.50	10°C	1.00	<0.05	0.20	11.72	7.64
	20°C	0.8945	<0.05	0.895	0.28	0.56	63.50	20°C	0.96	<0.05	0.09	8.98	10.17
	30°C	0.9636	<0.05	1.981	0.59	0.55	63.50	30°C	0.96	<0.05	0.06	5.54	9.43
	40°C	0.9866	<0.05	0.302	0.16	0.74	63.50	40°C	0.99	<0.05	0.11	11.56	10.26

	VANILLIC ACID							SYRINGIC ACID					
Spirit	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**
Spirit model	10°C	0.99	<0.05	0.25	17.72	8.37	40.00	10°C	0.98	<0.05	0.07	10.33	12.06
	20°C	0.99	<0.05	0.33	23.31	8.41	40.00	20°C	0.95	<0.05	0.34	40.27	10.92
	30°C	0.98	<0.05	0.24	18.18	8.72	40.00	30°C	0.95	<0.05	0.30	41.10	11.76
	40°C	0.96	<0.05	0.09	8.44	9.65	40.00	40°C	0.85	<0.05	0.03	5.91	14.97
	10°C	1.00	<0.05	0.38	18.30	6.94	48.00	10°C	0.99	<0.05	0.21	14.49	8.33
	20°C	1.00	<0.05	0.55	28.26	7.15	48.00	20°C	0.99	<0.05	0.13	11.39	9.32
	30°C	1.00	<0.05	3.75	283.29	8.69	48.00	30°C	0.95	<0.05	0.18	24.56	11.60
	40°C	0.99	<0.05	0.31	28.68	9.67	48.00	40°C	0.98	<0.05	0.07	16.14	14.94
	10°C	0.99	<0.05	0.28	18.90	8.26	56.00	10°C	0.99	<0.05	0.40	41.92	10.28
	20°C	0.99	<0.05	0.83	58.15	8.35	56.00	20°C	0.99	<0.05	0.27	29.15	10.32
	30°C	0.99	<0.05	0.55	31.33	7.56	56.00	30°C	0.99	<0.05	0.09	12.39	11.49
	40°C	0.99	<0.05	0.13	10.63	9.02	56.00	40°C	0.98	<0.05	0.05	8.76	13.05
	10°C	1.00	<0.05	0.61	28.90	6.88	64.00	10°C	0.98	<0.05	0.35	21.57	7.83
	20°C	1.00	<0.05	0.27	14.28	7.28	64.00	20°C	1.00	<0.05	0.17	12.26	8.59
	30°C	0.99	<0.05	0.24	15.49	7.99	64.00	30°C	0.99	<0.05	0.26	25.19	9.82
	40°C	0.96	<0.05	0.07	7.17	9.90	64.00	40°C	0.93	<0.05	0.03	5.84	14.51
Tequila	10°C	0.98	<0.05	0.19	9.17	7.00	40.00	10°C	0.94	<0.05	0.09	6.02	8.07
	20°C	1.00	<0.05	1.26	59.77	6.88	40.00	20°C	0.95	<0.05	0.63	39.53	7.89
	30°C	1.00	<0.05	0.33	19.63	7.66	40.00	30°C	0.99	<0.05	0.39	27.48	8.34
	40°C	0.99	<0.05	0.35	23.70	8.26	40.00	40°C	1.00	<0.05	0.19	19.54	10.26
Bourbon	10°C	1.00	<0.05	1.12	46.75	6.46	57.80	10°C	0.99	<0.05	0.60	29.41	7.02
	20°C	1.00	<0.05	0.20	12.94	8.02	57.80	20°C	0.98	<0.05	0.32	20.81	8.08
	30°C	1.00	<0.05	1.01	55.55	7.42	57.80	30°C	0.99	<0.05	0.33	26.24	8.98
	40°C	0.98	<0.05	0.27	17.64	8.09	57.80	40°C	0.95	<0.05	0.17	17.37	10.15
NMSA	10°C	1.00	<0.05	0.34	16.84	7.00	63.40	10°C	0.97	<0.05	0.13	8.81	8.18
	20°C	1.00	<0.05	1.54	71.47	6.80	63.40	20°C	0.98	<0.05	0.35	20.49	7.69
	30°C	0.97	<0.05	0.13	8.35	8.05	63.40	30°C	0.93	<0.05	0.06	5.85	9.80
	40°C	0.99	<0.05	0.20	11.94	7.73	63.40	40°C	0.94	<0.05	0.07	6.61	10.02
NMSB	10°C	1.00	<0.05	0.40	18.58	6.79	63.50	10°C	0.99	<0.05	0.16	9.49	7.77
	20°C	0.93	<0.05	0.07	5.18	8.87	63.50	20°C	0.87	<0.05	0.03	3.63	11.95
	30°C	0.98	<0.05	0.14	9.82	8.43	63.50	30°C	0.95	<0.05	0.07	8.85	11.02
	40°C	1.00	<0.05	0.34	23.67	8.40	63.50	40°C	0.88	<0.05	0.22	18.50	9.08

	ELLAGIC ACID							SYRINGALDEHYDE					
Spirit	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**
Spirit model	10°C	0.99	<0.05	0.08	10.43	11.13	40.00	10°C	0.98	<0.05	0.02	12.10	22.33
	20°C	0.96	<0.05	0.20	38.32	13.72	40.00	20°C	0.97	<0.05	0.03	17.00	22.83
	30°C	0.98	<0.05	0.04	13.41	17.46	40.00	30°C	0.93	<0.05	0.02	15.09	26.58
	40°C	0.91	<0.05	0.02	13.15	28.52	40.00	40°C	0.99	<0.05	0.03	27.61	28.19
	10°C	0.95	<0.05	0.05	8.18	12.88	48.00	10°C	0.98	<0.05	0.05	8.43	13.59
	20°C	0.95	<0.05	0.04	11.06	16.66	48.00	20°C	0.91	<0.05	0.04	10.14	16.35
	30°C	0.97	<0.05	0.01	8.96	40.38	48.00	30°C	0.94	<0.05	0.01	11.55	29.12
	40°C	0.97	<0.05	0.01	13.38	41.11	48.00	40°C	0.96	<0.05	0.01	10.43	43.88
	10°C	0.94	<0.05	0.02	6.88	17.76	56.00	10°C	0.97	<0.05	0.07	18.77	16.60
	20°C	0.98	<0.05	0.03	11.29	20.42	56.00	20°C	0.99	<0.05	0.03	12.91	20.63
	30°C	0.96	<0.05	0.01	10.19	34.18	56.00	30°C	0.95	<0.05	0.01	7.99	28.92
	40°C	0.99	<0.05	0.02	28.41	37.47	56.00	40°C	0.98	<0.05	0.01	11.53	30.53
	10°C	1.00	<0.05	0.06	15.21	15.64	64.00	10°C	0.98	<0.05	0.07	11.13	13.04
	20°C	1.00	<0.05	0.01	5.89	24.26	64.00	20°C	0.97	<0.05	0.03	8.62	15.75
	30°C	0.92	<0.05	0.01	5.39	22.46	64.00	30°C	0.99	<0.05	0.03	14.91	21.00
	40°C	0.96	<0.05	0.01	12.94	34.81	64.00	40°C	0.92	<0.05	0.01	8.23	35.24
Tequila	10°C	0.99	<0.05	0.07	10.73	12.48	40.00	10°C	0.73	<0.05	0.01	2.81	17.13
	20°C	0.96	<0.05	0.05	13.20	15.74	40.00	20°C	0.89	<0.05	0.07	13.50	13.72
	30°C	0.96	<0.05	0.04	27.95	25.11	40.00	30°C	0.95	<0.05	0.05	11.32	14.82
	40°C	0.96	<0.05	0.02	25.42	33.70	40.00	40°C	0.95	<0.05	0.02	9.71	21.56
Bourbon	10°C	1.00	<0.05	0.06	15.81	16.29	57.80	10°C	0.98	<0.05	0.11	11.85	10.47
	20°C	0.98	<0.05	0.02	15.39	25.40	57.80	20°C	0.98	<0.05	0.15	21.24	11.82
	30°C	0.95	<0.05	0.01	16.02	32.83	57.80	30°C	0.99	<0.05	0.03	11.12	19.30
	40°C	0.90	<0.05	0.01	22.07	51.70	57.80	40°C	0.96	<0.05	0.02	12.25	22.50
NMSA	10°C	1.00	<0.05	0.02	10.14	20.28	63.40	10°C	0.92	<0.05	0.03	6.14	14.87
	20°C	0.94	<0.05	0.01	9.24	30.17	63.40	20°C	0.97	<0.05	0.07	13.22	13.61
	30°C	0.95	<0.05	0.01	12.58	41.80	63.40	30°C	0.92	<0.05	0.01	4.65	22.00
	40°C	0.98	<0.05	0.01	30.80	46.62	63.40	40°C	0.88	<0.05	0.01	4.26	21.04
NMSB	10°C	0.96	<0.05	0.04	13.85	18.74	63.50	10°C	0.92	<0.05	0.04	5.69	12.48
	20°C	0.96	<0.05	0.02	17.12	27.04	63.50	20°C	0.94	<0.05	0.04	7.79	14.86
	30°C	0.99	<0.05	0.01	9.26	39.44	63.50	30°C	0.92	<0.05	0.03	13.30	21.50
	40°C	0.96	<0.05	0.01	19.98	53.44	63.50	40°C	0.85	<0.05	0.13	53.68	20.64

	CONIFERALDEHYDE							SINAPALDEHYDE					
Spirit	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**	**% ABV**	**Temp °C**	**R2**	**p**	**K (mg/L-days)**	**h (mg/L-days)**	**Conc.sat (mg/L)**
Spirit model	10°C	0.98	<0.05	0.01	4.25	19.99	40.00	10°C	0.98	<0.05	0.02	7.25	19.67
	20°C	0.96	<0.05	0.03	11.76	19.87	40.00	20°C	0.98	<0.05	0.03	16.33	22.45
	30°C	0.96	<0.05	0.02	14.47	24.10	40.00	30°C	0.99	<0.05	0.00	1.06	15.87
	40°C	0.99	<0.05	0.04	28.99	27.95	40.00	40°C	0.99	<0.05	0.05	46.31	29.59
	10°C	0.94	<0.05	0.02	2.92	13.38	48.00	10°C	0.93	<0.05	0.01	5.84	19.86
	20°C	0.94	<0.05	0.02	5.99	17.27	48.00	20°C	0.98	<0.05	0.03	14.90	21.77
	30°C	0.89	<0.05	0.03	19.94	24.92	48.00	30°C	0.98	<0.05	0.05	33.07	26.62
	40°C	0.93	<0.05	0.03	29.20	30.96	48.00	40°C	0.93	<0.05	0.02	22.84	34.03
	10°C	0.92	<0.05	0.02	5.77	18.66	56.00	10°C	0.97	<0.05	0.03	10.77	19.78
	20°C	0.98	<0.05	0.01	7.05	24.33	56.00	20°C	0.96	<0.05	0.01	10.71	30.31
	30°C	0.96	<0.05	0.01	9.98	30.10	56.00	30°C	0.99	<0.05	0.02	22.85	33.16
	40°C	0.98	<0.05	0.04	31.80	27.73	56.00	40°C	0.99	<0.05	0.06	66.06	34.62
	10°C	0.96	<0.05	0.03	5.30	13.51	64.00	10°C	0.97	<0.05	0.02	10.86	21.29
	20°C	0.98	<0.05	0.01	4.68	20.04	64.00	20°C	0.99	<0.05	0.01	9.32	29.89
	30°C	0.97	<0.05	0.04	20.93	23.86	64.00	30°C	0.97	<0.05	0.03	29.85	31.76
	40°C	0.97	<0.05	0.02	16.72	31.47	64.00	40°C	0.99	<0.05	0.02	32.82	37.67
Tequila	10°C	0.97	<0.05	0.04	2.62	8.45	40.00	10°C	0.80	<0.05	0.01	3.90	18.86
	20°C	0.99	<0.05	0.02	4.18	13.19	40.00	20°C	0.98	<0.05	0.03	10.70	19.51
	30°C	0.90	<0.05	0.03	12.75	21.92	40.00	30°C	0.97	<0.05	0.03	16.98	24.86
	40°C	0.93	<0.05	0.02	10.99	23.45	40.00	40°C	0.97	<0.05	0.06	39.34	25.75
Bourbon	10°C	0.89	<0.05	0.01	2.59	15.27	57.80	10°C	0.99	<0.05	0.04	9.45	15.51
	20°C	0.97	<0.05	0.02	4.33	15.52	57.80	20°C	0.98	<0.05	0.02	9.81	24.03
	30°C	0.93	<0.05	0.00	3.30	28.43	57.80	30°C	0.97	<0.05	0.02	21.98	30.67
	40°C	0.82	<0.05	0.01	3.53	21.23	57.80	40°C	0.85	<0.05	0.05	31.03	23.78
NMSA	10°C	0.92	<0.05	0.03	6.14	14.87	63.40	10°C	0.91	<0.05	0.02	6.68	21.04
	20°C	0.97	<0.05	0.07	13.22	13.61	63.40	20°C	0.91	<0.05	0.01	7.77	26.39
	30°C	0.92	<0.05	0.01	4.65	22.00	63.40	30°C	0.92	<0.05	0.00	7.95	41.73
	40°C	0.92	<0.05	0.01	4.26	21.04	63.40	40°C	0.93	<0.05	0.02	13.34	28.68
NMSB	10°C	0.95	<0.05	0.02	2.85	11.25	63.50	10°C	0.97	<0.05	0.04	11.31	17.06
	20°C	0.93	<0.05	0.04	5.55	11.39	63.50	20°C	0.97	<0.05	0.08	25.86	18.07
	30°C	0.96	<0.05	0.02	12.44	27.47	63.50	30°C	0.96	<0.05	0.01	13.26	35.21
	40°C	0.84	<0.05	0.05	20.91	20.84	63.50	40°C	0.89	<0.05	0.05	35.89	27.05